THE PHYSIOLOGIST

A Publication of the American Physiological Society Volume 31, Number 4 August 1988

GUEST EDITORIAL

That Animals Might Live . . .

. . . your patient, and certainly future patients, must die. That is the real message underlying the rhetoric with which animal rights proponents appeal to public sympathy.

Their tactics are varied, their arguments specious, but their actual aim is simple: to quickly bring the day when there are no animals in the lab. That this may not be the same day when all disease is readily cured or prevented by the methods at hand is not *their* problem—it is yours, and your patient's, and that of all future generations. The animal rights movement is a serious public health hazard.

The activists, whose premise is "a rat is a pig is a dog is a boy," (1) will claim all of the credit for animal lives saved. But they will accept none of the blame for human lives lost. Rather than admit the consequences of their position, they mask their goal with emotionally appealing arguments calculated to garner widespread support.

In public forums, "a rat is a pig is a dog is a boy" becomes, "we're simply asking people to extend their circle of consideration to all creatures with whom we share the earth." (2) Similarly, their abolitionist goal is modified publicly to, "We merely wish to stop needless animal suffering." (3) What they seldom admit is that, in their view, the mere caging of an animal constitutes "needless animal suffering."

The effectiveness of animal rights activists cannot be discounted. With extremist elements setting the pace, the movement has already measurably impeded research. And the activists have recruited media

(*Continued on p. 58*)

Travel Grant Applications Available for IUPS Congress in Helsinki

The U.S. National Committee for the International Union for Physiological Sciences is seeking applications for travel awards for the XXXI IUPS Congress in Helsinki, Finland, July 9–14, 1989.

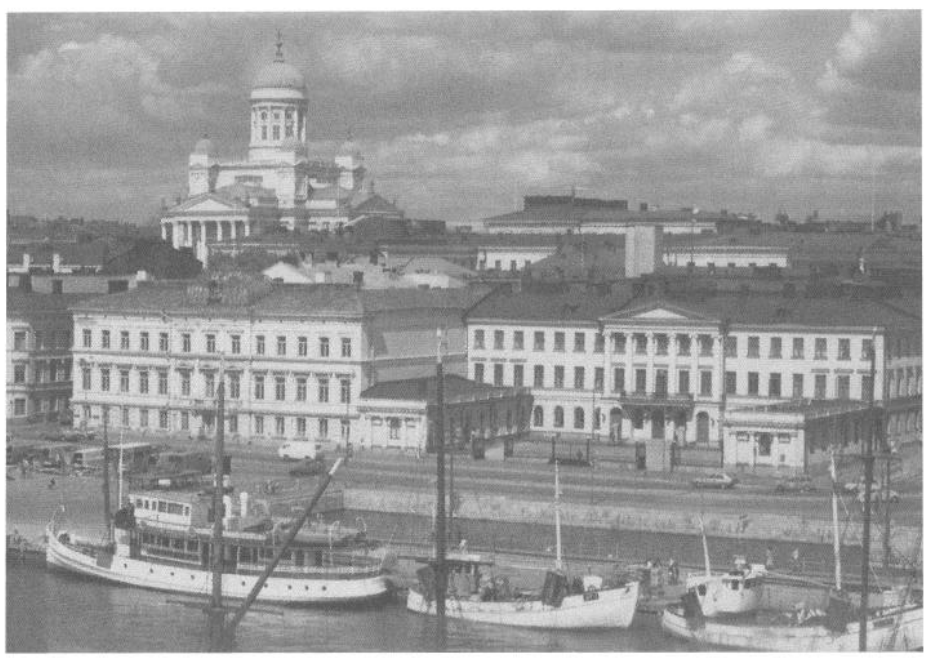
The Committee will screen the applications and the awards will be made by APS, which is raising funds for the travel. The travel awards will be ~$100 less than the lowest-cost round-trip air fare from the recipient's nearest gateway city to Helsinki.

The awards are intended for individuals who have no other source of funds to attend the Congress. Federal employees are eligible.

It is anticipated that more applications will be received than can be funded. To achieve as high a rank as possible, these factors should be considered:

- Complete all questions on the application.
- Provide copies of letters of invitation if you have been invited to the Congress to make a presentation.
- Provide an indication of participation in the Congress including presentations and attendance for most or all sessions.
- Have travel plans that include other professional visits or work.

Deadline for submission of application for travel award is December 15, 1988. **The application is on p. 89.** All applicants must submit six copies of the application to USNC/IUPS, National Academy of Sciences, attn June Ewing, 2101 Constitution Avenue, NW, Washington, DC 20418.



CONTENTS

The Physiologist
Published bimonthly and distributed by
The American Physiological Society
9650 Rockville Pike
Bethesda, Maryland 20814
ISSN 0031-9376

Martin Frank, *Editor* and *Executive Director*

Aubrey E. Taylor, *President*
Harvey V. Sparks, Jr., *Past President*
Vernon S. Bishop, *President-Elect*

Councillors
Beverly Bishop, Shu Chien, Allen W. Cowley, Jr., Gerhard H. Giebisch, Norman C. Staub, and Peter D. Wagner

Ex Officio
Carl V. Gisolfi, Francis J. Haddy, Paul C. Johnson, R. Blake Reeves, and W. S. Spielman

Publications Committee: *Chairman*, Paul C. Johnson; *Members*, Francois Abboud, John S. Cook, Melvin J. Fregly, and Stephen H. White. *Publications Manager*, Brenda B. Rauner; *Editorial Staff*, Laura North and Lorraine Tucker.

Subscriptions: Distributed to members as part of their membership; nonmembers and institutions, $25.00 per year in the United States; elsewhere $35.00. Single copies and back issues when available, $5.00 each; single copies and back issues of Fall Abstracts issue when available, $20.00. In 1988 subscribers to *The Physiologist* will receive it and the abstracts of the Fall Meeting of the American Physiological Society. The American Physiological Society assumes no responsibility for the statements and opinions advanced by contributors to *The Physiologist*.

Deadline for submission of material for publication: Dec. 5, February issue; Feb. 5, April issue; April 5, June issue; June 5, August issue; Aug. 5, October issue; Oct. 5, December issue. **If you change your address or telephone number, please notify the central office as soon as possible.**

Headquarters phone: (301) 530-7164. TELEFAX: (301) 571-1844.

EDITORIAL

(*Continued from p. 57*)

stars, public figures, and even some doctors. By selective use of facts, they have aroused the sentiments of a minority of pet lovers, and humane society contributors, as well as the moral indignation of legislators, whom they urge to ban all research on "sentient beings"—except sick patients.

The public does not view this cause as a high-priority issue, and is only vaguely aware that medical and surgical advances are tested—somehow—before being given to humans. If healthy, the average person is not concerned about his future claims on the benefits of biomedical research.

He may have only distant fears of AIDS or cancer, but he responds readily to the suggestion that his pet dog is not going to be disposed of for mascara safety testing—the reality evoked by the activists. Thus, the movement has successfully solicited middle-of-the-road Americans to write their congressmen decrying the rare instances of animal abuse that activists present as the norm, and protesting "pound seizure." From a position of moral superiority, the animal rights activists claim to be raising our consciousness about "speciesism"—a term linked to racism to make it equally pejorative.

I, too, have deference for all species, and no researchers whom I know are cavalier about experimenting on animal life. Human responsibility—not animal rights—requires concern for the welfare of animals: their feeding, housing, exercise, control of their reproductive functions, and prevention of needless suffering. This is entirely different from the position that no animal should ever be used for human benefit. If there is a conflict, and harm must come to some sentient being, chief among those whose interests I represent are patients.

How should we physicians as patient advocates react to this health menace? By penetrating the activists' specious arguments, and by translating the consequences of their position for the public.

Aware that there is not a large "rat lobby," and that 90% of all research animals are rodents, the activists focus on "companion animals"—dogs and cats—thereby arousing pet lovers against "pound seizure." No matter that fewer than 1% of experimental animals are dogs (to which few we owe most of what we know about cardiovascular disease, the number one killer of humans in the U.S.). As long as they can link the laboratory animal with people's love for their pets, they will seek

(*Continued on p. 91*)

Fall Meeting Mini-Theme on Molecular Biology

Shu Chien

The dramatic advances in molecular biology in recent years have opened new ways to probe the structural and functional bases of physiological events at the molecular level. As a result, new methods have been developed for the study of cellular properties and interactions and the elucidation of the mechanisms by which neural and hormonal factors affect physiological functions. Scientific advances resulting from the application of molecular biological approaches have been particularly notable in research on several areas of cellular physiology, e.g., nerve, muscle, and endocrine system. Important information on physiological regulation at the organ-system levels also begins to emerge as a result of such applications.

An example of the utilization of DNA technology to study physiological functions is the method of in situ hybridization, which allows the probing of many different organs and tissues to detect the presence of the mRNA coding for a given protein that has a specific physiological function. Another example is the use of cDNA coding for a particular protein obtained from one tissue to clone cDNA from other tissues and to deduce the nucleotide sequence of these cDNAs. Such studies often show that a protein thought to be specifically present in one tissue actually has a relatively wide distribution and that the analogous proteins in different tissues often have minor molecular variations that account for their different functional manifestations. These results indicate that molecular biology, which is usually considered as a science using a reductionist's approach, actually can offer a global picture of the physiological roles of specific proteins in different organs and tissues.

The modern technologies of molecular biology have made it relatively easy to clone a gene of interest, but it is often difficult to determine its function. While the application of molecular biological techniques will help to open new horizons for physiological research, molecular biology is in search of new problems to solve, especially those of physiological relevance. In physiology, the scope of investigations ranges from molecules and cells to tissues and organs, and the application of the molecular biological techniques to elucidate physiological functions can best be implemented by physiologists. Hence physiologists are in a unique position and have the responsibility to bridge the gap between molecular biology and organ-system research.

Since most physiologists were trained before the advent of modern molecular biology, many may not be familiar with these new developments. My own experience is that the main difficulty in becoming familiarized with a new discipline, such as molecular biology, is the initial barrier in terms of terminology and basic concepts. Therefore, several of us in the American Physiological Society have endeavored in the last few years to help physiologists to overcome this barrier through APS meetings.

At the APS Centennial Meeting in Washington, DC in March–April 1987, Dr. Jay Gargus and I organized a Symposium on Molecular Biology in Physiology sponsored by the Section on Cell and General Physiology of APS. After two introductory talks, six papers were given on the application of molecular biology to solve problems related to anion transport protein, Na^+-K^+-ATPase, atrial natriuretic peptide and renin, β-adrenergic receptor, cholinergic receptor and sodium channel, and long-term memory. The feedback we received after the symposium was that it would be helpful to have a practicum workshop with laboratory sessions to demonstrate the various basic techniques used in molecular biology.

In accordance with these suggestions, Dr. Gargus and I, with the help from Dr. Martin Frank, Executive Director, and Dr. Carl V. Gisolfi, Chairman of the Program Executive Committee of APS, organized a Mini-Theme on Molecular Biology at the 1987 Fall Meeting in San Diego, CA. This mini-theme was composed of a symposium and a practicum workshop. The workshop consisted of five sessions: DNA Isolation and Quantitation; Restriction Enzyme Digestion & Electrophoresis; Southern, Northern, and Plaque Transfers; Sequencing DNA; and Vectors. These sessions were given by five biotechnology companies. We would like to express our sincere thanks to the participating companies: Beckman Instruments, Inc., Bethesda Research Labs., Bio-Rad Labs., International Biotechnologies, Inc., and Stratagene Cloning Systems.

On each of three afternoons, all five workshops were given. A rotation system was set up, so that a scientist can attend all five sessions in sequence. Altogether approximately 200 scientists attended these workshops. After the workshop a survey was conducted. Most of the responses were very positive, illustrated by the data in Figure 1. Many of the respondents wrote in comments such as, "It is truly an excellent move from the APS. Please keep such quality courses going," "Excellent idea. I personally benefited from just seeing the techniques. Thank you for assembling such a worthwhile program," "a very positive experience overall," "Having started with almost no background in molecular biology, I learned a great deal. Actual demonstration of methodology was helpful," "This was an enjoyable and very useful experience, the highlight of the Fall Meeting," "Very good. Appropriate activity for the Society," "Excellent service. I wish I had been able to attend all workshops."

There were many constructive suggestions and some critical comments. These are summarized below.

1. More introductory talk and/or materials. Each session would be aided by having a hand-out on the general principle

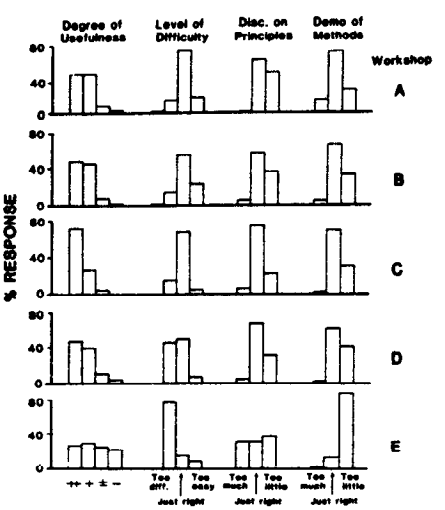


Fig. 1.

Department of Physiology and Cellular Biophysics, Columbia University College of Physicians and Surgeons, New York, NY 10032; Departments of A.M.E.S. (Bioengineering) and Medicine, University of California at San Diego, LaJolla, CA 92093; and Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan 11529, R.O.C.

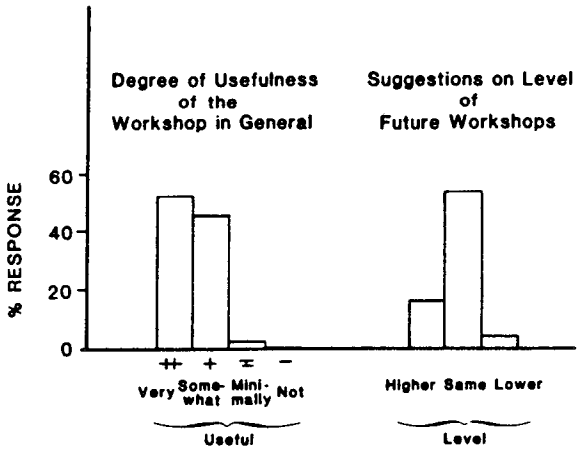


Fig. 2.

with a bibliography. Integrate the various techniques and illustrate how they can be used to answer physiological questions.

2. More hands-on experience. In this regard, the respondents contrasted the various sessions and praised those with hands-on opportunities. This is reflected in the uneven ratings received by the various workshops (Fig. 2).

3. More time for each session. While the workshops were useful, they were too brief and too crowded in time to impart learning information. Better to lengthen the session or to cover a narrow range of topics.

4. Have two types of workshops aimed at scientists with different backgrounds, i.e., sessions that are elementary as well as those that are more advanced.

5. Less emphasis on specific instruments made by the companies.

Based on these comments and also encouraged by the resolution by the APS Council to promote cellular and molecular biological approaches to the study of physiology, we have organized another Mini-Theme on Molecular Biology at the 1988 APS Fall Meeting in Montreal. This time we have changed our approaches to take into account the comments and advices by the attendees at the 1987 Fall Meeting.

The schedule of the Mini-Theme is as follows.

October 10, 1988

P.M.: Tutorial

Chair: J.J. Gargus
1:30–3:30 S. Chien: Introduction to Molecular Biology
3:30–3:40 Intermission
3:40–5:00 Discussion (J.J. Gargus and panel of experts from participating companies)

October 11, 1988

A.M.: Symposium on Molecular Biology of the Cardiovascular System

Chair: S. Chien
8:30–8:40 S. Chien: Introduction
8:40–9:15 C.P. Emerson: Genes Controlling Myogenic Determination
9:15–9:50 E. Morkin: Regulation of Cardiac Myosin Heavy Chain Genes by Thyroid Hormone
9:50–10:25 P.C. Simpson: Hypertrophy and Gene Expression
10:25–10:35 Intermission
10:35–11:10 C. Seidman: Studies on ANF Gene Expression: Insights into Molecular Basis of Cardiac Development and Myocardial Disease
11:10–11:45 J.F. Riordan: Molecular Biology of Angiogenin
11:45–12:00 General Discussion

P.M.: Workshop A

1:30–4:30 Practicum
A. DNA Isolation and Quantitation
B. Restriction Enzyme Digestion & Electrophoresis
C. Southern, Northern, and Plaque Transfers
D. Sequencing DNA

October 12, 1988

A.M.: Workshop B

8:30–11:45 Practicum (Each of the four simultaneous sessions will be ~90 min. The first half will be for beginners and the second half will be for scientists with more advanced knowledge on the subject.
A. DNA and RNA Isolation and Quantitation (Beckman Instruments, Inc.)
B. Restriction Enzyme Digestion & Electrophoresis (New England Biolab.)
C. Southern, Northern, and Plaque Transfers (Bethesda Research Labs.)
D. Sequencing DNA (International Biotechnologies, Inc.)

The structuring of this mini-theme program has been greatly aided by the results of the survey conducted after the mini-theme held at the 1987 Fall Meeting. The salient features are as follows (with the numbers corresponding to the comments summarized previously).

1. The mini-theme will begin with a relatively long (two hours) tutorial talk in the morning of October 11, 1988. This is specifically designed for people who have had little or no prior knowledge on molecular biology, so that they would gain sufficient information in terms of basic concepts and terminology as a preparation for the workshops. There will be hand-outs on the general principles of molecular biology, including a glossary and some references. The tutorial talk will be followed by a Discussion Session when Dr. Gargus and the workshop instructors will form a panel to answer questions raised from the audience and further amplify the materials given in the tutorial talk. All "beginners" are advised to participate in this afternoon session as a "prerequisite" to be able to benefit from the workshops that follow. This afternoon session is not for people who are already well versed on the subject.

2. The participating companies have agreed to try to have more hands-on experience for the participants, but in some cases practical limitations may make some of these procedures as a demonstration.

3. In the 1987 practicum, we had only about 30 minutes for each session. The time available for each session will be tripled at the 1988 Montreal meeting; this will allow a more in-depth treatment of each topic. The corollary is that each participant will only be able to attend one session each day (two of the four sessions during the week).

4. We will have two types of workshops on each subject for scientists with different backgrounds. That is, the first half of each session is for beginners and the second half is a more advanced session. Conceivably, in some cases the participants after finishing the first half may become sufficiently prepared for the second half. If there is a space limitation in the second half, however, preference will be given to those with advanced knowledge and have not attended the first half.

5. We have requested the companies to stress the general principle and put less emphasis on specific instruments, whenever possible.

By making these changes in the organi-

zation of the practicum, we hope to improve the results over those obtained last year in San Diego. Because of the space limitation in these workshops, we have to use the principle of first come, first served. Therefore, please sign up for the sessions in the registration area of the Montreal Convention Centre to be able to attend.

The symposium in the morning of October 11, 1988 is focused on the molecular biology of the cardiovascular system. After a very brief introduction, five papers will be given on the application of molecular biology to studies on the cardiac muscle (including genetic determinants, thyroid hormone regulation and cardiac hypertrophy), the atrial natriuretic factor (in relation to cardiac development and myocardial disease), and angiogenin (a protein that causes angiogenesis). This symposium is sponsored by the Cardiovascular Section of APS and the topics have conceivable relevance to the main theme of the 1988 Fall Meeting, viz. growth and development. Although the speakers are all experts working at the forefronts of these fields, they will endeavor to make their talks understandable to physiologists who are not familiar to molecular biology except for the exposure to the tutorial session on the previous day. It is hoped that the symposium will serve to illustrate how molecular biological techniques can be used to solve problems of physiological interests.

The repeat of the workshops on the morning of October 12 will allow the participant to select a different topic, and this time with the added knowledge gained from the symposium.

Physiologists are in a unique position to bridge the gap between molecular biology and organ-system research. To do so effectively, we physiologists must have a basic understanding of molecular biology. The aim of this mini-theme is not to convert physiologists into molecular biologists. On the contrary, the purpose is to strengthen our position as physiologists. We will continue to work on organ-system physiology, but we will make use of these powerful new tools to our advantage. History has proven that physiologists have adapted very well to changes in life science technology and have always been able to effectively apply new methods to answer their quests for understanding physiological functions. Therefore, I am confident that we will be able to succeed in making use of molecular biology, as we did so many times before with other new techniques and concepts.

As we enter into the second century of the American Physiological Society, physiology is poised at the threshold of a golden period. If we can take advantage of the new technologies developed in molecular biology, we will not only make significant advances in physiology per se but also place physiology at the center stage in this new era of development in biomedical sciences.

—Ask not only what molecular biology can do for physiology, but also what physiology can do for molecular biology.

John F. Perkins, Jr., Memorial Award

The American Physiological Society invites applications for the John F. Perkins, Jr., Memorial Fellowships. The fund is designed to provide supplementary support to the families of foreign physiologists who have arranged for fellowships or sabbatical leave to carry out scientific work in the United States. Applications by U.S. physiologists who require supplementary assistance to work abroad will also be considered.

It is the interest of the Perkins Fund to develop the full potentialities for cultural benefit associated with scientific exchange. Preference will be given to physiologists working in the fields of respiratory physiology, neurophysiology, and temperature regulation.

Each application should be made by both the visiting scientist and his host. Ordinarily, the joint applicants will have made financial arrangements for the visiting scientist before applying to the Perkins Fund for family support. The application should contain an account of these arrangements with a description of the proposed scientific work and a brief account of how the visitor and his family intend to make use of the cultural benefits.

The amount available for each award will be in the range of $3,000 to $7,500, depending on the estimated needs of the family over and above the amount already available to the visiting scientist. Ordinarily, two to four awards will be available in any one year.

Applications are reviewed by a selection committee in February and July of each year. The deadline for receipt of applications is January 1 and June 1, respectively. Forms for host and visiting scientist may be obtained from the Executive Director, American Physiological Society, 9650 Rockville Pike, Bethesda, MD 20814.

Travel Fellowships for Minority Physiologists

The American Physiological Society has been awarded a grant by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to provide fellowships for young underrepresented minority students and physiologists to attend the Annual APS/FASEB Meeting in New Orleans, Louisiana, March 19–23, 1989. At the national meeting, fellows will be hosted, introduced to prominent investigators, and exposed to a variety of research areas. Funds will provide transportation, meals, and lodging. The specific intent of this award is to increase participation of pre- and post-doctoral minority students in the physiological sciences. Applicants need not be members of the American Physiological Society but should be United States residents.

Advanced undergraduate and pre- and postdoctoral scientists interested in research in the physiological sciences may apply. Underrepresented minority students from all institutions are eligible. Those who have obtained their undergraduate education in MBRS- and MARC-eligible institutions as well as students in the APS Porter Development Program are encouraged to apply. Applications may also be submitted by minority faculty members at the above institutions.

Applications should include information on

1) academic background and experience;

2) a written statement of interest in research in physiology;

3) a letter of recommendation from the applicant's mentor;

4) a list of publications, if available;

5) a statement indicating the ethnic minority with which the applicant identifies himself/herself;

6) an estimate of required travel and per diem expenses.

Submit applications to NIDDK Travel Fellowships, c/o Dr. Martin Frank, Executive Director, American Physiological Society, 9650 Rockville Pike, Bethesda, MD 20814.

The deadline for receipt of completed applications is November 15, 1988.

American Physiological Society 139th Business Meeting

Time: 10:00 A.M., Wednesday, May 4, 1988
Place: Las Vegas Hilton Hotel, Las Vegas, NV

I. **Call to Order**

The meeting was called to order by the President, H. V. Sparks, Jr., who welcomed the members to the 139th Business Meeting of the Society and announced that Carl Gisolfi would be the parlimentarian. The agenda and ballot for the Election of New Members were distributed to the members along with Proposed Amendents to the Bylaws, Article III., Membership (Nominations and Elections of Members), and Article V., Standing Committees (Publications and Finance Committees), which had been published in the December 1987 issue of *The Physiologist.*

II. **Report on Membership**

The current status of the membership and deaths since the last meeting was reported by the President-Elect, Aubrey Taylor.

A. **Summary of Membership Status**

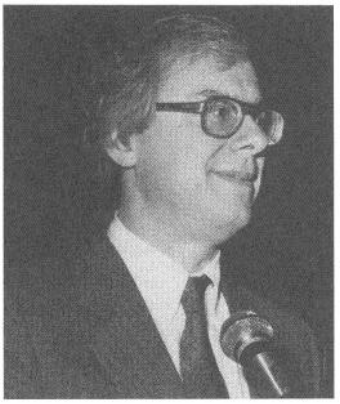
The Society, which continues to grow, is very healthy, and Dr. Taylor expressed appreciation for the hard work of the Membership Committee in its efforts to increase the membership. Since the Fall Meeting, the total membership reached 6,562, which includes 4,767 Regular, 19 Honorary, 188 Corresponding, 771 Associate, 657 Emeritus, and 160 Student members (see p. 82).

B. **Deaths Reported Since the Fall Meeting**

The names of 17 deceased members, who had dedicated their lives to physiology, were read by Dr. Taylor. The membership stood for a moment of silence in tribute to these physiologists (see p. 84).

III. **Election of Officers and Society Affairs**

The Executive Director, Martin Frank, was pleased to report that the procedure for the election of officers, under the new Society governance, ran smoothly. A Nominating Committee consisting of Section chairpersons selected a slate of candidates from nominees advanced by the membership. He was pleased to announce that as a result of the Election of Officers by mail



ballot, Vernon S. Bishop was elected President-Elect. The four new Councillors, serving alternate terms to allow for the rotation of two Councillors each year, were Beverly Bishop (1991), Allen W. Cowley, Jr. (1989), Gerhard H. Giebisch (1990), and Peter D. Wagner (1991).

The Society staff has settled in the new wing of the Lee Building on the FASEB campus and Dr. Frank invited members to visit their Society Headqaurters when they are in Bethesda. As part of the Society reorganization, Brenda Rauner has been appointed Publications Manager following the death of Stephen Geiger.

IV. **Membership**

A. **Appointment of Tellers**

President Sparks appointed John Hall, Harold Modell, and James Terris as Tellers. Members were instructed to strike those names from the ballot for whom they did not wish to vote, and Tellers were asked to collect the ballots for the election of new members.

B. **Election of New Members**

Following the tally of the ballots, Dr. Taylor announced that all candidates, who had been recommended for membership by the Membership Committee and Council, were unanimously elected to membership (see p. 82).

V. **Awards and Presentations**

A. **Ray G. Daggs Award**

The Ray G. Daggs Award was presented to Horace W. Davenport, 34th President of APS (see p. 66).

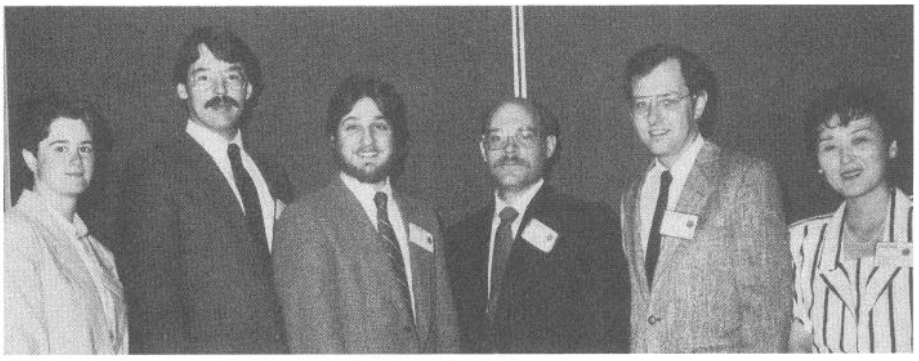
B. **Caroline tum Suden Professional Opportunity Awards**

Caroline tum Suden was one of the first women members of the APS, who had a long and productive career as an investigator and teacher. She left a sizable undesignated bequest to the Society, and six Caroline tum Suden Professional Opportunities Awards are given each year to graduate students or postdoctoral fellows presenting papers at the Spring FASEB Meeting. Selected by the Women in Physiology Committee, the recipients receive a $500 check to attend the meeting, a paid registration and placement service fees.

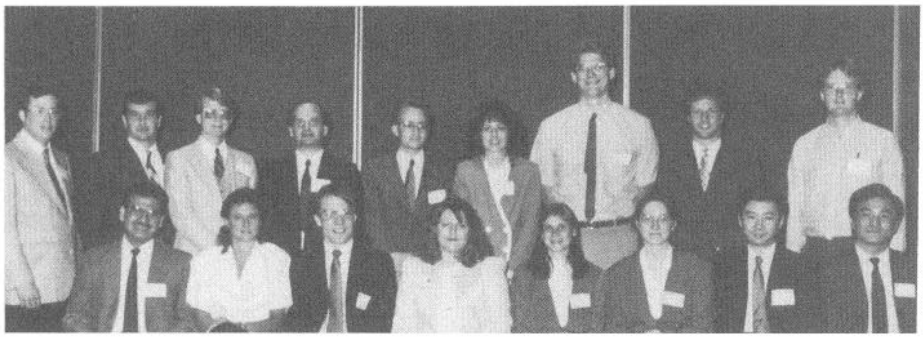
Helen Cooke, Chairperson of the Women in Physiology Committee, took pleasure in presenting the six awards to Hyung-Mee Han (Columbia University), Jeffrey W. Kiel (University of Texas), Douglas Light (Dartmouth Medical School), Kenneth D. Massey (University of Texas), Karen M. McGillivray (University of North Carolina), and Mark S. Siskind (Boston University).

C. **Procter & Gamble Professional Opportunity Awards**

The Society provides support (sponsored by the Procter & Gamble Company) for 17 predoctoral students who are within 12–18 months of completing their Ph.D. degrees. Candidates, presenting papers as first authors at the Spring Meeting, must be U.S. citizens or hold a permanent resi-



Caroline tum Suden Professional Opportunity Awards.



Recipients of 1988 Procter & Gamble Professional Opportunity Awards.

dent visa. Dr. Cooke announced that each awardee receives a $500 cash award and a paid registration fee to attend our Spring Meeting. The recipients of the 1988 Procter and Gamble Professional Opportunity Awards are Bryan F. Cox (University of Iowa), William Durante (University of Toronto), Nicholas S. Gantenberg (University of Alabama), Laura L. Hall (Uniformed Services University of Health Sciences), Christopher Hardin (University of Cincinnati), Yu Ru Kou (University of Kentucky), Ingrid K. Krampetz (University of Manitoba), Fred S. Lamb (University of Michigan), D. L. Mattson (Medical College of Wisconsin), David L. Osborne (East Carolina University), Anthony J. Pacitti (University of Florida), Nayel Moh'd Rawashdeh (Bowman Gray School of Medicine), Hal A. Skopicki (The Chicago Medical School), Holly Van Remmen (University of Texas), Allison K. Wilson (University of Illinois), Jamie Young (University of Louisville), and Menggia Zhao (Albert Einstein College of Medicine).

VII. Amendent to the Bylaws

The proposed amendments to the Bylaws, published in *The Physiologist* 30:284, 1987, were approved by Council. Article III. *Membership*, Sections 9 and 10, allows for the immediate acceptance of associate and student membership applications by the Executive Director if all the established criteria are met. The membership will continue to elect regular, corresponding, and honorary members by secret ballot at the Society Business Meeting.

A motion to amend the Society Bylaws Article III. Membership, Section 9, Nominations for Membership, and Section 10, Election of Members was seconded.

An amendment to the motion to insert "proposed" before regular and corresponding members (line 2) in Section 9(a) of Article III. Membership was adopted.

The motion was unanimously approved as amended.

It is important that the Council is represented in the APS Headquarters Office by the Executive Director. Therefore, the proposed amendment to Article V. *Standing Committees*, Sections 1. *Publications Committee* and 2. *Finance Committee* to assure the Publications and Business Managers are responsible to the Executive Director.

A motion was unanimously approved to amend Article V. Standing Committees. Section 1, Publications Committee and Section 2, Finance Committee of the Bylaws as presented.

V. State of the Society

Dr. Sparks stated, "I am glad to report that your Society is entering a second century with a full head of steam. Almost every committee is working hard to make our Society the best it can be. I will only report on a few of the most prominent changes at this point in time. Six months from now in Montreal, Aubrey Taylor will be able to report on many more.

Finance

A $7 million budget in the Society has been ably managed by Francis Haddy and his Finance Committee. We are in good financial shape and are strengthening the Society by building a $1 million program endowment fund to assure that the proper resources are available to provide programs of top quality for our members. At present we are approximately three-quarters of our way to this goal. We hope that a combination of giving by Society members and by our friends in industry will get us to our goal in the immediate future. We now have a much more aggressive stand in managing our investments to be sure that the return on our reserves is the best possible gain given our conservative fiscal philosophy.

Publications

Our journals continue to lead the field, with more pages of high quality work being published each year. We are starting a new journal, *The American Journal of Physiology: Lung Cellular and Molecular Physiology*. We feel that this will fill a significant need for the publication of pulmonary literature. Donald Massaro has already begun to assemble his editorial staff. Another exciting development is a new publication dealing with advances in physiology education. This will be a semiannual supplement to the *American Journal of Physiology* with Harold Modell as editor.

Paul Johnson, chairman of the Publications Committee, has led the book program away from possible financial disaster into a new era of fiscal restraint. Oxford University Press has now taken on the responsibility of publishing our books. This arrangement protects the American Physiological Society from the financial risk of new book ventures, yet gives us freedom to be more innovative in our book program. The *Handbook* series and the clinical physiology series will continue. There will also be a new technique series and a continuation of People and Ideas books.

Program

The Chairman of the Program Committee, Carl Gisolfi, has organized an exciting meeting for Montreal, where the theme will be growth, development and aging. We will be meeting jointly with pharmacologists, and this promises to be an excellent effort for both parties. The Program Committee is experimenting with a number of innovative ideas in the program. One of the most significant is a tutorial series on molecular biology organized by Shu Chien. This series started in San Diego and was a great success and will continue in Montreal. The Program Committee is

Second row, left to right: W. S. Spielman, C. V. Gisolfi, R. B. Reeves, N. C. Staub, S. Chien, and F. G. Haddy. *First row, left to right:* M. Frank, H. V. Sparks, Jr., F. G. Knox, V. S. Bishop, and A. E. Taylor

making a number of other innovative approaches to programming that are intended to make the meetings more useful. We recently wrote to you to encourage you to work with your section to state the type of program you want at our meetings. The sky is the limit.

Public Affairs

We have been taking a more pro-active stance on the animal issues. My letter urging you to write your legislators regarding the Pet Protection Act has paid off! Apparently, Senator Ford has decided to withdraw the Senate bill because of your letters. This is only one small victory, but it shows that it is important to educate our lawmakers. Thank you for writing. David Ramsay, chairman of our Animal Care and Experimentation Committee, and our new GRIP Committee (Government Relations Initiative Program) has a series of plans to help you in presenting our case to lawmakers, your peers, and your students. These will be bearing fruit over the next few months. In the meantime, keep writing your congressmen, senators, and president.

Long-Range Planning

Ernst Knobil, Chairman of our Long-Range Planning Committee, is leading a long-range study of the relationship between physiology as a discipline, physiology departments, and our relationship with other sciences. This is an important undertaking because it will help us define the directions of the Society over the next few decades.

Education

We have a second group of minority students supported by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) at this meeting. This program developed by Martin Frank has allowed us to bring a number of outstanding minority students to our Fall Meeting in San Diego and now our Spring Meeting here in Las Vegas. This is an excellent opportunity for us to recruit bright young minds into the field of physiology.

Harvey V. Sparks, Jr., APS president, introducing George K. Radda, Oxford University, The Walter B. Cannon Memorial Lecturer.

Sections

Our new governance system has begun to work. The sections provided a nominating committee and a slate of officers of this year's elections. Our new Councillors are evidence of the strength of the new system. The sections should be the powerhouse of our Society. It is up to you to work through your section to be sure that you are getting what you want from the Society in terms of leadership, publication, and meetings. The Society also encourages the formation of special interest groups that enhance your interactions at the meetings. Once you have formed an interest group, you will have input in the programming as well as access to the resources of the main office in Bethesda.

International Programs

Professor Vladamir Skok, Vice President of the All Union Pavolvian Physiological Society, is visiting us here in Las Vegas. This represents a continuation of a linkage with Society physiologists initiated in 1985 by John West and Orr Reynolds. The next step in building this relationship will come when Frank Knox and Martin Frank visit the USSR later in May.

You will remember that we recently opened the Society to all qualified physiologists living in The Americas. This was met with great enthusiasm by the Latin American physiologists and they cosponsored our meeting in San Diego. On that occasion a delegation of Latin American physiologists met with us. Aubrey Taylor and I will attend the meeting of the Latin American Association of Physiological Sciences, which will be held later this month in Buenos Aires, Argentina. Finally, we are in the midst of developing a number of initiatives to help our colleagues in developing countries. It is too early to give you the details, but I suggest by the time of the Fall Meeting you will have more information.

Summary

In summary, the Society is in a state of change, but the snapshot at this moment shows a healthy and powerful force promoting physiological science. It is safe to say that we are well positioned for a second century of progress."

VI. **New Business**

A. **Organization of a New Section**

Carlton Hazelwood, Baylor College of Medicine, expressed an interest in looking into the possibility of forming a new section on water and cellular function. The scope of the section is to present and study water as it relates to water organization and the dynamic aspects of cellular function. Dr. Sparks encouraged Dr. Hazelwood to discuss the proposal with Dr. Frank, who would be helpful in providing a mailing to APS members who might be interested in affiliating wtih such a section.

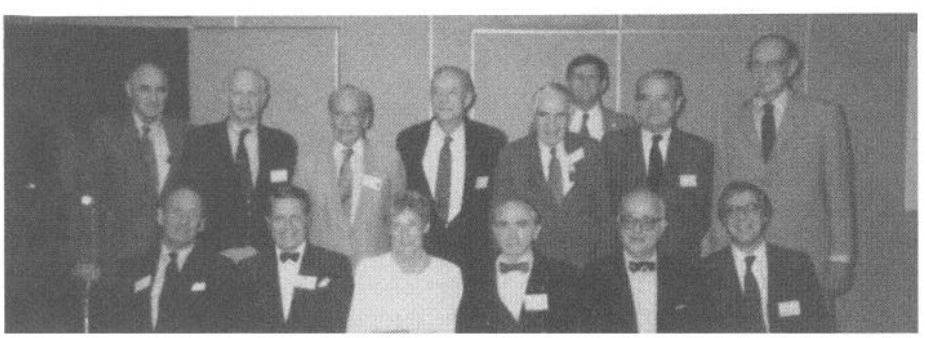
B. **Publications Review Process**

Another member expressed concern about the Society's review process. In his opinion, the referee should evaluate whether a paper is properly done. It is not the responsibility of the referee to agree or disagree with the evaluation or results of the paper. However, for many years, if a referee disagrees, the paper is rejected with the suggestion that the author read the referee's last publication. The member emphasized that this is a very serious business because too often, the best papers are rejected. (There was applause from the audience.)

Dr. Sparks said that this is a serious issue. Our journals are to serve our members. If this is not being done, the Council needs to know about it. The Chairman of the Publications Committee, Paul Johnson, commented that the editors are there to hear complaints and, he, too, has received letters that the overall review process is not working right. The Publications Committee is concerned and welcomes your comments on ways of improving the review system.

C. **Undergraduate Physiology Programs**

Another member suggested that the Society should promote undergraduate physiology programs, which could be done by proposing curriculum. Also, promoting the



APS Presidents. *Second row, left to right:* A. C. Guyton, R. M. Berne, D. H. Bohr, H. Rahn, E. H. Wood, A. E. Taylor, W. F. Ganong, and H. W. Davenport, *First row, left to right:* J. B. West, E. Knobil, B. Schmidt-Nielsen, A. C. Barger, A. P. Fishman, and H. V. Sparks, Jr.

President's Reception.

active participation of undergraduate research would be most useful. Unless there are a lot of undergraduate students in the pipeline interested in physiology, one cannot expect to have many students going into graduate programs. It is time for the APS to recognize that physiology does exist outside of the medical schools. Dr. Sparks expressed thanks and said he would pass the recommendation on to the Education Committee Chairman, William Spielman.

Harold Modell agreed with the criticism and interpreted the suggestion that the Society provide a session for undergraduate students to present their undergraduate projects at the Spring or Fall Meetings. An important step in recognizing the educational needs of the Society has been the initiation of the Education Supplement to the *AJP* where peer-reviewed scholarly work in the educational fields of physiology will be published. Another issue to be addressed along these lines is the membership policies, which should be directed to the Membership Committee to consider teaching and education development as a much more scholarly effort than is presently done.

D. **XXXI IUPS Congress**

Dr. Frank reminded the group that the XXXI International Congress of Physiological Sciences will be held in Helinski, Finland, July 9–14, 1989. Secretary General Osmo Hanninen has provided a list of Congress sessions, symposia, and satellite programs. Also, as in previous years, the APS will manage a travel grant program. These materials will be appear in the August issue of *The Physiologist*. In the interim, information may be obtained from the APS Headquarters Office.

There being no other business, the 139th Business Meeting was adjourned at 10:45 A.M., May 4, 1988.

Aubrey E. Taylor
President-Elect

APS/FASEB Spring Meeting New Orleans, LA March 19–23, 1989

APS Sponsored Symposia

Myocardial Function in Shock and Sepsis. F. L. Abel, Organizer

Congestive Heart Failure: Molecular Mechanisms and the Rationale for Inotropic Intervention. N. R. Alpert, Organizer

G Proteins and Ionic Channels. A. M. Brown, Organizer

The Cardiac Gap Junction: Protein to Pathologies. M. M. Burt, Organizer

Bronchial Circulation in Lung Edema. J. Butler, Organizer

Function and Modulation of Glutamate Receptors. E. Costa, Organizer

Genetic Determination of Ingestion. D. Denton, Organizer

Regulation and Identification of Transporters Involved in Epithelial Na and Cl Absorption. M. Donowitz, Organizer

Receptor Mechanism in the Development of Respiratory Control. J. P. Farber, Organizer

Human Colonic Fermentation of Dietary Carbohydrate: Physiological, Nutritional and Clinical Implications. C. L. Kien, Organizer

Physiological Mechanisms of Hypertonic Saline Resuscitation. G. C. Kramer, Organizer

Endothelial Barrier Function. A. B. Malik, Organizer

People and Ideas in Endocrinology. S. M. McCann, Organizer

Regulation of Synthesis of Membrane Transporters. A. McDonough, Organizer

The Proximal Tubule Interactions With the Renin-Angiotensin System. L. G. Navar, Organizer

Membrane Mechanisms of Ischemic Brain Damage. E. M. Nemoto, Organizer

The Influence of Temperature on Muscle and Locomotory Performance. L. C. Rome, Organizer

Integrative Factors in Gut Function. S. K. Sarna, Organizer

Recent Advances in the Physiology of the Vascular Endothelium. S. C. Silverstein, Organizer

Sexual Dimorphism in Regulation of Blood Pressure and Water and Electrolyte Homeostasis. L. Share, Organizer

Signal Transduction in Renal Cells. W. S. Spielman, Organizer

Functions of the Purine Nucleotide Cycle in Skeletal Muscle. R. L. Terjung, Organizer

Biologic Responses to Prolonged Infusions of Atrial Natriuretic Factor. N. C. Trippodo, Organizer

Cellular and Molecular Aspects of Growth and Contractile Activity in Vascular Smooth Muscle. R. C. Webb, Organizer

Tutorial

Using the Microcomputer in the Classroom. H. I. Modell, Organizer

Guest Society Symposia

Cellular and Molecular Basis for the Influence of Nutrition on Aging and Longevity. R. A. Good (SEBM), Organizer

Development and Evaluation of Chemically Modified Hemoglobins as Blood Substitutes. H. W. Kim (BMES), Organizer

Fractal Analysis of Bio-Medical Systems. J. E. McNamee (BMES), Organizer

Modern Analysis of Complex Systems, II. R. Sclabassi (BMES), Organizer

"Mechanisms of Adaptation to the Environment" Thematic Symposia

Central Nervous Mechanisms of Host Defense Responses. C. M. Blatteis, Organizer

The Uptake, Synthesis, and Physiological Function of Organic Osmolytes in Biological Systems. T. J. Bradley, Organizer

Factors Determining $VO_{2\,max}$ in Humans. P. Cerretelli, Organizer

Adaptations to Asphyxia—Lessons From Diving Animals. S.-K. Hong, Organizer

Response and Adaptation to Hypoxia: Organ to Organnelle. S. Lahiri, Organizer

Frontiers in Environmental Physiology. E. R. Nadel, Organizer

1989 FASEB Spring Meeting Abstract DEADLINE: November 1, 1988

The Ray G. Daggs Award, 1988

Horace Willard Davenport was the 34th President of the American Physiological Society from 1961 to 1962. He was President after serving on Council for five years from 1951 to 1955 and again in 1959–1960. He served on the Central Committee for the Survey of Physiology, the Membership Advisory Committee, the Committee on the Use and Care of Animals, the Porter Fellowship Committee, the Education Committee, and the Committee on Matters Related to Loyalty. He was also a member of the Editorial Board of the *American Journal of Physiology* and the *Journal of Applied Physiology*, on the Senior Physiologists Committee, the Centennial Committee, and the Honorary Membership Committee. As President of the Society, he restructured the finances and founded the Publications and Finance Committee, which have served us well over the past 25 years. In addition, the *Journal of Neurophysiology* was brought under the wing of the Society during his Presidency.

Dr. Sparks said he would not dwell on his successes as a teacher or as a scientist, since these were well documented. Almost every physiologist is familiar with the ABCs of Acid-Based Chemistry, and his many research accomplishments are evidenced by his membership in the National Academy of Sciences.

"When I was becoming involved in the affairs of the Society," stated Dr. Sparks, "Horace took me into his office and explained to me that I would find that participating in the affairs of the Society would be like becoming a member of a family. He told me I would find that each member of Council contributed his/her own unique skills and that the blend of these produced a positive effect for the Society. He emphasized that the most rewarding part of service to the Society is the deep friendships which would result. In his Past President's address, delivered in Buffalo in 1962, he ended by raising his arms and saying, 'I love you all'. Horace, the Ray Daggs Award is our way of saying that we all love you."

Dr. Davenport expressed thanks to President Sparks for the Ray G. Daggs Award.

Plus ça Change . . .

When Harvey Sparks gave me the Daggs Award at the Society's business meeting on 4 May 1988 he spoke of some of the things I had told him when he himself began to be involved in the Society's affairs. He said I had told him that the officers and the committee members make up a family and that each brings something unique to the Society. This is not the place for me to say what I think I contributed, but two topics that came up at the meeting reminded me of what others had done in my time, contributions now forgotten by all except us gray-beards.

Harvey Sparks said the budget was something like $10 million and that Council was trying to build a reserve of $1 million. Once long ago at a business meeting the president, I forget who, said the dues would be raised from something like $3.50 to perhaps $4.00. Ajax Carlson, who was sitting in the front row, got up to denounce the officers for extravagance; such high dues, he said, would keep the younger and poorer members out. Louie Katz, who was on Council and who was sitting next to Ajax, rose to say: "I'm not eighty, but I must be getting senile too." Then he spoke about adequate support for the Society's work. When he himself was president he did more than anyone before him to put the finances on a sound foundation. Julius Comroe, who was also on Council, helped too. Julius passionately believed that physiology had done much for medicine and that it is medicine's duty to repay in cash. That resulted in the category of institutional members, and each of us had the job of trying to persuade a drug company or an instrument maker to contribute. Julius started *Physiology for Physicians*, a four-page sheet that eventually became a department of the *New England Journal of Medicine*. The subscriptions by physicians went directly to the Society's treasury. Julius organized teaching ses-

Orr E. Reynolds Award

The Orr E. Reynolds Award is given annually by the American Physiological Society for the best historical article submitted by a member of the Society.

Articles may deal with any aspect of the history of physiology including the development of physiological ideas and their application, instrumentation, individual and collective biography, departmental and institutional history, history of societies including APS, and physiology in its public context. Manuscripts submitted for the award should represent original research and be adequately documented. Articles published in APS journals or books during the prior calendar year are also eligible for the award upon request by the author(s). The award is open to all classes of APS membership except for those members who have advanced degrees in the history of science and medicine. A member may receive the award only once.

The awardee will receive $500 plus expenses to attend the APS Spring Meeting. If the awardee wishes, and there is a suitable place on the program, an oral presentation will be made at the Spring or subsequent Fall meeting at the beginning of an appropriate scientific session. It is hoped that, after appropriate peer review, the article will be published in one of the APS journals.

Manuscripts will be evaluated by a committee consisting of three members of APS appointed annually by Council in consultation with the Chairman of the Section of the History of Physiology. At least one of the members will be a professional historian.

Manuscripts should be typed and double-spaced with wide margins on 8½ × 11 paper and should conform to the style used in APS journals. (Instructions will be sent on request.) Three copies should be submitted for use of the review committee. To be considered for the 1989 award, manuscripts should be sent to Orr E. Reynolds Award, American Physiological Society, 9650 Rockville Pike, Bethesda, MD 20814, by December 1, 1988. The recipient of the award will be announced at the 1989 Spring Meeting.

Recipients of Ray G. Daggs Award

1974	H. H. Brookhart
1975	M. B. Visscher
1976	J. D. Hardy
1977	J. H. Comroe
1978	H. Rahn
1979	J. R. Pappenheimer
1980	J. R. Brobeck
1981	A. C. Guyton
1982	R. W. Berliner
1983	C. L. Prosser
1984	E. F. Adolph
1985	A. C. Barger
1986	D. B. Dill
1987	O. E. Reynolds
1988	H. W. Davenport

sions at medical meetings, which physicians paid to attend. I well remember 500 crowding into a room in Philadelphia to hear us talk about some practical applications of physiology. Those of us who participated paid our own way or, as I once did, used a Project Site Visit to get us to where Julius was giving his course for physicians.

Someone at the meeting made a plea for an effort to each college undergraduate. In the days Ed Adolph was on Council and was president, that was one of the Society's major concerns. Ed Adolph and Ladd Prosser and Ruth Conklin and others I forget prepared laboratory exercises suitable for undergraduate use that were widely distributed. Many of us visited college and cultivated the dedicated men and women who taught physiology courses. I can't tell all that was done, but I can cite what we did in Michigan where there are a couple of dozen first-class colleges within two hundred miles of Ann Arbor. We brought likely undergraduates to visit our department, and some, Rusty Johnson among them, became our graduate students. One weekend we brought 30 or 40 college professors to Ann Arbor for a refresher course. On the last evening my wife and I gave him a dinner at our house, and on that occasion one said to Dorothy Luciano, "Are you the wife of the man who wrote that wonderful book?" That wonderful book, *Vander, Sherman and Luciano's Human Physiology,* really has done something for college teaching of physiology, and it is the product of our effort to reach the very large undergraduate population in our university. As a result, we had 500 students a year in what was the second most popular course in the university, History of Art being the first. I am sure many other departments of physiology in medical schools had similar programs.

At that dinner one college professor said he had an arrangement with his wife: on his birthday or other anniversary she was to give him another volume of one of the Society's *Handbooks of Physiology.* Others joined him in expressing their debt to the *Handbooks.* When Maurice Visscher, another past president, persuaded Council and the Board of Publication Trustees to risk their $300,000 on the *Handbook* venture, he wanted the *Handbooks* to replace the German *Handbuche* that had been killed by the war, and he thought the *Handbooks* might be a vein from which textbooks could be mined. I don't think he anticipated their reaching into the colleges, but I am sure he would have been pleased to know they did. Does that still happen, or have the *Handbooks* become so specialized that they serve only the most recondide physiologist and not the college teacher?

Perhaps these memories have two morals: that officers of the Society have tried to serve physiology in many ways and that what they have tried to do is easily forgotten.

H. W. Davenport

Committee Reports

ANIMAL CARE AND EXPERIMENTATION AND PUBLIC AFFAIRS

The joint meeting of the Animal Care and Experimentation Committee (ACEC) and the Public Affairs Executive Committee (PAEC) was held May 1, 1988, at the Las Vegas Hilton Hotel in Las Vegas, NV.

The first item of business was a review of the minutes of the Committee on Government Relations Initiative Programs (GRIP), which held its first meeting in March. David Ramsay said that the APS Council formed GRIP because there had been a lack of movement by either the ACEC or the PAEC and that there was confusion as to the roles of the two committees since both were concerned with the animal issues. One of the purposes of the GRIP Committee is to develop initiatives for both the ACEC and the PAEC so that each would have defined objectives.

The consensus was that this is a good plan and that initiatives relating to the animal issues should be given to the ACEC and that the initiatives for the PAEC should be concerned with broad public education and information programs.

William Samuels reviewed federal legislation involving animals. Among the recent actions by APS was the mailing of a letter in April to all APS members by the Society's President, Harvey V. Sparks, Jr., requesting the members write or telephone their congressional delegations to oppose the Pet Protection Act. Mr. Samuels reported that the Senate has since withdrawn its version of the bill but that the House version was likely to be added as an amendment of the NIH reauthorization bill. He also reported that the Society had testified before a Senate appropriations committee urging the funding for the Animal and Plant Health Inspection Service's enforcement program be increased, and he said APS would submit a statement for the record to a House subcommittee citing the Society's concerns about the consumer products safe testing bill.

In the discussion of the pet protection bills, Norman Marshall said he had sent 550 letters to industrial physicians urging them to tell their congressmen of their opposition to the bill and that he planned to send more letters to industrial physicians through the Pharmaceutical Manufacturers Association. He also noted that pet protection legislation was on the agenda for his meeting with the APS Liaison With Industry Committee.

William Samuels reported that in January Sen. Howell Heflin (D AL) is expected to introduce a bill that would make breakins and thefts at federally funded animal facilities a federal offense. The bill would be similar to the bill drafted by APS and introduced in the House in 1985, but its scope would be broader so that it would also include all facilities wherein animals are housed or used, including farms. Both committees urged that APS support this bill when it is introduced.

A discussion was held on how to develop materials that would have a positive impact upon the public concerning the use of laboratory animals. Several suggestions were made, including the development of video tapes, programs honoring well known scientists, researchers, and investigators who use animals in their work, and public forums for discussing accomplishments of physiologists. No action was taken on any of the suggestions.

Being no further business the meeting was adjourned at 1:45 P.M.

David J. Ramsay, Chairman

EDUCATION

The meeting was convened Monday, May 2, 1988, in Las Vegas.

1. Bill Spielman said the Education Committee would consider the charge adopted by Council at its October 1987 meeting. *1)* Develop a model curriculum leading to the Ph.D. in physiology. New approaches to graduate education should take into account the need to integrate systems physiology and cellular and molecular biology. In this regard the Education Committee should approach the ACDP for input. *2)* Develop tutorials, seminars and workshops designed to introduce graduate students and established investigators to the use of the new tools such as cell and molecular biology.

A. The development of a model curriculum that incorporates contemporary approaches of molecular and cellular biology into the training of physiologists without abandoning the perspective of organ/system integration. It was decided that Bill Spielman will develop a draft document that outlines the principles, objectives, and general plan (including timetable) for the development of such a curriculum. This draft will be distributed to the Education Committee members before the next meeting. The principle task of the Education Committee at the next meeting will be to generate a document for presentation to Council.

It was decided that to develop a useful curriculum, it was first necessary to generate a list of Educational Objectives for the training of graduate students. It was acknowledged that this was a major undertaking that should involve the various Sections of APS, including the Teaching Section/Interest Group, and will require several iterations of review and revision.

B. Seminars, tutorials, and workshops to introduce graduate students and members to new techniques and approaches. It was decided that the Education Committee should have official representatives to the Program Advisory Committee (PAC). Jerry Herlihy and Frank Powell will serve as the Education Committee PAC representatives. Ideas for symposia, workshops, and tutorials will be developed and forwarded to the Program Committee at the Fall and Spring Meetings of the Society.

Suggestions will be solicited from the membership via announcements in *The Physiologist*. Attempts will be made to coordinate topics with the themes of the upcoming meetings. Financial and technical support from pharmaceutical/biotech companies should be explored.

Jerry Herlihy will develop a symposia/workshop involving the intracellular imaging of fluorescent indicators to be submitted to the PAC at the upcoming Fall Meeting of the APS.

2. FASEB High School Science Teacher Program. The Federation has undertaken to support a program to support high school teachers for summer research experiences in active laboratories of its members. While the concept was felt to be outstanding, it was generally thought that APS could support our own program. Chris Barney stated that he was aware of similar programs supported by national agencies (i.e., NSF, NIH, Hughes) and indicated he would be willing to develop a program for submission to Council. The possibility of using the program for minority high school teachers was also discussed.

3. Overlap of Education Committee mission with the mission of other standing committees. Bill Spielman suggested that the Education Committee mission overlaps to some degree with the Career Opportunities Committee, the Program Committee, and possibly the Industrial Liaison Committee. Other areas of apparent overlap are the Teaching Section/Interest Group and the publication of an Educational Journal for the APS. This issue was raised by Bill Spielman in a letter to Norman Staub, Chairman of the Committee on Committees. The Committee on Committees is currently attempting to more clearly define the mission of the various standing committees. It was decided that as a first step to ensure coordination of activities between committees, the Education Committee would send representatives to the other committees meetings: Career Opportunities, Bill Spielman; Liaison with Industry, Bill Spielman; Program Advisory, Frank Powell and Jerry Herlihy. It was also decided that a representative from the Teaching Section/Interest Group would attend the meeting of the Education Committee as an Ex Officio member.

4. Declining scores on the Physiology section of the National Boards given to medical students. Concern has been expressed that scores of the physiology section of the national board taken by medical students is falling and is among the lowest of the basic sciences. Bill Spielman will contact Don Jewett of the National Board to determine if indeed that this is a problem and report back to the Education Committee.

5. Recognition of efforts to utilize molecular/cellular biology approaches in physiological research. A suggestion was made to explore the possibility of awarding someone for their efforts in the use of molecular biology or cellular biological techniques to address physiological research issues. It was suggested that this might serve to promote and encourage others to expand their approach to these newer technologies. No action was taken.

6. Chairman of Education Committee to be on sabbatical from August 1988 to August 1989. During the above period, Jerry Herlihy offered to serve as deputy Chairman of the Education Committee and will attend council at the Fall meeting in Montreal.

7. Next meeting of Education Committee. It was decided to have a meeting of the Education Committee on June 14, 1988 at APS headquarters in Bethesda. The meeting will be followed by selection of the Wellcome Fellows.

The meeting was adjourned at 1:30 P.M.

William S. Spielman, Chairman

FINANCE

The charge to the Finance Committee at its Spring Meeting is to review and modify the 1988 budget that was presented to Council in October 1987. Together with the Executive Director, the Committee reviewed the Society's performance in 1987, revised the 1988 budget, and submitted it to the Council for revision and adoption. Based on the performance of the Society in 1987, the Finance Committee recommended an overall budget for fiscal year 1988 of $7,514,690.

During 1987, the Society's journal operations ended the year with income over expenses in the amount of $703,386, a portion of which was directed to the Contingency and Reserve Account. The Society's operating fund, derived from direct membership activities, ended the year with income over expenses of $71,642. The Society's book operations ended the year with expenses in excess of income of $96,836. The Centennial Celebration Fund was closed out with an income over expenses of $30,090, which was allocated to the Program Endowment Fund.

The Finance Committee is also responsible for reviewing the performance of the Society's managed accounts. As a result of a review of previous performance, the managed accounts were divided among three different managers under the direction of E. F. Hutton in February 1987. Overall, the managed accounts weathered the October 19 crash well, experiencing less damage than the Dow Jones Composite Index. Over the year, the total value of the accounts was reduced by approximately $100,000. As of December 31, 1987, the accounts had the following market value: Operating Reserve Investment Account = $2,486,213; Publications Contingency and Reserve Account = $2,122,412; Caroline tum Suden Account = $257,946; IUPS Account = $170,967; and the Perkins Memorial Fund = $170,327.

The Publications Contingency and Reserve Fund is a long-term fund established by the publications trustees of which the income can be used by Council for emergencies of the Society including publications. Its utilization is determined each year at the Spring meeting, keeping in mind that the primary goal is to return as much as possible to capital investment. The Operating Reserve Investment Fund was authorized in 1976. These are funds derived primarily from advance subscription fees received for Society publications; hence they are diverted from the current operating cash account in an attempt to generate capital gains. Our long-term goal is to have sufficient funds in properly managed accounts to underwrite the activities of APS for one to one and a half years.

The annual audit of APS by Coopers and Lybrand was received and reviewed by the Finance Committee as part of its responsibility. The audit found the operations of the Society to be "in conformity with generally accepted accounting principles" and the statements "present fairly the position of the American Physiological Society."

F. J. Haddy, Chairman

APS Balance Sheet, December 31, 1987

ASSETS		LIABILITIES	
Cash, including savings accounts	$1,736,813	Accounts payable and accrued expenses, including $68,727 in 1987 and $77,843 in 1986 due FASEB	329,600
Certificates of deposit	2,300,000	Unearned income	
	4,036,813	Subscriptions	3,704,933
U.S. Treasury Bills, at cost which approximates market value	1,451,350	Dues	248,922
Marketable securities, at cost, market value: 1987, $4,530,082; 1986, $4,658,874	4,076,980		3,953,855
Accounts receivable, including $20,000 in both 1987 and 1986 due from FASEB	470,849		4,283,455
Advances to section editors	145,500	Unexpended grants and programs	1,118,407
Prepaid expenses	7,116	Commitments	5,401,862
Inventories	1,384,367		
Deferred audiovisual costs	93,475	**FUND BALANCES**	
Furniture, fixtures and equipment, net of accumulated depreciation of $28,992	120,601	Publications general fund	4,903,726
	11,787,051	Publications special fund	607,045
Net assets restricted and allocated for unexpended grants and programs:		Society general fund	179,672
Cash, including savings accounts: 1987, $128,651; 1986, $108,494	212,060	Publications contingency and reserve fund	
Certificate of deposit	100,000	Program Endowment Fund	500,000
U.S. Treasury Bills, at cost which approximates market value	197,098	Principal	1,098,651
Marketable securities, at cost, market value: 1987, $598,981; 1986, $556,170	610,855	Income	214,502
Accounts receivable (payable) net	(1,606)		7,503,596
	1,118,407		$12,905,458
	$12,905,458		

GOVERNMENT RELATIONS INITIATIVE PROGRAMS (GRIP)

The second meeting of the Committee on Government Relations Initiative Programs (GRIP) was held May 3, 1988, at the Las Vegas Hilton Hotel in Las Vegas, NV.

The first item of business was a review of the minutes of the March 10–11, 1988, meeting, which were approved without change.

In response to the request of the GRIP Committee, Louis Ramazzotto, who is the APS representative to the American Association for Accreditation of Laboratory Animal Care (AAALAC), was invited to discuss AAALAC policies and procedures. Ramazzotto gave the history, composition, and role of AAALAC and he agreed with Committee members that there are concerns and problems about AAALAC procedures. He said he would be willing to voice APS' concerns to the AAALAC governing board.

David Ramsay appointed a subcommittee—Drs. Arthur Guyton (chairman), Francis Haddy, and Howard Lowensohn—to identify APS members' concerns and to develop recommendations to be presented to AAALAC. The subcommittee is to invite input from Dr. Ramazzotto and is to present its findings and recommendations at the fall meeting of the GRIP Committee.

The chairman then told of his meeting earlier in the day with the APS Council and reported that the Council had endorsed all of the GRIP Committee recommendations from its March meeting. He also noted that many of the recommendations already have been started by staff, including brochures on animal usage and a handbook for dealing with the Congress. (The GRIP Committee members are to review the handbook before it goes to the printer).

One of the approved recommendations is the development of a source book/newsletter. A subcommittee—Drs. Richard Malvin (chairman), Allen Cowley, Francis Haddy, and David Ramsay—was appointed to develop a source book/newsletter format, including kinds of information to be collected, who should receive the materials, cost factors, etc. This information is to be presented to the GRIP Committee at its fall meeting.

In preparing for the 1989 Spring Meeting in New Orleans, Dr. Fred Zechman was named chairman of the subcommittee to develop a workshop program to be conducted the afternoon before the start of the meeting. Other members of the subcommittee are Drs. Thomas Burks and Sidney Solomon. The subcommittee is to coordinate its planning with the APS staff to avoid possible conflicts and to assure adequate meeting space. The subcommittee is to report its progress at the Committee's fall meeting, which is Sunday afternoon, October 9, 1988, in Montreal, Canada.

David J. Ramsay, Chairman

INTERNATIONAL PHYSIOLOGY

The International Physiology Committee on Tuesday, May 3, 1988. The meeting was chaired by Alfred Fishman. After some opening remarks, the committee considered a series of initiatives that Harvey Sparks and Martin Frank have undertaken during the past year. They reported on a meeting with Mr. William Walsh, Jr., Vice President for Operations of Project Hope. We found two areas of possible interaction. First, Project Hope will be able to arrange to distribute a number of the Society's books. We have an overstock of several of our *Handbooks* and Clinical Series and Project Hope feels these would be useful to a number of their clients in developing countries. They will take the books from our warehouse and arrange for their distribution. Project Hope is also interested in collaborating with the American Physiology Society to obtain support to send physiologists to developing countries to participate in the training programs of Project Hope. In a number of instances they have found that the training of practitioners in developing countries is less than optimum because the practitioners do not have an adequate basic science background. They would like to be able to come to us for names of individuals who have expertise in particular areas who would be willing to spend four-to-six weeks in a developing country putting on a course on the specific area needed to prepare practitioners for advanced training. After some discussion of this, the Committee endorsed the plan and recommended that we continue to explore the possibility of extramural funding of this activity.

Dr. Sparks reported on a request from AAAS to provide the Society's journals for distribution for subsaharan African countries through a program that AAAS has worked out with the U.S. Agency for International Development. The plan is that we will provide the journals to USAID, which will ship the journals to libraries in subsaharan Africa as specified by AAAS.

Brenda Rauner, Publications Manager, has estimated that the cost of a run of an additional 100 copies of the *American Journal of Physiology* and the *Journal of Applied Physiology* would be $6,000. The International Committee endorsed the idea of proceeding with this project and recommended that Council should give approval on this expenditure.

Dr. Sparks then described an exploratory letter that had been sent to several funding agencies that proposes that the APS be supported to be a clearing house for American physiologists who wish to teach and do research in developing countries. The Society is also prepared to foster linkages that would provide training for predoctoral students in physiological sciences.

The International Committee members encouraged Dr. Sparks and Dr. Frank to go ahead with these fund-raising efforts. However, the members encouraged Drs. Sparks and Frank to do a survey to find out how much of this type of activity is already going on in Departments of Physiology in the United States. Drs. Sparks and Frank agreed to prepare a questionnaire for Physiology Chairmen.

LONG-RANGE PLANNING

The Long-Range Planning Committee (LRPC) met on Wednesday, March 16, 1988, in the APS Headquarters. Dr. Ernst Knobil said that the LRPC will consider the charges adopted by Council at its October 1987 meeting.

1. Develop a "white paper" on the future of physiology and the ways in which the Society can be useful to the progress of the discipline. This should include a consideration of strategies to foster the use of tools of cellular and molecular biology to solve the important questions of systems physiology.

2. Make recommendations on how APS should relate to FASEB and other societies, e.g., the Society of Neuroscience, the Biophysical Society, the American Society for Cell Biology, and the Endocrine Society.

3. Develop a plan for more active leadership in the development of programs. The Long-Range Planning Committee should consider the success of the Publications Committee as a model for what may be possible in the area of Program.

4. Make recommendations on how the Society can best serve those Sections that currently have minimal participation in its meetings.

5. Make recommendations about the number and characteristics of the meetings of the Society.

It was reiterated that the agenda for this first meeting of the LRPC was the deter-

mination of how the group would approach these five charges. An animated, freewheeling discussion followed that ranged over all the issues confronting physiology in the past, present, and future as well as its opportunities. It was emphasized that before substantive progress could be made by the Committee, agreement must be reached regarding the definition of physiology, because the word means different things to different people at different times. It was recognized that, when discussing the future of physiology, its several aspects must be kept clearly in mind lest confusion result:

1. *The Science of Physiology.* The science of physiology uniquely deals with issues in regulatory or integrative biology at all levels of complexity from the molecular to the organismic. All biologists concerned with these issues may be considered physiologists. Viewed in this light, physiology is thriving at an unprecedented pace and will undoubtedly represent the next revolution in biology, the last having been molecular biology.

2. *The Discipline of Physiology.* Because of physiology's rapidly advancing and changing frontiers, it is no longer possible to view physiology as an easily encompassable and circumscribable discipline as it once was thought to be. When we speak of the discipline of physiology what exactly do we mean?

3. *Physiology Departments.* Departments of physiology are largely in medical school and exist principally because of their curricular responsibility of teaching courses in medical physiology to medical students and other health professionals. Much of the research conducted in these departments is no longer directly relevant to their teaching mission and much of what may be considered to be research in physiology is pursued in other university departments and nonteaching enterprises. Some major medical schools have considered departments of physiology to be superfluous and have abolished them in substance if not in name. Major problems exist in this arena.

4. *The American Physiological Society.* The mission of the APS is to serve physiology and physiologists in the broadest sense. Its future needs to be considered in the light of the future of regulatory biology as a scientific endeavor (very bullish), the future of physiology as a traditional discipline (bleak), and the future of physiology departments and their missions in medical curricula (problematic). In any case, these several futures may not necessarily have parallel courses and each must be discussed in its own right.

It is expected that all charges to the Committee will be executed within the next three years, culminating in the publication of the "White Paper on the Future of Physiology."

It was decided that the five charges to the LRPC be initially addressed by small subcommittees that will generate discussion papers for consideration by the group as a whole.

Charge 1

The Committee limited the "future" to be addressed to the year 2,000. This, however, may well be extended as the work progresses. It was agreed to have Dr. Schultz generate a discussion paper encompassing possible contents of the "White Paper" in consultation with Dr. Knobil. This discussion paper will then be circulated to the members of the Committee for emendation and redrafting. This reiterative process, along with periodic discussions at Committee meetings, should eventually result in a document that reflects the views of the LRPC. The deadline for the preparation of the initial discussion paper is August 1, 1988.

Charge 2

The relationship between the APS and FASEB have been thoughtfully considered by Council for many years. The last Task Force to address this issue, chaired by F. E. Yates, made its report in 1973. The historic conflicts between FASEB and its constituent Societies have recently intensified once again and a FASEB retreat to consider current issues has been scheduled for September. Following the retreat Drs. Frank and Knobil will prepare a discussion paper for distribution to the members of the LRPC.

Charges 3 and 4

It was pointed out that the Program Committee of the APS has customarily dealt with very immediate programming issues, while our Group is charged with addressing broad long-term matters such as the number and nature of our annual meetings.

The Program Committee, in response to a request by Council, will recommend that the APS reduce its annual meetings from two to one and that this singular event be held in conjunction with the FASEB meeting. While withholding judgement regarding the nature and venues of future meetings, the LRPC, after brief discussion, voted unanimously to endorse the recommendation of the PC that the APS have but one Society meeting per year.

In an attempt to delineate the task of the LRPC in this arena, Dr. Knobil will invite Dr. Gisolfi, the chairman of the PC, to meet with it in Las Vegas in April.

Charge 5

This charge, like most of the others, is inextricably linked with Charge 1. To help identify some of the issues inherent in this charge, Dr. Knobil will invite Dr. Blake Reeves, the Chairman of the Section Advisory Committee, to meet with the LRPC in Las Vegas. Further discussion with Dr. Reeves and his committee should assist in defining a point of departure for further activity in this arena.

To facilitate the work of the Committee, Dr. Frank agreed to supply it with background information relating to the affairs of the APS not readily available in the recently published history of the Society. In addition, he will attempt to procure position and policy papers of the American Association of Anatomists and the American Association of Zoologists who confronted many years ago some of the same problems of current concern to the APS. It would be useful for the LRPC to peruse the recent scientific programs of these two organizations, which are now seemingly alive and well despite earlier predictions of their certain demise.

Ernst Knobil, Chairman

PORTER DEVELOPMENT

The report of the Porter Development Committee to the Society again provides the opportunity of informing the members that fellowship funds are available for able minority students both at the pre- and post-doctoral level in keeping with the legacy of William Townsend Porter to "encourage and assist more young men and women of promise in the study of physiology." The current predoctoral fellows being supported by the Porter Development Committee are

- Karen Anderson, a candidate for the Ph.D. degree in the Department of Physiology and Biophysics at Colorado State University;
- J. Michael Gonzales-Campoy, a candidate for the Ph.D. degree in the Department of Physiology at Mayo Medical School;

- Jean A. King, a candidate for the Ph.D. degree in the Department of Biology at New York University;
- John Okwusidi, a candidate for the Ph.D. degree in the Department of Physiology and Biophysics at Howard University College of Medicine;
- Darlene K. Racker, who recently received the Ph.D. degree from the Department of Physiology and Biophysics at Chicago Medical School;
- Paulene Washington, a candidate for the Ph.D. degree from the Department of Physiology at the University of Western Ontario.

The Committee has continued funding for the Atlanta consortium, a program organized with the assistance of the Department of Physiology at Emory Medical School. Two former Porter Development Committee Fellows, Drs. Pamela Gunter-Smith and John C. S. Fray, have been Visiting Porter Lecturers in the Atlanta Program. The Dillard Program in New Orleans has been assisted by the staff of the Departments of Physiology at Louisiana State University and Tulane University.

The Committee is also providing support for a Summer Student Research Program for Native American Indians in the Department of Physiology and Biophysics at the College of Veterinary Medicine and Biomedical Sciences at Colorado State University and for a minority summer math-science program at Arizona State University. The Porter Development Summer Research Fellowship Program at the Marine Biological Laboratories at Woods Hole provided support for W. Hernandez, B. Dwight, K. Brewton, A. Freepong-Boudo, and E. Cruise.

Note the publication of an important book on science education among native American Indians: *Searching, Teaching, Healing: American Indians and Alaskan Natives in Biomedical Research Careers,* edited by Edwin W. Haller and Ruth A. Myers of the University of Minnesota-Duluth. It is available from Futura Medical Services, Inc., 295 Main Street, Mount Kisco, NY 10549.

We again express our appreciation to the Harvard Apparatus Foundation for its continuing support of the Porter Development Program. We should also like to acknowledge gifts from the Lederle Laboratories of the American Cyanamid Company and individual members of the American Physiological Society.

Dr. A. Clifford Barger
Dr. Eleanor Ison Franklin
Co-Chairmen

PUBLICATIONS

In 1987 the journal program continued to operate in the black despite the added cost of publishing 5% more pages and a 3% loss of subscriptions. Subscription prices for 1988 were increased 9% for the *AJP* journals, *JN*, and *PRV* and 15% for *JAP* to cover the expected publication of even more pages in 1988 and expected increases in mailing and paper. Submitted manuscripts increased by 9% in 1987.

On the recommendation of the Committee, the Council approved the founding of a new journal, *American Journal of Physiology: Lung Cellular and Molecular Physiology*, which will begin as a bimonthly in 1989 with Donald J. Massaro as editor. The first issue of the journal is scheduled for August 1989. The Committee is grateful to Alfred P. Fishman for continuing to serve in 1988 as editor of the *Journal of Applied Physiology*; the search continued for a new *JAP* editor.

The Committee approved the publication of an educational periodical to be distributed twice yearly to APS members with *The Physiologist* and *NIPS*. It will be indexed as a supplement to the *American Journal of Physiology* and also be available by subscription. Harold I. Modell, University of Washington, was chosen as editor.

The transition of editorship for *News in Physiological Sciences* from Knut Schmidt-Nielsen to John T. Shepherd went very smoothly. Dr. Shepherd's able group of assistant editors at Mayo and approximately 60 international associate editors will ensure the continued success of this prestigious journal. Dr. Shepherd's first issue as editor will be August 1988. The Society owes a debt of gratitude to Knut Schmidt-Nielsen, whose editorship was responsible for the excellence of this well-received IUPS/APS publication. Dr. Schmidt-Nielsen has agreed to serve as Consulting Editor beginning with the August issue. The Publications Office is cooperating with IUPS in locating subscribers in developing countries who could benefit from the $10,000 IUPS has available for subsidized subscriptions.

In response to the concerns of the journal editors and the Publications Committee over the length of time from receipt of manuscript to publication, the Committee has asked the Publications Manager to investigate the production time for comparable journals in the field and look into ways of reducing APS production time. Manuscripts on disks will be solicited through announcements in the journals in an effort to develop the benefits of the new technologies. The use of FAX machines and computers is already shortening communication time between the Publications Office and Editors.

Six books were published by the Society in 1987: two volumes of the *Handbook* (*Higher Functions of the Brain*, vol. V in the *Nervous System*, and *Gas Exchange*, vol. IV in the *Respiratory System*), one Clinical Series book (*Atrial Hormones and Natriuretic Factors*), and three history books (*Physiology in the American Context, 1850–1940*; *History of the American Physiological Society: The First Century, 1887–1987*; and *Renal Physiology: People and Ideas*).

In December 1987 a contract was signed with Oxford University Press under which Oxford agreed to take over publication of new APS-sponsored books in the future and the distribution of all current titles as of January 1988 (see the April 1988 issue of *The Physiologist*, p. 25). The transitional period in the book program has gone very well. Book orders have been fulfilled by Oxford since January with a significant increase in sales as books on consignment were paid for by dealers. Domestic and overseas stock has been moved from Williams & Wilkins to Oxford's warehouses. Oxford is developing an aggressive promotional campaign for all APS books, both old and new. Members are being offered a 35% discount on all books.

It was agreed that the four *Gastrointestinal System Handbooks* should be finished in-house, as well as the Clinical Series book, the *Clinical Physiology of Sleep*, and the two history books, *Endocrinology: People and Ideas* and *Membrane Transport: People and Ideas.*

The *Renal Physiology Handbook* will be the first *Handbook* published by Oxford University Press for APS. The Editor, Erich E. Windhager, is working closely with OUP to arrange royalty contracts and expedite publication.

The Publications Committee plans to appoint a committee to oversee the planning of *Handbooks* and one to oversee the new technique book series; the Clinical Series Book Committee is already in existence. Once the in-house books are completed, the financial burden on the Society of maintaining an expensive book program will be alleviated. Already the Publications Committee, Executive Director, and Publications Manager are concentrating their efforts on streamlining the journal program to counter the escalating publications costs

faced by all publishers.

1987 was an eventful year for publications, with the unexpected death of Stephen R. Geiger and the appointment of Brenda B. Rauner as Publications Manager and Executive Editor, the signing of the book contract with Oxford University Press, the search for editors for the new *American Journal of Physiology* journal, the *Journal of Applied Physiology*, and *News in Physiological Sciences*. The Publications Committee held many extra meetings and conference calls, and I thank them for their efforts. Amid all this activity, the flow and quality of the APS books and journals continued without interruption throughout the year because of the dedicated commitment of our editors, editorial board members, reviewers, and especially of our headquarters staff.

Paul C. Johnson, Chairman

Formation of Hypoxic Interest Group

The APS is in the process of formally establishing a multidisciplinary **Hypoxic Interest Group** and invites any member of the APS who is interested in any aspect of the physiology of low oxygen to join. We have been meeting informally at lunch time at the last two FASEB meetings with great success. Speakers have discussed diverse subjects such as control of erythropoietin, fluid balance, and cardiovascular adaptation to hypoxia. We will continue these lunch time discussions at future FASEB meetings as well as possibly programming "hypoxia" scientific sessions.

You can join the Hypoxic Interest Group without affecting your affiliation with an APS section. Reed Hoyt and Hershel Raff are the organizers of the Interest Group. If you are interested in joining, please write to Dr. Hershel Raff, St. Luke's Medical Center, Medical College of Wisconsin, 2900 W. Oklahoma Avenue, Milwaukee,WI 53215.

New *AJP* Journal

The Publications Committee of the American Physiological Society announces the establishment of a new journal, the *American Journal of Physiology: Lung Cellular and Molecular Physiology*. Donald J. Massaro has agreed to serve as Editor. He is joined by a distinguished group of Associate Editors and Editorial Board members. The new journal is scheduled for publication in August 1989. The decision to establish the new journal followed extensive review of the continuing growth of respiratory physiology and the demands it placed on current journals of the Society, specifically the highly successful *Journal of Applied Physiology*.

The *American Journal of Physiology: Lung Cellular and Molecular Physiology* is intended to provide a publishing outlet for a growing segment of the respiratory physiology community with interests in the cellular and molecular area. At present no journal has emerged to meet the needs of this group, and the work is dispersed in a wide variety of publications. The new publication will enable workers in this area to obtain peer review of their research by others whose interests are closely related to their own. The journal should also be of benefit to other respiratory physiologists who should find this type of research more readily accessible in a sectional journal of the *American Journal of Physiology*.

The *American Journal of Physiology: Lung Cellular and Molecular Physiology* will publish original investigative and theoretical papers dealing with molecular, cellular, and morphological aspects of normal and abnormal function and response of cells and components of the respiratory system; this is meant to include the nose and sinuses, the conducting airways, lung parenchyma and pleura, neural cells involved in the control of breathing, neuroendocrine and immunological cells in the lung, and cells of the diaphragm and thoracic muscles. Areas of interest include the following: metabolic control at a cellular level, regulatory and informational molecules, gene expression, the structure, composition, and turnover of macromolecules, cell-to-cell and cell-matrix interactions, cell motility, secretory mechanisms, membrane function, surfactant, matrix components, mucus and lining materials, lung defenses, macrophage function, electrolyte and water transport, development and differentiation of the respiratory system, and the response of the respiratory system and its components to the environment. Reports of research using innovative techniques and approaches of molecular and cell biology, cell physiology, molecular genetics, biochemistry, biophysics, and morphology are especially welcome.

Additional features of the new journal will include *1)* Invited Reviews that are devoted to an in-depth review of important topics; *2)* Commentaries that are short comments on timely topics, topics that have been overlooked and warrant attention, and reviews of workshops, seminars, and meetings of interest to readers; *3)* Historical Vignettes that are intended to provide a perspective on the development of the field of lung cellular and molecular physiology, including essays on those aspects of classic respiratory physiology that form the basis of contemporary lung cellular and molecular physiology; *4)* Letters to the Editor generally dealing with articles that appeared in the journal, but readers' views on other topics of interest are also solicited; and *5)* Rapid Communications.

Until now the needs of the respiratory physiology community have been served by the *Journal of Applied Physiology*. That journal has a long and distinguished history; it is one of the largest and most rapidly growing journals in the APS family of journals. The *Journal of Applied Physiology* has served and will continue to serve as the premier outlet for research on a broad variety of topics in respiratory, exercise, and environmental physiology. It has attracted relatively few papers in the area of emphasis of the new journal and, as a consequence, the two journals will complement one another. The new journal is expected to have little effect on the content of the *Journal of Applied Physiology*.

Further information about the new journal will appear periodically in the journals of the Society. **Manuscripts are now being accepted for review.** Authors are directed to the current *American Journal of Physiology* Information for Authors for manuscript submission requirements. Dr. Massaro invites authors to provide the names of four to six individuals whom they consider appropriate reviewers for their manuscripts. Although the editors will seriously consider these nominees, they reserve the right to use any or none of the proposed names for reviewers.

Submitted manuscripts and subscription inquiries should be directed to the American Physiological Society, 9650 Rockville Pike, Bethesda, MD 20814, and inquiries about scientific content to Dr. D. J. Massaro, Pulmonary Research R-120, University of Miami, P.O. Box 016960, Miami, FL 33101.

Paul C. Johnson

NIDDK Travel Fellowships for Minority Students Program

A most successful APS/NIDDK sponsored travel fellowship program provided an opportunity for 18 highly qualified minority students and scientists to attend the 1988 Spring FASEB Meeting. To enhance their experience at the meeting, the fellows were introduced to mentors at an orientation reception on preceding the scientific sessions. Throughout the week, the mentors assisted the fellows in selecting the appropriate scientific sessions. At an American Cyanamid Company–sponsored luncheon, held on the last day of the meeting, L. Gabriel Navar addressed the group, and there was an enthusiastic exchange of experiences. The program, which has been extremely well received, will be provide funds for 10 fellows to attend the Fall APS Meeting in Montreal, Canada.

Recipients of the spring 1988 fellowship program were Ricardo Brown (Howard University); Margaret Colden-Stanford (Baylor College of Medicine); Barbara A. Davis (Tucson, AZ); Dominique Delma (City College of New York); Carla Harwell (University of California, Los Angeles); Lucretia A. Hernandez (University of South Alabama); Cynthia Ann Jackson (University of California, Davis); John Okwusidi (Howard University); Hilda M. Osorio (University of Puerto Rico); Darlene Racker (University of Health Sciences/The Chicago Medical School); Alfredo Rego (Georgetown University); Jose Romero (University of Puerto Rico); Lisa Rubero (University of Puerto Rico); Annabell Segarra (New York University); Amilcar Toro (University of Puerto Rico); and Thomas B. Trice (North Carolina Agricultural and Technical State University).

1988–1989 Standing Committees and Sections

APS Council

A. E. Taylor, President
H. V. Sparks, Jr., Past President
V. S. Bishop, President-Elect
M. Frank, Executive Director
B. Bishop, Councillor
S. Chien, Councillor
A. W. Cowley, Jr., Councillor
G. H. Giebisch, Councillor
N. C. Staub, Councillor
P. D. Wagner, Councillor
C. V. Gisolfi, ex officio
F. J. Haddy, ex officio
P. C. Johnson, ex officio
R. B. Reeves, ex officio
W. S. Spielman, ex officio

Society Standing Committees

Animal Care and Experimentation
D. J. Ramsay, Chair (1989)
S. M. Cain (1990)
H. Lowenshon (1991)
R. L. Malvin (1991)
R. A. Murphy (1990)
L. Ramazzotto (1989)
N. B. Marshall, ex officio (1991)
W. M. Samuels, ex officio

Career Opportunities in Physiology
S. A. Nunneley, Chair (1989)
F. G. Hempel (1989)
N. Manson (1991)
R. S. Moreland (1990)
J. T. Reeves (1989)
M. Townsley (1991)

Committee on Committees
N. C. Staub, Chair (1989)
K. H. Berecek (1989)
H. J. Granger (1990)
L. R. Johnson (1991)
H. A. Kontos (1990)
C. L. Seidel (1989)
J. Van Liew (1989)

R. G. Daggs Award
R. G. Berne, Chair (1989)
O. E. Reynolds (1990)
D. F. Bohr

Education
W. S. Spielman, Chair (1988)
C. C. Barney (1990)
P. C. Churchill (1990)
J. P. Filkins (1991)
J. C. Fray (1989)
J. T. Herlihy (1989)
M. G. Levitzky (1991)
F. L. Powell, Jr. (1990)

Finance
F. J. Haddy, Chair (1989)
N. R. Alpert (1991)
D. Rennie (1990)

Honorary Membership
E. H. Wood, Chair (1989)
R. M. Berne (1990)
J. R. Pappenheimer (1991)

International Physiology
A. P. Fishman, Chair (1989)
C. Blatteis (1991)

S. K. Hong (1990)
D. Jennings (1991)
L. B. Rowell (1990)
N. L. Stephens (1990)

Liaison With Industry
N. B. Marshall, Chair (1991)
M. Blaustein (1991)
J. W. Fara (1991)
S. F. Flaim (1990)
L. Hamilton (1991)
P. M. Vanhoutte (1990)
C. V. Gisolfi, ex officio (1991)
W. S. Spielman, ex officio (1991)
S. Nunneley, ex officio (1989)

Long-Range Planning
E. Knobil, Chair (1990)
G. Giebisch (1991)
J. P. Granger (1991)
J. E. Greenleaf (1990)
R. R. Llinas (1990)
J. Mitchell (1991)
E. R. Nadel (1990)
S. G. Schultz (1989)

Membership
C. Levinson, Chair (1990)
N. S. Cherniack (1990)
I. G. Joshua (1990)
R. Korthius (1991)
C. M. Tipton (1989)

Perkins Memorial Fellowship
H. V. Sparks, Jr., Chair (1991)
S. Chien (1989)
R. H. Kellogg (1989)
C. Paganelli (1991)
J. R. Pappenheimer (1989)
J. Hauck, ex officio

Porter Physiology Development
A. C. Barger, CoChair (1989)
E. L. Ison-Franklin, CoChair (1990)
A. B. Craig, Jr. (1990)
D. L. Crandall (1989)
J. C. Fray (1990)
P. J. Gunther-Smith (1989)
L. G. Navar (1991)
J. G. Townsel (1991)

Program
C. V. Gisolfi, Chair (1991)
P. D. Harris (1990)
S. K. Hong (1989)
L. G. Navar (1989)
V. S. Bishop, ex officio (1989)
M. Frank, ex officio

Program Advisory
(Section Appointments)
Cardiovascular—J. W. Downey (1988) and H. J. Granger, ex officio
Cell and General Physiology
L. Mandel (1990)
Comparative Physiology
L. I. Crawshaw (1989)
Endocrinology and Metabolism
D. Wasserman (1988)
Environmental, Thermal and Exercise Physiology—E. Nadel (1990)
Gastrointestinal Physiology
J. D. Wood (1990)
Nervous System
R. Lydic (1989)
Neural Control and Autonomic Regulation—M. I. Phillips (1988)
Renal Physiology—R. G. O'Neill (1989) and L. S. Costanzo (1990)
Respiratory Physiology
R. A. Klocke (1991)
Teaching of Physiology
R. Carlin (1989)
Water and Electrolyte Homeostasis
E. H. Blaine (1988)
Clinical Physiology Group
J. F. Biebuyck (1988)
Epithelial Transport Group
R. A. Frizzell (1988)
History of Physiology Group
D. Gilbert (1990)
MYOBIO Group—M. Siegman (1988)

Public Affairs Executive
R. L. Malvin, Chair (1989)
A. C. Barger (1989)
S. Solomon (1990)
N. B. Marshall, ex officio (1991)
W. M. Samuels, ex officio

Publications
P. J. Johnson, Chair (1991)
F. Abboud (1990)
J. S. Cook (1989)
M. J. Fregly (1991)
S. H. White (1990)
A. E. Taylor, ex officio (1989)
M. Frank, ex officio
B. Rauner, ex officio

Section Advisory
B. R. Reeves, Chair (1990)
Cardiovascular Section
N. R. Alpert (1989)
Cell and General Physiology Section
L. Reuss (1989)
Comparative Physiology Section
A. F. Bennett (1991)
Endocrinology and Metabolism Section
L. S. Jefferson (1989)
Environmental, Thermal and Exercise Physiology Section—E. R. Buskirk (1991)
Gastrointestinal Physiology Section
J. A. Williams (1991)
Nervous System Section
R. R. Llinas (1990)
Neural Control & Autonomic Regulation Section—P. G. Schmid (1988)
Renal Physiology Section
W. J. Arendshorst (1990)
Respiratory Physiology Section
R. W. Hyde (1990)
Teaching of Physiology Section
H. Modell (1990)
Water and Electrolyte Homeostasis Section—L. Share (1988)

Senior Physiologists
R. O. Greep, Chair (1990)
R. W. Berliner (1989)
J. R. Brobeck (1990)
H. W. Davenport (1990)
S. M. Horvath (1990)
R. E. Johnson (1989)

Women in Physiology
C. Chew, Chair (1989)
H. V. Carey (1990)
H. J. Cooke (1991)
B. Horwitz (1989)
S. Opava-Stitzer (1990)
M. Frank, ex officio

Society Representatives to Other Organizations

American Association for Accreditation of Laboratory Animal Care
L. Ramazzotto (1989)

American Association for the Advancement of Science
M. I. Phillips (1989)
L. C. Senay, Jr. (1989)

American Institute of Biological Sciences
M. Frank (Indefinite)

Council of Academic Societies of the Association of American Medical Colleges
G. A. Hedge 91990)
J. L. Kostyo (1989)

Federation of American Societies for Experimental Biology
Board
H. V. Sparks, Jr. (1989)
A. E. Taylor (1990)
V. S. Bishop (1991)
Executive Committee
V. S. Bishop (1991)
Executive Officers Advisory Committee
M. Frank (Indefinite)
Education Committee
W. S. Spielman (1990)
Finance Committee
H. E. Morgan (1988)
Life Sciences Advisory Committee
W. B. Seevers (1991)
Meetings Committee
C. V. Gisolfi (1990)
Program Committee
M. Frank (Indefinite)
Public Affairs Committee
R. L. Malvin (1991)
Public Information Committee
M. Cassidy (1991)
Publications Committee
L. S. Jefferson (1991)
Research Conference Advisory Committee
C. V. Gisolfi (1988)
D. N. Granger (1990)
3M Life Science Award Committee
W. E. Crill (1990)

National Association for Biomedical Research
M. Frank (Indefinite)

US National Committee for IUPS
A. P. Fishman (1989)
H. V. Sparks, Jr. (1989)
A. E. Taylor (1990)
V. S. Bishop, ex officio
M. Frank, ex officio

US National Committee on Biomechanics
J. S. Petrofsky (1990)

Society Sections

Cardiovascular
N. R. Alpert, Chair and Secion Advisory Committee (1989)
D. M. Griggs, Treasurer (1989)
A. L. Mark, Secretary (1989)
J. S. Janicki, Cardiac Mechanics Subsection (1988)
D. N. Granger, Splanchnic Circulation Subsection (1988)
H. J. Granger, Program Advisory Committee and Nominating Committee (1990)
J. W. Downey, ex officio, Program Advisory Committee (1988)
H. Kontos, Nominating Committee (1989)
J. B. Bassingthwaighte, Nominating Committee (1991)

Cell and General Physiology
L. Reuss, Chair and Section Advisory Committee (1989)
C. Pace, Secretary-Treasurer (1989)
A. Fabiato, Councillor (1990)
N. K. Willis, Councillor (1991)
L. Mandel, Program Advisory Committee (1990)

Comparative Physiology
A. F. Bennett, Chair and Section Advisory Committee (1991)
W. K. Milsom, Secretary (1989)
W. M. Danttzler, Treasurer (1990)
L. Crawshaw, Program Advisory Committee (1989)

Endocrinology and Metabolism
L. S. Jefferson, Chair and Section Advisory Committee (1989)
C. Desjardins, Secretary-Treasurer (1989)
G. A. Hedge, Councillor (1990)
J. L. Kostyo, Councillor (1991)
A. D. Cherrington, Program Advisory Committee (1989)
D. Wasserman, Program Advisory Committee, ex officio (1989)

Environmental, Thermal and Exercise Physiology
E. R. Buskirk, Chair and Section Advisory Committee (1991)
E. R. Nadel, Program Advisory Committee (1990)
B. A. Horwitz, Councillor (1990)
C. Tipton, Councillor (1990)

Gastrointestinal Physiology
J. A. Williams, Chair and Section Advisory Committee (1991)
J. Fondacaro, Secretary-Treasurer (1991)
J. A. Christensen, Councillor (1990)
H. J. Cooke, Councillor (1989)
P. Rayford, Councillor (1991)
J. D. Wood, Program Advisory Committee (1990)

Nervous System
R. Lydic, Chair and Program Advisory Committee (1989)
R. R. Llinas, Section Advisory Committee (1990)
S. M. Barman, Councillor (1989)
L. A. Campfield, Councillor (1989)
L. T. Landmesser, Councillor (1990)
E. Taub, Councillor (1990)

Neural Control and Autonomic Regulation
P. G. Schmid, Chair and Section Advisory Committee (1988)
C. M. Heesch, Secretary (1988)
M. P. Kaufman, Treasurer (1988)
M. I. Phillips, Program Advisory Committee (1988)

Renal Physiology
W. J. Arendshorst, Chair and Section Advisory Committee (1990)
W. S. Spielman, Secretary (1989)
T. C. Welbourne, Treasurer (1991)
R. G. O'Neill, Program Advisory Committee (1989)
L. S. Costanzo, Program Advisory Committee (1990)

Respiratory Physiology
R. W. Hyde, Chair and Section Advisory Committee (1990)
M. P. Hlastala, Secretary (1991)
N. H. Edelman, Treasurer (1990)
J. R. Rodarte, Councillor (1990)
R. A. Klocke, Program Advisory Committee (1991)

Teaching of Physiology
H. Modell, Chair and Section Advisory Committee (1990)
E. Rosenberg, Secretary (1989)
D. Richardson, Treasurer (1990)
D. Bruce, Education Committee Liaison (1989)
R. Carlin, Program Advisory Committee (1991)

Water and Electrolyte Homeostasis
L. G. Navar, Chair (1991)
J. E. Hall, Councillor (1990)
E. Blaine, Program Advisory Committee (1988)
L. Share, Section Advisory Committee (1988)

Epithelial Transport Group
R. A. Frizzell, Chair and Program Advisory Committee (1988)
P. Aronson, Steering Committee (1988)
D. Dawson, Steering Committee (1988)
E. Wright, Steering Committee (1988)

History of Physiology Group
J. B. West, Chair (1988)
D. Gilbert, Secretary-Treasurer and Program Advisory Committee (1990)
R. H. Kellogg, Member-at-Large Steering Committee (1988)

MYOBIO Group
M. Siegman, Chair (1988)
R. S. Eisenberg, Steering Committee (1988)
R. L. Moss, Steering Committee (1988)
J. A. Rall, Steering Committee (1988)
F. S. Fay, Steering Committee (1988)
A. W. Jones, Steering Committee (1988)
A. Fabiato, Steering Committee (1988)
M. Lieberman, Steering Committee (1988)
R. J. Solaro, Steering Committee (1988)
M. Siegman, Program Advisory Committee and Section Advisory Committee (1988)

Section Reports

CELL AND GENERAL PHYSIOLOGY

For the past four years, the Cell and General Physiology Section of the American Physiological Society has offered research awards to undergraduate and postdoctoral students. The awards are made based on the quality of a recipient's research in the field of cell physiology as represented by an abstract submitted to the Spring FASEB meeting. This year cash awards and certificates of recognition were made to two students.

Dr. Burton Horowitz, a postdoctoral student, presented an abstract in collaboration with Dr. R. A. Farley from the Department of Physiology & Biophysics, University of Southern California, Los Angeles, entitled "Synthesis of mammalian Na, K-ATPase: Alpha and beta subunits in the yeast *S. cerevisia*."

The undergraduate award was given to Paul A. Negulescu, who, in collaboration with Drs. W. W. Reenstra and T. E. Machen, from the Department of Physiology-Anatomy, University of California, Berkeley, submitted an abstract entitled "Intracellular Ca requirements for stimulus-secretion coupling in the parietal cell."

The awardees were recognized during the banquet/lecture on May 4 at the Las Vegas Hilton Hotel. Dr. Susumu Hagiwara, from the Department of Physiology, University of California, Los Angeles, gave a lecture entitled "Biophysics in strange animals." Dr. Hagiwara, who is one of the foremost membrane biophysicists in the world, delivered an inspiring lecture covering his scientific accomplishments and personal, often humorous, scientific experiences.

Members of APS who are primarily interested in Cell and General Physiology are encouraged to inform APS, by letter, that you wish to have your primary affiliation in APS-Cell. In addition, encourage your students to submit an abstract for the APS-Cell Research Award, as soon as possible, after the deadline for receipt of abstracts for the 1989 FASEB meetings and before Feb. 1, 1989, to Dr. Lazaro Mandel, Department of Physiology, Duke University Medical Center, Durham, North Carolina, 27710.

Caroline S. Pace
Secretary/Treasurer

COMPARATIVE PHYSIOLOGY

The Comparative Physiology Section held its annual business meeting at the FASEB Meetings in Las Vegas in May. Several items of note were discussed at this meeting.

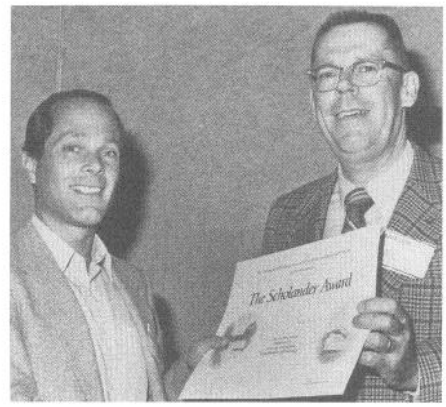
1. It was the consensus of all present that the Section should continue to have a keynote speaker begin the Scholander Award Session at the FASEB Meetings. Such an address at the beginning of this session was considered to be potentially valuable and the Program Committee of the Section is currently soliciting suggestions for future speakers.

2. It was also the consensus of those present that the Section should pursue the idea of hosting a Comparative Theme at the APS Fall Meeting every fourth year beginning with the meeting scheduled for San Antonio in 1990. This year was chosen so that these meetings would not conflict with the IUPS Meetings scheduled for 1989 or the IUBS meetings scheduled for 1991. Both international meetings will also convene every fourth year after these dates. It was envisioned that these "comparative" Fall Meetings would be held jointly with the Division of Comparative Physiology and Biochemistry of the American Society of Zoologists, allowing us to continue the successful collaboration that has developed over the years (APS Meetings in Toronto in 1980 and San Diego in 1982 and 1987).

3. Alan Hargens, Ron Millard, and Steve Wood reported that a Kjell Johansen Commemorative Symposium has been set for July 6–8, 1989, at the Krogh Institute in Copenhagen, Denmark. This Symposium, which will be a satellite symposium of the IUPS Congress in Helsinki, will feature speakers chosen by their association with Kjell Johansen in the areas of metabolism, circulation, gas exchange, and blood gas transport. There is still room available in the program for a number of poster presentations; anyone wishing to make a presentation or simply attend this symposium should contact one of the organizers as soon as possible.

4. Finally, at the completion of the business meeting, Al Bennett assumed the chair of the Section from Roger Fedde and is joined by Bill Danzler (Treasurer), Larry Crawshaw (Program Advisory Committee Representative), and Bill Milsom (Secretary) as the executives for the coming year.

At the FASEB Meetings in Las Vegas, the Comparative Physiology Section sponsored one symposium and two contributed paper sessions. There were eight contestants in the Scholander Award Competition, which was won by Gary Malvin. Gary received his B.A. from the University of Michigan (1976) and his Ph.D. from the University of New Mexico (1983). He is currently completing postdoctoral work in the Department of Physiology and Biophysics at the University of Washington before taking a position as an Associate Scientist in the Biomedical Research Division of the Lovelace Medical Foundation in Albuquerque, New Mexico. Gary was presented the Scholander Award by the Chairman of the Section, Roger Fedde, at the Comparative Section Social. The Social also featured a presentation by Gerry Kooyman of his associations with Pete Scholander. The Section wishes to thank Gerry as well as Al Bennett and Stan Hillyard for their efforts in organizing the activities of our Section at this meeting and all the participants in the Scholander Award Competition. The judges also wish to acknowledge the overall high standards of all the contestants in this year's competition.



Roger Fedde, Chairman of Comparative Physiology Section, presenting Scholander Award to Gary Malvin.

William K. Milsom, Secretary

GASTROINTESTINAL

The Steering Committee of the Gastrointestinal Section has continued to communicate by phone and informally at the FASEB Meetings. Communication with the membership has included mailing of three newsletters and an annual business meeting held after the GI Dinner at the FASEB Meetings.

A nominating committee consisting of Drs. Norman Weisbrodt, Jean Morisett, and Travis Solomon was chosen to identify candidates for election to the Steering Committee. Dr. John Williams was elected Chairperson and will replace Dr. Helen Cooke, who will continue for an additional year as councilor. Dr. Williams will serve as Section Advisory Committee Representative and will replace Dr. Norman Weisbrodt. Dr. Joseph Fondacaro was elected secretary/treasurer to replace Dr. Herbert Ormsbee, and Dr. Phillip Rayford will assume the position of councilor.

The Steering Committee has been involved in the revision of bylaws for the section to comply with the new guidelines of the APS. These were approved unanimously by the membership.

The GI Section sponsored two symposia at the Spring FASEB Meetings entitled "Physiology and pathophysiology of reactive oxygen metabolites in the digestive system" organized by Drs. Neil Granger and Mark Grisham and "Neural control of the intestinal epithelium" organized by Dr. Helen Cooke. Dr. Jack Wood has solicited topics for symposia and has presented three symposia titles to the Program Advisory Committee for consideration for the 1989 FASEB Meetings.

The Gastrointestinal Prize for meritorious research was awarded to Dr. Gabriel Makhlouf who spoke on "Stimulus transduction in smooth muscle of the gut" at the GI Dinner.

The recipients of the Young Investigator Awards for 1988 included Mr. John Mendlein, a graduate student under the direction of Dr. Ernest Wright from the University of California at Los Angeles. His abstract was entitled "In vitro translation and glycosylation of the intestinal Na^+-glucose transporter." Dr. Leslie Reinlib received the Young Investigator Award in the postdoctoral category. He was recognized for his work "Single cell microfluorescence analysis indicates a prolonged elevation of cytosol-free Ca^{++} is responsible for carbachol-induced Cl^- secretion in an intestinal cell line." This work was carried out in the laboratory of Dr. Mark Donowitz.

Helen J. Cooke, Chairperson

RESPIRATORY PHYSIOLOGY

The annual Business Meeting of the Respiration Section of the American Physiological Society was held in Ballroom F of the Hilton Hotel, Las Vegas, Nevada on May 4, 1988, Chairperson Richard W. Hyde presiding.

1. The first item of business was a report on the new journal entitled *AJP: Lung Cellular and Molecular Physiology*. The new Chief Editor is Don Massaro. Members were informed that the new journal would appear bimonthly starting in the fall of 1989. The scope was clearly defined as being cellular and molecular physiological research pertinent to the lung and its supporting structures (chest wall, neurocontrol mechanisms, etc.). As such, it was per-

ceived that it would have minimal impact on the *Journal of Applied Physiology*. It was lamented that the American Thoracic Society has essentially identical plans in progress with its publications. This means that two almost identical new journals will appear simultaneously from these two Societies.

2. Dick Hyde communicated a message from Sue Hurd at NIH requesting Section members to provide input to the Lung Division at NHLBI for areas in need of further research.

3. The next report was from the Nominating Committee chaired by Joe Rodarte (other members were Bob Johnson and Don Massaro). The nomination process was discussed. Currently, the Nominating Committee proposes two candidates for each position up for election, and these are voted on the membership through a mail ballot. Members of the Section have always been able to submit additional candidates to be placed on the voting ballot provided they can obtain at least three signatures of endorsement, submit the name at least two months before the ballot is to be mailed, and secure an agreement from the candidate that he/she is willing to serve if elected. Dr. Rodarte suggested that the interests of the Section might be better served if the Nominating Committee proposed a single candidate for each position. Of course, members of the Section would still have the opportunity to submit their own candidates in the manner described above. It was, however, pointed out that at all other levels within the APS, a slate of candidates is proposed, among whom members must choose in the manner that we currently also employ within the Section. It was decided that this issue should be brought to the membership by means of the next Section newsletter. This year, two Steering Committee members were to be elected (a Treasurer to fill the unexpired term of Harold Menkes, and a Secretary to replace Peter Wagner as of 7/1/88). The ballots had been circulated in the January 1988 newsletter, and of the 493 Respiration Section members, 182 returned properly marked ballots. This is about double the turnout of previous mail ballots. Dr. Norman Edelman was elected to the position of Treasurer and Dr. Michael Hlastala to the position of Secretary. Dr. Edelman will also be Dinner Chairperson in 1989, and Dr. Hlastala Dinner Chairperson in 1990.

4. Dr. Klocke, who is just beginning his tenure as Program Advisory Committee representative from the Respiration Section, discussed programming. He emphasized that the major influence that a Section has in the APS is through programming at the Spring and Fall Meetings by means of proposing symposia, etc. He noted the poor input from membership in the past in this regard and urged members to be more active in suggesting symposia and other means of communication at the Spring and Fall Meetings. In this vein, he noted that special forms must be filled out to submit to the Program Committee when proposing symposia and that these forms must be in the hands of the APS by March 1 and September 1 of the year preceding the meeting at which the symposia is to take place for the Spring and Fall Meetings, respectively.

Finally, Dr. Klocke emphasized that the next opportunity for programming input according to this schedule will be the 1989 Fall Meeting. This is particularly important to the Respiration Section because it is a joint meeting between the APS and the ATS. The membership was encouraged in the strongest possible way to provide ideas for symposia and any other format of communication for the fall meeting next year.

5. The Secretary-Treasurer's report was next given by Peter Wagner. He reported that, with a very low voter turnout (20 ballots), the new bylaws of the Section were passed unanimously. A financial report was given. As part of the financial report, it is noted that the Respiration Dinner this year sold 232 tickets, about double the turnout from last year when we had mailing list difficulties.

6. A letter from Karl Wasserman proposing a memorial function in the name of Harold Menkes be considered was tabled since Dr. Wasserman could not be present. This will be reviewed sometime in the future.

7. The Section Chairperson Dr. Hyde reported that the Long-Range Planning Committee of the APS is seriously considering the future of the Fall Meeting. There are apparently suggestions to reduce the number of meetings per year to one from the current two, although this is the subject of much discussion and no decisions have yet been made. Dr. Hyde noted that next year the Respiration Section will have direct input into APS Council nominations and asked the membership to become involved in that. He also mentioned that the Committee on Committees strongly requests the Sections to provide names of people to fill the many committee positions that are continually opening up in the APS structure. He also mentioned the need to continue to recruit members to the Respiration Section of APS.

8. In the absence of any new/old business, Dr. Hyde adjourned the meeting.

Peter D. Wagner, Secretary/Treasurer

1989 FASEB Spring Meeting Abstract DEADLINE:

NOVEMBER 1, 1988

RENAL

Johnathan Bortz and Leslie Lescale-Matys.

The Renal Section has announced its 1988 Awardees for Research Excellence. The recipients of the graduate study and postdoctoral fellow categories after judging at the past FASEB meeting were Leslie Lescale-Matys and Dr. Johnathan Bortz. Ms. Lescale-Matys' study was entitled "Serum repletion increases Na^+, K^+-ATPase synthesis pretranslationally in MDCK cells via sodium flux"; she is a third-year graduate student from the University Southern California School of Medicine working in the laboratory of Dr. A. McDonough. Dr. Bortz's study was entitled "Co-localization of insulin-like growth factor I mRNA in rat kidney collecting duct"; Dr. Bortz is a postdoctoral fellow in nephrology/endocrinology/metabolism at Washington University in St. Louis working in collaboration with Dr. Hammerman. The recipients of this coveted award receive an honorary plaque and a check for $200.00. Announcements for the 1989 competition are mailed to all members and affiliated members of the Renal Section.

Call for Symposia Topics—Spring 1989

Members are urged to submit proposals for APS symposia to their Section Program Advisory Committee representatives. Organizers should consider multidisciplinary approaches with other sections and the contribution by experimentation at multiple levels of investigation.

What specific questions will the symposium address? Are there two or three conflicting issues that warrant presentation and discussion? What does the symposium offer to the intended audience? Are further directions considered in the material to be presented?

Symposia proposals are welcome for the Spring or Fall Meeting. For the 1989 Spring FASEB Meeting, submit proposals to the appropriate Section Program Advisory Committee representative by January 15, 1989. All proposals should include the following: 1. Title; 2. Organizer and address; 3. Abstract (150 words); 4. Number of half-day sessions; 5. Names of session chairperson(s); 6. Presentors/Discussants—approximately six per half day (list the participant's name and title of presentation as it would appear in the program); 7. Brief biographical sketch (2–3 sentences) of each speaker in the symposium; 8. Budget information—symposia are evaluated on the basis of their scientific merit; however, to coordinate fund-raising efforts by the national office, the anticipated costs to support the travel and lodging of symposia speakers are needed. Organizers will be notified immediately on acceptance of the symposia and given a budget for nonmember participants.

Section Program Advisory Committee Representatives:

Chair
Carl V. Gisolfi
Dept. of Exer. Sci. & Phys. Ed.
Univ. of Iowa
N420 Field House
Iowa City, IA.52242
319-335-9494

Cardiovascular
James M. Downey
Dept. of Physiology
Univ. of South Alabama
College of Medicine
Mobile, AL 36688
205-460-7004

Harris J. Granger
Dept. of Med. Physiology
Texas A&M Col. of Med.
College Station, TX 77843
409-845-7816

Cell & General
Lazaro Mandel
Dept. of Physiology
Duke Univ. Med. Ctr.
Box 3709
Durham, NC 27710
919-684-6638

Comparative
Larry Crawshaw
Dept. of Biology
Portland State Univ.
P.O. Box 751
Portland, OR 92707
503-464-4209

Endocrinology & Metabolism
David Wasserman
Dept. of Molec. Physiol. & Biophysics
Light Hall, Rm. 613
Vanderbilt Univ. Sch. of Med.
Nashville, TN 37232
615-322-7014

Environmental, Thermal & Exercise
Ethan R. Nadel
John B. Pierce Fndn. Lab.
290 Congress Ave.
New Haven, CT 06519
203-562-9901

Gastrointestinal
Jackie D. Wood
Dept. of Physiology
Ohio State Univ. Col. of Med.
333 West 10th Aven.
Columbus, OH 43210
614-292-5449

Nervous System
Ralph Lydic
Pulmonary Div.
Dept. of Medicine
Hershey Med. Ctr.
Hershey, PA 17033
717-534-5625

Neural Control & Autonomic Regulation
M. Ian Phillips
Dept. of Physiology
Univ. of Florida Col. of Med.
JHMHC, Box 274
Gainesville, FL 32610
904-392-3791

Renal
Roger G. O'Neil
Dept. of Physiol. & Cell Biol.
Univ. of Texas Hlth. Sci. Ctr.
P.O. Box 20708
Houston, TX 77225
713-792-5282
Linda S. Costanzo
Dept. of Physiol. & Biophysics
Med. Col. of Virginia
Box 551, MCV Station
Richmond, VA 23298
804-786-7559

Respiratory
Robert A. Klocke
Dept. of Medicine & Physiol.
SUNY at Buffalo
462 Grider St.
Buffalo, NY 14215
716-898-3988

Teaching
Joel Michael
Dept. of Physiology
Rush Med. Col.
1750 W. Harrison St.
Chicago, IL 60612
312-942-6426

Water & Electrolyte Homeostasis
Edward H. Blaine
Dept. of Pharmacol.
Box 8103
Washington Univ. Sch. of Med.
660 S. Euclid Ave.
St. Louis, MO 63110
314-362-2724

Clinical Physiology Group
J. F. Biebuyck
Dept. of Anesthesia
Penn. State Univ. Col. of Med.
Hershey, PA 17033
717-534-5697

Epithelial Transport Group
Ray Frizzell
Dept. of Physiol. & Biophysics
Univ. of Alabama
Birmingham, AL 35294
205-934-7210

History of Physiology Group
Dan Gilbert
Biophysics Lab.
NINCDS, NIH
Bldg. 9, Rm. 1E124
Bethesda, MD 20892
301-496-3204

MYOBIO Group
Marion J. Siegman
Dept. of Physiology
Jefferson Med. Col.
1020 Locust St.
Philadelphia, PA 19107
215-928-7893

Liaison With Industry
Stephen F. Flaim
Dept. of Pharmacol.
Squibb Inst. for Therapeutic Res.
P.O. Box 4000
Princeton, NJ 08540-4000
609-921-4743

Physiology and FASEB 1988

The 1988 FASEB Meeting in Las Vegas was a joint meeting of the six FASEB member societies and several guest societies. Over all, 9,093 abstracts of volunteered papers were submitted, an increase of 30% over 1987. Of this total, 2,418 papers were submitted by the APS membership and three APS guest societies: the Society for Experimental Biology and Medicine (SEBM), the Biomedical Engineering Society (BMES), and the Society for Mathematical Biology (SMB). The physiology component of FASEB '88 represented 27% of the short communications presented by APS members and guests.

Of the APS-sponsored papers, 17% (408) were the scientific efforts of women physiologists as first authors and 7% (172) were by members from outside of the Americas. Also, 9% (219) were received from U.S. government laboratories and 2% (56) were received from physiologists employed by industry. Table 1 shows the various departmental abstracts received by APS.

Of the 2,418 APS member-sponsored abstracts, 21% (504) were designated by the authors for inclusion in topics programmed by other FASEB member Societies (Table 2).

Tables 3 and 4 show the distribution of volunteered papers, programmed by APS and its guest societies, in relation to Society sections. Of the 2,330 papers programmed by the Program Advisory Committee, 1,511 (65%) were scheduled for poster sessions, 733 (31%) for slide sessions, and 86 (4%) for poster-discussion sessions. Over all, there were 99 poster sessions, 64 slide sessions, 35 symposia sessions, and 5 poster-discussion sessions. A total of 203 physiology sessions were scheduled, an increase of 2% over FASEB '87 and a 19% increase over FASEB '86.

TABLE 1. **Author Affiliations of Programmed Volunteered Papers**

Department	No. of Papers	% Total
Physiology	676	28
Physiology & Biophysics	172	7
Medicine	212	9
Pharmacology	128	5
Biology	50	2
Surgery	68	3
Anesthesiology	101	4
Pediatrics	64	3
Pathology	28	1
Biochemistry	39	2
Engineering	67	3
Kinesiology	11	
Other	802	33

TABLE 2. **Volunteered Papers Sponsored by APS, SEBM, and SMB for FASEB '88**

Society	Total Received	FASEB Program Designation						Total
		APS	ASBMB	ASPET	AAP	AIN	AAI	
APS	2,275 (94%)	1,771 (73%)	53 (2%)	175 (7%)	188 (8%)	70 (3%)	18 (1%)	2,275
SEBM	100	36	9	17	17	16	5	100
BMES	38	29	3	0	6	0	0	38
SMB	5	2	1	0	1	1	0	5
Total	2,418	1,838	66	192	212	87	23	2,418

TABLE 3. **APS Scientific Sessions at FASEB '88**

Section	Slide	Poster	Poster Discussion	Symposia	Total
Cardiovascular	21	20		4	45
Cell & general	3	8		2	13
Comparative	1	1		0.5*	2.5
Endocrinol & metabolism	6	7		2	15
Environmental, thermal & exercise	5	8		0.5*	13.5
Epithelial	3	2		1*	6
Gastrointestinal	2	7		2	11
History		1		0.5*	1.5
Muscle	1	6		2.5*	9.5
Nervous system	1	7		1	9
Neural control and autonomic regulation	5	4			9
Renal	6	8		2*	16
Respiration	4	10	4	2	20
Teaching		1	1		2
Water & electrolyte	3	4		1.5*	8.5
BMES	2	2		2	6
SEBM				2	2
SMB				2	2
SGP				0.5*	0.5
Clinical				2	2
Theme	1	3		4	8
Special				1	1
Total	64	99	5	35	203

* Session sponsored jointly by two or more sections.

TABLE 4. **Programming of Volunteered Papers by Sections/Groups**

Section	Slide	Poster	Poster Discussion	Total
Cardiovascular	238	347		585
Cell & general	37	91		128
Comparative	10	16		26
Endocrinol & metabolism	73	125		198
Environmental, thermal & exercise	50	83		133
Epithelial	38	72		110
Gastrointestinal	33	67		100
History		2		2
Muscle	9	105		114
Nervous system	11	42		53
Neural control and autonomic regulation	59	45		104
Renal	61	63		124
Respiration	48	315	79	442
Teaching		9	7	16
Water & electrolyte	35	56		91
BMES	19	24		43
Theme	12	49		61
Total	733	1,511	86	2,330

News From Senior Physiologists

Letters to Horace Davenport:

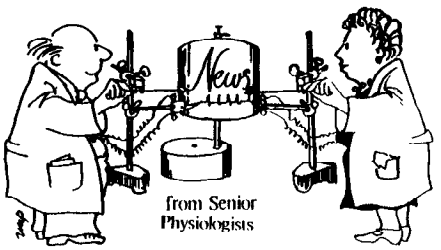
Kao Liang Chow reports that he still keeps an office in the department of neurology at Stanford and participates in the department's scholarly and social functions despite having retired three years ago after working there for 23 years. In retirement he has been chairman of one of the NIMH study sections, served on several committees, and co-edited a book. Now he has decided to pursue only avocational activities by spending only a few hours in the office reading and talking with colleagues and at home swimming, practicing Chinese calligraphy, listening to classical music, and reading Chinese poetry and the classics.

Kenneth G. Kohlstaedt writes, "We moved from Indianapolis to Palm Springs in 1977. I have maintained my interest in research and medical education by an association with the Eisenhower Medical Center. I am a member of the institutional review board and the hospital medical ethics committee. I also serve as an advisor to the Annenberg Center for the Health Sciences, which is located on the campus of the Eisenhower Medical Center."

Letters to Roy O. Greep:

Harold Copp gave up his headship of the Department of Physiology at the University of British Columbia in 1980 but still retains his office, laboratory, and research grant. "Science is a great hobby," he writes, "and I am now working on a previously unrecognized calcium regulating hormone from the corpuscles of Stannius of fish which we have named Teleocalcin." His laboratory isolated the pure hormone in 1986, and last year Donna Butkus and her colleagues at the University of Melbourne isolated the gene and by cDNA analysis determined the full structure. It contains 231 amino acids and 15 cysteines and has no homologies with any other known protein. Among other activities, he is involved in establishing the university's first clinic in postmenopausal osteoporosis and has recently joined the Board and Science Advisory Committee of Science World of B. C., which is in the process of building a world class Science Center.

David E. Goldman and his wife are living on Cape Cod and "ageing as gracefully as we can manage." He is learning to use a personal computer and writing up some experimental material on it. Completion of one project on the population dynamics of small rodents is delayed until that he tries to keep up with the literature, he finds it hard to stay in touch with the ever increasing pace of developments leading to more specialization. "This is not merely a matter of age." He remarks in closing, "The American Physiological Society is now 100 years old. Does that mean it is ready for a geriatric consultation? Perish the thought!"



Charles Sawyer writes: "For 45 years prior to retirement I led a double life; doing research on physiological aspects of neuroendocrinology and teaching medical gross anatomy. My neuroendocrine friends would never believe I taught "gross," and our med students thought I did nothing else." Having closed his research laboratory at UCLA three years ago, Sawyer is still teaching "gross" as a volunteer and writing up "ancient research data" and historical essays for publication. Retirement provides more time for listening to classical music, his principal avocation.

Werner P. Koella, who retired in 1982 from the Friedrich Miescher Institute in Basel, Switzerland, wrote "... and this is my 'word of wisdom' for my younger colleagues—if one comes closer to the day when one has to vacate the office and the laboratory, one should carefully plan the many years ahead and 'organize' the kind of activities from which one still is well suited in spite of failing vision or hearing, reduced capabilities of memory read-out, and a general slowing down of the mental processes. Still, one should organize one's retirement activities in a way that one still has time for a few hobbies that help keep the mind and body in shape. I have taken up hiking. In the course of the last five years I have crossed Switzerland on foot four times. And I still have time to practice my trumpet playing, for my weekly rehearsals with the Basel Brass Choir, and for an occasional jam session with a Dixieland band." But what has kept him busy during the last two years was writing a book: *The Physiology of Sleep—An Introduction.*

Estelle R. Ramey retired last year from Georgetown University and reports that she has been active on the lecture circuit, consulting, and generally enjoying life. "I am often asked how I developed the ability to speak without nervousness to big audiences without notes. I point out that for more than 40 years I lectured to medical students, the most hostile audience in the world. After that, no audience intimidates me."

Clara M. Szego writes, "There seems less time to carry out the various work-and-play-related projects I has so confidently anticipated. Nevertheless, I keep up with tennis, a bit of piano, and quite a healthy amount of literary aspiration—with inspiration striking occasionally, especially in the middle of the night." She reports that she presently has two scientific papers in press, one which appeared in the June issue of *AJP.*

Carl A. Bunde writes, "January 1, 1988, I agreed to come out of almost complete retirement to take over a fulltime job. A 24-bed clinical pharmacology facility (Hill Top Pharmatest, Inc.), which I started in 1976 and retired from in 1981, had lost its general manager and medical director; and I was asked to assume the job until a replace could be found. It is now a 54-bed unit and twice as many employees." He also has been chairman since 1981 of the Hill Top Research Institutional Review Board, which allows him to delve into new areas of research.

Donald F. Proctor reports that he is still writing and that he has "two chapters in the recent *Handbook of Physiology* and two chapters in the forthcoming *Textbook of Respiratory Medicine.* My book *Breathing Speech and Song* has been translated into Japanese." His one regret is that not having sources for travel funds, he cannot attend the scientific meetings which he enjoyed and that he especially misses the respiration dinners.

Herbert J. Spoor writes that he has not been able to do very much for the last seven years as a stroke has reduced his visual field and some recognition and recall capacity.

James T. Irving reports that he retired as an editor of the *Archives of Oral Biology* last year and does nothing more than empty the dishwasher and help make the bed. He also reports that he will soon have a hip replacement.

Membership Status
(March 1988)

Regular	4,767
Emeritus	657
Honorary	19
Corresponding	188
Associate	771
Student	160
Total	6,562

Newly Elected Members

The following, nominated by Council, were elected to membership in the Society at the Spring Business Meeting, 1988, Las Vegas, NV.

Regular

Latifeh Amini-Sereshki
Univ. of Pennsylvania Sch. Vet. Med.

Georgia Andrianopoulos
Univ. of Illinois, Chicago

Daniel E. Atkinson
Univ. of California, Los Angeles

George P. Biro
Univ. of Ottawa

Jesse W. Bowen
Univ. of Missouri, Columbia

Bruce D. Butler
Univ. of Texas Med. Sch., Houston

Peter J. Campbell
Vanderbilt Univ.

Shih-Wen Chang
Univ. of Colorado Hlth. Sci. Ctr., Denver

Stefan H. Constable
USAF Sch. of Aerospace Med., Brooks AFB, TX

Leslie H. Cronau
Univ. of Texas Med. Sch., Houston

Deborah M. Drechsler-Parks
Univ. of California, Santa Barbara

Gary A. Dudley
The Bionetics Corp.

Larry J. Findley
Univ. of Virginia Med. Ctr., Charlottesville

Theodore Garland, Jr.
Univ. of Wisconsin, Madison

David L. Geenen
Montefiore Med. Ctr., Bronx

Joey P. Granger
Eastern Virginia Med. Sch.

Matthew B. Grisham
LSU Med. Ctr., Shreveport

Harvey J. Grill
Univ. of Pennsylvania

Steven R. Gullans
Brigham & Women's Hosp.

David D. Gutterman
Univ. of Iowa Hospitals

Bernard M. Hitzig
Harvard Medical School

Eileen M. Hasser
Univ. of Missouri, Columbia

Steven R. Hays
Univ. of Texas/Southwestern Med. Ctr.

Raymond P. Henry
Auburn Univ.

David R. Hodgson
Washington State Univ.

Richard L. Hughson
Univ. of Waterloo, Ontario

Philip B. Hultgren
Kirksville Col. of Osteopath. Med., MO

Kenneth T. Izutsu
Univ. of Washington

Frederick J. Kaskel
State Univ. of New York, Stony Brook

Abram Katz
National Insts. of Health

Debra Ann Kirby
Harvard Sch. of Public Hlth.

Richard L. Lieber
VA Med. Ctr., San Diego

Michael P. Lilly
Rhode Island Hosp.

Robert F. Lodato
Univ. of Texas Med. Sch., Houston

Gary W. Mack
John B. Pierce Fndn. Lab.

Jane A. Madden
Zablocki VA Med. Ctr.

Danuta H. Malinowska
Univ. of Cincinnati

Scott Manaker
Hosp. of the Univ. of Pennsylvania

Martin J. Mangino
Washington Univ. Sch. of Med.

Daniel C. Marcus
Boys Town Natl. Inst., Omaha

Steven W. Mifflin
Univ. of Texas Hlth. Sci. Ctr., San Antonio

Suzanne Moreland
The Squibb Inst. for Med. Res.

Alan H. Morris
Univ. of Utah Sch. of Med.

Brian Mulloney
Univ. of California, Davis

Gary F. Nieman
State Univ. of New York, Syracuse

Keith B. Nolop
Univ. of Miami Sch. of Med.

Terry J. Opgenorth
Abbott Laboratories

Kaushik P. Patel
Univ. of South Dakota

Gregory De Loss Potter
Univ. of Texas Med. Sch., Houston

Patricia A. Preisig
Univ. of Texas/Southwestern Med. Ctr.

J. Usha Raj
Harbor-UCLA Med. Ctr.

Vazhaikkurichi M. Rajendran
Yale Univ. Sch. of Med.

Andrew J. Rankin
Memorial Univ., St. John's, Newfoundland

Peter S. Reinach
Washington Univ. Sch. of Med.

Diego Restrepo
Monell Chem. Senses Ctr., Philadelphia

Peter Richardson
Baylor Col. of Med.

Ellis B. Ridgway
Medical College of Virginia

Robert D. Roer
Inst. for Marine Biomed. Res., Wilmington, NC

Hariharan Sankaran
VA Med. Ctr., Portland, OR

Daniel P. Schuster
Washington Univ. Sch. of Med.

Steven S. Segal
Pennsylvania State Univ., University Park

Daniel I. Sessler
Univ. of California, San Francisco

Mohammad Shenasa
Hosp. Sacre-Coeur, Montreal

Sanjeev G. Shroff
Michael Reese Hosp., Chicago

Michael L. Smith
McGuire VA Med. Ctr., Richmond, VA

Lou Ann Stephenson
US Army Res. Inst. of Environ. Med., Natick, MA

Joseph Lee Unthank
Indiana Univ. Sch. of Med., Indianapolis

Joseph G. Verbalis
Univ. of Pittsburgh

Elizabeth M. Wagner
Francis Scott Key Med. Ctr., Baltimore

Benjimen R. Walker
Tulane Univ. Sch. of Med.

Lawrence C. H. Wang
Univ. of Alberta

Jean-Michel Weber
Univ. of Texas Med. Branch, Galveston

Jerry L. Wessale
Purdue Univ.

Randall C. Wetzel
Johns Hopkins Hosp.

Carin Wittnich
St. Michael's Hosp., Toronto

APS Membership Applications

Membership applications may be obtained from APS Membership Services, 9650 Rockville Pike, Bethesda, MD 20814. Applications received between February 1 and July 1 are considered for nomination by Council at the Fall Meeting, and those received between July 1 and February 1 are considered for nomination at the Spring Meeting of the Society.

(see p. 99)

Tadataka Yamada
Univ. of Michigan Med. Ctr.

Douglas A. Young
Sandoz Res. Inst.

John C. Young
Boston Univ.

Corresponding

Piergiuseppe Agostoni
Univ. of Milano

Angela Corcelli
Bari Univ., Italy

Bertrand Crozatier
Hosp. Leon Bernard, Limeil-Brevannes, France

Razi Dmi'el
Tel Aviv Univ.

Gerolf Gros
Med. Hochschule Hannover, FRG

Ann Harrison
Kuwait Univ.

Philippe A. Herve
Paris-South Univ.

Ryo Hosotani
Univ. of Arkansas for Med. Sci.

Heidrun F. Kiwull-Schoene
Ruhr Univ., Bochum, FRG

Jaime Requena
IDEA Ctr. for Biosciences, Caracas, Venezuela

Stefan Silbernagl
Univ. of Wurzburg, FRG

Peter A. Wieringa
Delft Univ. of Technol., The Netherlands

Jing Tian Xie
Nankai Univ., Tainjin, PRC

Derek M. Yellon
The Rayne Inst./St. Thomas's Hosp., London

Ahad N. K. Yusufi
Mayo Clinic & Fndn.

Associate

Frederic S. Bongard
Harbor-UCLA Med. Ctr.

William E. Dale
Univ. of Missouri, Columbia

James P. Dixon
USAF Sch. of Aerospace Med., Brooks AFB, TX

Mark B. Effron
Francis Scott Key Med. Ctr., Baltimore

Diane Eliades
Uniformed Services Univ. of the Hlth. Sci.

Gabriel P. Frommer
Indiana Univ.

Matthew R. Glucksberg
Roosevelt Hosp., New York

Drew A. Hildebrandt
Univ. of Mississippi Med. Ctr.

E. Heidi Jerome
Univ. of California, San Francisco

Jeffrey W. Kiel
Univ. of Texas Hlth. Sci. Ctr., San Antonio

Kevin C. Kregel
Univ. of Arizona

Henry A. Lester
California Inst. of Technol.

Edward D. Lewandowski
Baylor Col. of Med.

Elizabeth A. Miescher
Johns Hopkins Univ. Hosp.

Carol S. Packer
Indiana Univ. Sch. of Med.

Dietrich Walter F. Schwarz
Univ. of British Columbia

John A. St. Cyr
Univ. of Minnesota, Minneapolis

D. Lowell Stacy
Eastern Virginia Med. Sch., Norfolk

Joe R. Strader
Texas Col. of Osteopath. Med.

Terrie M. Williams
Sea World Res. Inst., San Diego

David C. Zawieja
Texas A&M Univ.

Student

Melisesa M. Cheeseman
Univ. of Kentucky Col. of Med.

Michael D. Delp
Univ. of Georgia

Kiran S. Deoras
Temple Univ. Sch. of Med.

Laurie R. Dodd
Univ. of Arizona Col. of Med.

Anne Folta
Univ. of Michigan

Ritchie Froehlich
Univ. of Delaware Sch. of Life & Hlth. Sci.

Suzanne Greenberg
Med. Col. of Wisconsin

Timothy W. Henrich
Texas A&M Univ.

Mary C. Hines
Univ. of Louisville

Vijayanand C. Kowtha
Rutgers Univ.

Ingrid K. Krampetz
Univ. of Manitoba

Eugenio A. Longo
Univ. of Puerto Rico Sch. of Med.

Donald J. Meyer
Univ. of Missouri, Columbia

Raymond B. Penn
Temple Univ. Sch. of Med.

Kathleen Z. Refinetti
Univ. of California, Santa Barbara

Jann Rhodes
Texas A&M Univ.

Kenneth J. Rodnick
Stanford Univ.

Christopher R. Ross
Univ. of Missouri, Columbia

Craig S. Stump
Univ. of Arizona

Catherine F. T. Uyehara
Tripler Army Med. Ctr., Honolulu

Barbara A. Vance
Dartmouth Med. Sch.

Ning Wang
Harvard Sch. of Public Hlth.

Jay H. Williams
Texas A&M Univ.

Cindy M. Wilson
East Carolina Univ.

Martin L. Wolf
Univ. of Nebraska Col. of Med.

Honorary

Setsuro Ebashi
Nat'l. Inst. for Physiol. Sci., Okazaki, Japan

Erwin Neher
Max-Planck-Inst. for Biophys. Chem., Gottingen, FRG

Ewald R. Weibel
Univ. of Bern, Switzerland

Fifty-Year Members

David I. Abramson, 1937
Errett C. Albritton, 1933
Willard M. Allen, 1934
Samuel B. Barker, 1938
S. Howard Bartley, 1935
Richard J. Bing, 1922
Emil Bozler, 1932
Chandler McC. Brooks, 1933
Paul C. Bucy, 1933
Hubert R. Catchpole, 1938
K. K. Chen, 1929
Robert W. Clarke, 1936
Madeleine F. Crawford, 1933
Phoebe J. Crittenden, 1937
Ray G. Daggs, 1935
Hallowell Davis, 1925
Louis B. Flexner, 1933
Florent E. Franke, 1934
Maurice H. Friedman, 1929
A. Pharo Gagge, 1937
Frederic A. Gibbs, 1935
Aruthur S. Gilson, Jr., 1927
Harold D. Greene, 1936
James B. Hamilton, 1938
Chester W. Hample, 1936
John W. Heim, 1936
Frances A. Hellebrandt, 1933
Charles B. Huggins, 1932
J. Raymond Johnson, 1938

Jane Sands Robb Johnson, 1925
Joseph L. Johnson, 1934
Frederic T. Jung, 1930
Nathaniel Kleitman, 1923
Arnold Lieberman, 1931
Donald B. Lindsley, 1937
Rafael Lorente de Nó, 1937
Horace W. Magoun, 1937
Ade T. Milhorat, 1934
Hayden C. Nicholson, 1932
Seward E. Owen, 1938
Irving H. Page, 1937
Herbert Pollace, 1933
C. Ladd Prosser, 1935
Paul Reznikoff, 1927
Oscar W. Richards, 1934
Curt P. Richter, 1924
John J. Sampson, 1932
Carl F. Schmidt, 1929
Francis O. Schmitt, 1930
Wilbur A. Selle, 1937
James A. Shannon, 1933
Herbert Shapiro, 1937
Herbert Silvett, 1933
Paul W. Smith, 1933
Fanklin F. Snyder, 1936
Samuel Soskin, 1930
J. Newell Stannard, 1938
Isaac Starr, 1929
George W. Stavraky, 1937
Theodore J. B. Steir, 1938
Eugene U. Still, 1928
Maurice L. Tainter, 1929
Sarah S. Tower, 1932
George E. Wakerlin, 1933
C. Beecher Weld, 1936
Harold C. Wiggers, 1937
Robert A. Woodbury, 1936
Clinton N. Woolsey, 1938

Deceased Members

Andre Cournand, New York, NY (02-19-88)
Thaddeus S. Danowski, Pittsburgh, PA (09-12-87)
Helge E. Ederstrom, Grand Forks, ND (10-02-87)
Robert C. Grubbs, Columbus, OH (Sept. 1987)
Rita Guttman, Brooklyn, NY (1984)
Reginald E. Haist, Toronto, ON (06-15-87)
A. Baird Hastings, La Jolla, CA (09-24-87)
John A. Johnson, Minneapolis, MN (11-05-87)
Jerzy Kaulbersz, Cracow, Poland (Dec. 1987)
George F. Koepf, Buffalo, NY (Dec. 1987)
G. N. Loofbourrow, Prairie Village, KS (12-10-87)
George R. Menelly, Shreveport, LA (09-06-87)
J. Eugene Millen, Richmond, VA (Feb. 1987)
Joseph M. Stinson, Nashville, TN (Feb. 1988)
George B. Theil, Nashville, TN (07-29-87)
Leland C. Wyman, Boston, MA (01-13-88)
John A. Zapp, Kennett Square, PA (04-02-87)

APS and Section Awards

The **APS Caroline tum Suden Professional Opportunity Awards**, which are presented at the Spring APS business meeting, include a $500 prize, paid registration and placement service fees, and a certificate. Six awards are given annually to graduate students or postdoctoral fellows presenting papers as first authors at the Spring FASEB meeting. Candidates are required to submit to APS an abstract with an accompanying letter from the sponsor certifying that the author is a student or postdoctoral fellow and the approximate date the nominee will be available for employment.

The **Cardiovascular Section** presents three annual awards—Fellowship, the Lamport Award, and the Carl J. Wiggers Award. Nominations for Fellowship Awards must be made by at least two existing fellows with supporting letters sent to the steering committee for vote. The total number of fellows cannot exceed 5% of the APS regular members who have published meritorious research in cardiovascular physiology. The Lamport Award is presented to a young investigator under the age of 36 showing outstanding promise in his/her field of cardiovascular research. The recipient, who receives a certificate and a $200 check, is selected by the Wiggers awardee of the previous year. The Carl J. Wiggers Award honors a founder of the section who has made outstanding and lasting contributions to cardiovascular research.

The **Cell and General Physiology Section** offers an award of $200 to an undergraduate student and a $300 award to a postdoctoral student after three years of obtaining an M.D. or Ph.D. degree. The award is based on research that is judged to be an outstanding contribution. Recipients are selected from those scientists who submit abstracts for the Spring FASEB Meeting in the field of cell physiology.

The **Comparative Physiology Section Scholander Award** is presented annually to recognize an outstanding young investigator presenting a paper as first author in a comparative physiology slide session at the Spring FASEB Meeting. Candidates must be graduate students or postdoctoral fellows, not more than five years beyond their highest degrees. The recipient receives a cash award of $100 and a certificate from the American Physiological Society.

The **Young Investigator Award of the Envrionmental, Thermal and Exercise Physiology Section** is for the recognition of excellence in research by a graduate student. Candidates must be first author on a paper presented at a previous APS Fall Meeting or the Spring Meeting at which the award is presented. Honoring Harwood S. Beling, an award of $150 is presented at the Temperature Regulation Dinner.

The **Gastrointestinal Physiology Section Student Prize** is designed to challenge and reward students and postdoctoral fellows who are conducting their research efforts in gastrointestinal physiology. Two awards—one for work done while enrolled as a student for a doctoral degree and the other for work performed during the first through the third postdoctoral years—are presented at the Spring FASEB Meeting. Applicants must be first author on abstracts submitted for the Spring FASEB Meeting, which are accompanied by a letter from the applicant's advisor indicating whether the applicant is a graduate student or postdoctoral fellow.

The **Van Harreveld Memorial Award of the Nervous System Section** is to honor the best APS student presentation at the Spring FASEB Meeting. The first annual cash award will be presented at the 1989 spring FASEB meeting at a section meeting, dinner, or reception.

The **Renal Physiology Section Award for Excellence in Renal Research** is to promote and develop excellence in research related to molecular, cellular, and organ mechanisms expressed by the kidneys. Annual awards are presented to a graduate and a postdoctoral students with judging based on abstract submission (25%) and meeting presentation (75%). Papers are evaluated by three judges in renal hemodynamics, epithelial transport, and metabolism. A certificate and prize of $200 are presented to the recipients at the annual renal dinner.

1989 FASEB Spring Meeting Abstract Deadline:
November 1, 1988

APS Members Got Their Two-Bits Worth As Letter Campaign Brings Withdrawal of Pet Bill

For only two-bits, it had to be the best buy of the year.

The quarters spent by APS members for a first-class stamp to send a letter objecting to Sen. Wendell Ford's proposed Pet Protection Act resulted in an avalanche of mail that soon convinced the Kentucky lawmaker to withdraw Senate Bill 1475.

Ford had been pushing the legislation since last year when a class B animal dealer in eastern Kentucky purchased for a research facility dogs that allegedly were pets. As a means to rectify the incident Ford introduced a bill that would have denied federal funds to researchers who use random source animals.

"I tried to attack the problem of pet theft from another angle, that of S 1475, the Pet Protection Act, but that became too embroiled in the highly controversial matter of pound seizure," Ford said in withdrawing the bill and introducing another bill that limits the source of animals available to class B dealers.

"Today, I separate the two issues, leaving pound seizure for some other person to deal with on some other day."

The letters to Ford were the result of a letter to the Society membership from APS President Harvey V. Sparks, Jr., who urged the members to express their concerns with the Pet Protection Act and its effect on research.

Ford's bill was virtually identical to the House version of the Pet Protection Act (HR 778). Although Rep. Robert Mrazek (D-NY), sponsor of the House proposal, has not indicated a willingness to withdraw his bill, enthusiasm for such legislation seems to have declined among House members with the withdrawal of the Senate bill.

Legislation Ford now is proposing, the Pet Theft Act (S 2353), would restrict class B dealers from obtaining dogs and cats from any source other than a state, county, or city owned and operated pound or shelter. Any dealer purchasing such animals from pounds or shelters will have to document the purchase and provide the recipient with a copy of the documentation.

Moreover, before the animal can be obtained by a dealer, the facility will have to have held the animal for at least one week to assure that the owner has had a chance to recover it or that the animal has had a chance for adoption.

Violation by the dealer would result in fines for the first two offenses and a permanent revocation of the dealer's license by the U.S. Department of Agriculture for a third offense.

The bill, which would be an amendment to the Animal Welfare Act, has been referred to the Committee on Agriculture, Nutrition, and Forestry.

American Physiological Society Statements on Animal Usage

The Use of Animals is Necessary for the Proper Teaching of Students of the Biomedical Sciences

The American Physiological Society believes the use of animals is important in the education of students in the biomedical sciences. The use of animals gives the student a direct understanding of how living systems work, an understanding that cannot be gained by reading a textbook, watching a video, or using a computer. To achieve the best biomedical education, students must have a complete learning experience including the use of laboratory animals.

October 1987

Animal Research is the Most Humane Response to Human Suffering from Disease

Depriving sick human beings of the benefits of animal research is inhumane and reprehensible. The American Physiological Society advocates the use of animals for research and teaching as the most human response to the need to relieve mankind from the suffering caused by disease. The use of animals is necessary if researchers are to combat illness, which affects both human beings and animals. The correct training of physicians and medical scientists also requires the use of animals for laboratory teaching. Textbooks, isolated cells, computer models, and other representations of the intact living organism can provide only a partial understanding of life processes for both the medical researchers and the student. Efforts to deny the human race the best possible curative power of modern sciences must be repulsed.

October 1987

Pound Animals

Unclaimed pound animals (random-source dogs and cats) have proved to be the most useful animals for the purposes of research and teaching. Medical advances benefiting both humans and animals were possible because of the availability of unclaimed pound animals for use in research. The American Physiological Society strongly believes that denial of the availability of random-source animals would be a catastrophic setback, and the Society strongly endorses the continued use of unclaimed pound animals for basic and clinical research and teaching.

April 1987

California Scientists Stage Demonstration in Support of Animal Research

Hundreds of supporters of animal research demonstrated at the University of California in Berkeley, but the media paid little notice, probably because there was no violence, no laws broken, nor outrageous acts.

The event was a "Celebration of Life and Health Day" and was sponsored by the Association For Animals and Animal Research (AFAAR), a newly formed organization composed of university students and staff who support biomedical research. It is believed to be the first time scientists have conducted a public demonstration to refute the animal activists.

Among the speakers were U.C. physiology professor Charles Nicoll, who told of the benefits to both humans and animals derived from animal research, and Steve Carroll, the director of the incurably ill For Animal Research. Nicoll also urged scien-

tists to become familiar with political issues such as Pet Protection bill in the Congress.

That so many scientists are compelled to conduct a demonstration in support of animal research is testament to the progress animal activists have made and the threat they represent to scientific progress, said Sharon M. Russel, a research physiologist.

Our organization and activities have lifted the spirits of the animal research community on campus, she added, and our celebration and subsequent activities have shown the Berkeley community-at-large another side to the issue, since citizens mainly hear only the anti-animal research position.

One of the major issues is the funding for the Northwest Animal Facility, a building needed to replace the antiquated facility now used for housing animals. Animal activists have been working to block construction, but AFAAR already has gained more than 1,100 signatures supporting construction of the new animal facility.

William M. Samuels

The More Things Change, The More Things Stay The Way They Are

An eminent physiologist's observations about animal activists:

". . . A few words should be said about the employment of living organisms for investigative purposes. Every student of physiology should be aware of the controversy over the justification for animal experimentation. Regardless of how irrational the position of the opponents of animal experimentation may be, this group of self-styled antivivisectionists is potentially important. Restrictive legislation preventing many types of experimentation is already in effect in several places and may spread.

"Scientists do not live and work in a vacuum, and scientific progress depends in many ways upon custom and popular opinion. Consequently, students of science have an obligation to consider such attitudes. The ethical basis of the antivivisectionist movement rests upon the assumption that either all, or some group of living animals, have equal rights to "life, liberty and the pursuit of happiness." It is an extension of the democratic political doctrine to species of animals other than man. There are many degrees of opposition to animal experimentation. Some partisans object only to the use of domesticated animals, others to all species. Various specific objections are raised, the commonest being that animal experimentation causes suffering and pain, the intentional infliction of which is said to be improper. Another common objection is that the moral standards of the experimenter himself are lowered by his actions.

"Ethically, there would seem to be no essential difference between the sacrifice of animal life for human food or for advancement of knowledge. Until the sacrifice of animal life for human food is abandoned, it would seem that the use of animals for the advancement of knowledge need not be abandoned. As to the effect of animal experimentation on the morals of the persons who conduct it, the obvious answer would seem to be to inquire as to whether the qualities of kindness, honesty and industry are conspicuously absent in the medical profession, which primarily sponsors animal experiments. I don't think they are. To the contrary, the kindest, most honest, most industrious people are in medicine.

"Not all antivivisectionists show consistency between their professed ethics and their own actions. To be logical, they should not eat meat, fish, fowl, milk, or eggs, because animals have had to suffer pain or inconvenience in order that such foods be made available. Further they should not wear leather, fur, feathers, wool or silk for the same reasons. There are probably no antivivisectionists who carry their ethical principles fully into effect in their own activities and some of them are flagrant violators, eating meat, wearing fur, hunting animals, etc.

"The science of physiology, and much of the rest of the science of medicine has grown out of animal experimentation. The ethical problem is really whether man is justified in his use of other animals to gain much of this knowledge. No person who answers this question in the negative has any right to use the fruits of modern medicine, either as a patient or a physician.

"Nevertheless, scientists generally, and physicians in particular, have an obligation to prevent pain and suffering. Therefore, it is a rule in physiological laboratories that animal experiments be conducted with every attention paid to the humane treatment of the animals employed. In the laboratory is posted a set rules concerning the treatment of animals. I would like you to read them and follow them."

Editor's note: The text is from the lecture given to University of Minnesota medical students in the 1940s by Maurice B. Visscher entitled, "Introduction To Physiology," thereby proving once again that there really isn't very much new under the sun.

APS Testifies on APHIS Funding and Consumer Product Testing Bills

Two major legislative statements were presented to the Congress this summer by APS.

In testimony before a Senate agriculture appropriations committee, APS Executive Director Martin Frank urged that the U.S. Department of Agriculture's Animal and Plant Health Inspection Service funding for the enforcement program of the Animal Welfare Act be increased to $10 million.

A statement for the record was submitted to a House subcommittee urging rejection of the Consumer Products Safe Testing Act (HR 1635). The bill would prohibit the use of laboratory animals for the LD50 and Draize tests.

APS Testimony for Animal and Plant Health Inspection Service

Mr. Chairman and Members of the Subcommittee:

My name is Martin Frank and I am the executive director of the American Physiological Society. I am here to speak on the behalf of the Society's 6,600 members who support the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) as the appropriate agency to assure that the standards and regulations of the Animal Welfare Act are properly enforced.

For nearly two decades the Society has been on record in its support of APHIS' role and since 1982 it has presented testimony and statements urging the Congress to assure that sufficient funds are made available to enable the agency to inspect facilities and to monitor the care and handling of animals. Adequate funding support for APHIS so that it can do its job effectively is the one area wherein the scientific community and the animal welfare organizations are in agreement despite a centuries-old disagreement over the use of laboratory animals for the purposes of research, teaching, and testing.

This year the Administration is proposing in the APHIS budget a 20% reduction in the funds for the enforcement program of the Animal Welfare Act. This proposed reduction is not new, however, as every year since 1982 the Administration has sought by some means to remove APHIS

Presented by Martin Frank before U.S. Senate Subcommittee on Agriculture, Rural Development, and Related Agencies, April 27, 1988.

from its responsibilities for animal welfare as mandated by the Animal Welfare Act and each year the Congress has rejected the Administration's proposal, for which the Society applauds the Congress for its wisdom.

The Administration's proposal for Fiscal Year 1989 would cut $1.3 million from last year's appropriation of $6.2 million. The Society's recommendation to the subcommittee is that APHIS funding should not be reduced, but rather it should be increased to $10 million, a figure that is in concert with the recommendations of other scientific societies and animal welfare organizations.

In making the recommendation the Society acknowledges the pressures the congress has in its efforts to reconcile spending for federal programs in light of a major budget deficit. However, to succumb to this pressure at this time very well could be the death knell of the Animal Welfare Act as a system for detecting animal abuse inasmuch as insufficient funding of this program would without doubt result in inadequate enforcement of the standards and regulations for the care, use, and treatment of animals.

The need for a strong system of enforcement of the standards and regulations for animal welfare, especially for the care of laboratory animals, is of major importance to the American Physiological Society, whose members are among the largest users of animal models for purposes of research and clinical teaching. Furthermore, there is a growing public interest in animal welfare which the Congress certified in 1985 by its amendments to the Animal Welfare Act that raised the standards for animal care and provided additional restrictions on animal use.

Continuation of the inspection functions by APHIS is of critical importance, especially if federally funded research is to continue to be conducted under standards prescribed by the federal government. Although there are laws in all 50 states and the District of Columbia that address animal abuse, none of these laws reflect the nature of the Animal Welfare Act. Collectively, these laws are a disparate body of rules, statutes, and regulations incapable—both technically and philosophically—of addressing violations of federal standards and regulations.

Moreover, the enforcement of the Animal Welfare Act not only is a legitimate function of the federal government, it is a function that is best served by the federal government. Any abdication of this responsibility—even if by insufficient funding—would be of serious consequence for the welfare of all animals.

The recommendation of $10 million is in the best judgment of the Society a necessary amount if the public, in general, and the scientific and animal welfare communities, in particular, are to be assured that the federal government does intend to have an effective program of enforcement of the Animal Welfare Act. The figure is based on the fiscal requirements to maintain current operations plus additional funds that will be needed in Fiscal Year 1989 to implement the standards and regulations the Congress has added to the Animal Welfare Act.

The standards and regulations added to the agency's inspection coverage include such diverse areas as institutional responsibilities for animal care and use, licensing of dealers, registration of research facilities, and requirements for adequate veterinary care at research facilities. If APHIS—or any other federal agency for that matter—is to be effective in its inspections it is most obvious that APHIS will need additional qualified personnel and will have to broaden its training programs to assure that the inspectors are capable of assessing proper veterinary care and institutional animal care and use as now prescribed by the law.

Additional personnel and training requirements and adjustments for inflation account for $3.8 million of the Society's recommendation. When this amount is added to the current appropriation level of $6.2 million, the funding recommendation total is $10 million.

It is the Society's belief that in the review of appropriations for programs of the Department of Agriculture, the funding of the program to enforce the provisions of the Animal Welfare Act must be given every consideration because an effective inspection program is essential to maintain public confidence in research involving the use of animals.

The Society is committed to the conduct of research and teaching under the optimum of scientific and ethical standards and conditions, including the humane and conservative use of animals. The Society believes that the Congress also shares in this commitment by its demonstrated legislative interests to assure the public that animals are cared for humanely.

Mr. Chairman, the American Physiological Society appreciates the opportunity given by the subcommittee to express its concerns as to the need at this time to increase the funding for the enforcement of the Animal Welfare Act. Should you or any member of the subcommittee have any questions, I will be pleased to answer them.

Thank you.

APS Statement on Consumer Products Safe Testing Act (HR 1635)

The American Physiological Society appreciates the opportunity to submit its comments for the record on HR 1635, the "Consumer Products Safe Testing Act."

Future Meetings

1988	
Joint APS/ASPET Fall Meeting	October 9–13, Montreal
1989	
FASEB Annual Meeting	March 19–23, New Orleans, LA
APS Fall Meeting	October 15–18, Rochester, MN
1990	
FASEB Annual Meeting	April 1–5, Washington, DC
APS Fall Meeting	October 6–10, Orlando, FL
1991	
FASEB Annual Meeting	April 14–18, Atlanta, GA
APS Fall Meeting	September 29–October 3, San Antonio, TX
1992	
FASEB Annual Meeting	April 5–9, Anaheim, CA
1993	
FASEB Annual Meeting	March 28–April 1, New Orleans, LA

While the Society commends the bill's sponsor, Rep. Barbara Boxer (D-CA), for her stated intention to clarify the federal government's position on the use of laboratory animals for consumer product testing, there are concerns among the Society's 6,600 members as to how HR 1635 would affect accepted testing practices in biomedical research should this legislation be enacted as currently written.

The purpose of the bill is to promote the use of acute toxicity testing methods that do not require the use of laboratory animals. To accomplish this purpose the bill would *1)* ban all federal agencies from accepting LD_{50} test results and *2)* require federal agencies to publish a justification for using any animal toxicity test when nonanimal alternative methods are said to be available.

Of major concern of the American Physiological Society is that the bill implies that the legislation is limited to the testing of consumer products, when in fact the bill, if enacted, also would affect some of the testing methodologies used to biomedical research.

Despite Rep. Boxer's claim that the proposed legislation would only prohibit federal agencies from considering the classic LD_{50} test when making regulatory decisions, the bill goes beyond the LD_{50} test and the public's concept of consumer products which, by and large, are cosmetics and household soaps, bleaches, polishes, cleansers, and the like. The reason for this, perhaps, is that it is difficult to separate the testing of cosmetics and household products from medical and pharmaceutical testing.

For example, the difficulty in separating cosmetics from topical medicine is that the ingredients used in the formulations of cosmetics are similar to those used for the application of drugs to treat skin or other diseases. The reason that the general cosmetic base is used is that the base has a low irritancy, nontoxic, and emollient properties that support the pharmaceutical agents. Not to test these agents could have serious medical consequences and potentially could represent an unnecessary threat to public health.

But what is important to understand is that drugs and support media cannot be tested separately but must be tested together. Because of this, animal testing is obligatory inasmuch as a single cell does not give the same physiological or toxicological information as what is learned from an intact animal. In both basic and applied research the only tool for predicting toxic effects in humans is animal testing, which provides information on the total effect on the animal's biological system, not just the target organs.

Should the Congress enact the Consumer Products Safe Testing Act much of the federally funded research and testing into the cause, treatment, and prevention of disease would be seriously curtailed or stopped because of delays imposed by the requirements for federal agencies to review and promulgate regulations specifing what nonanimal tests can be used and to prepare and publish justifications for continuing the use of animal tests. These bureaucratic delays virtually would halt research efforts to develop or improve therapies or vaccines for the treatment of maladies that range from cancer and AIDS to acute and chronic pain.

The American Physiological Society believes that the supporters of HR 1635 do not appreciate the complexities of toxicity testing nor understand the relationships between consumer product testing and biomedical research in assuring the public's continued state of well-being. Moreover, the bill itself is somewhat misleading in that it gives the impression that there are nonanimal tests readily available to replace the animals test. This simply is not the case.

It is true that some in vitro tests have been developed in recent years and other nonanimal toxicity tests are being developed. It also is true that the numbers of animals being used for toxicity testing has been greatly reduced in recent years as the classical LD_{50} test which required as many as 200 animals has been refined to where only 3–6 animals now are needed.

We still are some years away from having reliable nonanimal toxicity tests that can be used in place of animal tests. Scientists have been working and will continue to work in the development of reliable nonanimal testing methods. But the enactment of HR 1635 as currently written not only would delay progress toward the elimination of animal testing, it also would delay progress in the development of therapies and vaccines that require toxicity testing.

The American Physiological Society would be most willing to work with Rep. Boxer and the members of the subcommittee to develop responsible legislation that would achieve the desired goals without curtailing toxic testing that could seriously impact upon the development of therapies and vaccines.

Submitted to U.S. House of Representatives' Committee on Energy and Commerce Subcommittee on Health and the Environment, May 19, 1988.

IUPS NEWS

Kjell Johansen Commemorative Satellite Symposium

The Kjell Johansen Commemorative Symposium will be held July 6–8, 1989, at the August Krogh Institute in Copenhagen, Denmark. It will be a satellite symposium to the IUPS Congress in Helsinki the following week. The themes of the symposium, reflecting the interests of Kjell Johansen, are metabolism, circulation, gas exchange, and blood gas transport. Anyone wishing to present a poster on one of these topics may submit an abstract by September 1, 1988, to Stephen C. Wood, Lovelace Medical Foundation, 2425 Ridgecrest Dr., Albuquerque, NM 87108. Abstracts should not exceed two pages, double spaced. A selection committee will notify authors by November 1, 1988 whether their abstract was selected for presentation. The proceedings, oral communications, and poster abstracts will be published.

G. Edgar Folk, Jr., Senior Physiologists Fund

The G. Edgar Folk, Jr., Senior Physiologists Fund has been set up through the generosity of family and former graduate students and postdocs to provide modest but helpful assistance to senior physiologists 70 years or older who no longer have grant funds available to them. The awards might be used for such purposes as attending an APS meeting to present a paper, engaging in a series of modest experiments, or completing a manuscript (paying for typists or perhaps for page charges). Recipients will be selected with the assistance of the Senior Physiologists Committee throughout the year. Names of awardees will not be made public. Mary Folk writes that the purpose of the fund is for the Senior Physiologists Committee "to have *fun* assisting colleagues and for Emeritus APS members to keep in closer touch with APS."

Inquiries concerning the G. Edgar Folk, Jr., Senior Physiologists Fund should be made to Martin Frank, Executive Director, APS.

SURNAME_____

XXXI IUPS CONGRESS
Helsinki, Finland
July 9–14, 1989

1. Name and degrees: _____Year of highest degree: _____
2. Faculty position or employment title: _____Year of birth: _____
3. Address: _____
_____(Telephone # _____)
4. Country of Citizenship: _____Visa Status if not U.S. citizen: _____
5. Attending entire Congress? Yes _____No _____If not, which days will you attend? _____
Will you present an invited paper or poster at the congress? Yes _____No_____
If so, please indicate the sessions you will address. If invited, attach letter of invitation.
Invited to public lecture (give title) _____
_____
Invited to Congress symposium (give title; indicate if Chairman) _____
_____
6. Do you intend to submit a poster (title)? _____
_____
7. Please describe your area of specialty (i.e., cell physiology, cardiovascular physiology, neurophysiology, etc.) _____
8. Member of: APS _____; SGP _____; Div. Comp. Physiol. & Biochem., ASZ _____; Soc. Neurosci. _____; BMES _____; Microicirc. Soc. _____; Other _____
9. Are you employed by the Federal Government more than one-half time? _____
10. Travel: a. City of departure _____b. Support requested _____c. Amount of other travel support available (excl. personal) _____
11. Recent publications (not more than 5 titles giving full references). If listing abstracts or manuscripts in press, please indicate this.

_____
Signature

Deadline for postmark of applications—December 15, 1988
Submit six (6) copies to USNC/IUPS, National Academy of Sciences, 2101 Constitution Ave NW, Washington, DC 20418. Attn: June Ewing.

(over)

SURNAME_____

12. ABSTRACT (not more than 250 words on paper or poster you plan to present at the Congress, including names of author and coauthors and indicate presenter. If none, abstract of current work.)

13. Give a brief resume of the scientific purposes and goals of your trip, including other meetings, satellite symposia, laboratories you plan to visit, work on collaborations, etc., in addition to attending the Congress.

(*Continued from p. 58*)

to outlaw such research to "save the life" of a pound animal.

Fifteen million dogs and cats are destroyed every year. Some 250,000 of these animals are giving answers desperately needed for improved medical treatment, while 15 million are incinerated. It is in fact the researcher, not the activist, who assigns the greater value to those lives. What an enormous legacy from the pound dog used in the lab, as compared to 15 million of its mates.

"We don't have to have advanced techniques in surgery and medicine to treat established disease since it is mostly preventable by now, with healthier lifestyles, and improved nutrition, "the activists moralize. But in the imperfect world in which I practice, I don't know enough to successfully treat, let alone prevent, many of the problems I see. I wear a seat belt and try to drive safely, but I do not find time to preach about this during resuscitation in the emergency room.

To say that we can stop research because we already know enough, but just need to apply our knowledge better, is a cruel hoax to many accident victims, most patients with cancer, and all victims of AIDS. To give up on the most successful method of obtaining real results—immediately applicable to human and animal illness alike—is more than anti-intellectual. It is suicidal. And this lethal decision is the more detestable since it is not limited to one's self.

"We have alternatives to animal research, not being adequately used, to improve treatments," say the animal rights activists. Where? Enter the computer, a proposed substitute for flesh and blood in the laboratory. If it worked satisfactorily as a substitute, which of us as teachers/researchers/practitioners would forego a technique that produced such good results? We *do* use computers. What we get out of them is what we put into them, with some mixing of the information in between. Often, much of the input information is wrong. How do we know that, or how do we know that the output product doesn't work? By testing it in life. Life is not hypothetical, and it is continually correcting our mistakes. We welcome any technique that will help us to get to the truth. There are no *alternatives* that are satisfactory substitutes, only *adjuncts* to help ultimately test where we practicing life scientists work.

"After all," the animal rights advocates insist, "animals and humans are so different. All the tests are falliable. Use patients with disease, not healthy animals, to do your research." We do, within ethical bounds. Do any of your potential patients wish to step forward to generously offer to test an untried drug, device or operation? The only conscionable treatments I can employ in clinical research with patients are those for which there is proven promise of therapeutic results—proven in biomedical research models. Without that proof we cannot raise the hopes of patients. The activists' argument also ignores the use of animal-proven teatments for animal benefit.

"Experiment on criminals, or volunteers, or prisoners, or defective people, or limit the subjects to those people who would directly benefit from the research product, rather than experiment on innocent, healthy animals." All patients are innocent. All people alive have paid their dues. All deserve the benefits of health care science.

Such are the arguments voiced by those who have the luxury of discussing in the abstract the relative ethics of harming various species of sentient beings. But then the phone rings, and you and I have to respond to the real-world urgency of a patient and a problem. There is no debate now. To the extent that you are able to do anything medically to help solve the problem, the solutions are direct and immediate benefits of biomedical research that have produced, tested and taught us what we know and can do.

What if the patient is the animal rights advocate with whom you have argued? Assume that the vegetarian patient in plastic shoes and cotton clothes insists that, to be consistent with his position, no treatment be employed that has been developed through animal research. What in your black bag can still be used? Since there is, I believe no medical practice that qualifies, you might offer to treat the patient anyway despite his beliefs. As an individual, he might still refuse. That is his right. We do not impose treatment, nor do we impose our beliefs on him. But do not let him make that choice for you, for me, for all of our present patients, and for those yet to come.

That animals might live, we cannot let those patients die.

Glenn W. Geelhoed

References

1. Newkirk, Ingrid, as quoted in the *Washingtonian* magazine. August, 1986, page 115.
2. Ibid., "The Larry King Show," radio debate. July, 1985.
3. Ibid.

Glenn W. Geelhoed, an APS member, is a professor at George Washington University, Washington; DC.

Reprinted from *The Biomedical Investigator's Handbook* with permission from the Foundation for Biomedical Research.

BOOK REVIEWS

They Threaten Your Health
Ernest Verhetsel
Tucson, AZ: Nutrition Information Center, 1986, 115 pp., index, $13.95

For the first time, perhaps, there is a publication on the public book shelves that defends the use of laboratory animals in biomedical research. Usually, book marts are limited to those publications that condemn the use of laboratory animals; scientists seldom take the time to write for public consumption.

The publication is *They Threaten Your Health* and is described as a critique of the antivivisection/animal rights movement. The author uses the pseudonym Ernest Verhetsel.

If the reader is looking for a publication that trashes animal activists, there will be disappointment. What the publication does do is to examine the issues, in general, and the writings, in particular, concerning the now centuries-old conflict between the scientists and those who advocate the abolishment of the use of live animal models for biomedical research. And it is the latter that he does best, thus making the publication a worthwhile consideration for purchase.

In reviewing the literature Verhetsel provides references throughout that a reader may wish to check for the accuracy of the statements. In one chapter he examines 15 pamphlets and 11 books written by animal activists wherein "the truth" (the statement used by the animal activist) and "the whole truth" (the complete text from which the statement was taken) are presented to show how statements taken out of context are used to mislead the readers of those pamphlets and books.

While *They Threaten Your Health* does not rank as high in readability as some of the books published by animal activists, nevertheless it still is worth both the time to read it and to recommend its readings to those who are unsure about their beliefs regarding the use of laboratory animals in biomedical research.

William M. Samuels

(*Continued on p. 93*)

PEOPLE AND PLACES

Long-time member **Gerald H. Jacobs**, Ph.D., professor of psychology at the University of California, Santa Barbara, is one of 21 leading scientists working in the fields of optoelectronics or nutrition to receive one of this year's Rank Prizes. The prizes, established by the British industrialist Lord Rank in 1972, are intended to recognize and encourage scientific achievement in two fields that closely reflect his career and interests in flour milling and the film industry. Jacobs and three other scientists were jointly awarded a prize for their research into the retinal and genetic bases of color vision and color blindness. Jacobs, who has been conducting research that is broadly directed toward understanding the biology and evolution of color vision, received £7,500. He has shown that color vision varies among some other species in much the same way that it varies among individual humans. He has also studied the variations in photopigments that cause differences in color vision both in humans and in a variety of other species.

The Universita di Bologna conferred to **Richard J. Bing**, M.D., Huntington Memorial Hospital, Pasadena, CA, the Honorary Degree in Medicine and Surgery. The oldest university in Europe, the Universita di Bologna celebrated its 900th anniversary.

Clifton A. Baile, Ph.D., Monsanto Company, has promoted to Distinguished Fellow in Monsanto's prestigious Fellow program. In the history of the program there have only been 15 members, and Baile is currently one of eight to receive the highest honor in this program. A member since 1968, he is also the director of research and development in the animal sciences division at Monsanto, St. Louis; adjunct professor, department of medicine, Washington University; and adjunct professor, department of animal science, University of Missouri.

Carmine D. Clemente, Ph.D., University of California at Los Angeles, was one of 262 scholars selected from among 3,264 applicants to receive the John Simon Guggenheim Memorial Foundation fellowship. Fellows were selected "on the basis of unusually distinguished achievement in the past and exceptional promise for future accomplishment." Clemente, a member since 1957, received the honor for "studies in the regeneration of nerve fibers in the central nervous system."

David J. Ramsay has been elected chairman of the California Biomedical Research Association, a nonprofit organization defending the use of laboratory animals. He is senior vice chancellor for academic affairs at the University of California Medical School, San Francisco, and is chairman of the APS Committee on Governmental Relations Initiative Programs and the Committee on Animal Care and Experimentation.

APS member **Gerald E. Loeb**, M.D., has been appointed professor and director of special projects in the biomedical engineering unit and professor of physiology, Queen's University. Loeb has been appointed under the Queen's National Scholar Program to develop research and teaching in the general areas of neural prosthetics and sensorimotor control. For the past 15 years, he has been with the Laboratory of Neural Control, NINCDS, National Institutes of Health, most recently as section chief of neurokinesiology.

Gary Kamen, Ph.D., has recently moved from Indiana University school of health, physical education and recreation to the neuromuscular research center at Boston University.

Formerly with the Center for Alcohol Studies, University of North Carolina, APS member **Robert D. Myers**, Ph.D., has joined the department of pharmacology, East Carolina University.

Jerry Radziuk, M.D., C.M., Ph.D., has become director, clinical investigation unit, Ottawa Civic Hospital, Ontario. Radziuk was at the Royal Victoria Hospital in Montreal.

APS member **Andrew R. Labarbera**, Ph.D., Prentice Women's Hospital in Chicago, has accepted a position in the department of obstetrics and gynecology at the University of Cincinnati college of medicine in Cincinnati.

James P. Knochel, M.D., has been named chairman, department of internal medicine, Presbyterian Hospital of Dallas. Knochel was formerly chief, medical service, Veterans Administration Medical Center, Dallas.

Andrew P. Somlyo, M.D., has accepted the chairmanship of the Department of Physiology, University of Virginia School of Medicine, Charlottesville. Somlyo has been director of the Pennsylvania Muscle Institute and professor of physiology, University of Pennsylvania, Philadelphia.

The new department chairman at the University of Nevada School of Medicine, Reno, is **Kenton M. Sanders**, Ph.D., who has been professor at that institution.

John E. Zehr, Ph.D., professor, department of physiology and biophysics, University of Illinois, Urbana, has accepted the chairmanship of that department.

People and Places notices come almost exclusively from information provided by members and interested institutions. To ensure timely publication announcements must be received at least *three months* (by the 5th of the month) before the desired publication date. Send all information to Martin Frank, Editor, *The Physiologist*, APS, 9650 Rockville Pike, Bethesda, MD 20814.

Deadline Date for receipt of abstracts for the 1989 FASEB Spring Meeting is early: November 1, 1988

BOOK REVIEWS
(*Continued from p. 91*)

Hypoxia, Polycythemia, and Chronic Mountain Sickness
Robert M. Winslow, M.D. and Carlos Monge C, M. D.
Johns Hopkins University Press, $50.00.

Devotees of Gilbert and Sullivan will recall that the Duke of Plaza-Toro was "well-connected," and the same could be said of this welcome book on aspects of high altitude physiology and pathophysiology. One author is the son of Carlos Monge M, who developed the Peruvian Institute of Andean Biology at San Marcos University in Lima, Peru, and provided the first clinical description of chronic mountain sickness that bears his name. Many of the pioneering studies of Carlos Monge M appeared in a book *Acclimatization in the Andes* also published by Johns Hopkins University Press some 40 years ago. The other author, Dr. Winslow, is a hematologist who is well known for his research on native highlanders of Peru and Nepal and for his contributions on the 1981 American Medical Research Expedition to Everest.

The book begins with an interesting historical chapter and then goes on to deal with many aspects of human responses to high altitude including polycythemia and hemopoiesis, hemoglobin, cardiorespiratory function, renal function, exercise tolerance, and the effects of hemodilution. Chronic mountain sickness is an elusive clinical syndrome characterized by hypoventilation affecting the high altitude native or long-term resident that, by aggravating hypoxemia, leads to excessive polycythemia. The authors regard this as the tail of the normal distribution rather than any specific disorder. They point out that the original patient described by Monge M "is now (1986) living and well in Lima, living proof of the reversibility of chronic mountain sickness by descent to sea level."

The book raises many intriguing physiological questions including why the kidney is the chief site of production of erythropoietin that controls erythropoiesis. The authors note that a characteristic of the kidney is its high blood flow and therefore venous PO_2 and that the same is true of the carotid body, another organ that has important regulatory functions in hypoxia. The issue of the physiological advantage of polycythemia at high altitude is discussed. Certainly marked degrees of polycythemia appear to be deleterious. Presumably the genetic pressure for the development of the erythropoietin-hemopoiesis control mechanism was exerted over thousands of years at sea level rather than in response to man's attempt to go to high altitude. Whereas at sea level the regulatory mechanism is important in restoring red cells lost through trauma, parasites, etc., it may be that the polycythemia of high altitude is an inappropriate response.

An interesting related topic is whether the hemoglobin levels of Himalayan highlanders are lower than those of Andean natives at the same altitude. There is some evidence to suggest this, though it is disputed. One hypothesis is that the native population of Tibet has been at high altitude far longer than the relative newcomers in the Andes, and they therefore have a more advanced genetic adaptation to high altitude. For example, the incidence of chronic mountain sickness appears to be less in the Himalayan regions than in the Andes. The question of whether we are seeing human evolution in action here is a fascinating one.

This book will be of great interest to many physiologists, not only those who work on high altitude but also those who are concerned with other aspects of hypoxia and the respiratory functions of the blood. Certainly no biomedical library can afford to be without it.

John West

BOOKS RECEIVED

Progress in Biochemical Pharmacology. R. Paoletti (Editor). *Biologically Active Ether Lipids* (series, vol. 22). P. Braquet, H. K. Mangold, B. B. Vargaftig (Editors). Basel: Karger, 1988, 196 pp., illus., index, $110.

The Opiate Receptors. Gavril W. Pasternak (Editor). Clifton, NJ: Humana, 1988, 499 pp., illus., index, $79.50.

They Threaten Your Health. Ernest Verhetsel. Tucson, Arizona: Nutrition Information Center, 1986, 115 pp., index, $13.95. (Order directly from the Nutrition Information Center, 255 N. Granada, Suite 2058, Tucson, AZ 85701.)

pH Homeostasis, Mechanisms and Control. D. Haussinger (Editor). London: Harcourt Brace Jovanovich Limited, 1988, 479 pp., illus., index, $48.00.

Modern Cardiovascular Physiology (2nd ed.) Carl Honig. Boston: Little, Brown, 1988, 317 pp., illus., index, $22.00.

The Biology of Hearing and Deafness. Robert V. Harrison. Springfield, IL: Charles C. Thomas, 1988, 432 pp., illus., index, $67.50.

Neuromethods Volume 9, The Neuronal Microenvironment. Alan A. Boulton, Glen B. Baker, and Wolfgang Walz (Editors). Clifton, NJ: Humana, 1988, 732 pp., illus., index, $94.50.

Nature's Living Lights: Fireflies and Other Bioluminescent Creatures. Alvin and Virginia Silverstein. Boston: Little, Brown, 1988, 42 pp., illus., index, $12.95.

Processing of Environmental Information in Vertebrates. Milton H. Stetson (Editor). New York: Springer-Verlag, 1988, 261 pp., illus., index, $79.00.

Handbook of Research Laboratory Management. Virginia P. White. Philadelphia, PA: ISI Press, 1988, 240 pp., illus., index, $49.95.

Brain and Feeding Behavior. Wanda Wyrwicka. Springfield, IL: Charles C Thomas, 1988, 267 pp., illus., index, $42.75.

Animal Physiology: Mechanisms and Adaptations (3rd ed.). Roger Eckert, David Randall, and George Augustine. New York: Freeman, 1988, 683 pp., illus., index, $39.95.

The Alpha-1 Adrenergic Receptors. Robert R. Ruffolo, Jr. (Editor). Clifton, New Jersey: Humana, 1987, 568 pp., illus., index, $79.50.

Variations in Susceptibility to Inhaled Pollutants: Identification, Mechanisms, and Policy Implications. Joseph D. Brain, Barbara D. Beck, A. Jane Warren, and Rashid A. Shaikh (Editors). Baltimore, MD: The Johns Hopkins University Press, 1988, 502 pp., illus., index, $65.00.

Cell Separation: Methods and Selected Applications. Thomas G. Pretlow II and Theresa P. Pretlow (Editors). New York: Academic, 1987, 374 pp., vol. 5, illus., index, $75.00.

A History of Neurophysiology in the 19th Century. Mary A. B. Brazier. New York: Raven, 1988, 265 pp., illus., index, $69.00.

Differentiation Antigens in Lymphohemopoietic Tissues. Masayuki Miyasaka and Zdenek Trnka (Editors). New York: Dekker, 1988, 531 pp., illus., index, $135.00.

Molecules. P. W. Adkins. New York: Scientific American Library, 1987, 197 pp., illus., index, $32.95.

The Timing of Biological Clocks. Arthur T. Winfree. New York: Scientific American Library, 1987, 199 pp., illus., index, $32.95.

Brain, Mind, and Behavior (2nd ed.). Floyd E. Bloom and Arlyne Lazerson. New York: Freeman, 1988, 394 pp., illus., index, $29.95.

ANNOUNCEMENTS

AAAS/Westinghouse Award

Nominations are invited for the AAAS/Westinghouse Award for Public Understanding of Science & Technology. This is an annual award for working scientists and engineers from all disciplines who make outstanding contributions to public understanding of science and technology but are not members of the media. The award, a $2,500 prize, will be presented during the AAAS Annual Meeting in San Francisco January 15–20, 1989. Deadline for nominations is October 1, 1988. *Information:* Patricia S. Curlin, Administrator of the Award, AAAS Committee on Public Understanding of Science & Technology, 1333 H Street NW, Washington DC 20005. Phone: (202) 326-6600.

3M Life Sciences Award

The Federation of American Societies for Experimental Biology announces the 14th annual 3M Life Sciences Award. The award, a $25,000 prize, will be presented at the 1989 FASEB Annual Meeting in New Orleans, LA. Deadline for receipt of nominations and supporting letters is October 15, 1988. *Information:* Marge Averi, 3M Life Sciences Award Committee, Executive Office, FASEB, 9650 Rockville Pike, Bethesda, MD 20814. Phone: (301) 530-7092.

Science Scholars Program

The Division of Research Grants (DRG) announces its Science Scholars Program. A few senior scientists from outside the Federal Government will have the opportunity to participate

in analyses of extramural scientific merit review, in policy evaluation, and in the formulation of recommendations for DRG. Science scholars will work in DRG on short-term assignments, 3–6 months. *Information:* Jerome G. Green, Director, DRG, Room 450, Westwood Building, NIH, Bethesda, MD 20892. Phone: (301) 496-7211. Donald H. Luecke, Deputy Director, DRG, Room 448, Westwood Building, NIH, Bethesda, MD 20892. Phone: (301) 496-7461.

Pharmacology Congresses

The XII Congress of the Latin American Society of Pharmacology and the III Interamerican Congress of Pharmacology will be held in Caracus, Venezuela, on October 2–7, 1988. *Information:* Dr. Anita Stern-Israel, Org. Secretary, Apdo. Postal 69116, Altamira, Caracas 1062, Venezuela.

Research Proposal Competition

Research proposals that can lead to a Ph.D. dissertation are invited from doctoral candidates on the "Relationship of Diet and Environmental Contaminants to Disordered Behavior." Creative and innovative ideas are encouraged. A packet of information on the subject plus Guidelines for Submittal is available on request to John A. Wacker, Wacker Foundation, 10848 Strait Lane, Dallas, TX 75229. Phone: (214) 368-0150.

Submittal deadline is December 15, 1988. Winning entrees will be determined by a panel of eminent scientists and will receive favorable consideration for funding. First place prize is $2,500; second place, $1,500; third place, $500. Winners will be announced in February 1989.

ISHIB Call for Abstracts

The International Society on Hypertension in Blacks announces the Fourth International Interdisciplinary Conference on Hypertension in Blacks June 28–July 2, 1989, Kenyata International Conference Center, Nairobi, Kenya. The conference is hosted by Kenya Cardiac Society, Kenya Medical Association, Kenya Medical Research Institute, and Pan African Society of Cardiology. The conference includes three sections: Basic Science, Clinical, and Community Research in hypertension in blacks. Awards will be presented for Distinguished Research, Outstanding Community Service, and Outstanding Health Professional Student in hypertension in blacks. The deadline for submission of abstracts and nominations for awards is December 1, 1988.

Mail request for Abstract Rules and Guidelines, Rules for Awards, Nominations, and Conference Registration information to Ms. Cecile Cate, Executive Director, International Society on Hypertension in Blacks, 69 Butler Street, S.E., Atlanta, GA 30303 USA.

Scientific Award 1988

Hildegard Doerenkamp and Gerhard Zbinden Foundation for Realistic Animal Protection in Scientific Research will award a prize of DM 50000 for outstanding scientific contributions leading to the reduction of animal use in biomedical research (with preference given to large animals, i.e., dogs, cats, monkeys). This year's topic is "Reduction of Animal Use in Biomedical Research by Computer Modeling." Applications may consist of published or unpublished reports on computer use in all areas of biomedical research that are directly relevant to the topic. Computer programs for simulation of animal experiments in teaching and research are also acceptable. *Deadline*: December 31, 1988. Applications should be sent to: Professor G. Zbinden, Institute of Toxcicology, Schorenstrasse 16, CH-8603 Schwerzenbach, Switzerland.

Conference on Potassium Transport

The Department of Physiology and Biophysics at the University of Texas Medical Branch in Galveston is sponsoring a conference entitled "Regulation of Potassium Transport Across Biological Membranes: A Symposium" on October 24 and 25, 1988. It will be held in the Tremont House in Galveston. *Information:* Symposium Secretary, Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, TX 77551. Phone: (409) 761-1826.

Conference on Nuclear Analytical Methods

An international conference entitled "Nuclear Analytical Methods in the Life Sciences" is being organized by the National Bureau of Standards in cooperation with the International Atomic Energy Agency. It will be held in Gaithersburg, Maryland, USA on April 17–21, 1989. It is cosponsored by the American Nuclear Society, U.S. Department of Energy, and Food and Drug Administration.

Scientific contributions to this conference will be accepted on topics that are related to the

analytical utilization of nuclear techniques to determine the chemical and structural composition of biological materials or to the application of such techniques in research and development. Contributed and invited papers will be presented in oral sessions over five days. Sessions will be organized to include the following topics: new and emerging methodology; activation techniques; quality assurance; comparison of activation analysis with other methods; and applications of nuclear analytical techniques in biology, medicine, biotechnology, agriculture and nutrition, and in public, occupational, and environmental health.

For additional information: Rolf Zeisler, General Chairman. National Bureau of Standards, Center for Analytical Chemistry, Room B125 Building 235, Gaithersburg, MD 20899. Phone: (301) 975-6290.

ASCB/ASBMB Joint Meeting

The American Society for Cell Biology and the American Society for Biochemistry and Molecular Biology are pleased to announce their joint meeting to be held in the Moscone Convention Center, San Francisco, California, January 29–February 2, 1989, The deadline for receipt of abstracts in Bethesda is August 15, 1988. Forms and information about the meeting, registration, and lodging are available from ASCB/ASBMB Meeting Office, Room L3111, 9650 Rockville Pike, Bethesda, MD 20814. Phone: (301) 530-7153.

Bristol-Myers Awards Research Grants

Five $500,000 no-strings-attached research grants announced by Bristol-Myers Company are the first in a program to support basic research in the neurosciences, investigating how the brain and central nervous system work—and what goes wrong in disorders from Parkinson's and Alzheimer's diseases to schizophrenia and other major psychiatric illnesses. The first recipients are the Johns Hopkins School of Medicine, Yale University/Conneticut Mental Health Center, Cornell University Medical College, University of California-San Diego, and University of California-Irvine.

Executive Summary

In an effort to assess the health of the U.S. research system, The Conference Board and the National Governors' Association, with the support and participation of the National Science Foundation, undertook a joint project. The project solicited the views of the nation's Governors, senior officers of U.S. companies, and presidents and deans of U.S. colleges and universities on the relationship of U.S. competitiveness to our human resource base and research and development capacity. The views of these key leaders were obtained through a survey and three regional meetings held in April 1987. The study, titled The Role of Science and Technology in Economic Competitiveness, focused on three primary topics: the adequacy of our human resources and their relationship to our ability to compete; U.S. investment in research and development; and technology transfer, i.e., the ability to transform research findings into new products and processes. For copies of the study, contact National Science Foundation, Washington, DC.

Proposal Review at NSF

The National Science Foundation conducted a survey in late 1986 of more than 14,000 applicants whose proposals for research support had been awarded or declined during fiscal year 1985. The principal purpose was to seek the views of individual investigators at academic institutions about NSF's competitive proposal review and decision-making process. More than 9,500 people responded. Nearly half the applicants were satisfied with the review process, but a substantial proportion (38%) were dissatisfied. Declinees (two-thirds of the applicants) were much more likely to be dissatisfied. The report also contains results according to field of research, principal investigator, multidisciplinary research, and resubmission of proposals. It also contains the first cross-tabulation of applicants' field of research to the Foundation's research support divisions, and the initial chart of award rates by division for first proposals and resubmittals. Copies of the report are available from Forms and Publications Unit, Room 232, National Science Foundation, 1800 G Street NW, Washington, DC 20550 (Report NSF 88-4).

POSITIONS AVAILABLE

Director of Cardiovascular Research, University of Kentucky. The Division of Cardiovascular and Thoracic Surgery seeks applicants for a new tenure track position as Director of Cardiovascular Research. Applications will be considered from qualified cardiovascular physiologists, biomedical engineers, or biochemists with either a Ph.D or M.D. degree. Postdoctoral training is a prerequisite, with computer knowledge preferred. The development of a high-quality basic science research program will be expected. Laboratory interests should relate either to ventricular function, myocardial blood flow, electrophysiology, and/or cellular metabolism. The successful candidate will be expected to develop an independent funding program. Senior applicants, therefore, should show a good record of grant support. Considerable opportunities for interaction with basic science departments exist. Please send curriculum vitae, representative reprints, and three letters of recommendation to W. Randolph Chitwood, Jr., M.D., Division of Cardiovascular and Thoracic Surgery, College of Medicine, University of Kentucky, Lexington, KY 40536. [EOAAE]

Physiology Faculty Positions. The Department of Physiology, The University of Wisconsin-Madison, invites applications for tenure-track junior- and senior-level faculty positions in cellular and molecular physiology. The department especially encourages applications from individuals with research interests in regulatory or developmental aspects of cellular and subcellular function but will give serious consideration to outstanding applications in other areas. The search will favor individuals whose research entails the use of biochemical, biophysical, or molecular biological probes of physiologically relevant processes. Excellent opportunities exist for participation in campus-wide interdisciplinary research and training programs. These positions will be available after July 1, 1988. Women and minorities are specifically encouraged to apply. Applicants should send curriculum vitae, a summary of research interests and plans, and the names of three references to Dr. Richard L. Moss, Chair, Department of Physiology, University of Wisconsin, 1300 University Avenue, Madison, WI 53706. [EOAAE]

Positions Available

There is a $25 charge per issue for each position listed. A check or money order payable to the American Physiological Society must accompany the copy. Purchase orders will not be accepted unless accompanied by payment. Ads not prepaid will not be printed. Copy must be typed double-spaced and limited to 150 words. All copy is subject to the editorial policy of *The Physiologist*. EOAAE indicates Equal Opportunity/Affirmative Action Employer and appears only where given on original copy. Copy deadline: copy must reach the APS office before the 15th of the month, 2 months preceding the month of issue (e.g., before December 15 for the February 1987 issue). Mail copy to APS, 9650 Rockville Pike, Bethesda, MD 20814.

Know Your Sustaining Associates

The American Medical Association

The American Medical Association promotes the art and science of medicine and the betterment of public health. The AMA accomplishes this mission by advancing standards of medical education, promoting support for biomedical research, representing the medical profession, providing information about medical matters, and upholding professional conduct and performance.

Beckman Instruments, Inc.

Beckman Instruments, Inc. is a major international manufacturer of bioanalytical and diagnostic instruments and related products for science and medicine. The company was founded in 1935 by Dr. Arnold O. Beckman, who was inducted into the National Inventors Hall of Fame on Feb. 8, 1987. In 1982, the Orange County-based company merged with Smith Kline in Philadelphia to form SmithKline Beckman Corporation, a leading health care and life sciences company recognized worldwide.

Beckman's business is to combine chemistry and engineering. Its product lines include hundreds of instruments and related products. For the life sciences, Beckman manufactures centrifuges, liquid scintillation counters, spectrophotometers, and pH meters. For health care, the company produces clinical systems, diagnostic kits, reagents, and quality controls. A complete sales and service force supports Beckman customers.

Berlex Laboratories, Inc.

Berlex Laboratories, Inc. is a U. S. subsidiary of the multinational pharmaceutical and chemical firm, Schering AG West Germany (not connected with Schering-Plough Corp. or Schering Corp. of New Jersey). It conducts research and markets prescription drug products primarily for cardiovascular, diagnostic imaging, metabolic, endocrine, and central nervous system uses.

Coulbourn Instruments

Coulbourn Instruments, Inc. manufactures electronic instruments for in vivo life science applications. Products include the LabLinc Modular Instrument System for physiological signal conditioning, experiment control, and data acquisition, featuring over 100 modules, including computer interface ports, signal conditioning and processing, and counting and timing modules for chart and computer-based polygraphs.

The company also produces transducers, biotelemetry, signal processors, stimulators, and auditory and animal behavior test equipment.

Major markets include pharmaceutical, chemical, and biotechnological firms, universities, research hospitals, and government laboratories.

Dagan Corporation

Dagan Corporation manufactures electronic instruments used in electrophysiology. Dagan offers a full line of analog and digital products, including preamplifiers for use in intracellular and extracellular recording, single and two electrode voltage/current clamps, patch clamps/whole-cell clamps, signal averagers, programmable multichannel stimulators, and iontophoresis generators.

Glaxo, Inc.

Glaxo Inc., a leading research-based pharmaceutical company headquartered in Research Triangle Park, North Carolina, manufactures and markets prescription medicines including treatment for respiratory ailments, ulcers, hypertension, infectious diseases, and diseases of the skin. Glaxo is a wholly owned subsidiary of Glaxo Holdings p.l.c.

The Grass Foundation

The Grass Foundation underwrites the annual Walter B. Cannon Lectureship given at the Spring Meeting of the American Physiological Society. The naming of this lectureship serves two functions: to commemorate the enormous contribution of Dr. Cannon to the growth of knowledge of physiology and to pay a tribute to Dr. Cannon on behalf of many of the founding trustees of the Grass Foundation who were members of his research group at Harvard Medical School, early in their careers.

This lectureship is in accordance with the Grass Foundation's charter mandate to support research and education in neurophysiology. Other programs include funding for other annual and visiting lecturships, summer fellowship support for young students, and occasional relevant course support.

Harvard Apparatus

Harvard Apparatus, since its inception in 1904 at the Harvard Medical School, continues to design, develop, and supply the unique apparatus that has shaped the development of teaching and research in physiology and allied science, including syringe peristaltic and respiration pumps, recording systems, and research accessories.

ICI Pharmaceuticals Group

ICI Pharmaceuticals Group is comprised of two significant marketing arms—ICI Pharma and Stuart Pharmaceuticals. Worldwide research and development activities are conducted in behalf of the organization in two major research centers. One of these is located at our headquarters in Alderley Park, Cheshire, England and the other in Wilmington, Delaware. Drug discovery activities in cardiovascular, pulmonary, central nervous system, oncology, arthritis, metabolic disease and infection are conducted in these sites. In total, the company employs nearly 2,800 people worldwide in pharmaceuticals research and development, of which approximately 25% are located in the United States.

Jandel Scientific

Jandel Scientific designs and sells IBM compatible software for scientific research. Products include Sigma-Plot for publication quality scientific graphs (with automatic error bars, regression lines, and many other scientific graphing options); Sigma-Scan for x-y digitizing, morphometric measurement and analysis; and PC3D for generating three-dimensional reconstructions of objects from serial sections. JAVA, our latest product, is a video analysis system capable of image processing, densitometry, automatic object counting and edge tracking, and morphometric measurement. JAVA works with a video digitizing board and input from a video camera, VCR, or other video source.

Janssen Pharmaceutica

Janssen Pharmaceutica was founded in Belgium in 1953 by Dr. Paul Janssen. It is now an international company built on the foundation of research and a bedrock of innovation. The company remains under the direction of Dr. Paul Janssen and has an unparalleled record in the successful development and marketing of new pharmaceutical products. According to the Japan Drug Research studies, Janssen was responsible for more significant new drug discoveries during the period 1970–1983

than any pharmaceutical company in the world.

The company currently has approximately 6,000 employees worldwide. It is a world leader in medication used in the treatment of allergies, mental disorders, digestive and intestinal problems, cardiovascular conditions, and worm and fungal infections. Janssen's compounds have also enabled major advances in anesthesia and immunology. In addition, Janssen has also discovered many chemical compounds to identify and characterize receptors in the brain and the periphery that have played a prominent role in advancing our knowledge about neurotransmitters.

Nacro Bio-Systems

Narco Bio-Systems designs, manufactures, and distributes the Physiograph® physiological recording systems for use in clinical, research, and teaching applications. A selection of multichannel chart recorders are available with a complete line of modular input preamplifiers, signal conditioners, transducers, and accessories. This allows maximum flexibility to design your own system for recording physiological functions.

Ortho Pharmaceutical Corporation

Ortho Pharmaceutical Corporation, headquartered in Raritan, New Jersey, is a research-based pharmaceutical company engaged in the development and manufacture of a wide range of health care products marketed in more than 60 countries around the world. A wholly owned subsidiary of Johnson & Johnson, Ortho's four operating divisions produce a variety of contraceptives, gynecological therapeutics, prescription and proprietary skin-care products, self-care diagnostics, and a growing number of biotechnology-derived pharmaceuticals, including immunomodulators and monoclonal antibodies.

With more than 4,000 trademark registrations worldwide, Ortho continues its commitment to an intensive research and development program to ensure tomorrow's innovative health care products in the areas of conception control, immunobiology, and the treatment of gastrointestinal disorders and cardiovascular disease.

Pennwalt Pharmaceuticals

Pennwalt Pharmaceuticals Division, based in Rochester, New York since 1886, has continually been committed to provide physicians and consumers with excellent products. In addition to providing fine pharmaceuticals, Pennwalt has become a leader in the technology of controlled drug delivery systems. Existing or imminent consumer and prescription products include agents to treat cardiovascular and neurological diseases, pain, infections, immunological disturbances, eating disorders, colds, and allergy. Current research promises to yield additional and products targeted for these therapeutic areas.

Pharmacia

Pharmacia is the world's leading supplier of separation and purification products for the biotechnology industry as well as a research-intensive international manufacturer of products for use in areas of medicine, including gastroenterology, rheumatology, oncology, ophthalmology, blood volume replacement, allergy, and dermatology.

The Procter & Gamble Company

Procter & Gamble is a multinational, technically based consumer products cor-

APS Sustaining Associate Members

The Society gratefully acknowledges the contributions received from Sustaining Associate Members in support of the Society's goals and objectives.

* Second Century Corporate Founders

Abbott Laboratories
American Medical Association
Beckman Instruments, Inc.
Berlex Laboratories, Inc.
Boehringer Ingelheim
Burroughs Wellcome Company
Ciba-Geigy Corporation
Coulbourn Instruments, Inc.
Dagan Corporation
DuPont Critical Care, Inc.
E. I. du Pont de Nemours & Company
Glaxo, Inc.
Gould, Inc.
Grass Foundation
Harvard Apparatus
Hoechst-Roussel Pharmaceuticals, Inc.
* Hoffmann-La Roche, Inc.
ICI Pharmaceuticals Group
Jandel Scientific
Janssen Pharmaceutica
Lederle Laboratories
Lilly Research Laboratories
Marion Laboratories, Inc.
McNeil Pharmaceutical
* Merck & Co., Inc.
Miles Institute for Preclinical Pharmacology
NARCO Bio-Systems
Norwich Eaton Pharmaceuticals, Inc.
Ortho Pharmaceutical Corporation
Pennwalt Pharmaceuticals
Pfizer, Inc.
Pharmacia, Inc.
Pillsbury Corporation
Procter & Gamble Company
* Sandoz, Inc.
* Schering-Plough Corporation
G. D. Searle and Company
Smith Kline & French Laboratories
* Squibb Corporation
Sterling Drug, Inc.
Sutter Instruments Company
The Quaker Oats Company
* The Upjohn Company
Warner-Lambert/ParkeDavis
Waverly Press, Inc.
Wyeth Laboratories

poration with operations in 28 states and 36 foreign countries. It has four technical centers and its world headquarters in Cincinnati, Ohio. Technical centers are also located in Egham and Newcastle, England; Brussels, Belgium; Schwalbach, Germany; and Osaka, Japan.

Last year's R&D expenditures were $576 million. World-wide Ph.D. population is ~850, divided equally between chemists and life scientists, and total employees number 75,000.

Sales last year reached $17 billion in the paper, soap and detergent, health care, personal care, pharmaceutical, beverage, and food categories, making it the 18th largest U.S. corporation. *Fortune* magazine has recently named Procter & Gamble as the 4th most admired corporation in the United States.

Schering-Plough

Born out of a 1971 consolidation of two companies—Plough, Inc. and the Schering Corporation—Schering-Plough is dedicated to the discovery, development, and marketing of novel therapeutic entities. The company focused its research in the fields of anti-inflammatory, antiallergic, cardiovascular, and anti-infective disorders. The company has also attained a leading position in immunolog and recombinant DNA technology.

Smith Kline & French

A division of Smith Kline Beckman Corporation, Smith Kline & French Laboratories is a technology-intensive, worldwide healthcare company. Smith Kline & French is a leading supplier of pharmaceuticals to treat infectious, gastrointestinal, cardiovascular, and arthritic diseases and a leader in the research, development and marketing of innovative medicines.

Squibb Corporation

Squibb Corporation, a leading worldwide developer, manufacturer, and marketer of pharmaceutical and allied health care products, is organized into the Squibb Operating Group and the Science and Technology Group.

The Squibb Operating Group is responsible for the manufacturing, marketing and distribution of products and services. Squibb's pharmaceutical products are marketed by Squibb International and Squibb United States. The Medical Products segment consists of ConvaTec and the companies of Edward Weck Incorporated.

The Squibb Science and Technology Group is composed of The Squibb Institute for Medical Research, Worldwide Regulatory Affairs and Licensing. Celebrating its 50th anniversary in 1988, The Squibb Institute is among the nation's first industry-sponsored research centers. In recent years, it has focused on four main areas: 1) cardiovascular disease; 2) infectious disease; 3) diagnostics; and 4) inflammatory disease. It has recently broadened into molecular biology, the neurosciences and metabolic disorders.

The Upjohn Company

The Upjohn Company, a multinational corporation headquartered in Kalamazoo, MI, is celebrating its centennial year as a maker of fine pharmaceuticals. It is one of the 15 largest research-based pharmaceutical manufacturers in the world. It has research, production, and warehousing facilities in more than 45 countries and its products are sold in more than 150 countries.

Upjohn has long been committed to the research, development, manufacture, and marketing of pharmaceuticals. Human health care is the heart of Upjohn's endeavors.

Waverly Press, Inc.

Waverly Press, Inc., are printers of magazines and journals for the association marketplace.

Committed to servicing their customers through sharing knowledge, providing the best in modern technology, and establishing mutual respect, they offer full-range publishing services including design, editorial, composition, printing, binding, mailing, warehousing, subscription fulfillment, and ad sales.

Waverly practices team-concept management. Both client and staff are part of the team. Through this management concept each publication receives close personal attention.

Striving for excellence in the graphic arts industry is traditional at Waverly—one that continues. They believe in quality product and service through quality people.

Submit original and 7 copies of application and supporting documents.

APPLICANT'S LAST NAME _____

Date _____

THE AMERICAN PHYSIOLOGICAL SOCIETY
9650 Rockville Pike, Bethesda, MD 20814

MEMBERSHIP APPLICATION FOR:

REGULAR ☐
CORRESPONDING ☐
ASSOCIATE ☐
STUDENT ☐

CURRENT MEMBERSHIP CATEGORY; YEAR ELECTED _____

See Instructions

Name of Applicant: _____
First Middle Last

Mailing Address _____ Birth Date: _____

Citizenship: _____

Country of Permanent Residence:* _____

Telephone No.: _____

* Alien residents of Canada and Mexico see General Instructions. Alien residents of U.S. enter Alien Registration Receipt Card number _____.

1. EDUCATIONAL HISTORY

Dates	Degree	Institution	Major Field	Advisor

Doctoral Dissertation Title: (if any)

Postdoctoral Research Topic:

2. OCCUPATIONAL HISTORY

Present Position:

Prior Positions:

Dates	Title	Institution	Department	Supervisor

SPONSORS

#1. Name: _____ #2. Name: _____

Mailing Address: _____ Mailing Address: _____

Telephone No. Zip Code Telephone No. Zip Code

I have read the guidelines for applicants and sponsors and this application and attest that the applicant is qualified for membership.

#1 Signature _____ #2 Signature _____

Each sponsor must submit an original and 7 copies of a confidential letter of recommendation to the Society, under separate cover.

NAME _____

3. INTEREST IN THE SOCIETY

a. Have you attended meetings of the APS (Y/N)? _____

b. In the space provided state why you want to join the Society.

4. TEACHING

a. Do you teach physiology (Y/N)? _____

b. What percentage of your time/effort is devoted to teaching (lectures, conferences, etc.) physiology? _____

c. Do you supervise graduate and/or postgraduate students (Y/N)? _____

d. Have you produced teaching aids (textbook chapters, films, computer assisted instruction, etc.) (Y/N)? _____

5. RESEACH

a. What percentage of your time/effort is devoted to research? _____

b. If your research is funded state source: _____
Are you a principal _____ or co-principal investigator _____ ?

6. BIBLIOGRAPHY

Attach a list of your publications reported during the past 5 years. Star those in refereed journals.

Published Departmental Histories and Archival Materials

Issue	Author	Institution	Period
18(3): 99–103, 1975	Youmans, W. B.	University of Wisconsin, Madison	1906–1952
20(5): 26–27, 1977	Kaplan, H. M.	Southern Illinois University, Carbondale	1947–1977
21(5): 12–14, 1978	Jaques, L. B.	University of Saskatchewan, Saskatoon	1926–1978
25(1): 39, 1982	Aserinsky, E.	Marshall University School of Medicine, Huntington, WV	1976–1980
25(1): 39, 1982	Santos-Martinez, J.	Universidad Central del Caribe, Cayey, PR	1976–1980
25(1): 40–41, 1982	Brown, E. B., Jr.	Oral Roberts University, Tulsa, OK	1977–1981
25(1): Suppl. 1–96, 1982	Davenport, H.	University of Michigan, Ann Arbor	1850–1923
25(5): 414, 1982	Haddy, F. J.	Uniformed Services University of the Health Sciences, Bethesda, MD	1976–1982
25(5): 414–425, 1982	McGrady, A. V.	Medical College of Ohio, Toledo	1964–1982
25(5): 416–418, 1982	Tansy, M. F.	Temple University, Philadelphia, PA	1863–1982
25(6): 469–474, 1982	Hansel, W., E. P. Leonard, and H. H. Dukes	Cornell University College of Veterinary Medicine, Ithaca, NY	1868–1960
25(6): 474–478, 1982	Dill, D. B.	Medical Laboratories of the Army Chemical Corps, Edgewood, MD	1946–1961
26(2): 64–70, 1983	Chasis, H.	New York University School of Medicine	1841–1960
26(3): 119–120, 1983	Stinson, J. N.	Meharry Medical College, Nashville, TN	1876–1981
26(3): 120–121, 1983	Annegers, J. H.	Northwestern University, Chicago, IL	1894–1982
26(5): 260–266, 1983	Schmidt-Nielson, B. M.	Mount Desert Island Biological Laboratory, Salsbury Cove, ME	1926–1983
26(5): 269, 1983	Elwell, L. H.	Oregon Health Sciences University School of Dentistry	1899–1980
26(6): 366–368, 1983	Kline, D. L.	University of Cincinnati, Cincinnati, OH	1819–1983
27(1): 4–12, 1984	Loew, E. R.	Boston University, Boston, MA	1873–1948
27(3): 113–127, 1984	Rosenfeld, L. M.	Jefferson Medical College, Philadelphia, PA	1842–1982
27(5): 319–324, 1984	Elizondo, R. S., N. Jacobs, and W. W. Moore	Indiana University, Bloomington	1854–1983
27(5): 325–329, 1984	Selkurt, E. E.	Indiana University School of Medicine, Indianapolis	1958–1983
27(6): 385–389, 1984	Horres, A. D.	Medical University of South Carolina, Charleston	1824–1983
28(3): 139–140, 1985	Magee, D. F.	Creighton University School of Medicine, Omaha, NE	1892–1985
28(5): 402–406, 1985	Pitts, G. C.	University of Virginia, Charlottesville	1825–1985
28(6): 482–484, 1985	Boyarsky, L. L.	University of Kentucky, Lexington	1890–1985
28(6): 485–490, 1985	Weiss, A. K.	University of Oklahoma Health Sciences Center, Oklahoma City	1898–1985
28(6): 491–501, 1985	Lessler, M. A., and F. A. Hitchcock	Ohio State University, Columbus	1879–1985
29(5): Suppl. 1–6, 1987	Rahn, H.	State University of New York at Buffalo	1846–1986
29(5): Suppl. 7–20, 1987	Pace, N.	University of California at Berkeley	1860–1987
29(5): Suppl. 21–26, 1987	Copp, D. H.	University of British Columbia, Vancouver	1908–1987
29(5): Suppl. 27–33, 1987	Otis, A. B.	University of Florida, Gainesville	1956–1981
29(5): Suppl. 34, 1987	Baker, C. H.	University of South Florida, Tampa	1971–1987
29(5): Suppl. 35–45, 1987	Friedman, M. H. F.	Philadelphia College of Osteopathic Medicine	1883–1985
29(5): Suppl. 46–57, 1987	Smith, A. H., E. M. Bernauer, A. L. Black, R. E. Burger, J. H. Crowe, J. M. Horowitz, G. P. Moberg, and E. M. Renkin	University of California at Davis	1905–1985
29(5): Suppl. 58–62, 1987	Francesconi, R., R. Byrom, and M. Mager	United States Army Research Institute of Environmental Medicine, Natick, MA	1951–1986
29(5): Suppl. 63–73, 1987	Smith, J. J.	Medical College of Wisconsin, Milwaukee	1963–1987
31(4): 107–112, 1988	Adolph, E. F.	University of Rochester, NY	1924–1969
31(4): 113–127, 1988	Guest, M. M.	University of Texas Medical Branch at Galveston	1891–1987
31(4): 128–131, 1988	Solomon, S.	University of New Mexico, Albuquerque	1961–1987
Archives only	Tenney, S. M.	Dartmouth Medical School, Hanover, NH	1797–1983
Archives only	Guyton, A. C.	University of Mississippi, Oxford and Jackson	1903–1984
Archives only	Font, C.	San Juan Bautista School of Medicine, San Juan, PR	1978–1986

History of Department of Physiology at Rochester, 1924–1969

EDWARD F. ADOLPH†
Department of Physiology
University of Rochester
Rochester, New York 14642

The University of Rochester Department of Physiology started under two novel circumstances: it was founded by plan (without antecedents), and its first chairman was selected without interview by dean or president.

The University of Rochester was established in 1850. It was chosen as the site of an endowed School of Medicine and Dentistry in 1920. This choice was made by officers of the Rockefeller Foundation, partly in view of citizen George Eastman's interest in support of medicine and dentistry.

The Professor of Physiology was Wallace O. Fenn, who was brought to the attention of Dean George H. Whipple and President Rush Rhees. Since Wallace Fenn in 1924 was working in London (in collaboration with A. V. Hill), he was selected unseen, on the basis of the strong recommendations of senior persons who knew him.[1] As events turned out, Wallace Fenn was repeatedly entrusted with top responsibilities in American and international physiology during (and after) his 35 years as Department Chairman. Fenn was followed as Chairman by William D. Lotspeich (1959–67).

In 1924 most departments of physiology consisted of three faculty members: professor, assistant professor, and assistant. The professor did most of the lecturing; the others guided students in performance of experiments that were described in a laboratory manual. Most research was completed by the three working as a team.

In Rochester, Edward F. Adolph became Assistant Professor in 1925, sharing equally with Fenn in teaching and in independence of research. For seven years no faculty appointees were added.

The annual classes of medical students gradually climbed from 22 to 45 in the first decade. Graduate students were enrolled from 1928 onward; some young physicians came into the Department for one-year periods, and two technical assistants were at work. Certain medical students were fellows in physiology for one year each. They joined in the teaching activities that occupied four months every spring (1).[2]

Teaching

A Department of "Vital Economics" was founded in the Faculty of Arts and Science at Rochester seven years before the Department of Physiology came into existence. Its Chairman was John R. Murlin. In 1925 it was given space in the new School of Medicine and Dentistry and contributed to the instruction of medical and other students in areas of nutrition and metabolism. For this reason the Department of Physiology was able to concentrate its teaching in other areas.

In the first years, lectures in the new Department were few; laboratory projects were outlined orally, and group conferences offered opportunity for discussions of laboratory results and of reading topics. Fenn always planned personally the instruction of medical students. He varied the programs substantially from year to year; an instructor rarely restricted his responsibility to subject areas of his own specialty. I recall a conversation with the dean of another medical school in about 1930. He inquired how our topics of instruction were divided between two departments and then said, "No member of your Department would be considered for a chairmanship elsewhere, since you do not teach all of physiology." How much both physiologists and deans have changed in a half century!

In the first year of the new Department's work, every laboratory need had to be anticipated. Needs were curtailed by division of students into groups, each group rotating from one set of experiments and equipment to another set. After three or four years the laboratory procedures were written, and hand-outs enabled students to plan experiments ahead of time.

During the first decade the laboratories were always open (2), and a few students extended their laboratory observations into modest investigations that they "wrote up." Several such reports reached journal publication, standing as products of individual efforts encouraged by

[1] I have been asked how Fenn was appointed Chairman of this Department without interview by Dean Whipple or President Rhees. Fenn was in England in the two years 1922–1924. He was highly regarded by Walter B. Cannon and Simon Flexner, both of whom told Whipple about him. In London at the time, George W. Corner, already Chairman of the Department of Anatomy here, was asked to get acquainted with Fenn. J.R. Murlin, Chairman of our Department of Vital Economics, already knew Fenn, and A. V. Hill sponsored his work abroad. All these expressed unusually enthusiastic opinions of Fenn's suitability for the position. Conclusive action was taken by sea mail.

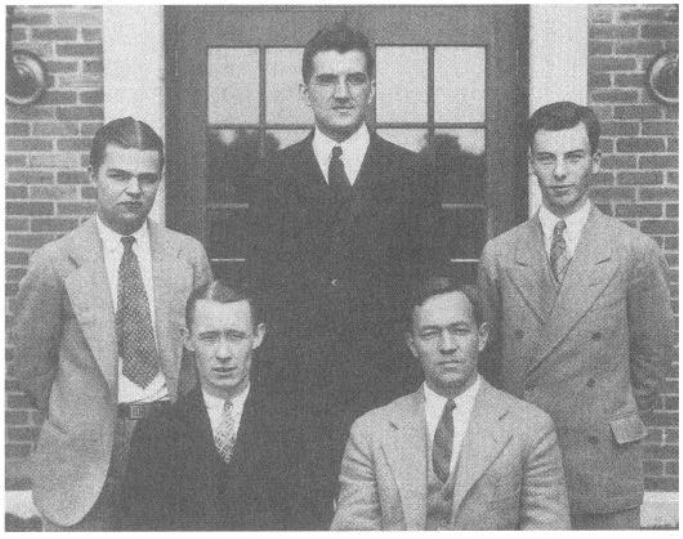
† Deceased.

[2] A few details about the initial plans for the Department may be of interest. Provisions were being made for teaching and for research and involved hard estimates for future space and equipment.

The School expected to instruct a maximum of 70 students in each medical class, a few graduate students, and 25 student nurses every year. No students for the degree D.D.S. were at any time accepted by the School, but dental graduates became candidates for Ph.D. or M.S.

In 1925 the Department had five members: Fenn, Adolph, P.L. Gray, assistant, W.B. Latchford and C.C. Smith, technicians. Fenn and Adolph each had research experiments in progress. Preparations for teaching began in January 1926; the first class started work in March. Each group of seven or eight students rotated through three laboratory units of one week each; by this arrangement only one-third as much apparatus was needed. Each day the group's experiment was explained orally. Results obtained were later discussed in conversations and in notebooks. Weekly conferences were held in each group. There were seminar type reviews of journal papers, and weekly class lecture.

Each first-year class of medical students spent half their time in the study of "physical physiology" in the Department of Physiology. In the second-year curriculum the same students spent a similar period of time studying "chemical physiology" in the neighboring Department of Vital Economics.



Department of Physiology, 1926. *Top, left to right:* Donald S. Martin (student fellow), Edward Adolph, and Colin C. Smith (technician); *bottom:* William B. Latchford (technician), and Wallace Fenn.

instructors. In later years each student chose a personal project, involving either designed experiments or journal searches or both. Oral and written presentations provided opportunity for initiative by the experimenter and discussion by fellow students.

In each year several clinical teachers were invited to present patients before the physiology class. These teachers were adept at showing the reasonings involved in diagnoses and treatment.

A library was, of course, essential to both teaching and research. Since the Medical School was under one roof, the medical library served all departments. Students as well as faculty used the stacks and requested purchases. Journals could be borrowed on the same terms as books. Little need was felt for a library within the Department of Physiology.

Decades

In the 1930s the number of medical students per class increased to 45, in 1944 to 60, in 1950 to 68, and in the 1970s to 96. Graduate students from various departments joined the medical class for the course in physiology.

Two features of education were early emphasized: laboratory methods and results and group discussion of those results and of journal publications.

At the end of the first decade (1936) there were 8 instuctors each working with 6–12 students. Class lectures had increased to five each week, with an occasional demonstration. Some demonstrations showed on-going research in faculty laboratories.[3]

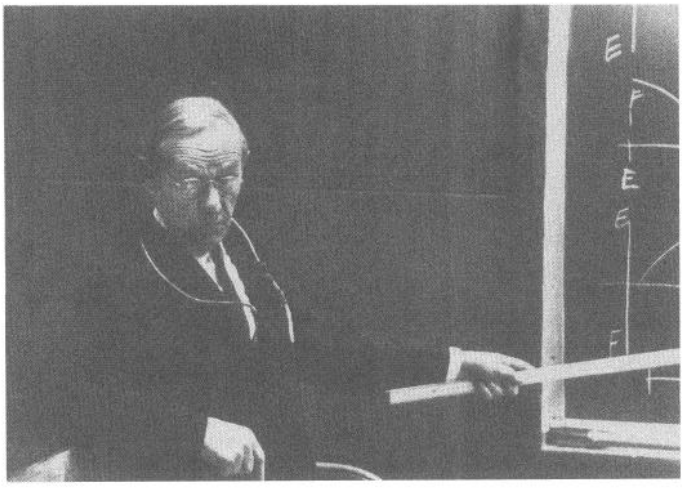
By 1946 the Department of Vital Economics (3) had fused with Physiology, almost doubling the Physiology teaching time available and the number of instructors. New topics were introduced from the experiences of wartime research (e.g., effects of altitude and of temperature stresses). Numerical problems were designed for student solution. Each student developed, as in earlier years, a library project of his choice. Faculty efforts continued to prevent the instruction from being stereotyped.

By 1956 the physiology course was divided into six independent units, some given in spring and others in autumn periods. Each period lasted four weeks and was attended by half the students, then repeated for the other half. Each instuctor and assistant thus taught for eight weeks. Even the lectures were repeated, becoming less formal. Laboratory experiments were emphatically the core of instruction, being somewhat more sophisticated.

By 1966 the instruction was radically revised, as planned by Lotspeich. Where possible, all students worked the same experiment on a given day. Topics were sequentially arranged. Group conferences were intensified, being designed to build student learning by means of topics in-depth. Neurophysiology had been transferred to a course in neurosciences in which several faculty physiologists participated.

What differences between the student experience in physiology in 1926 and that in 1966 now impress one? The teachers were less formal and more enthusiastic in 1926; each was present throughout the course. The logic of experiment was easier to follow when focused on a one-day problem. The student felt called upon to solve procedures and to reach conclusions alone, being one of only 30 individuals in the class and treated personally. By contrast, in 1966 the student knew more of the textbook. Students learned to correlate the multiple phenomena of hypoxia or the many factors in development of edema. Written examinations required more connected reasoning. Lock-step made learning and subject matter more alike for all, instead of bringing diverse insights to diverse individuals.

Fenn employed an efficient method of securing prompt grades on student examination papers. He invited staff members to work for an afternoon at the committee-room table. There each read and graded the answers to the question for which he was responsible; then he passed each paper to other staff members. Thus comraderie, promptitude, and occasional amusement over a students's misunderstanding accomplished what in separation would have been a drawn-out task. On one



Wallace Fenn, 1965.

[3] In the years after 1936, modest funds became available in the Department, which were used principally for the support of selected postdoctoral physiologists. These were promising persons who, in the depths of the financial "depression" (1930-1940), were turned loose from other universities with new degrees and no jobs.

occasion Fenn planted a fictitious examination paper with a worthless answer written in learned terminology. The faculty reader assigned a low grade, but not a zero, much to the enjoyment of the graders' circle.

When medical graduates speak of their days as first-year students, they most often recall practical aspects of laboratory work. They have indelible memories of the "smoked drum." They were impressed by being subjects of their own experiments, e.g., muscular exercise in hot room or breathing 7% carbon dioxide. They do not forget their observations of the exposed turtle heart, or the decerebrate cat, or the mouse in insulin shock. Drama was wherever they discovered it.

For faculty members and assistants, teaching was the core activity. Graduate students and postdocs from foreign and nearby lands contributed to the medical students' horizon. All saw education as a special mission of the Department.

In the 35 classes from 1926 to 1959, some 1,900 medical students and at least 140 graduate students in various biomedical sciences were educated in the annual physiology course. Over 100 instructors were present on various occasions in laboratory and conference sessions. From 1960 to 1969, the 10 classes consisted of 670 medical students, in contact with about 110 different instructors.

Instruction by means of additional departmental courses extended to undergraduate college students, to graduate students, and to student nurses. In each case, as planned usually by Adolph, laboratory experiments were emphasized. In the undergraduate courses the graduate-student assistants had more responsibilities and more varied experiences. By 1945, seminar-type courses were offered to graduate students, one each semester—aviation physiology, body fluids, endocrinology, neurophysiology, and other topics were explored.

Graduate students now reported the results of their thesis research in the weekly departmental seminar. Members of the faculty participated in the oral examinations of M.S. and Ph.D. candidates, not only candidates in physiology but also those in other branches of learning.

Teaching included invited visits of our faculty members to other institutions of learning. The medical school in Lagos (Nigeria) entered into an agreement with the school in Rochester for faculty exchanges, beginning in 1962. Two physiologists came from Lagos for study and research in Rochester during suitable periods. Four persons from the Department of Physiology in Rochester spent up to one year in residence in Lagos.

Service visits abroad were made, chiefly in the interests of teaching, as follows: India (Nasset, Adolph), Libya (Nasset), Lebanon (Nasset), Mexico (Cohen, Adolph), Japan (Honig), Norway (Craig), Philippines (Nasset), and Peru (Rahn). Department members also served briefly as visiting professors in many departments of physiology in the United States and Canada.

The enormous effort that goes into teaching is often underestimated in annual reports and other accounts of faculty activities. In part this is because much teaching is taken for granted. Also, the teaching is mostly done by schedule and on the home grounds. However, teaching by word and by example is, for some, the chief life of the faculty mind; its fervor often is no less than that of research.

Research

From the founding of the Department onward, it was taken for granted that everyone wanted to engage in research. In the early years no faculty members expected to receive specific funds to pay research expenses. The Department, however, was able to provide equipment and supplies for a majority of laboratory investigations. For most projects, specialized apparatus was not needed and not available. However, items used in teaching could be employed for researches during off seasons. "Make-do" was a virtue to be cultivated.[4]

Over the decades, specialized items came into use: galvanometers, stroboscopes, microscopes, and spectroscopes, then flame photometers, gas analysers, oscilloscopes, and isotope counters, and finally ink-writers and small computers. From 1942 research grants were available for all projects.

Topics of research that occupied faculty members of the Department varied widely, and each new member was encouraged to develop his own interests. Graduate students, medical student fellows, and postdoctoral individuals usually elected work related to ongoing pursuits of professors, since the initiates wished to retain the interest of their seniors in what they did. Also, equipment and methods were more available for related projects. When human subjects were required, team work resulted, but even then each worker was an investigator. During World War II, certain "conscientious objectors" served as subjects and observers in ongoing researches.

Some scientists regard research and teaching as two activities that compete for their time and effort. Only in small part has this been true here; instead, the two seemed to enhance one another. Fenn declared that "it is in the preparation of lectures that most ideas for creative work are born" (4). Moreover, concentration of anyone's teaching into a period of one to three months per year was usual and hardly allowed him to begrudge the effort expected in teaching.

Collaborations in research with faculty members in other departments developed in the early years. Medicine (exercise, muscle tonus), pediatrics (insensible losses), and microbiology (bacterial growth) furnished interdepartmental activities.

Services for hospital patients were developed, particularly in electroencephalography.

Other research projects are discussed next in connection with various faculty members. More complete accounts of research can be found in the published papers available in the bound collected publications of the Department (7).

Early Faculty Members

Brief notes on several faculty members are here given. For Fenn a mention of his chief research will suggest

[4] In the year 1954 only 41% of the Department's expenses came from the regular budget of the University; the remainder originated in 19 grants and contracts received within the Department. In 1958 there were 14 active grants and contracts; 29 of the 39 full-time members of the Department were mainly supported by funds from those sources. Ever since 1942 the number of Department members was partly determined by the extent of such funds. During the ensuing years the proposals for research that originated here were thus funded with exceptional regularity.

how he came to be considered outstanding among physiologists of the United States and of the world (5–7). Before he came to Rochester, Fenn completed highly original studies of phagocytosis by white blood cells and energy liberated in contractions of muscles. In Rochester he measured respiration in isolated nerve during stimulation, tension in human muscles. potassium and related electrolytes in muscles and other tissues, mechanics of breathing, and effects of high compression in several tissues. Each of these topics of research in Rochester occupied many years and was shared by collaborators. The mechanics of breathing has been summarized in some detail in a published history (8).

Faculty members were led to discuss unorthodox topics. At one stage they met periodically as "biotheorists," speculating about physical bases of life processes. At another stage they met with other scientists to learn about the basic assumptions being developed in their fields. One aim was to bring to light various philosophies about nature that go unrecognized in daily work.

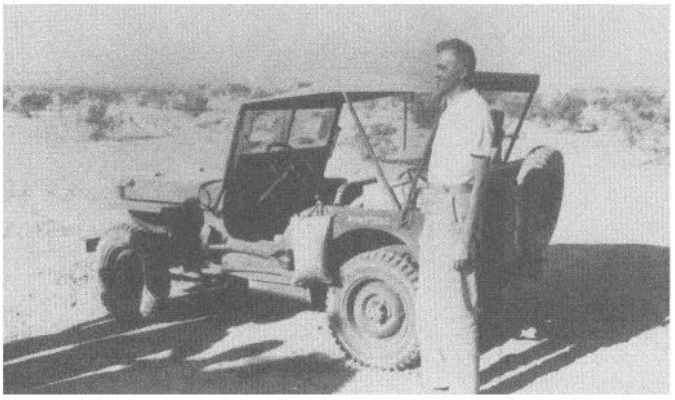
A former graduate student wrote, "Dr. Fenn impressed me with his ability to accomplish much and to concentrate completely on the subject of the moment. When I would discuss our research with him, at the appointed time he would turn aside from what he was doing, giving me his undivided attention. After our discussion, he would return to what he had been doing before I left the room."

The departmental atmosphere was one of self-help. Eleonore Ohr (later Associate Professor of Physiology in Buffalo) recalls the following incident of graduate student days. "Tomo Asano was typing a paper very slowly with the hunt-and-peck system. In sympathy I offered to type his paper for him. His response was: 'No, Dr. Fenn type, therefore Tomo type'."

Fenn indicated his judgments without "throwing his weight around." On the morning after a certain Ph.D. student passed his final examination, Fenn expressed the opinion that both the thesis and the experience of the student had been skimpy. The opinion was not pressed, but subsequent performances of graduate students were certainly scrutinized more carefully. His reticence in exercise of authority sharpened others' cooperation instead of emphasizing differences of judgment.

Fenn retired from the chairmanship of the Department in 1959, continuing as a Distinguished University Professor for 12 years.[5]

Adolph, before coming to Rochester, had inquired how frogs, earthworms, and protozoa could live in freshwater, a medium of low osmotic pressure, without endless swelling of the body. In new studies, both intake of water through skin and excretion through kidneys or



Edward Adolph, 1943.

vacuoles showed special controls over water movements. After any disturbance, the body's water content returned to the norm.

"Adolph recognized early in his researches the significance of regulatory controls," wrote M. J. Fregly and M. S. Fregly (9). "His book on *Physiological Regulations* of 1943 has become a classic in the field of regulatory physiology, as are his publications on the role of water in living organisms, physiological regulation of body fluids, body size, and body temperature. He has also published studies on development of regulations and adaptations in animals, self-regulation of heartbeats, and other characteristics." (9, 10).

Adolph officially retired in 1960, continuing his laboratory research for 15 more years. Thereafter he continued to write for publication.

Alfred M. Wedd was a member of the Physiology Department for 35 years, beginning in 1931. He practiced cardiology off campus for half of every day, appearing in the laboratory on campus in the remaining half-day with great regularity. He wanted to know what such drugs as digitalis did to excitability and contractility of hearts. He exposed turtle ventricles, recording mechanical and electrical contractions. He put great weight on the suggestions of others, especially Blair. However, he relied on his own suggestions in the area of pharmacology. His teaching was in lecture rooms, where he attracted intense student interest at his appearances during the courses both in physiology and pharmacology. He took a personal interest in the health of colleagues, always without intrusion (10).

Alfred M. Wedd, 1959.

[5] A major threat to the stability of this Department occurred in 1936. Fenn was offered the chairmanship of the Department of Physiology at Columbia University. He characterized that job as being "twice as large in every respect." He soon decided to stay in Rochester but went through the generous formality of asking for comments of colleagues. They agreed that his future here was unusually bright. For instance, all knew that the visions of the School's objectives and prospects were amazingly identical between Dean Whipple and Chairman Fenn. After his decision to stay here had been made, the University Trustees tendered a dinner in Fenn's honor. A silver bowl was inscribed for "the event that did not happen."

Wallace Fenn and Henry A. Blair.

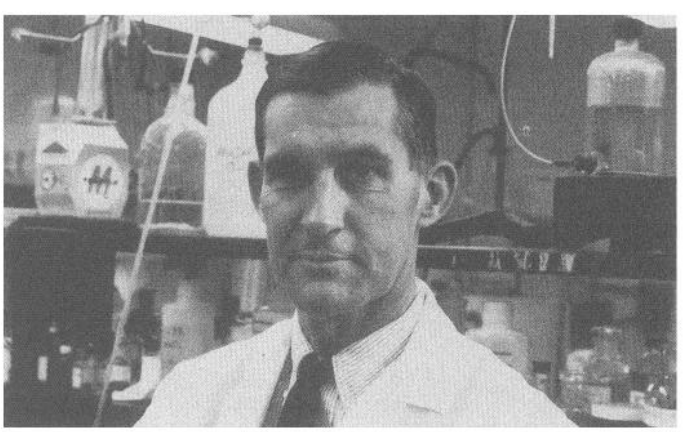
Melvin Fregly (now Professor of Physiology in the University of Florida) witnessed a quick Wedd encounter. Rebeca Gerschman walked down the corridor with some foil-wrapped candies in hand. She met Wedd and said, "Doctor Wedd, would you like a kiss?" Wedd quickly shifted his gaze upward and remarked, "That ceiling certainly needs repainting!"

Henry A. Blair joined the Department of Physiology in 1932. His background was in physics and biophysics. He taught a full share of the physiology course for medical students, emphasizing sensory phenomena and their analysis. In his research he measured electrical and mechanical excitabilities of isolated nerve and muscle, building new millisecond timing equipment for the purpose. He deduced the energies required for starting of a nerve impulse, which led to new understanding of membrane processes and of impulse conduction (12, 13). He collaborated with Wedd in the studies of excitability and conduction in turtle heart.

During the war Blair measured the mechanical effects of intestinal gases and methods of controlling their volumes during atmospheric decompression.

In 1948 Blair became Director of the "Atomic Energy Project" in the medical faculty and Chairman of the Department of Radiology Biology(14). He retired from the responsibilities in 1965 and died in 1971. For 17 years before retirement he continued to be a part-time member of the Physiology Department but was unable to share actively in its research or teaching.

In any scientific discussion, Blair usually created a hypothesis or a suggestion that he outlined clearly. At termination he warned, "But maybe not." These words left the door open to other ideas than those of this modest man.



William D. Lotspeich, ca. 1960.

Lotspeich came to Rochester in 1959 from the Chairmanship of Physiology at the University of Cincinnati. He had developed research interest in renal excretion and metabolism, which he enlarged at Rochester with the assistance of postdoctoral workers. He was especially known for his research on regulation of citrate metabolism and extracellular pH in kidneys. In 1959 he published a monograph on "Metabolic Aspects of Renal Function" (15). Subsequent studies included the effects of potassium deficit and renal hypertrophy.

Lotspeich devoted much energy and time to the teaching of medical students. conducting regular conferences and laboratory explorations with them (16, 17). He especially helped students to understand biochemical principles as applied to physiological problems; in this statement I quote colleague Julius Cohen (18).

Lotspeich had a background of interest in arts, literature, and religion. He and his family participated in domestic and overseas programs of the American Friends Service Committee. He resigned from the faculty of the University of Rochester in 1967 to become Executive Secretary of that committee. However, he was found to have a chronic disease, which ended his life in 1968.

Lotspeich was a concerned individual. One day he and I were walking downstairs to the medical library in earnest conversation. As we issued from the stairs into the hospital's outpatient area, Lotspeich suddenly saw a woman in distress. Without losing a step he went to her, supported her from falling, and placed her in a chair. Then he turned to the nurse in charge and secured her attention to the patient. It seemed entirely natural for him to aid an individual in a moment of need.

Other researcher-teachers in this Department should be characterized in the near future by their surviving colleagues. All contributed heavily to the sum of scientific efforts. This chapter is designed to end with 1969, when Paul Horowicz became the Department's third chairman.

Since it is not possible to record here the many activities that characterized this Department, I have written a more detailed supplement for the benefit of those who were intimate in the Department before 1969. The document includes lists of faculty, doctorates, fellows, publications, meetings, social activities, grants, honors, and memberships. It would take a book to record details about them all. Those persons who worked here substantiate the belief that this department was unique in its achievements.

The manuscript and footnotes from the supplement are in the archives of the Edward G. Miner Library, University of Rochester School of Medicine and Dentistry, FC 3, Box 60, Folder 28 (supplement, Folder 30). The photographs are courtesy of the Edward G. Miner Library. Many thanks to Christopher Hoolihan, History of Medicine Librarian, for his assistance in preparing this manuscript.

References

1. *Methods and Problems,* New York: Rockefeller Foundation. 1927, Vol. 7, p. 29–35.

2. *The First Decade.* U.R. School of Medicine and Dentistry. 1935, p. 54–59.
3. *The Quarter Century.* U.R. School of Medicine and Dentisty. 1950, p. 53–60.
4. Report to Alumni. By W. O. Fenn. 1957.
5. Rahn, H. Biographical memoir of W. O. Fenn. *National Academy of Sciences, Biographical Memoirs* 50: 141–173, 1979.
6. In memoriam, W.O. Fenn. 1971. (typed).
7. Collected Publications in Physiology. 14 volumes (library).
8. Otis, A. B., and H. Rahn. Development of concepts in Rochester. In: *Pulmonary Gas Exchanges.* New York: Academic, 1980, p. 33–66.
9. Fregly, M. J., and M. S. Fregly. Edward F. Adolph. *The Physiologist* 25: 1, 1982.
10. Adolph, E. F. *Physiological Regulations.* 1943 (library).
11. In memory of Alfred M. Wedd, 1887-1967 (typed).
12. Blair, H. A. Curriculum vitae and list of publications. 1965 (typed).
13. Memorial service for Blair, and remarks of W. F. Neuman. 1971 (typed).
14. Blair, H. A. *Brief History of the U.R.-A.E.P., 1943–68.*
15. Lotspeich, W. D. *Metabolic Aspects of Renal Function.* 1959, (library).
16. Lotspeich, W. D. Thoughts on teaching. 1963 (typed).
17. Lotspeich, W. D. Proposal for training grant. 1959 (typed).
18. Cohen, J. J. W. D. Lotspeich. 1983 (typed).

Orr E. Reynolds Award

The Orr E. Reynolds Award is given annually by the American Physiological Society for the best historical article submitted by a member of the Society.

Articles may deal with any aspect of the history of physiology including the development of physiological ideas and their application, instrumentation, individual and collective biography, departmental and institutional history, history of societies including APS, and physiology in its public context. Manuscripts submitted for the award should represent original research and be adequately documented. Articles published in APS journals or books during the prior calendar year are also eligible for the award upon request by the author(s). The award is open to all classes of APS membership except for those members who have advanced degrees in the history of science and medicine. A member may receive the award only once.

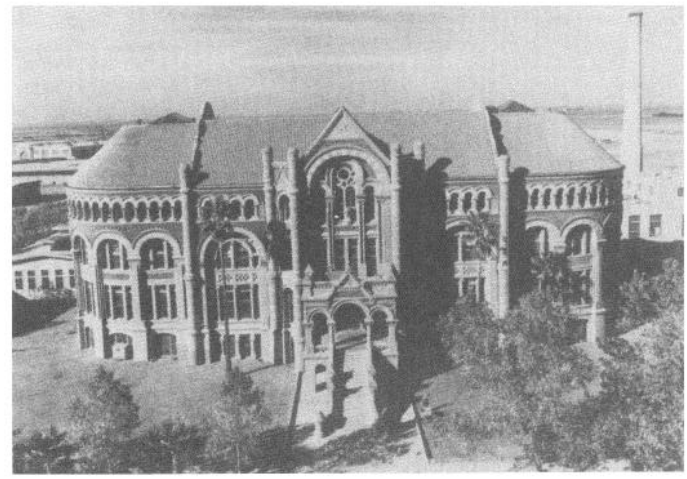
The awardee will receive $500 plus expenses to attend the APS Spring Meeting. If the awardee wishes, and there is a suitable place on the program, an oral presentation will be made at the Spring or subsequent Fall meeting at the beginning of an appropriate scientific session. It is hoped that, after appropriate peer review, the article will be published in one of the APS journals.

Manuscripts will be evaluated by a committee consisting of three members of APS appointed annually by Council in consultation with the Chairman of the Section of the History of Physiology. At least one of the members will be a professional historian.

Manuscripts should be typed and double-spaced with wide margins on 8½ × 11 paper and should conform to the style used in APS journals. (Instructions will be sent on request.) Three copies should be submitted for use of the review committee. To be considered for the 1989 award, manuscripts should be sent to Orr E. Reynolds Award, American Physiological Society, 9650 Rockville Pike, Bethesda, MD 20814, by December 1, 1988. The recipient of the award will be announced at the 1989 Spring Meeting.

History of Department of Physiology and Biophysics, University of Texas Medical Branch at Galveston

M. MASON GUEST
Shriners Burns Institute
Galveston Unit
Galveston, Texas 77550



Ashbel Smith Building (the Red Building).

Early History

In 1881 the Texas Legislature brought to fulfillment a prime objective of the Republic of Texas. This objective, to establish a state university, was consummated 45 years after Texas won its independence from Mexico and 36 years after it became the 28th state. The University of Texas was conceived as an academic institution with a medical department or branch. The medical department was to be located either in the community selected as the site of the main campus or in another city. The decision regarding the locations of the main university and the medical department was decided by popular vote. The citizens of Texas selected Austin, the state capital, as the site of the main university and they favored Galveston over Houston for the location of the Medical Department (1).

Galveston, with a population of about 23,000, was at the time of the university location vote the largest and most prosperous city in Texas. It had been the site of two or more medical schools. One of these, the Galveston Medical College, was the Medical Department of Soule University. Soule University's main campus was in Chappell Hill, Texas. The Medical Department of Soule University opened in Galveston on October 23, 1865, less than five months after the surrender of the Confederate Trans-Mississippi Forces in Galveston. The Galveston Medical College was terminated when Soule University moved to Louisiana in 1873. Coincident with closing of the Galveston Medical College, a charter for the Texas Medical College and Hospital was obtained from the state (March 1873). Ashbel Smith, the famed Texas physician, military leader, and politician, was elected President of the Board of Trustees (2).

When the state University was established in 1881, the Texas Medical College and Hospital closed its doors to permit the Medical Department of the University of Texas to begin operation. However, no financial support was forthcoming from the state until 1890, when construction of a Medical School building began. In the meantime (1888), to fill the gap, the Texas Medical College and Hospital was reorganized. However, it again disbanded when the Medical Department of the University of Texas opened in 1891.

The faculties of both the Galveston Medical College and the Texas Medical College and Hospital included a member with the title of Professor of Physiology. Instruction in physiology was given in the third year of the medical course in both schools (3); apparently it was presented entirely by lecture.

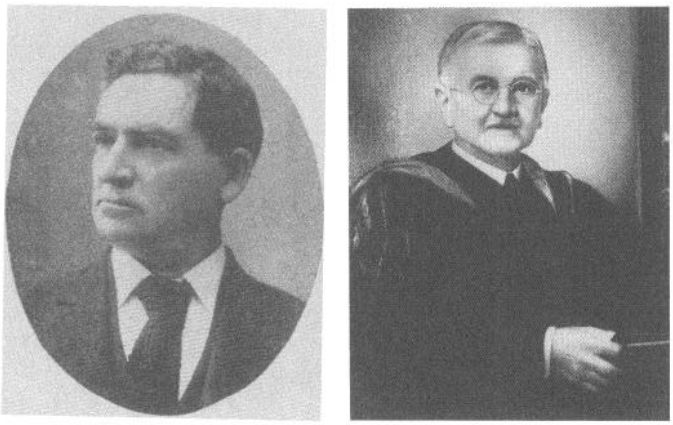
The initial Medical School building of the University of Texas Medical Department, designed by the famed architect Nicholas Clayton, was completed in 1891. For a number of years the strikingly conspicuous red brick structure, architecturally romanesque in design, housed the entire medical school. With the advent of other Medical School buildings it became known as the Red Building or Old Red. Later it was officially named the Ashbel Smith Building, thus honoring Dr. Ashbel Smith, the first Chairman of the Board of Regents of the University of Texas. The University is a monument to Ashbel Smith's tenacious campaign to foster public education in the State of Texas. Unfortunately though, Smith did not live to attend the dedication of the building that would later be named after him.

The laboratories and offices of the Department of Physiology continued to be located in the Ashbel Smith Building until 1953, when they were moved to the newly completed Gail Borden Building. In 1971 the Physiology Department was again relocated in the Libby Moody Thompson Basic Science Building.

In the 96 years the University of Texas Medical Branch has been in existence (1891–1987) there have been eight heads or chairmen of the Department of Physiology and approximately 70 academic staff members. During this period the Medical School graduates (physicians) have numbered 8,454. Since 1952, 51 master's degrees and 33 doctor of philosophy degrees in physiology have been awarded. The Department has also been involved from time to time in teaching physiology to student nurses and allied health science students.

Clopton, First Professor of Physiology

The original faculty of the University of Texas Medical Department, eight in number, included Albert G. Clopton, M.D., Professor of Physiology and Hygiene. Clopton, a native of Georgia, had served in the United States



Left: Arthur G. Clopton, first Head of Department of Physiology, 1891–1898; *right:* William Spencer Carter, second head of Department of Physiology, 1898–1922.

Army under General Zachary Taylor during the Mexican War. After the war Clopton obtained his M.D. degree (1852) at Louisiana Medical School (now Tulane). He then practiced medicine in several different communities in Arkansas and Texas, but in keeping with his ardent secessionist advocacy, when the war between the states began, he organized and originally commanded Company D of the First Texas Infantry Regiment in Hood's Brigade. On January 2, 1962, he was promoted to major and transferred to the Confederate Medical Department in Richmond, Virginia (7).

During Dr. Clopton's seven-year tenure as the Professor of Physiology he developed a reputation as a master of flowery oratory. In discussing an item of physiological information he was reported to have said, "At the foot of this great truth dead theories lie as thickly strewn as dead soldiers at the feet of a Grecian war god after battle." He also, the students claimed, graded examination papers according to their weight measured on a postal scale. One student stated that he had proven this by submitting instead of an answer to an examination question a voluminous description of the jetties, then under construction along Galveston's water front, and he had received a grade of 95 (4).

William Spencer Carter

The second head of the Department of Physiology at the University of Texas Medical Department was William Spencer Carter, M.D. At the time Dr. Carter was named Professor of Physiology and Hygiene he was a 28-year-old medical graduate of the University of Pennsylvania. He arrived in Galveston with his wife the year after the 1897 outbreak of yellow fever.

Before coming to Galveston Carter had served an internship in the Philadelphia hospitals and after his internship had been appointed to the faculty of the University of Pennsylvania as Demonstrator of Pathology and Assistant Professor of Physiology. Two years after receiving the M.D. degree Carter was awarded the Boylston Prize for his investigation of leucocytosis (5).

After his arrival in Galveston Carter organized one of the first physiology laboratories in the South for the teaching of medical students. At the completion of his tenure as head of the Department of Physiology he wrote a description of some features of physiology laboratory teaching at the University of Texas Medical Branch. The description was entitled, "The Physiology Department from 1898 to 1922" (8). Excerpts from his brief treatise follow.

> The space allotted to the department of physiology in the old original building of the Medical School of the University of Texas consisted of three rooms adjacent to the lower lecture-room on the first floor. The room on the north side of the hall was the only space available for a teaching laboratory. It will be well remembered by the students who received instruction there from October 1898 to February 1922, the period during which the writer was in charge of the department.
>
> When the new Laboratory Building on the south side of Strand [Street] was opened, the benches of the old lower lecture-room were torn out and the entire rounded west end of the first floor, together with corresponding space on the ground floor, was made available for a teaching laboratory.
>
> This made it possible for the first time for the entire class to work in laboratory exercises at the same time. That is still possible even though the size of the classes has been increased to one hundred members.
>
> The equipment of the laboratory of physiology in 1898 was very meager indeed and totally inadequate for giving systematic laboratory instructions. It consisted of a single kymographion with two small drums, which together with a few minor pieces of physiological apparatus, such as levers, tambours, etc. had been imported from Cambridge, England. The small drums could not be used for class demonstration as tracings could only be made for short periods of time. The frequent smoking and changing of drums would make effective teaching impossible.
>
> One of the first things to be done was to make drums carrying long stretches of glazed paper, so that tracings could be made over considerable periods of time. It was also necessary to provide driving power for moving the drums at different speeds. This was done by means of conical pulleys driven by the motor in the machine shop. Fortunately the machine shop was located in the basement directly under the laboratory. It was also necessary to arrange for recording the time in seconds on the tracings by means of an electro-magnet.
>
> Gradually improvised apparatus, which was made in the college machine shop, increased the amount of equipment so that laboratory demonstrations were carried on systematically during the first two or three years.
>
> Two courses of instruction were given: (1) a course for the freshman class consisting of two lectures and two laboratory exercises per week during the second half of the first year; and (2) the sophomore course consisting of five lectures and five laboratory periods of two hours each during the first semester of the second year.
>
> The first course included the properties of different foodstuffs, methods of separating them, and the changes which they undergo in the different digestive processes; also the physiology of blood. The laboratory exercises for this course were given for the most part in the laboratory of chemistry by providing a few additional reagent bottles on the chemical desks. The

entire class could work there at the same time. When the use of microscopes was necessary, as in the study of blood, the class was taken in sections to the laboratory of histology.

The sophomore course covered the remainder of the field of physiology. The limited size of the teaching laboratory made it necessary to divide the class into two sections as more than one-half of the class could not be accommodated in the laboratory at one time.

During the first few years of the period under consideration (1898 to 1922) all laboratory exercises in physiology for the sophomore class consisted of demonstrations. This was made necessary by the limited equipment and laboratory facilities. Gradually the amount of equipment was increased by purchases from the Harvard Apparatus Company, as far as the limited appropriations would permit each year. Much of the apparatus was made by the mechanic of the college, Mr. Michael Little. In these ways sufficient equipment was finally accumulated so that the individual students could themselves perform experiments and not merely observe others do so.

Beginning about 1902 we were able to provide thirteen work tables equipped with sufficient apparatus for the individual students to carry on their own experiments. Two students worked together at each table. They were able to make graphic records of such observations as could be made on each other, such as pulse tracing, respiration, etc. They also carried out experiments on frogs and turtles making graphic records when that was possible. In this way they become familiar with experimental methods and acquired manual dexterity.

Experiments on mammals continued to be conducted in the form of demonstrations in which the students assisted in rotation. The work in the laboratory was correlated as closely as possible with the didactic teaching by means of frequent quizzes.

The development of the laboratory exercises in which the individual students working in pairs performed experiments themselves was made possible by the enthusiastic co-operation and valuable assistance given by the late Dr. Oscar H. Plant. He became interested in experimental physiology during his undergraduate course and for two sessions acted as student assistant. After graduation he continued as full-time instructor for several years until he went to the University of Pennsylvania as assistant professor of pharmacology. He went from there to the University of Iowa as professor and head of the department of pharmacology and continued in that position until the time of his untimely death.

It has always been a matter of great pride and satisfaction to the writer that Dr. Plant laid the foundations for his professional career in the laboratory of physiology of the University of Texas.

Hurricane

Two years after Dr. Carter arrived in Galveston the 1900 hurricane (September 8) devastated the city and greatly damaged the Medical School building (the Red Building) and the hospital. It completely destroyed the nurses' residence, but fortunately when the building collapsed all of the nurses were in the hospital. The storm caused the greatest loss of human life of any single natural disaster during the history of this country. More than 6,000 people were reported to have been drowned or otherwise destroyed. Galveston, a barrier island, and at that time not protected by a seawall, was defenseless against the powerful winds and the even more devastating high water. Furthermore, the lack of (or indifference to) advance warnings precluded preparation for the storm or significant evacuation.

At the time of the storm Carter and most of the other members of the faculty were away from the city on vacation. Perhaps because the Medical School was not in session, no faculty member or member of the student body lost his or her life.

Alan J. Smith, M.D., Professor of Pathology, was in Galveston preparing for his vacation when the hurricane struck the city. He surveyed the damage after the storm and reported his observations in the University Record of March 1901 (9). Excerpts from his description of the damage to the Medical Department building (the Red Building) follow.

> The walls of the college building stood, but much of its adventurous architecture was gone; the great dome was down and a huge gap in the middle part of the roof and front wall marked where formerly it had stood. The ornaments of the side roofs, the cornices and gutters, the slates from nearly the whole broad covering of the building, the minaret-like caps of the ornamental pillars of the structure—all were gone or wrecked. . . . To one climbing over this rubbish and wading into the basement, one great jumble and tangle of chemical tables, gas and water pipes, bottles and apparatus impeded the passage, in some places piled high, in others in low heaps of broken lumber. The floor of the west end, where the concrete pavement did not extend, rose in hills as high as the waist and partitions and tables were thrown into an indescribable mess. . . .
>
> In the library, several of the alcoves were penetrated by the water and the books soaked; and these not discovered for several days, were soon covered by a luxuriant growth of mould and only saved in their harmed state from total ruin by being at once taken out, cleaned and dried. . . .
>
> Looking out of the north doors (of the Red Building) the dead house could be seen unroofed and swept clean of its contents and here and there among the rubbish its uncanny inhabitants, intended for the dissecting room, were found, unfit for use when opportunity came for their recovery and storage. The great smokestack, one hundred and twenty feet high originally, was broken off at about the middle of the first floor and in its fall it had utterly destroyed the roof of the boiler room and crashed in masses upon the remaining boiler and could be seen as piles of rubbish lying with (in) the enclosure. . . .

In *A History of Galveston, Texas* by C.S. Griffin, some of the destructive effects of the storm on the city are described (10).

> Fully 3,600 buildings were completely destroyed and in the area from Avenue P to the Gulf, the entire

length of the city, no one house was found on its original location. Many were completely demolished, no vestige remaining by which to identify them. In the extreme western end of the city, the force of the water had swept entirely across the island, leaving nothing but death and desolation in its path. Because of the indescribable conditions existing afterwards, no actual count of the dead was ever made. The lowest estimate places the number at 5,000. The weather bureau in its report dated Sept. 23rd states that 3,536 bodies had been identified, with possibly 1,500 more disposed of without identification, such procedure being imperative. The Galveston News in its issue of October 7, 1900, just one month afterwards, listed 4,236 identified dead.

Although before the storm the support from the state for the Medical Department had been relatively penurious, after the storm the Board of Reagents became more generous and legislative appropriations were increased. Money for rebuilding the Red Building and for the replacement of equipment was almost immediately appropriated. The people of Texas were proud that the Medical Department of their University had survived such a great disaster. Help also came from all over America. Doctors and nurses, including the famed Clara Barton, came to the stricken city to assist with its sudden plethora of medical problems.

Medical school classes were resumed by order of the Board of Regents on November 15, 1900, and the 1900–1901 session terminated on June 29, 1901, just one month late (6).

Carter, Dean of Medicine

In 1903 Dr. Carter was made the Dean of the Medical Department. He also continued as head of the Department of Physiology. Throughout almost all of the 24 years Carter served as Head of Physiology he taught the freshman and sophomore physiology courses, lectures and laboratories, with usually only one associate faculty member to assist him. He also organized (1899) and taught (1899–1904) a course in hygiene involving aspects of public health, biostatistics, sanitation, and epidemiology. When this course was transferred to the Department of Bacteriology in 1904 Carter introduced a course in pharmacodynamics, which he taught for several years.

Carter was reported to have been one of the first to point out the danger of drinking raw milk, and he vigorously advocated the pasteurization of milk. He also worked for the establishment of sanitary water supplies in communities. Although Dr. Carter's bibliography was not voluminous, his research publications provided important information. Among his published studies were the pathologic effects of the various species of amanita (mushrooms), the changes in cerebrospinal fluid pressure with withdrawal of the fluid and with intraspinal injections, the use of citrated blood in transfusion, and the functional features of renal epithelium.

In 1917 Carter was elected and served as President of the Association of American Medical Colleges. During his tenure as Dean the name of the Medical Department was officially changed in 1919 to the University of Texas Medical Branch.

Dr. Carter's stint as Head of Physiology ended in 1922 when he took a leave of absence and became Associate Director of the Medical Sciences Division of the Rockefeller Foundation. After serving in the Philippines, Australia, South Africa, Java, New Zealand, China, and India, Carter returned to Galveston in 1935 to resume the position of Dean. He retired in 1938 and moved to Auburndale, Massachusetts.

The third head of the Department of Physiology was Charles Cullom Gault, M.D. Gault had held an appointment as Assistant Professor of Physiology at the University of Minnesota before coming to Galveston. He served for only two years (academic years 1922–1923 and 1923–1924).

Porter Named Chairman in 1924

Eugene Lyman Porter, Ph.D., was appointed head of the Department of Physiology in 1924. He and his family arrived in Galveston in November and had their first Galveston Thanksgiving Day dinner on the Gulf shore beach.

Porter, a native of western Massachusetts (Springfield), obtained his baccalaureate degree from Harvard University, his master's degree from the University of Michigan, and his Ph.D. degree from Harvard University. He had successive appointments on the Physiology Department staffs of the University of Pennsylvania Medical School and Western Reserve Medical School before coming to Galveston.

Porter's primary interest in physiology was the nervous system. Most of his research was in this field and his carefully planned classical demonstrations of neural phenomena to the student body were colorfully instructive. In addition to his demonstrations of decorticate, middle ear disrupted and decerebellate pigeons, he skillfully presented before the student body mammals with various nervous system lesions and human patients with neurological disorders. His presentations of George Prater, a quadriplegic who had suffered a spinal transection in an automobile accident, were lessons, not only in the functional features of the nervous system and the neural control and mechanics of respiration, but also a demonstration of the art of showmanship in teaching.

Porter's tenure as Head of the Department appears to have been reasonably tranquil until 1939. Understandably, there were problems before 1939, such as lack of

Eugene Lyman Porter, third head of Department of Physiology, 1924–1941, 1943–1948.

funds for equipment and for staff salaries plus difficulties in teaching the laboratory portion of the course because of space and scheduling inadequacies. The alloted space for the physiology student teaching laboratory was divided between the first floor of the Red Building and its basement. With usually not more than two assistants and the class divided into a first floor and basement contingent, coordination of the teaching was difficult.

The Spies Interval

January 1939 to August 1942 was a tempestuous period at the University of Texas; the storm centered at the Medical Branch. John W. Spies, M.D., a native Texan and brother of the famed Tom Spies,[1] was appointed Dean of the Medical School by the Board of Regents; he arrived at the Medical Branch as its chief administrator in January 1939. Spies, a University of Texas alumnus and a medical graduate of Harvard University, came to Galveston from the position of Director of Tata Memorial Hospital in Bombay, India. He was an advocate of strong central authority in the operation of a medical center and in this philosophy he was encouraged and supported by the President of the University and the Board of Regents. On the other hand, the faculty had become accustomed, with Dr. Carter as the Dean, to a democratic format in the operation of the Medical School. Under Spies, faculty meetings, which had a bearing on the academic and administrative processes, were dispensed with and the faculty executive committee was chosen by the President of the University rather than elected by the faculty.

As a result of the conflict between Spies and most members of the faculty, the Medical School remained in constant turmoil. In the late winter and spring of 1942 the controversy reached its climax. The Board of Regents held hearings in which members of the faculty and Dean Spies were asked to testify. Throughout the University the issue became highly emotionalized. The President of the University, Dean Spies, and the Chairman of the Board of Regents were all hung in effigy by students on the Austin campus (11).

Porter, who supported the concept of faculty input in the management of the Medical School, during the academic year 1940–1941 had been supplanted as Head of the Department of Physiology by Carl August Nau, M.D. Nau had originally joined the Department of Physiology as an adjunct professor in the fall of 1928 and served in this capacity through the spring term of 1929. He returned to the Medical Branch during the Spies period and became one of the few active supporters of Dean Spies among the faculty. In the interim between his initial sojourn at the Medical Branch and his return he had obtained the M.D. degree from Rush Medical School in Chicago.

An interesting although nefarious episode enveloped the Department of Physiology during the Spies period. A young man who said he was David Walton Fell, B.M., was interviewed in New York City (1940) for a faculty position by Dean Spies. Spies, it was reported by members of the UTMB faculty, recommended Fell's employment without asking for credentials. He was given an appointment as Assistant in Pathology. Later he was advanced to Assistant Professor in Pathology and in Physiology and then to Associate Professor of Physiology.

Porter, who had continued to supervise the student physiology laboratory after the appointment of Nau as Head of the Department, recognizing that Fell was incompetent as a teacher of physiology, sent a memo indicating this to Nau. At about the same time, Howard Swann, who was in Galveston for only a short period before he left to join the Army Air Corps, recognized Fell as a former student at the University of Chicago. Fell denied the connection with the University of Chicago and he continued as a member of the physiology staff until December 24, 1941. It was reported in a medical student publication that a group of students, becoming suspicious of Fell's credentials, asked him during a clinical rounds to examine an artificial eye in a patient. Fell examined the interior of the glass eye with an opthalmascope, describing in detail the nerves and blood vessels of the fundus oculi.

The jig was up for the bogus Dr. David Walton Fell. Through inquiry to the University of Chicago it was discovered that the man claiming to be Fell had been a medical student who was dismissed because of inadequate grades. His real name was found to be Charles Peter Wisotsky and his original home had been in New York City. According to word-of-mouth information from faculty members who were at UTMB then, Wisotsky during the Spanish Civil War had roomed with an English physician, named Fell, who died during the period of the conflict. The real Dr. David Walton Fell had been educated in England and had graduated from the University of London with the bachelor of medicine degree; hence Wisotsky attempted to assume the mannerisms and speech of the English surgeon-physician as well as his medical degree.

Since the Second World War was in progress, Wisotsky was prosecuted by the federal government for false draft registration and for violation of the Harrison narcotic laws. His prison sentence was suspended on the condition he immediately join the armed forces of the United States (12).

In the summer of 1942 Spies was asked to resign and Chauncey D. Leake, Ph.D., a world renown pharmacologist, was appointed as a vice president of the University of Texas and Dean of the Medical School. In January 1943 Dr. Porter was reinstated as the Chairman of the Department of Physiology. (In line with Leake's democratic philosophy the title Head of Department was changed to chairman.)

Porter's Staff

The faculty member who remained for the longest period was Wilbur A. Selle, Ph.D. He had received his doctorate from Stanford University and came to the Medical Branch in 1929 as an associate professor. While at the Medical Branch his major research interest was related to cancer. Selle continued on the physiology staff until 1949 when he became Professor of Biophysics at the University of California School of Medicine, Los Angeles.

[1] Tom Spies, M.D., at about the time of the appointment of John W Spies had been widely recognized by popular communication media for his research on pellagra in people of the southern states with marginal incomes and deficient diets.

During 1925–1926 Associate Professor W.T. Dawson served on the physiology staff. Dawson transferred to the Department of Pharmacology at UTMB where he later became Professor and Chairman.

Francis Joseph Mullin, Ph.D., was a member of the physiology staff from 1936 to 1938 as Assistant Professor. He later became President of Shimer College in Mt. Carroll, Illinois.

W(illiam) Doyne Collings, who had obtained his Ph.D. degree from Princeton University, was a member of the physiology staff from 1942 to 1945 with the rank of Assistant Professor. From Galveston he went to East Lansing, Michigan, where he served as Associate Professor and then Professor of Physiology at Michigan State University.

The Ogden Chairmanship

During the academic year 1948–1949 Eric Ogden, with bachelor of science and medicine degrees from the University of London, replaced Porter as Chairman. Several years later Porter was made Professor Emeritus. He continued to teach until his death after a short illness in 1962.

Dr. Ogden, a Sharpey Scholar at the University of London, had been brought from the University of California School of Medicine, San Francisco, to the Medical Branch in 1943 by Chauncey D. Leake. His fields of research included the physiology of the heart, respiration, hypertension, and the kidney. During his tenure at the Medical Branch, graduate courses in physiology were developed and taught by the physiology staff.

Ogden resigned from the chairmanship of physiology at UTMB at the end of the academic year 1948–1949 to become Chairman of the Department of Physiology at Ohio State University College of Medicine. He later moved to the National Aeronautics and Space Administration's Ames Research Center, Moffat Field, California, where he was Chief of the Division of Environmental Biology.

Among the staff members in physiology during Ogden's chairmanship, in addition to Porter, Selle, Collings, and Swann, were M. Brucer, Charles Eric Hall, Mario Gaudino, and Ruven Greenberg. Brucer, who had obtained the M.D. degree from the University of Chicago, served as Instructor and then Assistant Professor before he moved to Oak Ridge, Tennessee, where he became Chairman of the medical division of the Oak Ridge Center for Nuclear Studies.

Hall, who came to the Medical Branch in 1947, continues as a member of the physiology staff, although as of 1987 on a partly retired basis. He obtained his Ph.D. degree under the supervision of Hans Selye at the University of Montreal. He and his talented wife Octavia, who served for many years as a research associate and then as a research scientist in the Department, have published widely on the endocrine control of the cardiovascular system. In addition to their professional activities in teaching and research the Halls are avid lay ornithologists. They have made an avocation of photographing birds, not only on Galveston Island but also in many other locales throughout the world.

Goudino, with an M.D. degree from the University of Buenos Aires and a Ph.D. degree from New York University, was a member of the departmental staff for two

Left: Eric Ogden, Chairman of Physiology, 1948–1949; *right:* Howard G. Swann, Acting Chairman of Physiology, 1949–1950.

academic years (1948–1950). After leaving Galveston he joined the Ciba-Geigy Pharmaceutical Corporation where he became Director of Medical Compliance.

Greenberg, Ph.D., Ohio State, a neurophysiologist, was a member of the physiology staff from 1949 until the summer of 1952, when he moved to Chicago to join the physiology staff at the University of Illinois College of Medicine.

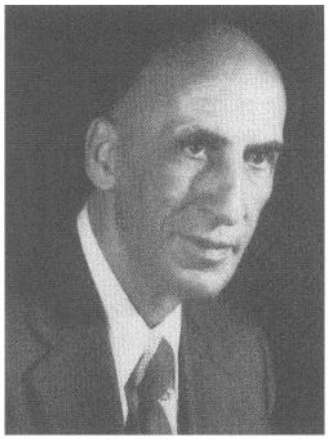
Howard G. Swann, who obtained his baccalaureate degree from Harvard University and his Ph.D. from the University of Chicago, was given an appointment as Assistant Professor of Physiology at UTMB in the spring of 1942. Later in the academic year 1942–1943, as a result of volunteering for military service with the U.S. Army Air Corps as an aviation physiologist, he received orders to report for duty at the School of Aviation Medicine in San Antonio, Texas. He was granted a leave of absence from the Medical Branch and continued on active military duty until the academic year 1945–1946, when he return to Galveston. In the summer of 1949 after Ogden had resigned to take the chairmanship at Ohio State, Swann was named Acting Chairman.

Swann, an effective teacher and researcher, continued to serve as Professor of Physiology until his untimely death in 1971. His research contributions included studies on the function of the adrenal gland, investigation of the mechanisms bringing about death in hypoxia, pathophysiologic changes in freshwater and saltwater drowning, procedures for resuscitation of drowned animals, including humans, and rheological studies of blood. He was a remarkably dynamic and versatile individual.

Guest Appointed Chairman

M. Mason Guest, Ph.D., accepted the appointment as Professor and Chairman of Physiology at UTMB during the fall of 1950, and in January 1951 he moved to Galveston from Detroit, Michigan, where he had been a member of the physiology staff at Wayne State University College of Medicine. Before this he had served in the Army Air Corps as an aviation physiologist. His undergraduate education had been completed at the University of Michigan and his graduate studies at the College of Physicians and Surgeons of Columbia University. His supervisory professor at Columbia was Ernest L. Scott, the scientist who some medical historians consider to be the discoverer of insulin.

The Guest family, Mason, Alice, and their two sons



M. Mason Guest, Chairman of Physiology, 1951–1973.

Avery and John, arrived in Galveston by automobile in late January 1951. The weather, in contrast with the cold and the ice and the snow in Detroit, was warm and sunny; the grass was green and throughout the city flowers were blooming. Then suddenly, about a week later, a "blue norther" hit Galveston. Everything changed. The temperature fell from the high sixties into the teens; exposed water pipes froze throughout the city; freezing rain made the streets almost impassable; it formed a thick coat of ice on windows and windshields. The Guests became popular with their neighbors because of their supply of windshield ice scrapers, which had been given away with the purchase of gasoline at service stations in Detroit.

The rented house in which the Guests resided at that time, like most of the houses in Galveston, did not have a basement. It stood on concrete piers and the cold north wind whistled through the open space between the ground and the floor. The house was heated by unvented gas stoves. Although several windows were left partly open, water vapor from the burning natural gas condensed on the floors and formed a slick coating of ice, even though the air temperature in the rooms was above 70°F. This was the Guests' initiation into life in a semi-tropical city.

Physical Facilities

When Guest arrived in Galveston, the Department of Physiology was still housed in the original Medical School building, the Red Building. Lectures were given in an amphitheater patterned after an amphitheater at the University of Pennsylvania. Student laboratory sessions were conducted in space provided on two different floors as in the later period under Carter. The laboratories had been named the Carter Physiology Laboratory and an engraved copper plate acclaimed this designation. A circular stairway connected the two floor levels.

Much of the equipment had been designed by Carter or Porter and had been crafted by the school's machinists. When Guest arrived the Department employed Carl Maynes, a self-taught machinist. Carl was a talented and resourceful Maine Yankee who had migrated to Galveston to obtain care and treatment for a brain-damaged (cerebral palsy) son. Maynes cleverly constructed many items of equipment that were used both in the student laboratory and in research.

In 1951 the Physiology Department occupied the basement student laboratory on the west end of the Red Building and all of the first floor with the exception of the dean's office on the east end of the building. However, a new building, the Gail Borden Building, which would house the medical school library, pharmacology, physiology, microbiology, and biochemistry, was under construction. In 1953 the Physiology Department moved into the Gail Borden Building.[2] It occupied the second floor of this block-long building.

In 1963 the physiology student laboratory in the Gail Borden Building was remodeled to provide offices and laboratories for the research activities of the departmental faculty. The student physiology laboratory was moved back into renovated space in the Red Building.

Physiology Teaching: 1950s and 1960s

The major teaching responsibility of the Department was instruction of first-year medical students. The class size varied between 120 and 200 students. Instruction in physiology was also given to graduate students and allied health science students. The course in physiology for first year medical students was (and still is) given during the second half of the academic year. In the 1950s and 1960s it consisted of lectures, lecture-demonstrations and clinical correlation presentations (about 80 hours), student conferences in groups of 16 to 20 students (30 hours) and laboratory exercises (about 118 hours). The total contact teaching hours for all courses (clock hours) during the freshman year was reduced from 1,362 to 1,092 for the 1951–1952 academic year and during the succeeding years. The Physiology Department was allotted 224 hours for teaching freshman medical students compared with 270 hours during the 1940s. In each of the years from 1951 to 1973, the physiology teaching faculty consisted of 7–10 members.

Faculty Members in Physiology While Guest Was Chairman

Only individuals with their primary appointments in physiology are listed. Each name is followed by the degree, where it was obtained, and year of appointment at UTMB. If the faculty member left UTMB, the year he resigned and where he went is indicated. Those marked with an asterisk are discussed later.

E. L. Porter, Ph.D., Harvard; 1924; 1962, deceased
Howard G. Swann, Ph.D., Chicago; 1942; 1971, deceased
Charles Eric Hall, Ph.D., Montreal; 1947
Ruven Greenberg, Ph.D., Ohio State; 1949; 1952, Univ. of Illinois, Chicago
Harold Diserens, M.A., Univ. of Texas; 1951; medical practice, San Antonio
Gerald R. Seaman, Ph.D., Williams College; 1951; 1958, Barnard College, Columbia Univ.
Melvin Schadewald, Ph.D., Minnesota; 1952; 1954, deceased
Bruce D. Fallis, M.D., Univ. of Washington; 1952; 1955, medical practice, Plano, TX
Victor C. Calma, M.D., UTMB; 1952; 1959, Memorial Hospital, Corpus Christi, TX
Robert C. Barnett, Ph.D., Chicago; 1955; 1958, New Guinea
Sidney Ochs, Ph.D., Chicago; 1956; 1958, Univ. of Indiana, Indianapolis
Warren Johnson, M.A., Boston Univ.; 1956; 1959, medical practice, Dallas

[2] Gail Borden developed methods for preserving meat, milk, and milk products; he was the first collector of customs at the Port of Galveston.

Arnold Nevis, M.D., Harvard; 1957; 1958, Univ. of Florida
Samuel N. Kolman, Ph.D., UTMB; 1958;* 1975, Wright State Univ. School of Medicine, Dayton, OH
Edgar A. Blair, Ph.D., Vanderbilt; 1958;* 1970, retired
F. Hermann Rudenberg, Ph.D., Chicago; 1959*
Emil Burger, M.D., UTMB; 1959; 1960, medical practice, Downey, CA
R. David Baker, Ph.D., Iowa State; 1960*
Luddo B. Nanninga, Ph.D., Univ. of Amsterdam; 1961; 1983, retired
Charles H. Wells II, Ph.D., Michigan State; 1962; 1964, South Carolina Medical College; 1967, returned to UTMB; 1979, Kriticon, Los Angeles, CA
Donald W. Stubbs, Ph.D., UTMB; 1963*
James R. Walker, Ph.D., Univ. of Mississippi; 1965*
Daniel L. Traber, Ph.D., UTMB; 1966*
Henry A. Germer, Jr., Ph.D., Univ. of Houston; 1968; 1974, DuPont, Wilmington, DE
James L. Rae, Ph.D., Michigan State; 1968; 1979, Rush Medical College, Chicago
Edward L. Beckman, M.D., Northwestern Univ.; 1969; 1972, Texas A & M Univ., College Station
Marvin E. Turbow, Ph.D., Univ. of Illinois; 1970; 1971, medical practice, Fountain Valley, CA
Robert E. Barrow, Ph.D., Wayne State Univ.; 1970; 1977, Marine Biomedical Inst., UTMB
James E. Blankenship, Ph.D., Yale; 1970; currently Associate Dean Graduate School, UTMB
H. Lowell Stone, Ph.D., Univ. of Illinois; 1971; 1977, Univ. of Oklahoma Health Science Center, Oklahoma City
Robert Feinstein, Ph.D., Univ. of Michigan; 1971; 1978, anesthesiologist, St. Louis, MO
Harold M. Pinsker, Ph.D., Univ. of California, Berkeley; 1971; 1986, deceased.

In part because it was impossible to present by lecture in the time available all of the physiologic information students needed as a foundation for their clinical studies, an attempt was made to make the time provided for lectures intervals for intellectual stimulation and illustration while depending on assigned reading for an adequate knowledge of physiology. Hence during much of the time allotted for lectures, lecture-demonstrations or in some cases demonstrations with little or no didactic embellishments were presented. Also, on the average, one clinical correlation session per week was included in which a patient was presented by a member of the clinical faculty. After the patient returned to the hospital, a panel consisting of the clinical faculty member and several members of the physiology staff involved the students in diagnostic and prognostic discussions with emphasis on the pertinent basic physiology.

In addition to demonstrations by members of the then-current physiology staff, including Dr. Porter, former physiology faculty members were invited to return to UTMB to give demonstrations in the areas of their special interests. For example, Eric Ogden returned yearly for a number of years to give his demonstration and keen analytical evaluation of Starling's heart-lung preparation.

While a member of the physiology staff at Wayne State University College of Medicine, Guest had been impressed by the teaching acumen of Arthur (Bill) Derbyshire, Ph.D., who was then a fellow member of the physiology faculty at Wayne State. Consequently, he invited Derbyshire to give a series of lecture-demonstrations to the freshman medical students at UTMB. Derbyshire's demonstrations on electroencephalographic-recorded phenomena, especially epileptic disorders, and on the middle ear and cochlea (cochlear microphonics) were received with enthusiasm by both staff and students. Derbyshire continued his colorful presentations at Galveston through most of the 1950s and 1960s.

Among the full-time members of the faculty who were especially adept at demonstrating physiologic phenomena were R. David Baker (gastrointestinal tract), Howard G. Swann (cardiovascular and respiratory systems), Edgar A. Blair, and Donald W. Stubbs (hemodynamics). Blair, who joined the Department in 1958, had been, before he volunteered for service in the U.S. Army, the faculty member in the Department of Physiology at Washington University in St. Louis who worked with Erlanger on the electronics used in the research leading to the Nobel Prize (Erlanger and Gasser 1944). At UTMB, although he did not pursue a specific research project, Blair was exceedingly helpful in advising and assisting other faculty members who were involved in active research. He was also an effective purveyor of physiologic information to students and faculty. He was named Professor Emeritus when he retired in 1970.

About the time when F. Hermann Rudenberg, Ph.D., joined the staff in 1959, the Department began to employ the use of television in the presentation of demonstrations to the students. Rudenberg worked diligently in the application of television to the teaching of physiology. It was found that with television screens strategically placed in the lecture hall, visual information could be effectively presented to the entire class, which otherwise could only be seen by three or four students at one time. Television screens were also effective conveyors of information that had been previously recorded on video tape.

Beginning in the 1950s, mimeographed study guides were given weekly to students during the physiology course. These emphasized information in texts and other sources that were important in the students' acquisition of physiologic knowledge. At first the guides mainly indicated page assignments in textbooks and other source books, with annotations about the assigned material. Eventually, however, they developed into purveyors of specific information plus detailed descriptive evaluations of textbook and other assignments. By the late 1960s the guides had evolved into a syllabus-like format, which gradually expanded in their coverage of physiologic information and in descriptive details. In the 1970s during the chairmanship of Arthur (Buz) Brown the study guide–syllabus supplied the core material for the textbook *Medical Physiology*, edited by A. M. Brown and D. W. Stubbs.

Also in the 1950s teaching quizzes were introduced. These were objective in format and were made up of about 20 objective questions of the type used on National Board Examinations. The mimeographed questions relating to topics covered during that week were given to the students a day or two before the student conference sessions. Students were requested to answer the questions before the conference. During the conference the instructor gave the answers to the questions, which had

been agreed on by the physiology staff, or in more recent years, the instructor called on students to answer the questions and give the reason for their answers. In either case many of the questions served as focal points for discussion and the development of understanding of functional entities and their relationships to other aspects of living processes. A student's performance on a teaching quiz was either not used in the calculation of his overall grade in physiology or if used for credit, it constituted only an insignificant part of his total grade. Currently the quizzes have progressed from objective questions to a non-objective-type format.

In the 1960s, the Department of Physiology at UTMB initiated a laboratory learning experience that was entitled the Free Choice Physiology Laboratory. Its purpose was to stimulate interest in the acquisition of (physiologic) knowledge through experimentation. The laboratory experience was designed to promote maximal student participation in the learning process. The term "free choice" was based on a system in which each student team, consisting of four students, decided on an experiment that they wished to perform during the following week. The experiment could be one that was conventionally performed in physiology student laboratories or it could be, and frequently was, an original approach to obtaining an answer to a physiologic problem. In the planning session with an instructor and the other students in their section of 16 students, a representative of each research team described the team's proposed experimental protocol and obtained suggestions and criticisms from the assembled group. A techniques manual was supplied to the students by the Department. It outlined standardized procedures and described the available equipment. When the experimental project was generally agreed to be feasible and worth doing, the list of equipment and the animal requirements were turned over to the laboratory supervisor for assembly of the required items.

In addition to the planning session and performing the experiment, in the hour before the following-week planning session, the results of the performed experiments were described. These report-sessions followed the format of national (and international) scientific meetings. A student reporting for his team was given 10 minutes to make his presentation. The length of the discussion period that followed, however, was usually timed on the basis of the importance of the subject to the students' understanding of physiology.

Although the Free Choice Laboratory was successful in stimulating the students' interest and in expanding their knowledge of physiology, it was expensive with respect to cost of animals, supplies, and equipment and to time devoted to the procedures by the physiology staff. When the Department moved to the new Libby Moody Thompson Building (1970), in part because sufficient separately located independent departmental student laboratory space for each basic discipline was no longer provided, an integrated functional laboratory (IFL) was established that encompassed physiology, pharmacology, cell biology, and genetics with some laboratory exercises involving biochemistry and pathology. The integrative features usually bring together in one laboratory exercise important aspects of several basic disciplines. The same space is also used for laboratory exercises in neuroscience, histology, microbiology, and pathology.

The IFL, which is devoted primarily to physiology and pharmacology, consists of two 3- to 4-hour laboratory periods plus two 1- or 2-hour discussion periods per week, during two 14-week terms (last term of freshman year and second term of sophomore year). The instructor leads a discussion of the experimental laboratory results and questions the students concerning the significant physiology and pharmacology involved. Usually 16 students are assigned to a laboratory and discussion group (four teams of four students per team).

The integrated functional laboratory has been under the supervision of Daniel L. Traber, Ph.D., since its inception in 1970. Traber, who obtained his Ph.D. degree in physiology at UTMB and now holds a joint appointment as Professor of Physiology and Biophysics and Professor of Anesthesiology, has done an outstanding job in organizing and directing the IFL. He has been ably assisted in its supervision by its codirector, James L. Walker, who came to UTMB after obtaining his Ph.D. degree under Dr. Arthur Guyton at the University of Mississippi College of Medicine. In addition to Traber's administrative duties, his activities on University committees, and his teaching assignments, he conducts research in his productive laboratory at the Shriners Burns Institute and he is the coordinator of research for the Institute. His laboratory has gathered and published significant research-generated information about circulatory shock (primarily endotoxin produced), the effects of smoke inhalation on pulmonary circulation and pulmonary lymph flow and composition, and about the pathophysiologic effects of thermal trauma.

Traber's supervisory profession in his quest for the Ph.D. degree at UTMB was Samuel N. Kolmen. Kolmen received his Ph.D. degree in physiology from UTMB and he became an able administrator and a popular and effective teacher, especially in small group sessions. During the early 1970s he served as research coordinator at the Shriners Burns Institute. He left UTMB to become the Chairman of the Department of Physiology at Wright State University School of Medicine in Dayton, Ohio. In 1984 he moved to Philadelphia where he became Deputy Dean of Medicine at the School of Medicine of Hahnemann University. It is interesting to note that Kolmen and Kolmen's student, Traber, have both been very successful in the education of Ph.D. candidates.

Research During the 1950s and 1960s

Research in the Department of Physiology throughout the 1950s and 1960s was diversified and in most cases supported by grants to individual staff members. Guest believed that the prime responsibility of a basic medical science department was instruction to medical students; hence faculty members were selected to cover, with reasonable competence, as many of the major subdivisions of the science of physiology as possible. The research projects of Guest, Swann, Hall, Kolmen, Seaman, Baker, Rae, Traber, and Barrow were mostly supported by grants from the National Institutes of Health. Other members of the teaching staff either did their research through collaboration with grant-supported colleagues or, in a few cases, limited funds were supplied from the departmental budget.

In the middle and late 1960s and the early 1970s the

major departmental research effort was supported by a program project grant. This grant involved several other departments in addition to physiology. The other departments included internal medicine, biochemistry, cell biology, and pathology. Guest was the principal investigator and W. C. Levin, M.D., an internist who later became President of UTMB, was the coprincipal investigator. The grant was for studies on the blood and the microcirculation. It was the first NIH program-project grant to be funded at UTMB.

Graduate School at UTMB

Although graduate-level courses had been initiated during Ogden's tenure as Chairman, the basic medical science departments had not been approved for doctorate programs by the Graduate School of the University of Texas. Some members of the faculty on the Austin campus considered the Medical School to be a nonacademic branch of the university ("a trade school"); this attitude was deeply resented by the faculty and administration of the Medical Branch. In consequence, a series of faculty meetings soon after Guest arrived in Galveston were devoted to the planning of a campaign to achieve approval for doctorate programs. Donald Duncan, Ph.D., the Chairman of the Department of Anatomy, and Guest were elected to represent the UTMB faculty in efforts to promote graduate education and to obtain approval for graduate degree programs at UTMB. After much correspondence and numerous trips to Austin during the early 1950s, the Departments of Anatomy and Physiology were given equal standing with the departments of the University of Texas at Austin in the operation of programs leading to the awarding of the Ph.D. degree.

When the other basic science departments were approved for doctorate programs a short time later, Duncan was appointed to be the Associate Dean of the Graduate School at Galveston and Guest was elected as the representative (with Duncan) for UTMB in the University of Texas Graduate School Legislative Assembly. Duncan and Guest continued to hold these positions until the UTMB graduate program was granted essentially complete autonomy in the mid 1960s. With autonomy, the UTMB Graduate School was headed by a dean with equivalent functions to the dean of the Graduate School of the University of Texas at Austin. After establishment of the essentially autonomous UTMB Graduate School Guest was elected to be its representative on the Graduate Council of the Biomedical Institutions of the University of Texas System; he served in this position until he retired from the Chairmanship of Physiology in 1973.

Graduate Degrees Conferred 1953–1987

Individuals who obtained graduate degrees (primarily Ph.D. degrees) following study and research in the department of physiology (and biophysics) at UTMB are listed below. The name is followed by the degree, the year it was conferred, and the position (or positions) held after the degree was conferred.

Stanley R. Mohler (M.D.), M.A., 1953; vice chairman, department of community health, director aerospace medicine, Write State College of Medicine, Dayton, OH

Earl T. Carter (M.D.), Ph.D., 1955; recently retired from chairmanship, division of preventive medicine, Mayo Clinic College of Medicine, Rochester, MN

Samuel N. Kolmen, Ph.D., 1957

Sidney Cassin, Ph.D., 1957; professor of physiology, Univ. of Florida, Gainesville

Fred S. Sanders (M.D.), Ph.D., 1958; adjunct assistant professor of geriatrics, Univ. of Indiana School of Medicine; medical practice, Indianapolis

Larry Joe O'Brian (M.D.), Ph.D., 1958; chairman of physiology, Texas Tech College of Medicine; currently medical practice, Lubbock, TX

Sheldon F. Gottlieb, Ph.D., 1960; dean of graduate school, Univ. of S. Alabama; currently professor of biological science, Univ. of S. Alabama, Mobile

Jess M. McKenzie, Ph.D., 1960; chief of stress analysis research, Civil Aeromedical Institute, Oklahoma City, OK

Richard G. Cooper, Ph.D., 1964; associate professor of physiology, Univ. of Missouri, Columbia

Daniel L. Traber, Ph.D., 1965

Jackie G. Weatherred, Ph.D., 1965; professor and coordinator of physiology, School of Dentistry, Medical College of Georgia, Augusta, GA

Mary Jean McNabb-George, Ph.D., 1966; assistant professor of biochemistry, Lamar Univ., Beaumont, TX

Elbert J. McCoy (M.D.), Ph.D., 1966; associate professor of physiology, Temple Univ., Philadelphia; more recently medical practice, Millinocket, ME

David D. Michie, Ph.D., 1966; chairman of physiology and bioengineering, Eastern Virginia Medical School; more recently president, Clinical Physiology Associates, Fort Myers, FL

Lona Claire Parson, Ph.D., 1968; professor of nursing and assistant professor of physiology, Univ. of Virginia School of Medicine, Charlottesville, VA

James P. Noone (M.D.), Ph.D., 1968; assistant professor of physiology, Univ. of Pennsylvania School of Medicine; more recently medical practice, Towanda, PA

Mary E. Guinen (M.D.), Ph.D., 1969; clinical research investigator of venereal disease, U.S. Center for Disease Control, Atlanta, GA

Margaret Young (M.D.), Ph.D., 1969; instructor of pathology, UTMB

Earl W. Ferguson (M.D.), Ph.D., 1970; commandant U.S. Air Force Hospital, Little Rock, AK

Larry Priano (M.D.), Ph.D., 1970; associate professor of anesthesiology, Univ. of Oregon School of Medicine, Portland, OR

Malcolm J. Wall (M.D.), Ph.D., 1970; associate professor of physiology, Marquette Univ. School of Medicine; more recently medical practice, Gainesville, TX

Roger A. Maunz, Ph.D., 1973; postdoctoral fellow Rockefeller Univ., New York, NY; more recently faculty member, Univ. of City of New York

Robert E. Schoen (M.D.), Ph.D., 1973; research associate of physiology, Univ. of South Florida College of Medicine in Tampa; currently medical practice, New York, NY

Salah Ayachi, Ph.D., 1974; associate professor of interdisciplinary studies, School of Allied Health Science, UTMB

Nick C. Trippodo (M.D.), Ph.D., 1974; postdoctoral fel-

low with Arthur Guyton, Univ. of Mississippi School of Medicine; currently associate professor, L.S.U. School of Medicine, and coordinator of research, Ochsner Research Foundation, New Orleans, LA

Walter Hugh Vance, Ph.D., 1975; rehabilitation engineer, Valley Children's Hospital, Fresno, CA

Carl T. Bohs, Ph.D., 1976; postdoctoral fellow, anesthesiology, UTMB; currently medical librarian for law firm, Portland, OR

Michael K. Rock, Ph.D., 1977; associate professor of interdisciplinary studies, School of Allied Health Science, UTMB

Thomas A. Miller (M.D.), Ph.D., 1977; assistant professor of anesthesiology, Baylor College of Medicine, Houston, TX

M. J. Denn-Young (M.D.), Ph.D., 1977; postdoctoral fellow, Marine Biological Institute, UTMB; currently medical practice, Orlando, FL

Michael E. Andresen, Ph.D., 1978; assistant professor of physiology and biophysics, UTMB

Richard F. Martin (M.D.), Ph.D., 1978; postdoctoral fellow and more recently resident anesthesiology, Univ. of Washington School of Medicine, Seattle

George C. Kramer, Ph.D., 1979; postdoctoral fellow and more recently assistant professor, department of human physiology, Univ. of California School of Medicine, Davis

J. B. (Duke) McHugh (M.D.), Ph.D., 1979; postdoctoral fellow, internal medicine, Univ. of Colorado School of Medicine, Denver

Elizabeth A. Murray, Ph.D., 1979; postdoctoral fellow, NIH, Bethesda, MD

Aldo J. Castiglione, Ph.D., 1980; postdoctoral fellow, Univ. of California, Los Angeles; more recently assistant professor of veterinary anatomy, Texas A & M Univ., College Station, TX

Gerald W. Davis, Ph.D., 1980; postdoctoral fellow, bioinformation systems, California Institute of Technology, Pasadena

Thomas H. Adair, Ph.D., 1980; postdoctoral fellow, physiology and biophysics, Univ. of Mississippi; more recently associate professor of physiology and biophysics, Univ. of Mississippi School of Medicine, Jackson, MS

David B. Butler, Ph.D., 1980; postdoctoral fellow, Wolfson Institute, Univ. of Dundee Medical School, Dundee, Scotland

Eric M. Lasater, Ph.D., 1980; postdoctoral fellow, department of biology, Harvard Univ., Cambridge, MA

Harold D. Shone, Ph.D., 1980; assistant professor, department of neuroscience, Children's Medical Center, Boston, MA

Jack P. Douglas, Ph.D., 1981; research assistant, Baylor College of Medicine, Houston, TX

Lewis B. Eberly, (M.D.), Ph.D., 1981; resident, neurology, Univ. of Texas School of Medicine, Dallas

Mark S. Ifshin, Ph.D., 1981; postdoctoral fellow with Dr. Simon Lewis, department of physiology, Yale Univ. School of Medicine, New Haven, CT; more recently with Smith, Kline and Beckman, Philadelphia, PA

Kai Sing Lee, Ph.D., 1981; research staff member, physiology, Yale Univ. College of Medicine, New Haven, CT; more recently with Upjohn Co., Kalamazoo, MI

Kenneth P. Madden (M.D.), Ph.D., 1981; resident, internal medicine, San Antonio, TX

Franca Sant'Ambrogio, Ph.D., 1981; research instructor of physiology and biophysics, UTMB

Karin Westlund High, Ph.D., 1981; associate professor of anatomy, UTMB

Michael H. Droge, Ph.D., 1982; assistant professor of biology, Texas Women's Univ., Parkland Campus, Dallas

Kevin D. Gerhart (M.D.), Ph.D., 1982; resident, rehabilitation medicine, Univ. of California, Irvine

Greg Redmann, Ph.D., 1982; laboratory of neurophysiology, NIH, Bethesda, MD

Steven W. Mifflin, Ph.D., 1983; postdoctoral fellow, Univ. of Heidelberg, Heidelberg, Germany; more recently postdoctoral fellow, Univ. of Iowa School of Medicine, Iowa City

Erwin Shibata, Ph.D., 1984; postdoctoral fellow, Canadian Heart Foundation, Univ. of Calgary, Calgary, Alberta, Canada

David Packey, Ph.D., 1984; instructor, department of chemistry, Univ. of Alaska, Fairbanks

Paul Munch, Ph.D., 1985; NIH postdoctoral fellow, Univ. of California, San Diego

Thomas Anastasio, Ph.D., 1986; postdoctoral fellow, Wilmer Institute, Johns Hopkins Hospital, Baltimore, MD

Donald Campbell, Ph.D., 1986; postdoctoral fellow, Canadian Heart Foundation, Univ. of Calgary, Calgary, Alberta, Canada

Michael Curran, Ph.D., 1986; NIH postdoctoral fellow, Bethesda, MD

Randall Newman, Ph.D., 1986; postdoctoral fellow, physiology and biophysics, Univ. of Miami, Miami, FL

Alan M. Frace, Ph.D., 1987; postdoctoral fellow, Emory Univ. Medical School, Atlanta, GA

Carla

In early September 1961 another devastating hurricane struck the Texas Gulf Coast. This one was named Carla. There had been other hurricanes that visited Galveston between the 1900 storm and Carla, but Galveston's seawall, constructed by the U.S. Corps of Engineers after the 1900 storm, protected the city from major damage. Carla, however, was different. When it came close to the Texas coast, its center didn't cross the shoreline as most hurricanes did, but it remained over water where the storm continued to grow in strength and size until the wind raking the coast reached a velocity of 175 miles/h in some of its major gusts. Finally after four days of sashaying unpredictably up and down the Texas Coast, the center of the storm came ashore at Port Lavaca, south of Galveston. The major damage to Galveston resulted from three tornados that took their toll as the hurricane moved inland. The tornados ripped buildings apart and uprooted trees. High water from the abnormal tides and the torrential rains also caused significant damage.

As the storm progressed, the Department of Physiology on the second floor of the Gail Borden Building became a refuge for physiology staff members, their families, and in some cases their friends. Wives of staff members worked in the hospital kitchens helping to prepare food for the patients and the many refugees who had come to the hospital to escape the raging winds and high water. Physiology staff members with other faculty

members and students formed a human chain to move library books to a safe, dry location above the basement and first floor of the Gail Borden Building when seawater mixed with rain water began to flow in.

Most of the damage to the Medical Center was caused by the high water. The electrically operated equipment including air conditioning, heating systems, and booster pumps in the basements of the college and hospital buildings were damaged or destroyed. Fortunately the tornados missed the medical complex, although one of them cut a swath through the town a few blocks away.

After the storm, teaching, research, and patient care were resumed, albeit often under trying circumstances. Air conditioning and heating were finally restored. Efforts were made to better locate and protect these amenity-producing paraphernalia in case of another Carla-type storm.

Brown Appointed Chairman in 1973

In 1971 Guest was named Acting Chairman of Physiology in line with a ruling that an administrative position requiring regental approval could not be held after an individual had reached the age of 65. He continued as Acting Chairman until the arrival of the newly appointed Chairman, Arthur M. Brown, M.D., Ph.D., in 1973. At about the time when he retired from the acting chairmanship Guest was elected Ashbel Smith Professor of Physiology. After Brown arrived Guest moved his office and research laboratory to the Shriners Burns Institute (on the UTMB campus). However, he continued to teach medical and graduate students in physiology. As of 1987, on a part-time basis, he does some teaching and continues to direct a research laboratory.

Arthur M. (Buz) Brown, M.D., Ph.D., came to Galveston from the College of Medicine at the University of Utah in Salt Lake City (Departments of Physiology and Internal Medicine). A Canadian by birth, he had obtained his M.D. degree from the University of Manitoba Faculty of Medicine and his Ph.D. degree from the University of London (England). Before his appointment at the University of Utah Brown had successive academic sojourns in the Department of Physiology, Middlesex Hospital Medical School, London, England, and in the Cardiovascular Research Institute, University of California, San Francisco. His major research interests while at Galveston involved the cardiovascular system, neurophysiology, and cell membrane function. Brown brought with him from Utah, and recruited from other centers, a capable and highly motivated group of young scientists, most of them with primary interests in the cell membrane field. Under Brown the number of physiology faculty members and the departmental name were expanded; it became the Department of Physiology and Biophysics.

During Brown's chairmanship, although the number of academic faculty members of the Department of Physiology and Biophysics increased, more individuals than previously were supported completely or in part from funds supplied by grants. A number of the staff members named next have (or had) appointments in both physiology and biophysics and a second department at UTMB; however, only faculty members with their primary appointment in physiology and biophysics are listed here.

New Faculty Members During Brown's Chairmanship

Names of new faculty are followed by the academic degree, where it was obtained, year of appointment at UTMB, year of resignation, and where he or she went. Only individuals with their primary appointment in physiology and biophysics are listed.

Arthur M. Brown, M.D., Ph.D., Univ. of London (England); 1972; 1985, Baylor College of Medicine, Houston, TX

Diana L. Kunze-Brown, Ph.D., Univ. of Utah; 1973; 1985, Baylor College of Medicine

Harvey M. Fishman, Ph.D., Univ. of California, Berkeley; 1973

Douglas C. Eaton, Ph.D., Univ. of California, San Diego; 1973; 1985, Emory Univ., Atlanta, GA

R. Thomas Dowall, Ph.D., Univ. of Iowa; 1973; 1977, Univ. of Oklahoma Health Science Center, Oklahoma City, OK

John M. Russell, Ph.D., Univ. of Utah; 1973

Malcolm S. Brodwick, Ph.D., Univ. of California, Los Angeles; 1974

Giuseppe Sant'Ambrogio, M.D., Univ. of Milan Medical School (Italy); 1975

Brian A. Hills, Ph.D., Adelaide Univ. (Australia); 1975; 1980, Univ. of Texas Health Science Center, Houston

Gabor Szabo, Ph.D., Univ. of Chicago; 1976

Norio Akaike, M.D., Univ. of Kumanimoto (Japan); 1975; 1976, Japan

Lee E. Moore, Ph.D., Duke Univ.; 1976

Syozo Yasui, Ph.D., M.I.T.; 1976; 1979, Japan

Paul R. Adams, Ph.D., Univ. of London (England); 1977; 1981, Stony Brook, NY

Burgess N. Christensen, Ph.D., Univ. of Utah; 1977

Susan L. Hamilton, Ph.D., Univ. of Colorado; 1979; 1985, Baylor College of Medicine, Houston, TX

Aileen K. Ritchie, Ph.D., Johns Hopkins Univ.; 1979

King Wai Yau, Ph.D., Howard Univ.; 1980; 1986, Howard Hughes Institute, Baltimore, MD

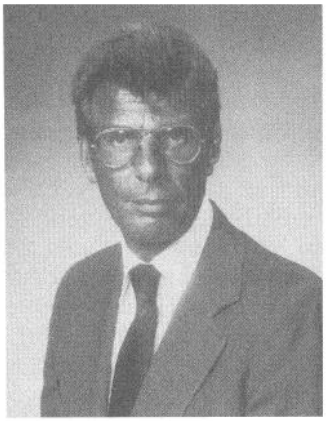
Michael C. Andresen, Ph.D., UTMB; 1981

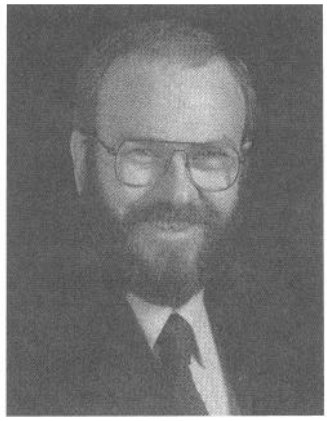
Robert K. S. Wong, Ph.D., Univ. of Alberta; 1981; 1986, Columbia Univ.

Teaching During the 1970s and Early 1980s

Teaching medical students during the period when Brown was Chairman was under the supervision of Donald W. Stubbs, Ph.D. This assignment is still extant (1987). Stubbs obtained his Ph.D. degree at UTMB in biochemistry and physiology. He is an excellent teacher and an able administrator. In 1983 the textbook *Medical Physiology* (edited by Brown and Stubbs; New York: Wiley) was published. The contributors were members of the UTMB physiology and biophysics faculty. The book has gone through a third printing.

During the late 1960s the faculty of medicine at UTMB began an in-depth evaluation of the curriculum. The study extended over a three-year period and the new curriculum was initiated in 1971. Its major feature is a division of the curriculum into three parts with provision for selected applying students to graduate in three years.





Left: Arthur M. (Buz) Brown, Chairman of Physiology and Biophysics, 1973–1985; *right:* John M. Russell, Jr., Acting Chairman of Physiology and Biophysics, 1985–1986.

The three parts are a basic science core over a period of about a year and one-half, a clinical core occupying a second year, and one half period and a so-called "track" program, which is scheduled as the fourth year of medical training. During the third year students may apply for graduation at the completion of that year. A faculty committee screens the applicants, approving those who have demonstrated maturity and a good academic record during their medical training. If students are required to take the track program (and the majority are), the time is spent on elective programs, on graduate study toward a M.S. or Ph.D. degree, or on a remedial program.

The basic core consists of 14 or 15 courses. Among these are physiology and biophysics, neuroscience, and endocrinology. About 64 lectures are given in physiology; they cover biophysical phenomena and cardiovascular, pulmonary, renal, and gastrointestinal physiology. Each week at a Wednesday lecture the students are handed a 15-question, essay-type, quiz that they take home to answer at their leisure. They are free to use textbooks and lecture notes to find answers. On Friday morning of the same week, a two-hour conference is held. The instructor calls on computer-selected individuals in the group of 16 students to answer questions that had been preselected by the staff. Usually six or seven students are asked to give answers and each of these students receives a grade; however, it only counts a fraction of 1% of the total grade for the course.

The courses in neuroscience and endocrinology are taught primarily by the staff members in physiology and anatomy with input from other basic science departments and some clinical departments. The organization and presentation of neuroscience and endocrinology are under the aegis of faculty committees. The integrated functional laboratory, described previously, is an important feature of the basic science curriculum. The current curriculum is again under evaluation and changes will doubtlessly be made in the near future.

Research During the 1970s and 1980s

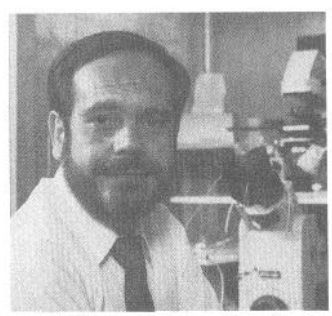
Brown's "game" plan was to put together a faculty with research strengths focused on physiologic problems related to the function of cell membranes. He was successful in recruiting a staff that amply met this goal. In general, the early wave of new faculty members reflected the state of membrane physiology at the time, in that the group was heavily committed to the study of membrane electrical phenomena (i.e., nerve potentials and fundamental studies of how electrical charges cross biological membranes). Over the next 10–12 years the membrane physiology theme of research at Galveston became clearly dominant, and as more new faculty members were added the Department gained additional technical wherewithal in membrane physiology.

Because of common interests in the field of research being pursued in the Department of Physiology and Biophysics at UTMB, communication among the faculty members was effectively promoted. One result of this intellectual intercourse has been the relatively large number of research projects involving two or more staff members. The Department during Brown's tenure had been the most successful of all academic departments at UTMB in obtaining extramural funds for its research

Brown Moves to Baylor

When Brown resigned in 1985 to take the Chairmanship of Physiology and Biophysics at Baylor College of Medicine in Houston, John M. Russell, Jr., Ph.D., was appointed Acting Chairman of Physiology and Biophysics at UTMB. Russell, who came to Galveston in 1974 after serving a postdoctoral fellowship in the Department of Physiology and Biophysics at Washington University in St. Louis, had obtained his Ph.D. degree in pharmacology in 1971 at the University of Utah. He ably served as Acting Chairman until the arrival of Dr. Reuss in the summer of 1986.

When the search committee was appointed to find a replacement for Brown, there was a strong effort by the members of the Department who had been brought to Galveston during Brown's chairmanship to have the search committee recommend for appointment a person with a background in cell membrane research. After considering a number of highly capable physiologists, the one who has invited to take the position was a well recognized biophysically oriented physiologist with a background of research primarily in epithelial transport and in the cell membrane field. Luis Reuss, M.D., originally a resident of Santiago, Chile, had obtained his medical degree from the University of Chile. In 1972 he came to the University of North Carolina as a Fogarty fellow. Then, after also serving as a Louis G. Welt fellow at North Carolina he was appointed in 1975 to the academic staff in the Division of Nephrology, Department of Medicine, University of North Carolina. From 1976 to 1986 Reuss was successively Associate Professor and Professor in the Department of Physiology and Biophysics at Washington University School of Medicine in St. Louis.



Luis Reuss, Chairman of Physiology and Biophysics, 1986–present.

Dr. Reuss has recruited several new staff members. These include Simon A. Lewis, Ph.D., University of California, Los Angeles; Nancy K. Wills, Ph.D., University of Virginia; Michael L. Jennings, Ph.D., Harvard University;

and Brian E. Peerce, Ph.D., University of Alabama. Dr. Pompeo Volpe and Dr. Bruce Simon are joining the staff in 1988.

The Department continues with its major research drive in the fields of electrophysiology and membrane transport. Under the capable leadership of Luis Reuss and with the addition of the new faculty members Physiology and Biophysics is again one of the strongest research-oriented departments at the University of Texas Medical Branch.

References

1. Faculty and Staff UTMB. *The University of Texas Medical Branch at Galveston. A Seventy-Five Year History.* Austin: Univ. of Texas Press, 1967, 13–14.
2. Ibid, p. 6–7.
3. Ibid, p. 9–10.
4. Ibid, p. 40.
5. Ibid, p. 78–80.
6. Ibid, p. 62–63.
7. Simpson, H. B. *Hood's Texas Brigade: A Compendium.* Hillsboro, TX: Hill Junior College Press, 1977, 11, 552.
8. Carter, W. S., N.d. *The Department of Physiology From 1898 to 1922.* The Truman G. Blocker History of Medicine Collection, Moody Medical Library, The University of Texas Medical Branch at Galveston. Typescript (unpublished).
9. Smith, A. J. *The Medical Department and the Galveston Storm.* University Record, 1901, Vol. 3, p. 53–67. Filed in the Truman G. Blocker History of Medicine Collection, Moody Medical Library, UTMB.
10. Griffin, C. S. *History of Galveston, Texas.* Galveston, TX: A. H. Cawston, 1931, p. 67–70.
11. Most of the information concerning the Spies interval was obtained from the Ainsworth File in the Truman G. Blocker History of Medicine Collection, Moody Medical Library, UTMB.
12. *Daily Texan,* Austin, Texas, May 12, 1942.

Appendix

I am making some comments and suggestions based on my half century as a working physiologist. At the beginning of my career (middle 1930s) physiology in medical schools was largely taught by individuals with broad training in physiology and the other basic sciences. During the graduate training period knowledge in-depth about a specialized topic was accomplished through an extensive perusal of the literature in the field of the dissertation research. When young physiologists received their graduate degrees they were equipped with the information needed to teach others and they were versed in the accepted ways to obtain additional information, both that which had already been recorded and that which are still essentially unknown.

In contrast to the status of medical physiology in the 1930s and into the 1950s many physiology departments in the 1960s, 1970s, and 1980s developed into research organizations, depending on funds from the National Institutes of Health for their continued existence. Salaries of the departmental staff members were usually paid, at least in part, by grants (federal funds); tenure depended on ability to write grant proposals and the number of publications and not on ability to teach physiology to medical and graduate students.

As a holdover from the ancient days of physiology departments in medical schools in which the prime function of the departments was teaching, supplemented by a search for additional basic knowledge, I look back with a degree of nostalgia. The research done in those days was not the means for obtaining salaries for the departmental staff. Salaries in medical schools before the 1950s were paid by the schools. Funds were limited and in many schools the departmental chairmen raised money by appealing to philanthropic sources. Thus the chairmen among other duties were frequently money raisers. The teaching staffs were free to effectively teach and to do research. Faculty members were scientific investigators because they were curious individuals, or in some instance because pressure was exerted on them to publish by the chairman or the dean. In those years faculty members did not spend their potentially productive time in writing grant applications and progress reports.

Today the cost is high for maintaining a teaching faculty with ancillary personnel and facilities in a basic science discipline such as physiology. Hence, deans are on the hunt for departmental chairmen who are adept at obtaining funds from grants and who can recruit successful grant-writing staff members. These grants supply a part (often most) of the salaries of the departmental personnel. However, each grant is commonly funded for the investigation of a topic that deals with only a small fraction of the science of physiology. Consequently, individuals recruited to staff a grant project are often trained in only a limited aspect of physiology. Unfortunately these recruits have little or no familiarity with the other features of physiological science and they find it difficult to present coherent, meaningful physiologic information to medical, dental, nursing, and allied health science students. Furthermore if most or all of the departmental grant support is for only one facet of physiology, the training and orientation of recruited faculty members tend to be limited to the one facet. The teaching in many physiology departments is relegated to a secondary position in the departmental activities.

We cannot (and should not) move back into the pattern of the 1940s, but I believe that teaching and research should be realigned in the future medical school setting. Major research projects should have the full attention of the principal investigators, and this leaves little time for teaching and the sharpening of teaching skills.

Based in part on the preceding observations, I am suggesting that two categories of medical school basic science faculty members be formally recognized. The first group would be scientist-teachers with an acceptable and recognized limitation on the amount of time devoted to research. The second group's function would be primarily research. To formalize the dichotomy, which currently actually exists in some physiology departments, a change in funding for health science instructional schools is required. Funding for the basic science departments to cover the cost of teaching, including salaries for instructional staffs, could continue to come from institutional or state or federal sources, and in most cases a combination of these sources. These funds should be sufficient to cover the costs of research projects that require no more than one half of the scientist-teacher's time and involve the services of no more than one or at the most, two technicians. A major change in the funding procedure would be that the scientist-teacher–generated research projects would be under the aegis of local committees and application for funds for such research would be made to the local committee.

The amount of federal funds to cover the salaries of scientist-teachers would not necessarily be greater than that currently used for salaries of departmental staff members now paid by the NIH. Furthermore the routine allotments of funds to the medical school administration (for disbursement by a local committee) for covering the cost of the supplemental research performed by the scientist-teachers might actually reduce NIH central expenses.

More extensive (and intensive) research projects supported by the NIH or NSF would continue to be handled as they currently are. However, the primary researchers, i.e., the principal investigators in such projects, would not be counted on to provide a significant amount of instruction to medical students, but they might contribute a limited amount of specialized types of information during the medical student teaching program. On the other hand, the primary researchers would be responsible, as would the other departmental faculty members, for providing instruction to graduate students. I also believe that the primary researchers should be active laboratory participants in the research and not just desk-oriented supervisors. The fact that they would be relieved of medical school teaching duties should permit active involvement in the laboratory phase of the research.

The retrieval of excellence in the teaching of physiology could lead, I believe, to a revitalization and a unification of an inherently coherent scientific discipline. Scientist-teachers who have had training in many facets of physiology and who participate in the instructional presentation of the entire subject to the students (in contrast to giving a few lectures relating to one organ or one organ system) should bring physiology back into focus; teachers could form a dedicated corps of scientists with an objective to reestablish the discipline of physiology in a parallel position with morphology as one of the two primary basic disciplines in biology and medicine.

History of Physiology at University of New Mexico

SIDNEY SOLOMON
Department of Physiology
School of Medicine
University of New Mexico
Albuquerque, New Mexico 87131

Early Days

In the legislative session of 1961, an enabling bill was passed to establish a medical school at the University of New Mexico. The first person to be appointed was the first Dean of the Medical School, Dr. Reginald Fitz. Dean Fitz had been an assistant dean at the University of Colorado and was familiar with the problems and assets of educational institutions of the Southwest. Only a courageous, optimistic, and adventurous dreamer such as he could have accepted the appointment and really thought that it was possible to build a quality medical school in an economically lagging state with a population less than 1 million. These characteristics were general characteristics of the faculty who had early appointments at this institution. Dean Fitz's attitudes about the way to build a medical school were shown by the sequence in which he picked his first chairpersons. Solomon Papper, M.D., became Chairman of Medicine in 1962 and Sidney Solomon, Ph.D., became Chairman of Physiology in 1963. The school accepted its first class in September 1964. It was the view of Dean Fitz that these were the two disciplines that formed the backbone of medicine in the broadest general terms. As a result, he was responsible in a large measure for the early growth and whatever successes were enjoyed by this Department during the early years of the school.

Curricular decisions were left to a consensus established by the faculty. However, Dr. Fitz and all following deans have had a strong influence on the outcome of these decisions through the appointments they have made. All of the new chairpeople were committed to try to find a new and better curricular form, and all were interested in developing some kind of integrated curriculum. After much deliberation, we ended our first years with a curriculum similar to that developed at Western Reserve University Medical School but with some significant differences.

The strong emphasis placed on teaching during the formative years of the school influenced Dr. Solomon with regard to the appointments he would make in the Department. He thought, as did other chairpersons, that the students would be best educated if the instructors were doing research in the areas in which they were teaching, or else at one time or another had an interest in those areas of physiology. His own areas of interest were renal physiology, peripheral nerve, and cell physiology.

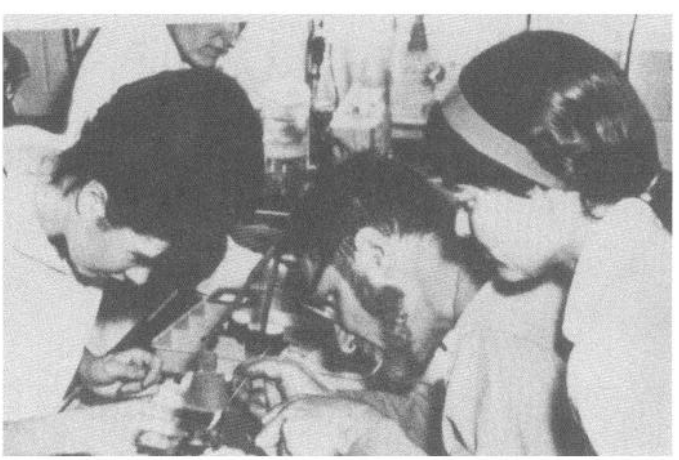
The first classes had only 24 students; this grew to 48 over the next two years. In the beginning each basic science department had only two positions supported by school money. It was decided that during the first year the areas of greatest need were cardiovascular physiology and neurophysiology. Consequently, in 1964, Solomon appointed Agammemnon Despopoulos, M.D., to teach cardiovascular physiology. Although Dr. Despopoulos' primary interest was in organic acid transport with emphasis on the kidney, he had previous experience with research on cardiovascular problems while at the National Institutes of Health. The second appointment, also in 1964, was Donald Frazier, Ph.D., a recent graduate from University of Kentucky and a neurophysiologist.

The appointment of Dr. Despopoulos proved to be fortuitous. He came to New Mexico from the Department of Pharmacology at Louisville, Kentucky. Since no pharmacologists were appointed until two years after the start of the school, Dr. D (as he was called) filled a major function in teaching this discipline with the assistance of whomever could give him some help.

Although he was not a full-time faculty member, Harold Sonnenberg, Ph.D., was another important appointment. He came for postdoctoral training with Dr. Solomon and was helpful in setting up certain of the teaching exercises, as well as in doing research.

Both teaching and research were given equal priority. It was easier to get grants for medical research in the 1960s than in the mid 1980s, but awards were not automatic. Nevertheless, all of the physiology faculty obtained support from various agencies and all were productive and publishing by the third year (1966) of this school's existence.

To build a strong research effort, it was quickly recognized by all departments that a graduate program was required. In those days, a new graduate program first required approval by the general faculty, approval by the Board of Regents, and finally, approval by the Board of Educational Finance—a creature of the state legislature that was responsible for evaluating the economic implications of new programs. The board then approved or



Agammemnon Despopoulos and two technical associates.

Left: Sidney Solomon; *right*: Harold Sonnenberg.

disapproved of the programs in accordance with its judgment as to whether the state could provide the necessary support.

It was recognized that no group would ever approve programs in the sciences where only three faculty would provide training. Yet the school had a great deal of talented faculty, who by working together could do a very acceptable job. This characterization was true not only of the basic science faculty but was an equally valid statement for the individuals in the so-called clinical departments. Since the school was only a two-year medical school for the first three years, the clinical faculty had relatively little teaching responsibilities outside of cooperating with basic science faculty in teaching fundamental biology and clinical correlations. Nearly all had research interests with many of them active in areas that were considered to be physiology. It was decided to take advantage of this situation.

Three faculty were assigned by Dean Fitz to develop and present a plan for a graduate program. They were Sidney Solomon, Chairman of Physiology; Leonard Napolitano, Ph.D., from Anatomy (now Dean of the Medical School); and Joseph Scalletti, Ph.D., from Microbiology. The program that was approved in 1965 was a Ph.D. in Medical Sciences rather than in departmental disciplines. In this way the basic scientists could take advantage of the interests of appropriate clinical faculty and involve them in the graduate program. Whether such an approach was wise may be debatable, but it worked during the early years of this institution.

Collaborative arrangements were best expressed by the joint training grant awarded to Solomon Papper and Sidney Solomon in nephrology in 1965. At this time of the formative years of this school (1964–1970), interdepartmental training grants were relatively rare. This grant provided funds not only for postdoctoral trainees in medicine and physiology but also for qualified faculty of other departments (later trainees in anatomy, pharmacology, and pathology were supported from this source). Undergraduate medical students, graduate Ph.D. students, and postdoctoral trainees with both M.D. or Ph.D. were all supported by this grant. This collaboration and mutual support was one of the strongest features of the early days of this Department. Not only were we involved with each other in research and teaching of basic scientists and medical students, but basic scientists were also involved with clinical teaching.

Very often we were invited to participate in various departmental rounds. We were also asked to participate in Grand Rounds and to formally present information to clinicians, interns, and residents. It was not important as to whether one had an M.D. or a Ph.D.; it was the knowledge that any of us could bring to a given situation that was valued.

Intermediate Years and Changing Emphasis

The attitudes and philosophy that underscored the early years were prevalent from 1964 to about 1969. At the end of this period, there were signs developing that the nature of the school was changing. It was getting larger. In 1968 Albert Ratner, Ph.D., was appointed to fill the teaching gap in endocrinology. Other departments were also expanding. It now became almost impossible to know all the faculty as we had in earlier years. Certainly, the spirit of intimacy and mutual support between members of different departments was being reduced and even lost. The faculty started to withdraw behind departmental identity, a process that manifested itself in several ways.

There was a withdrawal from an integrated curriculum to a more conventional structure by departments other than Physiology. It is unfortunate, but there is a lesson that comes from this retreat from a collaborative structure. If one is to have integrated teaching, one needs integrated teachers. We had deluded ourselves, and what we had was a juxtaposed curriculum. Although there were some individuals who had broad biological knowledge in other departments, physiologists in general have a wider appreciation and understanding of biology. As a result, this Department supported the continuation of an integrated curriculum, but in the long run it was unable to successfully oppose the increasing departmental parochialism that developed.

These attitudes also expressed themselves in research. Whereas there was lot of collaborative and interdisciplinary research in the early years, these activities tended to decrease as time went on. Faculty members no longer needed each other. There was a movement toward an increasing number of individual departmental seminars, accompanied by a decrease in attendance by faculty and students based outside of the concerned departments.

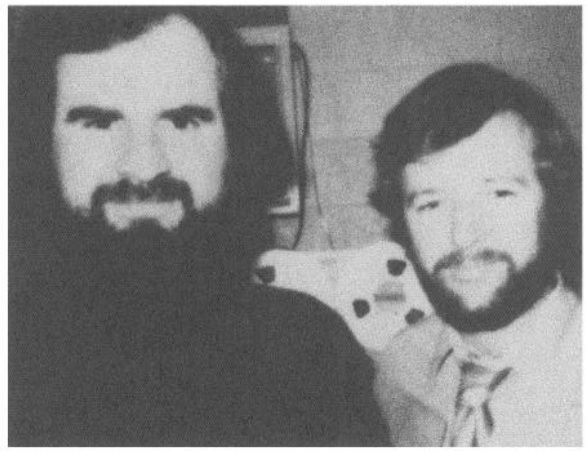
Front: Albert Ratner; *back*: Harold Spurgeon.

Other factors also contributed to further compartmentalization into individual, less interactive departments. Most of these factors related to increased responsibilities that were identified with individual departments. For example, allied health programs were developed that had courses in them given by a single departmental faculty. Physiology developed a course for physical therapists.

Beginning about 1970 collaboration did, however, develop between individuals working at different national laboratories in New Mexico and our faculty. Certain employees at Los Alamos requested that we give a course in physiology at that institution. The course was first established as an overall review of the discipline. The Los Alamos students wanted more physiology and the establishment of a Master's program. Eventually the single course evolved into a complete Master's program. Required allied courses (chemistry, biochemistry, physics, statistics, and others) were already offered by some Los Alamos personnel, while members of additional medical school departments offered courses in biomedical sciences (e.g., histology and immunology).

For those of us in physiology this period (1970–1975) was one of the most exciting and satisfying periods in our history. In 1971, the American Council of Higher Education released its evaluation of graduate programs, and our Department was named as one of the better ones in the country. We were producing Ph.D.s on the Albuquerque campus and M.S. degrees at Los Alamos. The latter activity was a particular source of pleasure. The students were highly motivated, very bright, and imaginative. Many were already well trained in science and held advanced degrees including the Ph.D. Most of these were in physical sciences, but we also had biologists, social scientists changing careers, and one pharmacist. Although the program has all but disappeared, there were still a few students finishing degrees in 1986. The residual benefit to this Department is that it resulted in an increased collaborative research with scientists at Los Alamos, much of which still continues.

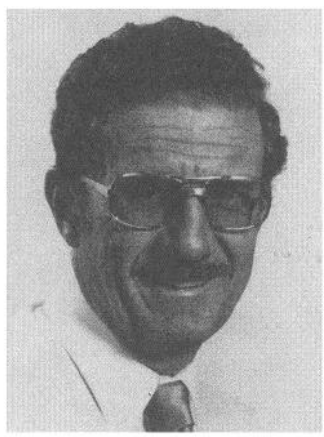
More faculty were added during this period. Gerry Weiss, Ph.D., was recruited in 1969 for his expertise in neurobiology and cardiovascular physiology. He taught and did research in those areas. We also lost faculty. Donald Frazier moved to Kentucky and was replaced by Kenneth Kastella, Ph.D., in 1971, who later moved back to Alaska and was replaced by Dr. Donald Partridge (1976). Donald Priola, Ph.D., transferred from our Department of Pharmacology, wherein he was first recruited (1970). William Galey, Ph.D., was recruited in 1972 to teach cellular and gastrointestinal physiology, and Roger Shannon, Ph.D., became the respiratory expert in 1970. He later moved to the University of South Florida and was replaced by Stephen Wood, Ph.D. (1974). Dr. Despopoulos resigned and moved to Europe in 1976. He and his wife were lost at sea while trying to cross the Atlantic from England to the United States in his sailboat. He is the only faculty member who is deceased.



Left: William Galey; *right*: Roger Shannon.

The Department at this time had expertise in most areas of physiology. It had seven members, a number that according to several articles represented a minimal critical mass, whatever that means. Although our research was in different subareas of physiology, we had enough breadth, as individuals, to understand each other's work, to talk to each other, and to collaborate when opportunities presented themselves. Although there was a lessening of interdepartmental dependency and cooperation, there was a strengthening of intradepartmental bonds between faculty. Many of the programs that were initiated strengthened both the Department of Physiology and the institution as a whole, while the same program could produce negative effects on one or another administrator or person. As one would predict, divisiveness between departments developed over implementation of new programs, assignment of time in the curriculum, and allocation of space, personnel, and money. As might also be expected, on individual issues, some departments were hurt and some would reap benefits. An institution seems to reach maturity when changes produce both positive and negative results.

The final end of the intermediate years is probably marked by July 1978, when Dr. Sidney Solomon resigned as chairman after serving 15 years. He was replaced by Dr. Donald Priola, who still serves in this capacity.



Left: Gerry Weiss; *right*: Donald Priola.

Latest Years—Maturation of Department

Physiology at the University of New Mexico is only 23 years old, very young when compared to other institutions. Nevertheless, it has reached a mature stage of development in that it probably is hard now to distinguish its structure and role from Departments of Physiology at other institutions. We have had all of the indi-

cations of success that are thought to show departmental achievement. The research of the Physiology Department at the University of New Mexico has been recognized. Faculty are invited to present papers at symposia and small meetings. They have served and continue to serve on study sections of NIH, review panels of the National Science Foundation, as well as other research review and award groups of other agencies.

The quality of individual faculty has been recognized in that other institutions have tried to recruit them and in some instances have been successful. "Second-generation" scientists who have spent time at this institution are starting to make their mark on physiology. Dr. Harold Sonnenberg has already been mentioned as being an early postdoctoral appointee. He is better known for his pioneering work on atrial natriuretic factor. Harold Spurgeon, Ph.D., a postdoctoral associate of Dr. Priola, has reached a responsible position in the NIH geriatric institute at Baltimore while Yang Park, Ph.D., another postdoctoral appointee, is Chairman of Physiology at Yonsei University in Seoul, Korea. Some of our locally produced doctoral awardees are also starting to be known. three of them are William Alter, who is now Chief of the Chemical Defense Division at Brooks Air Force Base in Texas; Jay Lyons, who has become Professor and Chairman of the Southeastern College of Osteopathic Medicine at North Miami Beach Florida; and Gary Malvin, who will be returning to Albuquerque as an associate of the Lovelace Foundation.

These statements lend a positive note to this history. However, there are problems facing this Department that are also the problems facing other departments. One can mention increased difficulty of research funding at this time, curricular changes that interfere with the quality of teaching of physiology, difficulty in obtaining adequate support for the University as a whole (and indirectly, thereby, of the Department), and reduced applications for graduate school (although this trend may be changing). Another view that may be forthcoming from this accounting is that a department has developed to maturity when societal problems such as anti-intellectual attitudes and changes in funding patterns are predominant in determining academic activities, rather than the philosophy and idealism of the early years. Many of the problems that we face result for the most part from factors outside the University, Medical School, and Department.

One additional development has occurred within this Medical School that may have impact on the future development of all departments. A new teaching program, The Primary Care Curriculum, has been instituted at this Medical School on an experimental basis. It is based on a problem-solving approach, with the teaching unit being a five-student tutorial. In 1986, 25 students were bring served by this program. The tutors are all faculty who are involved for a period of eight weeks. Students learn the basic sciences through studying those topics that are necessary to understand and resolve the clinical problem. As might be expected, such a program is controversial. As might also be expected, the Department of Physiology was one of the early ones where faculty became involved extensively. The breadth of biological knowledge needed by physiologists make them well suited for their jobs as tutors. More important the program has served as a vehicle for enabling faculty members from different departments to get to know each other. The program could well serve to provide a focal point that could produce an increase in interdisciplinary research as well as teaching. In any event, although we are now a mature organization, we are not so conservative that we cannot participate in radical change. The writing of the next history of this Department will answer the questions as to whether the experiment improved teaching, the quality of the students graduated from our school, and the quality and breadth of research done by the faculty.

Special Events

APS Business Meeting

Wednesday, October 12, 5:30 P.M.–6:30 P.M.
Montreal Convention Centre, Room 408A

Bowditch Lecture

Wednesday, October 12, 4:30 P.M.–5:15 P.M.
Montreal Convention Centre, Room 408A

APS Public Affairs Workshop

Tuesday, October 11, Noon–1:30 P.M.
Montreal Convention Centre, Room 406B
Title: Influences of Animal Care Committee on Research
Moderator: David J. Ramsay

Practicum of Molecular Biology Techniques

(A series of four hands-on workshops presented in cooperation with a number of biotech companies)

Tuesday, October 11, 1:30 P.M.–4:45 P.M.
Wednesday, October 12, 8:30 A.M.–11:45 A.M.
Montreal Convention Centre, Rooms 404A, 404B, 405A, and 405B

John V. Croker Memorial Lecture

Monday, October 10, 11:30 A.M.–12:30 P.M.
Montreal Convention Centre, Room 407C

Bernard B. Brodie Award in Drug Metabolism Lecture

Monday, October 10, 5:45 P.M.–6:45 P.M.
Montreal Convention Centre, Room 407C

Torald Sollman Award in Pharmacology Oration

Tuesday, October 11, 11:30 A.M.–12:30 P.M.
Montreal Convention Centre, Room 411B

ASPET Society Business Meeting

Tuesday, October 11, 4:45 P.M.–6:00 P.M.
Montreal Convention Centre, Room 411B

Otto Krayer Award in Pharmacology Lecture

Wednesday, October 12, 11:30 A.M.–12:30 P.M.
Montreal Convention Centre, Room 411B

Social Program

Opening Reception

Sunday, October 9, 8:00 P.M.–10:00 P.M.
Complexe Desjardins, adjacent to the Montreal Convention Centre

APS Past President's Address and Societal Banquet

Tuesday, October 11, 6:45 P.M.–10:00 P.M.
Queen Elizabeth Hotel, Duluth Room
Topic: A Physiologist in Africa Lending a Helping Hand in Developing Countries
Speaker: Harvey V. Sparks, Jr.

Luncheon Meeting of APS History Group

Wednesday, October 12, Noon–1:30 P.M.
Montreal Convention Centre, Room 410C
Topic: Aspects of the History of Soviet Space Flights
Speaker: Vanessa Koslovsky

French Canadian Night

Wednesday, October 12, 7:00 P.M.–Midnight
Alza Restaurant, Complexe Desjardins

Montreal Symphony Orchestra

The Montreal Symphony Orchestra, conducted by Charles Dutoit, with guest soloist Emmanuel Ax, will be performing in Montreal on Wednesday evening, October 12. The program includes *Three Preludes de Palestrino* by Psitzner, *Symphonette* by Zemlinski, and *Brahm's Concerto No. 2* for piano. Discounted tickets are available at the box office on a space availability basis.

Theme: Growth, Development and Aging

Symposia, Tutorials, Lectures, and Workshops

Monday AM

Theme Symposium
- Nutritional and physiological approaches to the study of aging

Symposia
- Molecular biological approaches to the study of pharmacologic sciences
- Neurotransmitters in opioid analgesia I
- Current concepts in gravitational physiology

Tutorial
- Blood volume flow measurement in the rat
- Application of spreadsheet software to the analysis of physiological parameters

Lecture
- John V. Croker Memorial Lecture

Monday PM

Theme Symposia
- Angiogenic polypeptide growth factors
- Theories of aging

Symposia
- Interactions of xenobiotics with cytochromes P-450: implications for the perturbation of heme biosynthesis
- Neurotransmitters in opioid analgesia II
- Receptor-effector system of ANF

Tutorials
- Introduction to molecular biology (Mini-Course)
- Physiological biotelemetry—systems for wireless monitoring of unrestrained animals

Lecture
- Bernard B. Brodie Award in Drug Metabolism Lecture

Tuesday AM

Theme Symposia
- Age-related changes in excitation-contraction coupling metabolism in the heart
- Changes in organ systems with age

Symposia
- Targets for the pharmacologic and toxicologic modulation of cellular energy metabolism
- Molecular biology of the cardiovascular system (Mini-Course)
- Neurotransmitters in opioid analgesia III
- Altered vascular noradrenergic innervation and function in hypertension

Recent space flight results in gravitational physiology

Lecture

Torald Sollman Award in Pharmacology Oration

Tuesday PM

Theme Symposia

Age-related changes in adrenergic control of the cardiovascular system

Cellular mechanisms in the development of respiratory control

Renal growth and development

Symposia

Reactions of hydroperoxides in biological systems

Neuropeptides and thermoregulation

Recent advances in coronary blood flow pharmacology

Workshops

Integrative study in physiology and medicine

Influence of Animal Care Committee on research

Practicum of molecular biology techniques (Mini-Course)

Lecture

Behavioral interventions for somatic disorders: biofeedback and techniques from the deafferentation laboratory

Wednesday AM

Theme Symposia

Age-associated alterations in the metabolism and disposition of foreign compounds: relation to toxicity

The development of motor control

Thermoregulation: development and decline with age

Symposia

Modern developments in platelet pharmacology

Microgravity and the lung

Workshop

Practicum of molecular biology techniques (Mini-Course)

Lecture

Otto Krayer Award in Pharmacology Oration

Wednesday PM

Theme Symposia

Factors influencing drug disposition and drug response in the elderly

Intestinal growth and development

Pharmacological treatment of Alzheimer's disease

Symposia

The teaching of undergraduate, nonprofessional pharmacology

Thromboxane receptor antagonists in acute myocardial infarction

Workshop

Integrative study in physiology and medicine

Lecture

Bowditch Lecture: Control of hypertrophic versus hyperplastic growth of vascular smooth muscle

Thursday AM

Theme Symposia

Changes in receptor responses and neurotransmitters with age I

Regular exercise, growth and development

Symposium

Evolving concepts in liver injury

Thursday PM

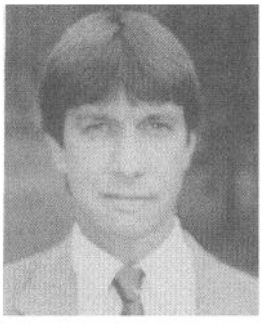
Theme Symposia

Changes in receptor responses and neurotransmitters with age II

Oxygen stress and aging

Ovarian follicular development and regression

APS Bowditch Lecture



Gary K. Owens, University of Virginia, Charlottesville, will present the Bowditch Lecture entitled "Control of Hypertrophic Versus Hyperplastic Growth of Vascular Smooth Muscle" at the APS Fall Meeting, Montreal, Canada, on Wednesday, October 12, 1988, at 4:15 P.M. in the Montreal Convention Centre.

Sessions and Symposia With Associated Abstracts

Integrative Study in Physiology and Medicine
APS Fall Meeting, Montreal, Canada
October 11, 12, 1988

Joseph Engelberg
Department of Physiology and Biophysics, Albert B. Chandler Medical Center, University of Kentucky, Lexington, Kentucky

The fourth annual "Workshop on Integrative Study in Physiology and Medicine" will be held at the 1988 Fall Meeting of the American Physiological Society on Tuesday and Wednesday, October 11, 12, 1988, from 12:00 to 2:00 P.M. The workshop is organized by a group of physiologists from seven universities in the United States, Canada, and the Phillipines. The focus of discussion will be the fetal and neonatal state, specifically, a medical case history describing multiple disease processes in a newborn child.

The principal purpose of these workshops is to provide a congenial atmosphere for the discussion of scientific questions free from limitations imposed by areas of specialization. Medical case histories serve as natural integrative devices since during a major illness an initial pathologic perturbation ultimately spreads its baneful influences to all parts of the organism. This transmission occurs in part via coupled feedback cycles as they maintain homeostasis. The involvement of the entire human body in disease allows medical case histories to serve as bases for discussion of phenomena from the molecular through the cellular, organ, organ-system, whole-body, and social levels.

A second purpose of these workshops is to work up medical case histories to introduce students in the health professions and graduate students to physiology at the level of the whole organism. Case histories selected for these workshops are among the most complex published in the medical literature. They are not simplified for teaching purposes. Rather, frameworks for thought are developed that enable individuals with even modest backgrounds in physiology to obtain significant insights into the physiology of the human organism and the physiologic basis of health, illness, dying, and death.

The medical case history to be discussed at the Montreal APS meeting follows.

Patient: A twenty-hour-old cyanotic infant suffering from congenital heart disease.

Source: *New England Journal of Medicine* 278: 496–504, 1968. Case 9-1968.

Speakers:

Robert L. Vick, Baylor College of Medicine

Edith Rosenberg, Howard University College of Medicine

Andrew M. Roberts, University of Louisville School of Medicine

Sidney Solomon, University of New Mexico School of Medicine

George P. Biro, University of Ottawa

Wayne Carley, Lamar University

Juliet Ver-Bareng, University of the East Ramon Magsaysay Memorial Medical Center

A twenty-hour-old male infant was admitted to the hospital because of respiratory distress and cyanosis.

The patient was the product of an uneventful forty-one-week pregnancy in a twenty-two-year-old primigravida. The vaginal delivery had been normal, and the infant weighed 8 pounds, 6 ounces. At the time of birth his color was fair, but he seemed to be in mild respiratory distress, with grunting respiration. Three hours later his color became dusky, and the respiratory distress increased. He was put into an oxygen atmosphere, with little change in his appearance until eight hours before entry, when the cyanosis deepened, and the cardiac rate rose to 200. The temperature was 102.2°F. Digoxin, 0.05 mg, penicillin, kanamycin sulfate and vitamin K were administered.

The mother and father were in good health. There was no family history of diabetes, heart disease, respiratory disease or congenital anomalies.

The patient was a cyanotic infant who appeared moribund. The respiration was gasping, with sternal and subcostal retraction. The head was normal; the fontanels were open. The skin was dry, without loss of subcutaneous tissue; the fingernails were long. The configuration of the chest was normal; a few breath sounds were audible, and there were fine rales in both lung fields; the respiratory excursion was poor. No cardiac murmur was heard. The abdomen was soft; the edge of the liver was felt 2 cm below the right costal margin, and the spleen was palpable at the left costal margin. The femoral pulses were of good quality. There was minimal response to painful stimuli.

The temperature was 97°F, the pulse 140, and the respirations 80.

The urine had a specific gravity of 1.016 and gave a ++ test for protein; the sediment contained 3 to 5 red cells per high-power field. The hematocrit was 64%, and the white-cell count 50,000, with 55% neutrophils, 7% band forms, 26% lymphocytes, 9% monocytes, 2% eosinophils and 1% basophils: there were 5 late erythroblasts and 1 normoblast per 100 white cells. The sodium was 140 mEq, and the potassium 5.5 mEq per liter. The urea nitrogen was 33 mg, and the fasting glucose 58 mg per 100 ml. A specimen of arterial blood, with the patient receiving 100% oxygen by funnel, taken via an indwelling umbilical-artery catheter, revealed that the partial pressure of oxygen was 35 mm of mercury, the partial pressure of carbon dioxide 110 mm of mercury, and the pH 6.82. An electrocardiogram demonstrated a sinus rhythm at a rate of 150, with an axis of +120°; the P waves were prominent in Leads 2, 3 and a VF; in Leads V_1 and V_2 the R waves were prominent but within normal limits for the age of the patient. X-ray films of the chest disclosed total homogeneous opacification of both lung fields with prominent air bronchograms; the diaphragmatic leaves lay at the level of the tenth ribs; the liver appeared normal in size.

Digoxin, penicillin and kanamycin sulfate were continued. A nasotracheal tube was inserted, curare was administered, and the lungs were ventilated with humidified 100% oxygen by a mechanical respirator. Culture of blood-stained material aspirated from the trachea yielded a moderate growth of klebsiella sensitive to tetracycline, chloramphenicol, streptomycin and colistin. Sodium bicarbonate was given intravenously. Four hours after entry, with the patient breathing 100% oxygen, a spec-

imen of the arterial blood showed that the partial pressure of oxygen was 26 mm of mercury, the partial pressure of carbon dioxide 71 mm of mercury, and the pH 7.06. Two hours later a Grade 2 systolic murmur was heard along the left sternal border.

On the *second hospital day* the patient's color was unchanged. The murmur was audible over the back and left upper portion of the chest. The arterial partial pressure of oxygen was 36 mm of mercury, and the partial pressure of carbon dioxide 62 mm of mercury; the pH was 7.36. Meralluride, 0.1 ml, was administered, but a diuresis did not result. An x-ray film of the chest showed marked clearing of the lung fields, with a few persistent mottled densities in the hilar regions; the heart appeared moderately enlarged; the pulmonary vascularity was not congested. The hemoglobin was 9.4 gm per 100 ml, and the hematocrit 29%; the white-cell count was 14,600, with 79% neutrophils and 6% band forms. The sodium was 143 mEq, the potassium 4.8 mEq, and the chloride 101 mEq per liter. Packed red cells, 40 ml, were given intravenously. An electrocardiogram showed a sinus rhythm at a rate of 125; the QRS axis was +140°; digitalis effect was demonstrated. On the *third hospital day* the right upper extremity and the right side of the thorax and head were less cyanotic than the remainder of the body, with a sharp line of demarcation down the midline of the sternum separating the pink skin on the right side from the blue on the left side. The sodium was 117 mEq, the potassium 4.0 mEq, and the chloride 83 mEq per liter. The urea nitrogen was 32 mg per 100 ml. A specimen of arterial blood, taken with the patient breathing 100% oxygen, revealed that the partial pressure of oxygen was 28 mm of mercury, the partial pressure of carbon dioxide 39 mm of mercury, and the pH 7.42. The inspired oxygen concentration was decreased to 40%, without change in the infant's color or condition.

On the morning of the *fourth hospital day* the nasotracheal tube was removed; the patient was able to breathe with minimal retraction. Four hours later the partial pressure of carbon dioxide was 41 mm of mercury, and the pH 7.40. X-ray films of the chest showed no change. In the evening the respiration became more labored, and the breath sounds were fainter; the cyanosis increased, and he became unresponsive. The partial pressure of carbon dioxide was 70 mm of mercury, the carbon dioxide content was 33 mEq per liter, and the pH was 7.28. The nasotracheal tube was replaced, with resumption of artificial ventilation. On the *following morning* seizures involving the left side were observed. A specimen of blood from the right radial artery, taken with the patient breathing 60% oxygen, revealed that the partial pressure of oxygen was 67 mm of mercury, the partial pressure of carbon dioxide 24 mm of mercury, and the pH 7.55; in a specimen of blood simultaneously obtained from the umbilical artery the partial pressure of oxygen was 25 mm of mercury, the partial pressure of carbon dioxide 33 mm of mercury, and the pH 7.51. The calcium was 5.6 mg, the phosphorus 5.6 mg, and the protein 4.4 gm per 100 ml. After the administration of calcium glucoheptonate the seizures subsided. He remained moderately cyanotic while on the respirator.

On the *sixth hospital day* the sodium was 134 mEq, the potassium 6.5 mEq, and the chloride 110 mEq per liter. The urea nitrogen was 9 mg per 100 ml, and the hematocrit was 58%. An electroencephalogram was within normal limits. On the *following day* an x-ray film of the chest showed areas of consolidation with slight loss of volume in each upper lobe. Artificial ventilation was continued. On the *eighth hospital day* a specimen of blood from the right radial artery, taken during the administration of 60% oxygen, showed that the partial pressure of oxygen was 65 mm of mercury, the partial pressure of carbon dioxide 35 mm of mercury, and the pH 7.43. The calcium was 9.3 mg per 100 ml. Curare was discontinued. The patient was unable to maintain adequate ventilation, with striking intercostal retractions and gasping respiration. On the *tenth hospital day* the cardiac rate slowed from 150 to 120 and diminished during the evening to 60. An electrocardiogram demonstrated complete bundle-branch block, atrioventricular dissociation and ectopic ventricular complexes. The peripheral pulses disappeared, and the infant died.

Normal Values at Birth

Test	*Birth*
Hemoglobin	15–24 g/dl
Hematocrit	44–64%
WBC count	9–30/mm^3
Neutrophil	45%
Lymphocytes	30%
Immature cells	10%
Sodium	134–144 mM/L
Potassium	3.7–5.0 mM/L
Calcium	7.0–12.0 mg/dl
Phosphorus	3.5–8.6 mg/dl
Chloride	96–106 meq/L
Fasting glucose	20–90 mg/dl
Heart rate	120–160/min
Respiratory rate	60/min for 1–2 days 25–40/min thereafter
Temperature	36.5–37.3°C (97.7–99.1°F)

Fall Meeting Mini-Theme on Molecular Biology

October 10–12, 1988

Montreal Convention Centre

Shu Chien

(see p. 59)

5.1

ISOLATION AND CHARACTERIZATION OF A NOVEL DIURETIC, NATRIURETIC AND HYPOTENSIVE PROTEIN FROM RAT ATRIA. T.G. FLYNN*, ANOOP BRAR*, LINDA TREMBLAY*, CHRISTINA LYONS*, DAVID HYNDMAN*, KATHY BENNETT*, INDER SARDA* and D.B. JENNINGS. Queen's University, Kingston, Ontario, Canada K7L 3N6.

During the purification of atrial natriuretic peptides (ANP's) from rat atria we isolated and purified a 20-residue peptide that had a unique amino acid sequence. This peptide was chemically synthesized and used to generate antisera for the development of a specific radioimmunoassay (RIA). The RIA allowed us to monitor the purification by gel-filtration and reverse phase high performance liquid chromatography of a larger protein (Mr = 10,500) than was originally detected. This protein exhibited diuretic, natriuretic, hypotensive and smooth muscle relaxing activity and the amino acid sequence revealed that a portion of the protein bore considerable homology to rANP (1-28). We have named the ANP homologous peptide iso-rat ANP (iso-rANP) since it seems to be an isomeric form of rANP. The presence of this peptide in atria and the discovery of brain natriuretic peptide clearly indicates that atrial natriuretic factor is comprised of a family of ANP's all of which are distinct gene products. Supported by Queen's University.

5.2

ANP-LIKE RAT ATRIAL FACTOR (iso-rANP) HAS SIMILAR BUT LESS POTENT CARDIOVASCULAR AND RENAL EFFECTS AS ATRIAL NATRIURETIC PEPTIDE. D.B. JENNINGS, I.R. SARDA* AND T.G. FLYNN*. QUEEN'S UNIVERSITY, KINGSTON, ONTARIO, K7L 3N6.

We have isolated, purified, sequenced and chemically synthesized a novel peptide (30 amino acids) from rat atria which exhibits homology with rANP. The native peptide and synthetic peptide were examined physiologically. For bioassay, Sprague-Dawley rats were anesthetized with sodium pentobarbital and given a constant infusion of saline. After control measurements, a bolus injection of vehicle, native peptide or synthetic peptide was administered I.V. and measurements obtained over 20 min. In this preparation, 2 μg rANP (1-28) gave a decrease in mean arterial pressure of -21%, a mean decrease in heart rate of -10%, and a renal response with increased urine volume (+687%) and Na+ excretion (+1574%). A comparable hypotension was obtained with 10 μg of the new atrial peptide, iso-rANP, but there were slightly lesser heart rate and renal responses than with rANP. There was also a less potent vasorelaxant effect of the native peptide or iso-rANP on rabbit aortic smooth muscle in comparison with rANP. These studies indicate that the atria produce at least two genetically different peptides, exhibiting homology within the disulfide ring, which have similar cardiovascular and renal activities. Supported by Queen's University.

5.3

EFFECTS OF INTRAVENOUS NOREPINEPHRINE ON URINE FLOW, HEMODYNAMICS, AND PLASMA LEVELS OF VASOPRESSIN AND ATRIOPEPTIN IN NORMAL AND CARDIAC-DENERVATED DOGS. Jialong Zhu,* Bin C. Wang, Robert J. Leadley, Jr.* and Kenneth L. Goetz. St. Luke's Hospital and Foundation, Kansas City, MO 64111.

Norepinephrine (NE) infused intravenously into anesthetized dogs produces a decrease in plasma vasopressin (AVP) and a water diuresis. It has been suggested that the decrease in plasma AVP may cause the diuresis. We now describe the effects of intravenous NE on plasma AVP and urine flow in conscious dogs. After a 30 min control period, NE (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused into sham-operated (SO) and cardiac-denervated (CD) dogs for 30 min. Norepinephrine increased urine flow ($P < 0.05$) in the CD dogs but not in the SO dogs; plasma AVP levels, however, did not change in either group. The NE infusion increased mean arterial pressure (MAP), left atrial pressure (LAP) and central venous pressure (CVP), and decreased heart rate (HR) in SO dogs; in CD dogs NE infusion increased MAP and HR, decreased LAP, and caused no change in CVP. Plasma levels of atriopeptin tended to increase during NE infusion in SO dogs, but decreased in CD dogs. These results demonstrate that NE infusion does not alter plasma AVP in conscious dogs. Therefore the diuresis that occurs during NE infusion is not caused by AVP in these dogs. Our results also suggest that the change in plasma atriopeptin induced by the infusion of NE is mediated by the concomitant change in atrial pressure.

5.4

OPPOSITE EFFECTS OF SHORT TERM (1 HR) OR LONG TERM (18 HRS) ANG II INCUBATION ON ANF-STIMULATED cGMP IN VASCULAR SMOOTH MUSCLE CELLS. Pierre Roubert*, Pierre-Etienne Chabrier*, Pascale Plas* and Pierre Braquet. I.H.B. Res. Labs. 1, av. des Tropiques, 91952 LES ULIS, France.

Atrial natriuretic factor (ANF) and angiotensin II (ANG II) act as physiological antagonists in the control of blood pressure and fluid homeostasis. The biological action of ANF in vasculature (i.e. vasorelaxation) is attributed to a stimulation of cGMP production although only a small number of ANF receptors present in vascular cells is coupled to guanylate cyclase. The present study investigated the effect of ANG II preincubation on ANF-induced cGMP production in rat cultured smooth muscle cells.

cGMP production induced by ANF and binding of ^{125}I-ANF were measured after 1 hr and 18 hrs preincubation of the cells (10^6 cells/dish) at 37°C with ANG II (10^{-6}M). After 1 hr preincubation, ANG II attenuated ANF (10^{-7}M)-induced cGMP production (control and ANG II : 13 and 10 pmol/10^6 cells/5 min, respectively) without significantly modifying the number of ANF binding sites. On the contrary, 18 hrs preincubation with ANG II enhanced the action of ANF (10^{-7}M) on cGMP production (control and ANG II : 14 and 24 pmol/10^6 cells/5 min, respectively) and paradoxally diminished the total number of ANF binding sites by 66 %.

These results suggest that short term effect of ANG II tends to decrease ANF-stimulated cGMP whereas long term effect, corresponding to an adaptation of the cells to ANG II, increases the biological response of ANF by down-regulating the non guanylate cyclase-coupled ANF receptors.

5.5

HOMOLOGOUS ANF RECEPTOR REGULATION : DISTINCT REGULATION OF COUPLED AND NON-COUPLED GUANYLATE CYCLASE RECEPTORS. Pierre-Etienne Chabrier*, Pierre Roubert*, Michèle Harle*, Pascale Plas* and Pierre Braquet. I.H.B. Res. Labs. 1, av. des Tropiques, 91952 LES ULIS, France.

Two subtypes of atrial natriuretic factor (ANF) receptors are present in vascular cells : one that stimulates cGMP production, the other (about 95 % of the total number of ANF binding sites) not coupled to guanylate cyclase. Since ANF receptors are sensitive to homologous down-regulation, we examined the time course of the effect of ANF preexposure of rat cultured vascular smooth muscle cells on ANF binding and cGMP responsiveness.

Preincubation of the cells in DMEM at 37° C with ANF (10^{-7}M) showed a rapid diminution of the number of ANF binding sites (- 70 % from control binding) as measured by ^{125}I-rANF binding assay, without modification of the K_d value (0.3 nM). The maximal effect appeared after 30 min and persisted at 18 hrs. A slower decrease of the response of ANF-stimulated cGMP production was observed after ANF preexposure - cGMP (pmol/10^6 cells/5 min) induced by 0.1 μM ANF were from control cells vs ANF treated cells ; after 2 hrs : 15.2 vs 12.0, after 6 hrs : 14.3 vs 9.8, after 18 hrs : 13.7 vs 4.9, respectively.

After 18 hrs preincubation with ANF (10^{-7}M) followed by an extensive washing and a reincubation in DMEM, about 85 % recovery of ANF binding was measured after 2 hrs whereas almost no recovery of cGMP responsiveness was noted after 6 hrs.

These results suggest a different regulation of the two ANF receptors subtypes reflecting probably distinct physiological roles.

5.6

KINETICS AND DYNAMICS OF rANF(99-126) IN CONSCIOUS RABBITS. S. Marleau,* H. Ong*, A. DeLéan and P. du Souich. Faculty of Pharmacy and Department of Pharmacology, Université de Montréal, Canada.

The aims of this study were 1) to compare the dynamics of a bolus of rANF(99-126) (ANF) to those of an infusion, and 2) to relate the effects to ANF plasma concentrations in conscious rabbits. After the bolus, ANF kinetics were first-order, with an estimated half-life of 0.8 $\pm$ 0.1 min (SEM), an apparent volume of distribution of 139 $\pm$ 9 ml/kg and a systemic clearance (Cl_{ANF}) of 132 $\pm$ 10 ml/min/kg. A reduction in Cl_{ANF} to 59 and 70 ml/min/kg ($p < 0.01$) was observed following ANF infusion at rates of 81 (x 140 min) and 126 ng/min/kg (x 480 min), respectively. A bolus up to 300 ng/kg produced a transient dose-independent increase in diuresis from 8 to 28 ml/h ($p < 0.05$). During ANF infusion, a good correlation was observed between the percentage reduction in mean arterial pressure, renal plasma flow and the change in the concentration of ANF. ANF effects on renal excretion parameters were negligible. ANF infusion decreased plasma renin activity but was followed by an abrupt increase after 5 hours of infusion. We can conclude 1) the kinetics of ANF are zero-order after an infusion, 2) this non-linearity is secondary to a reduction in Cl_{ANF}, and 3) the renal effects are minimal, possibly due to activation of counter-regulatory mechanisms. (Supported by a grant from Canadian Heart Foundation).

5.7

BRAIN AND PITUITARY ATRIAL PEPTIN AND ITS ROLE IN OSMOREGULATION OF EURYHALINE FISH. Sara M. Galli*, Birgitta Kimura* and M. Ian Phillips. Department of Physiology, University of Florida, Gainesville, FL. 32610.

We have reported the presence of AP-like peptide in plasma and brains of teleost fish (Galli et al., Fed. Proc., 1988). To further investigate the possible participation of AP in fish osmoregulation, we studied two euryhaline species, Opsanus tau (toadfish) and Mugil cephalus (mullet). We determined the main site of brain AP production and the forms produced. Second, we correlated brain, pituitary, spinal cord and plasma AP responses in fish from seawater adapting to lower salinities for 24 hours, 4, 7 and 10 days. The highest levels of brain AP were found in the hypothalamus (HYP) of mullet (100-150 ng/g) and 40-70 ng/g in toadfish HYP. Adaptation of toadfish for 24 hours to 50% SW increased HYP-AP from 29.96 ± 1.89 to 43.80 ± 4.29 ng/g tissue. Plasma AP decreased from 47.85 ± 11.95 to 21.38 ± 8.71 pg/ml. Longterm adaptation (7-10 days) to 50% SW enhanced these responses. If fish are back to SW, plasma AP returns to control SW levels. In mullets adapted to 75 and 50% SW for 24 hours or 4 days, HYP-AP did not change significantly. After 10 days in fresh water, AP in the HYP increased from 57.32 ± 4.97 to 70.74 ± 6.50 ng/mg tissue. Pituitary AP increased after adaptation to 50% SW and freshwater. Gel filtration of extracted brain AP revealed a low and a high molecular weight AP. Our results indicate high concentrations of AP in the HYP region and suggest that pituitary, HYP and plasma AP are involved in the osmoregulation control of euryhaline fish. (Funded by: Am. Heart Assoc.)

5.8

NEUROMODULATORY EFFECT OF ATRIAL NATRIURETIC FACTOR ON PURINERGIC NEUROTRANSMISSION IN RABBIT ISOLATED VASA DEFERENTIA. J.G. Drewett*, G.R. Marchand, and G.J. Trachte. Depts. of Pharmacology & Physiology, Univ. of Minnesota-Duluth, School of Medicine, Duluth, MN 55812.

Previously reported results from this laboratory (FASEB J. 2: A310, 1988) showed that ANF (rat, 101-126) had an inhibitory neuromodulatory effect on adrenergic neurotransmission in the vas deferens. The present study considers the ANF effect on purinergic neurotransmission in the rabbit vas deferens. Vasa deferentia were placed in organ baths containing Krebs-bicarbonate buffer at 37°C, passed through platinum electrodes and electrically stimulated over the frequency range of 0.5-12 Hz. ANF concentrations of 10^3, 10^4, & 10^5 pM shifted the frequency-response curve to the right of control in a statistically significant manner. ANF (10^1-10^5 pM) inhibited electrically-induced purinergic force at 4 Hz in a concentration-dependent manner, for every 10-fold increase in ANF concentration there was a 5% decrease in purinergic force. ANF (10^2-10^5 pM) had no effect on adenosine 5' triphosphate(ATP)-induced contractions. Therefore, the inhibitory effect of ANF on purinergic neurotransmission appears to be prejunctional on the release of ATP from the nerve. At present attempts are being made to measure electrically-induced ATP release from the vas deferens. (Support: PHS RO1 HL35934 and BRSG SO7 RR05896)

5.9

VAGOTOMY AFFECTS RENAL AS WELL AS CARDIOVASCULAR RESPONSES TO ATRIAL NATRIURETIC PEPTIDE. S.E. Robertson*, T.G. Flynn*, and D.B. Jennings. Queen's University, Kingston, Ontario, K7L 3N6.

Ackermann et al. (Can. J Physiol. Pharmacol. 62:819-826,1984) reported that vagal afferents, in inactin anesthetized rats, were involved in the cardiovascular, but not the renal, responses to I.V. injection of atrial extracts containing ANF. We studied 250 to 300 g Sprague-Dawley rats anesthetized with sodium pentobarbital. Vagotomy not only completely abolished the hypotension and bradycardia associated with injections of ANP (1-28) between 2 to 6 μg, but reduced increases in urine flow, and Na+, K+ and Cl- excretion to about one-third those seen in intact rats. We therefore examined the effects of infusions of rANP (2 μg·kg-1·min-1) in similar groups of intact and vagotomized rats anesthetized with inactin. In intact rats, infusion of rANP caused a transient 2000% increase in urine volume, compared to controls, within the first 10 min. In contrast, vagotimized rats did not exhibit a significant increase in urine volume in this same time period compared to sham vagotomized rats. Unlike Ackermann et al. (1984), we conclude that the vagus nerve is important in mediating the effects of ANP on water and electrolyte excretion in the kidney. Supported by Queen's University.

5.10

RELEASE OF ATRIAL NATRIURETIC FACTOR IN RESPONSE TO VOLUME EXPANSION IN CONSCIOUS, DIABETIC RATS. R.A. Hebden*, M.E. Todd*§, S. Sanderson* and J.H. McNeill. Div. Pharmacology & Toxicology, Fac. Pharmaceutical Sci., & §Dept. Anatomy, Fac. Medicine, Univ. British Columbia, Vancouver, Canada, V6T 1W5.

We have examined the effect of a 25% blood volume expansion on the release of Atrial Natriuretic Factor (ANF) in conscious Wistar rats (13-15 weeks old) treated 6 weeks earlier with streptozotocin (STZ, 55 mg/kg) or saline (Sal). The STZ-treated rats showed a significant ($P<0.05$) resting hypotension (132±2/91±1 mmHg, systolic/diastolic) and bradycardia (340±5 beats/min) compared to the controls (143±2/98±2 mmHg; 377±8 beats/min). Resting plasma ANF levels were slightly, but significantly ($P<0.05$) elevated in the STZ-treated rats (STZ: 87±4 pg/ml; Sal: 72±4 pg/ml) although resting right atrial pressures (RAP) were not different (STZ: 5.2±0.6 cmH_2O; Sal: 6.0±0.8 cmH_2O). Volume expansion with donor blood significantly elevated plasma ANF levels in both groups, but the increase in the control group (+527±80 pg/ml) was significantly ($P<0.05$) greater than that of the diabetic group (+323±45 pg/ml). Both groups showed similar elevations in RAP (Sal: +1.8±1.3 cmH_2O; STZ: +1.6±0.4 cmH_2O). Morphological studies are currently in progress in order to determine the possible cause of the impaired release of ANF in the diabetic animals.

This work was supported by the MRC (Canada) and the B.C. Heart Fdn. RAH is a B.C. Heart Fdn Post-Doctoral Fellow.

5.11

INDICES OF FLUID STATUS DURING PROLONGED PHYSICAL AND PSYCHOLOGICAL STRESS. Konstantine Kalogeras*, Bonnie L. Smoak*, George P. Chrousos*, Philip W. Gold* and Patricia A. Deuster. National Institutes of Health and Human Performance Laboratory, Uniformed Services University of the Health Sciences, Bethesda, MD 20892.

Indices of fluid status were assessed in 37 US Navy men (age 21.9 ± .5 yrs; mean ± SEM) before (B) and after (A) a 5 day period of psychological stress, physical exercise, and sleep deprivation known as "Hellweek" (HW). Fasting blood samples and 24-hour urine collections were obtained, and dietary intake was monitored. Body weight increased by 2.1 ± 0.2 kg during HW despite an energy surplus which could explain a gain of only 0.24 kg. Mean sodium (Na) intake increased from 304 mmol/d BHW to 375 during HW, and plasma volume increased by 11.9%. Further, urinary Na excretion increased from 168 ± 14 mmol/24h BHW to 306 ± 30 AHW. Plasma atrial natriuretic factor increased from 31.4 ± 2.1 pg/ml BHW to 82.7 ± 5.9 AHW and aldosterone decreased from 21.0 ± 2.3 ng/dl BHW to 14.1 ± 1.7 AHW. There were no changes in serum osmolality, but edema was noted in the extremities of most subjects. In conclusion, indices of fluid status were markedly altered in men undergoing 5 days of psychological stress, continuous physical activity, and sleep deprivation. Whether these changes reflect adaptive responses to severe prolonged stress or simply an increase in Na intake remains to be elucidated. Supported by NMRDC NOOO2487WR13039.

5.12

ACUTE EFFECT OF CANCER CHEMOTHERAPY ADMINISTRATION ON PLASMA ANP AND AVP SECRETION. Lawrence G. Granger*, Konstantine Kalogeras*, Olga Zegla*, George Fountzilas*, Spyros Papaioannou*, George P. Chrousos* and Philip W. Gold* (Spon: Patricia A. Deuster). Ahepa General Hospital, Thessaloniki, and BPB/NIMH.Bethesda, MD 20892.

The effect of alternate monthly cycle administration of two cardiotoxic chemotherapeutic agents Epirubicin and Mitoxantrone on plasma atrial natriuretic peptide (ANP) and arginine vasopressin (AVP) secretion was studied in 7 patients with metastatic breast cancer. A 20 to 40 min infusion of either drug in 100 ml of 5% dextrose was administered (80 mg/m2, and 12 mg/m2 respectively). Blood samples were collected in EDTA 20 min before the infusion (BI), at the end of the infusion (EI) and 20 min after the end of the infusion (PI), for determination of plasma ANP and AVP levels by RIA. Plasma concentrations of Na, K, Ca remained in the normal range. Both agents induced an increase in ANP with a parallel decrease in AVP.

	Epirubicin		Mitoxantrone	
	ANP(pg/ml)	AVP(pg/ml)	ANP(pg/ml)	AVP (pg/ml)
BI	25.5±4.0	3.7+0.5	23.6±2.7	3.8±0.6
EI	50.3±10.8	2.9+0.5	42.9±13.1	3.2±0.6
PI	56.4±10.7	2.9+0.5	43.1±5.7	3.5±0.6
P value	< 0.01	< 0.05	< 0. 10	< 0.05

Administration of two different cardiotoxic chemotherapeutic agents was associated with ANP secretion. Whether this represents a direct cardiac effect of these agents or is a result of the fluid infusion remains to be elucidated.

6.1

EFFECT OF ENDOTHELIAL CELL INJURY ON VASODILATION OF SKELETAL MUSCLE ARTERIOLES IN VIVO. Akos Koller*, Michael S. Wolin, Edward J. Messina and Gabor Kaley, New York Medical College, Valhalla, N.Y. 10595.

To demonstrate the role of endothelial cells (EC) in dilation of skeletal muscle microvessels, we injured EC in a 40-50 μm segment of third order arterioles (16.6±2.7 μm) of rat cremaster muscle by employing the light/dye (L/D)technique (Rosenblum et al. Stroke;1987:18:927). Impairment of EC and selectivity of the injury were assessed by topical administration (100μl) of acetylcholine (ACH), known to cause EC-dependent relaxation of large vessels in vitro and adenosine (ADO), whose actions are not EC-dependent. Increases in arteriolar diameter before injury were 5.3±2.0, 7.9±1.8, 15.3 ±5.0μm to 10^{-7}, 10^{-6}, 10^{-5}M ACH, and 7.3±3.5, 11.9±2.9, 15.9 ± 4.1μm to 10^{-6}, 10^{-5}, 10^{-4}M, ADO, respectively. After L/D injury 10^{-7}M ACH caused vasoconstriction (-2.8±2.5μm); vasodilation to 10^{-6} and 10^{-5}M ACH was inhibited by 82% and 77%, respectively. In contrast, responses to ADO were unchanged, indicating that vascular smooth muscle reactivity was unaffected. In addition, after EC injury, arteriolar dilation to arachidonic acid was completely abolished. However, responses to prostaglandin (PG)E_2 were still present. Arteriolar dilation to bradykinin (0.1ug) was decreased 57% by indomethacin (10μg/ml) and the remaining dilation was completely eliminated following L/D injury. These results indicate the L/D injury is specific for EC and that EC can mediate arteriolar dilation, via prostaglandins and/or other EC-dependent processes. Supported by NIH HL37453 & Westch. Heart Assoc.

6.2

CHARACTERIZATION OF DIFFERENT ENDOTHELIUM-DEPENDENT RELAXING FACTORS RELEASED FROM CANINE ARTERIES

N.A. Flavahan * and P.M. Vanhoutte, Dept. of Physiol., Mayo Clinic, Rochester, MN 55905, U.S.A.

The present experiments were performed in order to assess possible differences in the endothelium-derived relaxing factors released from canine arteries. Rings of canine femoral and left circumflex coronary arteries were suspended for isometric tension recording in organ chambers filled with physiological salt solution, gassed with 95% O_2/5% CO_2 (37°C). In femoral arteries, endothelium-dependent responses produced by acetylcholine, bradykinin, UK 14,304 (alpha$_2$-adrenergic agonist) and calcium ionophore (A23187) were not affected by incubation of the rings with a phospholipase A_2 antibody (courtesy of L.M. Popescu), or by blockade of cytochrome P450 with metyrapone or SKF 525A. In coronary arteries, these interventions inhibited the endothelium-dependent responses to acetylcholine, but did not affect the endothelium-dependent relaxations evoked by bradykinin, UK 14,304 and A23187. Bioassay experiments suggest that the endothelium of both arteries release a cytochrome P450 metabolite in response to muscarinic stimulation but that the femoral artery is relatively insensitive to the factor. In both arteries, A23187 and activation of alpha$_2$-adrenergic and bradykinin receptors stimulate the release of a distinct factor, unrelated to the metabolism of arachidonic acid.

6.3

MODULATORY ROLE OF MAGNESIUM AND CALCIUM ON VASCULAR SMOOTH MUSCLE TONE BY ENDOTHELIUM-DEPENDENT AND ENDOTHELIUM-INDEPENDENT MECHANISMS.

M.E. Gold*, G.M. Buga*, R.E. Byrns*, K.S. Wood*, L.J. Ignarro. Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024-1735

The objective of this study was to determine the role of magnesium (Mg) and calcium (Ca) on EDRF formation/release and cGMP formation in bovine intrapulmonary artery (A) and vein (V). Rapid removal of extracellular (XC) Mg (1.2 mM) elicited E-dependent transient relaxation of precontracted rings followed by sustained E-independent contraction. Relaxation and increased cGMP levels were blocked by hemoglobin and methylene blue. Relaxation was enhanced by superoxide dismutase and M&B22948 and inhibited by pyrogallol. Removal of XC Ca (1.5 mM) reversibly inhibited relaxation produced by removal of Mg; readdition of Ca produced EDRF-dependent relaxation with elevated cGMP levels. Removal of XC Ca (but not Mg) caused smooth muscle relaxation, not associated with an increase in cGMP levels. In bioassay studies, perfusates from A or V were superfused over 3 E-denuded strips of A or V. Removal of Mg from the perfusion medium enhanced EDRF formation/release. Removal of Ca inhibited EDRF formation/release. The data suggest that Mg and Ca function as physiological antagonists in the endothelium to modulate vascular smooth muscle tone elicited by EDRF and cGMP. (Supported by HL35014)

6.4

SUPEROXIDE ANION MEDIATES ENDOTHELIUM-DEPENDENT CONTRACTIONS TO CALCIUM IONOPHORE A23187 IN THE CANINE BASILAR ARTERY.

Z.S. Katusic and P.M. Vanhoutte. Mayo Clinic, Rochester, MN 55905.

The calcium ionophore A23187 causes endothelium-dependent contractions in canine basilar artery. The present experiments were designed to determine whether or not superoxide anion is the endothelium-derived contractile factor mediating this response. Rings with and without endothelium were suspended for isometric tension recording in Krebs-Ringer bicarbonate solution. In rings with endothelium contracted with uridine 5'-triphosphate (UTP), A23187 caused concentration-dependent, endothelium-dependent further increase in tension. Removal of the endothelium, or treatment with indomethacin, superoxide dismutase, or superoxide dismutase plus catalase did not affect the contractions to UTP, but abolished the endothelium-dependent contractions to the calcium ionophore. Catalase and deferoxamine did not affect endothelium-dependent contractions to A23187. Superoxide anion generated by xanthine plus xanthine oxidase, in the presence of catalase, caused contractions of the rings without endothelium, which were abolished by superoxide dismutase, or inactivation of xanthine oxidase by heat. These results suggest that superoxide anions generated by the hydroperoxidase function of the endothelial cyclooxygenase, is the endothelium-derived contracting factor released by A23187 in the canine basilar artery.

6.5

ENDOTHELIAL DEPENDANT VASOCONSTRICTION (EDCF) IN HUMAN SAPHENOUS VEIN IS PROSTAGLANDIN MEDIATED.

Olav Thulesius & Hani Shuhaiber*, Faculty of Medicine, P.O. Box 24923 Safat, 13110 KUWAIT.

Human saphenous veins were obtained from donor vessels for cardiac by-pass surgery. Control (C) and de-endothelialised (DE) ring preparations were tested in organ baths with isometric tension recording. Dose-response curves were calculated for norepinephrine (NE, 10^{-8}-10^{-3}M). In contrast to arteries and as shown before (Thulesius et al.FASEB J 2:A 1481, 1988) the dose-response curves to NE were shifted to the left in C and the maximum response higher compared to DE, moreover acetylcholine (ACh) often induced a further constriction in precontracted rings. In 5 out of 6 rings addition of indomethacin shifted the dose-response curve to the right and reduced the maximum response, moreover it inhibited contraction to ACh and unmasked a relaxation. A possible candidate for EDCF in veins is the new 9 alpha, 11 beta-Prostaglandin F-2, a vasoconstrictor and inhibitor of platelet aggregation (cf.Pugliese et al. Adv.Prostagland., Thromboxane & Leucotriene Res. 17: 50, 1987). The PGI_2-analogue iloprost did not induce contraction.
Supported by Grant KU MR 019/KFAS 87-07-02.

6.6

PERTUSSIS TOXIN INHIBITS ENDOTHELIUM-DEPENDENT RESPONSES IN CANINE CORONARY ARTERIES

P.M. Vanhoutte, H. Shimokawa* and N.A. Flavahan*. Dept. Physiol., Mayo Clinic, Rochester, MN 55905, U.S.A.

The present experiments were performed in order to evaluate the effect of pertussis toxin, an inhibitor of G_i protein function, on endothelium-dependent responses in canine left circumflex coronary arteries. Arterial rings were suspended for isometric tension recording in organ chambers filled with physiological salt solution, gassed with 95% O_2/ 5% CO_2 (37°C). In arterial rings contracted with prostaglandin $F2_\alpha$, pertussis toxin inhibited the relaxations evoked by UK 14,304 and by acetylcholine, but did not affect the relaxations produced by bradykinin, adenosine diphosphate or by the calcium ionophore A23187. The potassium channel antagonists, procaine, quinidine and 4-aminopyridine also inhibited the relaxations produced by UK 14,304 and by acetylcholine but did not affect the relaxations produced by bradykinin, adenosine diphosphate or A23187. The results suggest that the effects of certain endothelial receptors are mediated by activation of the pertussis toxin-sensitive G_i-protein. The G_i-protein may initiate endothelium-dependent relaxation by activating potassium channels.

6.7

EFFECT OF ENDOTHELIUM ON THE BLUNTED RESPONSES TO ADRENERGIC AGENTS IN MESENTERIC ARTERY RINGS OF TERM-PREGNANT RATS. A. Parent*, J. St-Louis and E.L. Schiffrin. Clinical Research Institute of Montreal, Montreal, Canada H2W 1R7

Concentration-response (C-R) curves to adrenergic agents were measured on rings of the mesenteric arteries of non-pregnant and 21-day pregnant rats. In the latter, the C-R curves to norepinephrine (NE) phenylephrine (PE) were significantly shifted to the right by comparison to rings of non-pregnant rats. Maximum responses to each agent were similar in the two groups of tissues. In PE (0.3 μM) pre-contracted vessels, the C-R curve to isoproterenol was not significantly different in the two groups. The pD_2's of PE and NE were inversely related to the presence of endothelium. A drop of one unit of the pD_2 of each agent was calculated from the total absence to the full presence of functional endothelium. This relationship (pD_2 vs. endothelium) was similar in slope in tissues from non-pregnant and pregnant rats, but the intercepts were significantly different. The sensitivity of the rings to isoproterenol was proportional to the presence of endothelium, but the curves were similar in both groups of tissues. These results demonstrate that the sensitivity of mesenteric artery to adrenergic agents depends on the presence of the endothelium in tissues of both groups of rats. The endothelium of mesenteric arteries is not involved in the blunted responses to α-adrenergic agents during pregnancy.

6.8

DIFFERENTIAL EFFECTS OF CHRONIC ESTROGEN-TREATMENT ON ENDOTHELIUM-DEPENDENT AND INDEPENDENT RESPONSES IN RABBIT ARTERIES. VM Miller and PM Vanhoutte, Dept. Physiol., Mayo Clinic and Fndn., Rochester, MN 55905, USA.

Chronic treatment with estrogens can alter endothelium-dependent relaxations, sensitivity of adrenergic receptors and synthesis of prostanoids. In order to determine whether such changes occur with uniformity throughout the vasculature, rings of aorta and carotid arteries from estrogen-treated and untreated rabbits were suspended for isometric force measurements in vitro. Estrogen-treatment increased the sensitivity of aortas but not carotid arteries to norepinephrine. Endothelium-dependent relaxations to acetylcholine (in the presence of indomethacin) were unaffected by estrogen-treatment in either artery. However, endothelium-dependent relaxations to the calcium ionophore A23187 were enhanced in aortas from estrogen-treated rabbits. Arachidonic acid initiated endothelium-dependent contractions in both arteries; these were augmented by estrogen-treatment only in aortas. Prostacyclin initiated contractions of the smooth muscle in both arteries, which were enhanced by estrogen-treatment in aortas. These results indicate that chronic treatment with estrogen affects adrenergic and endothelium-dependent responses differently depending on the anatomical origin of the blood vessel. Estrogen-treatment can alter both the production and the sensitivity of the smooth muscle to prostaglandins. (Supported in part by NIH 31183 and AHA MN 85-F12.)

6.9

DIETARY MODULATION OF ENDOTHELIUM-DEPENDENT RESPONSES TO AGGREGATING PLATELETS IN PORCINE FEMORAL VEINS. K. Komori*, H. Shimokawa* and P.M. Vanhoutte, Department of Physiology and Biophysics, Mayo Clinic, Rochester, MN 55905, U.S.A.

To study the effects of high-cholesterol diet or dietary fish oil on endothelium-dependent responses in veins, Yorkshire pigs were fed a regular diet, 2% high-cholesterol diet (for 10 weeks), or regular diet plus cod-liver oil (30 ml/day for 4 weeks). Endothelium-dependent responses were examined in vitro in rings of porcine femoral veins. In control pigs, aggregating platelets caused endothelium-dependent relaxations, which were mediated mainly by adenosine diphosphate (ADP) and serotonin. In cholesterol-fed pigs, the platelet-induced relaxations were not altered, while in oil-fed pigs the endothelium-dependent relaxations to platelets, ADP and serotonin were augmented. In quiescent rings, platelet-induced contractions were significantly reduced in rings with endothelium taken from oil-fed pigs, while they were comparable in rings without endothelium among the three groups. Relaxations to the calcium ionophore A23187 or sodium nitroprusside and contractions to potassium chloride were comparable among the three groups. These results indicate that in porcine femoral veins, hypercholesterolemia does not affect, but cod-liver oil facilitates the endothelium-dependent relaxations to aggregating platelets due to the augmented responses to ADP and serotonin.

6.10

ROLE OF ENDOTHELIUM IN EXPERIMENTAL HEART FAILURE: NITROGLYCERIN IN CORONARY & PERIPHERAL BLOOD VESSELS J.Main*, C. Forster, P. Armstrong*. Univ.of Toronto, Ontario, M5S 1A8.

The influence of the endothelium on vascular relaxations in Heart Failure (HF) is unknown. Accordingly, we investigated the role of the endothelium in modulating nitroglycerin (GTN) responses following pacing-induced heart failure. The potency of GTN on endothelial intact (I) & denuded (D) rings of canine circumflex artery (CX) & saphenous vein (SV) was examined in precontracted vessels (n) from 5 control (C) and 5 HF dogs. EC50 (x±sem:nM) & maximum % relaxation (x±sem) for GTN are shown:

	Control			HF		
	n	EC50	Max	n	EC50	Max
I/SV	13	500±150	66±6	17	61±12 ^	75±3 ^
D/SV	5	280±150	55±5	4	30±13*^	88±2*^
I/CX	13	7.7±1.3	100±0	16	7.6±1.1	100±0
D/CX	14	3.3±1.1*	100±0	16	4.4±1.1*	100±0

*p<0.05 D vs I, ^p<0.05 HF vs Control

Because denudation in both C & HF rings enhances the relaxant effect of GTN, we conclude that the endothelium plays an inhibitory role in GTN relaxations in the CX and SV. At HF, the high potency & efficacy of GTN in the CX is unaltered in contrast to the SV which becomes more sensitive. The greater selectivity of GTN for the CX in C & HF rings and the enhancement of the SV response at HF, may reflect a lower activity level of the cGMP messenger system in the SV, relative to the CX. Supported by the Ontario Heart & Stroke Foundation

CARDIAC MUSCLE PHYSIOLOGY

7.1

RYANODINE DOES NOT ELIMINATE THE SR Ca CONTENT IN RESTING RAT OR Na LOADED RABBIT VENTRICULAR MUSCLE. Donald M. Bers and David M. Christensen* University of California, Riverside, CA 92521.

In rabbit ventricular muscle (Rbt V) rest decay of twitches and loss of SR Ca content (based on rapid cooling contractures, RCCs) are greatly accelerated by ryanodine (Ry) ($t_{\frac{1}{2}}$~90 s + 1 s) and rapid loss of cellular Ca during rest with Ry is observed with Ca-microelectrodes (Bers et al., Can. J. Phys. Pharm. 65:610-618, 1987). Rousseau et al. (Am. J.Physiol. 253:C364-C368, 1987) also reported that Ry "locks" the SR Ca-release channel in a partially open state. We hypothesize that during rest, Ry allows the SR Ca to leak into the cytoplasm where it can normally be rapidly extruded by Na/Ca exchange. When Rbt V is Na-loaded by Na-pump inhibition, Ry no longer abolishes RCCs induced after rest. In rat V, rest results in potentiation of the twitches and RCCs and the cells gain Ca during rest. Rat V also has a higher resting Na_i activity than Rbt V (Shattock and Bers, Biophys.J. 53:608a,1988) favoring Ca entry via Na/Ca exchange at rest. Here we show that where Ry abolishes RCCs in Rbt V, Ry only partially inhibits RCCs in rat V. If rat V is exposed to low $[Ca]_o$ during rest (40μM), then rest decay occurs and RCCs are abolished by Ry. However, simultaneous reduction of $[Ca]_o$ and $[Na]_o$ (at constant $[Na]^3/[Ca]$) with Ry results in RCCs similar to those in control Ry solution. These results support the above hypothesis and suggest that Na/Ca exchange is a critical determinant of the loss of SR Ca in both the absence and presence of Ry. Furthermore, it appears that the SR Ca-pump can, under some conditions, keep up with the SR Ca "leak" induced by Ry.

7.2

BIOCHEMICAL CHARACTERISTICS OF HETEROTOPICALLY TRANSPLANTED HEARTS. L. Hornby*, N. Hamilton*, T. Salerno*, D. Marshall*, R. Superina*, C.D. Ianuzzo. Depts. of Physical Education and Biology, York Univ., Toronto, Ont., M3J 1P3, Dept. of Surgery, Univ. of Toronto, Toronto, Ont., Dept. of Surgery, Hospital for Sick Children, Toronto, Ont., M5G 1X8

Functional characteristics of cardiac muscle are determined by three major biochemical systems: contractile, Ca^{2+} regulating, and metabolic. These systems are regulated primarily by hormonal status and functional demand. To determine the effects of functional demand on the myocardium, hearts from inbred rats were heterotopically transplanted into the abdomen of recipient rats. The ventricles of these transplants are unloaded, making this a perfused, beating, essentially non-working heart. Rats were sacrificed 30-34 days following surgery. Mean weight of control hearts was 752 mg ± 37, and donor hearts 406 mg ± 22. Metabolic enzyme activities were all significantly lower in the transplanted heart. In contrast, sarcoplasmic reticulum Ca^{2+} ATPase activity was the same for both groups.

	PFK	PHOS	CS	HADH	SR Ca^{2+} ATPase
		($\mu mol \cdot g^{-1} \cdot min^{-1}$)			($\mu mol \cdot mg^{-1} \cdot min^{-1}$)
Control	57.6±1.7	37.3±1.0	141.2±2.6	58.4±2.6	0.32±0.03
Transplant	26.0±3.1	22.1±3.6	80.2±9.5	23.8±3.0	0.27±0.04

PFK=Phosphofructokinase, PHOS=Phosphorylase, CS=Citrate Synthase, HADH=Hydroxyacyl-CoA Dehydrogenase, Values are expressed as $\bar{X}$ ± SEM

The data suggests a close relationship exists between functional demand and metabolic potential but do not show this same relationship for the Ca^{2+} regulating system. Supported by Ontario Heart and Stroke Foundation.

7.3

CONTRACTILE DYSFUNCTION IN SKINNED RAT PAPILLARY MUSCLES FOLLOWING HYPOCHLOROUS ACID OXIDATION D.W. ELEY,* H. FLISS,* AND B. KORECKY. UNIVERSITY OF OTTAWA, OTTAWA, CANADA. K1H 8M5

Hypochlorous acid (HOCl) is a powerful oxidizing agent generated by activated neutrophils, and may contribute substantially to the cellular necrosis after reperfusion of ischemic myocardium. Isolated rat papillary muscles contracting isometrically developed contracture when exposed to HOCl (300 μM) for 80 minutes. These muscles were then chemically skinned (5 mM EDTA, 0.1% Triton X 100) and stored (50% Glycerol, 5 mM EDTA, 20 mM Imidazole) at -20° C for up to 4 weeks. Tension development was recorded (31° C) at increasing [Ca^{++}] (pCa 9 to 4) by altering the Ca/Ca-EGTA ratio. When compared to control (no HOCl) skinned muscles, the oxidized skinned muscles developed less maximal tension ($p<0.05$ at pCa 5.0), suggesting that the contractile proteins were damaged. The pCa/relative tension curves were not different, suggesting that the sensitivity of the myofibrils to calcium was not altered. Protein SH (P-SH) levels were significantly lower in the HOCl treated muscles. Dithiotreitol (DTT 1 mM) treatment produced a restoration of P-SH, which correlated with a significant restoration of contractile function. We conclude that the decline in mechanical function observed in this oxidized skinned papillary muscle preparation is due in part to an alteration of the P-SH redox status within the contractile proteins themselves.

7.4

CHANGES IN CARDIAC ACTOMYOSIN WITH HALOTHANE AND ISOFLURANE ANESTHESIA. Theodore J. Schmahai* and Mario A. Inchiosa, Jr., Departments of Pharmacology and Anesthesiology, New York Medical College, Valhalla, NY 10595.

Rat (Fischer 344) cardiac actomyosin (ACTO) was isolated following halothane (HAL) and isoflurane (ISO) anesthesia. Intrinsic ACTO turbidity and ATP-induced superprecipitation (SPPT) were measured. HAL exposure at 0.2-1.4% for 4 hr and cumulative days (1-3) of exposure to 1% HAL (4 hr/day) were studied. ACTO turbidity and SPPT showed dose- and time-related changes: initial turbidity increased ($p<0.001$), rate of aggregation increased ($p<0.05$), time to peak aggregation decreased ($p<0.001$), and area under the time-turbidity curve decreased ($p<0.001$), indicative of a more intense and rapid interaction. HAL-induced changes in ACTO turbidity and SPPT exhibited a time-dependent reversal over a 4-15 day period. Semi-log regression curves of recovery were all significant ($p<0.01$). The $t_{\frac{1}{2}}$'s of recovery ranged from 5 to 10 days; this is consistent with contractile protein turnover in the heart. Biochemical analyses (ionic strength precipitation, lipid content, gel filtration chromatography, SDS gel electrophoresis and ^{19}F NMR) revealed no differences between ACTO from control and HAL-exposed rats. Effects on ACTO turbidity and SPPT were less with ISO anesthesia (1.0-2.0% for 4 hr). HAL anesthesia induced a higher initial state of aggregation of ACTO and an accelerated SPPT, which reversed *in vivo* with protein turnover.

7.5

THE TIME COURSE OF MYOCARDIAL STIFFNESS: DIFFERENCES FROM THAT OF VENTRICULAR ELASTANCE. O. Nwasokwa* and M. Bodenheimer* (SPON: N. Gootman). Long Island Jewish Medical Center, New Hyde Park N.Y. 11042.

Defined as the slopes of isochronal curves, myocardial stiffness and ventricular elastance are analogous. We therefore studied the time course of isometric stiffness, S(t), in 7 *in-situ* canine papillary muscles and contrasted it with the *known* time course of ventricular isovolumetric elastance, E(t). We recorded the time course of isometric force, F(t), at successive lengths above and below Lmax under servo control; plotted isochronal F-L curves at 2msec intervals with a computer and fitted them with polynomials. For each twitch, we computed S(t) as the slope of isochronal curves, $(\partial F/\partial L)$, , at successive points, 2 msec apart, where they intersected its F(t) curve. Whereas isochronal elastance is constant, isochronal stiffness increased with muscle length over most length ranges. Developed stiffness peaked at a mean length (±SD) of 87.8±3.4% Lmax. Between 76.3±6.1% L_{max} (designated Lmin) and Lmax, S(t) increased with time during contraction and decreased with relaxation. Below Lmin and above Lmax, S(t) decreased with contraction and increased with relaxation. At Lmin and at Lmax, S(t) was constant throughout the twitch. Thus S(t) varies with length but E(t) is known to be almost invariant with volume.

7.6

Thermodynamic Studies on the **Interaction** of ADP with Normal and Thyrotoxic Cardiac Myosins. G. Kaldor & D. Hoak
VAMC, Allen Park 48101 & Dept. of Pathology
Wayne State University, Detroit, MI

We studied the heat production of rabbit normal and thyrotoxic cardiac myosins at various nucleotide (ADP) concentrations 25°C and 15°C. In the presence of a large ADP excess, and at 25°C, the enthalpy of ADP-myosin interaction was -13.0 Kcal $.mol^{-1}$ for the normal and -17.5 with the thyrotoxic myosin. The free energy change of the same interaction was -6.5 and -5.5 $Kcal.mol^{-1}$ with the normal and thyrotoxic protein respectively. The entropy change of the ADP-myosin interaction was 21.8 with the normal and 43.60 $cal.K^{-1}.mol$ with the thyrotoxic cardiac myosin. The heat capacity change was -240 and -350 $cal.K^{-1}.mol^{-1}$ with the normal and thyrotoxic proteins respectively. In general the ADP-cardiac myosin interaction was characterized by a large negative enthalpy change suggesting a conformational change. The large decrease of heat capacity and increase of enthalpy may be connected to a hydrophobic effect in connection with the conformational change. The thyrotoxic cardiac myosin-ADP interaction showed qualitatively similar but significantly larger changes of these thermodyamic parameters than did the normal protein.

Supported by a VACO Grant

7.7

HYPERTROPHY DOES NOT ENHANCE CARDIAC FUNCTION *IN VITRO* IN AGED RATS. Marvin O. Bohlyt*, Julie A. Opiteck*, Karyn A. Esser* and Timothy P. Whitè. Department of Kinesiology and Institute of Gerontology, The University of Michigan, Ann Arbor, MI 48109-2214.

Myocardial function *in vitro* and myosin heavy chains (MHCs) were studied in hypertrophied hearts of adult (9-10 mo) and aged (25-28 mo) female F344 rats. Seven days after constriction of the ascending aorta, hearts from experimental and age-matched control rats were removed under ketamine/rompun anesthesia. Functional measures were made in an isolated perfused working heart preparation. Left ventricular mass of aged control hearts was 127% of the adult control value (484 ± 12 mg; X ± SEM). Aortic-constriction increased left ventricular mass to 110 and 112% of control values in adult and aged rats, respectively. Peak left ventricular pressure development *in vitro* was 150 ± 7 mmHg in adult control hearts and did not differ due to age. Following aortic-constriction, there was a trend for peak left ventricular pressure to increase in adult rats ($p \leq 0.17$) and decrease in aged rats ($p \leq 0.08$), resulting in a significant interaction for this variable ($p \leq 0.05$). Maximum rate of pressure development in control hearts of aged rats was 75% of the adult value of 11,264 ± 1,527 mmHg/sec and was not affected by hypertrophy at either age. α-MHC accounted for 83 ± 1% and 46 ± 3% of the total left ventricular MHC for the adult and aged control groups, respectively. Hypertrophy had no effect on the percentage of left ventricular α-MHC in the adult cohort, but increased the variance 5-fold in the aged group. The adaptive responses of the myocardium to aortic-constriction differ in an age-related manner, and the capacity for pressure development *in vitro* is not enhanced in hearts of aged rats.

Supported by American Heart Association of Michigan and NIH AG-06130 and AG-00114.

7.8

MECHANISM OF NEGATIVE STAIRCASE IN RAT VENTRICULAR MYOCARDIUM. R. Bouchard* and D. Bose, Depts. of Pharmacol. & Therap, Anesthesiology and Internal Medicine, Univ. of Manitoba, Winnipeg, Man, Canada, R3E 0W3

A progressive increase in stimulus frequency (Fs) results in a positive tension staircase in the myocardium of most species studied. However, in rat cardiac muscle increasing Fs causes a progressive reduction in tension development. This raises the question of whether the negative staircase is i) due to decrease filling of the sarcoplasmic reticulum (SR) as a result of a reduction of transsarcolemmal (TSL) Ca entry with the faster drive rates or ii) due to overloading of the SR due to increased amounts of Ca entering the cell at the faster stimulation rates. To test this hypothesis we used post-rest tension development (RT) and rapid cooling contracture (RCC) at different external Ca concentrations to guage sarcoplasmic reticular (SR) Ca release and storage. Increasing Fs from 0.2 to 0.5 or 1.0 Hz resulted in a significant decline in peak isometric tension development in 2.5 mM external Ca. When the external Ca was lowered to 1.25 mM, the contraction amplitude in response to a steady stimulus train at different Fs was reduced but the pattern of response was similar to the one seen at the higher external Ca concentration. Increasing Fs was found to significantly enhance the RT and RCC after 30, 120 and 240 seconds rest at both external Ca concentrations which is consistent with increased loading of the SR with Ca rather than under-loading. In summary, the negative tension staircase accompanying increasing Fs is inversely related to the changes in SR Ca content and release as measured by RT and RCC. Since a period of rest is needed to demonstrate the Fs-dependent increase in SR Ca pool size in the rat, these data suggest that decreased filling of the SR may not be the cause of the negative staircase in the rat heart. The results are consistent with a frequency-dependent decrease in release of activator Ca from the SR release site in the rat. *Supported by the MRC of Canada and Manitoba Heart Foundation.*

7.9

HEART-CYCLE TRIGGERED JET VENTILATION (CTJV) DURING ACUTE PULMONARY VASCULAR HYPERTENSION (PVH). J.M. Maarek, R. Naeije, and H.K. Chang. Biomedical Engineering Dept., Univ. of Southern California, Los Angeles, CA 90089.

In order to examine the effects of CTJV on pulmonary hemodynamics during PVH, we gradually raised pulmonary artery pressure (PPA) to 25, 35 and 45 mmHg in 7 dogs by injecting controlled amounts of glass beads (0.15 mm diameter). Pressures in the 2 ventricles (RVP and LVP), aortic pressure, cardiac output (CO), RV end-diastolic volume (EDV) and pulmonary vascular impedance were measured during brief apneic pauses and while the animals were jet-ventilated. The timing of the jet was synchronized to the heart rate (HR) and adjusted to occur during 1 of 4 phases: early or late systole, early or late diastole. Although CO was invariant when PPA was raised, stroke volume (SV) decreased linearly while diastolic RVP and EDV nearly doubled when PPA was 45 mm Hg. Characteristic impedance dropped by 50% when PPA was raised to 25 mm Hg and changed more moderately afterwards. No marked difference existed between apnea and CTJV but HR increased slightly and RV diastolic pressure and EDV decreased when the inflations occurred during systole. We conclude that cardiac function is minimally disturbed by CTJV but that CTJV brings no hemodynamic improvement during PVH. (Supported by NHLBI Grant HL-36908).

7.10

MYOCARDIAL RESPONSE TO ISOTONIC CONTRAST MEDIA DURING CORONARY ANGIOGRAPHY IN THE DOG. P. Millet *, M. Sestier *, A.M. Donadieu *, B. Bonnemain *, F. Sestier. Notre-Dame Hospital, Montreal, Quebec, Canada, H2L 4MT.

Hemodynamic effects (HE) of a 10 ml slow injection of 3 low osmolar contrast media (LOCM): sodium-meglumine Ioxaglate (IOXA), Iohexol (IOHE), Iopamidol (IOPA) and an isotonic saline solution (ISS) were studied during left coronary angiography (LCA) in 10 anestized dogs. IOXA is an ionic LOCM with physiological sodium concentration. IOHE and IOPA are non ionic LOCM (NI) with no sodium. Aortic flow and heart rate were not significantly altered by any solution. A biphasic variation (short decrease followed by an increase) of myocardial contractility (MC), left diastolic ventricular pressure (LDVP), first derivative of LDVP (dP/dt max) and systolic aortic pressure (SAP) was observed. Every LOCM increased LDVP, dP/dt max and decreased SAP more than ISS. IOHE increased LDVP more than IOXA, and decreased SAP more than IOXA and IOPA. NI increased dP/dt max more than IOXA. NI increased MC, SAP and decreased aortic arterial resistance more than ISS, whereas IOXA did not. Ionic LOCM seem to be safer in animals than NI. We conclude that sodium concentration close to physiological values minimize HE during LCA.

7.11

REGIONAL AND GLOBAL LV PRESSURE VOLUME LOOP AREA IN IN SITU CANINE HEARTS USING FAST CT. Namsik Chung*, Xuesi Wu* and Erik L. Ritman. Mayo Medical School, Rochester, MN 55905

We have confirmed that the relationship (R) (described by Suga et al) between LV pressure volume loop area (PVA) and myocardial oxygen consumption (mVO_2) is linear in anesthetized, closed chest intact dogs (N. Chung et al: The Physiologist 30(4):154, 1987). To evaluate regional LV PVA and mVO_2 R, 11 dogs (8 control, 3 β-block) were anesthetized with fentanyl and droperidol and ventilated with an N_2O/O_2 mixture. Regional LV chamber volume (cross-sectional area of LV chamber x thickness of slice scanned) were measured from fast CT (DSR) images of the whole LV at 33msec intervals throughout a cardiac cycle. The cardiac chambers were opacified with a right atrial injection of nonionic contrast agent (Iohexol 30cc). LV pressure was measured using a catheter tip micromanometer. PVA was varied by inflating a balloon in aorta or in inferior vena cava. Myocardial blood flow (MBF) was measured by radiolabelled microspheres and mVO_2 was calculated from the product of coronary arteriovenous difference of blood O_2 content and MBF. Global PVA (Y) related to regional PVA (X) as Y=0.52 x +12.0, r=0.787. For an hypothetical, spherical, LV we would expect the slope of the R to be 0.67. Under control conditions $LVmVO_2$ (Y), in mjoule/gm/cycle, related to regional PVA (X) value as Y=2.47 X +4.33, r=0.701 (n=24). With β-block, Y=2.31 X -1.49, r=0.904 (n=9). We conclude that like global PVA to mVO_2 R a linear regional LV PVA to mVO_2 R exists and it changes similarly with β-block.

CONTROL OF BREATHING 1

8.1

LARYNGEAL CONTROL OF AIRFLOW AND VOLUME AFTER CESAREAN SECTION BIRTH IN LAMBS. Alastair A. Hutchison,* John A. Wozniak,* Randal A. Otto,* Haan-Go Choi,* Philip C. Kosch, Robert M. Abrams. Univ. of Florida, Gainesville, Fl. 32610.

The aim of this study was to determine the laryngeal and diaphragmatic control of airflow pattern in newborn lambs. In 6 fetal sheep, mean gestational age 133+/-0.2 (SE) days, thyroarytenoid [TA], posterior cricoarytenoid [PCA] and diaphragmatic electrodes and a brachial arterial catheter were placed. After 7 days, the lambs were delivered by cesarean section (with spinal anesthesia) and a face mask with a pneumotachograph applied. The lambs (3M:3F; mean birthweight 3.4+/-0.3Kg) were asphyxiated at birth and 4 required intubation. They were studied awake, prone with their heads in a neutral posture. After the onset of sustained spontaneous respiration, the airflow pattern in each lamb was characterized, in early expiration, by zero or near zero flow, coincident with TA activity but without diaphragmatic post-inspiratory inspiratory activity. Expiratory PCA activity was reciprocal to that of TA, decreasing during the period of minimal expiratory flow but evident when expiratory flow increased during the latter part of expiration. Sighs augmented the expiratory braked pattern and initially resulted in increases in end-expiratory volume (EEV). By 3.5 hours, in 5 of the 6 lambs, the markedly braked pattern was absent, being replaced by decreased TA activity and increased expiratory PCA activity. The change in respiratory pattern did not appear to be related to differences in temperature, pH, $PaCO_2$ or PaO_2. After birth, activities of laryngeal muscles are temporally related to the patterns of airflow and volume and to increases in EEV. (Supported by an ALAF grant and NIH grant HL39858-01.)

8.2

THE PULMONARY CHEMOREFLEX IN NEWBORN RABBITS. Teresa Trippenbach, Dept. of Physiol., McGill Univ., Montreal, Quebec, Canada H3G 1Y6.

This study was performed on six one-day-old rabbits anesthetized with urethane. Diaphragmatic EMG (EMGdi), tidal volume (V_t), esophageal pressure (P_{es}) and blood pressure (P_b) were recorded. 15 and 25 μl of lactic acid (LA) injected into a jugular vein only slightly increased minute ventilation (V_E), V_t, mean inspiratory flow (V_t/T_I) and EMGdi. 50 μl LA (769 ± 33 μl/kg) resulted in an increase in respiratory rate (f) with or without increase in end expiratory volume and a decrease in V_t (response I) followed by an intermittent phase of breath-by-breath increase in timing parameters (response II) and increased V_E, V_t, V_t/T_I and EMGdi (response III). The first two responses were vagally mediated, while response III remained qualitatively the same after vagotomy. A decrease in P_b was dose dependent. LA did not affect the dynamic mechanical properties of the lungs. Our results may suggest that the pulmonary chemoreflex is mature at birth. However, the effective LA dose when normalized for body weight was about 8 times higher in newborns than that reported by others in adult rabbits. This low C-fibre sensitivity may represent a protective mechanism against respiratory and cardiac disturbances during the first hours of life when the absorbtion of the amniotic fluid takes place in the lungs. (Supported by the MRC of Canada and the Hospital for Sick Children Foundation, Toronto).

8.3

EFFECTS OF XANTHINE AND ADENOSINE ANALOGS ON CONTRACTILITY OF THE NEWBORN RAT DIAPHRAGM. K.L. McGilliard and L.C. Farrell. Dept. of Zoology, Eastern Illinois Univ., Charleston IL 61920.

Methylxanthines (MX) are commonly used in the treatment of neonatal respiratory disorders. Certain MXs have been found to increase diaphragmatic contractility and reduce diaphragmatic fatigue in adult animals and man. The effects of xanthine and adenosine (ADO) analogs were tested on isolated, electrically-stimulated diaphragms from 4- to 7-day-old rat pups to determine the role of ADO antagonism on diaphragmatic contractility in the neonate. Caffeine (CAF) (0.05 to 5 mM) increased twitch tension in a dose-dependent manner in both directly and indirectly (phrenic nerve) stimulated diaphragms. Directly stimulated diaphragms had significantly larger responses to CAF than did indirectly stimulated diaphragms. Theophylline also produced a dose-dependent increase in tension in directly stimulated diaphragms. Enprofylline (ENP), which lacks ADO antagonistic properties, increased twitch tension at 1 mM. ADO (1 mM) and N^6-cyclohexyladenosine (CHA) (1 mM) significantly inhibited contraction of the diaphragm, although the effects of ADO were transient. CHA reduced the response to CAF at all CAF doses, suggesting an antagonistic relationship. These data suggest that the MXs may stimulate diaphragmatic contractility by antagonism of ADO A_1 receptors. The observation that ENP stimulates contractility suggests that a second mechanism, perhaps inhibition of cyclic AMP phosphodiesterase, may also be involved. Supported by a PMA Foundation Research Starter Grant.

8.4

DEVELOPMENT OF THE CHICK EMBRYO: EFFECTS OF EGG MASS. Jacopo P. Mortola and Lijing Xu*. Dept. of Physiology, McGill Univ., Montreal, Quebec, Canada, H3G 1Y6.

We asked to what extent, within a species, differences in egg mass, hence in eggshell surface area and O_2 conductance, could affect the development of the avian embryo. Large (L, about 70 g) and small (S, about 55 g) fertile chicken eggs were simultaneously incubated and the embryos studied at day 18, i.e. before the onset of lung ventilation. Embryo mass and O_2 consumption ($\dot{V}O_2$) (measured with a manometric technique) normalized per mass of the freshly laid egg were higher in S than in L, while no differences occurred after normalization by egg surface area (S.A.). Egg water vapour conductance, which is proportional to O_2 conductance, was also found to be directly proportional to egg S.A. Hence, the mass and $\dot{V}O_2$ of the embryo are more closely related to the O_2 conductance of the eggshell than to egg mass, giving support to the concept that the avian embryo's $\dot{V}O_2$ is not an invariable species characteristic, but a variable dependent on O_2 availability. Hatching and viability did not differ between L and S, and the specific mass of heart and lung and their cellular (DNA) concentration were also similar between the two groups of embryos. Therefore, differentiation of tissues and organogenesis do not seem to be affected by the differences in total O_2 availability determined by the differences in egg mass, while they are probably more important than total tissue mass in setting the time of hatching (Que. Lung Ass.).

8.5

AIRFLOW LIMITATION IN NEONATAL CALVES WITH PULMONARY HYPERTENSION FOLLOWING CHRONIC HYPOBARIC EXPOSURE S.C. Inscore*, K.R. Stenmark*, C.G. Irvin. Pulm. Phys. Unit, Natl. Jewish Ctr. for Immun. and Resp. Med. and CVP Res. Lab., U. of Colo. Hlth. Sci. Ctr., Denver, CO 80262

Marked airflow limitation has been observed in human neonates with pulmonary hypertension. The neonatal calf chronically exposed to altitude develops severe pulmonary hypertension with functional and structural changes in the pulmonary artery. We postulated that airways might be similarly effected. At birth, one of a pair of age-matched calves (N = 5 pairs) were continually exposed to hypobaric hypoxia (HH group) at 4300m simulated altitude; the other remained at 1500m (C group). Measurements of dynamic lung compliance (C_{dyn}), resistance (R_L), static end-inspiratory respiratory system compliance (C_{RS}) and mean pulmonary artery pressure (MPAP) were made in both groups at 4300m before and following cumulative doses of nebulized methacholine (0.6-100 mg/ml). After 2 weeks of hypoxia exposure, MPAP increased (HH:120±7 vs C:35±1.7 mmHg). R_L increased (HH:4.87±0.56 vs C:2.60±0.19 $cmH_2O/L/s$, $p < 0.005$); further, C_{dyn} decreased (HH:0.079±0.008 vs C:0.105±0.01 L/cm H_2O, $p < 0.05$) without significant change in Crs (HH:0.49±0.002 vs C:0.58±0.008 L/cm H_2O) or methacholine responsiveness. Histologically, circumferential increases of fibrous tissue and smooth muscle in large airway were observed with increases in smooth muscle in terminal bronchioles. Following chronic hypoxia exposure in neonatal calves and associated with pulmonary hypertension, marked airflow limitation without hyperresponsiveness was observed, presumably due to fibrous proliferation of the central airways.

8.6

VOLUME RECRUITMENT AND AIRFLOW RETARDATION AFFECT MEASUREMENTS OF RESPIRATORY SYSTEM COMPLIANCE IN THE NEWBORN. F. Ratjen*, R. Zinman*, AR Stark*, MEB Wohl. Department of Pediatrics, Harvard Medical School, Boston, MA 02115

To assess respiratory system compliance (Crs) over a volume range exceeding the tidal volume (Vt), we used the expiratory volume clamping technique (EVC) [JAP 62: 2107, 1987] to study Crs in 10 healthy sleeping newborn infants during regular breathing. Volume changes were measured with a pneumotachograph attached to a face mask and confirmed by respiratory inductance plethysmography. A two port valve system was used to allow selective expiratory occlusions. The pressure-volume relationship of the EVC maneuvers was compared to Crs during tidal breathing using the passive flow-volume technique (PFV) [ARRD 129: 552, 1984]. The volume increase achieved by EVC with 3-8 breaths was 77 ± 30% of Vt ($\Delta V \pm SD$). Crs measured by the EVC technique was unexpectedly greater than during tidal breathing ($\overline{Crs} \pm SD$ ml/cmH_2O: EVC 6.41 ± 0.94, PFV 4.05 ± 0.63 $p<0.0005$, paired t-test). In 6 infants we compared PFV to the multiple expiratory occlusion technique (MO) [S.Afr.J. Med. 50: 128, 1976]. In 5 out of 6 infants Crs measured by PFV exceeded Crs measured by MO ($\overline{Crs} \pm SD$ ml/cm H_2O: PFV 4.17 ± 0.73, MO 3.19 ± 0.32 $p<0.01$). In summary: (1) Crs increases above Vt, likely due to volume recruitment. (2) PFV gives higher Crs values than MO, possibly because of expiratory breaking following end inspiratory airway occlusions. Crs measurements must therefore be interpreted with caution in the unsedated newborn. (Supported by NIH HL 34606 + DFG).

8.7

THE EFFECTS OF CLINICAL DOSES OF A SURFACTANT SUPPLEMENTATION ON IMMATURE RABBIT LUNGS. Alan J. Mautone*, Mary B. Cataletto*, Mala Chinoy* and Emile M. Scarpelli. Children's Hosp. of N.J., Newark; Research Center, Schneider Children's Hosp-LIJMC, New Hyde Park, N.Y.

A bovine surfactant preparation (BSP) in clinical doses intratracheally was assessed in immature fetal rabbits both by initial air volume-pressure diagram in vitro during high speed cinephotomicrography and in vivo during mechanical ventilation at the onset of breathing. Comparison of BSP to control in vitro revealed: (1) Aeration of BSP group was more rapid and to higher volumes (>2x) comparable to mature fetal lungs. (2) Volume acceleration, an index of recruitment, was 2x control. (3) Untreated lungs were unstable, while stability was established in BSP lungs by formation of intrasaccular bubbles with surfactant films of zero surface tension. Comparison in vivo revealed: (4) BSP lungs retained ≥75% Vmax at operational pressures in vivo, indicating high-risk to reduced pulmonary blood flow, impaired ventilation and parenchymal damage. (5) Bubble formation was not restricted to saccules, so that airways were frequently occluded (possibly because BSP dose exceeded normal lung concentration of surfactant. Desired effects of BSP [(1-3)] result from formation of intrasaccular bubbles. Undesired effects [(4-5)] probably result from excess foaming of BSP and require further study.

8.8

THE EFFECTS OF AGING ON THE FUNCTION OF THE CAROTID BODY. Roland M. Ikuta, P.A. Rechnitzer, D.H. Paterson, D.A. Cunningham. The Center for Activity and Aging, The University of Western Ontario.

The carotid body is known to partially control the level of steady state ventilation during exercise and is an important determinant of the rate of attainment of steady state ventilation after the onset of exercise. The purpose of this study was to determine the effects of aging on the ventilatory control function of the carotid body. Subjects' consisted of 7 young (Y,24+0.5y), 7 elderly (E,71+0.8), and 7 trained elderly (TE, 70+0.9) men. Each subject performed two submaximal cycle ergometer tests at oxygen concentrations of 12%, 15%, 21% and by eight minutes of a constant workrate. (90% of the anaerobic threshold at 12% oxygen). The inspired gas was switched to pure oxygen for 30 second intervals at minutes 2.5 and 6 (modified Dejour's test). The kinetics of ventilation at the onset of the workrate and the magnitude of change of ventilation during the pure oxygen intervals were determined. A breath by breath system of analysis was used to determine the ventilation and pulmonary gas exchange levels throughout the exercise. The lower oxygen concentrations (12%,15%) resulted in a faster change toward steady state ventilation at the onset of the workrate, and a larger decline in the ventilation in response to the pure oxygen for all three groups. There was a significant difference in both of these responses between the Y and E (p=0.018). The TE, however, had a similar response to that seen in the Y. The results indicate that the carotid body function is reduced in the elderly, by both parameters used in this study. However, this change seems to be a reflection of inactivity rather than aging.

Supported by NSERC Grant (A2787)

8.9

THE EFFECTS OF INHALED SALBUTAMOL ON RESPIRATORY MECHANICS, DEAD SPACE AND GAS EXCHANGE IN THE HORSE. L.R. Soma and A. Baldock*. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA 19104

Salbutamol, a β_2-agonist was administered by inhalation during a 4-minute period of rebreathing through a 10L dead space. This was to assure a constant tidal volume. The first 3 horses were treated with the drug and the second 3 with the saline. The procedure was later reversed, and 4.25 and 8.5 mg were administered in this manner. Baseline (BL) measurements were made, followed by inhalation of drug or vehicle and measurements at 15, 30 minutes and 1, 2, 3, 4 hours. The rebreathing method of administering the saline vehicle produced a slight decline in resistance (R_L) which returned to BL within 1 hour. The dose of 4.25 mg produced a significant reduction from BL and from the saline controls for R_L and significant increases in respiratory minute volume ($\dot{V}_E$) and respiratory flow (RF). These changes lasted for 1 hour. There were no changes in compliance (C_{dyn}), tidal volume (V_T) or frequency. After the 8.5 mg, there were significant reductions in R_L and transpulmonary pressure (P_{tr}) and increases in $\dot{V}_E$, RF and V_T. The R_L changes were present for at least 4 hours; the other for only 1 hour. The inhalation of 8.5 mg produced a significant increase in dead space (V_T/V_D) for the 4-hour study period. No changes were seen in O_2 or CO_2 gradients, blood gases or oxygen consumption.

METABOLISM

9.1

CYTOCHROME P-450 ACTIVITY IN HEPATOCYTE MONOLAYER CULTURES (MLC) TREATED WITH EPIDERMAL GROWTH FACTOR (EGF). Thomas H. Dickinson*, Shu-Whei Tsai and Darrell R. Abernethy. Brown Univ., Division of Clinical Pharmacology, Providence, RI.

Adult mammalian hepatocytes when placed in culture tend to dedifferentiate and express functions associated with fetal liver. Albumin production and activity of liver specific enzymes can be maintained for several weeks in serum free chemically defined medium. Cytochrome P-450 amount is maintained by the addition of specific inducing agents, but adult isozyme pattern is not. Epidermal Growth Factor (EGF) is known to stimulate hepatocyte DNA synthesis, and prolong viability in MLC. To evaluate the effect of EGF on P-450 activity in MLC, ethoxycoumarin deethylation (ECDE) was compared in microsomes prepared from adult rat hepatocytes cultured with 20ng/ml EGF in addition to standard growth medium.

	Control	EGF
Day 0	152±28 (100%)	152±28 (100%)
Day 1	9.5 (6.3%)	5.2 (3.4%)
Day 2	6.8 (4.5%)	9.8 (6.4%)
Day 3	14.7±9.1 (9.7%)	8.9±5.5 (5.9%)
Day 4	10.0±7.7 (6.6%)	5.7±3.8 (3.8%)
Day 5	10.4±7.8 (6.8%)	4.9±.2 (3.2%)
Day 6	16.6 (10.9%)	3.5 (2.3%)

Microsomal protein and DNA content and protein to DNA ratio were similar in both groups, ECDE activity declined more rapidly in cells treated with EGF. This may be consistent with the previously described EGF mediated shift toward less differentiated forms of cellular function.

9.2

OXYGEN DEPENDENCE OF ACETAMINOPHEN METABOLISM IN HEPATOCYTES FROM RATS EXPOSED TO CHRONIC HYPOXIA. T.Y. Aw*, A.H. Sillau, and D.P. Jones. Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322.

Chronic hypoxia was induced in rats by exposure to a controlled atmosphere of 12% O_2 for 8-9 days. Freshly isolated hepatocytes were incubated with 5 mM acetaminophen for 30 min under steady-state O_2 concentrations. Glucuronidation, sulfation and conjugation with glutathione were markedly inhibited at low O_2 concentrations. Formation of the sulfate conjugate was half-maximal (P_{50} value) at 1.8 μM O_2 compared to cells from normoxic (control) animals (3.8 μM O_2), and the maximal rate (0.73 ± 0.05 nmol/10^6 cells/min) was 70% that of control. The P_{50} for glucuronide formation occurred at 2.0 μM O_2, which is lower than that for control cells (4.5 μM O_2), but similar to that for sulfation. Maximal rate of glucuronidation (1.33 ± 0.11 nmol/ 10^6 cells/ min) was 68% of control. The P_{50} for glutathione conjugation (4.0 μM O_2) was similar to the control (4.5 μM O_2), but the maximal rate was 40% higher, consistent with an induction of cytochrome P_{450} during chronic hypoxia. The lower P_{50} values for acetaminophen metabolism in hypoxic cells are consistent with a lower P_{50} for oxidation of mitochondrial cytochromes in these cells. The results suggest that during chronic hypoxia, impairment of glucuronidation and sulfation reactions may affect the disposition of other endogenous and exogenous compounds metabolized by these pathways. Supported by NIH grant GM 36538.

9.3

INFLUENCE OF AGING ON ETHANOL-INDUCED MICROSOMAL DRUG METABOLISM ACTIVITIES. L.E. Rikans and C.D. Snowden*. Dept. of Pharmacology, Univ. of Oklahoma, Okla. City, OK 73190.

The effects of ethanol on drug metabolism have not been examined previously with respect to aging. The purpose of this study was to determine how aging affects the induction by ethanol of the hepatic microsomal monooxygenase system. Female Fischer 344 rats, aged 4, 14, and 25 months, were fed ethanol-containing or control liquid diets for 15 days. Liver microsomes were prepared and analyzed for cytochrome P-450, cytochrome c reductase, aniline hydroxylase, p-nitrophenol hydroxylase, p-nitroanisole O-demethylase and benzphetamine N-demethylase activities. Microsomal drug metabolism activities of rats fed the control diet decreased with age and were 25 to 30% lower in old rats than in young rats. Ethanol-induced activities, however, were 50 to 60% lower in the old rats. When considered on a body weight basis, old rats consumed less diet and less ethanol than did young rats. Thus, the induction of drug metabolism activities was proportional to the intake of ethanol. In order to equalize ethanol intake, a group of young rats was fed a diet with the ethanol content adjusted to produce the same intake as that of the old rats. The induction by ethanol in this group of young rats was the same as in the old rats. The results suggest that old rats are as susceptible as young rats to the effects of chronic ethanol intake on hepatic microsomal drug metabolism. Supported by NIA Grant AG04984 and a contract from OCAST.

9.4

METABOLISM AND MUTAGENICITY OF 1,2,3-TRICHLOROPROPANE. N.A. Mahmood*, L.T. Burka* and M.L. Cunningham* (SPON: L.S. Birnbaum). Systemic Toxicology, NTP, NIEHS, RTP, NC 27709.

1,2,3-Trichloropropane (TCP) is used as an industrial solvent and degreaser. It has been reported to be a contaminant in some agricultural pesticides and some underground water supplies and has also been identified in sediments of the Great Lakes. Preliminary evidence from an NTP study of TCP toxicity and carcinogenicity indicates that TCP administration results in an increased incidence of tumors in male and female F344 rats and B6C3F1 mice. We have undertaken studies to (1) determine the metabolism and distribution of TCP(14-C) in F344 rats and (2) identify mutagenic metabolites. Following an acute exposure of TCP at 30 mg/kg (8-10 μCi/animal, p.o.), approximately 50,20,and 20% of the dose was excreted in the urine, feces and as carbon dioxide respectively, at 60 hr. TCP derived radioactivity was most concentrated in liver, kidney and forestomach. Two urinary metabolites were isolated by HPLC and identified as N-acetyl-S-(3-chloro-2-hydroxypropyl) cysteine (ACPC) and 3-chloro-2-hydroxypropyl cysteine by NMR, mass spectroscopy and comparison with synthetic standards. Studies of mutagenicity in the Ames assay using TA 100, demonstrated TCP to be mutagenic in the presence but not in the absence of S9 or microsomes. Mutagenicity was found to decrease upon addition of glutathione. No mutagenicity was observed when urine or synthetic ACPC was added in the assay. The results indicate the important role of glutathione conjugation in the biotransformation of TCP.

9.17

Effect of Various Chemotherapeutic Agents Against B16BL6 Melanoma After Glutathione Depletion Brian D. Thrall* and Gary G. Meadows. Washington State University, College of Pharmacy and Pharmacology/ Toxicology Program, Pullman, WA 99164-6510.

The growth response of B16BL6 (BL6) murine melanoma cells to levodopa methylester (LDME), 1,3-(2-chloroethyl)-1-nitrosourea (BCNU), dacarbazine (DTIC), and bleomycin (BLEO) was determined after depletion of intracellular glutathione by the thiol-modulating agent buthionine sulfoximine (BSO). Exposure of BL6 cells to 50 uM BSO for 24 hours depleted intracellular glutathione to less than 15% of untreated cells. BSO was not toxic alone after 24 hours exposure, but growth inhibition was observed at 48 hours (24 hours after the BSO was removed from cells). Pretreatment of BL6 cells with BSO potentiated the cytotoxic effects of all drugs tested. These studies emphasize the importance of intracellular glutathione in detoxification of chemotherapeutic drugs in melanoma cells and suggest a role for BSO as an adjuvant in chemotherapy of malignant melanoma. (Sponsored in part by American Institute for Cancer Research Grant #85B79 and National Cancer Institute Grant #CA 42465.)

9.18

DISPOSITION OF THE CALCIUM CHANNEL BLOCKER TIAPAMIL IN THE RAT. S. Nawoot*, K. Talluri*, D.C. Mays* and N. Gerber. The Ohio State Univ. Col. of Med., Columbus, OH 43210

Following a 10 mg/kg i.v. injection of 14-C tiapamil (specific activity 2.0 mCi/mmol) to the rat, an average of 30 and 67% of the total radioactivity was recovered in 72 hr urine and feces respectively. Highest concentrations of tissue total radioactivity 0.5 hr after i.v. injection of the same dose were found in the small intestine (32% of the dose) and the liver (23% of the dose). Tiapamil was extensively metabolized in the rat. Only a small amount of the dose (< 1%) was excreted in bile as tiapamil while 13% of the dose was excreted as tiapamil in urine in 24 hrs. Reverse phase HPLC with ammonium formate/ acetonitrile gradient separated six major radioactive peaks in unhydrolyzed urine and bile. Hydrolysis with ß-glucuronidase showed three major conjugated metabolites. Nortiapamil,a N-demethylated product of tiapamil, was a major metabolite recovered in urine and bile and accounted for 11% of the dose.

BIOTRANSFORMATION I

10.1

MULTIPLE PATHWAYS FOR NEPHROTOXIC CYSTEINE CONJUGATE METABOLISM IN RAT KIDNEY HOMOGENATE. James L. Stevens, Paul B. Hatzinger and Patrick Hayden (SPON: S. Lau). W. Alton Jones Cell Science Center, Lake Placid, NY 12946

We have shown previously that S-(1,2-dichlorovinyl)-L-cysteine (DCVC) is a substrate for L-amino acid oxidase (LAOx) and cysteine conjugate ß-lyase (CBL). In this investigation we have characterized the structure activity relationship for cysteine conjugate metabolism by LAOx. Also, the relative roles of LAOx and CBL in the metabolism of cysteine conjugates by kidney homogenate were studied using aminooxyacetic acid (AOA) and 2-hydroxy-3-butynoate (2H3B), selective inhibitors of CBL and LAOx, respectively.

S-(1,2,3,4,4-Pentachlorobutadienyl)-L-cysteine (PCBC) and DCVC were the best substrates for LAOx. Several aliphatic halogenated conjugates were poor substrates. AOA was ineffective as an inhibitor of LAOx but 2H3B was a potent antagonist while having no effect on CBL. Using these selective inhibitors, the relative roles of the two enzymes were assessed in rat kidney cortex homogenates. LAOx contributed 30% and 40% of the metabolism of DCVC and PCBC, respectively. However, 2H3B did not block binding of [^{35}S]label from [^{35}S]DCVC to cellular macromolecules.

Based on previous studies (JBC 261, 15529, 1986) and these results, we suggest that in rat kidney homogenate as much as 65% of DCVC may be metabolized to its corresponding α-keto acid and only 35% by β-elimination.

10.2

EFFECT OF THE MECHANISM-BASED INHIBITOR 1-AMINOBENZOTRIAZOLE (ABT) AND THREE OF ITS N-ARALKYLATED DERIVATIVES ON THE CYTOCHROME P-450 (P-450) MONOOXYGENASE SYSTEM OF GUINEA PIG LIVER. Kimberley J. Woodcroft*, Edward W. Szczepan* and John R. Bend. Department of Pharmacology & Toxicology, University of Western Ontario, London, Canada N6A 5C1.

We previously reported that N-benzyl-ABT (BBT) and N-α-methylbenzyl-ABT (α-MB) are more potent and isozyme selective suicide inhibitors of P-450 than ABT in rabbit lung (Mol. Pharmacol. 30, 25, 1986). In the present study the ability of N-α-ethylbenzyl-ABT (α-EB), a newly synthesized analogue of ABT, to destroy spectrally analyzed P-450 and to inhibit 7-ethoxyresorufin O-deethylase (ERF) and 7-pentoxyresorufin O-dealkylase (PRF) activities was compared with ABT, BBT and α-MB in hepatic microsomes from guinea pigs. When pre-incubated with NADPH for 45 min ABT, BBT, α-MB and α-EB (100 μM) all destroyed P-450 (50-70%). At lower concentrations (5 and 10 μM) α-EB was a less potent inhibitor of ERF activity than equimolar concentrations of BBT or α-MB. BBT or α-MB (100 μM) inhibited virtually all ERF activity whereas this concentration of α-EB only inactivated about 80% ERF. All three compounds were more potent suicide inactivators of ERF than ABT. Similar results were obtained for PRF activity; BBT and α-MB were more effective inhibitors than α-EB or ABT. In hepatic microsomes of the guinea pig neither ABT nor its N-aralkylated analogues showed marked selectivity for inactivation of the P-450 isozymes catalyzing PRF vs ERF activity. Supported by MRC of Canada.

10.3

BIOTRANSFORMATION OF 3,3'-DICHLOROBENZIDINE BY PURIFIED PORCINE LIVER FLAVIN MONOOXYGENASE. M.H. Chen* and M.M Iba* (SPON: R. Snyder). Rutgers Univ., Piscataway, NJ 08854.

Evidence from previous studies (Iba and Thomas, Carcinogenesis 9:717,1988) suggested the participation of the flavin-dependent monooxygenase (FMO) in the hepatic activation of 3,3'-dichlorobenzidine (DCB) to mutagens in the rat. Therefore, the present studies were carried out to assess the ability of the enzyme to metabolize DCB. Highly purified FMO from porcine liver microsomes (0.03 U/ml), ^{14}C-DCB (100 uM, 0.01 mCi/mmol), and NADPH (400 uM) were incubated in 0.1 M tricine buffer, pH 8.3 at 37 °C, followed by extraction of the incubation mixture with either ethyl acetate (A) or methylene chloride (B). Reversed phase HPLC analysis of the dried organic extracts revealed one major metabolite in either A (metabolite 1, retention time = 11 min) or B (metabolite 2, retention time = 26 min). The latter was identified as azo-DCB by mass spectrometry. Extraction studies with ^{14}C-ethyl acetate and comparative chromatographic and spectrometric studies with authentic N'-acetylDCB suggested metabolite 1 to be an addition product between the solvent ethyl acetate and a metabolite of DCB. Formation of the latter obligatorily required NADPH and was neither abolished by catalase nor accompanied by H_2O_2 formation. The data suggest that DCB may be oxidized directly to a reactive species by FMO. (Supported by EPA R-812459).

10.4

EFFECTS OF AGE ON HEPATIC DRUG METABOLISM *IN VIVO* AND *IN VITRO* IN MINIATURE SWINE. J.O. Peggins* and M. Weiner*. (SPON: G.G. Buterbaugh). Dept. of Pharmacology and Toxicology, School of Pharmacy, Univ. of MD at Balt., Baltimore, MD 21201.

Previous *in vitro* experiments demonstrated dramatic differences in hepatic phase I and phase II drug metabolism between young (10 mo - 4 yr) and middle-aged (5 - 7 yr) miniature swine. There were significant increases in the specific activities of various microsomal mixed function-oxidase enzyme activities as well as in the concentration of cytochrome P-450 in middle-aged pigs. This study was performed to determine whether the observed changes *in vitro* were predictive of changes *in vivo*. Acetaminophen(APAP) was chosen as a model drug. Young (2 yr) and middle-aged (6 yr) pigs were canulated and APAP administered (20 mg/Kg, IV bolus). Blood samples were drawn at times ranging from 1 min to 7 hr; plasma was separated and analyzed for APAP and its major metabolites by HPLC. The data was analyzed using ADAPT and PCNonlin, two non-linear regression programs. Initial estimates for the kinetic parameters were obtained using the curve stripping program JANA. Age did not affect the clearance(3.3 vs 3.4 ml/min/Kg), half-life(116.2 vs 119.5 min), or volume of distribution (0.635 vs 0.590 L/Kg) of APAP in young vs middle-aged pigs. The rate of production of the sulfate conjugate was 2-3 time faster in middle-aged compared to young animals. The plasma conc. of APAP-glucuronide was lower for the first 30 min in middle-aged compared to young pigs but this pattern reversed after 60 min and the middle-aged values became greater. The rates of elimination were not affected for either metabolite. The production of the cysteine conjugate, the primary phase I metabolite, was not affected by age; although the peak plasma conc.(Cmax) was 2 times higher in middle-aged pigs when compared to young animals. Analysis of the plasma metabolite data indicates that the *in vivo* metabolism of APAP is a combination of both first and zero order processes. Some of the age differences observed, such as the increase in the cysteine conjugate Cmax, can be predicted by the changes seen *in vitro*. Others, like the differences in glucuronide conc., are better explained by physiological changes, i.e. an increase in volume of distribution.

10.5

EFFECTS OF ADENOSINE, INOSINE AND HYPOXANTHINE ON HEPATIC GLUCURONIDATION OF PARA-NITROPHENOL IN SPRAGUE-DAWLEY RATS. M. Centra*, W. W. Day*, and M. Weiner* (SPON: E.E. El-Fakahany). Dept. of Pharmacology and Toxicology, School of Pharmacy, Univ. of MD at Balt., Baltimore, Maryland 21201

Previous studies in this laboratory have demonstrated that adenosine (ADO) inhibits the formation of para-nitrophenol (pNP)-glucuronide in rat hepatocytes. Studies were conducted to determine whether the ADO metabolites, inosine (INO) and hypoxanthine (HX), inhibit hepatic pNP- glucuronide formation. Hepatocytes were isolated from male Sprague-Dawley rats and incubated with ADO (500μM), INO (500μM), and HX (500μM). The formation of glucuronide and sulfate conjugates of pNP were measured. Control values (mean ± S.E.M.) at 15 and 30 min were 15.32 ± 1.04 and 28.09 ± 2.26 nmoles pNP-glucuronide /10^6 cells and 4.45 ± 0.61 and 8.90 ± 0.94 nmoles pNP-sulfate/10^6 cells. The formation of pNP-sulfate was not inhibited by ADO or its metabolites. ADO inhibited pNP-glucuronide formation by 40% at 15 and 30 min. A similar pattern of inhibition was observed for INO, with 23% inhibition of pNP-glucuronide maintained from 15 to 30 min. HX caused a 24% inhibition at 15 min; by 30 min, glucuronide formation returned toward control values. ADO increases hepatic endogenous cyclic AMP levels and it is this increase which has been postulated to mediate the inhibitory effects of ADO on hepatic glucuronidation. Cyclic AMP levels were determined at 3, 5 and 15 min. ADO stimulated basal cyclic AMP levels (4.87 ± 0.61 pmoles/10^6 cells) 2.3 fold, with maximal stimulation occuring at 5 min. INO and HX did not stimulate cyclic AMP at any of the three sampling times. ADO, INO and HX did not directly inhibit the activity of the conjugating enzyme, glucuronyl transferase. These data suggest that INO and HX inhibit hepatic pNP-glucuronide formation, but this inhibition occurs via a mechanism which differs from ADO.

10.6

EFFECT OF COMMONLY USED ANESTHETIC AGENTS ON PHASE I AND PHASE II METABOLISM IN RATS. C.T. Gombar, L. Gutzait*, L. Meunier*, G. Stelman*, J.P. Hsu* and M. Landi*, J. Maule-Schmidt*, M. Cyronak*. SK&F Labs, King of Prussia, PA 19406.

Anesthetics are frequetly used to prepare animals for investigations of the pharmacokinetics and metabolism of experimental compounds, but the effect of the anesthetics on the metabolism of these compounds is usually not known. Barbiturates are known to affect drug oxidation, and diethyl ether (E) is known to inhibit glucuronidation. In the present study, the effect of pretreatment of rats with E, methoxyflurane (M), pentobarbital (P), and ketamine/ xylazine (K) on drug oxidation and conjugation was determined. Drug oxidation was assessed by measuring the clearance of antipyrine (15 mg/kg, i.p.), sulfation was assessed by measuring the clearance of a low dose of acetaminophen (APAP)(40 mg/kg) and glucuronidation was assessed by the clearance of a high dose (300 mg/kg) of APAP. The difference in mean area under the plasma concentration-time curve (AUC) between each anesthetic pretreated group and an untreated control group for each drug was evaluated for statistical significance with Dunnett's test. Pretreatment with P significantly decreased the clearance of AP, and the clearance of high dose APAP was significantly decreased by E ($p < 0.05$). None of the other comparisons were statistically significant. Therefore, the positive controls showed the expected response, but the other anesthetics had no effect on phase I or phase II metabolism.

10.7

Metabolism of McN-5195 in Rats. W. N. Wu, K. T. Ng and J. Masucci (SPON: B. H. Dvorchik), McNeil Pharmaceutical and Janssen Research Foundation, Spring House, PA 19477-0776.

McN-5195-^{14}C HCl [3-(2-bromophenyl) octahydroindolizine-^{14}C hydrochloride], a potential analgesic, was administered orally (60 mg/kg) to 52 male and female Wistar rats as a solution. In the seven days following dosing, 59% of the dose was excreted in urine and 40% of the dose in feces. A quantifiable amount of unchanged McN-5195 was found only in fecal extracts (2-6% dose). Metabolites from plasma, urine, fecal, liver and brain samples were profiled and isolated using TLC, HPLC and column chromatography. Unchanged McN-5195 plus eleven metabolites were identified by MS and NMR. Several metabolites were derivatized prior to spectroscopic analyses. Identified metabolites accounted for >60% of the total ^{14}C in each sample. The most dominant pathway of McN-5195 in rat appeared to be oxidation at the octahydroindolizine ring to form 5-OH-McN- 5195. This was followed by ring-opening, oxidation, dehydration and N-methylation to form five carboxylic acid metabolites. Hydroxylation at the phenyl ring and oxidation at other sites of octahydroindolizine ring produced four minor metabolites. CI-MS and EI-MS fragmentation patterns for the important metabolites and the proposed metabolic pathways for McN-5195 in the rat will be presented.

10.8

THE DISPOSITION OF RECOMBINANT INTERLEUKIN-2 IN THE ISOLATED PERFUSED RAT KIDNEY. R.W. Nadeau* and D.J. Liberato*. (Spon: S.J. Kolis) Dept. of Drug Metabolism, Hoffmann-La Roche Inc., Nutley, NJ 07110.

Recent clinical studies of recombinant interleukin-2 (rIL-2) in cancer patients have demonstrated that rIL-2 is rapidly cleared from serum with a half-life of 1 to 2 hours. It was also recently shown in mice (J. of Immunol. 130:2203, 1983) that the rate of IL-2 clearance from serum was 20-fold less when the kidneys were removed from the circulation by ligation of the renal vascular pedicle. This study utilizing the isolated perfused kidney was undertaken to better define the role of the kidney in the clearance of rIL-2. Kidneys from male Sprague-Dawley rats (370-410 grams) were isolated and perfused at 32ml/min, 110 mmHg pressure in a recirculating system. Various concentrations of rIL-2 were added to the system after which urine and perfusate samples were taken every ten minutes for two hours. Perfusate and urine were assayed for biologically active rIL-2 by a sandwich capture antibody bioassay. Doses of 20, 200, and 2000 units/ml of rIL-2 were cleared from the perfusate in a linear fashion. Interleukin-2 bioactivity was detected in the urine only at the 2000 units/ml dose.

10.9

HUMAN LIVER TYROSYL SULFOTRANSFERASE (TST). W.F. Young, Jr.* (SPON: R.M. Weinshilboum). Div. of Endocrinology, Dept. of Pharmacology, Mayo Clinic, Rochester, MN 55905.

The enzyme(s) responsible for protein sulfation on tyrosine residues has(have) not yet been described in man. TST was characterized in normal human liver tissue with Boc-cholecystokinin-8 (Boc-CCK-8) as the sulfate acceptor substrate; 3'-phosphoadenosine-5'-phosphosulfate (PAPS) was the sulfate donor. Subcellular distribution studies showed that TST was localized to the microsomal (m-TST) and cytoplasmic (c-TST) fractions. Experiments with pooled homogenates showed that m-TST and c-TST were separate enzyme activities based on pH optima, sensitivity to inhibitors [2,6-dichloro-4-nitrophenol (DCNP) and SKF 525A], and effect of NaCl. c-TST appeared to be very similar or identical to the thermostable form of phenol sulfotransferase (TS PST) measured with p-nitrophenol.

	m-TST	c-TST	TS PST
pH optima	5.8	6.8	6.6
DCNP, IC-50	0.5 mM	5 μM	5 μM
SKF 525A, IC-50	0.5 mM	2 mM	2.5 mM
NaCl, 125 mM	↑ 335%	↓ 66%	
Boc-CCK-8, Km	246 μM	272 μM	

The "true" Km value for Boc-CCK-8 with purified liver TS PST was 304 μM. Therefore, at least two TST enzyme activities are present in human liver homogenates. Further studies should clarify the roles of these enzymes in tyrosyl sulfation.

11.1

FOOD IS A CRITICAL FACTOR IN BIOEQUIVALENCE STUDIES OF SUSTAINED RELEASE THEOPHYLLINE PRODUCTS.

M. Spino, G. Koren, M. Bologa*, and J.J. Thiessen*, Faculties of Pharmacy & Med, Univ. of Toronto, Toronto, Ont., M5S 2A2, and Div. of Clin. Pharmacol. & Tox., The Hosp. For Sick Children, Toronto.

Theo-Dur®, a sustained release (SR) theophylline product, maintains relatively constant blood levels over a 12 hr dosing period. Recently, some generic companies have begun manufacturing SR theophylline, with the intent of creating a "bioequivalent" product. We compared Theo-Dur® (TD) and a SR theophylline product manufactured by Forest (F-T) in 12 healthy young adult, non-smoking males. In random order, on 4 days at 1 week intervals, subjects took each product after an 8 hr fast or a standardized McDonalds' breakfast. Activity, beverages, meals, time of day, and other known variables affecting theophylline disposition, and/or drug absorption were carefully controlled and monitored. In a Clinical Research Unit, 20 blood samples were drawn at specified times over 48 hr and analyzed for theophylline by HPLC. The area under the curve (AUC), the maximum concentration achieved in the serum (C_{max}), the time to reach C_{max} (T_{max}) and the mean residence time (MRT) were compared and analyzed (ANOVA). The mean half-life (6.5 hr) and AUC's did not differ significantly on the 4 days, suggesting similar clearances and comparable total absorption. However, large and significant differences were seen for C_{max}, T_{max} and MRT. In the fasting state, C_{max} for TD was 23% > F-T ($p<0.05$). F-T had a long and highly variable T_{max} among subjects. After food, the mean T_{max} for F-T was 6.5 hours longer than for TD ($p<0.05$). Furthermore, food altered the rate of absorption for F-T, but not for TD, demonstrating bio-inequivalence.

11.2

AUTORADIOGRAPHIC LOCALIZATION OF OCTYLONIUM BROMIDE IN THE GASTROINTESTINAL TRACT. P. Baroldi*, F. Amenta, F. Ferrante, A. Meli* and P. Napoleone. (SPON: M.F. Lokhandwala). Università "La Sapienza", Dipartimento Scienze Neurologiche, Rome and *Laboratori Farmaceutici A. Menarini, Florence (Italy).

Octylonium bromide (SP 63), a quaternary ammonium derivative, shown to possess smooth muscle relaxing properties, is currently utilized in the irritable bowel syndrome. In an attempt to explain the mechanism of the antispasmodic activity of the drug, its autoradiographic localization was studied in in vitro and in vivo experiments.

(14C)-SP 63 was specifically bound to sections of rat colon and rectum. It was localized primarily within the inner (circular) and, to a lesser extent, the outer (longitudinal) smooth muscle layer. In contrast, no significant binding occurred at the level of the stomach or the small intestine.

The labelled compound was also bound, in a dose-dependent manner, to colonic smooth muscle after intraluminal and oral administration.

The whole of the present data suggest that the anti-spasmodic action of SP 63 is related to its direct interaction with large intestine smooth muscle.

11.3

EFFECT OF DIAZEPAM ON THE ABSORPTION RATE OF ATROPINE. Brian J. Lukey*, Connie R. Clark*, Gerald H. Daniel*, Robert C. Smallridge* and David H. Moore* (SPON: Larrel W. Harris). USAMRICD, APG, MD 21010-5425

This study was done to determine whether intramuscular co-administration of atropine (ATR) and diazepam (DIA) affects the rate of absorption of atropine. Six sheep were used in a cross-over design; each week, all received 2 mg ATR sulfate equivalent and half received 10 mg DIA in a single site via a special tandem syringe. Blood samples were taken from 0 to 300 minutes by jugular venipuncture. Serum ATR concentrations were determined by a radioimmunoassay. The average ATR concentration-time profile fits a two-compartment open model. Maximum ATR concentrations were 9.3 and 9.4 ng/ml in the absence and presence of DIA, respectively. The time to reach maximum ATR concentration was significantly shorter ($p<0.05$) when ATR was given alone (6.0 min.) than when co-administered with DIA (9.5). However, the ATR concentration at 6.0 minutes when co-administered was 98% of the maximum ATR concentration when given alone. Although atropine is absorbed more quickly when given alone the difference is minimal.

11.4

PHYSIOCHEMICAL CRITERIA FOR THE RETENTION OF DRUGS IN THE UVEAL TRACT OF THE EYE. P.A. Zane*, S.D. Brindle*, P. Robertson* and S.L. Tripp* (SPON: A. Kotake), Preclinical Drug Metabolism, CIBA-GEIGY Corp., Ardsley, N.Y. 10502

The relationship between the physiochemical characteristics of 40 new drug candidates and their retention in high concentration by an organ, such as the uveal tract (UT) of the eye, was examined. The ability to anticipate the disposition-al fate of a drug based on its chemical and physical characteristics, prior to administration to animals or man, may allow one to predict the organ(s) most likely to exhibit toxicity. For example, chloroquin is well-known to be taken up by melanin-containing tissues and this affinity is correlated with the ototoxic and retinotoxic effects of this drug.

In the present investigation, tissue distribution data were obtained from whole-body autoradiograms of pigmented Long-Evans rats sacrificed 96 hours after dosing. The physiochemical parameters considered include molecular weight, pK_a and octanol/water partition coefficient (Log $P_{O/W}$) determined using an HPLC method.

The results showed that UT retention in rats was a function of both pK_a and Log $P_{O/W}$, whereas no correlation with molecular weight was found. More specifically, retention is based on a combination of lipophilicity and the acidic or basic nature of the ionizable groups within the molecule.

11.5

ATROPINE LEVELS IN RAT PLASMA AFTER ADMINISTRATION OF ATROPINE BY JET SPRAY AND NEEDLE INJECTION. Claire N. Lieske*, Howard G. Meyer*, Robin T. Gepp*, Isaac J. Hayward*, and Brian J. Lukey*, (SPON: Larrel W. Harris). USAMRICD, Aberdeen Proving Ground, MD 21010-5425

The characteristics of atropine levels in rat plasma after jet spray injection were compared to needle injection (im) in 12 male rats, 6 per group. Blood samples were sequentially collected from the tip of the tail over a 7-hr period. Injection of atropine sulfate (8.0 mg/kg) with the jet spray resulted in mean peak plasma levels of 595 ng/ml (SD = 36.8) compared to 445 ng/ml (SD = 23.7) with needle. Times to reach maximum concentration were 26.4 min (SD = 5.1) and 49.1 min (SD = 4.4) for the jet spray and needle, respectively. Histopathologic examination (5 days post injection) of target muscle showed that minimal fiber damage resulted from using the low pressure setting on the jet spray. The results suggest that, in cases of anticholinesterase poisoning, jet spray may offer a means of increasing the antidotal benefit over that achieved with conventional techniques using presently available therapeutic formulations.

11.6

SECRETION OF NIZATIDINE INTO HUMAN BREAST MILK. B.D. Obermeyer*, R.F. Bergstrom*, J.T. Callaghan, A. Golichowsky*, M.P. Knadler*, and A. Rubin. Eli Lilly and Company, and Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Secretion of the H2-blocker, nizatidine (N), was studied in breast milk (M) of lactating women. Blood samples were collected after a single 150 mg dose and after the 5th 150 mg dose of a q12h regimen from five lactating and five nonlactating women. M samples were collected from the five lactating women over the same time period. N concentrations in M were directly proportional to the collection-interval averaged concentrations in serum. A total of about 90 µg N was secreted into M over a period of 12 hours after either one dose or after multiple doses; this 90 µg was less than 0.1% of the maternal dose. Mean pharmacokinetic values for N in serum were :

	$t_{½}$ hrs	App.Clear. L/hr	App.Vd L/kg
Single Dose			
• Lactating	1.3	42.0	1.3
• Nonlactating	1.6	37.6	1.4
5th q12h dose			
• Lactating	1.3	39.2	1.1
• Nonlactating	1.7	44.6	1.9

Data show that N is secreted into M and that the serum kinetics are similar in lactating and nonlactating women.

11.7

DISPOSITION OF A NEW ANTIISCHEMIC AGENT, LY256548, IN MICE, RATS AND DOGS. T.D. Lindstrom and K.J. Ruterbories*. Lilly Res. Labs, Eli Lilly and Co. Indianapolis, IN. 46285.

Mice, rats and dogs were given a single 50 mg/kg (po) dose of ^{14}C-LY256548. Plasma levels of radioactivity (RA) and LY256548 (LY) were determined as well as the excretion of RA. Peak plasma levels of LY occurred prior to those of RA in mice and dogs but were coincident in rats. The C_{max} of LY in rats, mice and dogs was 0.17, 0.3 and 0.05 µg/ml, respectively. However, the C_{max} of RA was ten fold greater than LY in rats and mice, and 24 fold greater in dogs. The T1/2's of LY were substantially less than those of RA in rats (5 vs 32 hr), mice (4 vs 29 hr) and dogs (2 vs 55 hr). Based upon iv administration of ^{14}C-LY to rats (10 mg/kg) and dogs (5 mg/kg), an oral absorption of 45% and 7% was derived, respectively. The bioavailability of LY in rats (6.4%) and dogs (0.4%) was low. Extensive biotransformation of LY was observed in all three species. Only 22% (iv) and 3% (po) of the plasma RA was LY in rats, 12% (iv) and 1% (po) in dogs and 2% (po) in mice. Fecal excretion of RA was the primary mode of elimination being 96%, 81% and 100% in rats, mice and dogs after oral administration, respectively, and 83% and 93% in rats and dogs after intravenous dosing, respectively. Single dose oral tissue distribution studies in rats indicated that RA was appreciable in the GI tract, organs of excretion, bone marrow and skin. Of 27 tissues evaluated, the mean tissue RA T1/2 was 16 ± 1 hr.

11.8

Disposition of [^{14}C]L-Buthionine-S,R-sulfoxamine (BSO) in Mice and Dogs Dosed Intravenously or Orally. S.M. El Dareer, K. Tillery and D.L. Hill. Southern Research Institute, Birmingham, AL 35255.

CD2F1 mice (males) and beagle dogs (males and females) were dosed with [^{14}C]BSO, an inhibitor of glutathione (GSH) biosynthesis. For mice dosed i.v. with 768 mg/kg, the α and β half-lives for elimination of radioactivity from plasma were 6 and 512 min, respectively. In 24 hr, 73.9% of the dose was excreted in urine; of this amount, 24.2% was metabolites. For mice dosed orally with 5000 mg/kg, the α and β phases were 40 min and 21 hr, respectively. For dogs dosed i.v. with 127 mg/kg, disappearance of [^{14}C]BSO from plasma also occurred in two phases, with half-lives of 34 min and 485 min. In 24 hr, urinary excretion of the unchanged drug was 93.1% of the dose with no detectable metabolites. After oral gavage of dogs with 739 mg/kg, radioactivity in plasma was highest between 1-3 hr. In 24 hr, 41.4-59.5% of the dose was excreted in urine and 13.9-44.6% in feces, indicating incomplete absorption from the gut. In both species, there was an inverse relationship between levels of BSO and the content of GSH in tissues. These results demonstrate that, after administration of [^{14}C]BSO to mice and dogs, most of the dose is rapidly excreted in urine, apparently unmetabolized by dogs but with appreciable metabolism by mice. Supported by Contract NO1-CM-67905, NCI.

11.9

Drug Interactions between H_2-blockers and Theophylline (T) or Warfarin (W). J.T. Callaghan, E.H. Nyhart, Jr.* Lilly Laboratories for Clinical Research, Eli Lilly and Co. and Dept. of Medicine and Pharmacology, Indiana University Medical School, Indianapolis, IN 46285.

Male volunteers (21 to 54 yrs), participated in two trials using T or W as probes to study whether nizatidine (N), a recently approved H_2-blocker, inhibited hepatic metabolism. N effects were compared to ranitidine (R), cimetidine (C), and placebo (P). For 4 to 6 days the daily dosing regimens were: 1. N 300 mg, 2. R 300 mg, 3. C 1 to 1.2 g, and P qid. The alterations in T and W kinetic variables during each regimen are listed below:

	T (4.0 mg/kg iv)		W (20 mg po)	
Rx*	Beta (hr^{-1})	Cl (L/hr)	Beta (hr^{-1})	Cl (L/hr)
N	0.20(.07)	6.4(2.3)	0.03(.02)	0.20(.09)
R	0.18(.07)	6.1(2.3)	0.03(.01)	0.20(.04)
P	0.18(.06)	5.8(2.1)	0.03(.01)	0.23(.03)
C	0.13(.06)	4.3(1.8)	0.02(.01)	0.18(.03)

*n= 7 and 7; no Rx changed Vd (T=.47 L/kg and W=.11 L/kg).

C inhibited T metabolism as indicated by a significant decrease in beta and apparent Cl, while N and R did not inhibit T metabolism. No Rx inhibited W metabolism significantly, although after C the apparent Cl and beta trended downward. Conclusion: At therapeutic dosages N like R should not profoundly affect hepatic oxidases.

METALS; HALOGENATED HYDROCARBONS

12.1

THE REGULATION OF THE SYNTHESIS OF METALLOTHIONEIN IN NEUROBLASTOMA IMR-32 AND FOR COMPARISON CHANG LIVER CELLS. M. Ebadi and T. Takahashi*. Depart. of Pharmacol., Univ. Neb. Coll. Med., Omaha, NE 68105

We have identified in the rat brain a metallothionein whose synthesis is stimulated following icv but not ip administration of zinc. Furthermore, the zinc-induced protein produces two isoforms by ion-exchange chromatography on DEAE-Sephadex A-25 columns and by HPLC; which have 60 and 61 amino acid residues and 17 and 18 cysteine residues, respectively, but no aromatic amino acids. One of the differences between this protein and the hepatic metallothionein is that the brain metallothionein binds selectively zinc and not cadmium. We decided to study this phenomenon further by comparing the induction of metallothionein in human neuroblastoma IMR-32 cells by zinc, cadmium, and dexamethasone using the human Chang liver cells as a control. Both cadmium (1 µM) and zinc (100 µM) significantly enhanced the incorporation of [^{35}S]cysteine into metallothioneins isolated from both neuroblastoma and Chang liver cells, whereas dexamethasone (2.5 µM) induced the metallothionein in only the Chang cells. The degree of induction of metallothionein in the Chang cells by cadmium was 500% and by zinc was 670%, whereas both metals induced the metallothioneins in the neuroblastoma cells to the extent of 240%. The neuroblastoma IMR-32 exhibited less tolerance to the toxicity of both cadmium and zinc than the Chang cells did, which may correlate with the inherent ability of these ions to induce metallothioneins in these dissimilar cells. The results of these studies are interpreted to indicate that the factor(s) regulating the synthesis of metallothioneins in the Chang cells and neuroblastoma cells is not identical, suggesting the presence of dissimilar mechanisms in the liver and in the brain. (Supported in part by a grant from USPHS ES-03949.)

12.2

ZINC METALLOTHIONEIN GENE EXPRESSION IN RAT BRAIN. V.K. Paliwal*, P.L. Iversen*, R.J. Imbra,*† and M. Ebadi. Dept. of Pharmacol, Univ. Neb. Coll. Med., Omaha, NE, 68105 and †Inst. of Env. Med. New York, Univ. Med. Ctr., Tuxedo, NY, 10987

Metallothionein isoforms I and II (MTI and MTII) have been identified in the rat brain, monkey brain, bovine retina, pineal gland and hippocampus and in the neuroblastoma IMR 32. Since the ip-administered zinc passes across the blood-brain barrier slowly the rat brain metallothionein can be induced in a time- and dose-dependent fashion by icv-administered zinc at a rate of 0.20 µmole/µl/hr for 24 hrs using Alzet minipump. The induced protein incorporates a large quantity of ^{35}S cysteine, and the protein that incorporates ^{35}S cysteine binds ^{65}Zn and has an identical elution profile to hepatic MT. In this communication, we report that icv-administered zinc in a bolus of 0.1, 0.5 and 1.0 µmole increased the synthesis of poly A+ RNA at 6.6, 8.0, and 9.6 µg/g brain tissue. Furthermore, we probed the poly A+ RNA with a ^{32}P-labelled 180 base pair BamH1/PvuII restriction fragment containing the cDNA information for hepatic MTII from the pHMTII-3 plasmid. Slot blot analysis revealed a dose dependent increase in brain MTII hybridizable mRNA under high stringency conditions. These data provide evidence that the zinc-induced MT synthesis in the brain is associated with the accumulation of mRNA which is analogous to the zinc-induced synthesis of hepatic MT mRNA. However, other evidence indicates that the factors regulating the synthesis and the function(s) of the brain and the hepatic metallothioneins are not identical. (Supported in part by a grant from USPHS ES-03949).

12.3

EFFECTS OF CADMIUM (Cd^{2+}) ON LLC-PK_1 CELLS IN CULTURE.
W.C. Prozialeck and R.J. Niewenhuis, Philadelphia College of Osteopathic Medicine, Philadelphia, PA 19131.

Although the renal toxicity of Cd^{2+} is well known, the mechanisms underlying this toxicity have yet to be elucidated. In the present studies, we have examined the effects of Cd^{2+} on the general morphology and on the structure of microfilaments in cultured LLC-PK_1 cells. This cell line is derived from porcine kidney and exhibits morphologic and functional properties similar to those of proximal tubular epithelial cells. LLC-PK_1 cells were grown to confluency in alpha-MEM supplemented with 10% calf serum, exposed to varying concentrations of $CdCl_2$, and then examined with a phase contrast microscope. In some instances, the cells were processed for the histofluorescent visualization of microfilaments. Non-treated cells were shaped irregularly and formed complete monolayers, with very tight intercellular junctions. Exposure to 40 microM Cd^{2+} for 4 hours caused many cells to shrink, detach, and assume a spherical shape. Interestingly, similar concentrations of several other agents including Hg^{2+}, Pb^{2+}, Al^{3+} and CN^- did not cause this effect even after 24 hours. Exposure to Cd^{2+} also altered the structure of microfilaments; fewer filaments were visible than in the non-treated cells and the filaments that were present often showed an atypical granular or tangled appearance. These results indicate that Cd^{2+} has a relatively specific damaging effect on LLC-PK_1 cells and that this effect may involve the disruption of key cytoskeletal elements and intercellular junctions.

12.4

ATTENUATION OF ACUTE N-(3,5-DICHLOROPHENYL)SUCCINIMIDE-INDUCED NEPHROTOXICITY BY CEPHALORIDINE.
G.O. Rankin, V.J. Teets and D.W. Nicoll. Marshall Univ. Sch. of Med., Huntington, WV 25704-2901

Recently, interest has developed within the scientific community in the interactive potential of subtoxic doses of toxicants with other toxicants. Previous studies in our laboratory have demonstrated that the experimental agricultural fungicide, N-(3,5-dichlorophenyl)succinimide (NDPS) induces renal proximal tubular necrosis following acute exposure in rats. The purpose of this study was to determine if subnephrotoxic doses of cephaloridine, a nephrotoxic cephalosporin, would alter the nephrotoxic potential of NDPS. Male Fischer 344 rats (4/group) were pretreated with cephaloridine (500 mg/kg, i.p.) in 0.9% saline (2 ml/kg) 1 hr before a single, i.p. injection of NDPS (0.2, 0.4 or 1.0 mmol/kg) or sesame oil (2.5 ml/kg). Renal function was monitored at 24 and 48 hr. Cephaloridine pretreatment did not enhance the nephrotoxic potential of NDPS (0.2 mmol/kg). However, cephaloridine markedly attenuated all aspects of NDPS (0.4 and 1.0 mmol/kg)-induced nephrotoxicity. These results indicate that cephaloridine (500 mg/kg) does not enhance NDPS-induced nephropathy but rather markedly inhibits the development of NDPS-induced renal effects. The mechanism of the interaction between these two nephrotoxicants remains to be determined. (Supported by NIH grant DK31210).

12.5

DICHLOROANILINE-INDUCED NEPHROTOXICITY IN THE FISCHER 344 RAT.
H.H. LO* AND G.O. RANKIN. Dept. of Pharmacology, Marshall Univ. School of Medicine, Huntington, WV 25704-2901.

Halogenated anilines are widely used chemical intermediates in the synthesis of industrial and agricultural products. Previous studies from our laboratory have demonstrated that aniline and monochloroanilines are capable of inducing nephrotoxicity in rats. The purpose of this study was to examine the nephrotoxic potentials of dichloroanilines, namely: 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-dichloroaniline (2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DCA), in the male Fischer 344 rat. Rats were given a single injection of a DCA (0.4, 0.8 or 1.0 mmol/kg, i.p.) or 0.9 % saline (2.5 ml/kg, i.p.). Renal function was monitored at 24 and 48 hr post-injection. The administration of 2,4-, 2,5- and 3,5-DCA at the dose of 0.8 mmol/kg produced proteinuria, hematuria, and elevated the blood urea nitrogen (BUN) concentration. Both basal and lactate-stimulated para-aminohippurate (PAH) accumulations were depressed. The administration of 2,6-DCA was capable of producing the same renal effects but at a higher dose, 1.0 mmol/kg. 2,3-DCA or 3,4-DCA (1.0 mmol/kg) administration altered some but not all of the renal parameters monitored. The administration of any DCA at the dose of 0.4 mmol/kg did not produce any evidence of nephrotoxicity. The results of these studies indicate that dichloroanilines are nephrotoxic at the dose of 0.8 or 1.0 mmol/kg in Fischer 344 rats. (Supported by NIH Grant DK 31210).

12.6

TOXICITY OF QUINONES, HYDROQUINONES, AND HYDROQUINONE-GLUTATHIONE CONJUGATES TO RABBIT RENAL PROXIMAL TUBULES (RPT).
R.G. Schnellmann, S.S. Lau and T.J. Monks. Univ. Georgia Vet. Med., Athens, GA; Univ. Texas, Austin, TX; and Univ. Texas M.D. Anderson Cancer Ctr., Smithville, TX.

Numerous compounds with a quinone nucleus are nephrotoxic. The goal of this study was to examine the toxicity of quinone (Q), hydroquinone (HQ), bromoquinone (BQ), bromohydroquinone (BHQ), glutathion-S-yl-hydroquinone (HQ-GSH), and 2,3,5-(triglutathion-S-yl)-hydroquinone (HQ-$(GSH)_3$) to RPT suspensions. The compounds (0.2 mM) were incubated with RPT for 15 or 60 min. At 15 min, BQ, Q, and BHQ decreased GSH content 92, 92, and 81%. HQ and HQ-GSH had no effect on GSH content. HQ-$(GSH)_3$ increased GSH content 19%. BQ, Q, and BHQ decreased nystatin-stimulated oxygen consumption (NYS-QO_2) 71, 64, and 26%. HQ, HQ-GSH and HQ-$(GSH)_3$ did not have an effect on NYS-QO_2. At 60 min, GSH contents and QO_2 were similar to those seen at 15 min. BQ, Q, and BHQ produced marked LDH release while, HQ, HQ-GSH and HQ-$(GSH)_3$ did not. The cytotoxic order of potency is BQ=Q>BHQ>>HQ=HQ-GSH=HQ-$(GSH)_3$. These results are in contrast to those found in the rat in which HQ-$(GSH)_3$>HQ-GSH>BHQ>HQ=BQ=Q. Differences in renal transport and/or biotransformation may account for this species variation.

12.7

ALT RELEASE BY ISOLATED SUSPENDED RAT HEPATOCYTES FOLLOWING EXPOSURE TO EIGHT HALOGENATED HYDROCARBONS.
L. Dahlstrom-King*, J. Couture*, C. Lamoureux*, T. Vaillancourt* and G.L. Plaa. Univ. de Montréal, Montréal, Québec, Canada, H3C 3J7

In rats, concomitant exposure to halogenated hydrocarbons (HH) can result in additive, supra- or infra-additive effects on liver injury. Such experiments require large numbers of animals. We wished to determine if isolated rat hepatocytes directly exposed to HH could mimic the in vivo phenomena. HH (5-40 mM) were added directly to vials containing isolated hepatocytes. ALT release was measured for 30-180 min as an indicator of cell integrity. 1,1,2,2-Tetrachloroethane and tetrachloroethylene caused the most severe cytotoxicity, followed by carbon tetrachloride, trichloroethylene and 1,1,2-trichloroethane. Low cytotoxicity occurred with chloroform, 1,1,1-trichloroethane, and 1,1-dichloroethylene. This ranking in cytotoxicity was dissimilar to in vivo results, where 1,1,2,2-tetrachloroethane and tetrachloroethylene had no effect and carbon tetrachloride was the most potent hepatotoxicant. ALT release from hepatocytes exposed to HH in vitro does not constitute an adequate endpoint for the study of HH hepatotoxicity. (Supported by NSERC and IRSST).

13.1

The Dissociation Between Aminoglycoside Serum Concentrations and Nephrotoxicity. G. Koren, M.D., J. Klein, M.Sc., S. MacLeod, M.D., Ph.D. From the Div. of Clinical Pharmacology, Dept. of Pediatrics & The Research Institute, Hospital for Sick Children, Dept. of Pediatrics & Pharmacology, University of Toronto.

Aminoglycosides (AG) are the most widely used antibiotics for the treatment of Gram negative infections. The therapeutic administration of any of the AG leads to some degree of renal injury. Current recommendations call for a daily dose which will produce a steady state peak concentratoin of gentamicin between 5 - 10 mg/L and a trough concentration of less than 2-3 mg/L. This is considered the "safe range". In the present study we tested the hypothesis that not the serum concentration of the AG but rather the amount reabosrbed by the renal brush border is the factor causing tubular damage. Using newborn and adult rats we tested the correlation between AG dose, resultant serum and renal cortical concentrations and nephrotoxicity evidenced by serum and urine creatinine, urine N-acetyl-ß-glucosaminidase (NAG), ß2-microglobulin and tissue sphyngolmyelinase (SM). Although serum concentrations in adult rats were much lower than in newborn rats (0.18 ± 0.07 vs 2.87 ± 1.02 mg/L at 40 mg/kg/day gentamicin) nephrotoxicity was significantly more evident in the adult rats than in the newborn rats. The renal accumulation of the same dose of gentamicin was substantially higher in the adult rats compared to the newborn rats (174 µg/g vs 94.4 µg/g tissue). These data indicate that high AG serum concentrations are not causing nephrotoxicity but rather reflect secondary accumulation after renal damage. ***Supported by MRC MA8544.***

13.2

MORPHINE-3-β-D-GLUCURONIDE PHARMACOKINETICS IN FETAL LAMBS. George D. Olsen and Karen M. Sommer*. Dept. of Pharmacology, The Oregon Health Sciences University, Portland, OR 97201

Morphine-3-β-D-glucuronide (M3G), an active hydrophilic compound, is the major metabolite of morphine in fetal lambs. A 46 mg dose of M3G was injected into the inferior vena cava of 5 fetuses of gestational age 122 to 127 days. (Term is 147 days). M3G was quantitated by a specific and sensitive high performance liquid chromatography assay with ultraviolet detection following solid phase extraction. Morphine was not detected in analyzed samples. Mean elimination half-life of M3G was 15 hr. Mean ± S.E.M. total body clearance was 2.5 ± 0.4 $ml \cdot min^{-1}$, steady-state volume of distribution was 3.0 ± 0.3 L, and estimated fetal weight was 2.2 ± 0.1 kg. Amniotic fluid M3G concentrations peaked at 21 hours and were 5 times that in fetal plasma. M3G was undetectable in all but one maternal plasma sample. In one fetus, renal clearance of M3G, which is not protein bound, and creatinine were 1.9 and 2.6 $ml \cdot min^{-1}$, respectively. Renal clearance of M3G was 82% of total body clearance. M3G elimination is primarily by glomerular filtration and excretion into amniotic fluid and not by placental transfer from fetus to ewe or hydrolysis of M3G to morphine. Slow elimination of rapidly formed M3G explains its extensive accumulation during morphine infusion. (Supported by NIDA grant DA03585.)

13.3

THE OCCURRENCE AND INDUCIBILITY OF CYTOCHROME P450IIIA DURING RAT FETAL DEVELOPMENT. Janis E. Hulla* and Mont R. Juchau. Department of Pharmacology, University of Washington, Seattle, WA 98195.

The focus of this study was to quantify cytochrome P450IIIA in fetal and maternal livers of uninduced and pregnenolone-16a-carbonitrile (PCN)-induced rats during fetal development. Immunoreactivity with anti-P450IIIA and triacetyloleandomycin (TAO)-inhibited debenzylation of benzyloxyresorufin were used to assess levels and activities in hepatic microsomes from fetuses at 15 to 21 days of gestation. As expected, P450IIIA was not detectable by immunoassay in uninduced livers. However, when maternal animals were induced with PCN, a range of 59.3 to 116 µg P450IIIA/mg protein, quantified by Western blot densitometry, was detected in the maternal livers. Changes in debenzylase activity of 15.9 to 46.5 pmol resorufin/mg protein/min were consistent with these findings as were the changes in TAO-inhibitable activity. In the induced fetal liver, debenylase activity increased steadily from 0.19 pmol resorufin/mg/min, at day 15, to 9.34 at day 21 and was paralleled by the TAO-inhibitable activity which ranged from 0.09 pmol resorufin/mg/min at day 15 to 3.33 at day 21. A corresponding increase in immunoreactivity, 0.5 to 28.7 µg P450IIIA/mg fetal microsomal protein was determined. When the dose of PCN used to induce the maternal animals was increased from 50 mg/kg, 1x daily for 3 days to 40 mg/kg, 2x daily for 4 days, fetal debenzylase activity was decreased by approximately 10-20% over the gestational period as was the TAO-inhibited debenzylase activity. Analogously, immunoquantifiable P450IIIA was also decreased. These data suggest that fetal livers are PCN-inducible as early as day 15 of gestation and that inducibility increases with fetal age. Our results also indicate that a higher dose of PCN diminishes this inductive response. (NIH grants ES-04041 and ES-03157)

13.4

ACUTE METHADONE DEPENDENCE IN THE 14-DAY-OLD CHICK EMBRYO. M.E. Bronson* and S.B. Sparber. U. Minn., Dept. Pharmacology, Mpls., MN 55455

Chick embryos injected with a single dose of methadone on day 3, 7 or 11 of embryogenesis fail to show dependence on day 14, measured as a significant overshoot in motility above baseline levels after challenge with naloxone (10 mg/kg egg). Constant infusion of methadone (1 or 10 mg/kg egg/day) from day 7 to 14 also failed to produce evidence of dependence on day 14. However, the $t_{1/2}$ for methadone in brain on day 14 is 2.5 hrs. (Seran and Sparber, Pharmacol. Biochem. Behav., in press), indicating rapid excretion and/or metabolism. To address the question of whether the 14-day-old embryo is capable of developing or expressing (via an increase in motility) dependence/withdrawal, isobutylmethylxanthine (5 mg/kg) was injected directly into the embryo, resulting in a significant increase in motility and thus demonstrating the capacity to express quasimorphine withdrawal. Moreover, if a low (2.5 mg/kg egg) dose of either methadone or morphine is given on day 14, followed by naloxone (10 mg/kg egg) one hour later, there is a significant overshoot in motility above control levels, indicative of withdrawal. These results suggest that the 14-day-old embryo is capable of becoming acutely dependent on opioids and of expressing opioid withdrawal. Timing, however, is very important as is choosing the correct combination of doses of both agonist and antagonist. (Supported in part by USPHS grant DA 01880).

13.5

PRENATAL EXPOSURE TO NICOTINE PERTURBS BLOOD PRESSURE CONTROL IN ADULT RATS. E. Mills, I. Tayyeb* and J. W. Bruckert*. Duke University Medical Center, Durham, NC 27710

Pregnant rats were infused continuously with nicotine (6 mg/kg/day) via osmotic minipump from the 4th through the 21st gestational day. Offspring were studied at 40-50 days of age. Unanesthetized rats exposed to nicotine prenatally responded to stress (restraint + air blast) with enhanced pressor responses. Blood pressure measured after ganglionic blockade with chlorisondamine or after pithing was elevated. In pithed preparations maternal nicotine enhanced the sensitivity of the pressor response to electrical stimulation of the SNS innervation of the vasculature (decreased EF_{50} = stimulus frequency for 50% maximum response). Heightened sensitivity was prejunctional in origin as sensitivity of the pressor response to injected NE (ED_{50}) was unchanged. Heart rate at rest, after stress or after ganglionic blockade was unaffected, but the accelerator response to electrical stimulation demonstrated decreased prejunctional sensitivity without a change in sensitivity to isoproterenol. We conclude that adult rats exposed to nicotine during gestation demonstrate (1) Hyper-reactive pressor responses to stress due to facilitation of impulse mediated neurotransmitter release in the SNS innervation of the vasculature; (2) Compensatory, possibly baroreflex, changes in the cardiac SNS that blunt the stress response; (3) Enhanced non-neuronal contribution to resting blood pressure. Supported by NIH HL-29403.

13.6

LITHIUM(Li)—CESIUM(Cs) INTERACTION AND THE NEWBORN. I. Geller and F.S. Messiha. Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; Southwest Foundation for Res. and Education, San Antonio, TX and Dept. of Pharmacology, Univ. of North Dakota School of Medicine, Grand Forks, ND 58201.

The contrasting pharmacological properties between Li^+ and Cs^+ salts were utilized to study their interaction as related to Li toxicity in the newborn. Female albino mice ingested 1 mEq of LiCl, CsCl or both combined at conception, during pregnancy and until weaning of the offspring. The offspring were then separated from maternal nursing for a subsequent 3 weeks prior to sacrifice. The maternal Li-treatment caused 8% ($p < 0.01$) reduction in offspring's brain weight compared to 6% ($p < 0.05$) decreases after maternal Cs exposure. By contrast, the combined alkali-metals treatment increased offspring's brain weight by 7% ($p < 0.01$) from controls. Maternal Li-exposure increased offspring liver weight ($p < 0.05$) which was nulified by coadministration of Cs salts. Maternal Li or Cs-treatment decreased offspring's testis weight by 34% ($p < 0.001$) and 13% ($p < 0.01$) from controls, respectively. The combined Li and Cs exposure negated this effect. Changes in offspring spleen weight were also observed by maternal exposure to these alkali metals. The results show a Li-mediated toxicity on the newborn and suggest a CS-mediated antagonism on certain organs. (Laboratory work was performed at the initial Institution)

13.7

AGE DEPENDENT EFFECTS OF DEXAMETHASONE (DEX) ON PERINATAL SERUM TESTOSTERONE (T) CONCENTRATIONS AND DEVELOPMENT OF HEPATIC STEROID METABOLISM IN THE MALE RAT. H. Cunny*, W. Slikker, Jr. and J.E.A. Leakey*. National Center for Toxicological Research, Jefferson, AR 72079 and University of Arkansas for Medical Sciences, Little Rock, AR 72205

Perinatal serum T concentrations are thought to influence the development of male-specific pituitary and hepatic function. Because exposure to glucocorticoids such as DEX suppresses T in adult male rats, it was postulated that perinatal serum T might be susceptible to glucocorticoid suppression during the critical period of prenatal and early postnatal development. To test this hypothesis, pregnant rats were treated during mid-gestation on gestational day (GD) 14 or perinatally on GD-21 or postnatal day (PND) 1 with 10 ug/g DEX and the effect on postnatal serum T was measured. GD-14 treatment significantly decreased the serum T peak which occurred 2 hours postpartum but no significant differences were seen 12, 24 or 36 hours postpartum. Neither GD-21 nor PND-1 treatment affected T during the perinatal period examined. Although DEX administered on GD-14 affected postnatal T profiles, this treatment did not significantly feminize hepatic male-specific cytochrome P-450h related activities toward T in 70 day old male offspring. This treatment did however significantly decrease female-specific 5a-reductase activity toward T. These data suggest that decreases in the birth peak of T does not affect androgen imprinting of the male-specific hepatic P-450h.

13.8

TERATOGENIC EFFECTS OF CHRONIC COCAINE EXPOSURE IN MICE. Michael P. Mahalik and Henry W. Hitner*. Philadelphia College of Osteopathic Medicine, Philadelphia, Pennsylvania 19131.

The purpose of this study was to observe the dose-response relationships of chronic maternal cocaine administration on fetal development. Gravid CF-1 mice received daily injections of 7.5, 15, 30, 60 or 120 mg/kg cocaine hydrochloride s.c. from days 5 through 18 of gestation and were compared to saline and untreated control animals. Fetuses were removed from the mother on day 18, sexed, weighed, and processed for subsequent skeletal and soft tissue examination. Maternal toxicity progressed in a typical dose-response fashion with 100% maternal lethality at 120 mg/kg. Maternal weight gain during gestation and mean fetal weight decreased as dosage of cocaine increased, while the incidence of premature births and both skeletal and soft tissue defects increased with dosage. There were no significant differences from controls in fetal, sex or resorption ratios. No maternal toxicity or significant teratogenicity was observed at 7.5 mg/kg. The specific anomalies observed in this study were similar to those reported previously and included CNS, ocular and genitourinary defects. Related malformations have been reported for human offspring of cocaine addicted mothers. Results suggest that higher doses of cocaine produce complications associated with enhanced maternal toxicity, whereas the defects occurring at lower doses appear to be due to alterations in maternal-fetal circulation or to direct effects of the drug on the fetus.

13.9

METABOLISM OF 2,6-DIAMINOTOLUENE -- A MUTAGENIC NONCARCINOGEN. M.L. Cunningham* and H.B. Matthews* (SPON. L.S. Birnbaum). Systemic Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

2,6-Diaminotoluene (2,6-DAT) is a major industrial chemical; approximately 100 million pounds are used annually in the synthesis of 2,6-toluene diisocyanate. 2,6-DAT is mutagenic in Salmonella typhimurium TA98 requiring S9 activation, but was found not to produce tumors in F344 rats fed 250 or 500 ppm or B6C3F1 mice fed 50 or 100 ppm in 2-year bioassays. 2,6-DAT is rapidly absorbed following oral administration and excreted primarily in the urine, 85% within 24 hours as four metabolites. Resolution of the metabolites excreted in the urine by reversed phase HPLC and analysis by electron impact and fast atom bombardment mass spectroscopy identified the metabolites as a) N-hydroxy-, b) N-hydroxyacetyl-, c) N-acetyl-, and d) N,N-diacetyl-derivatives of 2,6-DAT. The N-hydroxyacetyl metabolite was found to be highly mutagenic and the N,N-diacetyl metabolite was slightly mutagenic in TA98 with metabolic activation. The other metabolites were not mutagenic in the presence or absence of S9. Results of this work indicate that 2,6-DAT is metabolized by the rat to compounds which are proximate mutagens, but since 2,6-DAT is not carcinogenic, these proximate mutagens are either not carcinogenic or are not further activated in vivo.

13.10

ANTIMUTAGENIC ACTIVITY OF 1,3-INDANDIONE (IDD). Ching Y. Wang, Kim Zukowski* and Mei-Sie Lee*. Michigan Cancer Foundation, Detroit, MI 48201

It is believed that chemicals initiate carcinogenesis and mutagenesis *via* electrophilic reactions with DNA. Thus, IDD which can be ionized to an enolate or a carbanion may be a trapping agent of ultimate carcinogens and mutagens. This hypothesis was tested in the Ames' system using the direct-acting mutagens, methylnitrosourea and 2-nitrofluorene, and the indirect-acting mutagens, aflatoxin B_1 and benzo[a]pyrene, that require activation by rat liver S-9. IDD, 10 μmol/plate, inhibited the mutagenicity of 2-nitrofluorene and methylnitrosourea by 90%. However, it did not inhibit the mutagenicity of the latter when it was added 1 hr after the addition of the mutagen. At 20 μmol per plate, IDD completely inhibited the mutagenicities of aflatoxin B_1 and benzo[a]pyrene. When tested in an *in vitro* system employing the binding of these carcinogens to tRNA, 0.5 mM IDD inhibited rat liver microsome-catalyzed binding of aflatoxin B_1 to tRNA by 50%, and cytosol-catalyzed binding of *N*-hydroxy-2-acetylaminofluorene to tRNA by 90%. However, its effect on the binding of benzo[a]pyrene to tRNA catalyzed by microsomes was not as remarkable. Reaction of IDD with an activated 2-aminofluorene yielded a compound which,upon hydrolysis, produced IDD, and 1-hydroxy- and 3-hydroxy-2-aminofluorene. These data demonstrate that IDD can trap ultimate carcinogens, and suggest that IDD may possess anticarcinogenic activity. Supported by NIH grant CA23800 and CA37885

LIPID AND FATTY ACID METABOLISM

14.1

POST FIBER SUPPLEMENTATION EFFECT ON SERUM GLUCOSE, LIPIDS, AND LIPOPTOTEIN CHOLESTROL PROFILES IN DIABETIC AND NONDIABETIC SUBJECTS. John O. Ogunwole, David L. Trout, Esther E. Glover* and Jimmy L. Smith*. Alcorn State University, Lorman,MS 39096 and USDA, Beltsville, MD 20705.

The feeding and withdrawal effects of a dietary fiber supplement (FIBER EXCELL) were tested in 8 clinically diagnosed diabetic (fasting serum glucose $>$ 100 mg/dl) subjects (2 males and 6 females) and 8 nondiabetic subjects (2 males and 6 females). Subjects were fed 30 grams of supplement daily for two weeks. Blood samples were collected just before the beginning of supplement and at the end of week 1 and week 2 supplementation periods. Data are being analyzed for possible fiber-nutrient interaction effect on plasma glucose (G), total Cholestrol (TC), HDL, LDL Cholesterol, triaclyglycerol (TG) and Glycosylated hemoglobin (HbA_{1c}) Profiles. Average daily nutrient and caloric intakes were not significantly different ($P < 0.05$) between the two groups during the study period. The measured metabolites were consistently higher in the diabetic subjects (D) than the nondiabetics (ND) in the basal, supplemental, and withdrawal periods. Thus it appears that, (1) the metabolic effects of diatary fiber depends on the physiological state of individuals, (2) post fiber supplementation effect is not uniform on all metabolites and (3) while fiber intake resulted in a decrease in total cholestrol, LDL-Cholestrol level was not affected in diabetics.

14.2

INFLUENCE OF SEX HORMONES ON THE HYPOLIPIDEMIC ACTIVITY OF NICARDIPINE(NIC) IN FRUCTOSE-FED RATS (FFRs). **Morris J. Clarke*, Setrina Hunter*, Roy L. Hawke*, Jo S. Zulkoski*, and Richard M. Welch. Wellcome Res. Labs. Research Triangle Park, N.C. 27709.**

Oral administration of NIC lowers serum LDL-cholesterol (LDL-C) and raises HDL-cholesterol(HDL-C) in male rats fed a normal or cholesterol/cholic acid (CC) diet (Biochem. Pharmacol. 33:2199-2205, 1984). Serum LDL-C and HDL-C levels were 35% higher after 5 days of feeding a 60% fructose diet than after feeding a normal diet to rats. We have observed that oral NIC (10-100mg/kg/day for 3 days) reduced serum LDL-C levels 44 to 14 mg/dl without affecting HDL-C levels in male FFRs on day 8. NIC administered to female FFRs reduced LDL-C levels from 55 to 20 mg/dl and raised HDL-C levels from 49 to 74 mg/dl. Additional studies were undertaken to examine the sex difference in HDL-C response to NIC in gonadectomized animals(GFFRs) with and without hormone replacement. Serum LDL-C and HDL-C levels in GFFRs were the same as levels in sham-GFFRs. Gonadectomy did not alter the 60% reduction in LDL-C observed after NIC in intact FFRs. However, the 50% increase in HDL-C in female FFRs was completely blocked by ovariectomy. Estrogen replacement in female GFFRs restored the NIC effect on HDL-C. These influences of sex hormones on NIC induced hypolipidemia in GFFRs are consistent with reported antiatherogenic effects of estrogen with no or opposite effects of testosterone.

14.3

STOICHIOMETRIC ANALYSIS OF LDL LIPID PEROXIDATION.
M.L. Lenz,* H. Hughes,* C.V. Smith,* D.P. Via,* A.M. Gotto,* and J.R. Mitchell, Department of Medicine, Baylor College of Medicine, Houston, TX 77030.

Oxidation of low density lipoprotein (LDL) causes changes in the biological properties of LDL that may be important in atherogenesis. That LDL oxidation is accompanied by lipid peroxidation has been demonstrated but previous analyses of the products of LDL oxidation have not included measurement of specific lipid hydroperoxy and hydroxy acids. In this study LDL was isolated from plasma of normal volunteers and exposed to O_2 for up to 24 h in buffer containing 5 uM Cu^{++}. Oxidized LDL showed decreased linoleate (18:2) and arachidonate (20:4) content with increased concentrations of thiobarbituric acid reactive substances (TBARS) and hydroxy and hydroperoxy 18:2 and 20:4; the electrophoretic mobility of the LDL protein also was increased. Following reduction the hydroxy fatty acids were characterized by GC-MS analysis of the TMS ether Me ester derivatives. The hydroperoxy and hydroxy derivatives accounted for more than 70 percent of the linoleate consumed during LDL oxidation and represented greater than 100-fold more product than was measured by TBARS analysis. Conclusion: Examination of the stoichiometry of the peroxidation of LDL lipid revealed that hydroperoxy and hydroxy acids are the major products formed; numerous biological properties of these products have been reported, but the manner in which they contribute to atherogenesis requires further study.

14.4

STRESS EFFECTS ON SERUM TRIGLYCERIDE, NEFA, AND TOTAL CHOLESTEROL LEVELS IN MALE RATS.
D.Hershock* and W.H. Vogel. Thomas Jefferson University, Philadelphia, PA 19107

Serum triglycerides, nonesterified fatty acids (NEFA), and total cholesterol were measured after acute and chronic(4 day) immobilization stress (1 hour) in adult male Sprague-Dawley rats. Animals were fed either Purina Rat Chow or the same diet supplemented with 1% cholesterol and 10% peanut oil. Rats were either nonfasted, fasted (24hour), or nonfasted high cholesterol, high fat fed prior to stress. Blood was obtained from indwelling jugular catheters. During acute stress, nonfasted serum triglyceride values decreased, fasted levels increased, and nonfasted high cholesterol fed levels also increased. Nonfasted serum NEFA levels remained lower than baseline whereas fasted and nonfasted high cholesterol fed rats showed a significant decrease after 5 and 15 minutes of stress, increasing after 15 minutes. Serum total cholesterol levels were not altered by stress. After chronic (4 day) stress, nonfasted triglyceride levels remained constant, cholesterol did not change, but NEFA was consistently higher at all stress time points after day 3. It can be concluded that acute immobilization stress affected serum triglyceride and NEFA values. In contrast, cholesterol remained unaltered by stress. During chronic stress, NEFA seemed more sensitive to change whereas triglycerides and total cholesterol remained stable.

14.5

PLASMA LIPOPROTEIN CHANGES AFTER A LOW CALORIE HIGH SOLUBLE FIBER DIET IN OBESE SUBJECTS.
J.L. Durstine,* R.G. Sargent,* W.P. Bartioli,* S. Streater,* B. Boardman,* (Spon. S. Powers), Univ. of South Carolina, Columbia, SC 29208

Twenty one obese subjects (≥ 20% of ideal weight) were divided into Group I (GI) (n=10) and Group II (GII) (n=11). GI was given a low Kcal/fat diet (mean = 918 ± 45 Kcal/d) (54% carb.; 20% pro.; 26% fat) plus 80 g of oat bran supplement. GII followed a similar dietary plan (1025 ± 81 Kcal/d) (58% carb.; 20% pro.; 22% fat) with no oat bran supplement. After eight wks a cross over design was followed with GI following a low Kcal diet (948 ± 82 Kcal/d) similarly followed by GII (1055 ± 80 Kcal/d) plus the oat bran supple- ment. Plasma lipoproteins were determined at baseline, cross over and at 16 weeks. Total cholesterol (TC) did not differ between the groups at baseline (GI 176 ± 11; GII 177 ± 11). Triglycerides also did not differ at baseline (GI 163 ± 10; GII 159 ± 28). Although, reductions in TC were observed, no significant differences were found in either treatment group over the sixteen wk period. However, when those in each oat bran treatment group were segregated into those having TC greater than or lower than 175 (HI n=6, LO n=8) significant reductions were not found in the LO group, but were in the HI (LO pre 156 ± 6, post 150 ± 6) (HI pre 201 ± 7, post 187 ± 7). Similar reductions were found in LDL-C while no changes were seen in HDL-C. Therefore, a high soluble fiber diet may exert its affects in groups with high cholesterol levels.

14.6

CHOLESTEROL IS REQUIRED FOR THE SECRETION OF THE VERY LOW DENSITY LIPOPROTEIN (VLDL) BY THE RAT LIVER: IN VIVO STUDIES.
Bobby Khan, Henry G. Wilcox, and Murray Heimberg. Department of Pharmacology, College of Medicine, University of Tennessee-Memphis, Memphis, TN 38163.

We reported previously that cholesterol (C) is a necessary component for the secretion of the VLDL by the isolated perfused rat liver (FASEB J. 2:1620 (1988)). To reduce the putative hepatic metabolic pool of C, normal male rats were fed lovastatin (L, 0.1%) in the diet for one week. Control rats were fed unsupplemented ground chow for one week. Another group received chow containing both 0.1% L and 0.1% C. Rats in each group were then injected with Triton WR-1339 in 0.9% NaCl (50 mg/100 g body weight) or saline alone via the sacral vein. After 2 hours, the rats were exsanguinated, and the VLDL was isolated from the plasma by ultracentrifugation. Secretion of VLDL-triglyceride (TG) was 1.69 µmol/hr/g liver in the control group, as calculated from differences in plasma VLDL-TG concentrations between saline and WR-1339 treated groups. Addition of L to the diet reduced the output of VLDL-TG by 47% ($p<0.05$). Supplementation of the diet with both L and C returned the output of VLDL-TG to control values. The molar ratios in the VLDL of phospholipid, C, and C esters relative to TG did not change in any experimental group, suggesting the secretion of VLDL particles unchanged in composition. The data obtained in vivo appear to support prior observations with the isolated liver that C is required for the secretion of the VLDL and transport of TG.

14.7

IDENTIFICATION OF ATRIAL NATRIURETIC PEPTIDE (ANP) RECEPTORS IN BROWN ADIPOSE TISSUE.
Hiroake Okamura*, Paul A. Kelly*, Gérard Morel*, Marthe Belles-Isles* and Seymour Heisler. McGill University, Montreal, QC H3A 1A1, Université Lyon-Sud, 69600 Oullins, France and Laval University, Quebec, QC G1V 4G2.

The binding and metabolic effects of ANP were investigated in rat interscapular brown fat. Membranes from this tissue specifically bound [^{125}I]-ANP. The association of labelled peptide was very rapid; at 25°C equilibrium was achieved within 5 min. Analysis of binding data revealed a single class of high affinity ANP binding sites with a K_D value of 1.7 nM and a receptor density (Bmax) of 226 pmol/mg protein. Binding of [^{125}I]-ANP was completely inhibited by unlabelled ANP (IC_{50} = 0.86 nM) but not by other adipocyte-active drugs such as insulin, ACTH or (-)isoproterenol. ANP stimulated a concentration-dependent increase in the synthesis of cGMP ($EC_{50} \cong 2.5 \times 10^{-9}$ M) in isolated adipocytes. The atrial peptide, however, did not exert any in vitro effect on (-)epinephrine-induced glycerol production. The data demonstrate the presence of specific and biochemically-functional ANP receptors in brown fat which are not coupled to lipolysis in these cells. The physiological function of the atrial peptide in brown adipocytes remains unknown. (Supported by the Medical Research Council of Canada, the Quebec Heart Foundation and the Royal Victorial Hospital Research Institute)

14.8

COD LIVER OIL EFFECT ON DIET INDUCED RABBIT ATHEROGENESIS.
E. L. Beard, S. E. Skelly*, E. A. Michals* and J. Q. Touchy*. Loyola University, New Orleans, LA 70118.

Cod liver oil (CLO) supplemented atherogenic dieting reduced the severity of atherogenesis in rabbits. Cholesterol levels in the blood plasma and platelets of CLO treated rabbits were markedly reduced and those of lung and kidney lysosomes were below atherogenic control values. Phospholipid levels of plasma and platelets were lower in CLO supplemented animals in the second month of dieting than were control tissues. Plasminogen activator activity rose in the blood plasma and platelets of CLO supplemented rabbits in the second month of study.

Plasminogen activator activity increased in extracts of homogenized lysosomes from lung, liver and kidney lysosomes. Platelets of CLO treated rabbits responded to insulin exposure by taking up somewhat more glucose from the suspension medium than did non supplemented controls. In another experiment, rabbits fed normal diet (Purina Rabbit Chow) were compared to atherogenic dieted animals with half of the animals on each diet type receiving CLO supplementation. Diphosphoglyceric acid (DPG) levels of atherogenic dieted rabbits were lower than in animals maintained on normal diet compared to atherogenic dieted ones. DPG was higher in CLO supplemented atherogenic dieted rabbits than in those fed atherogenic diet alone. Atherogenic plaque development in CLO-AD rabbits was reduced in comparison to AD controls.

14.9

LIPOPROTEIN LIPASE ACTIVITY IN ADIPOSE TISSUES OF RATS FOLLOWING A MEAL HIGH IN STARCH OR SUCROSE. Marie-Josée Martineau* and Yves Deshaies* (SPON: J. LeBlanc). Dept. Physiology, Laval Univ., Québec, Canada G1K 7P4.

To further investigate the disparate effects of various types of dietary carbohydrates on lipid metabolism, serum lipids and lipoprotein lipase (LPL) activity in white (WAT) and brown (BAT)adipose tissues of rats were measured at various time points following a meal high in either starch (STA) or sucrose (SUC) (65% of energy). Diets were fed ad libitum for 4 weeks prior to the test meal. The postprandial triglyceride increase was maximal 2 hr after the meal, and it tended to be larger in SUC-fed (130% over fasting) than in STA-fed animals (87%). Insulin increased 3-4-fold over fasting levels 30 mn after meal intake. Total insulin response to the meal (i.e. area under insulin curve) was 3-fold larger in the SUC-fed rats than in animals given STA, whereas the small post-meal elevation in glucose was comparable in both groups. Meal intake increased LPL activity in WAT similarly in both groups (50% over fasting levels), but enzyme activity was 2-fold higher in SUC-fed animals compared to STA-fed rats at all pre- and post-meal time points. LPL activity in BAT was stimulated only by the high-STA meal (42% over fasting). This study showed that LPL in both WAT and BAT is sensitive to the type of dietary carbohydrates. The results agree with the notion that insulin may be a determinant of the LPL response to STA and SUC in WAT, but not in BAT. (Supported by NSERC of Canada).

14.10

ADIPOSE TISSUE LIPOPROTEIN LIPASE ACTIVITY IN EXERCISE TRAINED RATS TREATED WITH NADOLOL. Anne Paulin* and Yves Deshaies* (SPON: J. LeBlanc). Dept. Physiology, Laval Univ., Québec, Canada G1K 7P4.

Since catecholamines are known to decrease in vitro lipoprotein lipase (LPL) activity in white adipose tissue (WAT) of the rat, this study was carried out to verify whether the reduction in enzyme activity that occurs in exercise trained rats could be prevented by chronic beta-adrenergic blockade. One group of male rats was trained (T) (1 hr of daily treadmill running) for 30 consecutive days, and another group was kept sedentary (S). Half of each group received nadolol in their diet (25 mg/kg, sufficient to induce bradycardia). S rats were killed at rest and T animals immediately after their last running session, both groups being in the postprandial state. Exercise decreased LPL activity in WAT (63% below S levels) and in brown adipose tissue (-57%) as well as serum total triglyceride (-79%) and cholesterol (-24%) levels, but increased serum nonesterified fatty acid concentration (117%). Nadolol treatment did not affect either the exercise induced changes in serum lipid variables or LPL activity in WAT. This study suggests that in trained rats the beta-adrenergic pathway is not involved in the exercise induced reduction in LPL activity of WAT.
(Supported by NSERC of Canada)

INSULIN-GLUCAGON-CHO METABOLISM

15.1

ADENOSINE IS NOT NECESSARY FOR INSULIN-STIMULATED GLUCOSE UPTAKE IN INTESTINE. M.P. McLane*, W.R. Law, and R.M. Raymond Loyola University, Stritch School of Medicine, Maywood, IL. 60153, and the V.A. hospital, Hines, IL, 60141.

Recent studies have shown that basal adenosine is necessary for insulin-stimulated glucose uptake in the heart. To investigate whether basal adenosine is necessary for insulin-stimulated glucose uptake (GU) in the intestine, healthy mongrel dogs (20-25 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.), intubated and ventilated artificially with a Harvard respirator. A catheter was inserted into the portal vein. Inferior mesenteric and pancreaticoduodenal arteries were ligated. Blood flow was measured with an electromagnetic flow probe on the superior mesenteric artery. Intestinal metabolism was determined using the Fick technique.

After baseline measurements were calculated, a hyperinsulinemic (4U/min i.v.), euglycemic ($D_{20}W$) clamp (INS) was established in the presence or absence of adenosine receptor blockade via arterial infusion of 8-phenyltheophylline (10^{-5} mol/min, 8-PTH).

	Basal	INS	Basal	8-PTH	8-PTH+INS
GU (mg/min)	0.7 ± .4	1.1 ± .4	1.1 ± .4	0.7 ± .3	1.1 ± .3

Conclusions: 1) Insulin increased glucose uptake in the intestine.
2) Basal adenosine maintains a tonic intestinal glucose uptake.
3) Adenosine is not necessary for insulin-stimulated glucose uptake in the intestine. (Supported by NIH Grant HL-31163 and the V.A.)

15.2

BILE FLOW THROUGH THE JEJUNUM IMPROVES GLUCOSE UTILIZATION IN RATS. M. Rudnicki*, D.G. Patel, D.W. McFadden*, M.S. Nussbaum*, A. Balasubramaniam*, J.E. Fischer*. Depts. of Surgery and Pharmacol. & Med. Chem., Coll. of Pharmacy, University of Cincinnati, Cincinnati, OH 45267.

Jejunoileal bypass excludes approximately 90% of the small intestine which alters glucose homeostasis and inhibits postprandial insulin response. This study investigates the effect of blind ended short jejunal conduits (BJC) and bile flow through these conduits on glucose homeostasis and insulin response. Blind ended (7cm long) Roux-en-Y conduits (BJC) were created in 12 rats with intact biliary tracts. Another 11 rats had choledochojejunostomies (CJ) in which the bile ducts were anastomosed to the proximal blind ends of the jejunal conduits. 12 intact rats were used as matched controls. 2 hours oral glucose (0.75g/kg) tolerance tests (OGTT) were performed at 3 months. Insulinogenic indices were calculated using the ratio of the areas under the curves for plasma insulin and glucose after OGTT.

	Control	BJC	CJ
Ins. Index	0.178±0.016	0.132±0.013*	0.238±0.002*#

Mean ± SEM, *p<0.05 vs control, #p<0.05 vs BJC
The diabetogenic effect of an excluded, blind ended conduit is improved with bile flow through it. This part of the jejunum may play an important role in the bile mediated mechanism of glucose utilization in rats.

15.3

THE EFFECTS OF GLUCOSE AND OTHER SUGARS ON CULTURED ADRENAL MEDULLARY CELLS: IMPLICATIONS FOR DIABETIC NEUROPATHY. J.E. Fedyna*, I. Lopez*, and D.B. McKay* (SPON: A.M. Burkman) The Ohio State University, Columbus, Ohio 43210.

Evidence exists that elevated glucose concentrations can alter neuronal function or cause neuronal damage (e.g., diabetic neuropathy). Since cultured bovine adrenal medullary (BAM) cells are embryologically homologous to neurons, the effects of elevated sugar concentrations on BAM cells were investigated. Acute treatment (< 60 min) with glucose, galactose and ribose had little or no effect on cellular catecholamine content, on nonstimulated catecholamine release or on nicotine-stimulated catecholamine release. Acute sugar treatment with glucose and galactose (30 mM) inhibited the uptake of [^{3}H]myo-inositol (MI) by 58%±6% and 31%±5%, respectively. Fructose had no effect on uptake. For cells treated 7 days with glucose, galactose and fructose (30 mM), after removal of the sugar [^{3}H]MI uptake was reduced by 51%±15%, 24%±17% and 23%±14%, respectively. When cells were treated (40 mM) for 4 days with glucose, galactose and ribose, the total catecholamine content of the adrenal cultures was reduced by 18%±7%, 12%±9% and 49%±17%, respectively. The sugar effects were both time-dependent and concentration-dependent. These results a) demonstrate that chronic sugar treatment of cultured BAM cells produces alterations in MI uptake and catecholamine content and b) suggest that a parallelism exists between neuronal and BAM cell responses to elevated sugar levels. (PHS NS24814).

15.4

EFFECTS OF VANADIUM ON MODULATION OF GLUCOSE TRANSPORT IN VITRO. Ivan Bihler and Paul C. Sawh. Univ. of Manitoba, Winnipeg, MB R3E 0W3, Canada.

Vanadate and vanadyl (VO^{2-}) ions are known to have insulin-like effects in various systems. We have studied the effects of VO^{2-} on the membrane transport of glucose in isolated adult rat cardiac myocytes and in pigeon erythrocytes. Initial influx rates of the nonmetabolized glucose analog ^{14}C-3-O-methyl-D-glucose (3-MG) were measured by liquid scintillation counting. In the cardiac myocytes 10^{-8} to 10^{-9}M VO^{2-} increased 3-MG influx to a moderate extent, enhanced transport stimulation by submaximal or supramaximal concentrations of insulin or by hyperosmolarity (100 mM mannitol) and increased even more the stimulatory effect of $5x10^{-8}$M epinephrine. 3-MG transport in pigeon erythrocytes does not respond to insulin but is stimulated by $4x10^{-5}$M epinephrine and this was strongly enhanced by VO^{2-}. Stimulation by N_2 was not altered. VO^{2-} by itself also increased transport to a minor extent. These results indicate that the insulin-like effect of vanadium develops rapidly and may be studied in vitro. The ability of VO^{2-} to enhance transport stimulation by some agents but not by others suggests possible differences in their mechanisms of stimulation. Vanadyl may be a useful tool to study mechanisms of glucose transport modulation in various cell types. (Supported by grants from the Canadian Diabetes Association and the Manitoba Heart Foundation).

15.5

EFFECTS OF INSULIN AND CATECHOLAMINES ON RESPIRATION, LIPOLYSIS AND GLUCOSE TRANSPORT IN BROWN ADIPOCYTES ISOLATED FROM GENETICALLY OBESE ZUCKER RATS . A. Marette* and L.I. Bukowiecki Laval University, Medical School, Quebec, G1K 7P4.

The effects of insulin and catecholamines on oxygen consumption, lipolysis and glucose transport were investigated in brown adipocytes isolated from interscapular brown adipose tissue (BAT) of lean (Fa/?) and obese (fa/fa) Zucker rats. The results showed that tissue cytochrome oxidase activity was not altered in obese rats suggesting that BAT mitochondrial oxidative capacity is normal in this phenotype. Catecholamines (norepinephrine or isoproterenol) increased 8-12 times cellular oxygen consumption in both lean and obese rats. However, there was a small, but significant, decrease in norepinephrine (NE) sensitivity with obese BAT cells compared to lean (1/2 Vmax: 200 nM vs 50 nM). The lipolytic sensitivity to NE was also decreased but no significant differences were seen in NE-stimulated glucose transport. Dibutyryl cyclic AMP similarly stimulated oxygen consumption in both phenotypes suggesting that the decrease in NE sensitivity resulted from a deactivation of the receptor-adenylate cyclase complex. On the other hand, the antilipolytic and antithermogenic actions of insulin were markedly reduced in obese BAT cells (-15% and -45% respectively). Insulin-stimulated glucose transport was also significantly decreased in sensitivity (1/2 Vmax: 2 nM vs 0,3 nM) and responsiveness (4-fold vs 7- fold). These results demonstrate that: (a) insulin actions are highly impaired in brown adipocytes from obese Zucker rats, (b) the sensitivity of these cells to the lipolytic and thermogenic effects of NE is decreased, and (c) their maximal thermogenic capacity is not changed. (Supported by MRC, CDA and FRSQ).

15.6

MECHANISM OF ENHANCED LIPOLYSIS DURING COLD-ACCLIMATION IN ISOLATED RAT ADIPOCYTES. Louise Rochon* and Louis Bukowiecki. Dept. Physiology, Fac. Medicine, Univ. Laval, Quebec, G1K 7P4.

The aim of this study was to determine the effects of cold exposure (CE) and cold acclimation (CA) on the regulation of lipolysis in white adipose tissue (WAT). Rats were divided in 3 groups: cold exposed (CE) 7 days at 5°C, cold-acclimated (CA) 21 days at 5°C and warm-acclimated (WA) (25°C). Lipolysis was measured in isolated adipocytes as glycerol release in umoles/10^6 cells/15 min (U). Basal lipolysis was significantly higher in CE and CA groups than in WA animals (0.29 ± 0.02, 0.31± 0.04 and 0.18± 0.03 U respectively; $p<0.05$). Lipolytic responsiveness and sensitivity of adipocytes to catecholamines were significantly decreased in CE and CA rats. The inhibitory effect of cold on lipolysis was still observed in the presence of adenosine deaminase. Incubation of adipocytes with dcAMP and theophylline stimulated lipolysis to the same extent in all groups. Glucagon (GLU) and ACTH stimulated lipolysis significantly more in CE and CA rats than in WA controls (2.06± 0.2 and 0.91± 0.06 vs 0.37± 0.05; $p<0.05$ for GLU and 4.2± 0.18, 2.25± 0.42 vs 2.5± 0.24; $p<0.05$ for ACTH). The antilipolytic effect of insulin was found to be significantly decreased in both CE and CA groups. Altogether, these results suggest that the increased lipolysis in WAT observed during CA is the result of an increased sensitivity of the adipocytes to the lipolytic effects of glucagon and ACTH rather than to catecholamines.

15.7

EFFECTS OF 7 WEEKS OF ACARBOSE TREATMENT ON METABOLIC AND THERMAL PARAMETERS IN DIABETIC RATS. M.J. Katovich, M. Meldrum* and J.R. Vaselli. Department of Pharmacodynamics, University of Florida, Gainesville, FL 32610.

Streptozotocin (STZ) diabetes is characterized by polyuria, polydipsia, glucosuria, hyperglycemia and an altered tail skin temperature (TST) response to administration of isoproterenol (ISO) and heat stress. Acarbose (Bay.g-5421), a alpha glucosidase inhibitor of intestinal absorption of carbohydrates, was evaluated on the metabolic and thermal characteristics of STZ-induced diabetes. Three groups of male rats received STZ (65mg/kg, IV) while the remaining group received the vehicle. Two of the STZ groups received acarbose in the diet (20mg/100g and 40mg/100g). Metabolic parameters were measured daily in 6 animals from each group. Acarbose treatment significantly reduced the glycosuria, polyuria and polydipsia observed in the untreated STZ diabetic rats. Blood glucose was also significantly reduced in the acarbose treated groups during the 3rd-7th week of the study. The rise in TST associated with administration of ISO and heat stress (32°C) was significantly reduced in the STZ diabetic rat suggesting a defect in thermoregulatory vasodilatory responses. Although acarbose treatment did not restore any parameter to control values, the severity of the responses were attenuated. Collectively these results suggest acarbose may be beneficial in the treatment of diabetes related symptoms. (Supported in part by Miles Laboratories and NIH Grant HD18133).

15.8

IN VITRO REVERSAL OF INSULIN RESISTANCE IN MUSCLE FROM NIDDM RATS. D.A. Young, R.H. McIntosh*, and J.E. Foley. Sandoz Research Institute, East Hanover, N.J. 07936.

In a previous study it was found that muscles from hypoinsulinemic-hyperglycemic rats exhibited significant insulin resistance (IR), and that this IR could be reversed by prolonged incubation *in vitro* in the absence of insulin (Wallberg-Henriksson, JBC, Vol. 262:7665, 1987). We have repeated this experiment using a streptozotocin (STZ) rat of model of NIDDM. Rats were made diabetic by a single injection of 37.5 mg/Kg STZ, and exhibit hyperglycemia (>200 mg%) with normal insulin levels (~25 μU/ml). However, unlike animals used in the previous study, following an overnight fast NIDDM rats exhibit normal blood glucose values (~80 mg%). These animals still exhibit IR however, as judged by euglycemic clamps performed *in vivo* (average 90-120 min glucose infusion rates in normal and STZ animals were 25.4 ± 2.6 and 13.5 ± 1.4 mg/Kg/min respectively, $P<0.01$), or by insulin stimulated 3-methylglucose transport (3-MG) in epitrochlearis muscles *in vitro* (7.16 ± 0.70 vs. 3.35 ± 0.40 μMol/ml/hr, $P<0.01$). When muscles were incubated for 5 hours with 5 mM glucose in the absence of insulin, 3-MG transport reversed 50% of the way toward normal. The data indicates that NIDDM rats exhibit marked IR *in vivo* and *in vitro*, and that this resistance can be acutely reversed *in vitro*. This suggests that there is something unique about the diabetic state, other than hyperglycemia, which prevents reversal of IR *in vivo*.

15.9

EFFECT OF INSULIN AND GLUCAGON ON HEPATOCELLULAR ALANINE TRANSPORT IN RATS. Anwar B. Bikhazi, Habib Alloush*, Fadia Uthman*, Rula Abbud* and Najla Fakhreddine*. American University of Beirut, Faculty of Medicine, Beirut, Lebanon.

Hepatocytes from normal and streptozocin-treated diabetic rats were dispersed by collagenase perfusion. Cells were suspended in Ca-Mg-free phosphate buffer containing oxamic acid and treated with insulin, glucagon and/or cycloheximide prior to measurement of ^{14}C-alanine transport. Samples were withdrawn at specified intervals, centrifuged, and hepatocellular uptake was monitored by measurement of alanine intracellularly and in supernatant. Alanine uptake in insulin-treated normal cells increased (86%) compared to controls. However, in cycloheximide-treated cells, uptake decreased by 25%. Cycloheximide presence with insulin blocked increased uptake induced by insulin treatment. Diabetic cells showed 27% increase in uptake compared to normals. Insulin treatment enhanced uptake (67%), while cycloheximide decreased it by 13% compared to diabetic controls. No change in uptake was observed when diabetic cells were treated with insulin and cycloheximide simultaneously. Insulin enhances alanine transport in normal and diabetic hepatocytes contrary to the effect of cycloheximide. However, the data disagrees with the "down-regulation" concept. The increase in uptake exhibited by diabetic cells may be explained by increase in glucagon plasma levels. (Supported by RADAC, A.U.B., and the Nadim Andraos Foundation).

15.10

PROGESTERONE RECEPTOR-MEDIATED REGULATION OF INSULIN RECEPTOR BINDING ACTIVITY AND INSULIN ACTION IN THE ZR-75-1 HUMAN BREAST CANCER CELL LINE. R. Poulin* and F. Labrie, MRC Group in Molecular Endocrinology, Laval University Medical Center, Quebec G1V 4G2, Canada.

In order to gain better understanding on the function of the progesterone receptor (PgR) in mammary tumors, we have studied the effect of the synthetic progestin R5020 (promegestone) on cell proliferation and insulin receptor (InsR) content in the hormone-responsive ZR-75-1 human breast cancer cell line. No effect of the progestin (up to 100 nM) was observed on ZR-75-1 cell proliferation at the end of a 12-day growth period in the absence of estrogens, or in estradiol (E_2)-supplemented media (1 nM) containing insulin (0.5 μg/ml). However, when no insulin was added, R5020 inhibited the mitogenic effect of E_2 by about 65% (IC_{50} = 30 pM). The potent antiprogestin RU486 (mifepristone, 300 nM), but not the antiandrogen hydroxyflutamide (3 μM) reversed the effect of R5020 in a competitive manner, thus indicating a PgR-mediated effect. In the presence of E_2, increasing concentrations of insulin completely reversed the effect of R5020 (10 nM) with a half-maximal mitogenic effect similar to that observed in steroid-deprived (control) cells (EC_{50} = 0.5 and 0.6 ng/ml, respectively). InSR binding activity was increased by up to 3-fold after 4 days in intact ZR-75-1 cells incubated with 10 nM R5020 and 1 nM E_2. These results suggest that insulin action might be greatly potentiated by PgR-mediated up-regulation of InSR in breast cancer cells.

15.11

EFFECTS OF MATERNAL ALCOHOLISM ON FETAL BRAIN GLUCOSE METABOLISM. A.K. Snyder*, S.P. Singh, G. Pullen* and L. King*. V.A. Medical Center and The Chicago Medical School, North Chicago, IL 60064

We have reported reduced blood glucose (BG) and insulin (IRI) levels in term fetuses of ethanol-fed (EF) rats; fetal brain weight correlated with BG. In the present study, rats were fed liquid diet containing ethanol (30% of total caloric intake) or control diet throughout gestation. In term fetuses of EF rats, BG was 20 and 30 % lower than in those of pair-fed (PF) and ad libitum-fed (AF) controls, respectively. Glucose uptake by dissociated fetal brain cells, measured by incubation for 2 minutes with ^{3}H-2-deoxy-D-glucose, was reduced ($p<0.02$) in the EF group (2.29 ± 0.23 nmoles/min/mg protein, vs 3.64 ± 0.39 and 4.24 ± 0.26 in PF and AF controls, respectively). Using whole brain plasma membranes from EF, PF, or AF litters, maximum binding (B_{max}) of ^{125}I porcine IRI was 6.0 ± 0.7, 6.5 ± 0.5 and 6.4 ± 0.5 % and that of ^{125}I recombinant insulin-like growth factor-1 was 12.0 ± 0.7, 16.1 ± 1.0 and 15.6 ± 2.4 % respectively. Reduced BG and brain cell glucose uptake may contribute to the brain growth retardation we have observed in fetuses from ethanol-fed rats.

15.12

SUBREGIONAL MICROVASCULAR AND METABOLIC FEATURES OF THE RAT AREA POSTREMA. P.M. Gross, J.J. Pang*, D.S. Wainman*, S.W. Shaver* and M. Kadekaro[1]. Neurosurg. Res. Unit, Queen's Univ., Kingston, Ont. K7L 3N6 and [1]Div. Neurosurg., Univ. Texas Med. Branch, Galveston, TX 77550

The area postrema (AP) of rats is differentiated in cytoarchitecture and distribution of neuroactive substances. We tested the hypothesis in albino rats that the normal linkage between capillary density (CD) and tissue glucose metabolism (GM) would be topographically related within AP. CD was assessed by morphometry in 1 um-thick coronal sections at 3 rostrocaudal levels (obex -0.2 to -0.6 mm). GM was measured using [^{14}C]deoxyglucose and image processing. Four zones were discerned within AP in order of CD: medial = lateral > dorsal = ventral (range = 173 to 407 capillaries/mm^2 for vessels ≤ 7.5 um id). Corresponding topography of GM was in a range of .55 to .70 umol/g/min. In dehydrated rats, GM increased most (up to +38%) in the medial and lateral zones which contain the densest distributions of vagal afferent terminals, catecholamines, and neuropeptide Y. CD and GM reflect the complex cytoarchitecture and potential diverse neurochemical interactions within individual AP zones that may be functionally discrete.

ALCOHOL AND ANESTHETICS

16.1

ETHANOL-INDUCED DEPRESSIONS OF HUMAN CORTICAL NEURONS ARE ANTAGONIZED BY Ro15-4513: EVIDENCE FROM INTRAOCULAR TRANSPLANTS IN ATHYMIC RATS. B. Hoffer[1], M. Eriksdotter-Nilsson[2], I. Strombera[2], Philip Stieg[2], L. Olson[2], A. Seiger[3], M. Bygdeman[2], and M. Palmer[1]. [1]Dept. of Pharmacology, Univ. of Colo. HSC, Denver CO 80262, USA, [2]Dept. of Histology, Karolinska Institute, Stockholm, Sweden, [3]Dept. of Neurological Surgery, Univ. of Miami School of Med.

Human fetal tissue from the cerebral cortex was collected following elective abortions in the eighth to eleventh week of gestation using procedures approved by the Ethical Committee of the Karolinska Hospital and conforming to USPHS guidelines. The human cortical tissue was transplanted to the anterior eye chamber of athymic nude rats. The cortical transplants increased significantly in size in oculo and became vascularized from the host iris. Different classes of neurons were identified with immunohistochemistry. Extracellular recording of single unit activity was performed in 9 transplants after 3-7 months in oculo. Single action potentials showed a more immature waveform in younger transplants, with long durations and very slow spontaneous discharges. Firing rates ranged from 0.5 to 6.0 Hz. Superfusion with known concentrations of ethanol elicited predominantly inhibitions of baseline firing rates, with occasional excitations at low concentrations (1-10mM). The transplants could be divided into two groups with respect to neuronal ethanol sensitivity with EC50s of 28.9 mM (n=13) and 2.70 mM (n=4) respectively. Superfusion with Ro15-4513 caused a significant antagonism of ethanol-induced inhibitions. This antagonism lasted for 1.5-2 hours, whereafter the response to ethanol returned to control levels. In conclusion, ethanol elicits dose-dependent inhibitions of discharge in transplanted human cortical cells. This inhibition is antagonized by Ro15-4513.

16.2

SEROTONIN UPTAKE INHIBITORS (SUI) MAY INTERACT WITH THE NOREPINEPHRINE (NE) SYSTEM TO REDUCE ETHANOL (E) MICRO-DRINKING BEHAVIOUR IN RATS. Mary O Lawrin*, Edward M. Sellers and Claudio A. Naranjo. Clinical Psychopharmacology Program Addiction Research Foundation; Departments of Pharmacology and Medicine, University of Toronto, Toronto, Canada.

Brain serotonin (5-HT) pathways are thought to importantly regulate E consumption. For example, we show that all nominally selective SUI tested (1, 3, 10, 30 mg/kg i.p. zimelidine; 1, 3, 10, 30 mg/kg i.p. citalopram; 1, 3, 10, 30 mg/kg i.p. paroxetine; 1, 3, 10, 30 mg/kg i.p. fluvoxamine; 0.1, 0.5, 1, 5, 10 mg/kg i.p. fluoxetine; etc.) produce a dose-related decrease in E intake/preference and alter the pattern of E micro-structural drinking behaviour (increase latency to first E drinking episode; decrease number, size, duration of E drinking episodes) at 10- to 50-fold lower doses than reported using 24-hour E preference models (ANOVA, $p < 0.01$). Minute-to-minute characterization of male Wistar rat (250 g, n = 4/group) 60% to 100% free-choice E preference drinking pattern (6% E v/v) is assessed in a continuously-monitored computerized volumetric micro-drinking system. However, the rank order potency of SUI to reduce E micro-drinking does not correlate with 5-HT uptake specificity (IC_{50} [5-HT] nM) ($r = -0.64$) but does correlate with 5-HT uptake selectivity (IC_{50} [NE]/IC_{50} [5-HT]) ($r = 0.86$) and with SUI ability to block NE uptake into brain synaptosomes (IC_{50} [NE] nM) ($r = 0.87$). Furthermore, 1, 2, 5 mg/kg i.p. desmethylimipramine (DMI), a more selective NE uptake inhibitor, decreases ethanol micro-drinking by 30% to 60% in a dose-related manner and 1 mg/kg i.p. DMI produces a 4-fold potentiation in the the decrease in E micro-drinking both with selective and specific SUI: fluoxetine (1 mg/kg i.p.) and citalopram (3 mg/kg i.p.) and with selective 5-HT agonist, MK212 (3 mg/kg i.p.) (ANOVA, $p < 0.05$). NE may be a determinant of SUI drug action and may interact with 5-HT in the regulation of E consummatory behaviour.

16.3

BONE DEFECT ASSOCIATED WITH ETHANOL CONSUMPTION IN RATS. T-C Peng, Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill, NC 27599.

We have previously reported that acute administration of ethanol causes a fall in both total serum calcium (Ca) and serum ionized Ca while chronic treatment with alcohol results in alteration of bone. The color of dried bone from rats fed a liquid diet containing 8% ethanol (ELD) for 4-6 weeks was darker than that of rats fed control liquid diet (CLD), and scanning electron microscopy of femurs revealed defects in trabecular structure (the presence of thinner and more fragile trabeculae compared with controls.) We recently found that ethanol may act on osteoblasts to suppress serum levels of osteocalcin (255±12 [CLD] vs 172±13 ng/ml [ELD]; mean±SE; $p<0.001$). This noncollagenous bone protein has been implicated in the development and maintenance of bone tissue. With a 3-point bending test, we noted that the femurs of ELD-fed rats were weaker than those of controls. Linear regression analysis revealed a significant correlation ($p<0.001$--<0.05) between each of four mechanical properties (strength, stiffness, toughness, and ductility) and the ratio of ethanol dose to body weight. Preliminary bone histomorphometric study showed reduced cortical bone areas at the tibial junction, trochanter region, and mid femur levels compared to control bones. Our results indicate that ethanol causes a bone defect, and that the rat model facilitates research on osteopenia and osteoporosis in alcoholics.

16.4

ETHANOL DISCRIMINATION BY RATS AFTER SELF-ADMINISTRATION BY DRINKING. Harvey B. Hentelefft and Herbert Barry, III. University of Pittsburgh School of Dental Medicine, Pittsburgh, PA 15261

Male albino rats on a food deprivation schedule were trained to press different levers for food pellets depending on whether or not the 10-min. session was preceded by intake of ethanol (2 g/kg). The alternative fluids were 40 ml/kg tap water, containing either 5% w/v ethanol and 9.466% sucrose or an isocaloric concentration of sucrose (18.93%). One group (N=8) self-administered the fluid by drinking from a burette between 30 and 20 min. before the session. The fluid was gastrically intubated in another group (N=7) at 30 min. before the session. Most of the rats in both groups learned the discrimination. An important attribute of the discriminative stimulus for both groups was presence or absence of the systemic effect of ethanol. This was indicated by tests with intraperitoneal injection of ethanol or saline without the drinking or intubation of the usual fluid. The ethanol stimulus was stronger in the intubation group, presumably because of the more rapid intake or the absence of differential taste as an additional stimulus. Both groups were divided into two subgroups, with the alternative fluids in distinctively different environments or equally often in both environments. These preceding environments had little consistent effect on the discriminative lever pressing by either subgroup.

16.5

INSULIN RESISTANCE IN ISOLATED ADIPOCYTES FROM ETHANOL TREATED RATS. J.M. Harrer*, V.R. Grund, and D.G. Patel. Division of Pharmacology and Medicinal Chemistry, College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267-0004.

Ethanol (ETOH) has been demonstrated to adversely affect glucose tolerance in rats. The hypothesis was tested that insulin's antilipolytic action in adipose tissue might also be influenced by ETOH feeding. This study compares effects of insulin on isolated adipocytes from 3- and 13-month-old Fischer 344 rats. Rats were divided into three diet subgroups: ethanol, control ad libitum and control pair-fed. ETOH diet was isocaloric with control diets except that ETOH (30% of calories) was substituted for fat. Rats were fed the respective diets for two months. The effect of insulin (2-40 μU/ml) to inhibit norepinephrine (NE)-induced lipolysis was examined in isolated adipocyte preparations. NE (10^{-7}M) elevated free fatty acids 2-7 fold. ETOH feeding resulted in a right-ward shift in the insulin dose-response curve in both young and old rats. Insulin (4 μU/ml) inhibited lipolysis in old untreated animals by 43% whereas no inhibition of lipolysis was demonstrated in ETOH treated rats ($p < 0.05$). Fat cells from young rats appeared less responsive to insulin's antilipolytic effect compared to fat cells isolated from old rats. These results indicate that ETOH feeding reduces insulin's antilipolytic sensitivity in rat adipose tissue. (Funded by NIAAA #1 R01 AA6701.)

16.6

REDUCTION OF BILIARY PHOSPHOLIPID SECRETION BY ETHANOL IN RATS. S. Rhalmi,* I.M. Yousef,* B. Tuchweber,* M. Sharkawi, Département de pharmacologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

Chronic administration of ethanol results in increased concentration of cholesterol in blood and liver in rats and man. Such increase could result from increased synthesis, decreased excretion and/or redistribution of cholesterol. In this study we investigated the effect of acute ethanol administration on biliary lipid secretion, a major route for cholesterol excretion and catabolism. The bile duct and a jugular vein of male Sprague-Dawley rats weighing 200 g were cannulated. Bile was collected for 90 min. initial period. Ethanol was then infused at a rate of 80 mg/hr/100 g body weight for four hours and bile was collected every 30 minutes. Appropriate controls were given saline. Ethanol treatment increased bile flow without increases in secretion rates of bile acids or cholesterol. However, phospholipid secretion was significantly reduced when blood and biliary ethanol levels were about 1 mg/mL. The lithogenic index in ethanol-treated animals was consistantly higher as compared to controls. These data indicate that ethanol can be eliminated in bile and that acute ethanol administration reduces biliary phospholipid secretion which results in an increase in the relative biliary secretion of cholesterol. This imbalance may results in the precipitation of cholesterol in bile possibly leading to gallstone formation.

16.7

EFFECTS OF L-LYSINE ON THE HEPATIC REDOX STATES, ETHANOL AND ITS METABOLITES, AND LIPIDS OF RATS RECEIVING ETHANOL CHRONICALLY. C.A. Lau-Cam*, H.W. Char*, V. Romeo* and A. Kapoor* (SPON: B. Yoburn). St. John's University, College of Pharmacy and Allied Health Professions, Jamaica, NY 11439

The effects of L-lysine on the hepatic redox states, ethanol and its metabolites, and lipids were studied in male Sprague-Dawley rats pair-fed an ethanol-containing Lieber-DeCarli liquid diet for 53 days. L-Lysine significantly prevented the decrease in the cytosolic and mitochondrial NAD^+/NADH and $NADP^+$/NADPH ratios during the first 28 days of the study, and insignificantly at 53 days. Likewise, the amino acid significantly prevented the increases in hepatic ethanol, acetaldehyde, and acetate levels, while significantly protecting against the hepatic accumulation of cholesterol and triglycerides. The protective effects of L-lysine appear to be related to the ability of the amino acid to form a transient adduct (Schiff base) with acetaldehyde, and to the subsequent utilization of reducing equivalents for the stabilization of the adduct as N^{ϵ}-ethyllysine.

16.8

INDUCTION OF CHLORDIAZEPOXIDE METABOLISM AFTER CHRONIC ETHANOL OR CHLORDIAZEPOXIDE DIET INTAKE. Arthur W.K. Chan. Research Institute on Alcoholism, NYS Division of Alcoholism and Alcohol Abuse, Buffalo, NY 14203.

Chronic ethanol administration in C57BL/6J mice did not cause an induction of ethanol metabolism (Chan et al., Pharm. Biochem. Behav. 17, 1239, 1982). Using the same diet protocol, we now report an induction of chlordiazepoxide (CDP) metabolism in ethanol-dependent mice in response to a challenge dose of CDP (120 mg/kg) on day 3 of ethanol withdrawal. Significantly lower blood levels of CDP, but higher levels of N-demethyl-CDP (NDCDP), were observed in ethanol-dependent mice compared to pair-fed controls for the first two hours after CDP injection. Mice treated chronically with a CDP diet were tested on day 3 of CDP withdrawal with a challenge dose of either ethanol (3.5 g/kg) or CDP (80 or 120 mg/kg). CDP-dependent mice showed significantly lower blood levels of CDP and NDCDP than pair-fed controls. Although the former group also showed lower blood alcohol concentrations (BAC), the rate of fall of BAC was not different in the two groups. Thus, chronic CDP treatment affected the absorption and distribution of ethanol. These results provide a metabolic basis for the manifestations of CDP tolerance and ethanol cross-tolerance that have been reported in CDP-dependent mice. The design of a mouse model of combined CDP/ethanol dependence needs to take into account the induction effects of both drugs. (Supported in part by PHS grant #AA06016.)

16.9

EFFECT OF GOSSYPOL AND PYROGALLOL ON ENDOTHELIUM DEPENDENT TOLERANCE TO ETHANOL IN THE AORTA. Edward T. Knych. Department of Pharmacology, University of Minnesota-Duluth, Duluth, MN 55812

Two days of ethanol intoxication produces a tolerance in the aorta to ethanol (E) induced contraction. Expression of this tolerance is dependent upon a functional endothelium suggesting the mediation of endothelium-derived relaxing factor (EDRF). To test this hypothesis the effect of gossypol (G) and pyrogallol (P) on E-induced contractions of aorta with endothelium from control or tolerant male S-D rats was studied. G inhibits the formation or release of EDRF while P inactivates EDRF be generating superoxide radicals. E tolerance was produced by administering E, orally, twice daily for 2 days according to Majchrowicz (Psychopharm. 43:245,1975). Rings of thoracic aorta were suspended in tissue baths for 1.5 hrs and then tested for the presence of functional endothelium by measuring carbachol (C) induced relaxation. 1 hr after testing, G (10-7 to 10-5M) or pyrogallol (10-5 to 10-4M) was added to the tissue bath. 15 min later either E or C dose-response curves were generated. EC50 values for E contraction or C relaxation were estimated by linear regression for each individual curve. E treatment produced tolerance as manifest by a significant shift of the E dose-response curve to the left. *Pretreatment of tolerant aortic rings with either gossypol or* pyrogallol produced a significant shift of the E dose-response curve to the left. Both G and P induced effects on E dose-response were correlated with their ability to inhibit C induced relaxation. These data support the hypothesis that tolerance to E-induced contraction of the aorta is dependent upon EDRF. (Supported in part by NIAAA RO1-AA06272 and the Graduate School, Univ. of Minnesota).

16.10

EFFECTS OF VOLATILE ANESTHETICS ON RESPONSES TO NOREPINEPHRINE (NE) AND ACETYLCHOLINE (ACH) IN GUINEA PIG ATRIA. A. B. Seifen*, R. H. Kennedy, E. Seifen, J. P. Bray* and G. A. Bushman*. University of Arkansas for Medical Sciences, Little Rock, AR. 72205.

Myocardial sensitization to catecholamines by hydrocarbon anesthetics has been described in both patients and intact animals; however, the mechanism of this action is not firmly established. Dose-response curves for NE- and ACH-induced alterations in developed tension (DT) and spontaneous pacemaking activity (HR) were obtained in left and right atria, respectively, isolated from guinea pig heart. These preparations were suspended in Krebs-Henseleit buffer saturated with 95%O_2, 5%CO_2 plus varying concentrations (0, 0.6 and 1.2 MAC) of halothane (H), isoflurane (I) or enflurane (E). The chronotropic actions of NE and ACH were not significantly affected by any of the anesthetics. Of the three volatile agents only H affected the positive inotropic response to NE, causing a slight but statistically significant rightward shift (less than a 2.5-fold increase in ED_{50}) in the dose-response curve. The only anesthetic which *altered the inotropic action of ACH was I, causing a 2-fold* increase in the ED_{50} value for this response. These data suggest that commonly used volatile anesthetics have no appreciable direct effect on the inotropic and chronotropic actions of NE or ACH in isolated guinea pig atria.

16.11

MECHANISMS OF THE INHIBITION OF FAST AXONAL TRANSPORT BY LOCAL ANESTHETICS. P.-A. Lavoie, P.R. Filion* and T. Khazen*. Dép. pharmacologie, Univ. de Montréal, Montréal, Canada H3C 3J7.

Bullfrog spinal nerves were incubated in vitro with local anesthetics under conditions known to inhibit transport, and the effects of these exposures to local anesthetics on the content of adenosine triphosphate (ATP) in nerves and on the density of microtubules in unmyelinated axons were examined. Lidocaine, at concentrations of 14 mM or 20 mM, did not reduce significantly the ATP content, and the density of microtubules was also not affected by 14 mM lidocaine; some other mechanism must therefore be responsible for the inhibition of fast axonal transport by 14 mM lidocaine. Significant reductions in ATP content were observed with 1 mM or 2 mM tetracaine, and with 0.5 mM or 1 mM dibucaine; however, comparison with the effects of 2,4-dinitrophenol indicated that these reductions were insufficient to inhibit transport in the case of 0.5 mM dibucaine or could at best only partly explain the transport inhibition in the other cases. Since the density of microtubules was not affected by 1 mM tetracaine and was not sufficiently reduced by 0.5 mM dibucaine to inhibit transport, some other effect must again largely contribute to or be solely responsible for the inhibition of fast axonal transport by these concentrations of dibucaine and tetracaine. (This study was made possible by funds from the MRC of Canada)

16.12

PHARMACOLOGICAL PROFILE OF ORF 20085, A NEW POTENT, LONG ACTING LOCAL ANESTHETIC. B. Dubinsky, D. A. Shriver, P. J. Sanfilippo*, J. B. Press*, J. J. Schupsky* and A. J. Tobia. Research Laboratories, Ortho Pharmaceutical Corporation, Raritan, New Jersey 08869.

ORF 20085, 2-(4-dibutylaminopropoxyphenyl)-8-methylimidazo[1,2-a] pyridine HCl is a potent, long-acting local anesthetic. Regional infiltration of the mouse sciatic nerve showed ORF 20085 (ED_{50}=0.0078%; 90 min, duration) to be more potent and longer acting than bupivacaine (B; ED_{50}=0.035%; 30 min), etidocaine (E; ED_{50}=0.025%; 30 min) or lidocaine (L; ED_{50}=0.18%; 15 min). On the rabbit cornea, topical ORF 20085 (ED_{50}=0.023%: 300 min, duration) was more potent and longer acting than B (ED_{50}=0.056%; 90 min), E (ED_{50}=0.049%; 45 min) or L (ED_{50}~2.0%; 30 min). In the guinea pig pin-prick assay, intradermal ORF 20085 (ED_{50}=0.017%), B (ED_{50}=0.016%) and E (ED_{50}=0.02%) were equipotent; but L (ED_{50}=0.072%) was less potent. In vitro, frog sciatic action potential amplitudes were reduced by ORF 20085 (2.5×10^{-3}M) and L (5.0×10^{-3}M) with B or E being more potent (5.0×10^{-4}M). In mice, the acute i.m. therapeutic indexes (TI = LD_{50}/ED_{50}) of ORF 20085 (TI=77) and E (TI=112) were greater than B (TI=29) or L (TI=36) suggesting a margin of safety, comparable or superior to other agents. These results and its low potential for dermal irritation or contact sensitization suggests that ORF 20085 may have clinical utility as an injectable and/or topical agent.

16.13

CARBOXYHEMOGLOBIN PRODUCTION DURING THE IN VIVO METABOLISM OF VOLATILE ANESTHETICS VIA THE HEPATIC CYTOCHROME P-450 DEPENDENT MONOOXYGENASE SYSTEM. M.B. Dunning III*, Jeanne Seagard and Donna Van Wynsberghe. Univ. of Wisconsin and V.A. Medical Center, Milwaukee, WI 53201.

The in vitro and in vivo metabolism of volatile anesthetics has been the subject of considerable research. Cytochrome P-450 enzymes have been implicated and many of the biochemical pathways and metabolites elucidated. The current study was undertaken for two purposes: 1) Determination of carboxyhemoglobin (COHb) as a possible intermediate metabolite during the administration of the inhalation anesthetics halothane and isoflurane and 2) Elucidation of the mechanism responsible for any changes seen in COHb levels. Male Sprague-Dawley rats were anesthetized (sodium pentobarbital 50mg/kg.), intubated and the carotid artery cannulated. Blood samples were obtained at the onset and every 30 minutes for 150 minutes during exposure to 1.5 MAC halothane or 2.5 MAC isoflurane. Data collected included: body weight, liver weight, heart rate, PaO2, PaCO2, pH, base excess, HCO3, SaO2, Hb, metHb, COHb, and cytochrome P-450 concentration. Significant increases were seen in the COHb levels with halothane and isoflurane administration. Further investigation is underway to determine if the P-450 inducer sodium phenobarbital or the P-450 inhibitor diethyl maleate, will alter COHb production to determine if this CO production is mediated by way of the cytochrome P-450 oxidase system.

16.14

INDUCTION OF ATAXIA AND LOSS OF RIGHTING REFLEX (LORR) DUE TO HIGH PRESSURE ARGON IN ALCOHOL SHORT SLEEP (SS) AND LONG SLEEP (LS) SELECTION MICE. T.K. Akers and J.K. Belknap, Departments of Physiology and of Pharmacology, UND School of Medicine, Grand Forks, ND 58202.

Anesthesia, ataxia, sedation are due to alcohol and gaseous anesthetics. The site of action of these drugs is the membrane. Membrane binding drugs are thought to alter fluidity of the membrane. For poorly lipid soluble gases like argon, high pressure is needed to achieve concentrations which will allow for alteration in membrane fluidity. In the present experiment, we studied the effect of argon at high pressure on SS and LS mice. Animals of both lines were placed in an open cage in a hyperbaric chamber. Enough O_2 was added for maintenance. Argon was added at 10 lbs/min. In one series of experiments, the animals were allowed to roam free in their cage. Threshold of LORR was detected by observing ataxic walk and the pressure at which this occurred. In the second, a tumbling cage was used periodically at different pressure levels. Pressure at which the animals could no longer right themselves was threshold of LORR. Five signs were analyzed: weaving, staggering, LORR, return of righting reflex (RORR) and ambulation. There was significantly less sensitivity shown by the SS animals for all of these measures than shown by the LS animals, which roughly parallels results with alcohol on LORR and RORR, for which these mice were specifically bred. (Supported by AA06243 and BRSG 4314).

NEUROPEPTIDES

17.1

COMPARATIVE ONTOGENETIC DEVELOPMENT OF THE mRNA'S FOR CALMODULIN (CM), PROENKEPHALIN (ENK) AND PROSOMATOSTATIN (SS). M. Cimino*, F. Chen* and B. Weiss, Department of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129

To understand more fully the role CM and other neuropeptides play in regulating neurotransmission in vivo at the level of gene expression, we determined the distribution and ontogenetic development of the mRNA's for CM, ENK and SS in the developing brain of the rat using in situ hybridization histochemistry. The probes were synthetic oligonucleotides complementary to specific regions of the different mRNA's. They were radiolabeled with ^{35}S-dATP by terminal deoxynucleotidyl transferase and hybridized to fixed brain sections of rats one to 64 days of age. The formation of radioactive hybrids was detected by film autoradiography and quantitatively analyzed using a DUMAS Image Analysis System. CM mRNA increased markedly after birth in the granular layer of the cerebellum, coinciding with the migration of granular cells to the granular layer. In the adult relatively high levels were found in hippocampus, pyriform cortex, striatum and thalamic nuclei. ENK mRNA was highly localized to the corpus striatum. This increased between 1 and 16 days of age, then declined. SS mRNA was more diffusely distributed in brain, though highly localized to specific cell types. At birth it was present in high concentrations in the inferior colliculus and anterior olfactory nucleus. With increasing age the density of SS mRNA decreased in inferior colliculus. These results demonstrate that the mRNA's for different modulator peptides are differentially distributed in the brain and develop independently. (Supported by GM34334)

17.2

NEUROPEPTIDE Y AND PROENKEPHALIN-DERIVED PEPTIDES IN BOVINE PINEAL GLAND AND RETINA. C. Cherdchu*, T.D. Hexum and M. Ebadi. Department of Pharmacology, Univ. Neb. Coll. Med., Omaha, NE 68105

The mammalian retinas and pineal glands, having developed as diencephalic evaginations, share numerous morphological, immunological, biochemical, and biological properties including the possession of select peptidergic transmission which is being further investigated and reported in this communication. Fresh bovine pineals were minced, homogenized in 1M acetic acid containing 0.02 N HCl, 0.1% mercaptoethanol, boiled and centrifuged at 20,000xg for 20 mins. The pellets were reextracted with the same solution, and the supernatant combined, lyophilized, and assayed radioimmunochemically for neuropeptide Y (NPY) using antibody raised against a hemocyanin-NPY conjugate, and for enkephalin-like peptides (ME) using a C-terminal directed antiserum to a [Met^5]-enkephalin-hemocyanin conjugate. The radioimmunoassayed NPY and ME yielded values of 1.50 and 1.28 pmoles/mg protein respectively. Furthermore, studies involving gel filtration on Biogel P-10 revealed the presence of only one peak for NPY-like and multiple peaks for ME-like immunoreactivity. These peptides were undetectable in bovine retina. These and other data support the contention that the multiple functions of the mammalian pineal gland, in addition to melatonin synthesis, are brought forth by peptidergic transmission including NPY and ME-containing neurons, whose occurrence in the retina is apparently less important. (Supported in part by grants from Amer. Heart Assn. and USPHS ES-03949.)

17.3

DIURNAL VARIATION IN THE EFFECTS OF METHYLXANTHINES ON RAT BRAIN LEVELS OF β-ENDORPHIN. Magdi R. I. Soliman. Nabil S. Himaya* and Charles M. Winget. College of Pharmacy, Florida A&M University, Tallahassee, FL 32307.

Previous studies conducted in our lab have shown that caffeine and theophylline affect the levels of methionine-enkephalin in rat brains and that these effects are diurnally controlled. The present investigation was designed to study the effects of these methylxanthines on the levels of β-endorphin in rat brains. Male Sprague-Dawley rats (150 - 200 g) adapted to a 12h light: 12h dark illumination cycle were used in this study. Caffeine (30 mg/kg) or theophylline (30 mg/kg) was administered i.p. to rats either in the light phase (11:00 hr) or in the dark phase (23:00 hr). Control rats were injected with saline. Animals were sacrificed by decapitation one hour later. The brains were dissected and their β-endorphin levels were determined by radioimmunoassay. Caffeine administration in the light phase resulted in a significant decrease (43.9%) in the levels of β-endorphin in rat brains. A similar significant decrease (33.45%) in brain β-endorphin was observed following theophylline administration in the light phase. However, the administration of caffeine in the dark phase did not significantly alter brain levels of β-endorphin. On the other hand, a significant decline in brain β-endorphin levels (57.04% decrease) was observed following theophylline administration in the dark phase. These results clearly indicate that methylxanthines affect the levels of β-endorphin in rat brains in addition to their effects on rat brain levels of methionine enkephalin previously reported. Moreover, these effects are also diurnally controlled. (Supported by NIH grant RR02660)

17.4

AGE-RELATED CHANGES IN BETA-ENDORPHIN LEVELS IN SHR, WKY and SD RAT BRAIN. A.J. Ingenito and S-J. Li*, Dept. of Pharmacol., Sch. of Med. East Carolina Univ., Greenville, NC 27858

Brain beta-endorphin (BE) may have a pathophysiological role in SHR hypertension. We investigated age-related changes in BE levels in various brain areas of SHR and their normotensive, age-matched WKY and Sprague Dawley (SD) controls, using BE radioimmunoassay. No significant differences between rats were found in hypothalamus, midbrain and brainstem at equivalent ages, but statistically significant differences were found in pituitary, as below. Aging decreased anterior pituitary BE but increased it in posterior pituitary. These data are consistent with a role for pituitary BE in the development of SHR hypertension.

BE-like Immunoreactivity in Pituitary (nmol/mg protein)

	Age(wk)	SHR	WKY	SD
Anterior Pituitary	4	1.53±0.05	1.66±0.05	1.00±0.05
	8	1.00±0.06	1.08±0.05	0.76±0.02
	12	0.62±0.01	0.94±0.04 *	0.52±0.01
	16	0.68±0.04	0.97±0.04 *	0.56±0.03
Posterior Pituitary	4	7.67±0.79	8.03±1.04	7.01±0.91
	8	11.06±2.80	8.23±0.86	16.44±2.78
	12	12.80±1.10	5.96±0.42 **	11.92±1.54
	16	11.93±0.66	8.54±1.23 *	13.24±0.93

* $p<0.05$, ** $p<0.01$ as compared with SHR group. (n=7-8).

17.5

THE EFFECT OF SUBMERSION ON PLASMA β-ENDORPHIN LEVELS IN EXPERIENCED SCUBA DIVERS. H.L. Tripathi*, N.W. Eastman*, and W.L. Dewey. Dept. of Pharmacology/Toxicology, Medical Coll. of VA/VA Commonwealth Univ., Richmond, VA 23298 and Dept. of Health and Physical Education, Univ. of Richmond, Richmond VA 23173.

A recent study from our laboratories demonstrated that submersion in a state of buoyancy by relatively inexperienced divers produced a mean increase of 495% in plasma β-endorphin immunoreactivity (β-EIR). The present study was designed to measure the changes in β-EIR in more experienced (3-23 years) instructor-level subjects after submersion. Plasma β-EIR was measured by radioimmunoassay in eight male scuba divers before and immediately after remaining motionless 10 ft under water for 20 min in a state of neutral buoyancy. Seven subjects showed an increase in β-EIR. The average concentration rose from 30.1 ± 1.2 to 35.5 ± 1.6 pg β-EIR/ml of plasma. The percent increase in levels varied from 0 to 48% of the control, with a mean increase of 19%. All divers reported subjective feelings of "well being", "relaxation", or "euphoria" afterward. It is concluded that extensive experience in scuba diving produces adaptation to the increase in β-EIR but not to the feeling of well-being or euphoria. This work supported by NIDA Grant #DA01647-09.

17.6

HYPERTHYROIDISM AND μ, δ AND κ OPIOID RECEPTORS IN BRAIN REGIONS. **George A. Matwyshyn*, P. Ramarao*, A. Gulati* and H.N. Bhargava, Dept. Pharmacodynamics, Univ. Ill. at Chicago, Chicago, IL 60612.**

Chronic s.c. administration of thyroxine (T_4) (1 mg/kg) to male Sprague-Dawley rats on alternate days for 18 days resulted in development of typical symptoms of hyperthyroidism. They included decreased rate of body weight gain, increased colonic temperature, increased systolic blood pressure and heart rate and increased serum concentration of T_3 and T_4 in comparison to the euthyroid rats. The binding of ^{3}H-DAGO, ^{3}H-DSTLE and ^{3}H-EKC to μ, δ and κ opioid receptors in brain regions of hyperthyroid and euthyroid rats was determined. The binding of ^{3}H-DAGO to amygdala and hypothalamus, of ^{3}H-DSTLE to membranes of amygdala, hypothalamus, pons + medulla and striatum and of ^{3}H-EKC to pons + medulla of T_4 treated rats was greater than in vehicle treated rats. The binding of ^{3}H-EKC to amygdala membranes of T_4 treated rats was lower than in vehicle treated rats. The binding of ^{3}H-EKC to amygdala membranes of T_4 treated rats was lower than in vehicle injected rats. The changes in opioid binding was due to changes in the Bmax values. The results suggest that opioid receptors are generally up-regulated in hyperthyroid rats **(Supported by a grant from Chicago Heart Association and a grant DA-02598 from the National Institute on Drug Abuse).**

17.7

POSSIBLE INVOLVEMENT OF DELTA OPIOID RECEPTORS IN CHOLECYSTOKININ OCTAPEPTIDE-INDUCED ANALGESIA IN MICE. Eun K. Hong* and A.E. Takemori. Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

When given i.c.v., sulphated cholecystokinin octapeptide (CCK-8; Asp-Tyr-SO_3H-Met-Gly-Trp-Met-Asp-Phe-NH_2) produced analgesia in the writhing test in mice. When given i.t., CCK-8 produced partial analgesia at low doses (7.5-60ng/mouse) and hyperalgesia at doses over 120ng/mouse. There was no hyperalgesia shown even at high doses of CCK-8(i.c.v.). Analgesia produced by CCK-8 (i.c.v. and i.t.) was antagonized by naloxone (NLX) given s.c. but hyperalgesia induced by CCK-8 (i.t.) was potentiated. pA_2 value for CCK-8 (i.c.v.) against NLX (s.c.) was 5.88 which indicated that CCK-8-induced analgesia was mediated through non-μ opioid receptors. Studies using selective opioid antagonists showed that CCK-8-induced analgesia was most significantly antagonized by δ antagonists such as ICI 154,129 and naltrindol (NTI) which increased ED_{50} from 30 to 96 and 106 ng/mouse, respectively. There was little antagonism of CCK-8 (i.c.v.) by β-FNA, a selective μ-opioid antagonist and no antagonism by nor-BNI, a selective κ-opioid antagonist. δ-Opioid agonist binding study using [^{3}H]DADLE in mouse brain membrane preparations showed that there were no changes in B_{max} or K_d in the presence of CCK-8. These data suggest that CCK-8 (i.c.v.) produces analgesia via δ-opioid receptors indirectly through release of endogenous opioids that interact with δ-receptors. (Supported by USPH Service Grant DA00289.)

17.8

NICOTINE AND OPIOID INDUCED ANTINOCICEPTION IS ANTAGONIZED BY ICI 174,864. Nancy Neidt Rome* and A.E. Takemori ,Dept. of Pharmacology, Univ. of Minnesota Medical School, Minneapolis, MN 55455.

We have previously reported that (-)-nicotine di(+) tartrate(N) induces antinociception in mice that is antagonized by naloxone (NLX). Those results supported the hypothesis that N induces antinociception through an opioid mediated mechanism. To further test this hypothesis, ICI 174,864 (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-enkephalin), a highly selective δ antagonist, was used versus N, Morphine(M) or DPDPE ([D-Pen^2, D-Pen^5]-enkephalin). ICI 174,864 inhibited DPDPE-induced antinociception, raising the ED_{50} value (i.c.v.) by 2.3-fold in the hot plate (HP) assay and 3.9-fold in the tail flick (TF) assay. It also significantly increased the ED_{50} values of N (i.c.v.) in the HP and TF assays, by 1.7 and 5.7-fold, respectively. The ED_{50} value of N (i.t.) and DPDPE (i.t.)was increased 2.3-fold and 2.9-fold by ICI 174,864 in the HP assay. We also determined whether the nicotinic antagonist, mecamylamine (MEC), blocked N- , M-, or DPDPE-induced antinocieption. Regardless of the route of administration of N or the assay used, MEC significantly increased the ED_{50} values of N. MEC had no effect on either M- or DPDPE-induced antinociception. These results support the hypothesis that N induces antinociception through an opioid mediated mechanism and suggest that the opioid component may be a post-nicotinic receptor mechanism which involves δ opioid receptor. (Supported by USPH Service Grant DA00289.)

18.1

IN VIVO DECREASE OF GLUTAMATE IN THE FRONTAL CORTEX OF AGED RATS. J.M. Peinado and R.D. Myers. Dept. Biochem. Univ. Granada. Spain and Dept. Pharmacol. Univ. East Carolina, Greenville, NC 27858.

The "in vivo" release of amino acids neurotransmitters was examined in the frontal cortex of young and aged rats. Guide tubes for push-pull perfusion were implanted bilaterally in the frontal cortex of two groups of rats of 3 and 24 mo. of age. After 8-12 days postoperative recovery, perfusion sessions started. In each perfusate the content of Glu, Asp, Gln, Gly and Tau was analyzed by high presure liquid chromatography with electrochemical detector. The experimental protocol consisted in two consecutive sessions of perfusions. The first was used to determine the basal levels of amino acids. The second perfusion, containing either 25 or 50 mM K^+ added to the artificial CSF, was used as an artificial depolarization procedure.

The results show, that a selective decrease in the levels of glutamate (38%; $p < 0.001$) occurs in the aged rats when compared to control. In both groups of rats, K^+ at concentration of 50 mM, increases significantly the release of glutamate (149%, control; 143% aged, $p < 0.02$) and taurine (173%, control; 145% aged. $p < 0.02$).

These data support the hypothesis for a role of glutamate in the frontal cortex during the normal process of aging.

18.2

USE OF AN HPLC ON-LINE HYDROLYSIS PROCEDURE TO EVALUATE SOURCES OF GABA IN HUMAN CSF. Joanne M. Miller*, Thomas N. Ferraro* and Theodore A. Hare. Thomas Jefferson Univ., Philadelphia, PA 19107

GABA concentrations in human CSF become elevated by several orders of magnitude during hydrolysis. Part of this increase can be accounted for by release of constituents from GABA-containing peptides, e.g. homocarnosine. A novel HPLC system was developed which uses on-line hydrolysis to screen for additional compounds that liberate GABA. The system contains a C_{18} reverse phase column which is eluted with water/methanol. The column eluent is then combined on-line with 2N NaOH prior to entering a 36 minute delay coil located within a heating vessel maintained at 100°C to hydrolyze peptides and other components into constituent amino compounds. Primary amines present in the coil effluent are derivatized on-line with *o*-phthalaldehyde and are then detected fluorometrically. CSF from 4 individuals and a CSF pool (n=10 patients) were deproteinized in 5% sulfosalicylic acid, centrifuged, filtered and frozen until time of analysis. Reference standards were treated the same as the CSF samples and included physiological amino acids and related amines as well as 2-pyrrolidinone, the GABA lactam. Free amino acids present in the CSF samples generally eluted near the front. Measurement of 2-pyrrolidinone standards and CSF samples spiked with known quantities of 2-pyrrolidinone produced linear standard curves with a detection limit of 400 pmol/ml. 2-Pyrrolidinone was not detected in any of the CSF samples studied. Total GABA determined in acid hydrolyzed CSF by ion-exchange chromatography with fluorometric detection was greater than 5 nmol/ml, and therefore the contribution of 2-pyrrolidinone accounted for less than 8% of the total GABA pool . These data conflict with a recent report (*J. Neurochem.* 49:1402, 1987) which found CSF 2-pyrrolidinone concentrations to be in the range of 2-3 nmol/ml, i.e., one-half of the total GABA pool in human CSF.

18.3

LIPOPHILIC ENDOGENOUS SUBSTANCE WITH HIGH AFFINITY FOR THE CENTRAL BENZODIAZEPINE RECEPTOR. H.L. Komiskey, S.E. Demick,* and A. Rahman*. University of Illinois, College of Medicine, Rockford, Illinois, 61107-1897.

Several compounds have been suggested as possible endogenous ligands of benzodiazepine receptors. In this study a lipophilic compound was extracted from rat brain tissue, examined for affinity to central benzodiazepine binding sites, and for an ability to affect gamma-aminobutyric acid (GABA)-stimulated chloride (Cl^-) uptake by synaptoneurosomes.

The central benzodiazepine binding sites in homogenate of rat cerebral cortex were labeled with ^{3}H-flumazepil. Incubations were conducted at 0-4 °C. Specific binding was measured as the difference between the ^{3}H-flumazepil bound in the absence and presence of 1μM clonazepam. The lipophilic compound had slightly more affinity for the central benzodiazepine binding sites than diazepam.

Synaptoneurosomes were prepared from rat cerebral cortex. Five seconds following the simultaneous addition of drugs and $^{36}Cl^-$, the uptake of $^{36}Cl^-$ by the synaptoneurosomes was terminated. The lipophilic compound decreased GABA-stimulated $^{36}Cl^-$ uptake.

Based on the chemical characteristics of the lipophilic compound, it does not appear to be a benzodiazepine, a beta-carboline or a polypeptide.

18.4

GABA AND BENZODIAZEPINES MODULATE THE INHIBITION BY MORPHINE OF POTASSIUM-STIMULATED ^{3}H-NOREPINEPHRINE (^{3}H-NE) RELEASE IN RAT FRONTAL CORTICAL SLICES. R.W. Peoples and G.E. Isom, Dept. of Pharmacol. and Toxicol., School of Pharmacy and Pharmacal Sciences, Purdue Univ., W. Lafayette, IN 47907.

Agents active at GABA or benzodiazepine receptors can modulate certain effects of opioids, such as analgesia. In this study, interactions between morphine and GABA or diazepam affecting release of ^{3}H-NE from rat frontal cortical slices were examined. GABA, 10^{-4} and 10^{-3}M, reversed the inhibition of 27.5 mM potassium-stimulated ^{3}H-NE release induced by 1 μM morphine. GABA, 10^{-4}M, shifted the morphine concentration-response curve to the right, increasing the EC_{50} of morphine approximately 100 fold. The reversal of morphine inhibition by GABA was reduced by (-)-bicuculline methiodide, 10^{-4}M. Exposure of the slices to (±)-baclofen, 10^{-4}M, did not affect the release of ^{3}H-NE in the absence or the presence of 1 μM morphine. Diazepam, 10^{-4}M, enhanced spontaneous ^{3}H-NE release, but inhibited potassium-stimulated ^{3}H-NE release and augmented the inhibition produced by morphine. These results support a role of the $GABA_A$/benzodiazepine receptor complex in modulating the action of opioids on the noradrenergic system in the cerebral cortex of the rat. (Supported by PHS grant S075505586).

18.5

IN VIVO EFFECTS OF TACRINE (9-AMINO-1,2,3,4-TETRAHYDROACRIDINE, THA) ON RAT BRAIN CHOLINERGIC AND MONOAMINERGIC NEUROTRANSMISSION. Y-H. Shih*, S. Whetzel* and T. A. Pugsley. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

It has recently been shown that THA in combination with lecithin improved the symptoms of patients with Alzheimer's disease (AD). This study examines the effects of THA on the turnover of acetycholine (Ach), norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in rat brain. THA administration (0.63 - 10 mg/kg i.p.) caused a dose-dependent decrease in in vitro sodium-dependent high affinity choline uptake (HACU) into rat hippocampal synaptosomes, as did physostigmine (1 mg/kg i.p.). Unlike the latter, THA also inhibited HACU uptake in vitro. This indicates that both agents are increasing cholinergic function presumably due to a buildup of Ach which consequently activates pre- or postsynaptic cholinergic receptors resulting in decreased Ach turnover. THA increased the turnover of DA in DA enriched brain areas, as well as that of NE and 5-HT indicating increased monoaminergic neuronal activity. Whether the latter effects are indirectly mediated by increased cholinergic activity or by other mechanisms is unclear. In view of the neurochemical evidence that AD involves multiple neurotransmitter systems, the efficacy of THA in affecting monoaminergic as well as cholinergic neuronal systems in the CNS may contribute to its action in treatment of AD.

18.6

NORADRENERGIC RESPONSES IN RAT HIPPOCAMPAL GRANULE CELLS IN VITRO. Jean-Claude Lacaille. Universite de Montréal, Montréal, Québec, Canada H3C 3J7.

Short applications of norepinephrine (NE) produce long-lasting potentiation of extracellular responses of hippocampal granule cells (GC). NE and related compounds were applied by micropressure to the soma of intracellularly impaled GCs in hippocampal slices to determine the cellular actions of NE. 75% of NE-sensitive GCs were depolarized by NE. NE depolarizations were associated with increases in input resistance (R_{in}), reversed near -84 mV, and were blocked by timolol. In 38% of sensitive GCs, NE produced hyperpolarizations. NE hyperpolarizations were associated with decreased R_{in} and reversed near -99 mV. Both types of NE responses were observed in low Ca^{++}/high Mg^{++}. Isoproterenol (ISO) also produced GC depolarizations, which were blocked by timolol and associated with increased or unchanged R_{in}. NE may increase excitability of GCs by activating beta receptors and closing a K^+ conductance active at resting membrane potential.

Supported by CAFIR, FRSQ, Savoy Foundation and Sloan Foundation.

18.7

ROLE OF GABAERGIC NEUROTRANSMISSION IN THE SUBSTANTIA INNOMINATA/LATERAL PREOPTIC AREA (SI/LPO) IN THE HYPERMOTILITY (HM) RESPONSES PRODUCED BY EXCITATORY AMINO ACIDS (EAA) INJECTED INTO THE NUCLEUS ACCUMBENS (NA). P.E. Shreve* and N.J. Uretsky. The Ohio State Univ. Coll. of Pharmacy, Columbus, OH 43210.

The SI/LPO is a region of the ventral pallidum that receives a GABAergic projection from the NA. The injection of muscimol (MUS) into the SI/LPO has been shown to inhibit the HM responses to heroin and amphetamine suggesting that inhibition of this GABAergic projection may mediate these HM responses. The purpose of this study was to determine the role of GABAergic neurotransmission in the SI/LPO on the HM responses to EAAs and picrotoxin (PTX) after their injection into the NA. As shown previously, the EAAs, α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), kainic acid, and N-methyl-D-aspartic acid, as well as PTX produced intense HM responses after injection into the NA. The responses to all of these drugs were antagonized by the injection of MUS into the SI/LPO, while injection of MUS outside this region did not produce inhibition. MUS injected into the SI/LPO alone neither reduced locomotor activity (LMA) nor produced catalepsy as compared to controls. These results support the concept that the SI/LPO is a critical neural substrate for the stimulation of LMA produced by drugs injected into the NA and suggest that a decrease in GABAergic neurotransmission in the SI/LPO may mediate this effect. Supported by NS 22582.

18.8

ACUTE EFFECTS OF THE NEUROTOXIN β-N-METHYLAMINO-L-ALANINE ON CA1 CELLS IN THE HIPPOCAMPUS. A. Baskys, E. Grima*, D.R. McLachlan* and P.L. Carlen. Playfair Neuroscience Unit, Depts. of Physiology and Medicine, Univ. Toronto, Toronto, Ont. M5T 2S8, Canada.

The in vitro hippocampal slice preparation was used to investigate the neuronal effects of β-N-methylamino-L-alanine (BMAA), a plant neurotoxin presumably responsible for the high incidence of amyotrophic lateral sclerosis and parkinsonism/dementia complex of Guam. Intracellular recordings made from CA_1 neurons included measurements of membrane potential, R_{in}, Ca-dependent afterhyperpolarization (AHP) and synaptic potentials. Addition of 0.5-10 uM D,L-BMAA to the perfusate resulted in 2-18 mV membrane depolarization associated with cell firing and occurring in a concentration-dependent manner. D, L-BMAA had no effect on the AHP. This finding differs from N-methyl-D-aspartate (NMDA) actions which also included suppression of the AHP at 0.2-0.5 uM concentration (Baskys and Carlen, Soc. Neurosci. Abs., 1987). These preliminary data show that BMAA and NMDA do not have identical electrophysiological actions.

Supported by OMHF and MRC of Canada.

18.9

A NON-NMDA EXCITATORY AMINO ACID MEDIATES SUBTHRESHOLD SYNAPTIC ACTIVITY INFLUENCING CAT LUMBAR MOTONEURONS DURING QUIET AND ACTIVE SLEEP. P. J. Soja*, F. Lopez*, F. R. Morales*, and M. H. Chase *Physiology & Anatomy Depts., & the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.*

The present study was performed to determine whether an excitatory amino acid may be involved in mediating the spontaneous subthreshold synaptic potentials which influence lumbar motoneurons during quiet sleep (QS) and the non-REM periods of AS. In the chronic unanesthetized cat, discrete synaptic depolarizing potentials were detected during QS and AS using a recently developed computer system (*J. Neurosci. Meth. 13: 19-35, 1985*). Under drug-free control conditions, spontaneous depolarizing potentials present during QS were characterized by their relatively large amplitude and brief time course. The group mean (± SEM) waveform parameters during QS were 0.61mV ± 0.02, peak amplitude; 0.58ms ± 0.01,10-90% rise-time; 1.09mV/ms ± 0.05, maximal rate-of-rise; 2.77ms ± 0.15, 10-90% decay time; and 1.62ms ± 0.07, half-decay-width. During AS, the amplitude and rising phase were similar to those of the depolarizing potentials observed during QS ($P>0.05$); however, the 10-90% decay time and half-decay-width were reduced by 19.6% and 14.2%, respectively ($P<0.05$). The reduction in the decay parameters is probably due to the concurrent presence of postsynaptic inhibition which occurs during AS (*Brain Res. 225: 279-295, 1981*). Following juxtacellular microiontophoretic applications of kynurenic acid (KYN, 0.15M, pH8.0) during QS and AS, subthreshold depolarizing synaptic activity was markedly suppressed or abolished; the remaining potentials were characterized by their reduced amplitude and prolonged time course. The group mean (± SEM) waveform parameters of those potentials remaining after KYN were 0.49mV ± 0.03, peak amplitude; 0.67ms ± 0.02, 10-90% rise-time; 0.78mV/ms ± 0.06, maximal rate-of-rise; 3.09ms ± 0.14, 10-90% decay time; and 1.87ms ± 0.13, half-decay-width ($P<0.05$). Microiontophoresis of the N-methyl-D-aspartate (NMDA) antagonist, 2-amino-5-phosphonovaleric acid (APV, 0.2M, pH8.0) during QS and AS suppressed polysynaptic EPSPs evoked by sciatic nerve stimulation and motoneuronal depolarizations induced by juxtacellularly applied NMDA. However, during QS and AS, APV failed to block or modify the time course of the spontaneous subthreshold synaptic potentials when compared to those recorded under drug-free controls ($P>0.05$). On the basis of the documented selectivity of KYN and APV in antagonizing the postsynaptic actions of NMDA versus non-NMDA-like excitatory amino acids, the present results suggest that the subthreshold excitatory drive(s) that impinges on the soma and proximal dendrites of lumbar motoneurons during QS and AS are mediated primarily by a non-NMDA-like neurotransmitter. Supported by grant NS23426.

18.10

MODULATION OF PROCONVULSIVE BEHAVIOR OF EXCITATORY AMINO ACIDS (EAA) BY 2-AMINO-4-PHOSPHONOBUTYRIC ACID (AP-4). P. D. Williams* and D. R. Helton* (SPON: P. I. Eacho). Lilly Research Laboratories, Toxicology Division, Greenfield, IN 46140

The activation and antagonism of EAA receptor subtypes are of interest due to their possible involvement in neurotoxicity, epilepsy, and ischemia. The present study was designed to evaluate the proconvulsive actions of N-methyl-d-aspartic acid (NMDA), l-aspartic acid (ASP) and l-glutamic acid (GLU) and their antagonism by (AP-4). Male CD-1 mice were administered NMDA, GLU, ASP, or AP-4 intraperitoneally ten minutes prior to electroconvulsive shock. Additional groups of mice were given AP-4 (12.5 mg/kg) ten minutes prior to either NMDA, GLU, or ASP. Electroshock was administered through corneal electrodes and the number of tonic and tonic-extensor type seizures were recorded.

Compound (dose; mg/kg)	CD_{50}(mA)	CD_{50}(mA)+AP-4
Control	7.5-8.3	7.4-8.2
NMDA (6.25/12.5)	4.2/2.1	6.3/5.6
GLU (500/1000)	7.2/5.0	5.7/3.9
ASP (500/1000)	7.8/5.3	7.4/6.5

These results suggest that selective patterns of activation (glutamate) and inhibition (NMDA) may be associated with the functionality of the AP-4 receptor in modulating EAA activity.

18.11

COMPARISON OF THE BETHANECOL- OR GALANIN-INDUCED HYPERPOLARIZATION OF MUDPUPPY PARASYMPATHETIC POSTGANGLIONIC NEURONS. Lukasz M. Konopka* and Rodney L. Parsons. University of Vermont, Burlington, VT 05405.

Parasympathetic neurons in the mudpuppy cardiac ganglion are hyperpolarized by bethanecol or the neuropeptide, galanin. The present study compares the characteristics of the hyperpolarization induced by these two agents. With both bethanecol and galanin, the hyperpolarization reversed near -105 mV and was dependent on the extracellular potassium concentration. However, the time course of the hyperpolarization induced by bethanecol and galanin application was different; the galanin response developing more slowly and lasting much longer than the bethanecol-induced hyperpolarization. In addition, raising extracellular calcium potentiated the galanin-induced hyperpolarization without any consistent effect on the bethanecol-induced hyperpolarization. Substitution of manganese for extracellular calcium inhibited the galanin-induced hyperpolarization and either had no influence or potentiated the bethanecol-induced hyperpolarization. The hyperpolarization produced by either bethanecol or galanin is due to the activation of a potassium conductance. However, The galanin-induced hyperpolarization is dependent on extracellular calcium suggesting that this peptide may activate a calcium dependent potassium conductance. Supported by NS 23978 and NSF BNS 8605611.

18.12

INTRACELLULAR INJECTION OF AMINOPYRIDINE AND CESIUM INHIBITS ASSOCIATIVE LONG-LASTING POTENTIATION OF SYNAPTIC RESPONSES IN CAT MOTOR CORTEX. A. Baranyi*, M.B. Szente* and C.D. Woody, UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Associative long-lasting potentiation (LLP) of excitatory postsynaptic potentials and currents (EPSPs/EPSCs) was studied in intracellular microelectrode and single channel voltage clamp experiments in the motor cortex of conscious cats. EPSPs/EPSCs evoked by stimulation of the pyramidal tract and thalamic VL nucleus were paired with orthodromic, antidromic or current-induced action potentials (ISI: 0-200 ms, ITI: 0.1-0.5 Hz). EPSP-spike pairings (20-60x) induced LLP of the paired EPSP for 20 min or longer in 37 of 61 (60%) cells. More selective enhancement of explicitly paired than unpaired inputs was found in each conditioned cell tested conjointly. Comparable LLP was not observed following pseudorandom presentations of EPSPs and action potentials. In other experiments potassium channel blockers: 3-aminopyridine (3AP), Cs, apamin, and TEA were pressure injected intracellularly before pairings. Injection of 3AP induced increases in VL and PT EPSPs in 16 of 21 cells in wich both EPSPs could be elicited. Then pairings as above induced LLP in only 5 of 64 (8%, 3AP) and 0 of 6 (0%, Cs) injected cells. The ability to induce LLP was not affected significantly by apamin (8 LLP of 14 cells) or TEA (5 LLP of 8 cells). Our results suggest that induction of LLP in neocortical neurons depends on reduction of a 3AP-sensitive potassium conductance locally near the postsynaptic sites of the paired EPSCs. (Supported by AFOSR F49620 and HD 05958.)

19.1

COUPLING BETWEEN CARDIAC AND LOCOMOTOR RHYTHMS. PHASE LAG BETWEEN PEDAL THRUSTS AND HEART BEATS. R. Lee Kirby*, Donald A. MacLeod* and Alan E. Marble* (SPON: J.P. Finley). Dalhousie Univ., Halifax, NS B3H 4K4.

During some rhythmic exercises, the heart and exercise rates become identical and may remain so for up to 5 minutes. We hypothesized that, if the intraarterial and skeletal intramuscular pressure cycles were reciprocal, blood flow to exercising muscle would be maximized and cardiac load minimized. In this study 9 subjects pedalled, at a frequency natural to them, on an electronically-braked bicycle ergometer which held the power output constant regardless of pedalling rate. To assess the phase lag between pedal thrust and heart beat, pedal-by-pedal plots of the ECG channel were generated throughout the 5-min workload at which the rates were within 1.0% of each other for at least 30 s. While rates were within 1% of each other, each subject's phase lag gradually lengthened and shortened. The results of this study illustrate the importance of beat-by-beat analysis (rather than averaging data) when studying coupling phenomena and suggest that our preliminary assumption, that the apparent coupling between cardiac and locomotor rhythms was on the basis of a single ischemic muscle group, was unwarranted. (Supported by the Medical Research Council of Canada.)

19.2

SKIN ELECTRICAL PROPERTIES AND TRANSDERMAL IONTOPHORETIC DELIVERY OF ARGININE VASOPRESSIN (AVP). Parichat Lelawongs*, Jue-Chen Liu*, and Yie W. Chien*. (SPON: John K-J Li). Rutgers University, Piscataway, NJ 08855-0789

The objective of this study was to correlate the electrical properties of the excised hairless rat skin during a prolonged passage of pulse current with the iontophoretic skin permeation of AVP. The transmembrane potential during a constant-current application was measured by a pair of Ag/AgCl microelectrodes. An *in vitro* skin permeation study was performed under passive diffusion and iontophoresis. The experiment on current-voltage relationship for the skin revealed a nonlinear behavior when the applied current exceeded a certain value. At this region, the skin impedance decreased rapidly during the first ten-minute application, and then approached plateau. The decrease in impedance was found to be dirctly related with the applied current. This may be responsible for the enhanced AVP flux at higher current. The removal of the stratum corneum significantly reduced the skin impedance and no enhancement in the transport of AVP was observed through the use of iontophoresis. The result of *in vitro* iontophoretic skin permeation was compared with that of *in vivo*.

19.3

A LINEAR 2ND ORDER MODEL OF THE CAROTID SINUS WALL AND BARORECEPTOR PROPERTIES OF THE RABBIT. Henry O Stinnett. Physiol. Dept. UND Sch. Med., Grand Forks, ND 58202.

Using TUTSIM (Palo Alto, CA) computer simulation procedures a model was designed with 4 degrees of freedom (df) for wall and 6 df for receptor elements. Preliminary 2nd order models with less df were eliminated by comparison to mean (n=14) dynamic and near steady state responses of sinus wall and multifiber nerve activity following a critically damped step increase and decrease in intrasinus pressure. A set of receptor elements consisted of 3 units which simulated "slow" (positional responders), "fast adapting" and "resetting" (velocity responders) responses of baroreceptors. Two receptor sets were placed in model regions representing "media-adventitia" and more peripheral "adventitia" locals. Model wall elastic coefficients (K_1 to K_4) were equal at 400 N/m and viscosity coefficients (C_1 to C_4) ranged from 1.0E3 to 1.34E5 N(s)/m. Activity (a) transduction coefficients were, 1.36E6 a/m for positional and 4.0E7 & 7.2E8 a(s)/m for velocity responders. By comparison, model wall and receptor responses closely simulated experimental data. Baroreceptor characteristics of "diastolic silence", varied "threshold activation pressure" and "gain" were simulated by model receptors. Part support: UND Fac. Res. Com., ORPD and BRSG NSS 2-S07 RR05407 -27.

19.4

LOCAL CEREBRAL BLOOD FLOW INCREASED IN RAPID EYE MOVEMENT SLEEP. R.M. Abrams, J.C. Post*, D.J. Burchfield*, A.A. Hutchison*, K.J. Gomez* and M. Conlon*. Departments of OB/GYN, Otolaryngology, Pediatrics and Statistics, University of Florida, Gainesville, Florida 32610.

In fetal lambs a positive correlation exists between rates of local cerebral glucose utilization and time spent in REM sleep (Devel Brain Res 40:65, 1988). In the present research a heated thermocouple method was developed to determine if regional changes in fetal cerebral blood flow also rise during REM sleep. Four 36 guage Cu-Const thermojunctions (TJ) wound with 10-12 turns of 42 gauge urethane-coated copper heater wire were implanted in cerebral cortex and subcortex of 4 near-term fetal lambs. Electrocorticogram, electrooculogram and neck EMG leads were also implanted to determine fetal behavioral state, and at least 2 days allowed for recovery. A temperature difference (ΔT) between 2 TJs was measured continuously throughout several sleep cycles by applying 250 mAmps to one heater while using the contralateral unheated TJ as a reference. The ΔT in REM sleep was lower than in NREM sleep ($p<.02$) reflecting convective heat loss from increased blood flow. Differences in ΔT between sleep states depended on location of TJ ($p<.002$). The thermocouple method gives qualitative, continuous information on local fetal cerebral blood flow which will be useful in assessing the time course of the vascular response to changing behavioral states. Supported by NIH Grant HD-20084.

19.5

INTERPRETATION OF IONTOPHORETIC TRANSPORT USING AN EQUIVALENT CIRCUIT FOR SKIN

Jue-Chen Liu*, John K-J Li, Weimin Shi* and Ying Sun*, Rutgers University, Piscataway, NJ 08855-0789

Transdermal delivery of peptide/protein drugs by iontophoresis for systemic medication has been the subject of many studies for its potential applications. A new equivalent circuit was proposed to represent the electrical properties of skin and to predict transdermal iontophoretic process. This analog skin model encompasses an equivalent capacitor of stratum corneum, a switch controlled by threshold potential, and several resistors which are functions of size and physicochemical properties of the charged molecules. The current flow of charged molecules under pulse d.c. field was simulated at various conditions. The results of simulation indicated that the time constant of polarization should be smaller than that of depolarization in order to achieve high efficiency, i.e., the duty cycle of a periodic d.c. electrical field applied should be less than 50%. The competition factors between large and small molecules were found to be dependent upon the relative ionic compositions of the donor solution. A comparison was made between current model and Nernst-Plank/Poisson equations.

20.1

OLFACTORY RECEPTOR RESPONSE TO UPPER AIRWAY CO_2 IN THE BULLFROG. E. Lee Coates* and Garv O. Ballam Lovelace Medical Found. and Univ. of New Mexico, Albuquerque, NM 87131.

Previous studies have reported that bullfrogs and several species of reptiles (*Epicrates striatus, Thamnophis sirtalis,* and *Tupinambis nigropunctatus*) depress ventilation when exposed to upper airway (UA) CO_2. This response is abolished when the olfactory nerves are transected, indicating that CO_2-sensitive receptors are located in the olfactory epithelium. The purpose of the present study was to record the electro-olfactogram (EOG) of the olfactory receptors in the bullfrog, *Rana catesbeiana*, in response to a 1 sec pulse of 4% CO_2. The EOG, a slow change in the dc potential recorded on the surface of the olfactory epithelium, is the summed generator potentials of a population of receptor cells. It was found that 4% CO_2 caused a negative monophasic wave (mean duration = 7 sec) with a rapid rising phase and a slow return to baseline. The mean amplitude of the EOGs from 8 sites (N = 3) was 106μv. At two sites, 1.2% CO_2 was delivered to the olfactory epithelium. The EOG amplitudes in these cases were 20μv and 40μv. Pulses of air caused no apparent change in the dc potential. The mean amplitude of the EOGs recorded in response to a control odor (10^{-3}M amyl acetate) at the same 8 sites was 1.1mv. These data show that there is a measurable olfactory receptor response to CO_2 in the bullfrog. It is concluded from these data that the ventilatory depression observed in amphibians and reptiles in response to UA CO_2 is initiated with an olfactory receptor response to CO_2.

Supported in part by grants from the NHLBI #29342 and the Flinn Foundation #047-286-002-85.

20.2

ANION GAP DURING SEVERE SEPSIS WITH METABOLIC ACIDOSIS. Eric C. Rackow, Craig Goldstein,* Mark E. Astiz,* Carter Mecher,* David McKee,* and Max H. Weil. UHS/The Chicago Medical School, North Chicago, IL 60064

It is assumed that the development of metabolic acidosis during septic shock is secondary to lactic acidosis. To test this hypothesis, severe sepsis was induced in 5 rats by cecal perforation with 5 rats serving as sham operated controls. Arterial blood was sampled at 6 h for blood gases, and plasma Na^+, K^+, Cl^-, total CO_2, lactate (L), pyruvate (P), beta-hydroxybutarate (B), acetoacetate (A), citrate (C), urea nitrogen (BUN), albumin (ALB) and amino acids (AA).

	Septic	Sham	p
Na^+ mEq/L	143.1 ± 1.2	147.7 ± 1.6	<.01
K^+ mEq/L	5.9 ± 0.4	3.5 ± 0.1	<.01
Cl^- mEq/L	109.6 ± 1.6	108.9 ± 0.8	NS
Total CO_2 mEq/L	18.2 ± 1.6	29.2 ± 0.6	<.01
Anion gap mEq/L	21.6 ± 1.6	13.2 ± 0.5	<.01
$PaCO_2$ mmHg	24.9 ± 3.6	42.2 ± 2.4	<.01
pH units	7.41 ± .04	7.43 ± .02	NS
Lactate mEq/L	2.2 ± .03	0.9 ± .02	<.01

There were no differences in P, B, A, C, BUN, ALB or AA. Only 15% of the increase in anion gap in septic animals could be accounted for by lactate. Neither lactic acid nor the other metabolic intermediates accounted for the anion gap. This provides an explanation for the poor correlation of the anion gap and arterial lactate during clinical shock states.

20.3

RED CELL, GILL AND RENAL RESPONSES TO ANION EXCHANGE INHIBITION IN THE SHARK, Squalus acanthias. E.R. Swenson and P.A. King, University of Washington, Seattle, WA., Emory University, Atlanta, GA., and Mt. Desert Island Biological Laboratory, Salsbury Cove, ME.

Cl^-/HCO_3^- exchange is thought to be necessary in CO_2 elimination and acid-base regulation in fish. The effects of DNDS (4,4'-Dinitro-2'-2 stilbene disulfonic acid), a reversible nontoxic stilbene disulfonate were studied in a marine elasmobranch. In five resting sharks, 50 mg/kg, caused a mild respiratory acidosis by one hour (PCO_2 2.0 → 3.5 mmHg) but had no effect on urine pH or output. The rate of branchial HCO_3^- excretion following a 9 mmol/kg $NaHCO_3$ load was unaffected by DNDS either given systemically or in ambient seawater at 100 uM. Peak plasma levels of DNDS given systemically were 100-200 uM and the half time for drug clearance by the kidney was ~ 90 minutes. DNDS inhibited 50% of the ^{36}Cl efflux from red cells in vitro at 0°C with a K_I of less than 10 uM. These data show the role of red cell band 3 protein in CO_2 elimination but no demonstrable effect of anion exchange inhibition with this drug on gill or renal acid-base transfers.

20.4

DO LUNG MECHANICS EXPLAIN BREATHING PATTERN IN THE COATIMUNDI? D.F. Boggs, G. Baltopoulos* and C. G. Irvin. U. of Montana, Missoula, Mt. 59812; National Jewish Hosp., Denver, Co. 80206.

It has been shown that T_e/T_{tot} is an interspecific constant of 0.65 (Boggs & Tenney, Resp. Physiol.,1984). The coatimundi deviates from that pattern with an expiratory time (T_e) only 0.48 of total breath time (T_{tot}), and it has a large tidal volume (V_t = 56 ml) and low frequency (f = 22) for its body size (mean values for 5 coatis, 4.6 kg). To begin to investigate the basis of this pattern, we measured lung volumes, static and dynamic mechanics of 3 (5 kg) coatis in a volume-displacement plethysmograph.

Mean Lung Volumes (ml/kg)					
TLC	VC	IC	FRC	ERV	RV
154	118	93	54	26	25

R_L (cm H_2O/ml/sec)			Compliance (ml/cm H_2O)			
Insp.	Exp.	Total	$Lung_D$	$Lung_S$	R.S.	C.W.
0.0092	0.0108	0.0089	32	48	11	14.8

TLC is 2.5 times predicted for an animal this size, hence V_t is not an unusual proportion of TLC. Resistance, abit low for this body size, is appropriate to the large lung volume and does not explain the timing pattern. C_L is quite high and C_{CW} is low (which may be important to the coatis climbing behavior), but C_{RS} is appropriate to this lung volume. Inspiratory 'drive' (V_t/T_i) is not unusual for an animal this size, but chemosensory aspects of the control of T_e may represent an important avenue of study to explain the coatis peculiar intra-breath timing pattern.

20.5

BRANCHIAL SHUNTING IN THE RAINBOW TROUT, Salmo gairdneri.

N. Heisler*, I. Ishimatsu*, G. Iwama* and P. Neumann* (Spon: J. Piiper). Dept. Physiology, Max Planck Institute for Experimental Medicine, D-3400 Gottingen, FRG.

The fraction of cardiac output bypassing the systemic circulation and being returned directly to the sinus venosus was estimated in rainbow trout by application of a number of different techniques. Blood flow in ventral and dorsal aorta was determined by the Fick principle and multiple application of indicator distribution methods. Radio-labelled microspheres and inulin were injected and blood withdrawn for blood gas and radioactivity analysis at four different sites: caudal vein, bulbus arteriosus of the ventral aorta, proximal dorsal aorta, and the caudal artery. No significant differences could be found between gill blood flow estimated by the Fick principle, and ventral and dorsal aortic blood flows determined by indicator distribution techniques. These findings, suggesting insignificant afferent or efferent branchial shunting of the systemic circuit, were corroborated by data obtained from application of our recently developed new technique for in vivo chronic cannulation of the branchial vein. Using haemoglobin as an indicator, the flow through the central venous sinus (thus bypassing the systemic circulation) was estimated on the average to be less than 7 % of cardiac output. These data are in considerable contrast with those obtained from in vitro isolated head and gill preparations (30–70 % flow bypassing the systemic circulation). It is concluded that changes in intrabranchial pressure profiles and largely elevated circulating catecholamine levels may be responsible for the substantially elevated shunt fraction in vitro as compared to in vivo conditions.

20.6

TAUROPINE DEHYDROGENASE IN MOLLUSKS AND BRACHIOPODS. C.S. Hammen and S.C. Woodbury*. Dept. Zool. Univ. Rhode Island, Kingston, R.I. 02881.

The tissues of certain species of marine mollusks and brachiopods contain an enzyme that catalyzes the reductive condensation of pyruvate with taurine to form a compound first called rhodoic acid, but later re-named tauropine, by analogy with the formation of octopine from pyruvate and arginine in cephalopod mollusks. Enzymes such as octopine dehydrogenase (EC 1.5.1.11), alanopine dehydrogenase (EC 1.5.1.17), and tauropine dehydrogenase (TDH) apparently serve the same function in metabolism as lactate dehydrogenase (LDH), which is often present at very low levels of activity in marine invertebrates. In assays containing 2.0 mM pyruvate and 80 mM amino acids, tissue extract of the adductor of the abalone, Haliotis rufescens, had TDH activity more than twice as great as LDH. In the foot muscle of the same animal, ODH activity was twice as great as LDH, and TDH was lacking. Another gastropod, Littorina littorea, had ADH in the foot muscle in the same range as LDH, and lacked both ODH and TDH. Both internal tissues and pedicle of the small lingulid brachiopod, Glottidia pyramidata, were examined. There was very high TDH activity in both, moderate ADH, and extremely low LDH. Kinetic properties of TDH were the same in both tissues. More species should be examined, and the properties of these enzymes should be studied, in order to explain their distribution.

20.7
PHYSIOLOGICAL RESPONSES OF THE AVIAN EMBRYO FOLLOWING MICROWAVE-INCUBATION. Donald E. Spiers. John B. Pierce Foundation Laboratory, New Haven, CT 06519
This study determined if repeated exposure of the japanese quail embryo to microwaves results in abnormal development. Eggs were exposed (8 h/day) for the first 15 days of incubation to either sham or microwave (2450 MHz) conditions. Microwave power density was either 5 or 20mW/cm^2. Exposure temperature (T_a), set at either 35 or 30°C, respectively, resulted in an egg temperature of approximately 37.5°C (i.e., normal incubation level) for the microwave-exposed eggs. Embryos were individually tested in ovo at 16 days of incubation to determine thermal and metabolic responses to test T_a=30.0, 32.5, 35.0 and 37.5°C. Each egg was tested in a temperature-controlled chamber. Oxygen content of chamber effluent air was measured to estimate metabolic rate (Watt/egg) and an implanted thermocouple determined internal egg temperature. At 16 days, growth of sham-exposed embryos had slowed by 1 day for every 2°C reduction in exposure T_a below 37.5°C. Wet mass of embryos exposed to 5 and 20 mW/cm^2 microwaves was 106 and 86% of normal mass, respectively. Comparisons of thermal and metabolic responses as a function of wet mass showed no differences between the responses of microwave-exposed and normal embryos. Microwave incubation can be effectively utilized to increase growth of the avian embryo, without altering physiological development. (Supported by NIH Grant 5R23ES03769-03).

20.8
CREATINE KINASE (CK) AND LACTATE DEHYDROGENASE (LDH) IN MUSCLE AND SERUM OF EXERCISING PIGS.
F. Doizé*, R. Laporte and L. DeRoth. Faculté de Médecine vétérinarie, Université de Montréal, St.Hyacinthe, Qué. J2S 7C6 Canada.
In order to determine the response of skeletal muscle to exercise, six Landrace pigs were submitted to 10 min. running on a treadmill (0.5m/sec., 12%) and were compared to six controls. Blood samples were obtained just before, 24 and 48 h after the exercise. Muscle biopsies were taken from the m. longissimus dorsi (mLD) and biceps femoris (mBF) 24 h after the exercise. The total CK activity was not different in the two groups however total CK activity in mBF represented only 59% of that observed in mLD. A mean CK-MB value of 2.5% was found in the control group for both muscles but, it significantly increased with exercise to 9.5% ($P<0.05$) for mBF and 12.2% ($p<0.01$) for mLD. The total LDH was the same in both muscles. There was a tendency to a lower LDH5 ($P<0.1$) and a higher LDH1 ($P<0.1$) activity in both exercised muscles. Serum samples exhibited a similar pattern. Thus necrosis occured in the oversized fibers of pigs selected for muscularity, moreover, this is the first time that CK-MB is reported in skeletal muscle of pigs.

MICROCIRCULATION

21.1
GLUCOSE IS REQUIRED FOR THE MAINTENANCE OF ISOLATED BOVINE CEREBRAL MICROVESSEL ENERGY STATE (ATP/ADP RATIO)
Christopher Gaposchkin* and Anthony L. McCall. Boston University School of Medicine, Boston, Ma 02118
Isolated cerebral microvessels (ICMV) were incubated for up to five hours with various single metabolic fuels to determine their role in the maintenance of microvessel energy state. Ultrasensitive bioluminescent measurements of the ATP and ADP contents were made hourly. In fuel-free incubation, microvessel ATP/ADP ratios decreased from about 3 to 1. Glucose (5.5 mM) prevented the drop in ATP/ADP and frequently increased this ratio above the values of fuel-free controls. By contrast, pyruvate (2.0 mM), β-hydroxybutyrate(2.0 mM), and glutamate(2.0 mM) showed no effect, despite isotopic evidence of their oxidation. Incubation with oleate or palmitate(0.33 mM) increased microvessel ATP/ADP ratios slightly only when co-incubated with carnitine. Fuel-free incubations with 2-tetradecylglycidate, a specific inhibitor of long chained fatty acid β-oxidation, decreased ATP/ADP ratios by 75% when compared to drug-free incubations. These data suggest that: 1) glycolysis is a major source of ATP for ICMV's, and 2) their use of exogenous fatty fuels may limit loss of ATP content during fuel deprivation.

21.2
SURVIVAL OF STRESS-INDUCED MACROCYTES IS CRITICALLY DEPENDENT ON CELL GEOMETRY. James M. Norton. University of New England, Biddeford, ME 04005.
To investigate the role of red blood cell (RBC) geometry in the survival of stress-induced macrocytes, RBC volume, diameter, and filterability through 3 μ and 5 μ filters were measured on RBCs from control rats and from phenylhydrazine-(PHZ)-treated rats during a 28-day recovery period following the peak hematopoietic response to PHZ-induced anemia. Mean cell volumes (MCV) were calculated from volume distribution curves; RBC diameters were obtained from peripheral blood smears; surface area (SA), sphericity index (SI), and mean cylindrical diameter (MCD) were calculated using a biconcave erythrocyte model. At the time of the peak macroreticulocytic response to PHZ, both MCD and MCV were increased compared to controls (3.07 μ vs 2.57 μ, and 102.1 μ^3 vs 59.7 μ^3, respectively), SI increased (0.794 vs 0.741), SA/MCV was reduced (1.405 vs 1.670), and RBC filterability through 3 μ and 5 μ pores was significantly reduced. During the 28-day recovery period MCV, SI, SA/MCV, and filterability returned toward control values, MCD was inversely correlated with filterability through both 3 μ ($r = -0.861$, $p<.005$) and 5 μ ($r = -0.767$, $p<.01$) pores, and MCD fell below 3.6 μ in 97.5% of the animals despite the presence of large macrocytic subpopulations. These results demonstrate the correlation between RBC geometry and deformability, and support the existence of pore-like in vivo restrictions 3.6 μ in diameter limiting RBC lifespan. (supported by the AHA/Maine Affiliate, Inc.)

21.3
DIFFERENTIAL RESPONSES OF FEED AND TERMINAL ARTERIOLES TO EXERCISE OF RAT CREMASTER MUSCLE. David E. Mohrman and Lois J. Heller. University of Minnesota, Duluth, MN 55812.
The purpose of this study was to evaluate possible communication and coordination of action between consecutive vascular segments. Standard videomicroscopic techniques were used to record the time course of arteriolar diameter responses to exercise of rat cremaster muscle. In 15 preparations, diameters of 21 terminal arterioles and their respective feed arterioles were observed (maximum diameter with topical 1mM adenosine = 24 ± 2 μm and 54 ± 6 μm respectively). At rest, terminal arterioles were invariably closed whereas feed arterioles were at 26 ± 5% of their maximum diameter. Responses to tetanic stimulation of the motor nerve (2 sec @ 20 Hz) and twitch stimulation (3 min @ 1 Hz) were studied. Peak responses occurred at 25 sec with the tetanic and 3 min with the twitch stimulus. In each case, the two vessel segments followed an identical time course in reaching the peak response. Terminal and feed arterioles recovered from the twitch contraction bout with half times of 98 ± 11 ane 252 ± 56 sec respectively. Terminal arterioles recovered monotonically from the brief tetanic contraction with a half time of 38 ± 10 sec whereas their feed arterioles recovered with a much slower and biphasic time course. These results suggest coordinated vasodilation but uncoordinated vasoconstriction between immediately consecutive microvascular segments. (Supported by NHLBI HL32686.)

21.4
MICROVASCULAR AND PERIPHERAL HEMODYNAMIC RESPONSES TO NEUROPEPTIDE Y IN RATS. **P.L. Hester*, B.P. Fleming, and K.W. Barron. Dept. of Physiology and Biophysics, Univ. of Kentucky, Lexington, KY, 40536.**
The arteriolar responses to neuropeptide Y (NPY) were studied in the rat cremaster muscle. NPY in the suffusate produced vasoconstriction of 2A, 3A and 4A arterioles during alpha-adrenergic blockade with a threshold dose of $3x10^{-10}$ M. A 50% vasoconstriction of all vessels was produced by $3x10^{-9}$ M NPY. The 3A arteriolar responses to sympathetic nerve stimulation and exogenous application of norepinephrine were not affected by threshold doses of NPY. For comparison, the peripheral vascular effects of intravenous NPY were examined in 4 conscious rats. NPY produced dose-dependent (0.5, 1.0 and 2.0 nmoles/kg) increases in mean arterial pressure (MAP) and total peripheral resistance (TPR), and decreased cardiac output (CO). At 2.0 nmole/kg, NPY increased MAP to 120% of control and TPR to 144%, with CO falling to 85% of control. In summary, NPY produces non-adrenergically mediated arteriolar vasoconstriction in rat skeletal muscle consistent with its ability to produce increases in peripheral resistance. **Support: NIH HL36552.**

21.5

SUBSTANCE P (SP) PREFERENTIALLY DILATES PRECAPILLARIES IN FACIAL AND NASAL TISSUES OF DOGS. K. Pleschka, T. Ikeda* and M.Sugahara*.Max-Planck-Institut f. physiologische & klinische Forschung, W.G. Kerckhoff-Institut, 6350 Bad Nauheim, F.R.G.

Total blood flow and perfusion pressure (PP) of the internal maxillary artery (IMA) were recorded bilaterally at rest and during unilateral IMA infusion of SP at doses of 1, 2, 5, 10, 20 and 50 pmol/min in anaesthetized, paralyzed and artificially ventilated dogs. Distribution of IMA flow to precapillaries (CAP flow) and arteriovenous anastomoses (AVA flow) was determined by the tracer microspheres technique.

I.) Total IMA flow: Unilateral SP infusion caused a dose-dependent increase in ipsilateral, and at higher doses also in contralateral IMA flow. At 50 pmol/min IMA flow increased by 78% above resting level (32.0 ml/min). Flow increases were due to graded decreases in resistance to flow of the IMA. Heart rate, PP and catecholamines were barely affected by SP.

II.) IMA flow distribution: About 70% of IMA flow was drained as AVA flow and 30% as CAP flow. Increases in CAP flow were clearly dose-dependent in contrast to AVA flow increases. As a result fractional contribution of CAP flow increased, while that of AVA flow decreased during SP stimulated increases of total IMA flow.

III.) Flow compartmentalization: CAP flow revealed substantial differences in flow rate between the various compartments analyzed. The relationship between compartments with low and high flow rates which were found under control conditions largely persisted at all SP concentrations.

21.6

EVIDENCE OF STRETCHED PORES IN THE MICROVASCULATURE OF ISOLATED PERFUSED RABBIT HEARTS. R.E. Gosselin, D.R. Van Houten*, and C.J. Luneau*. Dept. Pharmacology & Toxicology, Dartmouth Medical Sch., Hanover N.H. 03756.

Interstitial fluid oozes continuously from the epicardial surface of non-beating, quinidinized hearts perfused with Ringers in the Langendorff manner. During steady-state perfusion the surface drip rate is a measure of the net filtration rate (J_v). Two lines of evidence suggest that raising the perfusion pressure (P_a) enlarges microvessel pores through which this filtration occurs. First, J_v is best described as proportional to $(P_a)^n$ where the mean n = 1.58 (±.04). Because the pressure gradient across the capillary wall in this preparation appears to be a linearly increasing function of Pa, we conclude that the capillary filtration coefficient (CFC) increased progressively with Pa between 0 and 250 mm Hg. At least in the upper range, it is unlikely that capillary recruitment was responsible for the rise. Second, in these abnormally leaky hearts, macromolecules even as large as Blue Dextran (mean 2 x 10^6 D) escape into the interstitium. The sieving ratio (SR) of Blue Dextran was evaluated as the steady-state concn. ratio of surface drip/perfusion fluid. In most hearts SR rose when J_v was increased by elevating Pa (in no case did SR fall). Thus BD transport appears to be purely convective. The rise in SR suggests pressure-induced increases in pore diameter. At all P_a levels, SR, J_v and CFC were much lower when the perfusion fluid contained bovine serum albumin (0.5 or 1%).

21.7

EFFECTS OF BRADYKININ ANTAGONISTS ON VASCULAR PERMEABILITY IN RAT SKIN. Larry R. Steranka, Richard Rodriguez* and Christopher J. DeHaas*. Nova Pharmaceutical Corporation, Baltimore, MD 21224

Bradykinin (BK) induces an increase in vascular permeability which has been reported to be inhibited by BK antagonists in rabbits, but not in rats (Whalley et al., NS Arch. Pharmacol., 336:430-433, 1987). Since the effects of the bradykinin antagonists alone were not examined in this study, and since they have been reported to degranulate mast cells, we examined the effect of the BK antagonist, D-Arg[Hyp^3,D-Phe^7]BK (NPC 567), on plasma extravasation in rat skin using the Evan's blue dye method and found it to induce a dose-related increase in vascular permeability in the range of 10^{-9} to 10^{-7} moles/injection. BK produced comparable extravasation at 10-fold lower doses. To examine the role of mast cell mediators, we examined the effects of diphenhydramine (DPH) and cyproheptadine (CYP) on extravasation induced by histamine, serotonin (5-HT), BK and NPC 567. CYP inhibited 5-HT- and histamine-induced extravasation with comparable potencies, while DPH inhibited only histamine-induced extravasation. CYP (5 mg/kg) produced a > 1000-fold rightward shift in the NPC 567-induced extravasation dose-response curve, indicating that the increased vascular permeability produced by NPC 567 is mediated by 5-HT and histamine, presumably released from mast cells by the compound. When rats were pretreated with CYP, NPC 567 markedly inhibited BK-induced extravasation.

21.8

TOTAL PROTEIN REFLECTION COEFFICIENT OF NORMAL AND ISCHEMIC MYOCARDIUM. Charles F. Pilati*(SPON: M. Maron). Dept. of Physiology. N.E. Ohio Univ. Col. Med. Rootstown, OH 44272.

In this study I determined the total protein reflection coefficient (σ) of canine coronary vessels. Myocardial lymph was collected from the anterior interventricular lymphatic trunk (located on the anterior surface of the LV) of anesthetized dogs, and σ determined from the lymph-to-plasma protein concentration ratio (L/P) measured during elevated coronary sinus pressure. L/P's were independent of lymph flow at lymph flows (Q_L) greater than 12 times baseline. It was necessary to infuse adenosine (0.4 μmoles/min) into the left anterior descending coronary artery (LAD) concurrently with elevated coronary sinus pressure to produce lymph flows of this magnitude. Mean L/P was 0.78 under baseline conditions and decreased to 0.33 ± 0.02 (σ = 0.67) during elevated coronary sinus pressure and adenosine infusion. Mean Q_L increased 18.5 times during this time. In a second group of dogs (n = 4), σ was determined during reperfusion following 60 min of ischemia produced by occluding the LAD. When coronary sinus pressure was elevated in this group (adenosine was not necessary), L/P decreased only slightly from 0.79 ± 0.05 to 0.70 ± 0.05 despite a 30-fold increase in Q_L. These results indicate that σ for coronary vessels is about 0.7 and decreases markedly following ischemic injury. (supported by AHA, Ohio Affiliate).

21.9

PHALLOIDIN PROTECTS THE STRUCTURE AND FUNCTION OF RETE CAPILLARIES AGAINST INJURY BY HYPOXIA AND REPERFUSION. E.A. Rasio, M. Bendayan*, C.A. Goresky, S.J. Alexander* and D. Shepro. Hôpital Notre-Dame, Montreal, Que., H2L4M1, Canada.

The effects of phalloïdin 10^{-6}M on blood capillary structure and permeability were studied in the countercurrent perfused rete mirabile of the eel swimbladder. In the normal rete, the addition of phalloïdin to the perfusion medium did not induce morphological or functional changes. In the rete subjected to stagnant hypoxia and reperfusion, cell membrane blebs and vacuolization, mitochondrial swelling and interstitial oedema were observed. A progressive breakdown of the intercapillary barrier during reperfusion resulted in an increase of the permeability coefficients to radiolabeled albumin, sucrose and sodium by a factor of 3.5 to 4.5 above baseline values. The permeability to water was not modified. When the same protocol was repeated with phalloïdin present in the medium throughout the experiment, the structural integrity of the endothelial cells was completely preserved; many pericytes were filled with filament-like structures. The permeability to albumin, sucrose and sodium increased more slowly and only by a factor of 1.5 above baseline values, a significant reduction by comparison with the experiments without phalloïdin. It is concluded that phalloïdin 10^{-6}M does not improve the capillary barrier of the normal rete but protects it against structural and functional damage induced by hypoxia and reperfusion. Supported by grants from MRC, QHF and USPHS.

21.10

NEUTROPHIL MEDIATED INJURY TO THE SKELETAL MUSCLE MICROVASCULATURE IN THE ABSENCE OF ISCHEMIA. D.L. Carden*, J.K. Smith*, and R.J. Korthuis. Department of Physiology, Louisiana State University Medical Center, Shreveport, LA 71130.

It is becoming increasingly apparent that neutrophils play a major role in producing ischemia/reperfusion (I/R) injury. However, the complexity of the chemical and physical alterations (hypoxia, acidosis, xanthine oxidase) accompanying I/R make this a difficult model to study the mechanisms involved in neutrophil-mediated changes in vascular permeability. The purpose of this study was to determine if activated neutrophils increase skeletal muscle microvascular permeability in the absence of ischemia. Granulocyte activation with zymosan activated plasma (ZAP) was associated with neutrophilic superoxide production and myeloperoxidase release. Intradermal ZAP injection produced a marked increase in tissue myeloperoxidase activity indicating that ZAP induced migration of neutrophils into the tissue. Perfusion of isolated canine gracilis muscles with ZAP (10 ml ZAP/100 ml blood in perfusion reservoir) produced a decrease in the osmotic reflection coefficient for total plasma proteins from 0.91±0.14 to 0.59±0.09 indicating a dramatic increase in microvascular permeability. Moreover, the ZAP-induced increase in microvascular permeability was very similar to that noted in canine gracilis muscles subjected to 4 hours of inflow occlusion and 1 hour of reperfusion. The results of this study indicate that 1) neutrophil activation, in the absence of ischemia, produces an increase in microvascular permeability which is very similar to that noted after prolonged I/R and 2) in vivo granulocyte activation with ZAP is a useful model to study neutrophil-mediated changes in skeletal muscle microvascular permeability. Supported by NIH HL-36069 and HL-07710 and AHA 880818. RJK is an EI of the AHA.

21.11

ROLE OF IRON IN ISCHEMIA/REPERFUSION INJURY TO SKELETAL MUSCLE MICROVASCULATURE. J. Keith Smith*, D.L. Carden*, M.B. Grisham, D. Neil Granger, R.J. Korthuis. Department of Physiology, Louisiana State University Medical Center, Shreveport, LA 71130

Iron catalyzed formation of hydroxyl radicals has been postulated to occur during reperfusion of ischemic tissues. To assess the role of iron-catalyzed oxidant production in ischemia/reperfusion (I/R) injury in skeletal muscle, we examined the effects of deferoxamine (DF, an iron chelator) and apotransferrin (ATF, an iron binding protein) on the increased vascular permeability produced by ischemia/ reperfusion in rat hindquarters. Osmotic reflection coefficients (σ) were measured in hindquarters subjected to 2 hours of ischemia and 30 minutes of reperfusion with either no pretreatment, or pretreatment with deferoxamine, apotransferrin, or iron-loaded deferoxamine(Fe-DF). I/R alone was associated with an increase in vascular permeability (as indicated by a fall in the osmotic reflection coefficient). DF or ATF pretreatment attenuated this permeability increase, while pretreatment with Fe-DF offered no protection.

GROUP	σ
Control	0.82±0.02
I/R	0.68±0.03*
I/R + DF	0.83±0.03
I/R + ATF	0.86±0.02
I/R + Fe-DF	0.71±0.02*

These findings lend support to the hypothesis that iron-catalyzed oxidant production is important in the production of microvascular injury following ischemia/reperfusion. This study was supported by grants from the NIH (HL-36069 and HL-07710) and AHA (#880818). RJK is a recipient of an AHA Established Investigatorship (#880197).

21.12

LIMITING OXYGEN AVAILABILITY DURING REPERFUSION ATTENUATES MICROVASCULAR INJURY IN POSTISCHEMIC SKELETAL MUSCLE. R.J. Korthuis, J. Keith Smith*, and D.L. Carden*. Department of Physiology, LSU Medical Center, Shreveport, LA 71130.

The purpose of this study was to test the hypothesis that molecular oxygen must be provided at reperfusion to produce ischemia/reperfusion (I/R) injury in skeletal muscle. Isolated, maximally vasodilated (papaverine) canine gracilis muscles were reperfused, after 4 hr of inflow occlusion, from reservoirs containing autologous blood equilibrated with either 95% O_2-5% CO_2 or 95% N_2-5% CO_2 gas mixtures. Arterial PO_2 fell from ~120 mm Hg to less than 3-5 mm Hg during the use of nitrogen. The osmotic reflection coefficient for total plasma proteins (σ) was determined for the following conditions: control (no ischemia), reperfusion with oxygenated blood after 4 or 5 hr ischemia, and reperfusion first with anoxic blood and then oxygenated blood. Reperfusion with oxygenated blood, after 4 hr of ischemia, significantly reduced σ from 0.93±0.02 to 0.63±0.02, indicating a dramatic increase in vascular permeability. When reperfused with oxygenated blood after 5 hr of ischemia, σ averaged 0.60±0.05. In muscles reperfused with anoxic blood, σ averaged 0.82±0.06. During the latter experiments, shifting the perfusate from anoxic blood to oxygenated blood further reduced σ to 0.60±0.05. This data indicates that 1) molecular oxygen, normally provided at reperfusion, is necessary to produce increased skeletal muscle vascular permeability during reperfusion and 2) reoxygenation of previously hypoxic skeletal muscle results in microvascular injury which extends beyond that which can be attributed to the ischemic process alone. Supported by grants from the NIH (HL-36069 and HL-07710) and the American Heart Association (#880818). RJK is the recipient of an Established Investigatorship from the American Heart Association.

21.13

LEUKOCYTE ADHERENCE TO MICROVASCULAR ENDOTHELIUM DURING ISCHEMIA-REPERFUSION (I/R). J. Russell*, J.N. Benoit, M.B. Grisham and D.N. Granger. LSU Medical Center, Shreveport, LA 71130.

Leukocyte adherence (#per 100 μm vessel length), leukocyte rolling velocity, vessel diameter, and red cell velocity were measured in cat mesenteric venules (≈30 μm). Venular blood flow, wall shear rate and the number of extravasated leukocytes (NEL) were also determined. All parameters were measured under control conditions, during 60 min. partial occlusion of the superior mesenteric artery (SMA), and following reperfusion. SMA occlusion led to a 75-80% reduction in venular blood flow, wall shear rate and leukocyte rolling velocity. Leukocyte adherence increased 3.5-, 6-, and 5-times during ischemia and 10 min & 60 min after reperfusion, respectively. NEL increased 3.4-, 5- and 8-times control during the same periods. Pooled data from all time periods produced a significant negative correlation between adherent leukocytes and wall shear rate, and a positive correlation between NEL and adherent leukocytes. The results of this study indicate that I/R promotes leukocyte adherence to microvascular endothelium as well as leukocyte extravasation. The low wall shear rates associated with I/R appears to be an important factor that facilitates leukocyte adherence and extravasation. (Supported by DK 33594).

21.14

XANTHINE OXIDASE INHIBITION ATTENUATES ISCHEMIA-REPERFUSION (I/R) INDUCED LEUKOCYTE EXTRAVASATION. D.N. Granger, J.Russell*, M.B. Grisham and J.N. Benoit. LSU Medical Center, Shreveport, LA 71130

Xanthine oxidase-derived oxidants have been implicated in the neutrophil infiltration associated with reperfusion of the ischemic intestine. We examined the influence of allopurinol pretreatment on leukocyte adherence and extravasation induced by I/R in cat mesentery, which contains 60-80 mU/g tissue xanthine oxidase activity. Leukocyte rolling velocity, vessel diameter and red cell velocity were also measured in ≈30 μm diameter venules. All parameters were measured under control conditions, during 60 min. partial occlusion of the superior mesenteric artery (SMA), and following reperfusion. The responses of venular blood flow, wall shear rate, and leukocyte rolling velocity to ischemia and reperfusion did not differ between control (untreated) and allopurinol-treated animals. However, the number of extravasated leukocytes following reperfusion, but not during ischemia, was significantly (≈50%) lower in the allopurinol-treated animals. The results of this study support the hypothesis that xanthine oxidase plays an important role in mediating the neutrophil infiltration initiated by reperfusion of ischemic tissues (Supported by DK 33594).

28.1

EFFECTS OF MICROGRAVITY ON THE DIFFERENTIATION OF GRAVISENSING CELLS IN THE LENTIL ROOT. Gérald Perbal and Dominique Driss-Ecole. Université P et M Curie, Paris, France.

A morphometric analysis of cell polarity of statocytes was performed on lentil roots grown for 25h : (1) on the ground, (2) on the 1g centrifuge on board, (3) in microgravity or (4) in microgravity and subjected to 1g for 3h. Dry seeds were hydrated in space and the seedlings were chemically fixed in microgravity. This analysis allowed us to distinguish : (1) the direct effects of microgravity on the distribution of amyloplasts and on the location of the nucleus, (2) the indirect effects of this factor on the number of amyloplasts per cell in section and on the morphometry of the aggregates of endoplasmic reticulum, (3) the role of a 3h stimulation on the number of mitochondrias. The comparison of the average area of the statocytes in section in the four samples showed that the volume of these cells was greater in seedlings grown in space, whatever the g conditions were. This could be due to other space factors as cabin atmosphere or cosmic rays. It is concluded that in space experiments on cell biology a 1g control is necessary to discriminate between the effects of microgravity and those of other space factors.

28.2

THE EFFECT OF SUSPENSION ON NICOTINIC ACETYLCHOLINE RECEPTOR NUMBER AND AFFINITY AT THE RAT NEUROMOSCULAR JUNCTION. Joyce E. Royland* and Lavern J. Weber. OSU Hatfield Marine Science Center, Newport, OR 97365.

We investigated a possible neuromuscular component to space induced muscle atrophy. Experimental rats were suspended by the Morey-Holton rat-tail suspension mode. Weight-matched, pair-fed controls were housed singly in identical cages. Animals were housed, fed and killed according to NIH guidelines. Using the specific nicotinic acetylcholine receptor ligand, α-Bungarotoxin, receptor number (B_{max}) and affinity (K_d) was measured in the tonic soleus and phasic gastrocnemius, triceps brachii, and tibialis. We found no change in B_{max} or K_d in any phasic muscle. In the tonic, antigravity soleus we found an apparent two-fold increase in B_{max} when measured as fmoles/gm wet wt tissue, but the increase disappeared when measured as fmols bound/whole muscle. Thus we found a concentrating effect due to decreases in muscle mass and nonmembrane protein, but no real increase in receptor number. About a 50% decrease in soleus K_d indicated an increase in receptor affinity. Work supported by OSU Pharmacology Department.

28.3

BIOCHEMICAL AND HISTOCHEMICAL OBSERVATIONS OF VASTUS MEDIALIS FROM RATS FLOWN IN COSMOS 1887 (EXPERIMENT K608). X. J. Musacchia, Joseph M. Steffen, and Ronald M. Fell. Univ. of Louisville, Louisville, KY 40292

Vastus medialis was obtained from rats exposed to weightlessness. Lyophilized samples were used for protein (mg/mg dry wt) RNA and DNA (ug/mg dry wt) and lactate dehydrogenase (LDH) and citrate synthase (CS) activity (u moles/min/gm). RNA in flight (F) subjects (5.5±.1) was significantly reduced below basal (B) controls (6.0±.1). DNA in F, vivarium (V) and B subjects were similar. Protein concentrations in F, B and synchronous (S) subjects were comparable (0.7 to 0.8 ug/mg dry wt). The level of LDH activity (>2100) is characteristic of fast twitch highly glycolytic (type II B) fibers. Conversely the oxidative capacity (10 u moles/min/gm.) as measured by CS activity was low and characteristic of fast twitch muscle. Frozen sections were stained for ATPase activity, fiber area and density measured and capillary distribution assessed. Composition was almost completely type II fast twitch fibers. F rats showed significant reductions (25%) in type II fiber area. Fiber densities (cells/mm^2) were inversely related to areas. F and V subjects showed an increase in cell density as compared to B controls. Although some morphological parameters indicate a degree of atrophy, biochemical analyses suggest these may be minimal. These results agree with previous observations of another type II fast twitch muscle, the EDL, from SL-3. (Supported by NASA NAG2-386 and COSMOS A53745C (CPL).

28.4

DECREASED SWELLING PRESSURE OF RAT NUCLEUS PULPOSUS ASSOCIATED WITH SIMULATED WEIGHTLESSNESS. Alan R. Hargens and Mubashar Mahmood*. Space Physiology Branch, NASA-Ames Research Center, Moffett Field, CA 94035 and Dept. of AMES-Bioengineering, Univ. Calif., San Diego, La Jolla, CA 92093

Intervertebral discs are important load-bearing tissues of the spine and lose height during upright posture. Although the disc is implicated in the etiology of back pain experienced by astronauts during weightlessness, little is known about its fluid balance with prolonged unloading. Therefore, this study examined swelling pressure within discs obtained from rats exposed to actual and simulated weightlessness and compared these pressures with those in several groups of control rats. Samples of nucleus pulposus were obtained from lumbar discs of : 1) five 300g male Wistar rats exposed to 12.5 days microgravity on Cosmos 1887, 2) two groups of ground-based controls (N=10), 3) seven 200g male Sprague-Dawley rats exposed to 7 days of tail suspension, and 4) another two groups of controls (N=14). Lumbar samples were pooled for each rat and equilibrium swelling pressure was determined in a compression-type osmometer for sample volumes of 5-10 microliters. Swelling pressures were 690, 675 and 622 mmHg for flight rats, synchronous controls and vivarium controls, respectively. For simulated weightlessness, swelling pressures were 295, 610 and 527 mmHg for tail-suspended rats, cage controls, and vivarium controls, respectively. Unfortunately, rats aboard Cosmos 1887 were re-exposed to normal gravity for over 50 hours prior to tissue harvesting and therefore, swelling pressures of their discs were probably readjusted to normal gravity. On the other hand, swelling pressure within discs of tail-suspended rats is about half that in controls, suggesting that fluid may move into the disc during prolonged simulated weightlessness. However, more studies of flight animals are needed to determine if tail suspension is a good model for disc physiology under conditions of actual weightlessness. (Supported by NASA and Cosmos 1887)

28.5

NORMALISATION OF BONE CELLULAR RESPONSES OCCURS BETWEEN 7 AND 14 DAYS OF SIMULATED WEIGHTLESSNESS IN RATS. Vico L., Alexandre C. Faculté de Médecine, Saint-Etienne, France.

Two experiments of rat tail-suspension, according to Wronski and Morey-Holton recommendations, have been performed in order to be further compared with results obtained from the Biocosmos 1667 (7d.) and 1887 (14d.) spaceflights. Rats used in both real and simulated microgravity experiments were of the same strain, age (~12 weeks) and weight (~310 g). Bone histomorphometric parameters were evaluated in proximal tibial metaphysis at the level of the secondary spongiosa where bone remodeling occurs. The bone volume is reduced to the same level in both suspension experiments. Resorption parameters i.e. osteoclast number (determined with a specific histoenzymologic method) and active resorption surfaces show a twofold increased after a 7d. suspension while they are normal after 14 days. Formation parameters i.e. mineralization rate (measured after double tetracycline labeling) and osteoid surfaces are decreased in the shorter suspension while they show normal values in the longer. We concluded that two bone remodeling phases occurs in this model: the first in which resorption activity increases whereas formation activity decreases, resulting in bone loss and the second in which bone cellular activities come back to equilibrium and bone tissue loss is no longer observed. Same dynamic evolution of bone mass could occur in weightlessness.

28.6

MODIFICATIONS OF BONE ATROPHY SEEN WITH HINDLIMB SUSPENSION BY EXERCISE AND DOBUTAMINE. S.Bloomfield*, B.Girten*, S. Weisbrode*, E.Eveland*, and L.Kazarian*. (SPON: D.R. Lamb). The Ohio State Univ., Columbus, OH 43210 and Armstrong Aerospace Medical Research Lab., WPAFB, OH. 45433.

This study evaluated effects of exercise(EX) and dobutamine(DOB), a synthetic catecholamine, on morphometric markers of bone loss seen with hindlimb suspension (SUS). Adult male S-D rats were randomly assigned to treadmill EX or sedentary(SED) treatments for 11 weeks. Half of each group were then suspended by tail traction for 14 days utilizing the Morey-Holton X-Y axis support system. During this period half of the SUS and half of the non-suspended (CON) rats received injections of DOB; others received saline(SAL). Fluorescent markers for bone growth were injected in all rats on days 0, 7 and 14 of SUS. Three-way ANOVA (<0.05) revealed a 17% decrease in cortical bone area(CBA) at mid-diaphyseal femur in the SED/SUS/SAL rats. Unexpectedly, 3 of the EX groups had osteopenic changes similar in severity to that of the SED/SUS/SAL rats; however, the CBA of the EX/SUS/SAL group was not different from baseline. DOB appeared to prevent osteopenic changes due to SUS in SED rats, but had variable effects in EX rats. It also had no effect on the decreased periosteal formation rate seen in SED/SUS/SAL and EX/SUS/SAL rats. In summary, EX was counterproductive in maintaining bone mass; DOB prevented osteopenic changes only in SED rats. Sponsored by AAMRL/BBD/AFOSR Project # 2312V6.

28.7

MYOCARDIAL DEGENERATION IN RATS EXPOSED TO 12.5 DAYS OF MICROGRAVITY. D. E. Philpott, K. Kato*, J. Stevenson*, W. Sapp*, I. Papova* and L. Serova*. Space Physiology Branch, NASA-Ames Research Center, Moffett Field, CA 94035

The flight of Cosmos 1887 provided an opportunity to examine hearts of rats exposed to 12.5 days in Earth orbit. Left ventricles (LV) were removed from five rats exposed to microgravity and two sets of five rats that acted as ground controls. Each LV was dissected into four parts, two of which were placed in fixative (Triple Fix) for electron microscopy and the remaining tissue frozen for later biochemical analysis. Tissues were shipped from Moscow, USSR to NASA Ames Research Center for processing, embedding in plastic, ultra-thin sectioning and ultrastructure examination. Volume density (VD) of the mitochondria was determined by point counting. VD of the flight tissue mitochondria was 9% less than the controls ($p<.05$). There was degeneration of mitochondria in some of the flight tissue myofibrils, an increase in glycogen granules and myeloid bodies. Blebbing also occurred on the inner walls of some of the capillaries. Generalized edema was also present. These preliminary results suggest that the myocardium undergoes degeneration in microgravity. (Supported by NASA and Cosmos 1887).

28.8

PLASMA STRESS HORMONES IN RESTING RATS. EIGHTY FOUR DAY STUDY. Vojin Popovic and Clegg Honeycutt*. Emory University School of Medicine, Atlanta, Georgia 30322.

Fifty six male white rats (Sprague-Dawley) with implanted cannulas (PE 10) in aorta and in ventricle of right heart as well as with aortic blood flow probes (pulsed doppler, Crystal Biotech) were used in this study. The implantation was done at a body weight of 180 ± 10 g. Microsamples of aortic blood (0.3 ml) were withdrawn once every 15 days from unrestrained, unanesthetized resting rats. During blood withdrawal the rats were placed in a nontransparent "sampling box" with slits on the top of the chambers for exteriorization of the cannulas. The aortic blood was withdrawn 30 minutes after the rat was placed in the sampling box, sufficient time for plasma stress hormones to return to resting values after being elevated due to handling of the animal. The experiment lasted 84 days (duration of cardiovascular experiments to be performed on NASA SLSI and SLSII flights). After 84 days, all animals were alive, their cannulas patent and their blood flow probes functional. The plasma levels of stress hormones (ACTH, corticosterone and prolactin) were at low resting values and stayed unchanged during the 84 day long study indicating that body weight growth, aging or seasonal changes do not have detectable effects on resting levels of stress hormones in rats. Thus, determination of the level of plasma stress hormones can be used to assess stresses occurring during and immediately after space flight. (Supported by NASA contract NAS2-10527.)

28.9

INFLUENCES OF SIMULATED WEIGHTLESSNESS AND CHEMICAL SYMPATHECTOMY ON THE $\dot{V}O_2$ MAX OF RATS. C.R. Woodman*, C.S. Stump, S.M. Beaulieu*, Z. Rahman*, L.A. Sebastian*, and C.M. Tipton, Dept. Exercise & Sport Sciences, University of Arizona, Tucson, AZ 85721.

Maximum oxygen consumption ($\dot{V}O_2$ max) has been shown to be reduced in humans and rats after periods of simulated weightlessness. To determine whether the sympathetic nervous system was associated with this process, female and male Sprague-Dawley rats were either chemically sympathectomized (Symx) with injection of guanethidine sulfate, or saline injected (Sham) after birth and assigned to one of three groups for 14 days; head-down suspension (HDS), horizontal suspension with all limbs bearing weight (HWB), or cage-control (CC). The rats were tested for $\dot{V}O_2$ max (ml/min) prior to suspension and on days 7 and 14. For comparison with our previous findings (**Med. Sci. Sport Ex.** 20(2):S48, 1988) only the results from females are listed ($\bar{X}$, SE, N, * statistical significance at 0.05 level).

Maximum Oxygen Consumption (ml/min)

GROUP	N	BEFORE	DAY 7	DAY 14
CC: Symx	6	21.1±1.3	22.3±1.4	22.6±2.1
Sham	8	22.9±1.2	24.2±1.4	22.8±1.2
HWB: Symx	7	23.0±1.1	22.0±0.8	22.5±1.2
Sham	8	21.9±0.9	20.0±0.7*	21.3±1.0
HDS: Symx	7	21.3±0.7	21.7±0.7	21.8±1.3
Sham	7	23.4±0.5	20.7±1.3*	20.6±1.0*

It was concluded that sympathectomy and weight bearing attenuate the reduction of $\dot{V}O_2$ max associated with simulated weightlessness. A similar trend was observed in males.

(Supported by NASA Grant, NAG-2-392.)

VASCULAR SMOOTH MUSCLE PHARMACOLOGY I

29.1

SHORT TERM VASCULAR REACTIVITY AND HISTOLOGICAL CHANGES OF AUTOLOGOUS VEIN GRAFTS. Adel S. Soliman*and Randall L. Tackett, Cardiovascular Pharmacodynamics lab, College of Pharmacy, Univeristy of Georgia, Athens, GA 30602

Autologous vein grafts have been used extensively to treat vascular insufficiency in angina. However, recurrence of anginal symptoms has been reported at different time intervals. This study was designed to investigate the short term changes that may relate to loss of graft patency. Saphenous vein graft was implanted into the carotid artery by the reversed end to end technique in mongrel dogs. After one week the graft was harvested, rings were cut from the mid portion of the graft and suspended in organ chambers for isometric force measurements. Dose response curves to various vasoactive compounds were determined and compared with those of the normal saphenous vein and carotid artery. Grafted vessels showed a significant decrease in maximal response to norepinephrine, tyramine, 5-HT, and BH-T933. Additionally, these vessels demonstrated a leftward shift of the norepinephrine and 5-HT curves. Several grafts developed vasospastic activity, spontaneously or after being exposed to tyramine or norepinephrine. Histologically these grafts showed proliferation of the subendothelial cell layer, and disorganization of the medial smooth muscle layer. According to our results, enhanced vascular reactivity, development of vasospasms and structural disorganization of the graft integrity may be contributing factors for the early failure of vascular grafts.

29.2

ROLES OF PROTEIN KINASE C, Na^+/H^+ EXCHANGE AND CALCIUM IN CONTRACTION INDUCED BY VASCULAR ALPHA-2 ADRENERGIC STIMULATION. T.K. Aburto and R.C. Deth, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA 02115

We investigated the involvement of protein kinase C (PKC), Na^+/H^+ exchange and extracellular calcium in contractions induced by the stimulation of alpha-2 adrenergic receptors α_2-AR) in rabbit saphenous vein. Staurosporine (0.1 uM; a PKC inhibitor) when preincubated for 20-25 min with the tissue preparations inhibited contraction elicited by the specific α_2AR agonist UK 14,304 (UK) by 80%. Direct activation of PKC by phorbol dibutyrate (PDBu; 1 uM) provoked a biphasic contraction and inhibited subsequent UK-induced contractions by 90%. Surprisingly, nifedipine (0.5 uM) potentiated PDBu-induced contraction and had little effect on UK-induced contraction (20% decrease). Studies undertaken in Ca^{++} free buffer, however, did indicate a requirement for Ca^{++} influx in order for contraction to be elicited via α_2AR stimulation. Amiloride, ethylisopropylamiloride and dimethylamiloride (50 uM) all potently inhibited both norepinephrine- and UK-induced contraction by up to 80%. Indomethacin (0.1 uM) did not affect UK-induced contraction. These findings indicate a possible role for PKC activation in α_2AR mediated arterial responses as well as PKC modulation of receptor coupling events. Amiloride and its analogs may inhibit these events either at the level of agonist binding or by blocking Na^+/H^+ exchange.

29.3

ADRENERGIC MODULATION OF THE NA^+ PUMP IN CANINE VASCULAR SMOOTH MUSCLE. J.C. Allen, S.S. Navran* and R.S. Masters* Baylor College of Medicine, Houston, Texas 77030.

α-and β-adrenergic agonists are well known modulators of smooth muscle tone. The agonists, phenylephrine (α-1), clonidine (α-2)and isoproterenol (β-)stimulate the Na^+ pump (ouabain sensitive ^{86}Rb uptake) in a receptor specific manner. The α-agonist stimulation is due to a Na^+ gating mechanism and is blocked by low Na_o, amiloride and Na preloading. The β-mediated stimulation does not involve Na^+ influx, since none of the above procedures affects isoproterenol stimulation of the pump. Thus the Na pump can be stimulated by both contractile and relaxing agonists, at concentrations where tone is also affected. In addition, the phorbol ester, TPA, stimulates the Na pump, suggesting a c-kinase mechanism. Since α-1 stimulation consists of generation of IP3 and DAG, α-1 receptor activation may induce an IP3 related contractile response coupled with a DAG relaxation response, suggesting complex multiple control of tension development. These data suggest that activation of the Na^+ pump by both α-and β-agonists may play a role in controlling the contractile response and that multiple mechanisms of Na pump stimulation are involved in the regulation of vascular smooth muscle tone. Supported by HL24585.

29.4

MITOGENIC AND A-1 ADRENERGIC MODULATION OF THE NA^+ PUMP IN PRIMARY CULTURES OF VASCULAR MUSCLE. S.S. Navran*, A.M. Kahn*, C.L. Seidel, and J.C. Allen. Baylor College of Medicine and University of Texas Health Sciences Center, Houston, Texas 77030.

We have demonstrated that phenylephrine (PE) stimulates the Na^+ pump of vessels by activation of an amiloride sensitive Na^+ influx. This α-1 response is present in isolated vascular muscle cells (VSMC) maintained in a serum-free defined medium. However, if VSMC are grown to confluence in serum, and then deprived of serum for 24 hrs, PE stimulates little or no inositol phosphate (IP) production, Na^+ influx or Na^+ pump activation. In contrast, the re-addition of serums, stimulates IP production, an amiloride sensitive Na^+ influx and Na^+ pump activation. Thus after proliferation in fetal calf serum, the α-1 receptor transducing mechanism linking Na^+/H^+ exchange and Na^+ pump control is altered, while the mitogenic activation of this transduction system remains intact. The data suggest common activation pathways for mitogen and α-1 receptors, and may be explained by heterologous desensitization of the α-1 adrenergic receptor pathway. (Supported by HL34280, HL35866, HL40624, and AHA Texas.)

29.5

RELAXATION IN BASILAR ARTERY (BA) AND THORACIC AORTA (TA) FROM NORMOTENSIVE (NR) AND COARCTATION HYPERTENSIVE RATS (CHR). F.M. Lai, A. Cobuzzi*, C. Shepherd*, A. Hoffman*, T. Tanikella* and P. Cervoni. Cardiovasc. Biol. Res., American Cyanamid Company, Med. Res. Div., Pearl River, NY 10965

Acetylcholine(ACh) and nitroglycerin(NG) relaxation of BA and TA of CHR at 1, 4 and 14 days post-surgery was studied in vitro and compared to time-matched, sham-operated normotensive rats(SNR) and in TA and BA with and without endothelium(ENDO) from NR. Blood pressure and plasma renin activity of CHR were significantly higher than time-matched SNR. ENDO removal from BA or TA of NR significantly diminished ACh-induced relaxation in BA and TA, had no effect on NG-induced relaxation in BA but potentiated NG relaxation in TA. ACh sensitivity did not change in 1 day CHR but decreased 4- and 9-fold in 4 and 14 day CHR, respectively. NG sensitivity increased 3-fold, did not change and decreased 9-fold in 1, 4 and 14 day CHR, respectively. Norepinephrine(NE) sensitivity in TA increased 3-, 15- and 13-fold in 1, 4 and 14 day CHR, respectively. In BA, ACh- and NG-induced relaxation was not altered in 1, 4 or 14 day CHR. The data suggest that 1) in CHR there is a complex progression of changes in the effects of NE, ACh and NG in TA at various stages of hypertension; 2) loss of ENDO-dependent relaxation in CHR is tissue specific (TA but not BA); 3) vascular response to NG in CHR is heterogenously altered; 4) TA ENDO may provide an acute protective mechanism to counteract the increased sensitivity of smooth muscle cells to NE. Prolonged hypertension appears to override this mechanism.

29.6

Responses of Isolated Fetal and Maternal Ovine Blood Vessels to Vasoactive Drugs. D. C. Dyer. Dept. of Veterinary Physiology and Pharmacology, Iowa State Univ., Ames, IA 50011.

Terminal aorta (FTA), intra-abdominal umbilical artery (IUA) and umbilical artery (UA, midpoint to placenta) from the fetus and the maternal terminal aorta (MTA) were removed from pregnant sheep within 3 weeks of term. Isotonic muscle activity was monitored from helically-cut strips bathed in a modified Krebs-Henseleit solution oxygenated with O_2:CO_2 (95:5). All fetal tissues were placed under 1 g tension while the MTA was under 3 g tension. Initially a dose response to KCl was determined on all strips followed by a dose response to a specific vasoactive agonist. The potency order for vasoconstrictors was as follows: FTA: angiotensin (A) > 8-arginine vasopressin (VP) > norepinephrine (NE) > serotonin (S) ≥ dopamine (DA) > KCl; IUA: A ≥ VP > NE > DA > KCl; UA: A ≥ VP > S > DA ≥ NE > KCl; MTA: A > NE > S > KCl (VP was inactive). The maximal response to each agonist differed on the three fetal tissues when compared to KCl with the agonist providing the greatest response as follows: FTA: NE; IUA: S, UA: A ≥ S. All responses to NE were antogonized by prazosin. Isoproterenol (I) did not relax KCl (30 mM) contracted MTA stips but did those of the IUA and UA. Relaxations to I were antagonized by propranolol. The data suggest that receptors to vasoactive drugs vary in their distribution in the main arterial conduit blood vessels of the fetus. Supported in part by the Iowa Heart Association.

29.7

EFFECT OF AGE ON α_1-ADRENERGIC RECEPTOR SUBTYPES IN RAT AORTA. Peter W. Abel* and Wanyun Zeng* (Spon: F.J. Dowd). Creighton Univ. School of Medicine, Dept. of Pharmacology, Omaha, NE 68178

Subtypes of α_1-adrenergic receptors causing contraction of rat thoracic aorta were studied in vitro. Ring segments of aorta from young (149 ± 3 gms) and old (391 ± 4.5 gms) male Sprague Dawley rats were prepared and contractile responses to norepinephrine (NE) and phenylephrine (PE) were measured. The irreversible α-adrenergic receptor antagonist chlorethylclonidine (CEC; 100 μM) had little effect on the contractile response to NE in aorta from young rats but caused a 240-fold decrease in the potency of NE in causing contraction and a decrease in maximal contraction in aorta from old rats. Preincubation of aorta from old rats with 1 μM prazosin, prevented these effects of CEC. Schild plots were constructed for inhibition of PE-induced contraction by the α-adrenergic receptor antagonist WB 4101. pA_2 values obtained from the Schild plots were significantly different and were 9.1 ± 0.1 in young and 8.6 ± 0.1 in old aorta. These data suggest that in young aorta, NE-induced contraction is caused by stimulation of α_{1a} receptors that are insensitive to inactivation by CEC and have a high affinity for WB 4101. In old aorta, NE-induced contraction is caused by stimulation of α_{1b} receptors that are inactivated by CEC and have a lower affinity for WB 4101. (Supported by a grant from the Health Future Foundation.)

29.8

DIFFERENTIAL SENSITIVITY OF K^+- AND AGONIST-INDUCED ARTERIAL CONTRACTIONS TO INHIBITION BY THE PROTEIN KINASE C INHIBITOR, H-7, AND THE CALMODULIN ANTAGONIST, W-7. P. H. Ratz. Eastern Virginia Medical School, Norfolk, VA 23501

This study investigated the mechanism of stress-maintenance in arteries by comparing the inhibitory efficacy of H-7 and W-7. H-7 more effectively inhibited KCl- than phenylephrine (PhE)-induced contractions (IC_{50} (uM): KCl; 4, PhE; 14). W-7 produced the opposite result (KCl; 60, PhE; 40). H-7 (10 uM) reduced KCl-induced stress and myosin phosphorylation (Mp) by, respectively, 74% and 22% compared to controls, while PhE responses were reduced by 36% (stress) and 44% (Mp). The level of steady-state Mp produced by PhE was about 1.7-fold greater than that produced by KCl (PhE; 61.2%, KCl; 36.3%, n=3), while stress values were more similar and at or near maximal (S_0) levels (%S_0: KCl; 87, PhE; 101, n=3). These results imply that protein kinase C may be involved more in KCl- than PhE-induced stress-maintenance. Alternatively, differences in sensitivity to inhibition of stress may have been caused by intrinsic differences in the level of KCl- and PhE-induced crossbridge activation, as measured by steady-state Mp. Support: PMAF Starter and Jeffress Trust Grants.

29.9

AMILORIDE PROMOTES RELAXATION VIA INTRACELLULAR ACIDIFICATION IN CANINE TRACHEAL SMOOTH MUSCLE. Ingrid K. Krampetz and Ratna Bose. * Dept. of Pharm. and Therap., Univ. of Manitoba, Winnipeg, Canada, R3E OW3.

We have previously demonstrated the relaxant effect of amiloride (a Na^+/H^+ exchange blocker) on isolated canine tracheal smooth muscle - an action which may be promoted by a decrease in calcium release and influx. Based on the documented properties of this compound in other tissues, and on our own observations that external acidification promotes a transient relaxation in tracheal smooth muscle, we hypothesized that these effects were mediated by an increase in intracellular proton concentration. To test this hypothesis, intracellular pH was measured simultaneously with tension in isolated muscle strips using the pH-sensitive intracellular dye 2',7'-bis-(2-carboxyethyl)-5(and -6) carboxyfluorescien (BCECF). Muscle strips were suspended isometrically under physiological conditions in the beam of a Perkin-Elmer fluorescence spectrophotometer and incubated for 30 min. in 2 uM BCECF acetoxymethyl ester in Krebs-Henseleit medium. Fluorescence was measured at an excitation wavelength of 503 nm, and an emission wavelength of 530 nm. After a 30 min. equilibration period, strips were contracted with carbachol (1 uM) or KCl (80 mM), and amiloride was added at plateau tension. The addition of amiloride produced intracellular acidification during both forms of stimulation. The time course and magnitude of this acidification closely paralleled the observed decrease in tension. Acidification was also observed with the addition of amiloride in uncontracted tissue, and in sodium-free medium. These data indicate that regulation of intracellular pH via the Na^+/H^+ antiporter may play an important role in respiratory smooth muscle contraction.

Supported by the Manitoba Lung Association.

29.10

SEXUAL DIMORPHISM IN VASOPRESSIN(VP)-INDUCED CONTRACTION OF RAT AORTA. J.N. Stallone, J.T. Crofton, and L. Share. Dept. of Physiology, University of Tennessee, Memphis, TN 38163

Previously, we reported that in the rat, pressor responsiveness to VP is higher in males than in females in most phases of the estrous cycle. To explore the role of the vasculature in this sexually dimorphic response to VP, we examined the reactivity of male and female rat thoracic aortae to VP. Aortic rings (3.0 mm width) were prepared from age-matched (13-15 wks) male and female Sprague-Dawley rats and mounted for isometric tension recording (in Krebs-Ringer-bicarb., 37°C, 2.50 g passive tension). After equilibration (2 hr), a cumulative dose-response curve (D-R) to arginine VP (10^{-11}-10^{-6} M) was obtained. The maximum response of female aortae to VP (3,888 ± 150 mg, n = 27; mean ± SEM) was nearly twice ($P<0.01$) that of male aortae (2,160 ± 254 mg, n = 13). Similarly, the sensitivity of female aortae to VP was substantially higher ($P<0.01$) than that of male aortae (EC-50: 10.88 ± 0.68 vs. 19.04 ± 1.63 nM, respectively). Maximum response and sensitivity of female aortae did not differ significantly during the estrous cycle. In contrast, when a cumulative D-R for phenylephrine (Phe) was obtained in separate aortae, maximum response of female aortae to Phe (1,023 ± 114 mg, n = 8) was less than half ($P<0.01$) that of male aortae (2,461 ± 204 mg, n = 7); sensitivity did not differ significantly (EC-50: 0.29 ± 0.04 vs. 0.34 ± 0.07 μM, respectively). These data suggest that vascular reactivity of the rat aorta to VP and Phe may be modulated by the gonadal steroids in a specific, independent manner. (Supported by NIH: HL-07339 and HL-19209)

29.11

S11701 INHIBITS NEURONAL UPTAKE AND FACILITATES RELEASE OF NOREPINEPHRINE IN ISOLATED BLOOD VESSELS M. Feletou, C. Vallet, J. Lepagnol, and B. Teisseire (SPON: P.M.Vanhoutte). Institut De Recherches Servier (Fondax) 92800 Puteaux, France

To determine the effect of S11701 [(morpholinyl-2)methoxy]-8 tetrahydro-1,2,3,4 quinoleine on adrenergic neurotransmission rings of canine saphenous veins were suspended in organ chambers for isometric tension recording. S11701 shifted the concentration-response curve to norepinephrine to the left in a dose-dependent manner. The shift induced by S11701 was similar to that produced by cocaine and imipramine. The effects of S11701 and the inhibitors of neuronal uptake were not additive. S11701 inhibited tyramine-induced contractions. The response to electrical field stimulation was potentiated both by S11701 and the inhibitors of neuronal uptake, with no additive effect. In quiescent tissues S11701 induced contractions which were blocked by the alpha$_2$-adrenoceptor antagonist rauwolscine or by 6-hydroxydopamine. Incubation with cocaine prior to the addition of S11701 did not prevent the contraction. Addition of S11701 in veins contracted with electrical stimulation caused a potentiation which was more pronounced than that obtained with inhibitors of neuronal uptake. These results suggest that S11701 inhibits the neuronal uptake of norepinephrine and releases norepinephrine from adrenergic nerve endings by a mechanism which does not involve the cocaine-sensitive neuronal uptake.

ARRHYTHMIAS I

30.1

ADENOSINE AND BRADYARRHYTHMIAS OF SLEEP APNEA. Larry J. Findley, U of Virginia, Charlottesville, VA 22908.

Hypoxemia augments bradyarrhythmias during sleep in patients with sleep apnea. Animal studies show that hypoxemia increases adenosine in cardiac tissue and produces bradyarrhythmias. Therefore, we hypothesize the bradyarrhythmias during sleep in patients with sleep apnea are caused in part by adenosine.

Two patients with obstructive sleep apnea received oral dipyridamole (75 mg TID), a drug which blocks the uptake of adenosine and increases plasma adenosine levels. Holter monitoring in these two patients showed multiple episodes of bradycardia (heart rate less than 45/min.), sinus pauses as long as 4.7 seconds, and AV block including complete heart block. The first patient stopped the dipyridamole and had no prolonged sinus pauses and fewer episodes of bradycardia (8 vs. 75 episodes/24 hrs.). The second patient then begun taking the adenosine antagonist theophylline in addition to dipyridamole. This patient had no episodes of heart block and fewer episodes of bradycardia (4 vs. 132 episodes/24 hrs.) while taking theophylline.

Blocking adenosine uptake by dipyridamole provoked bradyarrhythmias in these two patients with sleep apnea. The adenosine antagonist theophylline decreased bradyarrhythmias in one patient. These observations suggest the bradyarrhythmias occurring during sleep in patients with sleep apnea are caused in part by adenosine.

30.2

SMALL ANIMAL 3-D ELECTROCARDIOGRAPHY USING STANDARD EQUIPMENT. Wm. C. Van Arsdel III* and Tibor Balazs, FDA, Division of Drug Biology, Washington, DC 20204.

A small animal electrocardiographic system (ECS) was developed for animal toxicology equivalent to that used in clinical diagnosis by electrocardiologists who utilize 12 ECG leads (but can use a 3-lead orthogonal or orthogonal loop system), and where computers are utilized widely to separate probabilities. This small animal ECS adapts the human clinical ECS by simplification to provide, with only 4 electrode lead positions and standard ECG equipment, a 12 lead (orthogonal hexaxial) ECS which can be used like the human 12-lead (hexaxial limb leads and 6 chest leads) in lead pattern recognition, and/or with only 3 select leads, can also provide 3-dimensional orthogonal lead ECG recordings, vectors, vector constructions, vector projections, vector loops, special vector loop constructions, and/or complete computer analyses. We have with this animal orthogonal ECS collected over 600 control and treated animal ECGs. The ECS has provided reproducible control recordings, and has revealed changes after cardiac damage induced by various treatments. A computer program has been prepared (at NIH) and published for the analysis of the rat ECG utilizing this 3-dimensional system of ECG data collection.

30.3

TRIGGERED REPOLARIZATION AND THE INDUCTION OF VENTRICULAR FIBRILLATION. Michael MacConaill. Dept. Pharmacol., Univ. Ottawa, Ottawa, ON, Canada, K1H 8M5

It is well established experimentally that a single stimulus of adequate strength applied during the vulnerable period can induce a ventricular fibrillation even though current models of the process require a series of rapid stimuli to initiate the arrhythmia. Experiments in Langendorff-perfused rabbit hearts have shown that under appropriate conditions, a stimulus of diastolic threshold strength applied within the first half of the cardiac action potential can induce ventricular fibrillation, and that the characteristic high frequency irregular activity appears immediately following the extrasystole. While it could be postulated that the conventional 'early' or 'late' oscillatory afterdepolarizations are involved in the initiation of the fibrillation, both processes are too slow to serve as adequate mechanisms. Monophasic electrograms of the induction of fibrillation suggest that the inducing stimulus can abort an ongoing action potential, presumably by triggering repolarization. It is known that triggered repolarization can be followed by a rebound depolarization: if this rebound were weakly damped, it could oscillate at a time course matching that of an induced fibrillation. Such a mechanism would also explain how weak stimuli applied early in the cardiac action potential can elicit extrasystoles with a prolonged latency.

30.4

VERAPAMIL PROTECTS AGAINST COCAINE INDUCED VENTRICULAR FIBRILLATION

George E. Billman, Richard S. Hoskins*, Dept. of Physiology The Ohio State University, Columbus, Ohio 43210

There is increasing evidence that cocaine use can trigger lethal cardiac events, including ventricular fibrillation (VF). The mechanism responsible for these lethal cardiac arrhythmias remains to be determined. Therefore, 13 mongrel dogs were instrumented to measure heart rate, left ventricular pressure (LVP) and d(LVP)/dt. After a 3-4 week recovery period, the left circumflex coronary artery was occluded for 2 min., beginning during the last minute of exercise (EX) and continued for 1 min. after the cessation of EX. None of the dogs developed cardiac arrhythmias during the control EX plus ischemia test. On a subsequent day, the test was repeated after the injection of cocaine HCL (1.0 mg/kg). Cocaine significantly ($p<0.01$) elevated heart rate (19.5%), systolic LVP (30.2%) and d(LVP)/dt (28%) and elicited cardiac arrhythmias in 12 of the 13 animals during the EX plus ischemia test. In fact, 11 animals developed VF. Verapamil, a calcium channel antagonist (250 ug/kg) attenuated the hemodynamic effects of cocaine and prevented VF. Thus, these data suggest that cocaine can induce VF during myocardial ischemia and further, that these lethal arrhythmias can be prevented by a calcium channel antagonist. (Supported by NIH Grant HL36336).

30.5

BETA ADRENOCEPTOR BLOCKING ACTIVITY OF DIPRAFENONE (D), A NEW CLASS Ic ANTIARRHYTHMIC AGENT: A COMPARISON WITH PROPRANOLOL (PROP) AND PROPAFENONE (P). *Stan S. Greenberg and E. Cantor. Dept of Pharmacology, Berlex Laboratories, Inc. Cedar Knolls, N.J. 07927.

D is a new class Ic antiarrhythmic agent approximately 3x more potent than P (class Ic). We compared the hemodynamic and cardiac beta adrenoceptor antagonistic effects of D, to that of P and PROP (class II) in pentobarbital anesthetized dogs (N=4-5/group). Isoproterenol (ISO) (0.01 and 0.03 ug/kg) was given as bolus injections, iv, before and 20 min after iv infusions of antiarrhythmic doses of D or P (0.3, 1 and 3 mg/kg) or PROP (0.03, 0.1 and 0.3 mg/kg). The ability of D, P and PROP to displace ^{3}H-dihydroalprenolol (DHA) binding in isolated cardiac membranes was also examined. Each agent produced competitive mixed beta adrenoceptor blockade of the hemodynamic and heart rate (HR) responses to ISO. PROP was the most potent beta adrenoceptor antagonist, while D was more potent than P. The apparent pA2 values against HR were 0.05, 0.1 and 0.6 mg/kg, while those against diastolic pressure were 0.016, 0.1 and 0.6 mg/kg, respectively. The Ki values for displacement of DHA by PROP, D and P were 2.7, 14.3 and 252 nM, respectively, corresponding to the rank order of potency of in vivo beta adrenoceptor blockade. These data show that D is 6x more potent as a beta adrenoceptor blocking agent than P. The potent beta adrenoceptor blocking activity of D in combination with its class Ic antiarrhythmic activity may make D a more effective antiarrhythmic agent than P or PROP.

30.6

EARLY AFTERDEPOLARIZATIONS INDUCED IN A MODEL OF ISCHEMIA-REPERFUSION IN RABBIT CARDIAC PURKINJE FIBERS. George J. Rozanski and Richard C. Witt*. University of Nebraska College of Medicine, Omaha, NE 68105

The mechanisms of ventricular arrhythmias upon reperfusion of previously ischemic myocardium are poorly understood. Thus, the arrhythmogenic effects of ischemia (I) followed by reperfusion (R) were studied in vitro in seven isolated rabbit Purkinje fibers. Microelectrodes were used to record transmembrane potentials from fibers exposed first to control, oxygenated Tyrode's solution, to an ischemic-like solution (low P_{O2} and pH, high $[K^+]_o$) for 45-60 min, and again to control Tyrode's to mimic R. At the end of the I period, maximum diastolic potential (MDP), action potential amplitude (APA) and duration (measured to 90% of repolarization; APD_{90}) were reduced from control by 19.9 ± 2.3 mV, 23.6 ± 3.3 mV, and 119.8 ± 37.0 ms, respectively ($p<0.01$). Within five minutes of R, MDP and APA returned to control values but APD_{90} was prolonged by 140.6 ± 84.3 ms. This delay in repolarization favored the development of early afterdepolarizations (EADs) which elicited coupled premature beats (coupling interval = 500.9 ± 125.9 ms; n = 5) and sustained runs of repetitive responses (cycle length = 399.7 ± 57.0 ms; n = 4) at low levels of membrane potential (-63.3 ± 4.8 mV). Thus, these data suggest that triggered responses arising from EADs in Purkinje tissue may underlie the arrhythmias occurring in vivo upon reperfusion of ischemic myocardium.

30.7

INCREASED ANTIARRHYTHMIC POTENCY OF DANTROLENE SODIUM ON MULTIPLE ORAL ADMINISTRATION TO RATS. Robert R. Brooks, George E. Decker*, and John F. Carpenter*. Norwich Eaton Pharmaceuticals, Inc., Norwich, NY 13815, A Procter & Gamble Company.

Dantrolene sodium (DS), a clinically useful, direct-acting skeletal muscle relaxant, prolongs action potential duration of cardiac fibers in vitro, prevents or abolishes ventricular arrhythmias (VA) in animals, and ameliorates VA in man during malignant hyperthermia crises. In rats in the gastrocnemius muscle assay, the estimated single oral dose of DS for inhibiting twitch tension 50% (GTT-ED50) was 13.1 mg/kg. After 7 days of once-per-day dosing, the GTT-ED50 was 4.6 mg/kg, not markedly different. In contrast, in the anesthetized rat model of VA induced by coronary artery ligation and reperfusion (CALR), the CALR-ED50 (dose giving arrhythmia score of 50, where 100=no effect, 0=no arrhythmias) changed from 56.9 (1 dose) to 3.6 (7 doses) mg/kg. The CALR-ED50 values for quinidine under these conditions were 28.9 (1 dose) versus 18.2 (7 doses) mg/kg p.o. While these single determinations limit statistical analysis, the 15-fold increase in antiarrhythmic potency versus less than 3-fold increase in muscle relaxant potency on multiple dosing suggests that cardiac and skeletal muscle responses to dantrolene sodium are different.

30.8

EFFICACY OF PROPAFENONE IN VENTRICULAR TACHYCARDIA (VT)-INVERSE CORRELATION WITH EFFECT ON CONDUCTION TIME. T. Kus*, M. Dubuc*, C. Lambert*, R. Nadeau*, M. Shenasa, Sacré-Coeur Hospital, Univ. of Montreal, Montreal, Canada.

The efficacy of propafenone (P) in preventing VT induction by programmed electrical stimulation was studied in 17 patients (pts) with old myocardial infarction and spontaneous sustained (sust) VT. Right ventricular (RV) drive at 2 basic cycle lengths (BCL) and 1 to 3 extrastimuli at 2 RV sites was done without and on oral P (785±188 mg/day). P prevented induction of sust VT in 6 pts (Group A): 1 noninducible, 5 nonsust (<30 sec). In the other 11 pts (Group B), P slowed VTCL from 280±45 to 377±66 ms. P did not affect sinus CL but prolonged AH and HV intervals and QRS duration equally in both groups. With RV pacing, P also prolonged RV effective (E) and functional (F) refractory period (RP) equally in both groups. However, as shown below, there was significant inverse correlation between efficacy and % change (Δ) in paced QRS duration ($p<0.02$).

BCL 400	%Δ VERP	%Δ FRP	%Δ QRS	P	5-OH P
Group A	10±6	9±9	15±2	2.5±1.1	0.3±0.2
Group B	18±13	14±11	37±22	1.6±1.3	0.2±0.1

Neither P nor 5-OH P plasma levels were significantly different between group A and B. A critically balanced Δ in RP and conduction time may be necessary to interrupt a reentry circuit. Excessive phase 0 depression may be counter-productive.

30.9

ELECTROPHYSIOLOGICAL ACTIONS OF MONO-N-DEALKYLDISOPYRAMIDE. William Toy* and Betty I. Sasyniuk. McGill University, Montreal, Canada, H3G 1Y6

Mono-N-dealkyldisopyramide (MND), the major metabolite of Disopyramide (D), occurs in patients in significant concentrations; yet, little is known of its electrophysiology. We therefore assessed the sodium channel blocking properties of MND in canine Purkinje fibers superfused in vitro using $\dot{V}_{max}$ as an index of sodium channel blocking activity. Also, effects on action potential duration and effective refractory period (ERP) were determined. At a basic cycle length of 1 sec, MND (4-32 μg/ml), produced a conc dependent decrease of $\dot{V}_{max}$, amplitude and all phases of repolarization of the action potential. To assess frequency dependent block, fibers were stimulated with pulse trains at cycle lengths of 0.4, 0.6, 1 and 2 sec. MND produced a conc dependent increase in the magnitude and kinetics of onset of rate dependent $\dot{V}_{max}$ block. Magnitude of block increased as pulse train rates increased. Significant tonic block occurred only at the highest conc. Recovery from rate dependent block followed a single exponential time course with a τ_{rec} of 5.23±0.90 sec. MND, 4 μg/ml, shortened all phases of repolarization and ERP at cycle lengths of 0.25 to 1 sec with more pronounced effects at the slowest rates. Combination of MND with D produced additive frequency dependent $\dot{V}_{max}$ block with τ_{rec} intermediate to those of MND and D alone. Thus, MND must modify the clinical effects of D.

Supp. by MRC and Roussel Laboratories.

30.10

RATE AND VOLTAGE DEPENDENT EFFECTS OF DISOPYRAMIDE IN CANINE CARDIAC PURKINJE FIBERS. Betty I. Sasyniuk and Matthew Flemming*. McGill University, Montreal, Canada, H3G 1Y6.

The purpose of the study was to investigate the dose response relationships and kinetics of the rate dependent effects of disopyramide (D) on Vmax, conduction and effective refractory period. D (2-16 ug/ml) produced a conc dependent increase in rate dependent Vmax block (RDB) and rate independent tonic block. Hill plots for tonic and RDB showed different slopes. At therapeutic concs the magnitude of RDB did not change when extracellular potassium conc was increased from 4 to 8 mM whereas tonic block increased progressively. Similar effects were seen for conduction suggesting that D would produce greater conduction block in ischemic tissue independent of rate. Recovery from RDB was best fit by two exponentials with time constants of 506 ± 76 msec and 15.1 ± 1.6 sec. D produced a selective block of Vmax at steady state cycle lengths less than 400 msec. D prolonged the ERP of premature responses to double and triple extrastimuli significantly more than that of basic responses. The results may explain the effectiveness of D in preventing initiation of ventricular tachycardia by programmed electrical stimulation.

Supp. by MRC and Roussel Laboratories.

30.11

CAFFEINE-NOREPINEPHRINE INTERACTIONS IN CARDIAC PURKINJE FIBERS. Hiroyasu Satoh* and Mario Vassalle. SUNY, Health Science Center, Brooklyn, NY 11203.

The interactions between caffeine and norepinephrine on electro-mechanical events were studied in canine cardiac Purkinje fibers perfused in vitro. Caffeine (0.5-1 mM) increased and then decreased contractile force but had little effect on the action potential. In the presence of norepinephrine (NE, 1 uM), caffeine increased force more and then decreased it less (or not all). The force decrease was abolished by raising $[K]_o$ to 12 mM. In the presence of caffeine, NE increased force but gradually less as caffeine was increased from 0.5 to 2 mM. In 9 mM caffeine, NE still increased force but slowed the final phase 3 repolarization. Caffeine had the usual biphasic effects in 0.1, 1 and 10 uM NE. In NE plus caffeine, high $[Ca]_o$ (8.1 mM) increased force. In NE (1 uM) plus propranolol (1 uM), caffeine had only a positive inotropic effect. Caffeine had the usual biphasic effects in the presence of methoxamine, but methoxamine decreased force in the absence and increased it in the presence of caffeine. The initial increase in force by caffeine was eliminated by theophylline. In the presence of iodoacetic acid and 2-deoxyglucose, NE caused contracture and caffeine exaggerated it. Thus, within limits, NE appears to diminish the calcium overload induced by caffeine in the cytoplasm, presumably by overcoming the caffeine-induced impairment of calcium reuptake into the sarcoplasmic reticulum. Supported by NIH grant 627038.

CONTROL AND MECHANICS OF BREATHING

31.1

EFFECTS OF ALTERING LUNG MECHANICS ON THE INITIAL RESPONSE OF PULMONARY RAPIDLY ADAPTING RECEPTORS TO HISTAMINE IN CATS. Jun Yu* and Andrew M. Roberts. Dept. of Physiology and Biophysics, Univ. of Louisville, Louisville, KY 40292

Histamine stimulates pulmonary rapidly adapting receptors (RARs) directly, and indirectly by alterating lung mechanics. We did experiments to determine the relative importance of these two modes of stimulation in the time course of the response in anesthetized, open-chest, artificially ventilated cats. We compared the response of RARs to right atrial injection of histamine (50 μg/kg) with the response to mechanically decreasing dynamic lung compliance (C_{DYN}) by decreasing positive end-expiratory pressure (PEEP). Histamine increased the activity of 13 RARs from 1.3±0.5 to 5.2±1.4 imp/s at 10.6 s when C_{DYN} was decreased by 34.8%. This increase was similar to that (5.2±1.2 imp/s) caused by mechanically decreasing C_{DYN} by the same amount (34.8%). The firing pattern of RARs changed from a relatively irregular pattern to a more pronounced respiratory modulation when C_{DYN} was decreased by histamine injection or by decreasing PEEP. The response evoked by histamine reached its peak (7.2±1.5 imp/s) at 24.7 s after injection and the firing pattern further changed from the respiratory to a cardiac modulation. This increase in activity was greater ($p<0.05$) than that caused by mechanically decreasing C_{DYN}. Our experiments suggest that in cats, histamine initially stimulates RARs mainly by alterating lung mechanics. Supported in part by a grant from Univ. of Louisville Medical Research Committee.

31.2

BREATHING FREQUENCY AFFECTS DIAPHRAGM FATIGUE. Frank Cerny, Martin Lawler*, Jeff Mador*. Depts. Physical Therapy & Exercise Science and Medicine, SUNY/Buffalo & V.A. Med. Center, Buffalo, NY 14214.

The pressure developed across the diaphragm (Pdi=gastric(Pga)-pleural(Ppl)) and the duration of contraction (Ti) are primary contributors to diaphragm fatigue. The purpose of this study was to determine if breathing frequency (f) also had an effect on fatigue. Pdi, with esophageal balloons, and tidal volume (Vt), with a spirometer, were measured in 4 healthy males (22-41) breathing against a load requiring 60% of Pdi maximum. Vt, Ti/Ttotal (inspiration=0.5 of total breath) and Pdi were held constant at breathing frequencies of 15, 30, & 45/min, in random order on separate days. Subjects breathed with a metronome and inspired using diaphragm only such that Pga was 50% of Pdi, and so that flow approached a square wave. Pdi and Vt, with appropriate targets, were displayed on an oscilloscope. Fatigue was measured when subjects were unable to reach 60% Pdimax. If frequency has no independent effect total number of breaths to fatigue should be the same under each condition. Total "work" ((Vt*Pdi)*f) and number of breaths to fatigue were different (p 0.05) from 15 to 45 breaths/min with 30/min intermediate. These data indicate that breathing frequency has an independent, but probably small, effect on diaphragmatic fatigue.

31.3

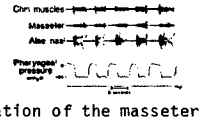
LOCALIZED VARIATIONS IN THE RESPONSE OF THE HUMAN UPPER AIRWAY TO APPLIED POSITIVE PRESSURE. Kingman P. Strohl, Peter L. Hoekje*, E. Mark Haacke* and Lee J. Brooks*. Case Western Reserve University, Cleveland, OH 44106

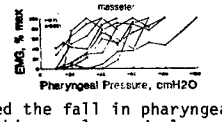
We used Magnetic Resonance Imaging to image the upper airway (oropharyngeal, hypopharyngeal, supraglottic, and extrathoracic tracheal regions) in 6 supine, awake, healthy male subjects before and during application of +10 cmH20 pressure via mouthpiece. All subjects showed increases in airway size in most of these regions during the application of positive pressure. In the oropharynx, the lateral walls and the anterior wall (genioglossus) were displaced outward in 5 of the 6 subjects, while in the sixth the tongue was displaced posteriorly, reducing airway area. Outward displacement of the lateral and anterior walls was also seen in the hypopharynx, though the position of the epiglottis was relatively stationary (n=6). The greatest increase in airway size occurred in the supraglottic regions and was characterized by unfurling of the aryepiglottic and lateral glossoepiglottic folds and expansion of the esophageal inlet (n=6); enlargements were observed in areas constraining the flowing airstream as well as in cul-de-sacs. The tracheal areas expanded more isotropically, and by smaller amounts (n=6). We conclude that there are substantial regional differences in the upper airway response to positive pressure due in part to anatomic features. These inhomogeneities may relate to dynamic features of upper airway flow resistance.

31.4

ACTIVATION OF MASSETER MUSCLES WITH INSPIRATORY RESISTANCE LOADING. Douglas E. Hollowell*, Dudley F. Rochester and Paul M. Suratt. U. Virginia, Charlottesville, VA 22908.

Closure of the jaw exerts traction on muscles which insert on the hyoid bone. We postulated that the masseter muscles, which close the jaw, would be activated when the patency of the upper airway is threatened. We therefore measured EMG's of the masseters during inspiratory resistance loading and compared it to EMG's of chin muscles and alae nasi in 10 normal subjects. EMG's were measured with surface electrodes and displayed as a moving time average. Subjects were studied supine, while breathing through their nose with a mask over their face and a catheter in their pharynx. We observed no masseter activation during quiet unloaded breathing but as pharyngeal pressure became lower there was a significant increase in masseter activation in all subjects which was similar to that of chin muscles and alae nasi.





Activation of the masseter preceded the fall in pharyngeal pressure as also occurred in the chin muscles and alae nasi. We conclude that the masseters are activated by inspiratory loading and may function as accessory muscles of respiration.

31.5

SYNAPTIC TRANSMISSION OF THE CENTRAL RESPIRATORY OUTPUT TO THE PHRENIC MOTONEURONS IN RAT. L. Fedorko* & K. Kranz *. (SPON: A. Charles Bryan) Mt. Sinai Hosp. Res. Inst. Toronto, Ont M5G 1X8.

In vitro studies suggest that glutamate receptors are involved in transmission of respiratory synaptic excitation to phrenic motoneurons (PN). An in vivo newborn rat (5-11 day) preparation was developed and glutamate agonists/antagonists were applied to the exposed spinal cord. Respiratory activity was determined from the vagal and PN neurograms in spontaneously breathing rats anesthetized with ketamine/acepromazine. The agonist kainic acid (1 mM) first increased and then irreversibly abolished phasic and tonic activity of PN. Its excitotoxic effects were prevented by kynurenic acid (KA). The antagonists KA and piridino dicarboxylic acid (2,5, 10 mM) reduced PN discharge by only 10-24%. Ketamine (1mM), a selective NMDA receptor antagonist, had no effect on PN activity. Aminophosphoro butyric acid (1-5mM) reduced PN discharge 20-50%. These studies, in contrast to in vitro studies, demonstrate that glutamate receptors play only a small role in PN synaptic excitation in the intact 5-11 day old rat.
Supported by MRC (Canada) Grant #MA9849

31.6

RESPIRATORY MECHANICS IN DOGS WITH HYDROSTATIC LUNG EDEMA. **F. Shardonofsky*, M. Skaburskis*, F. Robatto*, R.P. Michel and J. Milic-Emili. Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada, H3A 2B4.**

Five dogs were anesthetized, tracheotomized, paralyzed, and ventilated with a Siemens constant flow ventilator. Respiratory mechanics were studied with the end inspiratory occlusion technique. Dividing the immediate and subsequent tracheal pressure drops following flow interruption by the preceding flow gave Rrs,min and Rrs,diff, respectively. In the dog, Rrs,min reflects primarily airway resistance and Rrs,diff the viscoelastic properties and pendelluft of the respiratory system. Lung (l) and chest wall (w) mechanics were partitioned by using an esophageal balloon. Measurements were made before and up to 60 min after the administration of a buffered Ringer's lactate solution (40 ml/kg in 30 min). Histology showed interstitial edema in all dogs. Rrs,min and both lung (Est,l) and chest wall (Est,w) static elastances increased significantly with the fluid load. Rrs,diff did not change. Interstitial edema increases airway resistance and Est,l and Est,w, without producing significant changes in viscoelastic properties.
(Supported by the Medical Research Council of Canada and the Canadian Lung Association.)

31.7

STIMULATION OF PULMONARY C-FIBER AFFERENTS PRODUCES EXPIRATORY MUSCLE ACTIVATION. P.S. Clifford and R.L. Coon. Depts. of Anesthesiology and Physiology, Medical College of Wisconsin and VA Medical Center, Milwaukee, WI 53295

Stimulation of pulmonary C-fiber afferents with capsaicin has been shown to result in apnea followed by tachypnea, hypotension and bradycardia. However, previous investigations have utilized only respiratory measurements related to inspiratory muscle activity such as phrenic nerve activity, diaphragm EMG or airflow. We examined the response of an expiratory muscle - triangularis sterni - to intravenous injections of capsaicin at doses of 1,2,4,8,16 and 32 ug/kg in spontaneously breathing dogs anesthetized with sodium pentobarbital. Triangularis sterni and external intercostal EMGs were recorded from fine wire electrodes inserted into the muscle through the chest wall. At doses of capsaicin above a threshold for each particular dog (4-8 ug/kg), intercostal EMG was silenced whereas triangularis sterni EMG activity was markedly augmented. Both the amplitude and period of augmented triangularis sterni EMG activity increased with incremental doses of capsaicin. Cervical vagotomy abolished the early apneic period and the corresponding effects on both inspiratory and expiratory muscle activity. These findings show that stimulation of pulmonary C-fiber afferents results not in a total cessation of all respiratory activity but rather an inhibition of inspiratory muscle activity and concomitant activation of expiratory muscle activity.
(Supported by VA Medical Research Service)

31.8

FLOW DEPENDENCE OF PULMONARY RESISTANCE IN BRONCHOCONSTRICTED CATS. M. Skaburskis*, F. Shardonofsky* J. Milic-Emili. Meakins-Christie Labs, McGill University, Montreal, Quebec, Canada H3A 2B4

5 anesthetized and paralyzed cats were placed in the supine position, tracheostomized and mechanically ventilated with a Siemens 900C constant flow ventilator. Expiratory isovolume pressure-flow curves were constructed by pressurizing or depressurizing the expiratory limb of the ventilator for a single expiration. Pressures between +3 and -15 cmH2O were randomly selected. Animals were then treated with progressively increasing doses of intravenous serotonin (10,20,50 and 100 μg/kg/min) and all measurements repeated at each dose. Isovolume pressure-flow curves at 0.25, 0.5 and 0.75 tidal volume tended to be curvilinear under all conditions. In 3/5 cats volume-dependence of isovolume R_L was demonstrated. However, all cats showed significant flow dependence of R_L. Increases in isovolume R_L were proportionally different when calculated for different isoflows. Given this source of variability in isovolume R_L, resistance measurements using inspiratory data alone may be preferable when inspiratory flow can be kept constant.

Supported by the Canadian Lung Association.

SKELETAL MUSCLE PHYSIOLOGY I

32.1

URINARY 3-METHYLHISTIDINE EXCRETION INCREASES WITH REPEATED BOUTS OF WEIGHT TRAINING EXERCISE. **J.M. Pivarnik, J.F. Hickson, Jr.*, and I. Wolinsky*. University of Houston, Houston, TX 77004.**

We investigated the effect of weight training exercise on urinary 3-methylhistidine (3-MH) excretions in untrained subjects. For 19 consecutive days, 11 males were fed weight maintenance, lacto-vegetarian diets which contained the RDA (0.8 g· kg^{-1}· day^{-1}) for protein. No exercise was performed for the first 7 days of the study. Subjects were strength tested on day 8, and performed upper and lower body weight training exercises from days 9-19. Complete, 24-hour urine collections were obtained from each subject on a daily basis. Samples were assayed for creatinine and 3-MH. Stable baseline 3-MH values were present during the pre-exercise control period. Significant increases in 3-MH occurred by study day 11, which was the third day of weight training exercise. This was true regardless of whether the data were expressed by daily excretions (uM· day^{-1}, $p<0.01$), per unit of body weight (uM· kg^{-1}· day^{-1}, $p<0.005$), or per unit of creatinine excretion (uM· g $Creat^{-1}$· day^{-1}, $p<0.001$). Since urinary 3-MH is an index of actin and myosin catabolism, these data support the hypothesis that the rate of skeletal muscle degradation is increased during strength building exercises.

Funded in part by a University of Houston Research Initiation Grant.

32.2

EFFECTS OF SLEEP LOSS AND MENSTRUAL CYCLE ON RESPIRATORY MUSCLE FUNCTION. H.I. Chen and Y.R. Tang*. Department of Physiology, College of Medicine, National Cheng-Kung University, Tainan, Taiwan, R.O.C.

Sleep loss is common in patients with respiratory disorders. To determine whether sleep loss affects respiratory muscle function, we compared 30 normal male subjects' respiratory muscle and pulmonary functions after normal sleep with those measured after a 30-h sleepless period. We found that inspiratory muscle endurance was decremented from 673±46 to 501±49 s after sleep deprivation. 12-s maximal voluntary ventilation was also significantly reduced after sleep loss. Nevertheless, the respiratory muscle strength, forced expiratory volume in 1-sec and forced vital capacity were unaltered. In addition, to investigate the effect of menstrual cycle on respiratory muscle function, respiratory muscle and pulmonary functions were measured and compared in the mid-follicular phase and in the mid-luteal phase of the menstrual cycle on 20 healthy adult women. We observed that the inspiratory muscle endurance was greater in the mid-luteal phase than in the follicular phase (822±34 vs. 671±63 s respectively) while the respiratory muscle strength and pulmonary function were unchanged. We conclude that inspiratory muscle endurance decreases after 30-h sleep loss and increases in the mid-luteal phase.

32.3

MAXIMAL O_2 UPTAKE LIMITATION DURING CARBON MONOXIDE AND ANEMIC HYPOXIA. C.E. King. Queen's Univ., Kingston, Ontario K7L 3N6.

The present experiments examined the mechanisms underlying the O_2 limitation in contracting skeletal muscle during carbon monoxide hypoxia (COH) and anemic hypoxia (AH). The gastrocnemius muscle was surgically exposed in anesthetized mongrel dogs. The tendon was cut and attached to a force transducer. Muscle blood flow was controlled at constant perfusion pressure (100-120 mmHg). Three groups of animals were studied under conditions of normoxia (NOR) or AH (Hct=15) or COH (COHb=60%). Muscle O_2 uptake (VO_2), delivery, extraction, blood flow and tension development were measured at rest and during 5 min of isometric contractions at 1, 2, 4, and 6 Hertz (Hz). These frequencies represented 25, 50, 90 and 100% maximal O_2 uptake (MVO_2). At 2 Hz, VO_2 was reduced ($p<0.05$) during both COH (47%) and AH (25%). O_2 delivery was not different between the three groups at 2 Hz but O_2 extraction was lower ($p<0.05$) in the COH group as compared to both NOR and AH. At 4 Hz, MVO_2 values were 120 and 68 $ul \cdot g^{-1} \cdot min^{-1}$ during NOR and AH respectively; an MVO_2 of only 40 $ul \cdot g^{-1} \cdot min^{-1}$ was observed during COH. These data suggest that the lower VO_2 during COH and AH at 2 Hz was associated with a diffusion limitation. At 4 Hz, the difference in VO_2 between NOR and AH was primarily the result of the greater O_2 delivery during NOR. The greater VO_2 limitation during COH at 4 Hz was associated with a further diffusion limitation and/or increased O_2 affinity effect. Supported by Dean's MRC,ARC,PDF,Queen's U.

32.4

EFFECTS OF CHLORZOLAMIDE (CLZ) ON CONTRACTILE PROPERTIES OF ISOLATED PERFUSED CAT SOLEUS. M.I. Lindinger* and G. Gros* (SPON. L.L. Spriet). Abteilung Vegetative Physiologie, Medizinische Hochschule Hannover, Hannover, West Germany.

Effects of muscle carbonic anhydrase (CA) inhibition by CLZ on contraction of blood-free perfused cat soleii were studied at 4 [CLZ]s of 5x10-7, -6, -5 and -4 M. Maximal twitch (Pt) and tetanic (Po) tensions were illicited once/15 min prior to, during and after CLZ perfusion. After CLZ, perfusion with CLZ-free medium showed that Po and Pt were irreversibly reduced at all [CLZ]s. The largest decrease in Pt (-22%) occurred at the lowest [CLZ]; 5x10-4 M CLZ showed only a 9% decrease. Po progressively decreased with increasing [CLZ], showing a 50% decrease after 90 min of perfusion at 5x10-4 M. The time to Pt was reduced by 9% only at the high [CLZ].Time to Po was reduced by 13% and 70% at the 2 highest [CLZ]s, respectively; this effect was fully reversible at the lower [CLZ] but only 57% reversible at the highest [CLZ]. Twitch 1/2 relaxation times (RTs) were reversibly reduced at the two highest [CLZ]s by 10% and 20% respectively. CLZ was without effect on tetanus 1/2 RTs, but at the 2 highest [CLZ]s the time required for full relaxation to occur following the tetanus was increased from 320-420 ms (controls) to 540 ms (5x10-5 M) and >2000 ms at the highest [CLZ]. CLZ appears to have both reversible and non-reversible effects. Rapid effects may result from reversible inhibition of CA isozymes located within the intercellular matrix or externally on the sarcolemma. The irreversible and partially reversible effects at higher [CLZ]s are consistent with intracellular CLZ-binding and CA inhibition. Supported by MRC Canada and DFG.

32.5

MUSCLE GLYCOGENOLYSIS IS UNALTERED BY GLYCOGEN CONTENT DURING MAXIMAL ELECTRICAL STIMULATION. C.B. Campbell*, L.L. Spriet, D.R. Marsh*, L. Berardinucci*, and T.E. Graham. School of Human Biology, Univ. of Guelph, Ontario CANADA N1G 2W1.

To examine the effects of muscle glycogen content on glycogenolysis during maximal electrical stimulation, rats were randomly assigned to one of three groups: Control (C, stimulated only); Swim (S, stimulated 21 hr after a 3 hr swim); Fasted (F, stimulated after a 20 hr fast). Prior to stimulation, glycogen (umol glucosyl units/g dry muscle) in the white and red gastrocnemius (WG, RG) and soleus (SOL) muscles was increased in S (13-25%) and decreased in F (15-27%) as compared to C. Hindlimb blood flow was occluded 60 s prior to stimulation to produce a predominantly anaerobic environment and muscles were stimulated for 60 s at a train rate of 1.0 Hz (100 ms, 80 Hz). Glycogenolysis, estimated from the accumulation of glycolytic intermediates and the decrease in glycogen, was unaffected by the resting glycogen content (mean ± SE):

	WG	RG	SOL
C	96.5 ± 2.4	58.1 ± 4.2	8.4 ± 2.2
S	95.5 ± 4.8	58.8 ± 4.0	12.7 ± 2.5
F	88.6 ± 2.5	58.1 ± 1.6	12.6 ± 2.2

Hindlimb isometric tension and muscle anaerobic ATP production during contraction were also similar across groups. The results of this study suggest that in vivo phosphorylase activity is not regulated by its substrate glycogen during maximal contractions in an anaerobic environment. Funded by N.S.E.R.C.

32.6

RELATIONSHIP BETWEEN OXIDATIVE CAPACITY AND ANTIOXIDANT ENZYME LEVELS IN RAT SKELETAL MUSCLES. M.H. Laughlin, T. Simpson, J.K. Smith*, O. Brown, W. Sexton, and R.J. Korthuis*. Dept of Biomed Sci and Dalton Res Ctr, Univ of Missouri, Columbia, MO 65211 and *Dept of Physiol, LSU Med Ctr, Shreveport, LA 71130.

The purpose of this study was to characterize the relationship between oxidative capacity and antioxidant enzyme levels in skeletal muscles composed of different fiber types and to determine if this relationship was altered by endurance exercise training. Male, Sprague-Dawley rats were exercise-trained (ET) on a treadmill 2 hr/day at 32 m/min (15% incline), 5 days/week, or were cage-confined (C) for 12 weeks. In both C and ET rats, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) activities increased as functions of the percentage of oxidative fibers in the 6 muscles tested. Also, muscles of the ET rats had increased oxidative capacities and increased SOD and GPX activities as compared to the same muscles of the C rats. However, CAT activities were lower in the muscles of ET rats relative to C rats. Thus, these data indicate that while antioxidant enzyme activities are related to skeletal muscle oxidative capacity, the effects of exercise training on antioxidant enzyme levels in skeletal muscle are not solely determined by changes in oxidative capacity.

CARDIOTONIC DRUGS

33.1

LY249933: A POSITIVE INOTROPIC 1,4-DIHYDROPYRIDINE LACKING VASCULAR EFFECTS. Donald R. Holland*, James H. Wikel*, Jeffrey K. Smallwood*, Karen Zimmerman*, Barbara G. Utterback*, Mitchell I. Steinberg, and Raymond F. Kauffman*. Lilly Research Labs, Eli Lilly & Co., Indianapolis, IN 46285.

Calcium agonists (e.g., Bay K 8644) show little selectivity for cardiac vs. vascular smooth muscle. LY249933[1] (LY) and its diastereomers (RR and SR) displaced [^{3}H]nitrendipine bound to cardiac membranes (K_ds 3-7nM). In isolated rat ventricular strips RR and LY increased contractile force (EC_{50}s 1, 8 μM); SR was a weak negative inotrope. Inotropic effects of RR were not antagonized by prazosin, propranolol, or carbachol, and RR produced a leftward shift in the Ca^{2+} dose-response curve. In isolated canine cephalic veins, the contractile response to 20mM KCl was increased by RR, decreased by SR, and unaffected by LY. In pentobarbital-anesthetized dogs, effects of LY, RR, and SR (200 μg/kg/min, i.v.) on inotropy (dP/dt_{60}), stroke volume (SV), mean arterial pressure (MAP), vascular resistance (VR), and heart rate (HR) were (%Δ;*, $p<0.05$ vs. vehicle):

	dP/dt_{60}	SV	MAP	VR	HR
LY249933 (n=6)	43±15*	42±9*	0±5	-7± 4	-22± 3*
RR (n=4)	20± 9	5±9	18±1	41±16*	-18± 2
SR (n=4)	25±15	67±9*	-42±7*	-59± 6*	-12±12

Thus, combined effects of RR (agonist) and SR (antagonist) yield a cardioselective Ca^{2+} modulator (LY) that increases contractility without adversely effecting afterload. [1](R)-1,4-dihydro-2,6-dimethyl-5-nitro-4-thieno-[3,2-c]-pyridin-3-yl-3-pyridinecarboxylic acid, 1-phenylethyl ester.

33.2

DETERMINATION OF THE ORAL EFFICACY OF RG-12152 IN CONSCIOUS CHRONICALLY INSTRUMENTED DOGS.

RF WOLTMANN, JA BARRETT, RG PENDLETON AND MH PERRONE.
RORER CENTRAL RESEARCH HORSHAM PA

The effects of RG-12152, a new positive inotropic agent, and milrinone(MIL) were studied in a group of 5-6 conscious dogs prepared to monitor dP/dt, arterial pressure and heart rate (HR). RG-12152, MIL and 0.5% methylcellulose(MC) were administered via gavage in increasing doses at 1hr intervals in a crossover design with 6 days between treatments. RG-12152 0.3, 1 and 3 mg/kg,p.o. increased dP/dt (max: 13±7, 32±4 and 32±5%, respectively) while not significantly affecting MAP or HR. MIL 0.1,0.3 and 1mg/kg,p.o. increased dP/dt (max: 19±4,43±3 and 49±8%, respectively) and HR (max: 4±6,23±19 and 44±31%, respectively) while not affecting mean arterial pressure(MAP). The ED(25) for dP/dt for RG-12152 and MIL was 570 and 140 ug/kg,p.o., respectively. RG-12152 1mg/kg,p.o., MIL 0.3mg/kg,p.o. or MC were administered to the same group of dogs and the effects monitored for 5 hrs with >72 hrs between treatments. RG-12152 and MIL increased dP/dt 30-300 min posttreatment with the maximum increases of 29±5 and 20±6, respectively occurring at 75-90 min. RG-12152 did not affect HR or MAP while MIL increased HR 31±16% (15-240min) and decreased MAP (max: 11±3% 15-135 min). It is concluded that RG-12152 is an orally effective positive inotropic agent in the conscious dog.

33.3

EFFECT OF RG-12152, A NEW POSITIVE INOTROPIC/VASODILATOR AGENT, IN THE NORMAL AND DEPRESSED MYOCARDIUM OF ANESTHETIZED DOGS. JA BARRETT, RF WOLTMANN, R SWILLO, A SPADA, RG PENDLETON AND MH PERRONE.
RORER CENTRAL RESEARCH, HORSHAM PA

The effects of RG-12152 6-[3'-cyano-6'methyl-2'-oxo-(1H)-pyridin-5'-yl]-1-methyl-2H-pyrido [3,2-b]-1,4-oxazin-3 (4H)-one and milrinone(MIL) 30,100 and 300ug/kg,i.v. were studied in separate groups (N=6-8) of normal and cardiac depressed (mecamylamine(M) 2mg/kg,i.v. and propranolol(P) 1mg/kg,i.v. +0.3mg/kg/hr) anesthetized dogs prepared to monitor contractile force(CF), heart rate(HR), arterial pressure and aortic flow(AF). In normal dogs, RG-12152 and MIL increased CF and HR while decreasing mean arterial pressure(MAP) and not affecting AF. The ED(50) values for CF were 218 and 25 ug/kg,i.v., respectively. At the ED(50) HR increased 22 and 7% while MAP decreased 16 and 3%, respectively. In the cardiac depressed dog, RG-12152 and MIL increased CF and HR while decreasing MAP and not affecting AF. The ED(50) values for CF were 195 and 35 ug/kg,i.v., respectively. At the ED(50) HR was increased by 22 and 10% while MAP was decreased by 20 and 5%, respectively. It is concluded that RG-12152 is a selective direct acting positive inotropic/vasodilator agent which appears to possess more vasodilatory activity per unit of inotropy than milrinone.

33.4

THE EFFECTS OF MILRINONE IN RATS WITH CONGESTIVE HEART FAILURE INDUCED BY LONG-TERM PRESSURE OVERLOAD. Suzanne Desjardins* and Michael J. Cauchy* (SPON: Madhu B. Anand-Srivastava) Bureau of Drug Research, Health and Welfare Canada, Ottawa K1A 0L2.

The effects of iv milrinone (MIL) were measured in old rats with longstanding (82-93 weeks) pressure overload induced by aortic clipping. Based on heart weight and pathological findings, rats with an aortic clip were divided into 2 groups: with congestive heart failure (CHF) or without (CLIP) CHF. Three groups of anesthetized rats received MIL iv boluses of 0.1, 0.5, 1, 5 and 10 mg/kg at 20 min. intervals: Gr 1, SHAM (n=9), Gr 2, CHF (n=10) and Gr 3, CLIP (n=22). One group of 3 CHF and 5 CLIP rats received only the vehicle which had no effect on any hemodynamic or ECG parameters measured. MIL at doses of 1, 5 or 10 mg/kg induced ventricular fibrillation and death in a total of 10 rats (n=2,1, and 7 in Gr1,2 and 3 respectively, P=0.8, Chi-square analysis). The major effect of MIL on the ECG was a significant increase in QRS interval duration at all doses tested in Gr 1 and at the 2 highest doses in Gr 2 and 3. There was no effect of MIL on heart rate. MIL induced a significant decrease of arterial and left ventricular pressures and of dP/dt min. in all 3 groups. MIL had either no (Gr1 and 2) or a negative (Gr3,2 highest doses) effect on dP/dt max. It is concluded that MIL has no positive inotropic effect in old anesthetized rats and that rats with CHF may be less sensitive to the toxic action of MIL.

33.5

COMPARATIVE HEMODYNAMICS OF MILRINONE AND MEDORINONE IN ANESTHETIZED DOGS; K. Lee, P. Canniff*, D. Hamel*, G. Roth*, D. Fort*, A. Ezrin; Sterling-Winthrop Res. Inst., Department of Pharmacology, Rensselaer, N.Y. 12144

The hemodynamic effects of the selective phosphodiesterase III isozyme inhibitors, milrinone (a bipyridine) and medorinone (a 1,6 naphthyridine), were investigated in β-adrenoreceptor blocked (nadolol, 1 mg/kg, i.v.) anesthetized dogs. When compared with vehicle (n=8), milrinone and medorinone (0.01, 0.03, 0.1 and 0.3 mg/kg cumulatively, i.v., n=6 each) increased the rate of change in left ventricular pressure (+dP/dt) in a dose related manner (2570±440 and 1420±270 mm Hg/sec, max. changes, respectively). The increase in +dP/dt induced by milrinone was significantly greater than that induced by medorinone at doses higher than 0.01 mg/kg. Milrinone and medorinone decreased mean arterial pressure (-18±3 and -15±8 mm Hg), systemic vascular resistance (-1620±570 and -1700±270 dynes.sec.cm^{-5}), right atrial pressure (-2.1±1.0 and -1.5±0.3 mm Hg) and increased heart rate (34±5 and 24±4 b/min). Left ventricular end diastolic pressure was decreased by milrinone (-2.3±0.8 mm Hg) but not by medorinone. Cardiac output and stroke volume were not affected by either agent. In conclusion; milrinone is more potent than medorinone as a positive inotropic agent, both agents are equi-effective as vasodilators and milrinone is more effective than medorinone in reducing cardiac preload in β-adrenoreceptor blocked anesthetized dogs.

33.6

COMPARATIVE EFFECTS OF MILRINONE AND MEDORINONE ON RENAL FUNCTION AND REGIONAL BLOOD FLOWS IN ANESTHETIZED DOGS P. Canniff*, K. Lee, D. Hamel*, G. Roth*, D. Fort*, A. Ezrin; Sterling-Winthrop Res. Inst., Rensselaer, N.Y. 12144

The effects of the selective phosphodiesterase III isozyme inhibitors, milrinone (a bipyridine) and medorinone (a 1,6 naphthyridine) on regional blood flows and renal function were investigated in β-adrenoreceptor blocked (nadolol, 1 mg/kg, i.v.) anesthetized dogs. Milrinone and medorinone (0.01, 0.03, 0.1 and 0.3 mg/kg cumulatively, i.v. n=6 each) were equi-effective in decreasing mean arterial pressure in a dose-related manner (-18±3 and -15±8 mm Hg, max. changes, respectively). Cardiac output was not affected by either agent. When compared with vehicle (n=8), milrinone significantly increased renal blood flow (RBF) at 0.1 and 0.3 mg/kg (24±7 and 32±8 ml/min, respectively). Medorinone did not significantly affect RBF. Milrinone had no significant effect on femoral blood flow (FBF) but medorinone (0.1 mg/kg) significantly increased FBF (11±7 ml/min). When compared with vehicle, milrinone did not significantly affect urine output (UV) while medorinone (0.1 mg/kg) significantly increased UV (0.32±0.09 ml/min) Urinary Na and K excretion rates were not affected by either agent. In conclusion, milrinone and medorinone are equi-effective in decreasing afterload; milrinone selectively increases RBF whereas medorinone selectively increases FBF. Despite a decrease in renal perfusion pressure (indicated by hypotension), renal function was not altered by milrinone and was enhanced by medorinone.

33.7

Therapeutic Effects of Milrinone (M) in Rabbits with Chronic Aortic Insufficiency (AI). A. DeFelice*, S. Fein*, K. Daudiss*, P. Horan* and R. Frering* (Spon: P. Silver), Sterling-Winthrop Res. Inst., Rensselaer, NY, and Cardiol. Div., Albany Med. Cent., Albany, NY

The aim of this study was to evaluate effects of M in chronic AI induced in New Zealand rabbits (1-2 kg) via aortic valve puncture. After 24 mo., left ventricular (LV) size, aortic diameter, forward and reverse aortic mean flow velocity (F Fl Vel; R Fl Vel), and heart rate (HR) were measured by M-mode/Doppler echography, from which mean velocity of circumferential fiber shortening (Vcf), forward and regurgitant stroke volume (F St V; R St V), and total and forward LV output (TCO; FCO) were derived. Basal mean LV end-diastolic diameter and F Fl Vel of AI rabbits (n=8) were 139% and 154% of sham-operated values ($p < 0.05$). Basal F St V and TCO were elevated by 155-183% ($p < 0.01$), but FCO was normal. M (10 μg/kg/min) decreased TCO of AI rabbits by 25% ($p < 0.02$) but FCO remained normal; HR increased by 13%. M reduced LV regurgitation: R St V, R Fl Vel/F Fl Vel ratio, and LV end-diastolic diameter were 48% ($p < 0.05$), 84% ($p < 0.05$) and 93% of values seen after vehicle (saline/lactic acid) alone. Vcf rose from 2.8 to 3.9 circumf./sec ($p < 0.03$), i.e. that seen in sham-operated rabbits. Thus, M reduced total LV output and regurgitation, maintained normal forward CO, and restored Vcf in rabbits with chronic AI and eccentric LV hypertrophy. In further studies, M changed LV dP/dt and diastolic BP of normal rabbits by +23% and -18% ($p < 0.05$), respectively. Thus, beneficial effects of M in AI may derive from positive inotropic and vasodilating activities. These results suggest possible utility of milrinone in treating AI.

33.8

PIMOBENDAN INCREASES MYOFIBRILLAR RESPONSIVENESS TO Ca^{++} IN INTACT MAMMALIAN MYOCARDIUM. J.H. Kappler* and N.K.M. Lee* (SPON: J.R. Blinks). Department of Pharmacology, Mayo Foundation, Rochester, MN 55905

Pimobendan is an inotropic agent that increases myocardial cAMP levels by inhibiting PDE activity. However, the drug does not abbreviate contractions and increases myofibrillar Ca^{++}-sensitivity in chemically skinned bundles of heart muscle -- actions inconsistent with a primarily cAMP-dependent inotropic mechanism. We measured tension development and intracellular Ca^{++} transients simultaneously to determine whether a change in myofibrillar responsiveness to Ca^{++} plays a significant role in the inotropic effect of pimobendan in intact cells.

Multiple superficial cells of ferret (n=6) and cat (n=2) papillary muscles were microinjected with aequorin. Isometric contractions and aequorin signals were monitored, first as $[Ca^{++}]_o$ was varied from 2 to 8 mM (32° C; 0.5 Hz; 0.5 μM (±)-bupranolol throughout). Then muscles were exposed to 20 μM (-)-pimobendan (in 2.25 mM Ca^{++}), which approximately tripled tension development and doubled the amplitude of the aequorin signals, with little change in the time course of either signal. Plots of max dP/dt (or peak tension) vs. peak light were shifted in the direction of increased myofibrillar responsiveness to Ca^{++} in the presence of the drug. The pimobendan was then washed out, and after 10-20 min the aequorin signal had returned to or nearly to control levels, while contractile force declined relatively little. When $[Ca^{++}]_o$ was now varied, tension development was substantially greater at every $[Ca^{++}]$ than before exposure to the drug, and the curve relating max dP/dt to peak light was shifted more strikingly than in the continued presence of the drug. These results indicate that pimobendan does increase myofibrillar responsiveness to Ca^{++} in living myocardium, and that it does so by a mechanism different from that responsible for the increased amplitude of the Ca^{++} transient (which may be phosphodiesterase inhibition). Support: USPHS grant HL12186. Pimobendan was provided by Dr. W. Diederen, Karl Thomae GmbH.

33.9

CARDIOVASCULAR EVALUATION OF ORF 22867: A POTENT, LONG-ACTING POSITIVE INOTROPE AND PERIPHERAL VASODILATOR AGENT. Robert Falotico, Barbara Haertlein, Constance Lakas-Weiss, John B. Moore, Donald W. Combs and Alfonso J. Tobia. Research Laboratories, Ortho Pharmaceutical Corporation, Raritan, New Jersey.

ORF 22867, (ORF) [6-(3,4-dihydro-3-oxo-1,4(2H)-benzoxazin-7-yl)-2,3,4,5-tetrahydro-5-methylpyridazin-3-one] is a potent, orally effective cardiotonic agent with a long duration of action. In anesthetized dogs, ORF (ED_{50} = 5.4 µg/kg, i.v.) is equipotent to indolidan, four times more potent than milrinone and 15 times more potent than imazodan as a positive inotrope. In conscious dogs, ORF increases dP/dt_{max} 86-130% after oral administration of 30-300 µg/kg (ED_{50} = 10 µg/kg, p.o.). A long duration of action is observed with inotropic activity for up to 24 hr. ORF relaxes phenylephrine contracted aortic strips (ED_{50} = 0.29µM) and increases femoral blood flow (20 - 100%) in denervated canine hindlimb (0.02 - 2.0 µg/kg, i.a.). ORF is a potent, competitive and selective inhibitor of canine cardiac phosphodiesterase (PDE) fraction III, (K_i = 70 nM). The inotropic effect is not antagonized by propranolol pretreatment. In an acute heart failure model, ORF (25 µg/kg, i.v.) increases cardiac output and stroke volume, while reducing elevated filling pressures and afterload. ORF is currently undergoing clinical evaluation for the management of congestive heart failure.

33.10

[^{3}H]OUABAIN BINDING TO REGIONS OF RAT HEART AS DETERMINED BY AUTORADIOGRAPHY. R.L. Evans*, L.M. Plunkett*, R.H. Kennedy and E. Seifen. University of Arkansas for Medical Sciences, Little Rock, AR 72205.

[^{3}H]ouabain binding to left and right ventricular, septal, and papillary myocardium isolated from male Fischer-344 rats was analyzed by autoradiography. After removal, hearts were perfused with Krebs-Henseleit buffer, frozen, and cut into 8 um sections. Sections were incubated for 1 hr at 37 °C in a medium containing 100 mM NaCl, 5 mM $MgCl_2$, 50 mM Tris-HCl, 5 mM Tris-ATP, and a single concentration of [^{3}H]ouabain (950 nM; sp. act. 24.1 Ci/mM). Nonspecific binding (20-30%) was determined in the presence of 1 mM unlabeled ouabain. Following incubation, sections were exposed for 14 days to [^{3}H]-Ultrofilm. The amount of bound [^{3}H]ouabain was determined by computerized densitometry (DUMAS System) and expressed as fmol/mg protein, assuming a homogeneous distribution of protein across the sections. Binding sites were quantified in various myocardial segments. Under these conditions, septal tissue had a significantly higher level of specific ouabain binding (26.3 fmol/mg protein) than papillary or right ventricular muscle (20.5 and 18.0 fmol/mg protein, respectively). There was no difference in specific binding in septum vs. left ventricular muscle (22.6 fmol/mg protein). These results suggest that there are regional differences in specific ouabain binding in rat heart.

33.11

THE BINDING OF (^{3}H)-OUABAIN TO MYOCYTES ISOLATED FROM GUINEA PIG HEART. Sarah Samuelov* and Theodore M. Brody, Department of Pharmacology and Toxicology, Michigan State University, E. Lansing, MI 48824.

Increasing ouabain concentrations reduces the viability of isolated myocytes from guinea pig heart. The reduction in viability appeared to correlate with a non-linear Scatchard plot describing the specific binding of ouabain to myocytes. With low ouabain concentrations that had no significant effect on myocyte viability, an almost linear plot was observed. As the number of viable cells was reduced by higher ouabain concentrations, linearity of the plot was lost. For this reason, studies on the specific binding of (^{3}H) ouabain to non-viable myocytes were conducted. A Scatchard plot of non-viable, rounded cells (97%) was linear from 0.1-20 µM ouabain. Its slope, Kd and Bmax values were similar to those obtained with rod-shaped, viable cells (77%) at the high ouabain concentrations. This Scatchard plot is higher than that extrapolated from the linear portion of a similar plot observed with low ouabain concentrations in viable myocytes. These findings suggest that the non-linear behavior of the Scatchard plot of isolated guinea pig heart myocytes is a consequence of cell death resulting from the higher concentrations of ouabain. As the viability of myocytes is reduced, the population of receptors which specifically binds ouabain increases while their affinity is decreased. (Supported by USPHS grants HL-16052 and AG-02398).

33.12

EFFECTS OF DIGOXIN ON GLOBAL CARDIAC FUNCTION OF HYPERTENSIVE-DIABETIC RATS. Joseph M. Capasso, Bruce Halpryn, Laura M. Quattrocci, Giorgio Olivetti, Piero Anversa. New York Medical College, Valhalla, NY 10595

To determine whether cardiac glycosides ameliorate global cardiac hemodynamics in an animal model of myocardial dysfunction, adult male rats with two kidney, one-clip, renal hypertension and streptozotocin induced diabetes were treated with digoxin (500 ug/kg body weight) for a period of 8 weeks. Systolic arterial pressure (BP) and blood glucose concentration (BGC) were comparable throughout the study in the two experimental groups. However, BP and BGC were approximately 1.5-fold and 3.0-fold greater in these animals than in age-matched control rats. Because of the variation in body weight among the three animal groups, cardiac hypertrophy was determined by left ventricular weight-to-body weight ratio. In comparison with controls, this parameter was increased by 71% in digoxin treated rats and 52% in placebo subjected animals. Ventricular function measured at sacrifice showed that left ventricular peak systolic pressure (LVPSP) was 196 ± 23 and 186 ± 7 mmHg and left ventricular end diastolic pressure (LVEDP) was 27 ± 6 and 13 ± 5 mmHg in placebo and digoxin treated rats, respectively. The difference in LVEDP was statistically significant ($p<0.0002$). Corresponding values of LVPSP and LVEDP in sham operated controls were 129 ± 16 and 6 ± 2 mmHg. The cardiac glycoside had no effect on +dP/dt, and -dP/dt was depressed in both groups of hypertensive-diabetic rats with respect to controls. In conclusion, digoxin failed to enhance the inotropic state of the myocardium when hypertension and diabetes are simultaneously present.

33.13

EFFECTS OF DPI 201-106 ON ACTION POTENTIALS, Ca^{++} TRANSIENTS, AND CONTRACTIONS OF MAMMALIAN CARDIAC MUSCLE. Y.-d. Cai*, N.K.M. Lee*, and J.R. Blinks. Department of Pharmacology, Mayo Foundation, Rochester, MN 55905

DPI 201-106 (Sandoz) is an inotropic and antiarrhythmic agent that delays inactivation of the sarcolemmal Na^+ channel, thus prolonging the action potential and increasing the entry of both Na^+ and Ca^{++} into the cardiac cell during the action potential. This might be expected to increase the amount of Ca^{++} stored in and liberated from the sarcoplasmic reticulum, and thus the amplitude of the Ca^{++} transient. However, the drug has also been reported to increase myofibrillar Ca^{++}-sensitivity in chemically skinned bundles of heart muscle. We measured tension development and intracellular Ca^{++} transients simultaneously to assess the relative importance of these two actions in intact mammalian myocardium. Multiple (50-100) superficial cells of cat papillary muscles were microinjected with aequorin. Isometric contractions and aequorin (light) signals were monitored simultaneously in all experiments; in some, transmembrane potentials were also recorded before and after the administration of the drug. Muscles were studied at 30° and 37°C, 0.25 Hz, with beta-adrenoceptor blockade.

DPI 201-106 (0.3-10 µM) produced substantial concentration dependent increases in tension development and in the amplitude of the aequorin signal; the action potential was prolonged as much as threefold. There was little change in time to peak tension or peak light, but relaxation was slowed somewhat, and the aequorin signal remained perceptibly above baseline for the duration of the action potential. When the action potential was sufficiently prolonged, there was often a secondary rise in luminescence, and the twitch was followed by a low-amplitude shoulder of tension maintenance that ended with repolarization. High concentrations of DPI 201-106 increased the amplitude of the Ca^{++} transient less than did equally inotropic elevations of $[Ca^{++}]_o$, which indicates that increased myofibrillar responsiveness to Ca^{++} also contributes significantly to the inotropic effect of the drug. (Support: HL12186).

34.1
THERMOREGULATORY AND CARDIOVASCULAR (CV) RESPONSES TO PASSIVE HEATING IN FISHER 344 RATS. K.C. Kregel, J.A. Taylor*, C.M. Tipton and D.R. Seals. Depts. of Exercise & Sport Sciences and Physiology, Univ. of Arizona, Tucson, AZ 85721.

The purpose of this study was to compare the thermoregulatory and CV adjustments to acute heat stress in mature (MAT, 12 mo, N=6) vs senescent (SEN, 24 mo, N=6) unanesthetized Fisher 344 rats. On 2 separate days (48 hr apart) MAT and SEN were exposed to an ambient temperature of 42°C until a colonic temperature (T_C) of 41°C (T_{41}) was attained. Heart rate (HR), blood pressure (BP), and lactate (La) were obtained from a carotid artery catheter. Baseline T_C (37.4 ± .2 vs 37.2 ± .3, $\bar{X}$ ± SE, MAT vs SEN) was not different in the 2 groups; however, the rate of increase in T_C was greater (.09 ± .01 vs .06 ± .01°C/min, p = .07), and the time to T_{41} was shorter (46 ± 5 vs 69 ± 6 min, p = .02) in MAT during the 1st exposure. The increase in HR from baseline to T_{41} was 2-fold greater in SEN (38 ± 10 vs 80 ± 13 bt/min, p = .04), whereas the increases in AP (14 ± 7 vs 13 ± 7 mmHg) and La (1.15 ± .23 vs 1.75 ± .37 mmol/ℓ) were not different in the 2 groups. The rate of increase in T_C was greater in both groups during the 2nd heat exposure; however, the magnitude of the change was 4-fold greater in SEN (29 ± 10 vs 117 ± 35%, p = .02). Four of 6 SEN died ≤ 24 hr after the 2nd heating whereas only 1 of 6 MAT died during this period. These preliminary findings indicate that heat tolerance during an initial exposure is not impaired, and may be enhanced, with advancing age in Fisher 344 rats. However, older animals demonstrate a more marked reduction in heat tolerance and a greater mortality rate in response to a subsequent thermal challenge.

34.2
PERIODIC HYPERSENSITIVITY TO MICROWAVE IRRADIATION IN THE DEVELOPING RAT. Eleanor R. Adair and Donald E. Spiers. John B. Pierce Foundation, New Haven, CT 06519

To explore the potential for microwave incubation of infant mammals, we pinpointed critical periods in the first 16 days of life when the developing rat may be adversely sensitive to microwave exposure. Groups of 8 pups underwent daily 8-h sham or microwave (2450 MHz, 20 mW/cm²) exposure at an ambient temperature (T_a) of 30 °C, from 2 to 5 (Grp I), 6/7 to 11/12 (Grp II), or 11/12 to 15/16 (Grp III) days of age. Animals were weighed before and after each exposure. One day post-treatment, steady-state body temperatures (T_b) and metabolic rate (M) were measured at test T_a of 35, 32.5, 30, and 25 °C to assess thermoregulatory ability. Animals from Grps I and III showed a significant % loss of body mass during microwave exposure, re sham-exposed, and a few animals died during treatment. M and T_b of these rats deviated from sham-exposed only at certain test T_a. Growth rate, M, and T_b of Grp II microwave-exposed rats were similar to sham-exposed; all survived the treatment. Results indicate two critical periods of hypersensitivity to 2450-MHz microwave exposure, one immediately after birth and the other at 1 1/2 weeks of age. Both are related to thermal stress: a high rate of energy absorption in newborns and greater insulation in older rats. (Supported by USPHS Grant HD18002).

34.3
THERMOREGULATION IN DIABETIC RATS. T.H. Shalaby*, R.K. Dupre* and M.K. Yousef. Dept. of Biol. Sci., Univ. of Nev., Las Vegas, Las Vegas, NV 89154

Little attention has been given to thermogenesis in animals with major "chronic" diseases. Impairment of thermoregulation in diabetic individuals remains a scientific judgement that has not been fully substantiated by controlled laboratory studies. Thermal responses and skin microcirculation were measured in streptozotocin-induced diabetic (SD) rats during exposure to ambient temperatures ranging from about 5C to 35C. Exposure of the SD rats to 35C and 5C caused a sharp rise and decline in rectal temperature (Tre), respectively. At 35C, hyperthermia in the SD rats was associated with greater increase in VO_2 than controls. At 5C, VO_2 changes similarly in both the SD and control rats. Tail skin microcirculation (SKBF) was measured using a Laser-Doppler. At 28C, tail skin microcirculation SKBF measured by Laser-Doppler was greater in SD than in control rats. During exposure to 35C, the percent increase in tail SKBF was about the same in both SD and control groups. However, at 5C, the percent decrease in tail SKBF was greater in the control than in SD rats. The data suggest that hypothermia in SD rats may be associated with impairment of vasoconstriction and hyperthermia may be related to greater VO_2 that was not accompanied by higher vasodilation.

34.4
COMPARATIVE EFFECTS OF U-50,488H (U50) AND CHLORPROMAZINE (CPZ), ALONE AND IN COMBINATION, ON BODY TEMPERATURE (Tb) IN RATS AND GUINEA PIGS. M.W. Adler, R. Martinez*, and E.B. Geller. Temple University School of Medicine, Philadelphia, PA 19140

As reported in earlier studies from this laboratory, the combination of the selective kappa opioid agonist U50 (80 mg/kg, sc) and the neuroleptic CPZ (5 mg/kg, sc) causes a profound hypothermia in rats, amounting to over 8°C at 20°C ambient. Because of the greater density of kappa opioid receptors and the increased ratio of kappa to mu receptors in the guinea pig brain, the drugs were tested in this species. The subjects were young adult male Hartley guinea pigs weighing 350-400 g. Groups of 5-8 animals were injected sc with 2.5 or 5.0 mg/kg of CPZ and 40 or 80 mg/kg of U50, alone or in combination. The low dose of CPZ produced a drop in Tb of -1.37±0.10°C and the high dose, -1.58±0.24°C. These decreases in guinea pigs were virtually identical to those found in our earlier studies in the rat. The U50 alone, however, produced drops of -4.56±0.21°C and -7.10±0.69°C for the low and high doses, respectively, in the guinea pigs. These changes were markedly greater than the drop of approximately 2°C seen in rats. Though the combination of 5 mg/kg CPZ and 80 mg/kg U50 was found to produce a marked hypothermia, there was no addition or potentiation in the guinea pig; in fact, the effect was the same as with U50 alone. The combination of lower doses of the drugs (2.5 mg/kg CPZ and 40 mg/kg U50) produced a drop in Tb of -6.67°C, slightly greater than the additive effect of the drugs individually, but less than the potentiation seen with these doses in the rat. Other differences between the two species in the effects of U50 and the combination were also noted. For example, U50 produced escape behavior and excessive urination in the rat, but not in the guinea pig. In addition, the combination caused deaths in the guinea pig, but not in the rat. Although the reason for the differences between these species is not known, it is reasonable to speculate that it may be due to different ratios of opioid receptors in sites critical for temperature regulation. This study provides further support for our hypothesis that kappa receptors mediate the hypothermic actions of opioids. Supported by grant DA 00376 from NIDA.

34.5
RENAL CHANGES IN THE GROUND QUIRREL DURING ACTIVE AND HIBERNATION PERIODS. D. Anderson*, D. Bewernick*, S. Brazal*, J. Ponder*, J. Russom* and G. A. Lopez. California State University at Los Angeles, Los Angeles, CA 90032

In previous studies, animals hibernating for only brief periods of time have been compared to active counterparts regarding differences in renal glomerular morphology and physiology. In the present study, the golden-mantled ground squirrel, Spermophilus lateralis, is used to evaluate these differences at seven time-points during a one-year active-through-hibernation cycle. The hibernation period typically lasted from November to May. Renal cortical tissue was taken for transmission electron microcopy (TEM) and trunk blood was collected for plasma renin activity (PRA). Glomerular endothelial pore number decreased 70% from mid-active to mid-hibernation period and then increased 57% by the onset of activity. Epithelial slit pore number showed a similar pattern (26% and 30%, respectively). No changes occurred in podocytic foot process size or width of the glomerular basement membrane. Mesangial cells showed an increased phagocytic activity and PRA levels were greater during hibernation. These data suggest that during hibernation: 1) the decrease in membrane pore number is designed to maintain cellular hydration and sodium conservation; 2) mesangial cells may cleanse the basal lamina of increased toxins and; 3) decreased renal perfusion pressure leads to an uncompensated increase in PRA. (Supported by NIH #5-S06-RR08101).

34.6
OBSERVATIONS OF SKELETAL MUSCLE FROM A HIBERNATOR, SPERMOPHILLUS LATERALIS. J.M. Steffen[1], M.C. Steffen[1], T.E. Geoghegan[3], X.J. Musacchia[1], W.K. Milsom[2], and R.F. Burlington[3]. [1]Univ. of Louisville, Louisville, KY 40292, [2]Univ. of British Columbia, Vancouver, B.C. V6T 2A9, [3]Central Michigan Univ., Mt. Pleasant, MI 48859.

Hindlimb skeletal muscles (soleus, plantaris, EDL) from hibernating and winter-active golden-mantled ground squirrels (Spermophillus lateralis) were examined histochemically (myosin ATPase staining) and biochemically (protein, RNA and DNA contents as well as α-actin mRNA levels) to elucidate cellular alterations associated with hibernation. There were no significant differences in body weights, absolute or relative (mg/g body wt.) muscle weights, protein and DNA contents or concentrations (mg/g muscle wt.) between active and hibernating groups. Type I slow twitch fibers in soleus muscles from hibernators exhibited a 30% greater cross-sectional area than winter active controls. Absolute RNA content was reduced 30% ($P<.05$) in soleus muscles from hibernators while RNA concentrations were reduced from 2.6 to 1.8 μg/mg muscle (45%). α-actin mRNA levels, determined by hybridization of LiCl precipitated total RNA to a ^{32}P-labelled cDNA probe, were unchanged in the soleus but markedly elevated in plantaris muscles from hibernators. These results are suggestive of complex cellular alteration in muscle during the hibernating season.

34.7
METABOLIC RESPONSES TO CAFFEINE AND COLD EXPOSURE IN HUMANS. K.W. MACNAUGHTON* and T.E. GRAHAM. School of Human Biology, U. of Guelph, Guelph Ontario. N1G 2W1

Caffeine (Cf) and cold air (CA) have each been reported to increase serum FFA and VO_2 in resting humans. We examined the possible interaction of these stimuli in 6 non-caffeine consuming young men (22±3 yr). Each subject was tested twice while sitting in a 28 and 5°C environment for 2 h after receiving Cf(5mg/kg) or a placebo. VO_2, VCO_2, and venous FFA levels were determined before and 30 min after ingestion, and at 5, 30, 60, 90, and 120 min of either 28 or 5°C exposure. There were no significant differences between the initial and the 30 min post ingestion times in any measures. CA alone did not have a significant effect on serum FFA levels, but VO_2 and VCO were increased while mean skin and mean body temp. were decreased significantly throughout the CA trials. In contrast, Cf did not influence these parameters but significantly increased FFA in both the 28 and 5°C trials. Thus, Cf and CA appear to act independently on metabolism of resting humans.

Supported by Canadian Fitness and Lifestyle Research Institute and by NSERC

HYPERTHERMIA/HYPOTHERMIA

35.1
THE EFFECTS OF HYPOTHERMIA ON THE BLOCKADE OF MUSCARINIC RECEPTORS BY DIBENAMINE. CHENG S. TSAI* AND RICHARD F. OCHILLO. LABS OF PHARMACOLOGY AND TOXICOLOGY. XAVIER UNIVERSITY OF LA. NEW ORLEANS, LA. 70125.

Hypothermia facilitated a parallel rightward shift of the dose-response curve of ACh. To decipher the changes induced by hypothermia, we used dibenamine as the alkylating agent to measure the activity constants for muscarinic receptors of the smooth muscle. However, the results of our attempt were erratic. We have therefore hypothesized that the occlusion of the receptors and the recovery from the binding may be temperature dependent. The response of isolated longitudinal muscle to ACh at 37 and 24°C was recorded before and after incubation of the preparation with dibenamine for 20 min. After dibenamine treatment, the contractions were reduced to 60 ± 8.0 and 70 ± 7.0% respectively. The recovery at 37°C was not significant within 60 min. However, the recovery at 24°C was rapid and a complete recovery occurred after washing off the unoccluded dibenamine. We concluded that the lack of reversibility of the effects of dibenamine at 37°C is due to the predominance of covalent binding between the receptors and dibenamine whereas at 24°C the blockade is mainly due to simple occlusion. (Supported by grant N-00014-84-K-065 from U.S. Office of Naval Research and grant #RR-08008 and #MR 1 R 25 HL37736 from NIH).

35.2
XANTHINE OXIDASE D - O FORM CONVERSION AND FERRITIN IRON RELEASE. J. Skibba, A. Stanicka* and R. Powers*. Medical College of Wisconsin and Zablocki VAMC, Milwaukee, Wisconsin 53226

Hyperthermic liver perfusion has been proposed as a treatment for certain forms of inoperable liver cancer. A problem of this method is hepatotoxicity which appears to be a function of lipid peroxidation. We have shown that incubation of milk xanthine oxidoreductase (XO) with partially purified ferritin and xanthine results in the release of ferrous iron, a process which is inhibited by the inclusion of allopurinol, superoxide dismutase and increased by inclusion of catalase in the incubation. The rate of iron release in a similar incubation using rat liver XO was seen to be a function of time of pre-incubation of XO at 37 or 42.5°. This pre-incubation resulted in a significant conversion of XO from the dehydrogenase to the superoxide-generating O form. The ability of XO to cause release of iron from ferritin upon incubation with xanthine was correlated with the % O form. We suggest that conversion of hepatic XO from the D to O form as a function of hyperthermic liver perfusion contributes to the formation of superoxide and the release of iron from ferritin, conditions consistent with the processes of lipid peroxidation.

35.3
CHANGES IN PLASMA CATECHOLAMINES DURING HYPERTHERMIA. C.V. Gisolfi, R.D. Matthes, R.A. Oppliger, and K.C. Kregel. Exercise Science Dept., Univ. of Iowa, Iowa City, IA 52242

Our laboratory has provided evidence that a selective loss of compensatory splanchnic vasoconstriction may be an important mechanism signaling circulatory failure in heat stroke. Is this decline in splanchnic vasoconstriction associated with a decline in circulating catecholamines? Twenty-five male Sprague-Dawley rats (270-300 grams) were assigned to one of 5 groups based on their colonic temperature (T_c) (37,39, 41,43,44°C) at sacrifice. HR, mean blood pressure, T_c, and renal, caudal, and superior mesenteric (SM) artery blood flows were monitored during heat stress under α-chloralose anesthesia (12.5 mg/ml @ 1 ml/hr). At each predetermined T_c, an aortic blood sample was drawn and analyzed (X±SE) for norepinephrine (NE) and epinephrine (E). At T_c>41°C, renal and caudal blood flows fell as SM flow rose. From 41-43°C, NE rose markedly and E rose significantly. Thus, the elevation in SM flow can not be attributed to reduced levels of circulating catecholamines. (Supported by NIH HL38959-01)

	37°C	39°C	41°C	43°C	44°C
NE, pg/ml	308 ±27	398 ±69	376 ±26	989 ±293	4828* ±631
E, pg/ml	947 ±395	2161 ±556	2418 ±840	6856** ±877	10069* ±563

*Significantly different ($p<0.05$) from all other groups.
**Significantly different ($p<0.05$) from all groups but 44°C.

35.4
ALTERED THERMAL RESPONSE TO INTRAHYPOTHALAMIC INJECTION OF NOREPINEPHRINE AND 5-HYDROXYTRYPTAMINE DURING HYPERTHERMIA IN RATS. U. Sachdeva*, G.S. Chhina* and B. Singh* (SPON: D.B. Jennings). Department of Physiology, All India Institute of Medical Sciences New -Delhi 110029 (India).

Thermogenic and thermolytic responses have been demonstrated by injection of 5-hydroxytryptamine (5HT) and norepinephrine (NE) into the preoptic-anterior hypothalamus (POAH) of normothermic rats. To assess the role of 5HT and NE in hyperthermic rats (T_H), experiments were conducted in rats a) with PGE_2 centrally induced pyrexia, b) with direct hypothalamic heating, and c) in a control group (T_N). Rectal (T_{rec}) and skin (T_s) temperatures were monitored following injection of 5HT and NE (10 ugm in 0.2 ul) into the POAH. The results suggest that injection of 5HT in T_N rats induced a rise in T_{rec} (0.02 ± 0.4°C) associated with a fall in T_s (1.7 ± 0.16°C). NE injection induced a biphasic thermal response with an initial fall in T_{rec} (0.42°C) followed by a rise (1.18°C). This was accompanied by a fall in T_s which was preceded by a slight elevation. In contrast, injection of 5HT in T_H rats failed to induce the rise in T_{rec} and fall in T_s. NE application in T_H rats induced a potentiated monophasic hyperthermia with abolition of initial fall in T_{rec} and rise in T_s. The results suggest that the thermal responses of 5HT and NE are altered in T_H rats whereas NE acts as a predominant thermogenic neurotransmitter and 5HT effects are inhibited.

36.1

COMPARATIVE BIOAVAILABILITY OF TWO DIFFERENT FORMULATIONS OF DILTIAZEM (D) IN HEALTHY VOLUNTEERS. S. Boucher,* G. Caillé,* and P. du Souich. Département de pharmacologie, Université de Montréal, Montréal, Canada.

Diltiazem is usually administered in fractionated dose ranging from 60 to 90 mg t.i.d. or q.i.d. In this 2 X 2 Latin square design study, 24 healthy male volunteers received 240 mg D sustained release (D.S.R.) orally once daily or 2 x 60 mg (D.I.R.) twice a day, for 7 days. Plasma samples were collected on Day 0 and on Day 6 for determination of the kinetic profile and before dosing on Days 1 to 5. The plasma concentrations were analyzed by using a HPLC method with U.V. detection. The mean maximum plasma concentration (Cmax) for D.S.R. on Days 0 and 6 were 141.2 and 178.1 ng/mL respectively. For D.I.R. Cmax were 103.4 and 172.2 ng/mL respectively. The areas under the curve (AUC) for D.S.R. were 1811.3 and 2425.0 ng.h/mL^{-1} after single dose and after repeated dosing. For D.I.R. we obtained 1340.8 and 2390.3 ng.h/mL^{-1} respectively after normalisation. The minimum plasma concentrations (Cmin) were 30.1 ng/mL and 53.5 ng/mL for D.S.R. and D.I.R. on Day 5. The fluctuation (Cmax-Cmin/Cmin) was 2.2 for D.I.R. and 4.9 for D.S.R. According to those results, the 240 mg D.S.R. is a good formulation of D for a once daily regimen.

36.2

PHARMACOKINETICS AND DISPOSITION OF SK&F 103829 IN MALE SPRAGUE-DAWLEY RATS. G. Stelman*, G. Joseph*, M. Carbonaro*, J. Kao, and C. Gombar. SK&F Labs, King of Prussia, PA 19406.

SK&F 103829 (8-hydroxy-7-methylsulfonyl-2,3,4,5-tetrahydro-1H-3-benzazepine) is a serotonin agonist that has been shown to cause marked increases in lower esophogeal sphincter pressure in animals. Following oral administration of 10 mg/kg of ^{14}C-SK&F 103829, 74% of the ^{14}C was recovered in urine, and 18% was recovered in feces over 96 hr. After i.v. dosing of 5 mg/kg recovery of radioactivity was 79% and 10% in urine and feces, respectively. The plasma protein binding and blood to plasma concentration ratio, determined in vitro at concentrations ranging from 0.01 to 10 ug/ml, was about 5% and 0.99, respectively. The clearance of SK&F 103829 from plasma after i.v. administration of 5 mg/kg was 26 ml/min/kg, the steady state volume of distribution was 1.7 l/kg and the terminal half-life was 1.7 hr. SK&F 103829 was administered orally at doses of 50, 100, 300 and 1000 mg/kg and the plasma concentrations determined for 24 hr after dosing. The area under the plasma concentration-time curve was proportional to dose over the entire range. The apparent elimination half-life was about 4 hr, and the oral bioavailability was approximately 60%. Therefore, SK&F 103829 appeared to be rapidly and completely absorbed after oral administration with about 60% of the oral dose available to the systemic circulation.

36.3

DOSE-DEPENDENT INHIBITION OF CYCLOSPORINE METABOLISM *IN VIVO* IN MICE BY FLUCONAZOLE. I. La Delfa*, Y. Xia*, T.F. Blaschke, Stanford Univ Medical Center, Stanford, CA 94305.

Fluconazole (FZ), a new bis-triazole antifungal drug, is a more potent antimycotic agent (mg/mg) when compared to ketoconazole (KE), an imidazole antifungal. We have previously shown that FZ is a potent inhibitor of the cytochrome P-450 enzyme system (CP450) in mice. The CP450 is a major pathway of cyclosporine (CyA) metabolism and is inhibited by KE. We investigated whether FZ had any effect on CyA metabolism in mice. CD-1 male mice were assigned to 4 groups: control and 3 FZ groups (1, 5 and 20 mg/kg). Forty minutes after intraperitoneal (ip) injection of FZ, to allow plasma concentrations to peak, CyA (2.6 mg/kg with a radiolabelled tracer dose) was injected ip. Blood samples were obtained for 8 hr after CyA injection in each animal. The dpm/ml of unchanged ^{3}H-CyA in whole blood was determined by HPLC and scintillation counting. Terminal rate constants (k_{el}), half-lives ($t_{\frac{1}{2}}$) of unchanged ^{3}H-CyA were (mean ± SD):

Group	n	k_{el}(min^{-1})	$t_{\frac{1}{2}}$ (hr)	(% of Control)
A. Control	8	.0027±.0007	4.6±1.2	(100)
B. FZ 1 mg/kg	8	.0021±.0005	5.8±1.4[1]	(126)
C. FZ 5 mg/kg	8	.0016±.0005	7.9±2.2[1,2]	(172)
D. FZ 20 mg/kg	8	.0010±.0003	13.2±4.2[3]	(287)

[1] *p<0.05 vs A;* [2] *p<0.05 vs B, D;* [3] *p<0.01 vs A, B*

We conclude that FZ, at doses required for antifungal efficacy in mice, produces dose-dependent inhibition of CyA metabolism *in vivo* in mice. The effects of FZ on the CP450 in man are unknown and should be evaluated. (Supported by NIH Grant GM22209).

36.4

RELATIONSHIP BETWEEN SALBUTAMOL (SAL) HYPOKALIEMIC EFFECT AND ITS PLASMA CONCENTRATIONS. S. Perreault*, H. Ong* and P. du Souich. Univ. of Montréal and Hôpital Hôtel Dieu of Montréal, Québec, Canada.

To assess SAL plasma kinetics and SAL hypokaliemic effect, 6 rabbits received 120, 120 and 2400 mcg/kg of SAL, intravenous (i.v.), intratracheal (i.t.) and per os (p.o.), respectively. Multiple plasma samples were drawn to assay SAL (RIA) and K^+. Even though the bioavailability was 0.8% for the p.o. route and 15% for the i.t. route, SAL plasma kinetics were independent of the dose and route of administration, e.g. clearance 0.2 L/min and volume of distribution of 10 L. Maximal decrease in kaliemia was 1.31, 0.84 and 0.70 mmol/L for the i.v., i.t. and p.o. routes, and was observed between 30 and 45 minutes after SAL peak plasma concentrations (56.4 ± 3.9, 5.2 ± 0.9 and 2.8 ± 0.2 ng/mL, respectively). The hypokaliemic effect was not related to SAL plasma concentrations. Using an integrated model, defining the effect and the concentrations in the compartment model, the estimated values of Emax were 1.71 and 1.05 mmol/L with a CE50 of 5 and 0.7 ng/mL for the i.v. and the i.t. routes, respectively. This model did not fit the effect following the oral dose. Based on the decrease in kaliemia and the computer generated data, we conclude that SAL hypokaliemic effect is dependent on the route of its administration.

(Supported by grants of HDM (S.P.) and MRC)

36.5

ATRACURIUM PHARMACOKINETICS IN OBESE PATIENTS. J. Ducharme*[1], F. Varin[1], J. G. Besner*[1], Y. Théorêt*[1], D. R. Bevan*[2] and F. Donati*[2]. [1]Univ. de Montréal and [2]McGill Univ., Montréal, Canada, H3C 3J7.

All non-depolarizing muscle relaxants, including atracurium (ATRA), are polar compounds which have a volume of distribution nearly equal to that of the extracellular fluid (ECF). An increase in the effect and duration of ATRA-induced neuromuscular blockade is expected in obese patients, because of reduced fraction of the ECF volume over the total body weight. Blood samples were obtained for up to 2 hours after the injection of ATRA (0.2 mg/Kg) in 3 obese ($\bar{X}$: 104 Kg) and 4 non obese ($\bar{X}$: 68 Kg) patients, scheduled for elective surgery. Obese patients had values of CLtot and Vss which were respectively 30% and 27 % lower than those of non obese patients, while there was no difference in the MRT. The absence of correlation between Vss and the excess over ideal body weight, confirms that ATRA does not distribute into fat. Since ester hydrolysis and Hofmann elimination of ATRA are both independent of liver function, it is suggested that the decrease in CLtot observed in obese patients is related to a reduced excretion of ATRA into bile or to a decreased activity in non specific plasma esterases, which could be secondary to pathological changes (e.g. steatosis) observed in these patients. (Supported by MRC grant)

36.6

INFLUENCE OF FOOD (F) ON THE PHARMACOKINETIC PARAMETERS OF KETOPROFEN (K). D. PILON,* G. CAILLÉ,* P. du SOUICH and Y. LAVASSE* Département de pharmacologie, Université de Montréal, Montréal, Québec, Canada.

To assess how F affects ketoprofen plasma and urine concentrations, following single and multiple administration (twice daily for five days), twelve healthy volunteers received orally 100 mg of enteric-coated ketoprofen alone and after a meal according to a 2 X 2 Latin square design. Blood samples drawn and urine collected after single and multiple dosing were assayed by an HPLC method using an UV detector. After a single dose, F reduced the peak plasma concentration (Cmax) from 10.7 to 6.3 ng/mL; increased the time to attain Cmax (Tmax) from 2.8 to 7.1 h and decreased the area under the curve (AUC 0 → ∞) from 23.8 to 13.3 ng.h/mL. Similar changes were observed after multiple administration: Cmax from 12.1 to 8.0 ng/mL; Tmax from 2.8 to 7.6 h and AUC 0 → 12 h from 29.3 to 16.8 ng.h/mL. All these changes were statistically significant. However, in the urine, K recovery was not altered significantly by F. It is concluded that F affected the plasma kinetics of K administered either singly or repeatedly, whereas it did not affect the total amount recovered in the urine.

36.7
A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR ORAL ADMINISTRATION OF CONTROLLED RELEASE MISOPROSTOL. James Stolzenbach, Ken Kowalski, Paul Von Doehren, Grant Schoenhard. (SPON: F.M. Radzialowski). G.D. Searle & Co., Skokie, IL 60077

Misoprostol is a PGE1 analog known to be effective in decreasing the production of stomach acid. Previously reported results from in vitro experiments with isolated parietal cells demonstrate that the major metabolite, which is the free acid of misoprostol (SC-30695), is the compound which actually binds to parietal cell receptors and causes the biological effect. SC-30695 is also absorbed into the systemic circulation after oral administration of misoprostol and the concentrations of circulating SC-30695 are hypothesized to influence the extent of side effects of the drug. The goal of this computer model is to simulate the slow release of misoprostol into the stomach in a manner similar to a sustained release formulation which may minimize the circulating concentrations of SC-30695. This model allows prediction of the plasma and organ concentrations of SC-30695 that will occur after dose administration. The model was developed and implemented using a computer program called SimuSolv. The model incorporates estimates of the metabolic capabilities and partioning characteristics of several tissues including the gut, liver, kidney, lung, and uterus. Current use of the model involves determining low various gut metabolic rates may effect plasma and organ concentrations of SC-30695 during slow release into the stomach. The model may be applied to either the dog or the rat.

36.8
EFFECTS OF SOIL ADSORPTION ON KINETICS OF ORAL TRICHLOROETHYLENE IN FEMALE RATS. G.A. Skowronski*, A.M. Kadry*, R.M. Turkall* and M.S. Abdel-Rahman. Toxicol. Lab., Pharmacol. Dept., N.J. Med. School, UMDNJ, Newark, N.J. 07103

More informed evaluations of the potential health hazards following ingestion of soil-adsorbed trichloroethylene (TCE) can be achieved by the bioavailability data from these studies. Peak plasma levels of activity were similar for TCE alone (P) and sandy soil-adsorbed chemical (S) but higher for clay (C) soil. Although the half-life of absorption was statistically longer and the half-life of elimination was statistically shorter in S, the area under the plasma concentration-time curve was approximately equal for all groups. Kidney represented the main route of excretion following P or C treatment with lungs as a secondary route. Equal amounts of the dose were excreted in both urine and expired air for S with a statistical increase of radioactivity in expired air throughout the 72 hr study period. The highest tissue concentrations of radioactivity were found in liver and kidney. Trichloroethanol was the primary urinary metabolite for all groups. Supported by NSF/Industry/Univ. Center for Research in Hazardous and Toxic Substances.

36.9
KINETIC DIFFERENCES OF PURE AND SOIL-ADSORBED XYLENE IN ORALLY EXPOSED FEMALE RATS. R.M. Turkall*, A.M. Kadry*, G.A. Skowronski* and M.S. Abdel-Rahman. Toxicol. Lab., Pharmacol. Dept., N.J. Med. School, UMDNJ, Newark, N.J. 07103

The extent of risk which occurs following exposure to contaminated soil depends upon the chemical's ability to desorb from soil and enter the body. 5μCi of ^{14}C-m-xylene alone (P) or with 0.5 g of sandy (S) or clay (C) soil was suspended in aqueous gum acacia and administered by gavage to fasted female rats. Maximum plasma concentration of radioactivity for S was higher than for P and C. S also significantly increased the area under the plasma concentration-time curve. S and C demonstrated longer absorption half-lives, while S showed a significantly shorter elimination half-life versus P. Fat contained the highest tissue concentration of radioactivity followed by stomach in all groups. ^{14}C activity was excreted primarily in urine followed by expired air with <1% in feces. S produced a significant decrease of radioactivity in urine during the 0-12 hr collection period. Methyl hippuric acid was the major urinary metabolite detected in all treatment groups. Supported by NSF/Industry/Univ. Center for Research in Hazardous and Toxic Substances.

36.10
ANTI-LFA-1α MONOCLONAL ANTIBODY PHARMACOKINETICS IN RABBITS. S.H. Norris, J.N. Johnstone*, and H. Silverstein*. Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877.

Anti-LFA-1α, a mouse monoclonal antibody (MAb), blocks the adhesion function of human lymphocyte LFA receptors. The pharmacokinetics of ^{125}I-anti-LFA-1α were studied in rabbit, a species with which the MAb cross-reacts. Rabbits were dosed i.v. (0.3 or 3 mg/kg). A 2nd dose of ^{125}I-MAb was given 8 days after the 1st. Prior to the 2nd dose, plasma ^{125}I fell to undetectable levels, and ^{125}I excretion had plateaued. The time course of plasma ^{125}I levels (all TCA-precipitable) had 3 phases. An initial distribution phase ($t_{\frac{1}{2}}$=2.3 hr) preceded a slow elimination phase ($t_{\frac{1}{2}}$=55 hr). The 3rd phase, beginning 5 days after dosing, was a rapid elimination phase which seemed to accelerate. The initial volume of distribution (V_d) equaled the expected plasma volume of the rabbits; the steady-state V_d (prior to the onset of the 3rd phase) was 50% larger. Estimates of pharmacokinetic parameters were the same for both dose levels. After the 2nd dose of ^{125}I-MAb, only a single phase of very rapid decline in plasma levels was observed. The rapid elimination seen in the 3rd phase after the first dose and throughout the 2nd dose suggests the presence of an antigenic response. ^{125}I was eliminated almost entirely in the urine as TCA-soluble ^{125}I after both the 1st and 2nd dosings. However, ^{125}I was eliminated more rapidly after the 2nd dose than after the 1st. These results suggest that the pharmacokinetics of ^{125}-anti-LFA-1α are similar to those of other heterologous MAb's reported in the literature.

36.11
COMPARATIVE PHARMACOKINETICS OF LORACARBEF AND CEFACLOR IN THE DOG. J. F. Quay, L. S. Finch,* S. R. Johnston,* and J. F. Stucky II*., Lilly Research Labs, Div. of Eli Lilly & Co., Indianapolis, IN 46285.

Loracarbef (LY163892, KT3777)(I), 7-[D-(aminophenylacetyl) amino]-3-chloro-8-oxo-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, is the carbacephem analogue of the oral antibiotic cefaclor (II). The antibacterial spectrum of I and II are similar but I is more stable in aqueous solution. Following oral administration of single 3.75 mg/kg doses to female mongrel dogs, I and II are efficiently absorbed with apparent bioavailabilities of 97% and 115%, respectively, as measured by the oral/IV ratio of areas under the plasma concentration verses time curves (AUC). However, the post-absorption pharmacokinetics of I and II differ in this species. Both drugs undergo renal excretion modified by saturable reabsorption that approaches completion at low plasma drug concentration. The terminal plasma half-life of II (2 hr), limited by solution instability, is much shorter than that of I (8 hr). After a single 3.75 mg/kg IV dose, 51% of I survives to be excreted in urine and 30% of II. After IV administration of graded doses of I and II AUC increases as dose increases, but in a less than proportionate manner, and the percent of dose excreted in urine increases.

36.12
MODELING OF CYCLOSPORINE (CSA) PHARMACOKINETIC DRUG INTERACTIONS IN BEAGLE DOGS. D.Duprez*, G.Powis and I.Jardine*. Mayo Clinic, Rochester, MN 55905

Previous studies conducted in our laboratory demonstrated that clinically important drug interactions involving cyclosporine (CSA) and rifampin or ketoconazole could be duplicated in the beagle dog. The mechanism underlying these interactions appears to be an alteration in the metabolism of CSA. We have further evaluated the effects of cimetidine, erythromycin and diltiazem on the pharmacokinetics (PKin) of CSA using this "model" system. Cimetidine, a known inhibitor of oxidative drug metabolism, had no effects on the PKin of i.v. CSA nor on the disposition of the 2 major metabolites of CSA, metabolites "1" (M1) and "21" (M21) in beagle dogs. These results were consistent with the reported lack of interaction of these 2 drugs in humans. Diltiazem did not effect the PKin of CSA or the disposition of M1 and M21 in beagle dogs. This may differ from the situation in humans; however, the reports of interactions have not been conclusive. Erythromycin decreased the clearance and increased the terminal half-life of CSA in beagle dogs. Further there appears to be a selective inhibition of the formation of the N-demethylated metabolite, M21. The effects of erythromycin are much less than those of ketoconazole, which is consistent with the effects seen in humans. In addition, ketoconazole is more potent than erythromycin as an inhibitor of the in vitro metabolism of CSA. Our results suggest that clinically important pharmacokinetic drug interactions involving CSA and other drugs may be modeled in beagle dogs, and further that this system may be useful in the elucidation of the mechanism underlying these interactions.
Supported by NIH grant GM34866

36.13

DOSE-DEPENDENT ELIMINATION OF ETHYLDEOXYURIDINE BY THE ISOLATED PERFUSED RAT LIVER. J.M. Joly* and W.M. Williams. University of Louisville, Louisville, KY 40292

The kinetics of elimination of the antiviral drug 5-ethyl-2'-deoxyuridine (EdUrd) by the isolated perfused rat liver were investigated. EdUrd was injected into the perfusion fluid and serial blood samples were taken for HPLC determination of EdUrd and its metabolites 5-ethyluracil (EUra) and 5-(1-hydroxyethyl)uracil (OH-EUra). Experiments were performed at three EdUrd doses and a hepatic flow of 20 ml/min. The kinetic data are shown below:

Dose,	Kinetic Constants		Plateau Conc.,μM	
μmoles	$t_{1/2}$,min	Cl, ml/min	EUra	OH-EUra
3.9 (5)	18.9 ± 0.9	5.50 ± 0.2	17.8 ± 1.4	15.5 ± 0.8
12.9 (4)	25.3 ± 0.6	3.81 ± 0.2	57.6 ± 6.1	56.4 ± 4.7
39.0 (5)	36.4 ± 1.4	2.49 ± 0.2	121 ± 5.3	82.8 ± 10

Values are mean ± SE (n)

Semilogarithmic plots of conc. vs time showed apparent first-order (mono- or biphasic exponential) disappearance of EdUrd. However, with increasing dose, there was a progressive increase in terminal $t_{1/2}$ and decrease in clearance (Cl), indicating a saturable process. After EdUrd injection, EUra and OH-EUra concs. increased steadily and approached plateau levels, which were proportional to the dose. It was concluded that the hepatic elimination of EdUrd may involve both first-order and zero-order metabolic processes.

36.14

MORPHINE ANALGESIA AND DISPOSITION FOLLOWING BURN INJURY IN THE RAT A. Kazianis*, P.F. Osgood, J.W. Kemp*, N.E. Atchison*, D.B. Carr*, & S.K. Szyfelbein* Shriners Burns Institute, Harvard Medical School, Boston, MA 02114

Time after injury appears to effect the analgesic potency and elimination half-time of morphine (MS) in burned children. In order to reduce confounding variables that may effect our clinical studies, we assessed these pharmacological parameters at 2 and 5 days post burn (DPB) using a rat model. Venously cannulated male S.D. rats (n=29) were subjected to either a scald or sham burn (50% body surface area) while anesthetized. Analgesia was assessed by tail flick latency (TFL), and analgesic potency was determined by analyzing the area under the time-course curve of the TFL response to MS. Elimination half-time was assessed by measuring MS levels over time via HPLC (electrochemical detection). TFL and MS levels were obtained before MS (8.0 mg/kg), and 5, 15, 30, 60, 120, 180 and 240 minutes after the dose. At 2 DPB the analgesic potencies and elimination half-times of sham and burn rats were not significantly different ($p > 0.05$). However, at 5 DPB both analgesic potency and elimination half-time were significantly less in burn rats ($p < 0.05$). These results suggest that the analgesic potency and rate of elimination of morphine diminish as a function of time after burn.

(Supported by SBI grant)

36.15

TOXICOKINETICS OF 2-BUTOXYETHANOL (BE): EFFECTS OF AGE, AND INHIBITION OF METABOLISM AND ELIMINATION. Burhan I. Ghanayem*, J. M. Sanders*, Ann-Marie Clark*, John Bailer*, and H. B. Matthews (SPON: L.S. Birnbaum) NIH/NIEHS, RTP, NC 27709.

Acute exposure to BE causes severe hemolytic anemia with older rats being more sensitive than younger rats. Recently, we have shown that butoxyacetic acid (BAA) is the ultimate hemolytic agent, and inhibition of alcohol dehydrogenase by pyrazole protected rats against BE-induced hemolytic anemia. In contrast, pretreatment of rats with probenecid potentiated BE-induced anemia. The kinetics of ^{14}C-BE metabolism and clearance were studied in control adult (3-4 mo) and old (12-13 mo) male F344/N rats and in rats treated with pyrazole or probenecid. Results of these studies showed no difference in the half-life (t1/2) of BE in adult and old rats. However, t1/2, area under the curve (AUC), and maximum plasma concentration (MPC) of BAA were significantly higher in older rats. This difference was more pronounced at high doses. Pretreatment of rats with pyrazole resulted in a significant increase in the t1/2 and AUC of BE. In contrast, pyrazole significantly decreased MPC of BAA, presumably due to inhibition of BE metabolism to BAA. Probenecid had no effect on the t1/2, AUC, or MPC of BE, but significantly increased the t1/2 and AUC of BAA. These data suggest that compromised urinary elimination of BAA in old rats, rather than altered metabolism, may account for their higher sensitivity. Finally, compromised BAA elimination in old rats and treatment of adult rats with probenecid is thought to involve the cation transport system in the kidney.

36.16

EFFECT OF EXERCISE ON ATROPINE PHARMACOKINETICS IN YOUNG MEN. G.H. Kamimori*, R.C. Smallridge*, V.M. O'Donnell*, D.P. Redmond*, G.L. Belenky* and H.G. Fein* (SPON: S.M. Somani). Departments of Behavioral Biology and Clinical Physiology, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100

Seven healthy males (19-32 yrs) underwent each of four separate conditions in a repeated measures design. In each trial subjects received 2.0 mg atropine sulfate (A) i.m. in the antero-lateral portion of the left thigh: at rest (T1); prior to three exercise (EX) bouts (25 min cycling at 40% VO_2 max with 5 min rest) (T2); after one EX bout (T4); and after one and prior to three EX bouts (T5). T3 was similar to T2 with substitution of a placebo. Serum samples were collected over a 12 hr period and atropine concentration was determined by RIA. Kinetic parameters were determined by PCNONLIN and statistical analysis consisted of an ANOVA with orthogonal contrasts. All trials were compared to T1. EX prior to A (T4) significantly ($P<0.05$) decreased (277 vs 232 l) Vz. EX following A (T2) compared to T1, significantly decreased t1/2 (4.2 vs 3.5 hr), Vz (278 vs 198 l), and Cl (763 vs 638 ml/min) and increased Cp (6.7 vs 12.3 ng/ml) and AUC (44.1 vs 53.1 ng/hr/ml). EX prior to and following A (T5) compared to T1, significantly decreased t1/2 (3.4 hr), Vz (182 l), and Cl (630 ml/min, P=.054) and increased Ka (.482 vs 1.1), Ke (.0015 vs .0012), Cp (14 ng/ml), and AUC (53.3 ng/hr/ml). These results demonstrate that mild to moderate exercise affected the pharmacokinetics of i.m. atropine.

ENVIRONMENTAL PHARMACOLOGY AND TOXICOLOGY

37.1

POTASSIUM CYANIDE-INDUCED RELEASE OF NEUROTRANSMITTERS IN NEURAL MODELS. E.U. Maduh, R.W. Peoples, J.L. Borowitz and G.E. Isom, Dept. of Pharmacol. & Toxicol., Sch. of Pharmacy & Pharmacal Sciences, Purdue Univ., W. Lafayette, IN 47907.

Recent findings from our laboratory demonstrate that acute cyanide poisoning elevates total brain calcium in mice, which correlates with CNS-mediated symptoms of toxicity. Additionally, KCN induces ultrastructural changes in a neuronal cell model, characterized by depletion of secretory granules. In the present study direct alteration of catecholamine secretion by KCN was investigated in a rat pheochromocytoma cell line (PC12 cell) by high performance liquid chromatography with electrochemical detection. Exposure of PC12 cells to KCN (10 mM) markedly increased levels of norepinephrine (NE) and dopamine (DA) in the cell suspension media 10-30 min following addition of cyanide. Similarly, addition of KCN (1-10 mM) to rat neocortical slices loaded with ^{3}H-NE, increased the release of ^{3}H-NE over a 10-30 min period. In both systems, the extracellular release of NE and DA decreased in absence of calcium in the perfusion medium or with prior addition of 10^{-5}M diltiazem, a calcium channel antagonist. These observations indicate cyanide elicits exocytotic release of neurotransmitters in a calcium-dependent manner and this effect may contribute to the CNS mediated symptoms of intoxication. (Supported by NIH grant ES04140).

37.2

CHANGES IN THE GLUTAMATE NEUROTRANSMITTER SYSTEM FOLLOWING POTASSIUM CYANIDE TREATMENT. M.N. Patel, B.K. Ardelt, E.U. Maduh, G.K.W. Yim, J.L. Borowitz and G.E. Isom. Dept. of Pharmacol. & Toxicol., School of Pharmacy and Pharmacal Sciences, Purdue Univ., W. Lafayette, IN 47907.

Following exposure to cyanide, a marked increase in neuronal cytosolic free calcium occurs, leading to a number of neuronal events including release of neurotransmitters. Since cyanide produces convulsions and tremors, it was of interest to study the effect of KCN on the excitotransmitter, glutamate, in a number of neuronal models. Incubation of PC12 cells with 10 mM KCN for over a 30 min period increased the spontaneous release of glutamate from 0.33 μm/10^6 cells to 0.57 μm/10^6 cells. In mouse brain cortical slices, incubation with 10 mM KCN for 30 min increased the spontaneous release of glutamate from 4.27 μm/g wet weight to 7.14 μm/g wet wt. Pretreatment of cortical slices with 10^{-5}M diltiazem, a calcium channel blocker, inhibited the cyanide-induced increase of glutamate release. Thirty min after adm of KCN (7 mg/kg, sc) to mice, cerebellar glutamate levels increased from control levels of 8.9 μm/g to 15.4 μm/g, whereas cortex glutamate levels were not significantly elevated over controls. These observations indicate KCN directly interacts with the brain glutamate system and this may contribute to the excitatory manifestations of intoxication. (Supported by PHS grants 5T32ES07039, ES04140 and S075505586).

37.3

RESEALED CARRIER ERYTHROCYTES IN CYANIDE ANTAGONISM. Elizabeth P. Cannon*, Peter Leung*, Amina Nagi*, Carie Chui*, Lynn Baxter*, and James Way. Texas A & M University, College of Medicine, College Station, Texas 77843

Cyanide intoxication can arise from ingestion of cyanide-containing foods, from occupational exposure, and from drugs with cyanide-liberating moieties. Rhodanese (thiosulfate: cyanide sulfurtransferase (E.C. 2.8.1.1)), a mitochondrial enzyme catalyzes the formation of relatively nontoxic thiocyanate from cyanide in the presence of a sulfur-donor. Encapsulation of rhodanese in red blood cells by hypotonic dialysis provides a method to protect the enzyme in vivo from degradation and delay its excretion. Rhodanese isolated and purified from bovine liver was encapsulated into murine red blood cells by hypotonic dialysis techniques. After the cells were resealed and annealed, these carrier red blood cells were injected intravenously into the mouse tail vein. Approximately twenty four hours later these mice were challenged with a sublethal dose of cyanide (5 mg/kg) and blood samples were withdrawn by cardiac puncture at predetermined times after cyanide administration. When carrier red blood cells containing rhodanese were administered, rapid conversion of cyanide to the less toxic thiocyanate occurred. Sodium thiosulfate and encapsulated rhodanese were observed to reduce blood cyanide concentration substantially. (Supported by NIH Fellowship #1-F32-ES05456 and NIEHS Grant #03951).

37.4

ROLE OF CYCLIC GMP IN CARBON MONOXIDE-INDUCED RELAXATION OF AORTIC SMOOTH MUSCLE. J.J. McGrath, H. Lin and K. Ramos, Depts. Physiology and Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

We have shown earlier that carbon monoxide (CO) relaxes aortic smooth muscle (Lin and McGrath, 1988), and that this relaxation is associated with a decrease in calcium concentration (McGrath and Lin, 1988). In these studies, the effects of CO on c-GMP levels were investigated in cultures of aortic smooth muscle cells prepared from rat aortae. Confluent cultures were placed in chambers at 37°C and treated with air enriched with 5% CO_2 (N_2 + 21% O_2) or air enriched with 5% CO_2 and 5% CO (CO + 21% O_2). Methylisobutylxanthine (50 mM) was added to the cultures to inhibit cyclic GMP phosphodiesterase. After 30 and 60 min of incubation, the following cyclic GMP concentrations were obtained:

Treatment	Cyclic GMP(pmole/mg protein) 30 min.	60 min.
N_2 + 21% O_2	0.330±0.071	0.360±0.108
CO + 21% O_2	0.489±0.178*	0.634±0.195*

*P < 0.005. Students t-test $\bar{X}$ ± SE

These results suggest that CO may interact with guanylate cyclase to stimulate the formation of c-GMP and thereby generate the intracellular signal for initiating smooth muscle relaxation. (Supported by NIH Training Grant # HL07289).

37.5

ACUTE NEPHROTOXICITY OF THE ACROLEIN-GLUTATHIONE ADDUCT IN MALE SPRAGUE-DAWLEY RATS. J. Horvath*, C. Witmer*, and G. Witz*. (SPON: R. Snyder). Joint Graduate Program in Toxicology, Rutgers Univ. and UMDNJ Robert Wood Johnson Medical School, Piscataway, NJ 08855.

Acrolein (Acr) is an alpha,beta-unsaturated aldehyde that may be found in the environment as an air pollutant or formed in vivo as a result of metabolism of xenobiotics such as cyclophosphamide and allyl alcohol. Acr may express part of its toxicity through the alkylation of vital sulfhydryl groups of cellular constituents. One reaction shown to occur in vitro is formation of the acrolein-glutathione adduct (Acr-GSH). There is some evidence that this adduct is also formed in vivo. Until recently, formation of similar adducts has been considered a detoxification reaction. Our studies were undertaken to assess the in vivo toxicity of Acr-GSH in the male Sprague-Dawley rat. Rats given this adduct as a single intra-portal vein dose of 0.5 or 1 mmol/kg showed dose-dependent increases in serum urea nitrogen, 24 hr urine volume, and 24 hr urinary glucose and protein excretion. Histologic examination of these rats revealed proximal tubule degeneration associated with increased deposition of proteinacious-staining material in the distal and collecting tubules. No toxicity was evident in rats given Acr-GSH at 0.1 mmol/kg. Pretreatment of rats with an inhibitor of alcohol dehydrogenase (pyrazole) or aldehyde dehydrogenases (disulfiram) did not alter the course of the nephrotoxic response in rats subsequently given Acr-GSH at 1 mmol/kg.

37.6

DIETARY FLAVONOID, A POTENTIAL SOURCE OF OXY-RADICALS IN THE INTESTINE. Andrew Canada, David Watkins, and Toan Nguyen*. Duke Univ. Med. Ctr. Durham, NC 27710

Approximately 1 g of flavonoids are ingested by man daily. Although these compounds have been proposed to be antioxidants, there is now evidence that some of these compounds generate toxic oxy-radicals upon hydrolysis and possibly through intracellular recycling. Our studies are designed to 1. Confirm the presence of oxyradicals upon oxidation and 2. Investigate the effect of these compounds on isolated enterocytes.

The flavonoids with either dihydroxy- (quercetin) or trihydroxy- (myricetin) configurations on the b ring were found by EPR to generate both super-oxide and hydroxyl radicals on autoxidation. Enterocytes isolated from guinea pigs were incubated with 50, 150, and 450 uM of each of three flavonoids and viability determined. After 3 h incubation, significant toxicity was seen with 450 uM of each of the compounds. Toxicity was also observed with exposures as low as 50 uM, a concentration potentially achieved In vivo. Supported by a grant from the National Foundation for Ileitis and Colitis.

37.7

NERVE GROWTH FACTOR ENHANCES RESISTANCE OF PC12 CELLS TO REACTIVE O_2 SPECIES. J. O'Brien* and F.C. Kauffman. Dept. Pharmacol. and Exper. Therap., Univ. Md. Sch. of Med., Baltimore, Md. 21201

Reactive O_2 species have been implicated in a variety of physical and chemical injuries to neural tissue. Accordingly, rat pheochromocytoma cells, PC12, were employed as a model to examine alterations in sensitivity to oxidative stress that accompany neuronal differentiation. Studies were performed using control and NGF-treated cells. Neuron-like differentiation was achieved by exposure of cells to 100 ng 7S NGF/ml for 2 days. Reactive O_2 species were generated by exposure of cells to either H_2O_2 (250 μM) or methylene blue (1 μM), which undergoes redox cycling and forms H_2O_2 via a photosensitive mechanism. Decreases in cellular ATP and phosphocreatine (PCr) induced by both agents were attenuated by treatment with NGF. For example, ATP and PCr were about 30% lower in control compared to NGF-treated cells exposed to methylene blue for 4 h (ATP:12.1 ± 2.5 vs. 30.2 ± 1.6 nmole/mg protein; PCr: 12.1 ± 1.1 vs. 18.0 ± 1.1nmol/mg protein). Similar results were noted in cells treated with H_2O_2. Destruction of H_2O_2 occurred more rapidly in cells treated with NGF and recovery of high energy phosphate stores after destruction of H_2O_2 was faster in NGF-treated cells than in control cells. Collectively, these data suggest neuronal differentiation induced by NGF is accompanied by enhanced resistance to reactive O_2 species. (Supported in part by PHS Grant HD-16596.)

37.8

ARACHIDONIC ACID METABOLITES MODULATE THE INFLAMMATORY CELL RESPONSE TO ACUTE OZONE EXPOSURE IN SUSCEPTIBLE MICE. S.R. Kleeberger*(SPON: E.W. Spannhake). The Johns Hopkins Medical Institutions, Baltimore, MD 21205

We have demonstrated that C57BL/6J inbred mice are susceptible to acute ozone-induced airway inflammation. To test the hypothesis that selective metabolites of arachidonic acid (AA) contribute to airway inflammation, we pre-treated male mice (20-23g) with a cyclooxygenase blocker (indomethacin or sodium meclofenamate) or vehicle (control) 30 min before 3 hr exposure to 2 PPM ozone. Pulmonary inflammation was assessed 6 and 24 hr after exposure by differential cell count and total protein in bronchoalveolar lavage (BAL). Six hr post-ozone, the percentage of polymorphonuclear leukocytes (PMNs) in BAL of treated mice was significantly less than vehicle controls (2.8 ± 0.7 vs 17.2 ± 1.4%, P<0.001), although total BAL protein concentrations were not different (370 ± 23 vs 348 ± 21 ug/ml, P>0.05). Twenty-four hr post-ozone, the percentage of PMNs in treated mice was significantly less than controls (3.7 ± 0.7 vs 6.3 ± 0.7%, P<0.05), and total BAL protein concentrations were still not significantly different (462 ± 21 vs 421 ± 20 ug/ml, P>0.05). To determine whether thromboxane A_2 (TxA_2) contributes to ozone-induced inflammation, mice were pre-treated with a TxA_2 synthetase inhibitor (UK-37,248) or saline vehicle and exposed to ozone. No significant differences in lavagable PMNs or protein were detected between treatment and control groups at any time point. These data suggest that cyclooxygenase metabolites of AA other than TxA_2 play a central role in the ozone-induced influx of PMNs in mice. Supported by ES03505 and HL37061.

37.9

OXIDATIVE DESULFURATION OF PARATHION BY MOUSE BRAIN IN VIVO. T.M. Soranno*, L. Woods*, and L.G. Sultatos. Dept. of Pharmacology, New Jersey Medical School, UMDNJ, Newark, N.J. 07103

Oxidative desulfuration in vitro of the insecticide parathion O,O-diethyl-O-(4-nitrophenyl) phosphorothioate (PS) with a corresponding release of atomic sulfur produces diethyl phosphoric acid, p-nitrophenol, and the toxic acetylcholinesterase inhibitor paraoxon O,O-diethyl-O-(4-nitrophenyl) phosphate (PO). Since much of the atomic sulfur produced is known to bind covalently to cytochrome P-450 and/or surrounding macromolecules, covalently bound (^{35}S) can be used as an indicator of oxidative desulfuration after administration of (^{35}S)PS in vivo and in vitro. Brains from mice treated with (^{35}S)PS (specific activity: 17 mCi/mmol; 17 mg/Kg, ip, corn oil vehicle) were dissected into 8 regions. Covalent binding of (^{35}S) to protein was identified in all brain regions as well as various peripheral tissues. Three hours after (^{35}S)PS administration, the hypothalmus and olfactory bulb had the most sulfur bound - 360 and 258 fmol/mg tissue, respectively; whereas, the midbrain had the least at 134 fmol/mg tissue. While significant regional differences exist, these findings suggest that certain cytochrome P-450-dependent monooxygenases within mouse brain have the capacity to oxidatively desulfurate and therefore probably metabolically activate this insecticide. (Supported by Grant ES04335 from NIEHS).

37.10

MPTP AND MPTP ANALOGS INDUCED CELL DEATH IN CULTURED RAT HEPATOCYTES INVOLVING THE FORMATION OF PYRIDINIUM METABOLITES. R.K. Kutty*, Y. Singh*, E. Swanson*, E. Sokoloski*, and G. Krishna. NHLBI, NIH, Bethesda, MD 20892

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) which produces a Parkinson-like syndrome in humans and monkeys, also causes cell death in cultures of rat hepatocytes. Deprenyl, a specific inhibitor of monoamine oxidase B (MAO-B), the enzyme known to catalyze the conversion of MPTP to 1-methyl-4-phenyl pyridinium ion (MPP^+) prevented the toxicity of MPTP, but not that of MPP+. When analogs of MPTP were tested, N-acetyl amino MPTP was found to be virtually non-toxic, whereas N-butyl PTP, 4'-amino MPTP, and 2'-methyl MPTP were found to be toxic, but to a lesser degree than MPTP itself. The 4'-fluoro and 4'-chloro analogs evoked toxicities similar to that of the parent compound. Deprenyl decreased the toxicities of N-butyl PTP, 4'-amino MPTP, 4'-fluoro MPTP, and 4'-chloro MPTP, but not that of the 2'-methyl analog. The conversion of all of these compounds, except the N-acetyl amino analog, into their corresponding pyridinium metabolites by liver cells was confirmed by high pressure liquid chromatography and plasma desorption mass spectrometry. Deprenyl inhibited their formation to varying degrees. Moreover, MPTP and its analogs were substrates for monoamine oxidase in rat liver mitochondria to varying degrees. These findings indicate that the formation of pyridinium metabolites is essential for expression of toxicity of the analogs.

37.11

α-AMINO-β-METHYLAMINO PROPIONIC ACID AND ALUMINUM: ENVRONMENTAL TOXINS IMPLICATED IN GUAM AMYOTROPHIC LATERAL SCLEROSIS (ALS) AND PARKINSONISM WITH DEMENTIA (PD). Etienne A. Grima*, Catherine Bergeron* and Donald R. McLachlan* (Spon: P.G. Wells) U of T, Toronto, Ontario, M5S 1A8.

Exposure to either α-amino-β-methyl- aminopropionic acid (BMAA) or aluminum (Al) has been postulated as a factor in development of Guam ALS/PD neurological syndromes. BMAA is a neurotoxic plant amino acid found in the nut of *Cycad circinalis*, a traditional Guamanian food; Al is found in high levels in the Guamanian environment and is associated with neurofibrillary degeneration. Dose responses (0.04-5.0 mg/rat brain) have been established for intra-cerebroventricular/striatal administration of D,L-BMAA in adult male Wistar rats, and the resulting lesion(s) characterized by light microscopy. Systemic exposure to ligand bound Al (Al maltol) (7 mg Al/Kg body weight/day) is acutely toxic and results in differential tissue distribution. Rats given a sublethal dose of 26 mM D,L-BMAA into the right ventricle, after a 2 day intraperitoneal pretreatment of 7 mg Al/Kg body weight, exhibited a significantly higher (p=0.05) level of Al in the ipsilateral hemisphere than controls, 24 hours later. These studies indicate BMAA and Al are potent toxins when administred alone. BMAA increases brain uptake of systemically administered Al in test animals. These results indicate that exposure to multiple environmental factors may be important for the development of the Guamanian disorders. (Support: ALS Society of Canada)

37.12

INHALATION OF SOMAN: EFFECTS ON GUINEA PIG ALVEOLAR CELL FUNCTION. Fay K. Kessler*, Rosalind B. Coles*, James E. Lyddane*, Billy R. Martin, and Richard A. Carchman. Va. Commonwealth Univ./Med. College of Va., Richmond, VA 23298

Using a "nose only" inhalation chamber, guinea pigs were exposed to volatilized soman at 0.01, 0.05, or 0.1 LD50. Alveolar cells obtained via bronchoalveolar lavage were greater than 85% alveolar macrophages (AM) exhibiting 90% cell viability under all test conditions. The functional assays performed were as follows: 1) macrophage spreading PMA (phorbol-12,13-myristate acetate) showed small but significant increases within each treatment group; 2) at 0.05 LD50 soman produced a 2-fold increase in macrophage chemotaxis to PMA; 3) soman did not change the ability of the AM to recognize a normal (3T3) or tumor (P815) target cell line using a 51Cr release assay; 4) A23187 (10-6M) produced a maximal lysosomal enzyme secretory response (31%) in both sham and soman-exposed cells; 5) phagocytosis of Ab-51Cr-sRBC was enhanced 2-fold by 0.05 LD50 soman while the other concentratioins of soman were slightly inhibitory; 6) superoxide anion production PMA stimulation was virtually identical between sham and soman-treated groups. The specificity of the inhalation of soman on important AM functions such as chemotaxis and phagocytosis warrants further investigation. Supported by DOD contract DAMD17-85-5104

37.13

EFFECTS OF WASTE SITE LEACHATE ON BENZO(A)PYRENE-DNA ADDUCT FORMATION IN RAT LUNG. S. S. Bentivegna* and C. M. Witmer* (SPON: R. Snyder). Joint Graduate Program in Toxicology, Rutgers Univ. and UMDNJ Robert Wood Johnson Medical School, Piscataway, N.J. 08855.

Benzo(a)pyrene (BP), a widespread environmental pollutant, forms adducts with DNA which can be detected using the ^{32}P-postlabeling method of Randerath et al.(PNAS 78:10 1981, Carcinogenesis 7:9 1986) with a sensitivity of greater than 1 /10^7 bases. As an approach to studying the potential effects of mixtures, we have exposed male CD-1 rats simultaneously to BP (5 mg/kg i.p.) and a waste site leachate (500 ul i.p.) and analyzed the BP-DNA adducts in lung tissue for changes in number or amount. Animals were sacrificed 48 hours after dosing and DNA isolated from lung was subject to the postlabeling procedure. Relative adduct labeling values (RAL) were calculated from the cpm in the adduct spots and are presented as the number of adducts per 10^7 bases. Animals treated with BP alone showed two adduct spots, adduct A had an RAL of $5.8x10^7$, adduct B of $5.3x10^7$. Animals treated with both BP and the leachate had two adduct spots migrating in the same region with RAL values for A of $3.7x10^7$ and for B of $5.8x10^7$. Animals treated with leachate alone did not show adducts in this system. (Supported by the NSF Industry/University Cooperative Center for Research in Hazardous and Toxic Substances and EOHSI).

37.14

COMPARISON OF BROMODEOXYURIDINE AND TRITIATED THYMIDINE FOR IN-VIVO DETERMINATION OF CELL PROLIFERATION. William E. Ribelin*, Alan G.E. Wilson*, Dorothy A. Edwards*, and Marianne L. Kitchell*. (SPON: D.H. Will). Monsanto Environ. Health Laboratory, St. Louis, MO. 63167

Tritiated thymidine (H^3) is commonly used as a marker for thymidine incorporation into DNA during the S phase of cell proliferation. The technique requires radioactive nucleoside and several weeks tissue processing. Consequently 5-bromo-2'-deoxyuridine (BrdU) uptake demonstrated by immunoperoxidase techniques is becoming increasingly used as a non-radioactive substitute for H^3. This study shows that BrdU is a more sensitive marker than H^3. Fisher and Sprague-Dawley rats were given single I.P. doses of BrdU or H^3 18 hours post-hepatectomy and killed 6 hours later. BrdU labelled 3 to 4 times more hepatocytes than H^3 depending upon the rat strain used. The "window" during which BrdU or H^3 were available during the cell cycle is obviously limited as cells in mitosis were frequently unlabelled. Compounds were studied which both damage liver and cause proliferation of liver, thus many labelled cells are endothelial, biliary or sinusoided macrophages as well as hepatocytes. Cell counting should therefore include a distinction as to the cell type being counted.

39.1

PRENATAL ADMINISTRATION OF ACTH OR NICOTINE DELAYS SEXUAL MATURATION IN FEMALE RATS. Annabell C. Segarra, Cheung Wong* and Fleur L. Strand. New York University, Biology Dept., Washington Square, New York, N.Y. 10003

Previous studies in our laboratory have indicated that prenatal nicotine or ACTH administration has a negative effect on several male reproductive parameters. We decided to investigate if the reproductive system of the female progeny was affected also. Sprague-Dawley rats were ordered 3 days pregnant and injected with either nicotine hydrogen tartrate (0.25 mg/kg) or ACTH 1-24 (10 ug/kg) twice daily from gestation day 3-21. Onset of puberty was delayed and the estrous cycle was shortened in both the nicotine and ACTH treated groups. Ovarian weight was increased in the nicotine treated group. There are several mechanisms by which ACTH might delay puberty and shorten the estrous cycle. Various investigators have reported that ACTH decreases luteinizing hormone (LH) release by inhibiting gonadotropin-releasing hormone (GnRH) or indirectly via elevated glucocorticoid levels. In contrast, nicotine may exert its effect by interacting directly with the hypothalamic-pituitary-gonadal axis or by its well known ACTH-releasing effect. This study was supported by a grant from the Council for Tobacco Research and by a MARC fellowship to A.C.S.

39.2

EFFECT OF DOSE OF PREGNANT MARES SERUM GONADOTROPIN (PMSG) ON OVARIAN STEROID AND PROSTANOID LEVELS DURING OVULATION IN PMSG/hCG-PRIMED IMMATURE RATS. Yasuhiko Higuchi* and Lawrence Espey. Trinity University, San Antonio, TX 78284

Pregnant mares serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) are commonly used in sequence to induce ovulation in immature rats. Low doses of PMSG are necessary for ovulation, but high doses inhibit the process. In the present study, PMSG was administered to different groups of 23-day-old Wistar rats in graded doses of 5, 10, 20, and 40 IU, sc. On day 25 of age, all rats were given 10 IU hCG, sc, to initiate ovulation. Doses of 0, 5, 10, 20, and 40 IU of PMSG induced ovulation rates of 0, 19.9, 64.6, 30.5, and 2.5 ova/rat, respectively. At 0, 1, 2, 4, 8, and 12 h after hCG, ovaries from each PMSG group were homogenized and extracted for assay of progesterone (P), estradiol (E), prostaglandin E (PGE), and prostaglandin F (PGF). All doses of PMSG increased P during the first 12 h of the ovulatory process, but the lower doses caused the greatest increase. There was a transient increase in E, followed by a sharp decline at 4 h after hCG, especially with the lowest dose of PMSG. The two lower doses of PMSG caused a sharp increase in both PGE and PGF by 4 h after hCG, but the two higher doses caused very little change in the prostanoids during ovulation. In conclusion, the reduction in ovulation following high dosage of PMSG is associated with a slightly lower production of ovarian P and a substantial reduction in the synthesis of ovarian prostanoids. (Supported in part by NIH Grants HD21649 and P30-HD10202).

39.3

4-MA, A POTENT INHIBITOR OF PURIFIED HUMAN PLACENTAL 3β-HYDROXYSTEROID DEHYDROGENASE/Δ⁵-ISOMERASE. V. Luu-The*, M. Takahashi* and F. Labrie, MRC Group in Molecular Endocrinology, Laval University Medical Center, Quebec, G1V 4G2, Canada.

4-MA (N,N-diethyl-4-methyl-3-oxo-4-aza-5α-androstan-17β-carboxamide), a compound synthetized by Merck Sharp & Dohme Research Laboratories, is well known as a 5α-reductase inhibitor but devoid of estrogenic and progestational activity and has no significant gonadotropin inhibitory potency. Recently, Chan et al.[1] have reported that it is a potent inhibitor of FSH-stimulated progesterone synthesis in porcine granulosa cells and suggested that it may have a direct inhibitory action mediated by androgen receptor on the induction of 3β-HSD activity. Using purified enzyme, we have shown that 4-MA has a direct effect, in vitro, on purified human placental 3β-HSD. It is indeed a potent inhibitor of enzymatic activity with a Ki of 80 nM with pregnenolone as substrate and a Ki of 220 nM when dehydroepiandrosterone is used. Trilostane [(2α,-4α,5α-17β)-4,5- epoxy-17-hydroxy-3-oxoandrostane-2-carbonitrile), a well known inhibitor of 3β-HSD activity in rats has a Ki of 54 nM in our purified system. Dixon plot analysis indicates that the two inhibitors have the same active site. The present data indicate that in addition to its activity as inhibitor of 5α-reductase activity, 4-MA may play a useful role by blocking 3β-HSD activity.

[1]Chan, W.K., C.Y. Fong, H.H. Tiong and C.H. Tan (1987) Biochem. Biophys. Res. Commun. 114: 186.

39.4

ALTERED SERUM LUTEINIZING HORMONE (LH) CONCENTRATIONS IN MALE RATS EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD). W.N. Piper, L.L. Bestervelt* and D.W. Piper* Toxicology Program, School of Public Health and Department of Pharmacology, Medical School, The University of Michigan, Ann Arbor, MI 48109-2029.

Adverse effects of TCDD reported for the male reproductive system in laboratory animals include testicular hypoplasia and impaired spermatogenesis. Previous research in our laboratory has shown that administration of TCDD to male rats resulted in decreased activity of two microsomal cytochrome P-450 dependent enzymes in the testosterone biosynthetic pathway, 17-hydroxylase and 17,20-lyase. The decreases in the activity of these two enzymes paralleled decreases observed in testicular microsomal cytochrome P-450 after administration of TCDD. Decreases in the biosynthesis of and the amount of testicular heme, as well as a decrease in the serum concentration of testosterone have also been demonstrated after TCDD exposure. Since LH regulates the synthesis of heme and testosterone in the testis, it is important to determine the early effects of TCDD on serum LH concentrations in the rat. Male Sprague-Dawley rats (220-240g) were given a single oral dose of TCDD (50ug/kg). Serum LH levels were determined by RIA on days 1,2,3 and 7 following TCDD treatment. Serum LH concentrations were decreased to 60% of controls as early as day 1 and continued to be depressed on days 2 and 3 at 50% and 60% of control values respectively. Serum LH levels returned to control levels by day 7. It appears as if TCDD is capable of causing an early depression of serum LH levels in male rats. This early depression of LH may be related to the known subsequent decreases of hemoprotein, cytochrome P-450-mediated reactions participating in testicular testosterone biosynthesis. (Supported by ES-02423 and ES-07062).

39.5

INHIBITORY EFFECT OF DIHYDROTESTOSTERONE (DHT) ON THE GROWTH OF HUMAN BREAST CANCER CELLS (ZR-75-1) IN NUDE MICE. S. Dauvois* and F. Labrie, MRC Group in Molecular Endocrinology, Laval University Medical Center, Quebec, G1V 4G2, Canada.

Androgens have been used with success in the therapy of breast cancer, but their mechanism of action is poorly understood. For the first time, we have recently shown that DHT inhibits the growth of ZR-75-1 cells in culture in the presence as well as in the absence of estrogens. In the current study, we have studied the effect of DHT on the same cells in vivo in nude mice. The mice were ovariectomized (OVX) and supplemented with estradiol (E_2) implants before inoculation of ZR-75-1 cells. After tumor development, the original implants of E_2 were removed and four groups were studied: OVX, OVX + E_2, OVX + DHT and OVX + E_2 + DHT. E_2 and DHT were released constantly from Silastic implants of appropriate size. Tumor diameters were measured every 3 days. In the group of OVX mice, tumor volume decreased by 50% after 30 to 50 days. DHT accelerated the inhibition of tumor size, a 50% tumor regression being obtained after only 15 days. With E_2 implants, on the other hand, tumor volume remained stable during the whole treatment period. DHT, given in association with E_2 caused a 50% tumor regression after 20 days. The present data show that DHT is not only able to inhibit the growth of ZR-75-1 cells as observed in vitro but that it also decreases tumor volume of developed tumors and blocks the effect of E_2.

39.6

INFLUENCE OF DIETARY FAT AND CALCIUM ON GROWTH OF HUMAN BREAST CARCINOMAS MAINTAINED IN ATHYMIC NUDE MICE. C. Welsch, C. Heil† D. O'Connor† S. Wysong† M. Gonzalez† and L. Sheffield† Michigan State Univ., E. Lansing, MI 48824

The purpose of this study was to determine whether alterations in dietary fat or calcium can influence the growth of human breast carcinoma xenografts in athymic nude mice. Two human breast carcinoma cell lines, i.e., MCF-7 (hormone responsive) and MDA-MB-231 (hormone non-responsive) were grafted s.c. as 2 mm^2 slices to mature female athymic nude mice. Commencing 5 days after grafting, the mice were divided into 5 groups and fed for 8 weeks a diet containing standard levels of calcium (0.5%) but varying in fat content, i.e., (I)5% corn oil, (II) 20% corn oil, (III)20% butter, (IV)19% beef tallow-1% corn oil and (V)19% fish(Mendaden) oil-1% corn oil. Mean (±SD) volume (mm^3) of the breast carcinomas (19-36 carcinomas/group) at termination of diet feeding for MCF-7 was (I)852 ± 120, (II) 1006 ± 182, (III)591 ± 93, (IV)589 ± 102 and (V)335 ± 48 and for MDA-MB-231 was (I)262 ± 122, (II)771 ± 381, (III)577 ± 181, (IV)418 ± 195 and (V)275 ± 49. MCF-7 and MDA-MB-231 carcinoma growth was significantly ($P<0.05$) reduced in butter, beef tallow and fish oil fed mice compared to corn oil (20%) fed mice. In additional experiments, MCF-7 and MDA-MB-231 carcinoma growth, while responding to the fat diets as described above, did not significantly respond to changes in levels of dietary calcium (1.0% vs 0.1%). (Supported by Wisconsin Milk Marketing Board research grant #III-17)

39.7

HUMAN MAMMARY GLAND PHYSIOLOGY: SYNTHESIS OF MEDIUM CHAIN FATTY ACIDS (MCFA) IS INITIATED AT PARTURITION AND IS INDEPENDENT OF THE DURATION OF PREGNANCY. M. Hamosh, M.L. Spear*, J. Bitman, D.L. Wood*, N.R. Mehta* and P. Hamosh, Georgetown University, Washington, D.C. 20007, Medical Center of Delaware, Newark, DE, 19718 and USDA, Beltsville, Md 20705

MCFA amount to 10-17% of total fatty acids (FA) in human milk (Am J Clin Nutr 38:300, 983). Animal studies show that de novo synthesis of MCFA (C8-C12) is specific to the lactating mammary gland (MG) and does not occur in other tissues, because only in the former is the activity of FA synthetase terminated by the enzyme acyl thioester hydrolase II. To assess the onset of MCFA synthesis in the human MG we studied mammary secretion obtained before term parturition, and colostrum after full term (T) preterm (PT - 31-36 wks and VPT - 26-30 wks) gestation. FA were quantitated by wide bore capillary GLC.

SPECIMEN		FATTY ACID (%)			FAT
	N	C10	C12	C14	g/dl
Prepartum Secretion (-70 to -1 day)	12	0.10	1.70	4.90	1.20
Postpartum colostrum VPT	18	0.26	3.09	5.52	2.00
PT	26	0.31	3.14	5.87	1.80
T	6	0.27	3.10	6.81	2.20
Colostrum: blood FA ratio	31	16.23	17.11	4.35	1.60

Conclusion: 1. The similar concentration of MCFA in VPT, PT and T colostrum and markedly lower levels in pepartum secretion indicates that parturition is the stimulus for MCFA synthesis in the human MG. The high colostrum: blood ratio of MCFA compared to a ratio of ~ 1.0 for FA > C14 further indicates that MCFA are synthesized in the human MG whereas Long Chain FA are provided from the circulation. (Support NIH grant HD 20833.)

39.8

POTENT INHIBITORY EFFECT OF ESTROGENS ON BREAST GROSS CYSTIC DISEASE FLUID PROTEIN-15 (GCDFP-15) mRNA LEVELS IN ZR-75-1 HUMAN BREAST CANCER CELLS. J. Simard*, A.C. Hatton*, C. Labrie*, H.F. Zhao*, L. Petitclerc* and F. Labrie. MRC Group in Molecular Endocrinology, Laval University Medical Center, Quebec, Canada, G1V 4G2.

Androgens and glucocorticoids are known to stimulate expression of GCDFP-15 in human breast tissue. The concentration of this glycoprotein is correlated with androgen receptor concentration within breast tumors. Our group recently demonstrated the antiproliferative effect of androgens and glucocorticoids in the ZR-75-1 breast cancer cell line. In order to further characterize the hormonal control of GCDFP-15 gene expression, we studied the effect of a 9-day incubation with 17β-estradiol (E_2; 1nM) alone or in combination with dihydrotestosterone (DHT; 1nM) and/or dexamethasone (DEX; 300nM) on GCDFP-15 mRNA levels. Specific cDNA probes (kindly provided by Dr. Leigh Murphy and Dr. Tom Parrish) were used for dot blot hybridization. Treatment with E_2 doubled cell growth while cell number was decreased by about 40% following exposure to DHT or DEX. GCDFP-15 mRNA levels in cells incubated with DEX or DHT were increased by 280% and 600%, respectively. Moreover, GCDFP-15 mRNA levels measured in cells incubated with E_2, E_2 + DEX, E_2 + DHT or E_2 + DEX + DHT were measured at 15, 65, 125 and 325% of those of control cells, respectively. The present data clearly demonstrate that E_2, DHT and DEX have opposite effects on GCDFP-15 gene expression and cell growth.

39.9

EFFECTS OF THYROID STIMULATING HORMONE (TSH) AND ADRENOCORTICOTROPIC HORMONE (ACTH) ON THE DEVELOPMENT OF THE EYELIDS AND INCISOR TEETH OF NEONATAL RATS.

Leo G. Parmer, Long Island Jewish Medical Center, New Hyde Park, NY 11042

Thyroid hormone, glucocorticoids, mineralocorticoids, and epidermal growth factor have similar effects in producing precocious opening of the eyelids and eruption of the incisor teeth when injected into rodents. The purpose of this study was to determine whether the newborn rat's thyroid or adrenal cortex can be stimulated by pituitary tropic hormones to produce a supply of endogenous hormones to result in the same developmental precocity. Two day old rats of the Sprague-Dawley strain were injected sc into the upper dorsum of the animal. A dose of 0.5u/gm body wt of ACTH (Parke-Davis) was given. Despite subsequent gain in body weight, it was decided to give the initial dosage daily over a 7 day period. It was determined that an average total of 28U of ACTH resulted in the eyelids opening an averge of 1.6 days and incisor teeth erupted 1.8 days earlier as compared to littermates injected with only the diluent. Up to 48U ACTH per rat over 7 days was more effective and no gross evidence of toxicity was noted. TSH, 0.015u/gm, (Thyrotropar, Armour Pharm.) given over 7 days (average total 0.9u) produced comparable effects on eyelids and teeth. These studies suggest that the neonatal rat's thyroid or adrenal cortex can be stimulated to produce a quantity of endogenous hormone that results in precocious development of eyelids and teeth. The effects are similar to previously reported injections of (exogenous) thyroid and adrenal hormone.

39.10

MATERNAL ALCOHOLISM: CORRELATION BETWEEN FETAL FUELS AND GROWTH RETARDATION. S.P. Singh, G.L. Pullen*, and A.K. Snyder*, Chicago Medical School and Veterans Administration Medical Center, North Chicago, IL 60064

We have reported reduced blood glucose (BG) and hepatic glycogen stores in the fetuses of ethanol-fed rats. The present study examines growth and energy substrates of the term offspring of rats (EF) fed ethanol (30% of caloric intake) in liquid diet and of controls given isocaloric control diet by pair-feeding (PF) or ad libitum (AF). Weight gain was reduced equally in the EF and PF mothers, but only the EF group differed ($p<0.05$) from AF controls in mean fetal body weight. Brain weight was lower ($p<0.001$) in EF fetuses (Mean ± SEM = 195 ± 2 mg) than in PF (206 ± 2 mg) or AF (209 ± 2 mg) fetuses. BG levels were nearly 20% less in EF and PF than AF dams ($p<0.05$) but fetal BG was decreased only in EF pups ($p<0.05$). EF pups had an elevated ($p<0.001$) blood lactate:pyruvate (L/P) ratio (88.6 ± 6.4 vs 52.9 ± 3.9 in PF and 53.0 ± 3.6 in AF fetuses). Circulating acetoacetate was reduced 24% ($p<0.001$) in the EF fetuses. Brain L/P ratio was increased ($p<0.01$) in EF fetuses (63.3 ± 5.6 vs 45.9 ± 6.9 in PF and 38.4 ± 2.7 in AF fetuses, n = 30 in each group). Fetal body weight correlated positively ($r = 0.4075$, $p<0.0001$) with BG but inversely with lactate ($r = -0.2802$, $p<0.005$), and β-hydroxybutyrate ($r = -0.2089$, $p<0.05$). Conclusion: Alterations in the availability of fetal fuels may play a role in fetal growth retardation due to maternal alcoholism.

39.11

THE ROLE OF THE RENAL SYMPATHETIC NERVOUS SYSTEM DURING DEVELOPMENT. Francine G. Smith* and Jean E. Robillard. Dept. of Pediatrics, Univ. of Iowa, Iowa City IA 52242

Despite growing interest in factors modulating renal sympathetic nerve activity in the adult, there is little information available on the role of the renal nerves during development. It was the purpose of this study to record renal nerve traffic in the immature animal and thus provide direct evidence for a role of the renal nerves during the perinatal period. Chronically instrumented newborn lambs (aged 3-10 days, n=10) were studied. Following surgery, at least 24 h were allowed for recovery. During the experimental period, renal nerve traffic was amplified, filtered and recorded from conscious newborn animals using standard recording apparatus and an on-line computer. Inhibition of nerve traffic was achieved with the pressor agent norepinephrine (Levophed; iv bolus). This inhibition was dose dependent (0.05-10 μg/kg) and proportional to the rise in systemic arterial pressure. Nerve traffic was able to be increased with 5-10 μg/kg acetyl choline chloride (Miochol). Our study provides the first direct recording of renal sympathetic nerve activity in the immature animal and opens a new avenue for investigating the role of the renal nerves during the critical postnatal period.

39.12

RESPONSE OF PLASMA CORTISOL TO MEAL FEEDING IN INFANT, JUVENILE, AND ADULT BABOONS. Douglas S. Lewis and Eveyln M. Jackson*. Southwest Foundation for Biomedical Research, San Antonio, Texas 78284

The effect of meal feeding on plasma cortisol concentrations was examined in 24 infant (10-12 weeks old), 12 juveniles (24 weeks old), and 12 adult (> 6 years old) baboons. Infant baboons were bottle-fed four times daily, juveniles fed chow two times daily, and adults fed chow once daily. In infant but not adult baboons plasma cortisol and ACTH significantly decreased ($p < 0.05$) within 30 min after feeding. In juvenile baboons plasma cortisol decreased 60 min after feeding. The meal-associated decrease in cortisol was dependent upon the ingestion of food since fasting and presentation of either an empty or a water bottle did not influence plasma cortisol concentrations. The meal related decrease in plasma cortisol in infants was independent of time of day. A highly synchronized ultradian cortisol rhythm with periodicity of 3 hours was observed in infant baboons: zenith prior to feeding and the nadir at 60 min following feeding. These results demonstrate meal associated changes in plasma cortisol concentrations in infant primates reared with regular bottle feedings. These rhythms begin to disappear soon after weaning and are completely absent in the adult. It is not known whether differences in cortisol response to feeding are due to age, type of diet, or to feeding schedule.

39.13

REGIONAL DISTRIBUTION OF GLUCOSE UTILIZATION IN THE DEVELOPING CHICK HEART AT 11 DAYS OF INCUBATION. David R. Kostreva and James Wood. Med. Col. WI. and VA Med. Ctr., Milwaukee, WI 53295

Glucose is known to be one of the primary metabolic substrates of the developing heart. Fourteen developing chicks at 11 days of incubation were administered a single bolus of 6 uCi of [14C] 2-deoxyglucose (specific activity 55 mC/mmol) through the vitelline vein. After 45 minutes at 37°C, the chick fetuses were rapidly removed from the eggs and frozen in -40°C isopentane. The chicks were then frozen sectioned sagittally at 20 um and placed on glass coverslips, warmed, covered with film and stored in x-ray cassettes with a set of [14C] standards for 2 days. The autoradiographs were then developed and scanned using a computerized densitometer. The autoradiographic data revealed that glucose utilization is not uniform across the walls of the heart, nor from the base of the heart to the apex. The apex uses considerably less glucose than the base, and the right ventricle and septum uses more glucose than the left ventricle. However, there are some well defined regions of the left ventriclar wall which have relatively high glucose utilization. This study demonstrates that glucose utilization by the developing heart is not uniform.

39.14

CYSTEINE PROTEINASES IN RAT ORGANS DURING PREGNANCY. John C. Rose*, Ruth Kleinfeld* and James F. Lenney. Univ. Hawaii, School of Medicine, Honolulu, HI 96822

The uterine response to pregnancy involves major structural modifications characterized by regional variations in cell growth, cytodifferentiation, cell death and cell migrations. The roles proteinases may play in these processes are of great interest.

The concentrations of two lysosomal cysteine proteinases were measured in organs of the pregnant and stimulated pseudopregnant rat. Cathepsin B (M_r 25,000) and Cathepsin J (M_r 230,000) were separated by chromatography on Fractogel TSK-HW55s and assayed fluorometrically using benzyloxycarbonyl-phe-arg-aminomethylcoumarin as substrate.

The concentration of cathepsin B in the rat uterus increased 15-30-fold during pregnancy or pseudopregnancy, while the concentration of cathepsin J increased 3-6X.

Significant increases in the levels of these proteinases were observed in the liver and lung during pregnancy, but not in the kidney. These striking increases in the concentrations of these enzymes correlate in part to the major restructuring of the endometrium in the latter half of pregnancy and to the formation of the metrial gland.

39.15

ANALYSIS OF ENDOGENOUS NON-CONJUGATED AND ACYL STEROID CONCENTRATIONS IN THE LIPOPROTEIN FRACTIONS OF HUMAN FOLLICULAR FLUID. R. Roy*, A. Bélanger*, S. Moorjani* & S. Caron* (SPON: A. DUPONT), Laval Univ. Med. Center, Quebec G1V 4G2, Canada.

Transport of cholesteryl esters within lipoproteins has been well described. Within the human follicular environment, we have found elevated levels of pregnenolone acyl esters which may also be associated with endogenous lipoproteins. To study the relationship between acyl and free steroids and the lipoproteins present in follicular fluid (FF), 4 ml volumes of fresh FF were subjected to sequential ultracentrifugation. Fractions were collected and were then measured for cholesterol and protein content. Purity of the fractions was assessed by SDS-PAGE. 1.0 ml aliquots were taken from each fraction from which steroids were extracted using ethanol and hexane. Extracts were then treated using reverse-phase liquid chromatography. Non-conjugated and ester fractions were then chromatographed on LH-20 columns (Sephadex) to isolate steroids, the concentrations of which were thereafter determined using radioimmunoassay. In FF, we observed a low concentration of very low density (VLDL) and low density (LDL) lipoproteins. High density lipoprotein (HDL) concentrations are quite similar to those of plasma. 77.4 ± 10.1% of the pregnenolone (P) present in HDL fractions was esterified while 88.4 ± 3.3% of P in the fraction containing albumin was non-conjugated. The present results indicate that transport differs between free and acyl steroids and that acylated steroids favour a lipoprotein carrier.

39.16

SYNERGISTIC EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL, FOLLICLE-STIMULATING HORMONE AND EPINEPHRINE ON LACTATE SECRETION BY SERTOLI CELLS. S.C.Newton*, A.Bartke* and L.L.Murphy* (SPON: R. Browning) Southern Illinois Univ., Carbondale, IL 62901

Lactate, a product of the Sertoli cell and a preferred substrate for developing germ cells, is an excellent marker for Sertoli cell function. Sertoli cells contain receptors for both follicle-stimulating hormone (FSH) and catecholamines and direct effects of delta-9-tetrahydrocannabinol(THC) on Sertoli cell function have been reported. In the current study, the effects of Sertoli cell lactate secretion were investigated using immature rat Sertoli cells in culture.Sertoli cells were isolated from 18 day old Sprague-Dawley rats. After 1 week in culture the cell monolayers were treated with serum free media (Dulbecco Modified Eagles Media:Hams F-12, 1:1) containing 1mM MIX and 1 µg/ml of THC alone or in various combinations. Aliquots of the media were obtained 24 hr after initiation of treatment for lactate measurement. FSH and EPI are not stimulatory using this protocol and THC had no effect on lactate production when compared to untreated controls (25.0 ± 2.7 µg lactate/µg DNA). However, FSH + 0.8 or 3.1 µg THC significantly stimulated lactate secretion (108.9 ± 10.0 and 56.0 ± 7.5µg lactate/µg DNA, respectively;$p<0.05$) as did EPI + 0.8 or 3.1 µg THC (52.4 ± 5.4 and 76.7 ± 9.2 µg lactate/µg DNA, respectively; $p<0.05$). These results indicate that THC synergizes with FSH and EPI to induce lactate secretion from Sertoli cells,but the mechanism of THC action remains unclear at this time. (Supported by DA 03875.)

39.17

ESTROGEN ALTERS STEROIDOGENESIS ACTIVATOR POLYPEPTIDE (SAP) IN RABBIT CORPUS LUTEUM. JA Holt, SJ Cok*, RV Hay*, LM Mertz*, and RC Pedersen*. Depts of Obstetrics & Gynecology, of Biochemistry & Molecular Biology, and of Pathology, University of Chicago, Chicago, IL 60637, and of Biochemistry, SUNY, Buffalo, NY 14214.

Estradiol (E2) is believed to stimulate progestin production in the rabbit corpus luteum (CL) via induction in vivo (AJP 243:E188,1982). We tested whether expression of SAP (J Ster Biochem 27:731, 1987) is E2-sensitive in this model. The CL at day 9 of pseudopregnancy were E2-stimulated (E2-stim) or E2-deprived (E2-depr) using silastic capsules placed s.c. in vivo. As expected, over the 48 hr treatment period, serum progesterone (the principal source of which is the CL)

treatment	CL	adrenal	kidney	liver
E2-stim	1.2 ± 0.3*	2.9 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
E2-depr	2.9 ± 0.7*	3.0 ± 0.1	0.2 ± 0.1	0.3 ± 0.1

mean ± sem SAP ng/mg protein; * = different by $p < 0.05$, ANOV

dropped from 50 ± 5 to 1 ± 1 ng/mL (x ± sem) in E2-depri but remained elevated in E2-stim rabbits. SAP, measured by RIA on acid extracts of whole homogenates of selected tissues collected on day 11 of pseudopregnancy, was lower in the CL from E2-stim than from E2-depr rabbits ($p < 0.05$). Conversely, Western blot analysis of CL extracts with an antiserum directed against a C-terminal region of glucose-regulated protein-78 demonstrated higher levels of a small cross-reactive protein, but lower levels of a large cross-reactive protein in E2-stim CL than in E2-depr CL. SAP levels in the adrenals, kidneys, and liver were unaffected by E2. These data are consistent with a functional relationship between SAP and E2-sensitive progestin production in the rabbit CL. [CA27476, GM07183, AHA85-805, AM18141].

40.1

VENTRAL MEDULLARY SURFACE (VMS) INPUTS CONTRIBUTE TO THE APPEARANCE OF MAYER WAVES. M.A. Haxhiu*, E.C. Deal, E. van Lunteren, and N.S. Cherniack. Case Western Reserve University, Cleveland, OH. 44106

Increased discharge from peripheral chemoreceptors may induce the appearance of nonrespiratory related slow regular oscillations in arterial blood pressure (ABP) that occur at the rate of about 20-40 s (Mayer waves). Recently we showed that structures located near the VMS have profound effects on respiratory modulation of sympathetic activity (van Lunteren et al., Am. J. Physiol. 252:R1032-R1038, 1987). In the present work we examined the contribution of VMS inputs in production of Mayer waves. Experiments were performed in 18 chloralose anesthetized and paralyzed cats hyperventilated to phrenic neural apnea. Application of pledgets containing N-methyl-D-aspartic acid (NMDA), a synthetic excitatory amino acid, to the intermediate area of VMS raised ABP (32±6%, $\bar{x}$±SEM) and had variable effects on heart rate. Furthermore, NMDA caused instability of ABP manifested as "Mayer waves". The cardiovascular effects of NMDA were blocked by the topical administration of lidocaine (2%) on the VMS. In addition, lidocaine diminished Mayer waves induced by an increase of peripheral chemoreceptor discharge. These results suggest that VMS structures may produce slow regular nonrespiratory related oscillations of ABP, and modulate the fluctuations caused by stimulation of peripheral chemoreceptors. Support: HL-25830, HL-01600, HL-38701

40.2

SELECTIVE SENSITIZATION OF REFLEX BRADYCARDIA BY GLUTAMATE RECEPTORS IN ROSTRAL VENTROLATERAL MEDULLA OF CHLORALOSE-ANESTHETIZED RATS. Xin Zhang*, A-R. A. Abdel-Rahman and W. R. Wooles. East Carolina University School of Medicine, Greenville, NC 27858.

Glutamate (G) microinjection caused a dose-related and correlated increases in sympathetic efferent discharge (SED) and mean arterial pressure (MAP) and decreases in heart rate (HR). The glutamate antagonist, glutamate diethyl ester, abolished all 3 effects of G suggesting involvement of specific G receptors in these effects. Sinoaortic denervation and cardiac autonomic blockade by atropine (A) and propranolol (P) abolished the decrease in HR without influencing peak increases in SED and MAP evoked by G suggesting the bradycardia was baroreflex mediated. Further, the decrease in HR was attenuated by P and was converted to a small but significant increase following A pretreatment suggesting a major involvement of the vagal component in the response. The decrease in HR for any given increase in MAP evoked by G was significantly greater than that evoked by phenylephrine (PE) and the baroreflex slope evoked by PE was significantly greater after G. These findings suggest G receptors in the RVL sensitize the central mediation of the baroreceptor HR response. This effect is G-mediated since equal volume of vehicle had no effect. The data also suggest the effect of G is selective to the HR response since baroreflex control of SED was not influenced by G.

40.3

REGIONAL HEMODYNAMIC EFFECTS PRODUCED FOLLOWING MEDULLARY VENTRAL SURFACE APPLICATION OF L-GLUTAMIC ACID IN THE CAT. Philip J. Gatti* and V. John Massari. Howard University College of Medicine, Washington, D.C. 20059.

It is known that application of the neuroexcitant amino acid L-glutamic acid to the intermediate area of the cat ventrolateral medulla evokes an increase in arterial pressure. The aim of the present study was to determine the changes in regional vascular resistance which occur following the application of this amino acid to this site. Experiments were performed in chloralose anesthetized artificially respired cats. Blow flow (ml/min) was measured in the renal and femoral arteries using a transit time ultrasonic flowmeter. Regional resistance was calculated by dividing diastolic aortic pressure by the diastolic flow in each particular vessel. Results are expressed as percent changes in resistance from control. Bilateral application of L-glutamic acid (5 µl of a 1 M solution) produced an immediate rise in resistance in the renal artery (13 ± 4.6%, n=6) and a rise in femoral resistance (38 ± 7.8%, n=6). These effects lasted no more than 10-15 min. The difference between the rise in resistance in these two vessels was statistically significant ($p < .05$, Student's unpaired t-test). These data suggest that supraspinal control of femoral resistance may be greater than renal resistance. (Supported by the American Heart Association/Nation's Capital Affiliate).

40.4

NEUROKININ A AND NEUROKININ B-LIKE IMMUNOREACTIVITIES ARE PRESENT NEAR THE SURFACE OF THE VENTROLATERAL MEDULLA. E.C. Deal, Jr., T. Dick, A. Thomas, N.S. Cherniack, and M.A. Haxhiu. Case Western Reserve University, Cleveland, OH. 44106

The present study examined the distribution of two new mammalian tachykinins: Neurokinin A (NKA) and Neurokinin B (NKB)-like immunoreactivity in the ventral medulla of 7 normal, colchicine untreated cats. Findings were compared to the distribution of Substance P-like immunoreactivity in the same animals. Mapping of the three mammalian tachykinins was done between the caudal border of the corpus trapezoideum and the caudal bundles of the hypoglossal root. Using a modified technique of Sternberger and applying polyclonal antibodies, SP, NKA and NKB-like immunoreactive neurons were found in the ventral part of medulla in close relation to the surface. These large, multipolar and more sparse cells were situated 2-4 mm from the midline and anterior to inferior olivary nucleus, which contained small, unipolar and dense tachykinin-like immunoreactive cells. Furthermore, SP neurons were seen in the nucleus interfascicularis hypoglossi, pars ventralis. NKA and NKB-like immunoreactive cells had the same distribution as SP-like immunoreactive cells, but were slightly more frequent. SP, NKA and NKB-reactive fibers which extended to the ventral surface of medulla were also visible in caudal, as well as in more rostral levels, where no immunoreactive cells were found. These results suggest the possible importance of NKA and NKB, besides SP neurons in central control of respiration and airway tone. Support: VA Merit Review, HL-25830, HL-39921

40.5

CONTRIBUTION OF THE AMYGDALA AND ADRENAL MEDULLA IN STRESS-INDUCED CARDIOVASCULAR RESPONSES. Rod Casto, Raymond Henry, and Morton P. Printz. University of California at San Diego, La Lolla, CA 92093

Cardiovascular and behavioral responses to alerting stimuli (100 msec air puff) are exaggerated in spontaneously hypertensive rats (SHR). Wistar-Kyoto (WKY) exhibit bradycardia [-42 ± 8 bpm] accompanying a startle-induced pressor response, while SHR demonstrate tachycardia [20 ± 5 bpm]. The contribution of central cardiovascular centers such as the amygdala in this response is not well-understood. Radio-frequency lesions of the amygdaloid complex were performed prior to testing rats in a classical startle paradigm. Amygdala ablation had no effect on the magnitude or time course of the cardiovascular response. However, induced-motor activity was abolished. Sympathetic nervous activity appears enhanced in SHR and may extend to adrenal medullary function. To probe the sympatho-adrenal axis, we performed adrenal enucleation (Adx) three days prior to testing. Behavioral and pressor responses in both WKY and SHR were unchanged by Adx. In WKY, Adx produced and exaggerated bradycardia [-89 + 23 bpm]. The absence of bradycardia in SHR was maintained after Adx. We conclude that the amygdaloid complex does not play a major role in setting the level of tonic or phasic responsivity in this paradigm while adrenal medullary function has a clearly evident role in the cardiac response to novel stressors in WKY by not SHR.

40.6

CENTRAL CARDIOVASCULAR ACTIONS OF CORTICOTROPIN-RELEASING FACTOR IN SAFFAN-ANESTHETIZED RATS. L.A. Fisher and J.M. Overton. Dept. of Pharmacology, College of Medicine, Univ. of Arizona, Tucson, AZ 85724

Corticotropin-releasing factor (CRF), a 41-residue neuropeptide, acts within the central nervous system (CNS) to produce an integrated pattern of endocrine, autonomic, visceral and behavioral responses in conscious animals. Administration of CRF into the CNS elicits simultaneous elevations of arterial pressure (AP) and heart rate (HR) coincident with marked locomotor activation. To determine whether CRF-induced elevations of AP and HR are secondary to locomotor activation, cardiovascular responses to intracerebroventricular (icv) administration of CRF were compared in unrestrained, chronically instrumented rats receiving continuous intravenous (iv) infusions of saline or the anesthetic agent, Saffan (Glaxovet). Saline infusion (10 µl/min) did not alter resting AP or HR. In saline-infused rats, treatment with CRF (0.15 nmol) elicited immediate (within 5 min) and sustained (at least 60 min) elevations of HR (80-100 bpm) and AP (15-20 mm Hg) accompanied by gross locomotor activation, i.e., exploratory behavior, burrowing, grooming and chewing. Saffan infusion (120 µg/min) reduced resting AP (5-10 mm Hg), increased resting HR (10-20 bpm) and induced torpor. In Saffan-infused rats, CRF treatment elevated HR (60-100 bpm), but had variable effects on AP (increases, decreases and biphasic changes) and did not alter behavior. These results suggest that CRF-induced tachycardia is not secondary to behavioral or locomotor activation.

40.7

SIX-WEEK PAIR-FEEDING (PF) OF SHAM-OPERATED CONTROLS (CON) TO RATS WITH DORSOMEDIAL HYPOTHALAMIC LESIONS (DMNL): SOMATIC AND ORGAN GROWTH, EFFICIENCY OF FOOD UTILIZATION (EFU) AND BODY COMPOSITION. L. Bernardis, L. Bellinger, M. Kodis* and M.J. Feldman*. VAMC and SUNY/Buffalo, 14215 and Baylor Coll. Dent., Dallas 75246.

12 day-long PF of CON to DMNL has been shown to cause somatic and metabolic responses indicative of poorer coping by CON-PF. Weanling male Sprague-Dawley rats received DMNL. One CON group was fed ad libitum (CON-ADLIB), another CON group was PF to DMNL (CON-PF) for 6 post-op (POP) wks. DMNL vs. CON-ADLIB were hypophagic and had reduced body weight (BW) and linear growth but normal EFU and carcass fat (Lee Index). However, CON-PF weighed less than DMNL and CON-ADLIB throughout 6 wks. Linear growth was reduced in CON-PF vs DMNL at 6 wks but not at 3 wks POP. Carcass fat was reduced in CON-PF vs DMNL and vs CON-ADLIB. Absolute weight of liver, kidneys, epididymal fat pads (PADS) and spleen was lower in CON-PF vs DMNL (both groups had smaller organs than CON-ADLIB). Testes, adrenals and brain were comparable in CON-PF and DMNL but smaller in both groups vs CON-ADLIB. Organ growth/metabolic size of liver, pads and kidneys was comparable in DMNL and CON-PF whereas testes and brain were heavier and spleen lighter in CON-PF vs DMNL rats. Extension of the PF time exaggerates the poorer response of CON-PF vs DMNL and confirms past findings that lower food intake and BW may be "normal" for the DMNL rat. Supported by VA and Baylor funds.

CATECHOLAMINES

41.1

ALPHA-ADRENERGIC AGONIST PROPERTIES OF RING 2- AND 6-FLUORINATED ANALOGS OF EPINEPHRINE. Dennis R. Feller, Adeboye Adejare*, Urusa Intrasuksri*, Yangmee Shin* and Kenneth L. Kirk*. Coll. Pharm., The Ohio State Univ., Columbus, OH 43210, and NIDDK/NIH, Bethesda, MD 20892.

The effects of 2-fluoro- and 6-fluoroepinephrine [2-FE and 6-FE, respectively] and (-)-epinephrine (E) were examined in rat thoracic aorta and human platelets as representative $alpha_1$- and $alpha_2$-adrenoceptor systems. The comparative rank order potencies, EC_{50}'s for the compounds as agonists of human platelet primary wave aggregation and rat aorta contraction were E (0.53 μM) = 6-FE (0.55 μM) > 2-FE (3.35 μM) and E (0.13 μM) ≥ 6-FE (0.31 μM) > 2-FE (0.69 μM), respectively. Aggregation responses to these agents were blocked by yohimbine and phentolamine, but not by prazosin. The potencies of E and 6-FE, and rank orders of potency for these compounds were the same in the two systems. 2-Fluoro ring substitution of E, as in 2-FE, gave significant ($P < 0.05$) decreases of 6.1- and 2.2-fold in agonist potency as compared to 6-FE. Thus, fluorine atom ring substitution of E at the 2-position, but not at the 6-position, confers a negative effect on the biological activity of the parent drug in $alpha_1$- and $alpha_2$-adrenoceptor systems. These fluorine substituent effects with E are similar to those previously reported with norepinephrine on α-adrenoreceptors (J. Med. Chem. 22: 1493, 1979). [Supported in part by NIH GM 29358].

41.2

SYNTHESIS AND ADRENERGIC PROPERTIES OF 2- AND 6--FLUOROEPINEPHRINE AND 2,5- AND 2,6-DIFLUORONOREPINEPHRINE. C. R. Creveling, F. Gusovsky*, G. Chen*, A. Adejare*, K. L. Kirk*, and J. W. Daly. NIH, Bethesda MD. 20892

The synthesis of 2-, 5- and 6-fluoronorepinephrines (FNE) was reported in 1979 and led to the unexpected observation that, depending upon the site of ring fluorination, the adrenergic specificity of norepinephrine (NE) was markedly altered. In brief, the adrenergic activity of NE was changed so that 2-FNE was a relatively specific ß-adrenergic agonist and 6-FNE predominantly an α-agonist while 5-FNE retained both the α- and ß-adrenergic activity of NE. Most recently we have reported the synthesis and adrenergic properties of 2- and 6-fluoroepinephrines (2-FE, 6-FE). Fluorine subsitution of E on the 2- and 6-carbon of the aromatic ring alters the selectivity of E towards α- and ß-adrenergic receptors, in the manner seen with NE. Unlike the FNEs however, fluorine substitution of E also markedly increases potency at either α- or ß-adrenergic receptors. The fundamental mechanism(s) for the observed effects of ring-fluorination on the biological properties of these adrenergic agonists is still not fully understood. In an effort to illucidate the mechanism for the "fluorine effect" we have prepared the 2,5- and 2,6-difluoroderivatives of NE. The affinity of 2,6-diFNE (Ki = 0.75μM vs clonidine) for α_2-adrenoceptors is similar to the affinity observed for 2-FNE (Ki = 0.73μM) rather than 6-FNE (Ki = 0.012μM). Thus fluorine on the 2 carbon appears to negate the induction of α-selectivity of fluorine on carbon 6.

41.3

RELEASE OF CATECHOLAMINES FROM PERFUSED RAT ADRENAL GLANDS AND SLICES: EFFECTS OF CHRONIC HYPOGLYCEMIC STRESS. P.R. VULLIET, J.P. MITCHELL*, D.A. CARBONARO*, AND F.L. HALL*. UNIVERSITY OF CALIFORNIA, DAVIS CA 95616

Long term stress is known to produce a wide variety of biochemical changes within the mammalian adrenal medulla, including changes in the amount of epinephrine released by a standard dose of acetylcholine. The present study was designed to investigate the morphology of adrenal chromaffin cells in rats subjected to chronic hypoglycemia induced by long acting insulin, and to assess this morphology in terms of associated changes in catecholamine content and release. Surgically isolated, perfused adrenal glands and adrenal slice preparations were utilized to characterize the functional release of catecholamics from the rat adrenal medulla. Pretreatment with long acting insulin resulted in a selective depletion of epinephrine stores and acetylcholine-mediated epinephrine release, but did not appear to significantly alter either the levels or the release of norepinephrine. The biochemical effects of long acting insulin persisted for several days after termination of the treatment, exhibiting a gradual recovery over a period of approximately 5 days. Electron microscopic examination of the adrenal medulla revealed a progressive degranulation and vacuolization of numerous chromaffin cells followed by a gradual recovery toward the morphology of control cells. The results of these studies confirm that alterations in adrenomedullary reactivity following periods of chronic stress may be partially accounted for by changes occurring at the level of the adrenal medulla itself, independent of both the central nervous system and the sympathetic neurons innervating the adrenal gland.

41.4

MECHANISMS OF DOPAMINE REGULATION IN THE STRIATUM: CATABOLISM AND NEW SYNTHESIS VERSUS REUPTAKE AND RE-RELEASE. John W. Commissiong, Dept. of Physiol., McGill Univ., 3655 Drummond St., Montreal, Canada H3G 1Y6.

In conditions of minimum stress, electrical stimulation of the nigrostriatal, dopaminergic fibers does not cause an increase in the synthesis and metabolism of dopamine (DA). In conditions of normal stress, however, synthesis and metabolism of DA are increased by electrical stimulation. Infusion of TTX (3.0×10^{-7} M, 1.5 μl, 1.0 μl min^{-1}) into the medial forebrain bundle, causes a marked increase in the synthesis and metabolism of striatal DA. Therefore, paradoxically, under conditions in which DA release is increased, DA synthesis and metabolism are not increased, and under conditions in which DA release is reduced (TTX), the synthesis and metabolism of DA are increased. It is concluded that under physiological conditions, the principal mechanism of DA conservation is by reuptake and re-release. The synthesis and metabolism of DA seem to be increased under a wide variety of conditions, many of which may not be related to functional neurotransmission. Therefore, it follows that DOPAC and HVA are products of intracellular DA metabolism, and are not indicative of functional dopaminergic neurotransmission. High concentrations of intracellular monoamines may, however, affect neuronal function. However, the mechanisms of these postulated intraneuronal effects are not at present understood.

41.5

THE EFFECTS OF CHLORIDE ION ON THE ACCUMULATION OF ^{3}H-DOPAMINE BY SYNAPTIC VESICLES PURIFIED FROM RAT STRIATUM. Norman Weiner, Jeffrey K. Disbrow, Ellen M. Barnes and Joseph M. Masserano. Department of Pharmacology, University of Colorado Health Science Center, Denver, CO 80262.

The effects of Cl^- ion on the transport of ^{3}H-dopamine (^{3}H-DA) into synaptic vesicles purified from rat striatum has been evaluated. ^{3}H-DA uptake was abolished in synaptic vesicles incubated in vitro with reserpine (7 nM) and in vesicle preparations obtained from rats pretreated with reserpine (5 mg/kg, i.p., 18 hr). The inclusion of 10 mM Cl^- ion in the incubation medium produced an increase in ^{3}H-DA accumulation from 1.8 nmol/mg to 3.7 nmol/mg. Kinetic analysis of ^{3}H-DA uptake at 1.5 min indicated that uptake was saturated at 5 µM in the presence and absence of Cl^- ion. Uptake of ^{3}H-DA in the presence and absence of Cl^- ion was temperature sensitive, ATP dependent and was decreased by drugs that inhibit ATPase activity, quercetin (50 µM), DCCD (50 µM), and NEM (200 µM) or by drugs that disrupt the electrochemical proton gradient, FCCP (1µM). Compounds which influence Cl^- ion permeability across biological membranes were also examined for their effect on the steady-state accumulation of ^{3}H-DA. SCN^- ion (10 mM), SITS (100 µM) and duramycin (2 µg/ml) selectively inhibited (90%) the stimulatory effect of Cl^- ion on ^{3}H-DA uptake. SCN^- ion (40 mM), SITS (500 µM) and duramycin (10 µg/ml) inhibited ^{3}H-DA uptake in the absence of Cl^- ion. These data support a role of Cl^- ion as a regulator of the transmembrane electrochemical proton gradient of rat striatal vesicles. Supported by USPHS grants NS09199 and NS07927.

41.6

NICOTINIC RECEPTOR REGULATION OF [^{3}H]NOREPINEPHRINE UPTAKE IN CULTURED BOVINE ADRENAL MEDULLARY CELLS. D.B. McKay*, W.B. Hunter*, D. Del Paggio* and J.L. Burnside* (SPON: P.N. Patil). The Ohio State University, Columbus, OH 43210.

Cultured bovine adrenal medullary (BAM) cells possess a high affinity catecholamine transport system which is similar to the uptake$_1$ system of sympathetic neurons. We have found that [^{3}H]norepinephrine ([^{3}H]NE) uptake in cultured BAM cells is inhibited by nicotine. These studies were designed to investigate the mechanisms responsible for nicotine's inhibitory actions on adrenal catecholamine uptake. Nicotine caused a concentration-dependent inhibition of [^{3}H]NE uptake in BAM cells which paralleled nicotine's effects on release. Hexamethonium prevented nicotine's action on release and antagonized nicotine's action on uptake. Reserpine pretreatment (72 hr) or omission of calcium did not prevent nicotine's inhibitory effects on uptake, but did reduce or inhibit catecholamine release. Depolarizing concentrations of K^+ also produced a concentration-dependent inhibition of [^{3}H]NE uptake which paralleled the effects of K^+ on release. Other secretagogues (e.g., histamine, veratridine, barium) also inhibited [^{3}H]NE uptake in BAM cells. These results suggest that the high affinity catecholamine uptake system of cultured BAM cells (and, possibly, of sympathetic neurons) may be regulated by processes involved with stimulus-secretion coupling which are independent of release. (PHS NS24814).

41.7

EFFECTS OF CARBIDOPA ON THE PERIPHERAL AND CEREBRAL METABOLISM OF 6-[^{18}F]-L-FLUORODOPA IN RODENTS. W.P. Melega*, A. Luxen*, M. Perlmutter*, M.E. Phelps* and J.R. Barrio*(SPON: R. Pechnick). Division of Nuclear Medicine, UCLA School of Medicine. Los Angeles,CA 90024

6-[^{18}F]-L-fluorodopa (FD) is a probe used for the in vivo assessment of presynaptic dopaminergic function with positron emission tomography(PET). In this work we have characterized the kinetics and metabolism of FD in the presence of carbidopa, a peripheral aromatic aminoacid decarboxylase inhibitor. Sprague-Dawley rats (275-325 g) were pretreated with CD (5 mg/kg s.c.) 60 min prior to an FD injection (800 µCi/kg) via jugular cannulae. Plasma samples were obtained at 3, 10 and 30 min after the FD injection. The animals were sacrificed by decapitation at 30 min; the striatum was removed and prepared for HPLC analysis (C-18 reverse phase column, 80% 0.1M Na_2HPO_4, 2.6 mM OSA, 0.1mM EDTA; 20% MeOH, pH 3.3); the FD metabolites were identified by comparison with authentic standards. CD pretreatment increased the plasma levels of FD by150%, and 3-O-methylfluorodopa (3OMFD) by 350%; striatal levels of FD were unchanged, but 3OMFD increased 400% and fluorodopamine (FDA) increased 500%. CD inhibited peripheral metabolism of FD to FDA, fluorohomovanillic acid (FHVA), and their corresponding sulfates. In contrast,significant amounts of sulfoconjugated FDA and FHVA were detected in plasma (10% of total) and striatum (20% of total) from animals without CD pretreatment. Thus, the total radioactivity arterial input function obtained with CD can be resolved into FD (3 min, 80%; 10 min,61% and 30 min, 23% of total)and 3OMFD (3 min,20%; 10 min, 38%; and 30 min, 74% of total) components for tracer kinetic modeling of PET studies. Supported by DOE contract DE-ACO3-76-SF0012, NIMH grant RO1-MH-37916-02, NIH grant RO1-NS-20867-08,and PO1-NS-15654.

41.8

CARDIOVASCULAR RESPONSES TO CENTRAL ADMINISTRATION OF EPINEPHRINE (EPI) AND NOREPINEPHRINE (NE) IN THREE STRAINS OF RATS Yang Zhao* and Raymond F. Orzechowski, Phila. College of Pharmacy & Science, Philadelphia, PA 19104

Systemic arterial blood pressure and pulse rate were measured in conscious unrestrained rats prior to and up to 2 hours after intracerebroventricular (ICV) administration of EPI or NE, 20ug total dose in a volume of 1 ul. Normotensive Sprague-Dawley and Wistar-Kyoto rats, and the hypertensive SHR strain were studied. Catecholamines were delivered into the right lateral ventricle, via an indwelling 30 ga. stainless steel cannula, at 3 different rates: 20ug dose given in 10, 40, or 160 sec. Blood pressure and pulse rate were obtained from a chronically implanted aortic cannula; analog pressure signals were monitored on a Grass Model 7D polygraph and digitized for storage/data analysis on an IBM PC-XT computer. NE produced greater pressor responses than EPI in all three strains of rats. EPI typically elicited biphasic pressure changes (immediate increase followed by longer lasting decrease). Slower rates of EPI infusion (40 or 160 sec) resulted in more pronounced secondary hypotension; when injection time was 10 sec, little or no depressor response was noted. Our data illustrate the variation in cardiovascular responses to centrally-infused EPI and NE when the same total dose is given at different infusion rates.

41.9

ALPHA$_2$-ADRENERGIC REGULATION OF PLASMA CATECHOLAMINES IN GLUCOCORTICOID HYPERTENSION Katalin Szemeredi*, Gyorgy Bagdy*, Irwin J. Kopin and David S. Goldstein* (Spon: H.R. Keiser) NIH, Bethesda MD 20892

Alpha-2 adrenoceptor regulation of sympathoadrenomedullary function was assessed during glucocorticoid-induced hypertension in conscious, unrestrained Wistar-Kyoto rats. Cortisol administered for seven days using a subcutaneous resevoir pump produced hypertension, decreased body weight and suppressed sympathoadrenomedullary activity as indicated by plasma norepinephrine (NE) and epinephrine (E). Systemic iv administration of the alpha-2 adrenoceptor antagonist, yohimbine, increased plasma levels of NE and of dihydroxyphenylglycol (DHPG), the main intraneuronal metabolite of NE. Cortisol treatment abolished these responses to yohimbine. Systemic iv administration of clonidine, an agonist at alpha-2 adrenoceptors, decreased plasma NE, and cortisol treatment did not affect the proportionate decrease in plasma NE after clonidine. Cortisol treatment also did not affect inhibitory effects of clonidine on the reflexive catecholamine responses to nitroprusside-induced hypotension. The results suggest that hypercortisolemia indirectly interferes with the function of central neural alpha-2 adrenoceptors regulating sympathoneural outflow.

41.10

DIFFERENTIAL EFFECTS OF NOREPINEPHRINE ON PHOSPHOINOSITOL TURNOVER IN THE BRAINS OF LONG SLEEP (LS) AND SHORT SLEEP (SS) MICE. Eric A. Weiner, Thomas A. French and Joseph M. Masserano. Department of Pharmacology, University of Colorado Health Science Center, Denver, CO 80262.

We have previously reported that the ethanol-induced sleep times of the LS mice decreased and those of the SS mice increased following the intraventricular injection of norepinephrine (NE) (J. Pharm. Exp. Ther. 221: 404, 1982). These mice were selectively bred for differences in CNS sensitivity to ethanol with LS mice exhibiting much greater sensitivity to hypnotic doses of ethanol than SS mice. In the present study, the effects of NE on phosphoinositol (PI) turnover were evaluated in five brain regions of the LS and SS mice. PI turnover was measured by the method of Berridge et al (Biochem. J. 212: 473, 1983) utilizing ^{3}H-inositol. ^{3}H-PI turnover in non-stimulated tissues was significantly higher in the SS mice as compared to the LS mice in the locus ceruleus (22%), hypothalamus (22%), cerebellum (47%), hippocampus (56%) and cortex (233%). Likewise, the stimulation of ^{3}H-PI turnover by 10^{-4}M NE was also significantly higher in the SS mice as compared to the LS mice in the locus ceruleus (38%), cerebellum (109%), hippocampus (83%) and cortex (130%), but not in the hypothalamus (4%). Preliminary studies indicate that 80 mM ethanol has no effect on either basal or NE-stimulated ^{3}H-PI turnover in the hypothalamus and cerebellum of LS and SS mice. Additional studies are in progress to evaluate the effects of ethanol on NE-stimulated PI turnover. Supported by USPHS grant AA03527.

42.1

PHARMACOLOGICAL AND BIOCHEMICAL EFFECTS OF SZL49, AN ALPHA-1 ADRENOCEPTOR PROBE. John W. Kusiak*, Josef Pitha*, and Michael T. Piascik. (SPON: Stephen P. Baker). *Macromolec. Chem., NIH, NIA, Baltimore, MD 21224 and Dept. Pharmacol., Univ. Kentucky, College of Medicine, Lexington, KY 40536

1-(4-Amino-6,7-dimethoxy-2-quinazoliny1)-4-(2-bicyclo-[2.2.2]octa-2,5-diene-2-carbonyl)piperazine (SZL49) was used to characterize alpha-1 adrenoceptors in rat tissues. In binding studies, SZL49 exhibited high affinity for brain cortical (BC) ^{3}H-Prazosin binding sites and irreversibly blocked ~50% of these sites. It had no irreversible effect on ^{3}H-Dihydroalprenolol or ^{3}H-Yohimbine binding and blocked only ~15% of ^{3}H-Spiperone binding suggesting specificity at alpha-1 sites. In comparing SZL49 (10^{-7}M) and chloroethylclonidine (CEC) (10^{-5}M) (alpha-1 and alpha-2 selective), both irreversibly blocked ^{125}I-HEAT binding in BC membranes about 30% in high ionic strength buffer, but in 10mM HEPES, pH 7.4, CEC (10^{-6}M) blocked ~80% and SZL49 (10^{-7}M) about 36% of receptors. In either buffer, (-)Norepinephrine (NOR) was able to prevent completely the irreversible blockade of CEC. (-)NOR and (-)Epinephrine had little effect on the SZL49 blockade. In functional studies, SZL49 preincubated with aortic rings right shifted the phenylephrine (PE) dose-contractile response after a 1.5 and 4 hr washout. At low [PE] a plateau in the response was noted. At high [PE] a maximal response was obtained. SZL49 may be a useful alpha-1 subtype selective ligand.

42.2

CHANGES IN VASCULAR α_1-RECEPTOR RESERVE IN PACING-INDUCED HEART FAILURE. Christine Forster and Paul Armstrong* University of Toronto, Toronto, Ontario, M5B 1W8.

The dorsal pedal artery (a) and saphenous vein (v) show increased responsiveness to α_1-agonists from dogs with pacing-induced heart failure (HF). Accordingly we determined the α_1-receptor reserve (RR) using fractional inactivation in a and v from 4 dogs before and at HF. Concentration-effect curves to phenylephrine (PE) were constructed in the absence and presence of phenoxybenzamine (concentration causing a reduction in PE maximum by 25-30%). Individual EC_{50}'s (uM), dissociation constants (K_A:uM), % receptors occupied to give 50% response (%) and RR were determined. Results were expressed as $\bar{x}$ with 95% confidence limits or ±sem where appropriate and are shown in the table for a and v. *P<.05 before vs at HF

	Before HF (n=7 rings)		At HF (n=8 rings)	
	a	v	a	v
EC_{50}	4.6(2.8-7.6)	3.0(2.0-4.2)	1.9(1.3-2.6)*	1.5(1.2- 1.6)*
K_A	14 (9-24)	22 (15-34)	9 (5-12)	48 (29-81)
%	22.4±4.2	17.4±3.8	7.3±1.4*	5.6± 2.7*
RR	7.5±4.6	13.4±1.2	40.0±9.8*	37.5±10.8*

These results show that at HF there was 1) a significant reduction in EC_{50},2) a decrease in % occupancy and 3) an increase in RR in both a and v. Thus, at HF, there was an increased vascular sensitivity to PE associated with increased RR suggesting that the peripheral vascular contractility is enhanced.

42.3

ALPHA1 ADRENOCEPTORS IN MEN WITH SYMPTOMATIC AND ASYMPTOMATIC BENIGN PROSTATIC HYPERPLASIA (BPH). Herbert Lepor*, and Daniel I. Gup*. (SPON: K.A. Hruska). Washington University School of Medicine, St. Louis, MO 63110.

There is increasing clinical evidence that infravesical obstruction in men with BPH is relieved by alphal adrenergic antagonists. The role of alphal adrenoceptors in the development of bladder outlet obstruction has not been studied previously. Prostate adenoma specimens were obtained from 9 men with asymptomatic BPH undergoing cystoprostatectomy, 11 men with symptomatic BPH undergoing open prostatectomy and 12 men with symptomatic BPH undergoing transurethral resection of the prostate (TURP). A quantitative symptom score analysis and urinary flow rate determination documented the absence of bladder outlet obstruction in men undergoing cystoprostatectomy and confirmed the presence of bladder outlet obstruction in men undergoing prostatectomy. Saturation experiments using 125I-Heat were used to determine alphal adrenoceptor density (Bmax) and the binding affinity of 125I-Heat (Kd) in the above mentioned prostates. The mean Kd of 125I-Heat in TURP, open prostatectomy and cystoprostatectomy specimens were 95 pM, 72 pM, and 79 pM, respectively. The mean B_{max} of alphal adrenoceptors in TURP, open prostatectomy and cystoprostatectomy specimens were .17 fmol/mg wet wt, .30 fmol/mg wet wt, and .19 fmol/mg wet wt, respectively. The development of infravesical obstruction in men with BPH is not associated with up-regulation or altered binding affinity of alphal adrenoceptors in the prostate adenoma.

42.4

ALPHA$_2$-ADRENOCEPTOR BLOCKADE BLUNTS THE HYPERGLYCEMIC RESPONSE TO AN ORAL GLUCOSE CHALLENGE IN NORMAL RATS. J. Paul Hieble, Anthony C. Sulpizio*, Charles Sauermelch* and Robin Goldstein*. Smith Kline & French Laboratories, Philadelphia, PA, 19101.

Glucose stimulated insulin release from the pancreatic islet cell is known to be enhanced by alpha$_2$-adrenoceptor blockade. An oral glucose challenge (2 g/kg) produced an increase in plasma glucose from a basal concentration of 102 ± 6 mg/dl to a peak concentration of 195 ± 10 mg/dl in fasted (18 hr) male Sprague-Dawley rats, with the peak concentration occurring 30-60 min post dosing. Significant hyperglycemia was sustained for 120 min following dosing. Peak glucose concentrations were not significantly attenuated by tolbutamide (50 mg/kg, PO, administered concurrently with the glucose)(178 ± 8 mg/dl), however, plasma glucose was depressed by tolbutamide 60-240 min following glucose challenge (120 ± 10 and 92 ± 5 mg/dl in control and tolbutamide groups, respectively, at 240 min). Prazosin, a selective alpha$_1$-adrenoceptor antagonist, (3 mg/kg, PO) had no effect on the glucose levels during or subsequent to the glucose challenge (peak response = 193 ± 6 mg/dl). In contrast, the alpha$_2$-adrenoceptor antagonists, rauwolscine (5 mg/kg, PO) and SK&F 86466 (15 mg/kg, PO) significantly attenuated peak plasma glucose concentrations (168 ± 10 and 139 ± 12 mg/dl, respectively), but did not affect plasma glucose 120-240 min following the glucose challenge. SK&F 86466 was most effective in this regard, probably because of the rapid oral absorption of this agent. These studies show that SK&F 86466 blunts the peak hyperglycemic response to an oral glucose challenge in rats. Since similar effects have been reported with other alpha$_2$-adrenoceptor antagonists in normal and diabetic animals as well as in diabetic patients, this type of agent may be useful in maintaining normal plasma glucose concentrations in non-insulin dependent diabetics.

42.5

YOHIMBINE ENHANCES SPINAL BUT NOT SUPRASPINAL NOCICEPTIVE REFLEXES.

M. Bansinath, K. Ramabadran, H. Turndorf and M. M. Puig, Dept Anesthesiology, NYU Medical Center, 550 First Avenue, New York, NY 10016.

The tail flick response represents a monosynaptic spinal reflex, while the jumping response in hot plate test is a polysynaptic reflex involving higher centers. In the present study, the effects of Yohimbine (Y: 1, 3 and 10 mg/kg s.c.) were evaluated on nocieptive reactions in the tail immersion (water bath 50°C, cut off 15 sec) and hot plate (60°C, cut off 60 sec) tests. Drug and test naive mice (SW 20-25 G, 10-16/group) were used. The post-drug (30 min) reaction latency was expressed as % of control latency. Y significantly lowered the tail flick latency ($F_{3,57}$ = 14.3, p < 0.0001). The post-drug latencies were: 89 ± 7, 57 ± 8, 34 ± 6 and 27 ± 3 for control, 1, 3 and 10 mg/kg of Y, respectively. When compared to controls, all the doses of Y siginificantly (p < 0.05) lowered the reaction latency. On the contrary, Y did not affect the jump latency in hot plate test. The results demonstrate that adrenergic alpha-2 receptors influence the spinal but not supraspinal nociceptive reaction.

42.6

EFFECTS OF DIETARY LIPIDS ON α_2-ADRENOCEPTOR FUNCTION IN THE RAT VAS DEFERENS. Peggie J. Hollingsworth*, Walter R. Dixon and Charles B. Smith, Dept. of Pharmacol., Univ. Michigan, Ann Arbor, MI 48109 and Dept. of Pharmacol. and Toxicol., School of Pharmacy, Univ. Kansas, Lawrence, KS 66045.

Previous studies indicated that feeding rats with a diet enriched in saturated fat results in a subsensitivity of α_2-adrenoceptors on adrenergic neurons in rat tail arteries whereas a diet enriched in unsaturated fat results in a supersensitivity of these receptors. In the present study, the effect of a diet containing 16% (w/w) of either coconut oil (saturated fat) or sunflower oil (unsaturated fat) upon α_2-adrenoceptor function was assessed with the isolated, electrically stimulated rat vas deferens. The EC50 for clonidine for vasa deferentia from control rats (fed standard Purina Rodent Chow) was 11.7 nM±0.6 (n=4). The EC50's for clonidine were 8.7 nM ±0.8 (100% inhibition, n=3) and 10.5 nM ±1.6 (93.6 ±3.7% inhibition, n=4) for vasa deferentia from rats fed a sunflower oil or a coconut oil diet, respectively. In the presence of idazoxan, 10^{-7} M, the EC50's for clonidine in the two groups were 14.9 nM ±0.9 (n=3) and 20.1 nM ±5.7 (n=4), respectively. The present study shows that, unlike α_2-adrenoceptors on adrenergic neurons in the rat tail artery, α_2-adrenoceptors on adrenergic neurons in the vas deferens are not altered by either diets high in saturated or in unsaturated fats.

(Supported by NIDA grants DA 03504 and DA 025438)

42.7

(+)-AJ 76, A SELECTIVE ANTAGONIST OF DOPAMINE AUTORECEPTORS. M.F.Piercey, J.T.Lum*, and W.E.Hoffmann*. The Upjohn Company, Kalamazoo, MI 49001

Svensson et al. report that (+)-AJ 76 and (+)-UH 232 increase locomotor activity and dopamine (DA) metabolism (Naunyn-Schmeideberg 334:234, 1986). We describe here 1) the antagonism of DA autoreceptor stimulation in substantia nigra pars compacta (SNPC), and 2) the use of 2-deoxyglucose (2-DG) autoradiography to map the neuroanatomical distribution of (+)-AJ 76 effects. DA neurons of chloral hydrate rats were depressed by stimulation of autoreceptors with 100 ug/kg apomorphine (APO). (+)-AJ 76 and (+)- UH 232 reversed APO effects with ED50's of 480±233 and 103± 53 ug/kg, respectively. The greater potency for (+)-UH 232 as an autoreceptor antagonist contrasts with its weaker stimulant effects (Svensson et al., ibid) suggesting that (+)-AJ 76 may be a specific antagonist for autoreceptors. Using a standard 2-DG protocol (Sokoloff et al., J. Neurochem. 28:897, 1977), 15 mg/kg i.v. (+)- AJ 76 injected 5 min prior to 2-DG increased glucose metabolism in SNPC, VTA, globus pallidus, and n. accumbens, sites stimulated by DA agonists and depressed by DA antagonists. Locus coeruleus metabolism was also increased. Like dopamine postsynaptic antagonists, (+)-AJ 76 stimulated the lateral habenula. It is concluded that (+)-AJ 76 is a selective autoreceptor antagonist.

42.8

DA_2 DOPAMINE RECEPTORS AND ALPHA2-ADRENOCEPTORS IN THE CANINE STELLATE GANGLION. Y Satoh*, JD Kohli, LI Goldberg. Clinical Pharmacology, University of Chicago, Chicago, IL 60637

Receptors mediating the inhibitory effects of norepinephrine (NE) and dopamine (DA) on ganglionic transmission were studied. Cardioaccelerator nerves were electrically stimulated pre- or post-ganglionically in anesthetized open-chest dogs. Drugs were injected i.v., or into the arterial supply of the stellate ganglion i.a., monitoring heart rate for neuronal activity. UK-14,304 (UK), an alpha2 agonist, NE, phenylephrine (PE), a selective alpha1 agonist, dipropyl DA (DPDA), a preferential DA_2 agonist, DA, and fenoldopam (FE), a selective DA_1 agonist, i.a. inhibited ganglionic transmission. The potency order was UK>>NE>PE for the alpha agonists and DPDA> DA>>FE for the DA receptor agonists. The composite potency order was UK>NE>DPDA>DA>PE>FE. ED_{50}'s ranged from $1.5x10^{-9}$ to $>10^{-6}$ moles. SCH 23390, a DA_1 antagonist, and domperidone, a DA_2 antagonist, (both 10 µg/kg i.v.) had no effect, but rauwolscine, an alpha2 antagonist (300 µg/kg i.v.) markedly augmented the preganglionic frequency response curve. I.A. rauwolscine (20, 60, 200 µg) facilitated preganglionic, with significantly less effect on postganglionic, stimulation. SCH 23390 had no effect on DA or NE while domperidone antagonized DA but had no effect on NE. Rauwolscine antagonized NE at 60 and 200 µg i.a. but DA only at 200 µg. These results support the presence of DA_2 and alpha2-adrenoceptors in the canine stellate ganglion and suggest alpha2-adrenoceptors may be more important physiologically. NIH grant GM-22220.

42.9

INTERACTION BETWEEN PREJUNCTIONAL PURINOCEPTORS AND ADRENOCEPTORS IN REGULATION OF NOREPINEPHRINE RELEASE IN RAT CAUDAL ARTERIES.

K. Shinozuka*, R.A. Bjur* and D.P. Westfall. Dept. of Pharmacology, University of Nevada School of Medicine, Reno, NV, 89557.

The present experiments were performed to determine the influence of purinergic agonists on the inhibition of the release of norepinephrine (NE) produced by adrenergic agonists and also whether adrenergic agonists influence the inhibitory action of purinergic agonists. Rat caudal arteries were subjected, in vitro, to electrical field stimulation with 0.5 msec pulses for 3 min at 1 Hz. Endogenous NE was quantified by HPLC-electrochemical detection techniques. 2-Chloroadenosine at a concentration of 0.1 uM did not affect NE-release but markedly potentiated the inhibitory action of clonidine (1 uM) on the release of NE. On the other hand, clonidine at 0.01 uM did not alter NE-release significantly, but potentiated the inhibition of NE-release by 2-chloroadenosine (10 uM). These results suggested that there is a potentiative interaction between prejunctional adrenoceptors and prejunctional purinoceptors in the regulation of norepinephrine-release. (Supported by NIH grant HL38126).

42.10

EFFECT OF S-11701 ON ADRENERGIC NEUROEFFECTOR INTERACTION IN ISOLATED CANINE SAPHENOUS VEINS. H.-Y. Guo*, R.R. Lorenz* and P.M. Vanhoutte, Department of Physiology and Biophysics, Mayo Clinic, Rochester, MN 55905, U.S.A.

The effect of S-11701 [(morpholinyl-2)methoxy]-8 tetrahydro-1,2,3,4 quinoleine] on adrenergic neuroeffector interaction was investigated in isolated canine saphenous veins. Tissues were incubated with ^{3}H-norepinephrine (^{3}H-NE) for one hour in the absence or presence of S-11701. The content of ^{3}H-NE and its metabolites was determined after the incubation. S-11701 caused a concentration-dependent inhibition of the accumulation of ^{3}H-NE and its metabolites, except for 3-methoxy-4-hydroxy-mandelic acid (VMA). Helical strips of canine saphenous veins were incubated with ^{3}H-NE for two hours and then suspended for isometric tension recording and the measurement of the overflow of labeled transmitter and its metabolites. Under basal conditions S-11701 significantly increased the basal efflux of ^{3}H-NE and its metabolites (except for VMA) without affecting resting tension. During electrical stimulation, S-11701 increased the contractile response and the overflow of ^{3}H-NE and 3,4-dihydroxyphenylglycol (DOPEG). The present experiments indicate that in the canine saphenous vein: (a) S-11701 causes a concentration-dependent inhibition of neuronal uptake; (b) S-11701 enters the adrenergic nerve terminals and displaces ^{3}H-NE from its storage sites.

42.11

EFFECT OF CHRONIC NOREPINEPHRINE(NE) ADMINISTRATION ON NEUROTRANSMISSION IN THE ISOLATED PERFUSED RAT KIDNEY. D.C. Eikenburg and P. Glass*, Dept. of Pharmacology, Univ. of Houston, Houston, TX 77204-5515

Chronic epinephrine administration to rats produces prejunctional alpha-adrenoceptor desensitization and a 2-fold increase in stimulus-induced neurotransmitter overflow in the kidney (JPET 244,11-18, 1988). The present study examines the effects of chronic NE treatment on sympathetic neurotransmission in the rat kidney. Male rats were treated for 6 days with NE (100µg/kg/hr, sc). On day six, animals were anesthetized, the right kidney isolated and perfused with Krebs solution at 37°, 6 ml/min. Stimulus-induced (1Hz, 90 V, 1msec, 2 min) overflow of endogenous neurotransmitter was measured in the presence of cocaine (10µM) and corticosterone (40µM) using HPLC-EC. Plasma NE concentrations in the NE treated group during surgery on day 6 were approximately 33 nM. NE treatment increased NE content of the kidney from 525.0±19.7 pmol/g to 611.5±42.1 pmol/g($p<0.05$). Absolute stimulus-induced overflow of NE from the kidney was increased by NE treatment from 10.2±1.0 pmol/g to 14.5±1.1 pmol/g($p<0.05$). This increase was not simply due to the increased NE content of treated kidneys as fractional overflow was also increased (C: 1.70±0.14%; NE: 2.10±0.11%)($p<0.05$). Phentolamine (10-9-10-5M) produced dose-dependent increases in stimulus-induced overflow in both groups. However, the dose-response curve in the NE treated group was shifted slightly to the right. In conclusion, chronic NE treatment resulted in an increase in both absolute and fractional stimulus-induced overflow of neurotransmitter from the isolated perfused rat kidney which apears to be due to a decrease in prejunctional alpha-adrenoceptor function. (Supported by AHA and NIH HL38767)

42.12

CARDIOVASCULAR AND TACHYPHYLACTIC EFFECTS OF PHENYLPROPANOLAMINE IN BEAGLE DOGS. Sydney Ellis and James A. Vick*, FDA, Division of Drug Biology. Washington, DC 20204.

Blood pressure effects of i.v. phenylpropanolamine (PPA) yielded dose-response curves which are steep, but the responses to PPA injected i.a. into the femoral artery autoperfused with a roller pump produced very flat curves. Norepinephrine (NE), however, produced steep dose-response curves by both routes of administration. An additional contrast between central and peripheral cardiovascular effects is that PPA was found to potentiate i.v. administered NE but did not potentiate responses to i.a. NE in the perfused femoral bed. Marked changes in cardiac contractile force and nasal volume occurred at minimal pressor doses of PPA and showed sharp dose-response effects. Prazosin severely inhibited the pressor-response to PPA without modifying the increase in cardiac contractile force. Tachyphylaxis was demonstrable only after large single doses of PPA and was greater and more complete in the cardiac than in the pressor responses. After the pressor response to a large tachyphylactic PPA dose dissipated, potentiated responses to NE as well as to tyramine and isoproterenol persisted for several hours. During this period the pressor response to bilateral occlusion of the carotid arteries was reduced.

42.13

QUANTITATION OF BIOGENIC AMINES AND METABOLITES IN NEONATAL CSF BY HPLC AND MULTIELECTRODE ELECTROCHEMICAL DETECTION. M.C. Castle, J. DeWinkler*, T. Bass* and W.J. Cooke. Dept. Pharmacol. and Dept. Ped., Eastern Virginia Medical School, Norfolk, VA 23501.

Quantitation of chemicals in neonatal CSF has been hampered by small sample volumes and by the chemical diversity of the various neurochemicals, precursors and metabolites. The Neurochemical Analyzer (ESA, Inc., Bedford, MA) combines gradient elution HPLC with a 16-electrode coulometric electrochemical detector to permit the quantitation of a large number of elctrochemically-active compounds from a small sample (50 uL) with little or no sample preparation. A high degree of analytical specificity is derived from the HPLC separation and by setting each electrode of the detector to a specific oxidation potential (0 to 900 mv in 60 mv increments). This method enables the quantification of femptomole amounts of precursors, products and metabolites of biogenic amines. The technique has been employed for the analysis of CSF specimens to establish profiles for biogenic amines and other electrochemically active compounds as a function of age and disease. (Supp. by Eastern Virginia Medical Foundation).

42.14

INTRAVENOUS RITODRINE THERAPY AND PLATELET ACTIVATION. A. A. Saleh*, K. G. Fahey*, A. M. Farag*, E. F. Mammen* and S. F. Bottoms* (SPON: M. A. Marrazzi). Pontiac General Hospital, Pontiac, Michigan 48053

Recent reports link platelet activating factor (PAF) to the onset of preterm labor. Reports measuring the unique platelet activation markers, platelet factor 4 (PF4) and β thromboglobulin (βTG), in preterm labor are lacking. We measured PF4 and βTG by RIA in six patients with preterm labor before and after ritodrine (which is a β2 adrenergic agent used to inhibit labor). The levels were compared with those of six matched pregnant controls not in labor as shown below. Student and paired (t) tests were used and $p<0.01$ was considered statistically significant.

	BEFORE RITODRINE	AFTER RITODRINE	CONTROL
PF4 ng/ml	90 ± 40	36 ± 36	8 ± 7
βTG ng/ml	160 ± 43	90 ± 61	45 ± 34

PF4 and βTG levels before ritodrine were significantly higher than after ritodrine ($p<0.01$) as well as control levels ($p<0.001$). The after ritodrine levels were not statistically different from controls. Platelet activation takes place in preterm labor and is inhibited by ritodrine therapy.

42.15

SPECTRAL ANALYSIS OF MONOPHASIC AND BIPHASIC RENAL SYMPATHETIC NERVE ACTIVITY IN THE RAT. D. R. Brown, D. C. Randall, and J. D. Yingling*. Depts. Biomed. Engin. and Physiology & Biophysics, Univ. of KY, Lexington, KY 40536.

Monophasic and biphasic recordings of renal sympathetic nerve activity in urethane anesthetized rats (n=6) were digitized at 10,000 Hz and their spectral content analyzed using a Fast Fourier Transform (FFT). The power spectrum of the biphasic signals was roughly normally distributed with a center frequency of 200 Hz and decay to zero at 25 and 350 Hz with no "DC" offset. The upper limit of the power spectrum of the monophasic signal was similar to the biphasic signal, but there was no apparent decay to zero at the lower limit. Biphasic and monophasic recording techniques were modelled by summing randomly distributed, zero mean sine waves (biphasic) and positive mean sine waves (monophasic) with a frequency of 200 Hz. The power spectrum of the theoretical signals was determined using an FFT and found to closely approximate the experimentally measured biphasic and monophasic sympathetic nerve traffic. We find empirical and theoretical evidence of a smaller spectral band width for biphasic recordings of sympathetic activity compared with monophasic recordings, and we find sympathetic spectral activities at higher frequencies than usually reported. The spectra of biphasic and monophasic recordings of sympathetic activity can be explained by a summation of neural impulses with similar frequencies. (Supported by NIH Grant HL 19343)

OCULAR PHARMACOLOGY

43.1

ATYPICAL ALPHA-ADRENOCEPTOR MEDIATES PHENYLEPHRINE-INDUCED MYDRIASIS. John A. Hey, Tseggai Gherezghiher and Michael C. Koss. Univ. of Okla. Hlth. Sci. Ctr., Oklahoma City, OK 73190

Mydriasis and nictitating membrane (NM) contractions elicited by pharmacological activation of alpha-adrenoceptors in vivo were produced by a constant infusion of the $alpha_1$-adrenoceptor agonist phenylephrine, in anaesthetized cats. Phenylephrine was given at a constant rate (150 µg/min, i.v.). Steady-state mydriasis and NM contraction responses were attained about 20 minutes after starting the phenylephrine infusion. Following plateau-level pupillary and NM responses, administration of the $alpha_1$-adrenoceptor antagonist prazosin (.01-1.0 mg/kg, i.v.) produced a dose-dependent blockade of the NM contraction without altering phenylephrine-induced mydriasis. In contrast, treatment with the $alpha_1$-adrenoceptor antagonist WB-4101 (0.1-1.0 mg/kg, i.v.) or pretreatment with phenoxybenzamine (3.0 mg/kg, i.v) blocked both the NM and pupillary responses. These results suggest that in vivo mydriatic response to phenylephrine is mediated by an atypical alpha-adrenoceptor that cannot be readily classified as an $alpha_1$- or $alpha_2$-adrenoceptor. (Supported by Research to Prevent Blindness and NSF)

43.2

RESPONSES OF EQUINE, PORCINE AND FELINE IRIDES TO CHOLINERGIC DRUGS. Popat N. Patil and Joyce P. Griffiths*. The Ohio State University, Columbus, Ohio 43210.

Dose-response curves were constructed for the isometric contraction of excised irides, suspended in a tissue bath with carbachol (CARB) or pilocarpine (PILO). Histamine (HIS) was tested in equine iris strips,and dissociation binding constants (KB) for atropine (ATR) were determined in porcine and equine irides.

SPECIES	-log M ED50 CARB (mean)		-log M ED50 PILO (mean)		% max contraction* (* CARB = 100%) (mean)
Horse	n = 4	6.86	n = 4	5.39	67
Pig	n = 9	6.66	n = 6	5.65	50
Cat	n = 3	6.75	n = 3	5.48	61

The -log M ED50s for CARB and PILO were similar across species. PILO was a partial agonist in all three species. Histamine, a compound structurally related to PILO was a partial agonist; it produced 56% of maximum contraction in the equine iris (-log M ED50 5.79). K_B of atropine (ATR) against CARB in porcine iris (1.13×10^{-9} M) was similar to the K_B for ATR against PILO in equine iris strips (1.19×10^{-9} M), and the curves generated were consistent with competitive antagonism. Irides from horse, cat and pig reacted similarly to these cholinergic drugs.

43.3

ARGON LASER INDUCED OCULAR HYPERTENSION: ANIMAL MODEL OF OCULAR INFLAMMATION. T. Gherezghiher and M.C. Koss. Dean McGee Eye Institute, Oklahoma City, OK 73104

There is substantial evidence to indicate that prostaglandins (PG's) as well as non PG substances mediate acute elevations of intraocular pressure (IOP) due to laser irradiation of the iris. In the present study, we determined the optimal laser parameters needed to produce the maximal rise in IOP in anesthetized rabbits. Photocoagulation of pigmented rabbit iris produced an acute rise in IOP that was at least 11mm Hg higher in the experimental eye when compared to the control eye. The onset of hypertension occurred within 15 min and remained elevated for at least 90 min. The rise in IOP was accompanied by miosis and an increase in aqueous protein concentration. This hypertensive phase was followed by a delayed hypotony at 24 hours that lasted for at least 4 days. Indomethacin pretreatment (0.01-30mg/Kg, ip) produced a dose dependent block of the acute rise and the delayed fall in IOP, as well as the increase in aqueous protein concentration. Similarly, topical ibuprofen (0.03-0.3%) and aspirin suppository (600mg) prevented the laser induced rise in IOP. These results support the contention that iris photocoagulation induced ocular hypertension in pigmented rabbits could be used as a potential animal model of ocular inflammation to screen non-steriodal antiinflammatory agents (Supported in part by Research to Prevent Blindness).

43.4

RETINOID ANTAGONISM OF RETINOL EFFECTS ON RETINAL PIGMENTED EPITHELIUM RNA SYNTHESIS. J.D. Gabourel, J.M.B. Bradley*, T.S. Acott*. Oregon Health Sciences University, Portland, OR 97201

The effects of retinol and several other retinoids on RNA synthesis by cultured human retinal pigmented epithelium (RPE) was investigated. Retinol, complexed to serum retinol binding protein (R-SRBP), stimulated RNA synthesis when added to the cultures. At a retinol concentration of 1.0 µg/ml the average rate of ^{3}H-uridine ± s.e.m. incorporation was 142 ± 5.6% of control. The stimulation was concentration dependent over the range 0.25 - 1.5 µg/ml. Stimulation of RNA synthesis was seen with native R-SRBP as isolated from pooled human serum and with R-SRBP reconstituted by addition of retinol to apo-SRBP in vitro. Other retinoids complexed with apo-SRBP in vitro failed to stimulate RNA synthesis in this system. Free retinol and retinoids, complexed with serum albumin in the culture medium, also failed to stimulate RNA synthesis. Most of the retinoids when complexed with serum albumin selectively antagonized the R-SRBP induced RNA synthesis suggesting that they interfere with retinol action or transport rather than directly inhibiting RNA synthesis. The order of potency for this selective effect roughly parallels the incidence of ocular toxicity (night blindness and/or excessive glare sensitivity) reported when these retinoids are used clinically to treat various dermatological disorders.

Supported by a grant from Hoffman-LaRoche Inc.

43.5

IMPROVEMENT OF READING VISION BY FISH DIET

Richard F. Tislow, M.D. Philadelphia State Hospital, Philadelphia, PA 19103

This is a progress report of the effect of cold water fish in improving the reading (macular) vision of the author.

I observed blurring of visual acuity in June 1986 and the chart of examination at the Scheie Institute of the Univ. of Penn., recorded on 7-8-86, some RPE in the macula of both eyes, and confirmed it on 7-21-86.

Starting in the summer of 1986 I started eating flounder caught and flash frozen in the cold waters of the North Atlantic, for its beneficial effects on the Cardiovascular system. I noticed that my vision improved but did not connect it with the fish diet (3 - 4 oz. per meal about 3x/weekly). During the winter of 1987 I neglected the fish diet and in February and March noticed a worsening of my reading vision, eg. difficulties in reading the book section of the N.Y. Times. With some excitement I resumed the fish diet and within 1 week I was able to read the N.Y. Times; also after 6 weeks I was able to read small numbers stamped onto small tablets. Recent examination by an opthalmologist gave easy and clear reading of Jaeger print #1. This personal experience is communicated because of the importance of vision, in the aging population.

LUNG FLUID BALANCE

44.1

INCREASED PERMEABILITY FOLLOWING INTRAVASCULAR PARAQUAT IS POTENTIATED BY LACK OF INTERNAL CALCIUM IN ISOLATED RAT LUNG. J.W. Barnard*, W.A. Womack*, S.M. Smith*, and A.E. Taylor, Dept of Physiol., Univ of South Alabama, Mobile, AL 36688

Paraquat (PQ) causes pulmonary injury including increased permeability and edema formation. We examined the effects of 0.01M paraquat on the capillary filtration coefficient ($K_{f,c}$, ml/min/cmH_2O/100g) and on lung wet:dry weight ratio. In isolated rat lungs perfused with 5% human serum albumin and 2.5mM Ca^{++} in Krebs buffer, neither the $K_{f,c}$ nor wet:dry weight changed. In lungs perfused with Krebs buffer omitting calcium, both $K_{f,c}$ and the wet:dry weight were elevated. When the slow calcium channel was blocked with 200µM nifedipine, $K_{f,c}$ and wet:dry weight both rose.

	BASELINE DATA		+2.5 HOUR DATA	
	Kf,c	W:D	Kf,c	W:D
2.5mM Ca^{++}/0.01M PQ				
mean	0.48	7.87	0.51	8.11
±SEM	0.09	0.63	0.07	0.54
No added Ca^{++}/0.01M PQ				
mean	0.45	6.80	2.14	8.44
±SEM	0.07	1.39	1.11	0.71
2.5mM Ca^{++}/0.01M PQ/200µM Nifedipine				
mean	0.41	8.73	0.76	9.34
±SEM	0.09	1.67	0.13	1.99

We conclude that intracellular calcium plays a protective role in the etiology of paraquat-induced lung injury. Supported by NIH HL22549.

44.2

LEUKOCYTE MEDIATION OF PROTAMINE MICROVASCULAR DAMAGE IN DOG LUNGS. J.C. Parker*, L.A. Hernandez*, T. Shibamoto*, P. Coker*, and B. Buchanan*. Dept. of Physiology, University of South Alabama, Mobile, AL 36688

Protamine sulfate (PR) can cause increased capillary permeability to fluid and proteins. To determine the contribution of polymorphonuclear leukocytes to microvascular damage, two groups of dog left lower lobes were constant pressure perfused with either 5% albumin in Kreb's solution (ALB; n=5) or the same perfusate with 10^9 autologous dog white cells added (PMN; n=4). Lobe weight, perfusate flow and vascular pressures were monitored to calculate capillary filtration coefficient (Kfc) in ml/min/cmH_2O/100g, and vascular resistance (Rt) in cmH_2O/l/min/100g. Sequential doses of PR of 100, 1000, 3000, 6000, and 10,000 mg were administered. The following data were obtained (*=p<.05 vs. 0; **=p<.05 vs. ALB).

PR Dose (mg)	0		6000	
	ALB	PMN	ALB	PMN
Kfc	.18±.08	.19±.15	.55±0.18*	2.04±1.11**
Rt	7.7±4.1	8.1±4.1	12.2±2.3*	75.8±39.6**

These data indicate PR activated the PMN's which in turn augmented the increased Rt and vascular permeability in lung capillaries. Supported by HL24571.

44.3

EFFECT OF HYPEROXIA AND HYPOXIA ON PARAQUAT-INDUCED INCREASES IN PULMONARY VASCULAR PERMEABILITY. T. Shibamoto*, L.A. Hernandez*, A. Moise*, K. Peevy*, A.E. Taylor, and J.C. Parker. Univ. of South Alabama, Mobile, AL 36688.

Paraquat (PQ) is believed to induce lung damage by oxidant injury. The effects of hyperoxia (PvO_2 400-500 mmHg) and hypoxia (PvO_2 30-50 mmHg) on PQ-induced vascular damage were studied in isolated blood-perfused dog lungs. Vascular permeability was assessed using the filtration coefficient (Kf) and isogravimetric capillary pressure (Pc,i). In nonventilated lungs Kf was increased at PQ doses of 10^{-2}M (n=6) and $4x10^{-3}$M (n=5) at 3 hr and 5 hr, respectively. Lungs were also treated with $4x10^{-3}$M PQ and exposed to hyperoxia (Hyper+PQ, n=5) and hypoxia (Hypo+PQ, n=5) using 95% O_2/5% CO_2 and 95% N_2/5% CO_2, respectively, for 5 hr. The group data are summarized in the table.

	Kf(ml/min/cmH_2O/100g)			Pc,i(cmH_2O)	
Group	BL	3hr	5hr	BL	3hr
Control	.13±.05	.14±.03	.18±.02#	9.5±0.9	8.6±1.5
10^{-2}M	.11±.02	.60±.26*	.47±.08*	9.9±0.7	7.4±1.6*
$4x10^{-3}$M	.12±.01	.20±.09	.42±.15*	9.2±0.7	7.9±2.5
Hyper+PQ	.15±.02	.52±.28*#	1.86±.13*#	9.1±1.1	6.9±1.0*
Hypo+PQ	.15±.02	.24±.08	.90±.49*	9.7±0.7	8.9±0.9

*p<0.05 from baseline. #p<0.05 from $4x10^{-3}$M. Mean ± SD.

We conclude that PQ caused a dose related increase in pulmonary vascular permeability which was accelerated by hyperoxia but not prevented by hypoxia. Supported by NIH HL24571 and HL24459.

44.4

HIGH PEAK AIRWAY PRESSURES INCREASE MICROVASCULAR PERMEABILITY IN ADULT RABBIT LUNGS. K. Adkins*, L. Hernandez*, A. Moise*, K. Peevy*, B. Buchanan, and J.C. Parker, Depts. of Physiology and Pediatrics, University of South Alabama, Mobile, AL.

To determine the effect of intermittent positive pressure ventilation (IPPV) with high peak airway pressure (P_{aw}) on microvascular permeability in isolated rabbit lungs, groups of closed-chest adult rabbits were subjected to 1 hour of IPPV at peak P_{aw}'s of either 15 (n=5), 30 (n=5), or 55 (n=5) cmH_2O. The animals were then exsanguinated; the lungs were excised and perfused with a mixture of blood and 5% albumin in Kreb's solution using constant flow perfusion at 37°C. Microvascular permeability was evaluated using the capillary filtration coefficient (K_{fc}) in ml/min/cmH_2O/100g. Vascular resistance (R_T) in cmH_2O/ml/min/100g and pre/post-capillary resistance ratios (R_a/R_v) were calculated from vascular pressures, flow and double occlusion pressures. The following data were obtained:

Peak Paw	15cmH_2O	30 cmH_2O	55cmH_2O
K_{fc}	0.146±.015	0.215±.038*	.310±.029*
R_T	0.0113±.0023	0.0117±.0015	.0200±.0039*
R_a/R_v	1.22±.15	1.43±.17	2.47±.53*

*significant difference from 15 cmH_2O group (p<0.05).

These data indicate that IPPV with high P_{aw} increases pulmonary microvascular permeability probably as a result of overdistention. Supported by NIH HL24571.

44.5

EFFECT OF VASCULAR AND ALVEOLAR PRESSURES ON PERIALVEOLAR INTERSTITIAL PRESSURES IN ISOLATED DOG LUNG. M. Glucksberg and J. Bhattacharya. St. Luke's-Roosevelt Hospital Center and Departments of Physiology and Medicine Columbia College of Physicians and Surgeons, New York, NY, 10019.

Lung perialveolar liquid clearance may depend on the interstitial pressure gradient from alveolar junctions (Pjct) to microvessel adventitia (Padv). We reported that the gradient falls with lung expansion (Fed. Proc. 44: 1911, 1985). To determine the effect of vascular pressure, in each of 4 excised lobes of dog lungs we filled the vasculature with Microfil to abolish filtration. Then by micropuncture, in each lobe we measured Pjct and Padv at different of vascular (Pvas) and alveolar pressures. The data are shown as cmH_2O above pleural pressure (mean±SD).

Alveolar Pressure:	7		15	
P_{vas}	P_{jct}	P_{adv}	P_{jct}	P_{adv}
5	-.6±.8	-2.2±.2	-3.3±.5	-3.5±.4
15	-.7±.7	-3.3±.2	-3.4±.7	-4.7±.8

Increase of vascular pressure always increased the P_{jct}-P_{adv} gradient. The gradient increased from 1.6±0.8 to 2.6±0.8 cmH_2O at low alveolar pressure (P<0.05), and from 0.2±0.2 to 1.2±0.3 cmH_2O at high alveolar pressure (P<0.01). Therefore high vascular pressure prevented the gradient from being abolished during lung expansion. We speculate that when vascular pressure increases, an interstitial mechanism for edema prevention operates by widening the P_{jct}-P_{adv} gradient for improved perialveolar liquid clearance. (Support: HL36024, HL01696, HL07652 and AHA860681).

44.6

HYALURONAN DETERMINES EXTRAVASCULAR WATER CONTENT IN RABBIT LUNG. Bhattacharya, S.*, T. Cruz, B. Anderson Bray and J. Bhattacharya. St. Luke's-Roosevelt Hosp. Ctr. and Depts. Physiol. & Med., Columbia College of Phys. & Surg., New York, N.Y. 10019.

We have investigated the effect of reducing lung hyaluronan content on extravascular lung water in unanesthetized rabbits. In 8 pairs of rabbits we gave hyaluronidase (Bovine Testicular, Sigma) intravenously at 750 units/[Kg·min] over two hours to one animal and an equal volume of saline (~1% body weight) to the other. In each rabbit, we estimated extravascular water content in one lung by the wet-dry method. We analyzed the other lung for hyaluronan content by our reported methods (Fed. Proc. 45:283, 1986). Lung hyaluronan of hyaluronidase treated rabbits (73±34 ug/g dry) was 40% lower than that of controls (122±50 ug/g dry) (P<.01). Nine other pairs received similar doses of saline or hyaluronidase, followed by volume expansion with intravenous saline amounting in volume to 24% of body weight given over two hours. The table shows extravascular lung water content (mean±SD g/g dry).

Condition	Saline	Hyaluronidase
Baseline	4.1±.4	3.5±.4
Volume Expansion	4.3±.5	6.3±2.5

In hyaluronidase treated rabbits, extravascular lung water was 14.6% lower than control at baseline (P<.01), but 47% higher than control after volume expansion (P<.01). We interpret that in lung, hyaluronan content determines the extravascular water content, possibly by direct water binding, as also by an effect on the microvascular barrier (Supported by HL36024, AHA860681).

44.7

INJURY OF ISOLATED RAT LUNGS BY PHORBOL MYRISTATE ACETATE (PMA)-ACTIVATED HUMAN MONONUCLEAR CELLS. M.L. Perry*and A.E. Taylor, Dept. of Physiology, University of South Alabama, Mobile, AL.

Isolated rat lungs were perfused with 5% human serum albumin in Krebs buffer and $3x10^7$ isolated human mononuclear cells (MN) (recirculating volume - 30 ml). Lung weight (LW, g) and vascular pressures were continually monitored. Predicted pulmonary capillary pressure ($P_{pc,p}$, cmH_2O) was used to estimate hydrostatic pressure changes while the capillary filtration coefficient ($K_{f,c}$, ml/min/cmH_2O/100g) was used as an index of permeability. MN-perfused lungs were then challenged with either 5µg PMA in dimethylsulfoxide (DMSO) (n=7) or DMSO (n=4). Both substances were added to the perfusate. DMSO-treated lungs showed no change 90 minutes after challenge versus control, while all measured parameters were increased 90 minutes following PMA (mean ± SE; *:p<0.05 versus control):

	$K_{f,c}$		$P_{pc,p}$		LW	
	Control	90 min	Control	90 min	Control	90 min
DMSO	0.33 ±0.03	0.33 ±0.04	5.9 ±0.2	5.7 ±0.2	1.8±0.2	2.2±0.3
PMA	0.38 ±0.08	1.00* ±0.28	6.1 ±0.3	56.9* ±25.6	2.2±0.2	3.5±0.7*

We conclude from these results that PMA-stimulated MN are capable of producing an acute lung injury. Supported by NIH HL22549.

44.8

EFFECTS OF IV SALINE INFUSION ON FETAL LUNG LIQUID SECRETION. T.A. Davis*, G. Gause*, M. ter Riet*, H. Kuck*, A.M. Perks and S. Cassin. Dept. of Physiology, College. of Medicine, Univ. of Florida, Gainesville, FL 32610.

Suggestions were made previously from this laboratory that in anesthetized, exteriorized fetal goats, the lungs may play a role in regulating fetal body fluid volume and electrolytes. Since fetal lungs are responsible for the secretion of large amounts of lung liquid (LL; 200-400 ml/day), variations in body fluid volume or osmolarity may be reflected in the rate of LL secretion. To test this hypothesis further, chronically catheterized fetal sheep (131-142 days gestation) were infused intravenously with isotonic saline at 4 different rates. After 1 hr of mixing, LL secretion rates were measured for 1 control hr, 2 hrs during infusion, and 1 hr after infusion. Infusion rates of 0.076 ml/min were followed by significant increases in secretion rate after infusion in 3 out of 5 expmts. Higher infusion rates (0.76 ml/min (N=5), 1.0 ml/min (N=1) and 1.25 ml/min (N=1)) produced no significant changes during the 2 hrs of infusion. However, at 0.76 ml/min there was a significant decrease in secretion rate in the hr after infusion had ceased (hrs 1, 2, 3 (4.35 ± 0.60, 5.08 ± 0.98 and 4.48 ± 0.56 ml/hr.kg respectively) vs hr 4 (2.47 ± 0.16 ml/hr.kg). This suggests that saline infusions may have some influence on LL secretion rate, but that the effects are less clear and consistent than in the acute goat. The disparity between these and earlier studies may be due to a) species variation, b) use of the anesthetic chloralose, or c) the state of hydration of mother and fetus. (Supported in part by NIH HL10834)

44.9

EFFECT OF ALBUMIN CONCENTRATION ON HYDRAULIC CONDUCTIVITY OF PULMONARY INTERSTITIUM. N. L. Rochester*, S. J. Lai-Fook and L. V. Brown*. Biomedical Engineering Center, University of Kentucky, Lexington, KY 40506.

To study the interstitial conductivity of rabbit lungs (Fed. Proc. 46:330, 1987), we inflated degassed lungs with silicon rubber through the airways and vessels at 15 cm H_2O pressure. We sliced the lung into 1 cm thick slabs and bonded two reservoirs to opposite ends of the slab enclosing a vessel, 1-3 mm diam. We filled the reservoirs with normal saline and applied a driving pressure of 15-25 cmH_2O across the interstitium surrounding the vessel. The flow rate of the following solutions was measured in sequence: saline, a 3, 8 or 15% albumin solution, 0.02% hyaluronidase solution and albumin solution. Albumin-to-saline flow ratios averaged 1.00 ± 0.23 (SD), 1.63 ± 0.68 and 1.54 ± 0.36 for the 3, 8 and 15% albumin solutions, respectively. Despite an increased fluid viscosity, flow increased with albumin concentration. Hyaluranidase increased flow 27-fold (± 24, n=21). After hyaluronidase, albumin-to-saline flow ratios decreased; thus, flow of albumin became more viscosity dependent. Hyaluronan was responsible for the increase in interstitial conductivity with protein concentration. This might be attributed to a reduced excluded volume by hyaluronan to flow of albumin. (Supported by HL 40362).

44.10

PAF INCREASES PROTEIN EXTRAVASATION IN RAT AIRWAYS: EFFECT OF BN-52021 AND L-655,240. M.G. Sirois*, G.E. Plante*, S. Jancar*, P. Braquet* and P. Sirois* (SPON: J. Barabé). Dept. Pharmacology, Fac. Med., University of Sherbrooke, Sherbrooke, P. Q., Canada.

The effect of PAF (platelet activating factor), a potent inflammatory mediator was studied on vascular protein extravasation in rat airway tissues using the Evans blue dye (EB) as a marker. EB was injected with doses of PAF (1.0 and 5.0 µg/kg) in the caudal vein. The animals were killed, the dye was extracted using formamide (4 ml/g wet weight tissues) and the content was expressed as EB µg/g dry weight. Significant increases of EB extravasation as a function of time (from 5 to 60 min) and doses (1.0 and 5.0 µg/kg) of PAF were noted in the trachea, upper and lower bronchi. However, the EB extravasation was not seen in the parenchyma. BN-52021, a PAF-antagonist (10 mg/kg), produced a dose-dependent inhibition of the PAF effects on the trachea, upper and lower bronchi (80-100%). This antagonist given in the absence of exogenous PAF reduced significatively the EB extravasation below basal levels in the parenchyma. L-655,240, a thromboxane antagonist (1.0 and 5.0 mg/kg), slightly reduced the protein extravasation induced by PAF in the airway tissues. These results confirm the role of PAF in inflammatory reactions and underline the difference between the effects of PAF on protein extravasation and its effects on fluid extravasation (oedema) reported in other studies. (Supported by the MRC)

44.11

INHIBITORY EFFECT OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) ON CONTRACTION OF SMOOTH MUSCLES OF THE RAT AIRWAYS. A. Cadieux, C. Lanoue, P. Sirois, and J. Barabé. Dept. Pharmacology, Fac. of medicine, Univ. of Sherbrooke, Sherbrooke, Que. Canada J1H 5N4

Calcitonin gene-related peptide (CGRP) is localized to afferent nerve terminals both in human and rat airways. In vitro, human α-CGRP contracts isolated human bronchi, producing equivalent contraction to carbachol. In the present study we have compared the effects of rat and human α-CGRP in rat isolated airways. Our results revealed that the resting tension of trachea, stem bronchus and parenchymal strips were not affected by the addition of either rat or human α-CGRP in concentrations up to 10^{-6}M. Similarly, both peptides had no relaxing effect on the contraction developped by 10^{-7}M carbachol. However, rat α-CGRP (but not human α-CGRP) caused a shift to the right of concentration response-curves for carbachol and 5-HT in the three preparations. This inhibitory effect of rat α-CGRP was found to be concentration dependent, was greater in peripheral tissues than in trachea and was accompanied by a decrease of the maximal response. The addition of propranolol (10^{-6}M), indomethacin (10^{-6}M) tetrodotoxin (10^{-6}M), as well as the absence of extracellular calcium in the Krebs solution did not influence the action of the peptide, suggesting a direct effect of rat α-CGRP on airway smooth muscles. (Supported by the MRC of Canada)

44.12

LUNG SCANNING WITH RADIONUCLIDES:REGIONAL RELATIONSHIP BETWEEN TISSUE ATTENUATION AND GAS VOLUME. M.L. Groth *, J.A. Silver *, and W.M. Foster. VA Northport and SUNY at Stony Brook, NY 11794.

Regional differences in tissue density permit noninvasive scanning of soft tissue organs, eg. renal and pulmonary tissues, using gamma emitting radionuclides. We hypothesized that regional differences in gas volume and/or pulmonary capillary blood volume should correlate to tissue attenuation during transmission scans of the lung. For 10 healthy subjects in an erect position planar images of the lung at functional residual capacity (FRC) were acquired from posterior aspect with Anger camera under 2 conditions: 1)with liquid filled, uniform radionuclide source (Tc 99m in 45.7 cm X 45.7 cm X 2.5 cm lucite phantom) held parallel to the thorax, and 2) with rebreathing gas technique after equilibrium of radio-tracer gas (Xe 133) was achieved. Planar images were analyzed for distribution of count per unit time by dividing the right lung field from apex to base into 10 rectangular areas of equal height and lung width. The distribution of radioactivity imaged within each area was expressed as percent activity of the entire lung field. Both scanning technics demonstrated a relationship between the distribution of counts and lung height. For instance, from lung apex to base, regional gas volume increased (Xe 133 activity) at a constant rate and attained a maximum volume at approximately 30% of the lung height, and below this height, regional volume in most cases gradually decreased. With respect to tissue attenuation, a similar association to height was observed for the transmission scan. For transmission and equilibrium gas scans, the ratio of % cts (Tc/Xe) for all ten areas of the lung ranged between 0.566 and 1.535 with a mean of 1.003 +/-0.157 SD. Ratio values near unity indicate that on a % basis the regional tissue attenuation was equated to regional gas volume. These data indicate that in the upright position differences in regional gas volume known to be related to lung height, correlate also to regional differences of tissue attenuation. The influence of pulmonary capillary blood volume on the relationship between regional gas volume and attenuation, or whether similar relationships exist for other lung volumes above and below FRC, need to be evaluated. Supported by VA and NIH HL31429-04, Washington, DC.

ADRENAL CORTEX/METABOLIC DISEASES

45.1

SIMILAR SITES OF ACTION OF ATRIAL NATRIURETIC FACTOR AND OF GUANABENZ ON ALDOSTERONE BIOSYNTHETIC PATHWAY. M. Brochu‡, J. Féthière‡ C. Coderre‡, H. Ong‡ and A. De Léan. Lab. Pharmacologie Moléculaire, IRCM, Montréal, Québec, H2W 1R7.

This study was performed to determine the site of action of ANF and guanabenz in adrenal steroidogenesis. We compare the effect of these two compounds on the precursors of aldosterone (A) i.e. pregnenolone (Preg), progesterone (Prog), deoxycorticosterone (DOC) and corticosterone (B) with or without trilostane (T) which blocks the step between pregnenolone and progesterone. The steroids from culture medium of zona glomerulosa cells were separated by HPLC and then measured by radioimmunoassay. In the absence of T, AII increased 4 to 10-fold A, B and DOC levels while Prog and Preg levels were increased by less than 3 times. In the absence of T, both ANF and guanabenz profoundly and dose-dependently inhibited A and B with an ED_{50} of 40 pM for ANF and of 35 µM for guanabenz. When trilostane is added the two compounds inhibited the synthesis of pregnenolone. In conclusion, the results obtained document that guanabenz mimics the effect of ANF on A biosynthetic pathway in zona glomerulosa cells.

45.2

REGULATION OF PROSTATIC SPERMINE-BINDING PROTEIN GENE EXPRESSION BY DEHYDROEPIANDROSTERONE AND ANDROSTENEDIONE IN THE RAT. C. Labrie*, J. Simard*, H.F. Zhao*, A. Bélanger*, G. Pelletier* and F. Labrie, MRC Group in Molecular Endocrinology, Laval University Medical Center, Quebec, Canada G1V 4G2.

Human adrenals secrete large quantities of dehydroepiandrosterone (DHEA) and androstenedione (Δ⁴-dione) which are converted into the potent androgen 5α-dihydrotestosterone (DHT) in the prostatic tissue. We have studied the effect of the continuous administration of DHEA and Δ⁴-dione on two sensitive and specific parameters of androgenic activity in the castrated adult rat, namely ventral prostate weight and prostatic spermine-binding protein (SBP) gene expression. Steroid-releasing implants were used to obtain plasma concentrations of DHEA and Δ⁴-dione similar to those found in the sera of adult men. SBP mRNA were measured by in situ hybridization using a [³⁵S]-labelled cDNA probe generously provided by Dr. M.G. Parker. Treatment of castrated rats with Δ⁴-dione, Δ⁴-dione + DHEA or testosterone resulted in SBP mRNA levels similar to those found in intact animals while DHEA alone was less potent (50% relative to control intact animals). These stimulatory effects were reversed by concomitant administration of the pure antiandrogen flutamide (5 mg, b.i.d.). Similar effects were observed on ventral prostate weight and prostatic steroid-binding protein C1 component mRNA levels. These results clearly demonstrate the stimulatory effect of DHEA and Δ⁴-dione on androgen-dependent gene expression in the rat prostate and support their important role in prostatic cell function.

45.3

LIPID PEROXIDATION IN THE ADRENAL GLANDS OF MALE RATS EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD). L.L. Bestervelt*, D.W Piper* and W.N. Piper Toxicology Program, School of Public Health and Department of Pharmacology, Medical School, The University of Michigan, Ann Arbor, MI 48109-2029.

Toxic effects of TCDD such as chloracne, hirsutism, anorexia, weight loss and hypoglycemia suggest alterations in endocrine function, and potential effects on the adrenal gland. Previous studies in our laboratory have shown that exposure of rats to TCDD results in decreased adrenal 21-hydroxylase activity associated with depressed levels of microsomal cytochrome P-450. It has been shown that increased porcine adrenal microsomal lipid peroxidation is associated with decreased cytochrome P-450 levels and loss of 21-hydroxylase activity. This study was undertaken to determine whether TCDD could induce microsomal lipid peroxidation in the adrenal glands of male rats. Male Sprague-Dawley rats (220-240g) received a single oral dose of TCDD (50ug/kg) on day 0 and were killed at days 1,2,3,5, and 7. Adrenal glands were removed, trimmed of adhering fat and weighed. Microsomes were obtained by differential centrifugation. Malondialdehyde (MDA) production, as measured by the thiobarbituric acid test, served as an index of lipid peroxidation. The amount of MDA formed was found to be significantly higher than controls at days 1,2,3 and 5 following administration of TCDD. The amount of MDA produced was increased 1.9 fold relative to controls on day 1, whereas at days 2,3 and 5 the amount produced was increased 5, 3.5 and 2.7 fold, respectively. No difference in MDA production was observed at day 7. These results indicate that TCDD causes early increases in adrenal microsomal lipid peroxidation. It is possible that the early increases of lipid peroxidation seen in rat adrenal microsomes after exposure to TCDD are associated with the destruction of microsomal cytochrome P-450 causing subsequent decreases in 21-hydroxylase activity. (Supported by ES-02423 and ES-07062)

45.4

DIURNAL CHANGES IN PLASMA ACTH, CORTISOL, AND ADRENAL RESPONSIVENESS TO ACTH IN SWINE. H.G. Klemcke, J.A. Nienaber*, G.L. Hahn*. USDA-ARS RLHUSMARC, Clay Center, NE 68933.

A study was conducted to measure diurnal rhythms of ACTH and cortisol (F) in castrated male pigs (barrows). Fourteen barrows with indwelling jugular catheters were bled at 6 hr intervals for a 24 hr period. Significant changes in plasma ACTH were evident with peak levels (61 ± 6 pg/ml) evident at 0100-0700 hr, and a trough (38 ± 4 pg/ml) at 1900 hr. Changes ($P<.05$) in plasma F were also present in barrows with a peak (44 ± 6 ng/ml) at 0700 hr, and a trough (21 ± 5 ng/ml) at 1900 hr. Plasma norepinephrine and epinephrine were measured concomitantly and did not differ among hours. The ratio of F to $\log_{10}$ ACTH at 0700 hr (25.3 ± 3.0) was greater than the ratio at 1900 hr (12.9 ± 2.7; $P<.05$), and suggests an enhanced adrenal responsiveness to ACTH in the morning. Sequential blood samples were taken on four of the barrows 12 and 26 days later. Plasma ACTH did not differ among hours ($P>.05$), but plasma F on both dates was greater ($P<.05$) at 0100 or 0700 hr than at 1900 hr. The ratio of F to $\log_{10}$ ACTH at 0700 hr was always greater ($P<.05$) than at 1900 hr. These studies are the first to demonstrate diurnal changes in plasma F in barrows, and are the first direct measurements suggesting similar changes in plasma ACTH in pigs. The apparent diurnal change in adrenal responsiveness to ACTH suggests that it is in part responsible for diurnal changes in plasma F.

45.5

CARDIAC CONTRACTILE PERFORMANCE OF BB/WOR DIABETIC RATS. D.J. Paulson, S.J. Kopp, R.C. Jurak*, J.P. Tow *, and M.D. Galle*. Dept. of Physiol., Chicago Coll. Osteopathic Med., Chicago, IL.

Studies of patients with insulin dependent diabetes mellitus (IDDM) as well as drug-induced animal models have shown that myocardial contractile function is impaired. The purpose of this study was to determine whether the BB/Wor diabetic rat exhibits similar alterations. Cardiac performance was assessed 1) in vivo by measuring left intraventricular pressure and its derivatives under basal conditions and in response to various doses of i.v. dobutamine; and 2) in vitro perfused hearts by progressively increasing the volume of a balloon inserted into the left ventricle. Hearts were paced at 300 beats/min and perfused with normal (0.4mM) and elevated (1.2mM) levels of palmitate plus 5.5mM glucose. For 8 weeks the rats were maintained on PCI insulin (0.8 to 1.2 U/day). The BB/Wor rats were then divided into 2 groups based on the levels of blood glycosylated hemoglobin (% Hb). No alterations in in vivo cardiac function were found under basal conditions, but BB/Wor rats with high % Hb exhibited a depressed response to dobutamine. No differences were found between either of the BB/Wor groups or control group in the in vitro perfused heart protocol. These findings indicate that 8 weeks of IDDM in the BB/Wor rat does not cause significant alterations in cardiac contractile function but may impair the B_1-adrenergic receptor system. (NIH grant DK39200 and RR05972).

45.6

EXPERIMENTAL MODELS OF DIABETIC AUTONOMIC NEUROPATHY: ALTERATIONS IN BLADDER FUNCTION AND PHARMACOLOGICAL USE OF GANGLIOSIDES. M. Paro*, G. Italiano*, L. Petrelli*, M. Prosdocimi, R.A. Travaglì*, R. Zanoni*, A.F. Sima* (1). Fidia Research Laboratories, 35031 Abano Terme - Italy and (1) University of Manitoba, Winnipeg, Canada

Previous studies in the experimental models of diabetes have demonstrated functional and structural polyneuropathy involving the autonomic nervous system. The purpose of this study was to assess the effects of chronic diabetes and pharmacological treatment with gangliosides on urinary bladder function in alloxan diabetic rats and BB rats. We investigated bladder function in an in vivo situation in animals with 3-6 months of diabetes. Rhythmic contractions evoked by the micturition reflex were recorded following controlled bladder distension. In alloxan diabetic rats the rythmic bladder contractions of micturition were irregular and their frequency was reduced to 58% and 34% of the normal value after 3 and 6 months respectively. In BB rats the frequency was reduced to 33% and 26% of the normal value after 4 and 6 months of diabetes. In both models this functional impairment was associated with marked hypertrophy as reflected by an increase in bladder weight and threshold volume for the micturition reflex. The functional findings in both models were correlated to morphological and morphometric changes in autonomic nerves (pelvic and hypogastric) constituting the neural reflex arc. Pharmacological treatment with insulin restored the metabolic condition and, partially, the functional alterations of urinary bladder. Bovine brain gangliosides (10 mg/kg i.p.) treatment improved the bladder function in alloxan diabetic animals studied at three months, as shown by a reduction in threshold volume and an increase in rate of bladder contraction (90% of normal value).

45.7

DIFFERENTIAL UPTAKE AND DISTRIBUTION OF RADIOIODIDE BASED ON I_2 and I^- PRETREATMENT. K.D. Stout* and R.J. Bull. Pharmacology/Toxicology Program, College of Pharmacy, Washington State University, Pullman WA. 99164-6510

Various inorganic forms of iodine have been considered more or less equivalent in the body. To challenge this notion, we have undertaken studies to determine whether pretreatment of rats with I_2 alters the distribution of a radioiodide tracer ($125\text{-}I^-$) relative to pretreatment with equivalent doses of I^-. Rats were pretreated for seven days with water supplemented with 1, 3, 10, 30 or 100 ppm I^- or I_2. At the end of seven days the rats were dosed with $125\text{-}I^-$ and sacrificed 1 hour later. Thyroid uptake decreased in a dose-dependent manner in both I^- and I_2 pretreated animals, but the effect was greater in the I^- group. $125\text{-}I^-$ accumulation in peripheral tissues was significantly greater in I_2 pretreated animals versus both control and I^- animals at the dose of 1 ppm. Peripheral distribution of $125\text{-}I^-$ in 100 ppm I^- pretreated rats converges with levels observed in the I_2 group. This suggests that a physiological adaptation in iodine metabolism is induced by low doses of I_2 that is not observed with I^-. (Supported by NASA Grant NO. NAG 9-226).

49.4

REGULATION OF CARDIAC MYOSIN HEAVY CHAIN GENE EXPRESSION. Eugene Morkin, Joseph J. Bahl* and Bruce E. Markham.* University Heart Center, University of Arizona, Tucson, AZ 85724

3,5,3'-Triido-L-thyronine (T_3) is an important regulator of cardiac myosin heavy chain (MHC) gene expression. Recent studies have established more clearly the molecular basis of this control by transfection of plasmids containing α-MHC 5' flanking sequences fused to the chloramphenicol acetyltransferase (CAT) gene into cultured fetal rat heart cells. The response time and doses of T_3 required for induction of CAT activity and α-MHC mRNA were similar, suggesting that the synthetic and endogenous genes have common mechanisms of control. The role of the product of the proto-oncogene, c-erb-A, in control of this process has been established by showing that the activity of α-MHC/CAT can be blocked by co-transfection of a clone encoding the anti-sense message for c-erb-A. Progressive deletions of α-MHC 5' flanking sequences indicate that more than one region is required for control. A protein factor which binds to one of these regions has been identified as have several possible binding sites for the c-erb-A product.

49.6

MOLECULAR ANALYSES OF THE ATRIAL NATRIURETIC FACTOR GENE C.E. Seidman*, J.A. Jarcho* and R. Fenton* (SPON. S. Chien). Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115.

To understand the genetic basis for regulated expression of Atrial Natriuretic Factor (ANF), we have analyzed sequences 5' of the ANF gene for their ability to promote cardiac-specific expression and appropriate developmental regulation of the prokaryotic marker protein chloramphenicol acetyltransferase (CAT). Hybrid genes containing putative regulatory sequences derived from the rat ANF gene were fused to CAT gene sequences and transfected into primary cultured atrial or ventricular cells or used to produce transgenic mice. A region bearing 1.5 kb of sequences 5' of the ANF gene was found to direct high level CAT expression in adult atria, lov level expression in the ventricles and even lower levels in the hypothalamus. Developmental analyses of transgenic mice bearing rat ANF-CAT sequences showed early, high level transgene expression in fetal atrial and ventricular tissues but marked reduction of ventricular expression following birth. Further studies showed differences in the perinatal ventricular expression of the rat ANF transgene compared with the endogenous murine ANF gene. These data demonstrate that the cis-acting signals required for correct tissue specificity and developmental regulation of the rat ANF gene are encoded in 5' sequences which act in a dominant fashion in the transcriptional milieu of the murine ventricle.

49.7

THE MOLECULAR BIOLOGY OF ANGIOGENIN. James F. Riordan* (SPON: S. Chien). Ctr. Biochem. Biophys. Sci. & Med., Harvard Med. Sch., Boston, MA 02115

Angiogenin, a single-chain polypeptide (M_r=14,124) is a potent inducer of neovascularization on the chick chorio-allantoic membrane. Its amino acid sequence is highly homologous to that of pancreatic ribonuclease and it exhibits a specific ribonucleolytic activity that is related to its biological function. Originally obtained from medium conditioned by human HT-29 tumor cells, angiogenin was subsequently isolated from normal human plasma. In order to obtain sufficient material for detailed studies, mammalian cell expression systems were investigated. Baby hamster kidney cells were transformed with DNA sequences derived from the gene for angiogenin and expression was controlled by the mouse metallothionein I promoter. The recombinant protein isolated from the cell culture medium was identical with authentic angiogenin. Even greater quantities of material have been obtained from E. coli cells transformed with a synthetic gene coding for angiogenin. The availability of these recombinant expression systems has made it possible to examine structural features of angiogenin responsible for its function. Site-directed mutagenesis has been used to identify residues involved in both enzymatic and angiogenic activity. In particular, replacement of Asp-116 by Asn, Ala, or His markedly enhances both activities while replacement of Lys-40 by Gln abolishes them. These data support a critical link between the two activities of angiogenin.

CORONARY

53.1

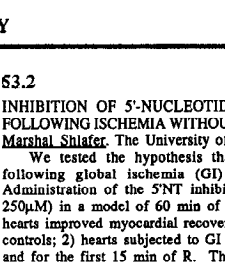
MYOCARDIAL INFARCTION DOES NOT PRODUCE APICAL DENERVATION. John Bianchi, James M. Levett, and Candyce A. McClernan. Deborah Research Institute, Browns Mills, NJ. 08015

Myocardial Infarction (MI) has been reported to denervate myocardium apical to the MI. This study was to determine biochemically, electrophysiologically, and histologically if this is true. Eight dogs received latex injection ($0.1\text{-}0.3cm^3$) in medial branch of left anterior descending artery producing a homogeneous MI. Epicardial wires were placed in a line from base to apex. Strength interval curves were produced by extra stimulus technique, 1-2 x/wk. After 28d, hearts were removed under pentobarbital anesthesia and samples of 4 areas, base to apex were taken. Biochemical and histological studies were performed. It was determined that infarction produced no decrease of norepinephrine, expected if neural processes were destroyed. Nor was there evidence of changes in receptor density in tissue apical to MI. Adenylate cyclase activity did not increase in apical myocardium when compared to normal. O_2 consumption indicated that denervation did not occur as adrenergic stimulation of this tissue did not produce an increase. Histology studies confirmed that intact neural processes were depleted of neuronal content, calcitonin gene related peptide and neural peptide y. Our study confirms that infarction does not result in denervation of myocardium, persay; but, histological evidence suggests a derangement in neuronal activity which can contribute to arrhythmogenesis.

53.2

INHIBITION OF 5'-NUCLEOTIDASE (5'NT) IMPROVES MYOCARDIAL RECOVERY FOLLOWING ISCHEMIA WITHOUT INCREASING ATP CONCENTRATION. John R. Forder*, Marshal Shlafer. The University of Michigan, Ann Arbor, MI 48109

We tested the hypothesis that inhibiting 5'NT would improve myocardial function following global ischemia (GI) and reperfusion (R) by increasing ATP repletion. Administration of the 5'NT inhibitor alpha-ß-methyleneadenosine-5'-diphosphate (AMP-CP; 250μM) in a model of 60 min of normothermic GI and R of isolated buffer-perfused rabbit hearts improved myocardial recovery. We studied three groups of hearts: 1) 2hr nonischemic controls; 2) hearts subjected to GI and R; and 3) administration of AMP-CP 5 min before GI and for the first 15 min of R. This AMP-CP concentration inhibited (>95%) purified 5'NT in vitro. AMP-CP reduced amounts of hypoxanthine (HX) and inosine (INO) efflux (measured in the coronary effluent) before GI, compared to untreated hearts (see graph), suggesting an alteration in purine degradation; effluent adenosine (ADO) levels were elevated in the AMP-CP group. Upon R, there was a significant increase in HX and INO efflux in all ischemic hearts. There were no between-group differences in HX or ADO efflux during R; INO efflux remained lower in the AMP-CP group. Left ventricular developed pressure (LVDP) declined from 92±5 mmHg preischemic period to 1±1 mmHg during R in nontreated hearts ($p<0.001$). Postischemic recovery of LVDP of AMP-CP treated hearts was greater (23±3 mmHg; $p<0.05$), as was the recovery of dP/dt (45±6% vs 3±2%; $p<0.01$). AMP-CP significantly reduced creatine kinase loss from the hearts (900±56 vs 2100±480 μg PO4/min/g tissue wet wgt; $p<0.01$). There were no between-group differences in intracellular ATP, phosphocreatine, or pH during GI or R (simultaneous 31P-NMR assessment). We conclude that: AMP-CP provides modest cardioprotection under conditions of severe GI; the beneficial effect of AMP-CP is not due to increased ATP concentration; and under the conditions imposed, AMP-CP may increase ATP synthesis (but not concentration) upon R.



(Supported by a grant from the American Heart Association of Michigan)

53.3

PROSTAGLANDIN E1 (PGE1), SIGNIFICANTLY ATTENUATES PROLONGED POSTISCHEMIC CONTRACTILE DYSFUNCTION IN THE STUNNED MYOCARDIUM. Neil E. Farber* and Garrett J. Gross. Medical College of Wisconsin, Milwaukee, WI 53226

Previous work has shown that treatment with certain vasodilators (eg., iloprost and papaverine) results in enhanced myocardial postischemic functional recovery after a brief coronary occlusion (OCC) and subsequent reperfusion (REP) which, following other vasodilators (eg., sodium nitroprusside) or in untreated animals, exhibits prolonged contractile abnormalities in the absence of tissue necrosis (stunned myocardium). While the prostanoid vasodilator, PGE1 has been shown to decrease infarct size, its role in the stunned myocardium is unknown. In this study, the effect of intravenous PGE1 (1.0 μg/kg/min) on the recovery of segment shortening (%SS) after 15 min OCC and 3 hr REP was compared to a control, saline-treated (SAL) group in anesthetized dogs. PGE1 was infused 15 min prior to and throughout OCC. Risk areas, myocardial blood flow and %SS prior to and during OCC were similar in all groups. PGE1 decreased arterial pressure during OCC and early reperfusion (30 min) and significantly improved %SS throughout REP without affecting normal area %SS. Ischemic %SS; * P<0.05 vs C group.

	CONTROL	OCC	30 min	1 hour	2 hour	3 hour
SAL	19±1	-7±1	7±2	8±2	6±2	4±2
PGE1	24±1	-9±2	17±1*	17±2*	15±2*	14±2*

PGE1 attenuates the stunning phenomenon possibly by both hemodynamic and cytoprotective effects and may have potential therapeutic benefit for the treatment of transient ischemic episodes.

53.4

ALLOPURINOL IMPROVES REPERFUSION IN A XANTHINE OXIDASE FREE MODEL (PIG) OF REVERSIBLE MYOCARDIAL ISCHEMIA. You Su Sun*, Roshanak Etemad-Moghadam*, Ron F. Morrison*, Robert M. Lust. East Carolina Univ. School of Medicine, Greenville, NC 27858

Allopurinol (ALO) has been shown to reduce reperfusion injury following myocardial ischemia, suggesting involvement of xanthine oxidase (XO) mediated substrates. To determine if the protective effects of ALO were due to inhibition of XO, and whether pretreatment was necessary, reversible ischemia was produced in 11 domestic pigs. An 8 minute occlusion/4 hr reperfusion of the circumflex coronary artery was used. LV and aortic pressure, ekg, and regional wall motion (sonomicrometry) were monitored throughout. Regional blood flow (microspheres) were obtained before, during and 5, 10 and 30 minutes after ischemia. At the time of occlusion, 45 minute i.v. infusions of ALO (5mg/kg, n=6) or saline (equal volume, n=5) were begun. Occlusion decreased transmural flow at the midpapillary level by 75% (0.28 vs 1.10 ml/min/g, p<.01). ALO was associated with a mild, generalized hyperemia at five minutes (ischemic zone: 1.44 vs 1.10 ml/min/g, p<.01) which had returned to control levels at 10 and 30 minutes. In contrast, saline was associated with only 80% restoration of resting flow at 5 mins. (0.84 vs 1.10 ml/min/g, p<.05) which decreased and stabilized at 63% of control at 10 and 30 mins. Since pigs have no detectable levels of XO activity, ALO must exert protection by some other mechanism. Since protection was observed without pretreatment, beneficial influences are not necessarily the result of ALO degradation products.

53.5

FERRITIN-BOUND IRON CATALYZES THE DEGRADATION OF DEOXYRIBOSE BY HYDROGEN PEROXIDE. W. Inauen*, P.R. Kvietys and M.B. Grisham. Dept. of Physiology and Biophysics, LSU Medical Center, Shreveport, LA 71130.

We have recently demonstrated that hydrogen peroxide (H_2O_2) interacts with intracellular iron (Fe) to generate a potent oxidant that injures cultured endothelial cells. Because cells contain very little, low molecular weight Fe we proposed that ferritin, the Fe-storage protein, may act as an intracellular source of Fe. The objective of this study was to assess the ability of ferritin-bound Fe to catalyze the H_2O_2-dependent formation of hydroxyl radical ($^{\cdot}OH$) in vitro. Hydroxyl radical was detected by its ability to degrade deoxyribose (DR) to yield malondialdehyde (MDA). Incubation of ferritin (0.5 mg/ml) with H_2O_2 (1mM), DR (5mM), EDTA (0.1mM) and phosphate buffer (10mM; pH 7.4) for 30 min, 37°C produced 9.0 ± 0.16 nmoles MDA/ml. Omission of H_2O_2, ferritin or EDTA produced approximately 80% inhibition of MDA formation. Control studies demonstrated that the requirement for EDTA was not due to its ability to remove Fe from ferritin. Addition of catalase (15 μg/ml), SOD (100 μg/ml), deferoxamine (0.05mM), DMSO (20mM) or DMTU (20mM) resulted in 70-80% inhibition suggesting that iron, H_2O_2 and superoxide ($O_2^{\cdot-}$) were required for $^{\cdot}OH$-mediated degradation of DR. Taken together, our data suggest that H_2O_2 interacts with ferritin-associated Fe to produce $^{\cdot}OH$ via the formation of $O_2^{\cdot-}$. We propose that ferritin-bound Fe may act as an intracellular catalyst for the production of cytotoxic oxidants. This work was supported by a grant from the NIH (DK 39168).

53.6

PROTECTION AGAINST SUPEROXIDE INACTIVATION OF CA PUMP IN ENDOPLASMIC RETICULUM OF PIG CORONARY ARTERY. A.K. Grover & S.E. Samson. Department of Neurosciences, McMaster University, Faculty of Health Sciences, Hamilton, Ontario. L8N 3Z5

Superoxide inactivates the 100 kDa subunit Ca pump in the endoplasmic reticulum (ER) enriched fraction of pig coronary artery smooth muscle. Pig coronary artery smooth muscle also contains superoxide dismutase (SOD). Cu-Zn-SOD is distributed primarily in the soluble (SOL) fraction. Some Cu-Zn-SOD and a small amount of Mn-SOD are also present in a fraction enriched in mitochondria but also containing other fractions. The plasma membrane (PM) and the ER enriched fractions contain little SOD. Addition of SOL to ER caused protection of the inactivation of the Ca-pump by two mechanisms. The first mechanism is the superoxide dismutation by the SOD present in SOL. The second is due to the presence of DTT used in the homogenization medium and thus present in SOL. The protection due to DTT is not due to its action as a scavenger for superoxide. We conclude that superoxide inactivates the Ca-pump by acting directly or indirectly on one of the sulfhydryl groups of this protein since PCMB and DTNB also inactivate the pump and DTT protects from the superoxide inactivation. Supported by Heart and Stroke Foundation of Ontario.

53.7

COCAINE'S CORONARY AND CARDIOVASCULAR ACTIONS. Gary F. Merrill, Hwu Meei Wei* and Greg Fredericks* Rutgers University, New Brunswick, N.J. 08903

We have investigated the coronary and cardiovascular actions of cocaine in anesthetized, instrumented dogs (n=5) and pigs (n=5) and in isolated, Krebs-Ringer perfused guinea pig hearts (n=15). In dogs and pigs we have compared the intravenous (iv) and intracoronary (ic) routes of administration employing a dose range of 0.02-20.0 mg/kg. Under the latter conditions, no animal survived a cumulative dose greater than 400 mg (iv) or 10 mg (ic) for more than 30 minutes. Cumulative doses of 5-10 mg (ic) produced sudden cardiac death within one minute of administration . In dogs and pigs the coronary vascular response to cocaine was biphasic and dose-dependent. Initial, transient vasodilation was followed by more sustained, but temporary, vasoconstriction. In isolated guinea pig hearts, cocaine caused both coronary vasodilation (n=5) and vasoconstriction (n=5). The cardiac and coronary vascular responses to isoproternol in the latter preparation were intensified by cocaine. We conclude that the coronary and cardiovascular effects of cocaine are 1) multiple, 2) dose-dependent, 3) temporally related and 4) probably mediated by both direct and indirect mechanisms. More work is needed in this area.

53.8

COMBINATION OF THE THROMBOXANE RECEPTOR ANTAGONIST, SULOTROBAN (BM 13.177), WITH STREPTOKINASE: DEMONSTRATION OF THROMBOLYTIC SYNERGY. G.A. Kopia* and L.J. Kopaciewicz* (SPON: R.R. Ruffolo, Jr.) Smith Kline & French Laboratories, King of Prussia, PA 19406.

We examined the ability of the thromboxane/endoperoxide antagonist, sulotroban (BM) to enhance the thrombolytic efficacy of a minimally effective thrombolytic dose of streptokinase (SK). Thrombi were formed in the left circumflex (LCX) coronary artery of anesthetized open chest dogs by applying a 150μA anodal current to a wire placed within the lumen of the LCX. A critical stenosis sufficient to just abolish the hyperemic response to a 20 sec total occlusion was placed on the LCX just distal to the current wire using an adjustable screw occluder clamp. Animals were then given either SK (20,000 IU bolus + 2000 IU/min X 180 min, n=10) or SK+BM (5 mg/kg bolus + 5 mg/kg/hr, n=10). A second study was performed wherein animals received either the same dose of SK used above + heparin (H, 300 IU/kg bolus + 100 IU/kg/hr, n=9) or SK+H+BM (n=9). Of 10 animals receiving SK alone only 1 reperfused at 55 min after the start of SK infusion. Conversely, 9 of 10 animals receiving SK+BM reperfused at 79.4±10.5 min post-SK (p<.05). In the second study, 8/9 animals receiving SK+H (89%) reperfused in an average of 66.8±8.6 min after the start of SK infusion. Reperfusion incidence was similar in animals receiving SK+H+BM (9/9, 100%, p=NS) but occurred more rapidly after the start of SK infusion (25.4±5.2 min, p<.05 from SK+H group). BM alone failed to produce reperfusion in this animal model (0/6 animals reperfused). We conclude that BM can enhance the efficacy of even minimally effective thrombolytic doses of SK and can significantly shorten the time to SK-induced reperfusion in the presence of H.

53.9

PERSISTENT RIGHT CORONARY FLOW RESERVE DURING SEVERE NONISCHEMIC MYOCARDIAL HYPOXIA. H. Fred Downey, Hironori Murakami,* and Arthur G. Williams, Jr.* Tex. Col. Osteopath. Med., Ft. Worth, TX 76107

We recently described persistent right coronary flow reserve at low coronary perfusion pressure (FASEB J. 2:303,1988). The present study was conducted to determine if a flow reserve persisted during severe hypoxemia. The right coronary artery of 5 anesthetized dogs was cannulated and perfused at 100 mmHg with either normal arterial blood (control condition) or with blood containing less than 1 ml O_2/100 ml blood (hypoxemia). Blood was deoxygenated by a pediatric oxygenator supplied with 5% CO_2, 95% N_2. Venous blood was collected from a right coronary vein, so right ventricular oxygen consumption could be determined. Under control conditions, right coronary blood flow was 39±7 ml/min/100 g, and right ventricular oxygen consumption was 3.5±0.4 ml O_2/min/100 g. During hypoxemia, right coronary blood flow increased by 533±72%, but right ventricular oxygen consumption fell by -80±4%. Although oxygen supply was not sufficient to meet right ventricular oxygen demand, intracoronary infusion of adenosine caused a further 21±7% increase in coronary blood flow. Although severe hypoxemia caused pronounced dilation of the right coronary vasculature, a significant flow reserve remained. (Supported by HL-35027)

53.10

THE EFFECTS OF AGE ON CORONARY COLLATERAL DEVELOPMENT IN THE MINIPIG. S. Dobbs-Soanes*, F. White, D. Roth, C. Bloor. UCSD School of Medicine, La Jolla, CA 92093.

We studied growing [G] and mature [M] minipigs to determine the effect of age on coronary collateral development. Coronary collateral reserve [CCR] was measured using three methods (exercise [Ex], adenosine infusion [AI], and pacing [P]). Nine G (0.7±0.1 year) and eleven M (4.4±.09 years) pigs were studied 9±1 weeks following placement of an ameroid occluder on the left circumflex [LC] coronary artery. We measured endocardial blood flow [Qen] with radiolabeled microspheres during EX (heart rate 263±4 beats per minute [BPM], mean arterial blood pressure = 138±7 mmHg), AI (1mg/kg/min), and pacing (180 BPM). CCR during AI [AIR], the ratios of Qen in the ischemic and nonischemic myocardium, and transmural infarct percentages [% INFt] as a % of the LC bed were as follows:

	P Qen (ml/min/g)	EX Qen	AIR (mmHg/ml/min/g)	% INFT
G	0.33±0.04(n=4)	0.38±0.08(n=5)	3.15±0.45 (n=7)	3.03±0.45(n=9)
M	0.38±0.06(n=6)	0.27±0.07(n=5)	3.25±0.43(n=11)	3.33±0.39 (n=10)

There were no signficicant differences between G amd M pigs in any of the measured parameters. Thus normal myocardial growth does not affect coronary collateral development in the minipig.

53.11

EFFECT OF HALOTHANE ON HUMAN CORONARY ARTERY RINGS VASOMOTION. G. Blaise*, D. Girard*, C.Hollmann*, J. Buluran* and R. Meloche* (SPON: H. Brunnengraber). University of Montreal, Notre-Dame Hospital, Dept. of Anesthesiology, Montreal, Quebec, H2L 4M1, CANADA.

Increase in coronary tone and spasm in response to different vasoconstrictors are important features of coronary artery disease. These events can occur during anesthesia. We studied the response of human coronary artery rings to 3 mediators of spasm (serotonin, histamine and PGF2-alpha) in the presence and absence of halothane, a volatile anesthetic. Human hearts were harvested during organ transplantation. Coronary arteries (RCA, LAD and LCX) were dissected, cut into rings, suspended between two stirrups and introduced in organ chambers filled with oxygenated Krebs-Ringer solution. The rings were connected to a transducer for isometric force measurements and stretched to their optimal tension. The response to serotonin, histamine, PGF2-alpha were determined in control and halothane treated rings. At the end of the experiment, the rings were sent for histological studies. The results showed that serotonin, histamine, and PGF2-alpha are potent vasoconstrictors of human coronary artery rings; The potency being PGF2-alpha > histamine > serotonin. Halothane depresses the response to these agonists, suggesting that halothane can prevent or treat spasm during anesthesia. (Funded by the Medical Research Council of Canada and the Canadian Heart Foundation Grants).

53.12

A METHOD FOR CHARACTERIZING THE SPATIAL DISTRIBUTION OF REGIONAL TRANSCORONARY TRANSFER FUNCTIONS USING FAST CT. Malcolm R. Bell,* Wolfgang J. T. Spyra,* Xuesi Wu,* Paul J. Thomas,* and Erik L. Ritman. Mayo Clinic, Rochester, MN 55905

The transfer function (TF), or impulse response, of an organ describes the probability density function of all transit times through a vascular bed. The frequency distribution of these transit times is characterized in part by mean transit time (MTT). A deconvolution technique to estimate canine transcoronary (global) TF was reported by Knopp et al. (Ann Biomed Eng 1976;4:44) using indocyanine-green whereby dye dilution curves (DC) of aortic root and coronary sinus were used as input (C_{in}) and output (C_{out}) functions, respectively. To test whether Fast CT can characterize TF in an analogous manner, 6 closed-chest dogs were anesthetized and 30 cc of Iohexol injected over 2 s into the aortic root during a 20 s sequence of 60/s scans. DCs were generated from the aortic root (C_{in}) and in 4 quadrants of myocardium (C_{out}) during control and maximally vasodilated states. Employing the Knopp method, regional TF and MTT in 24 different regions (mean 3.7±0.5 s during control and 2.4±0.2 s during vasodilated states) were derived. Skewness of the TFs was similar to those described by Knopp and, if normalized for MTT, regional TF curves were coincident to each other. The amplitude of TFs of vasodilated regions were greater than control with a greater proportion of shorter pathways represented. We conclude that Fast CT may be used to determine regional transcoronary TF.

MECHANICS OF BREATHING I

54.1

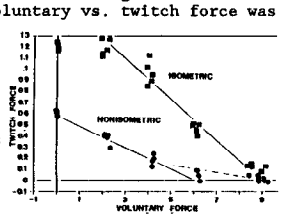
DIAPHRAGM BLOOD FLOW (Q_{di}) DURING NORMOXIC AND HYPOXIC CONDITIONS AT ISO-DIAPHRAGM WORK. **A. Comtois*, F. Hu* and A.E. Grassino. Meakins-Christie Laboratories, McGill University, and Notre-Dame Hospital, University of Montreal, Quebec, Canada.**

Hypoxia has been shown to increase Qdi. However, these increases in Qdi were caused mostly by augmented work of breathing induced by the hypoxic stimulus. The purpose of this study was to investigate the effect of hypoxia on Qdi under iso-work conditions. We hypothesized that low levels of P_{O2} would increase conductance to Qdi. In 5 anaesthetized dogs, left phrenic artery blood flow (Qpha) was measured (Doppler) during various levels of phrenic nerve stimulation. Pacing frequency was set at 10/min and duty cycle at 0.25, 0.50 or 0.75 during inhalation of room air or 7.6% O_2 in N_2. Changes in Qpha during normoxia was parabolic function of TTdi. This relationship was also seen for hypoxia, but was slightly shifted upward and to the left. TTdi optimal at which Qdi reached a maximum was thus lower during hypoxia. Vascular conductance was slightly higher during hypoxia while VO_2 of diaphragm remained unchanged from normoxia. We conclude: 1) Qpha is a function of TTdi both during normoxia and hypoxia; 2) Hypoxia affects directly smooth muscles of the diaphragmatic vascular bed and 3) Vascular conductance change maintains constant diaphragmatic VO_2. (Supported by the Medical Research Council of Canada.)

54.2

RELATIONSHIP OF TWITCH FORCE TO VOLUNTARY FORCE DURING NON-ISOMETRIC CONTRACTIONS. S.H. Loring, and M.B. Hershenson*, Harvard Schools of Medicine & Public Health, Boston, MA 02115

Activation of the diaphragm has been assessed by phrenic stimulation. The increase in pressure caused by a supramaximal shock to the phrenic nerve (P_{di} twitch) decreases linearly with increasing voluntary effort, so that the extrapolated value of voluntary P_{di} for zero P_{di} twitch equals maximal voluntary P_{di} ($P_{di}max$). Linear extrapolation might not be valid, however, when muscle contraction is not isometric. To test this, we examined the relationship between voluntary and twitch force of the adductor pollicis during ulnar verve stimulation in "isometric" contractions against a steel wire and non-isometric contractions against stretched rubber tubing (compliance = .2 cm/N). Muscle length was held constant during voluntary contractions. During non-isometric contractions (Fig.) the plot of voluntary vs. twitch force was concave upward, so that if voluntary force were limited to submaximal values, the extrapolated maximal force would be falsely low. We speculate that diaphragmatic contraction against a compliant rib cage may lead to the erroneous conclusion that that diaphragm is maximally active during inspiratory efforts when P_{di} is submaximal. HL33009, HL07633.



54.3

MECHANICAL ARRANGEMENT OF THE PARASTERNAL INTERCOSTALS IN THE DIFFERENT INTERSPACES. A. De Troyer and G.A. Farkas. Thoracic Diseases Research Unit, Mayo Clinic and Foundation, Rochester, MN.

We have previously demonstrated that the shortening of the canine parasternal intercostals during inspiration results mostly from the muscles' own activation (JAP 64:1546, 1988). When a parasternal intercostal is selectively denervated in phrenicotomized animals, however, it continues to shorten with inspiration. In the present studies, we have tested the hypothesis that this persistent inspiratory shortening results from the action of the parasternals located in the other interspaces. The third right parasternal was denervated in eight supine phrenicotomized dogs, and changes in length of the muscle were measured by sonomicrometry. The inspiratory muscle shortening increased after denervation of the third left parasternal. However, it gradually decreased as the parasternals situated in the second, fourth, and fifth interspaces were successively denervated. Stimulating selectively the third left parasternal in the apneic animal produced muscle lengthening, whereas bilateral stimulation of the parasternals in either the second or the fourth interspace promoted muscle shortening. We conclude that: 1) The two parasternals situated in the same interspace on both sides of the sternum are mechanically arranged in series; 2) The parasternals located in adjacent interspaces are mechanically arranged in parallel. (Supported by NIH grant HL21584).

54.4

CONTRIBUTION OF RIB CAGE AND ABDOMINAL EXPIRATORY MUSCLES TO TIDAL VOLUME IN HEAD UP DOGS. G.A. Farkas, M. Estenne, and A. De Troyer. Thoracic Diseases Research Unit, Mayo Clinic and Foundation, Rochester, MN; and Erasme Univ. Hospital, Brussels, Belgium.

When anesthetized dogs are tilted from the supine to the head up posture, there is considerable recruitment of the rib cage and abdominal expiratory muscles. However, when this postural change is produced over a 2-3 sec. period, there is an initial apnea during which all muscles are silent; quiet breathing subsequently resumes with phasic expiratory muscle activation. In the present studies, we took advantage of this initial electrical silence to establish the change in end-expiratory lung volume (relative to passive FRC) produced by the expiratory muscles in head up posture; this change, in turn, represents the expiratory muscle contribution to tidal volume (V_t). Eight animals were studied. V_t in head up posture was (mean $\pm$ SE) 515 $\pm$ 77 ml, and the expiratory muscle contribution amounted to 329 $\pm$ 70 ml (62 $\pm$ 6% V_t). When the internal intercostal nerves in interspaces 3 to 8 were sectioned at the rib angles so as to denervate the rib cage expiratory muscles, the expiratory muscle contribution to V_t was still 243 $\pm$ 84 ml (49 $\pm$ 10% V_t). Therefore, the contribution of the rib cage expiratory muscles initially was only 54 $\pm$ 19 ml (11 $\pm$ 4% V_t). We conclude that in head up tilted dogs the abdominal muscles, but not the rib cage expiratory muscles, contribute a very substantial fraction of tidal volume. (Supported by NIH grant HL21584).

54.5

SHIFTS IN THE MAXIMUM PRESSURE-FLOW RELATIONSHIP AFTER INSPIRATORY MUSCLE FATIGUE. T.L. Clanton, B.T. Ameredes, and S.G. Kelsen, The Ohio State University, Columbus, OH, 43210 and Temple University Sch. of Med., Philadelphia, PA 19140.

Four normal human subjects performed maximum dynamic inspirations from a constant flow generator at mouth flows ranging from 0 to 3.5 L/sec. Changes in thoracic volume and thoracic flow were measured with a volume plethysmograph and plotted against mouth pressure. Measurements were made in the resting state and in the sustainable portion of an isoflow endurance test. The endurance test consisted of repeated maximum dynamic inspirations at a mouth flow of 1.0 L/sec (T_I = 2 sec; T_{TOT} = 4 sec). After 10 min, a sustainable pressure development was reached and flow was changed, for single breaths, in a random fashion every 3rd to 5th breath. RESULTS: At a sustainable level of inspiratory muscle fatigue, the maximum pressure-flow relationship was shifted, at each lung volume studied, down and to the left, in a parallel fashion. There were no significant changes in slope. However, the y-intercept (max. flow) was reduced from 9.5±2.6 SD to 6.3±2.4 L/sec ($p < 0.05$) at a lung volume of 0.5 L above relaxation volume, and from 8.6±4.0 to 5.5± 2.0 L/sec ($p<0.5$) at 1.0 L above relaxation volume. CONCLUSIONS: At a sustainable level of inspiratory muscle fatigue, the maximum pressure-flow relationship is shifted in a parallel fashion such that the capacity to generate peak flow is greatly reduced. NIH HL34770-03.

54.6

THE RELATIONSHIP OF EXERCISE INDUCED DYSPNEA TO CHANGES IN PLEURAL PRESSURE IN NORMALS AND COPD PATIENTS. **S. Burke*, P. Begin* and A.E. Grassino. Notre-Dame Hospital, University of Montreal, Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada.**

Previous studies have found a direct relationship between dyspnea and pleural pressure (Ppl) swings. This study examines the sensitivity (the Ppl) at which dyspnea is first perceived and response, the ratio between dyspnea level and Ppl swings. Six COPD and five normal subjects performed graded exercise tests on a cycle ergometer with inspiratory flow, pleural and gastric pressures measured. Rib cage (RC) and abdominal (Ab) excursions were measured as well. Dyspnea was rated inspiratory effort sensation (IES) levels using a Borg scale. All subjects demonstrated a direct relationship between IES and Ppl. Sensitivity thresholds were similar amongst normal subjects and in patients were equal or higher than normals. Although response was similar in all normal subjects, COPD patients showed two main patterns: low and high responders. The low response seems to be related to the ability of the patients to suddenly release abdominal muscle tone at the onset of inspiration. We conclude that normal subjects have similar sensitivity thresholds. Dyspnea is linearly related to Ppl. COPD patients demonstrate different sensitivity thresholds and varying response. The causes for such behaviour may be related to the chest wall/lung impedance, degree of hyperinflation and mainly to coordination of chest wall muscles.
(Supported by Medical Research Council of Canada.)

54.7

DIAPHRAGMATIC CONTRIBUTION TO TIDAL VOLUME ESTIMATED FROM FLUOROSCOPY OF THE DIAPHRAGM: Knight H*, Petroll WM*, Rochester, DF. University of Virginia School of Medicine, Charlottesville VA 22908. We hypothesized that the contribution of the diaphragm to inspired volume could be estimated from the product of the diaphragm's descent and the cross-sectional area (CSA) of the thorax at the level of the diaphragm. In 4 supine, anesthetized dogs, AP fluoroscopy of the right hemidiaphragm was recorded during two tidal breaths (range 270-330 ml). From fluoroscopic images at FRC and end-inspiration we measured the vertical descent of the diaphragm dome in the midline, and of the lateral costophrenic angle (CPA). CSA was approximated by πr^2, where r was the horizontal distance between the midline and the lateral chest wall. Diaphragm contribution to tidal volume was calculated as the product of CSA and descent of either the dome or the CPA. Mean values for the descent of the dome and CPA were 0.89 $\pm$ S.D. 0.32 cm and 1.50 $\pm$ S.D. 0.45 cm respectively. Diaphragmatic contribution to tidal volume based upon dome descent ranged from 26-84% of V_T (mean 50%). Volume contribution calculated from CPA descent was significantly greater in each case and on occasion exceeded 100% of V_T. Calculations done from CT scans in a single dog support the validity of the estimates from dome descent. The fact that CPA descends more than the dome may imply a change in shape of the diaphragm. We conclude that fluoroscopy of the descent of the diaphragm dome can be used to estimate diaphragmatic volume contribution.
Supported by BRSG-2-507-RR05431-26

54.8

BRONCHIAL VASCULAR HYPERPERMEABILITY ACCOMPANIES HYPERPNEA-INDUCED BRONCHOCONSTRICTION (HIB) IN GUINEA PIGS DW Ray*, CM Doerschuk*, M Jackson*, C Hernandez,* S Eappen*, AR Leff, and J Solway. Dept. of Med., Univ. of Chicago, Chicago, IL; and Dept. of Path., Univ. of British Columbia, Vancouver, B.C.

To evaluate whether vascular hyperpermeability accompanies HIB in guinea pigs (GP), we performed 5 types of experiments in 23 anesthetized GP given 0.5 ml Monastral Blue (3%) suspension (MB) iv. (i) *Negative Control*: 3 GP were mechanically ventilated (6 ml/kg, 60 br/min) for 20 min, then exsanguinated and their lungs removed. During this period, no change in respiratory resistance (Rrs) occurred and no MB staining of the central airways was found. (ii) *Positive Control*: 3 GP also received capsaicin 50 mg/kg sc at the start of the 20 min ventilation period. Rrs rose 7.5-fold, and MB staining was evident from the trachea through generations 3-4. (iii) *Dry Gas Hyperpnea (HYP)*: 12 GP received 5 min of eucapnic HYP (f=150 br/min) with V_T 3-6 ml, followed by 15 min control ventilation. Peak Rrs after HYP rose with V_T during HYP. MB stained the central airways, but neither the intensity nor extent of staining correlated with V_T during HYP. 2 GP ventilated with 3 and 4 ml had little bronchoconstriction and no MB staining. (iv) *Humid Gas HYP*: 3 GP received eucapnic HYP with humid gas (5 ml), and displayed reduced HIB (peak Rrs less than 1/5 that of dry gas GP) and little or no MB staining. (v) *Tachykinin Depleted*: 2 GP received capsaicin 50 mg/kg sc 1 week prior to study, and received dry gas HYP using 5 ml V_T. Peak Rrs was reduced to 1/4 that of non-pretreated GP, but similar MB staining was observed. These results show that bronchial vascular hyperpermeability accompanies HIB in GP, and that vascular and airway responses to dry air hyperpnea have differing stimulus-response relationships. Supp. by HL34702, HL07432, HL32495, the Trudeau Scholar Award, and the Whitaker Fnd.

54.9

INTRAVENOUS PAF-ACETHER CAUSES BRONCHOCONSTRICTION WITHOUT BRONCHIAL HYPERRESPONSIVENESS IN SHEEP. Tahir Ahmed and Alfredo Fernandez*. Mount Sinai Medical Center, Miami Beach, FL 33140.

We studied the effects of intravenous PAF on airway mechanics and bronchial responsiveness to carbachol in sheep. We measured specific lung resistance (SR_L=lung resistance x thoracic gas volume) in ten unsedated sheep before, immediately after and serially up to 2 hours following an intravenous injection of PAF (0.3 ug/kg). Bronchial responsiveness to carbachol was expressed as the cumulative provocating dose of carbachol (in breath units) which increased SR_L to 4 cm $H_2O \cdot sec^{-1}$ (PD_4); one breath unit was defined as one breath of 1% carbachol solution. PD_4 was determined on a control day, 2 hours and 48 hours after PAF. Mean $\pm SD$ SR_L increased from 0.85±.14 before to 4.38±2.07 cm $H_2O \cdot sec^{-1}$ immediately after PAF, while Cdyn decreased from 0.08±.03 to 0.04±.02 L·cm H_2O^{-1} ($P<.05$). SR_L returned to baseline by one hour, while Cdyn was still 79% of baseline at 2 hours. Platelet and WBC counts decreased to 56% and 39% of baseline, respectively ($P<.05$). PAF had no significant effect on carbachol responsiveness: Mean PD_4 was 33±15 breath units on the control day, 33±16 breath units at 2 hours and 42±24 breath units at 48 hours post-PAF. We conclude that in sheep, intravenous PAF causes marked bronchoconstriction, leukopenia and thrombocytopenia without an increase in non-specific bronchial reactivity to carbachol.

54.10

COMPARATIVE DISTRIBUTION OF MUSCARINIC AND PARASYMPATHETIC CONTRACTILE RESPONSES IN MAJOR DIAMETER AIRWAYS OF DOGS. N.M. Munoz*, T. Shiova*, and A.R. Leff. Pulmonary and Critical Care Med. and Comm. Clin. Pharmacol., Univ. of Chicago, Chicago, IL 60637.

The distribution of muscarinic and parasympathetic contractile responses within airway generation (Gen) 0-5 was examined by tantalum bronchography in 10 anesthetized dogs in vivo. Responses were assessed as change in airway diameter (dDaw) at functional residual capacity and as change in lung resistance (R_L) obtained at the plateau of the response to each stimulus. Dose-response curves to 10^{-10} to 10^{-7} mol/kg i.v. methacholine (MCh) were generated in 5 dogs, and stimulus-response curves were generated in 5 other dogs by bilateral electrical stimulation (1-20 Hz; 20 V) of the vagus nerves. The dDaw elicited by maximal vagus nerve stimulation (10 Hz; 20 V) increased progressively from 28.8 ± 4.4% for Gen 0 to 42.4 ± 3.6% for Gen 5 airways. In contrast, a dose of MCh (10^{-7} mol/kg) causing comparable dDaw (45.6 ± 7.4%) in Gen 5 caused substantially less dDaw in Gen 0 (8.1 ± 2.6%; $P < 0.001$) and Gen 1 (21.6 ± 2.1%; $P < 0.001$) vs vagal stimulation. Vagal stimulation causing ~ 45% dDaw in Gen 5 caused increase in R_L to 1634 ± 173% of baseline value vs 981 ± 107% ($P < 0.01$) for MCh-treated dogs having the same dDaw in Gen 5. We demonstrate substantial differences between post-junctional muscarinic and parasympathetic stimulation in major diameter airways. These data indicate that parasympathetic innervation is greatest among tracheal airways and mainstem bronchi. Parasympathetic activation also differs substantially between central and peripheral airways of canine lung. [Supported by NHLBI HL-35718, HL-32495, and HL-01398].

54.11

CLINICAL UTILITY OF PARTIAL EXPIRATORY FLOW-VOLUME CURVE (PEFV) MEASUREMENTS IN THE HORSE. D.B. Tesarowski, T. Whiting*, W. McDonell* and L. Viel*. Clin. Studies, O.V.C.; Guelph, Canada N1G 2W1.

PEFV curves have been successfully used in children and to evaluate the response to bronchodilators in adults. The present study was designed to assess the stability and clinical utility of these tests in non-sedated, standing horses. Expired airflow was recorded when horses (n=21) were challenged with inhalation of 10% CO_2 in room air to simulate the forced vital capacity. The PEFV curve was evaluated by measuring expired volumes in the time domain, mean transit time, peak flow and time constant. All values with the exception of the time constant were repeatable ($r > 0.52$) and the most stable measurement was total expired volume with a within day, and between day within horse r value of 0.799 and 0.756 respectively. The measured forced vital capacities approached the values expected on the basis of comparison with other species although peak flows were 65% of predicted. Habituation to the testing procedure was not observed. The technique demonstrated good stability and is currently in use in the laboratory measuring response to therapy.

BIOTRANSFORMATION II

55.1

CHARACTERIZATION OF CYTOCHROME P-450I ISOZYMES IN HUMAN LUNG. C.W. Wheeler, S.S. Park, and T.M. Guenthner, Dept. of Pharmacology, U. of IL College of Med., Chicago IL 60680 & NCI, Bethesda MD 20205

Monooxygenase activity in the lung is of great toxicological significance. We have therefore used metabolic (Ethoxycoumarin O-deethylase (EROD) activity) and immunochemical (solid phase RIA) methods to study the pattern of cytochrome P-450 isozymes, specifically those of the P-450I (polycyclic aromatic hydrocarbon inducible) family in human lung biopsy samples. Monoclonal antibody 1-7-1, which recognizes P-450I isozymes in rat liver, recognized corresponding isozyme(s) in all human lung samples. The RIA-detected levels of these isozymes correlated highly with EROD activity (r=0.949), indicating the presence, at low levels, of cytochrome P-450I isozymes in human lung. Total cytochrome P-450 levels are low in human lung compared to nonprimate lung, but the ratio of type I isozymes to total P-450 is relatively higher in human lung. EROD activity, P-450I isozyme levels, and total P-450 content in human lung are all similar to those values found in lung microsomes from untreated baboons. Therefore, human lung does not appear to reflect a highly "PAH-induced" state. Supported by PHS CA-46129 and CA-01287.

55.2

EVIDENCE FOR STEREOSELECTIVE INHIBITION OF FERROCHELATASE (FC) BY N-ALKYLATED PORPHYRINS. S.A. McCluskey*, R.A. Whitney* and G.S. Marks. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Mechanism-based inactivation of cytochrome P-450 (P450) by 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) and related analogues results in the formation of a mixture of N-alkylprotoporphyrin IX (N-alkylPP) regioisomers. The FC-lowering activity of 4-ethyl- and 4-propyl-DDC is attributed predominantly to the formation of the A (N_A) and B (N_B) pyrrole ring substituted regioisomers of N-propylPP and N-ethylPP, respectively. The goal of this study was to determine if N_A + N_B regioisomers of synthetically and biologically generated N-alkylPPs differ with respect to their FC-inhibitory potency. Biological N-ethylPP and N-propylPP were isolated from the livers of phenobarbital-pretreated rats while synthetic compounds were prepared by heating PP and ethyl- or propyl-iodide at 100°C. The N-alkylPPs were separated into their regioisomers by HPLC. The N-alkylPP regioisomers obtained from rat livers were found to be optically active using circular dichroism spectropolarimetry; the synthetic regioisomers were optically inactive. The N_A + N_B regioisomers of synthetic N-ethylPP and N-propylPP were found to be less potent inhibitors of chick embryo hepatic FC-activity than the optically active N-alkylPP regioisomers obtained from rat livers. This suggests that the FC-binding site can better accommodate the enantiomeric form of N-ethylPP and N-propylPP derived from the inactivation of P450. (Supported by the MRC of Canada)

55.3
FERROCHELATASE (FC) INHIBITION PRODUCES A GREATER INDUCTION OF δ-AMINOLEVULINIC ACID SYNTHASE (ALAS) ACTIVITY THAN DOES SUICIDAL INACTIVATION OF CYTOCHROME P-450 (P450).
Jane E. Mackie* and Gerald S. Marks. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

This study compares the effects of three groups of compounds related to 3,5,-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) on the activity of ALAS; ALAS is controlled by heme through feedback repression. The first group of compounds lowers cellular heme levels by inhibiting FC activity and destroying the heme of P450 through suicidal inactivation. The second group lowers heme levels through FC inhibition. The third group lowers cellular heme levels by suicidal inactivation of P450. An hepatic cell culture was prepared from 17-day-old chick embryos, and the medium was changed 24 hr later. Drugs were added after a further 24 hr and ALAS activity was determined at various time points. 4-Ethyl DDC, a member of the first group, caused a peak increase in ALAS activity at 12 hr after administration (565% of control levels). N-ethylprotoporphyrin IX, a member of the second group which is produced by P450-mediated oxidation of 4-ethyl DDC, caused a peak induction of ALAS activity at 3 hr (444% of control). 4-Isobutyl DDC, a member of the third group, produced a peak induction of ALAS activity at 3 hr (210% of control). These data suggest that the inhibition of FC is the more important factor involved in induction of ALAS activity by DDC analogues. (Supported by MRC of Canada)

55.4
CYTOCHROME P-450c AS A TARGET FOR DESTRUCTION BY 4-ALKYL ANALOGUES OF 3,5-DIETHOXYCARBONYL-1,4-DIHYDRO-2,4-6-TRIMETYL-PYRIDINE (DDC). D.S. Riddick*, G.S. Marks, S.S. Park*, and H.V. Gelboin. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, K7L 3N6, and Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, MD 20892.

DDC analogues cause mechanism-based inactivation of cytochrome P-450 (P450) via heme destruction. The goal of the present work was to examine the selectivity of DDC analogues with respect to the major phenobarbital (PB)- and β-naphthoflavone (βNF)-inducible P450 isozymes of rat liver. Hepatic microsomes from uninduced, PB-induced (PB microsomes), and βNF-induced (βNF microsomes) rats were incubated with NADPH and a DDC analogue, and then analyzed for P450 content, isozyme immunoreactivity, and enzymatic activity. 4-Isopropy DDC and 4-hexyl DDC caused less P450 destruction in βNF microsomes and PB microsomes, respectively. Western blots probed with monoclonal antibodies (MAbs) to the major inducible P450 isozymes revealed that, in βNF microsomes, DDC analogues cause formation of lower molecular weight proteins showing reactivity to MAbs directed against the major βNF-inducible isozyme (P450c). DDC analogues also cause mechanism-based, NADPH-dependent loss of enzyme activity selective for P450c (7-ethoxyresorufin O-deethylase), but not that of the major PB-inducible isozyme, P450b (7-pentoxyresorufin O-dealkylase) These data suggest that P450c is a target for mechanism-based inactivation by DDC analogues. (Supported by MRC of Canada)

55.5
MOLECULAR CLONING AND SEQUENCING OF A RAT LIVER MICROSOMAL CARBOXYLESTERASE TRIFLUOROACETYLATED BY HALOTHANE. R.M. Long[1], H. Satoh[1#], B.M. Martin[2#], S. Kimura[3*], F.J. Gonzalez[3#], and L.R. Pohl[1]. [1]Lab. Chem. Pharmacol., NHLBI, [2]Clin. Neurosci. Br., NIMH, and [3]Lab. Molec. Carcinogen., NCI, NIH, Bethesda, MD 20892.

One of the immunogens associated with halothane-induced hepatitis is a trifluoroacetylated 59 kDa liver microsomal carboxylesterase (ISSX 88, Kobe, Japan, Abs. III-403-P12). To characterize this enzyme, molecular cloning and sequencing of the cDNA encoding the protein have been initiated. Several clones were isolated from rat liver lambda gt11 libraries screened with polyclonal anti-59 kDa antibodies. Clones that subsequently hybridized with oligonucleotides prepared from amino acid sequences of tryptic fragments of the purified 59 kDa protein were subcloned into pGEM and M13 and sequenced by the dideoxy chain termination method. The largest clones hybridized to an mRNA of 2 kb, coded for a peptide containing the catalytic active site regions of serine esterases, and had 66% amino acid homology to a rabbit liver carboxylesterase of 60 kDa (JBC 263:3486, 1988). (RML is supported by a PRAT Fellowship from NIGMS, NIH.)

55.6
CHRONIC ETHANOL TREATMENT INCREASES MORPHINE GLUCURONIDATION IN RABBITS. Shamla S. Narayan* and Garold S. Yost* (SPON: William K. Nichols). Washington State University, Pullman, WA 99164-6510 and University of Utah, Salt Lake City, UT 84112.

Male New Zealand white rabbits were injected ip with 15 mg/kg morphine sulphate in normal saline, treated with ethanol (10% in drinking water for 14 days) and were again injected ip with morphine. Blood samples were collected from ear arteries from 5 min to 360 min after the ip injections. Plasma samples were analyzed by HPLC for morphine and morphine glucuronide. Plasma concentration curves from four rabbits showed an increased area under the plasma concentration curve (AUC) for morphine glucuronide by an average of 23% (range 10-47%) and a decreased AUC for the parent compound morphine by an average of 32% (range 10-75%) after ethanol treatment. Thus, morphine metabolism after chronic ethanol treatment was increased due to an increase in its glucuronidation. Morphine disposition studies after acute alcohol treatment with chronically alcohol-induced and untreated animals will also be presented. Supported by USPHS Grant AA06555. GSY is a USPHS Research Career Development Awardee (HL02119)

55.7
VARIATION BETWEEN ANIMALS AND BETWEEN HEPATIC LOBES IN THE COLLAGEN CONTENT OF LIVER SPECIMENS IN THE RAT CCl_4-INDUCED CIRRHOTIC MODEL. M. Gascon-Barré*, P.M. Huet*, J. Belgiorno*, V. Plourde*, P.A. Coulombe*, (SPON: P. DuSouich) Ctre rech clin A.-Viallet, Hôp. St-Luc, and Univ. de Montréal, Montréal, Qué. Canada

Numerous studies have been reported on the handling of xenobiotics using a CCl_4-induced model of micronodular cirrhosis. In this model, the extent of hepatic collagen infiltration has not, however, been evaluated. A study was undertaken 1) to analyze the variation a) between animals and b) between lobes of the same liver in hepatic collagen content following CCl_4-induced cirrhosis, and 2) to evaluate the correlation between morphometric and colorimetric evaluations of hepatic collagen content. The results revealed a significant correlation ($r=0.9458$, $p<0.001$) between the morphometric and colorimetric methods of collagen evaluation; both methods also distinguished data obtained from controls and cirrhotic rats ($p< 0.0005$). Following induction of cirrhosis, a highly significant variation in hepatic collagen content was observed between animals ($p<0.0001$). By contrast, no significant difference was observed ($p>0.05$) between hepatic lobes of a given animal. Our results point out, that in this model of liver cirrhosis, the interpretation of metabolic data would benefit by being related to the severity of hepatic collagen infiltration of each animal. Our data also show that representative values on the total hepatic collagen content can be obtained from a single liver specimen.

55.8
THE INTACT HEPATOCYTE HYPOTHESIS APPLIES TO THE C-25 HYDROXYLATION OF VITAMIN D_3 IN MICRONODULAR CIRRHOSIS. V. Plourde*, M. Gascon-Barré*, P.Coulombe*, S. Vallières*, C. Dubé*, and P.M. Huet*, (SPON: P. DuSouich) Hôp. St-Luc, Univ. de Montréal, Montréal, Qué. Canada

In liver diseases, a wide spectrum of vitamin D (D) status as well as responses to D challenge are reported, but no unifying concept as to the liver capacity to metabolize D at C-25 is yet clearly emerging. Using isolated-perfused liver preparations, the uptake and C-25 hydroxylation of D_3 were studied in a CCl_4-induced cirrhotic model. Cirrhotic rats had a lower fractional hepatic D_3 uptake than controls (0.2 ± 0.03 vs 0.3 ± 0.05, $p<0.05$), and lower 25-hydroxyD_3 ($25(OH)D_3$) in liver (1.1 ± 0.3 vs 3.6 ± 0.9 pmol, $p<0.01$) and perfusate (2.5 ± 0.8 vs 6.3 ± 0.8 pmol, $p<0.01$) following 150 min of perfusion. Histomorphometric analysis allowed calculation of the relationship between $25(OH)D_3$ production and the hepatic lesion. Linear relationships were observed, in controls and cirrhotics, between the volume density (V_V) of normal hepatocytes and the liver ($r=0.8612$, $p<0.005$), perfusate ($r=0.8457$, $p<0.005$) and total $25(OH)D_3$ ($r=0.7703$, $p<0.01$). Evaluation of the D_3-25 hydroxylase in hepatocytes isolated from cirrhotic and control livers revealed similar activities in both groups. Our data show that the D_3-25 hydroxylase is unimpaired in the remaining hepatocytes of the diseased liver, and that the Vv normal hepatocytes is the major factor of the $25(OH)D_3$ production by the cirrhotic rat liver.

55.9

CHARACTERIZATION AND IMMUNOREACTIVITY OF HUMAN LIVER DEHYDROEPIANDROSTERONE SULFOTRANSFERASE. Charles N. Falany*, Mary E. Vazquez*, and John M. Kalb* (SPON: P.M. Hinkle). University of Rochester, Rochester, NY 14642.

Dehydroepiandrosterone sulfotransferase (DHEA-ST) was purified from human liver cytosol by chromatography on DEAE-Sepharose 4B and adenosine 3',5'-diphosphate-agarose. The resolved enzyme was capable of sulfating a number of different steroids, including pregnenolone, β-estradiol and androsterone, but was inactive towards p-nitrophenol and dopamine. DHEA-ST displayed a subunit molecular weight of 35,000 daltons following SDS-polyacrylamide gel electrophoresis. Antibodies raised in rabbits against the subunit form of the enzyme recognized a single band in human liver cytosol following Western blotting and did not react with purified M- or P-phenol sulfotransferases. Also, antibodies raised in rabbits against the human platelet M-phenol sulfotransferase did not react with DHEA-ST. Of several human tissues tested by Western blotting, immunoreactive proteins were detected by the anti-DHEA-ST antibody only in cytosol prepared from liver and the adrenals. In adrenal cytosol, two bands were observed with molecular sizes of 35,000 and 30,000 daltons. These results indicate that the DHEA-ST in human liver is distinct from the sulfotransferases which react with small phenols and catecholamines and is immunologically similar to the form of the enzyme present in human adrenals. Supported by NIH grant GM-38953.

55.10

METABOLISM AND PHARMACOKINETICS OF TRIPROLIDINE HCL IN RATS. Richard M. Welch, Steve Weller* and Chacko Verghese*. The Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Although triprolidine [(E)-2-{3-(1-pyrrolidinyl)-1-p-tolylpropenyl}pyridine HCL] is a widely used antihistamine, little is known regarding its pharmacokinetics and metabolism in animals and man. The present study examined the pharmacokinetics and metabolism of triprolidine in male and female rats following the oral administration of 10 mg/kg, 30 mg/kg, 60 mg/kg and 120 mg/kg of triprolidine HCL. Marked non-linearity in AUC for triprolidine was observed in rats between oral doses of 10 mg/kg and 120 mg/kg. Although the dose was increased 12-fold, the AUC for triprolidine increased more than 100-fold indicating saturation of metabolism. This phenomenon was very apparent even at doses between 10 mg/kg and 30 mg/kg. After a po dose of 10 mg/kg of C^{14}-triprolidine HCL, the major plasma metabolite was the carboxylic acid formed by oxidation of the tolyl-methyl group. This metabolite comprised over 40% of the total radioactivity in plasma. Comparisons of the AUCs for triprolidine after oral and iv doses of 10 mg/kg indicated an absolute oral bioavailability of 5% and 13% in male and female rats respectively. After an iv dose of 10 mg/kg, the plasma $t_{\frac{1}{2}\beta}$ was 45 min. and no sex difference was observed. These results indicate that triprolidine undergoes extensive first-pass metabolism in the rat and that its metabolism is easily saturable.

55.11

IN VITRO PROTEIN BINDING OF SULFAMETHAZINE AND ITS MAJOR METABOLITE. Yu Chung Tsang* and Jake J. Thiessen* (Spon: J.P. Uetrecht). Faculty of Pharmacy, University of Toronto, Toronto, Ontario M5S 1A1.

As part of a study examining dose-dependent drug acetylation, the possibility of nonlinear protein binding of sulfamethazine (SMZ) was studied in rabbit serum. Serum solutions comprising various concentrations (~0.01 – 1 mM) of SMZ and its acetylated metabolite, N^4-acetylsulfamethazine (AcSMZ), were prepared. These 'control' samples were also mixed via a Latin square design to study the interactive binding of SMZ and AcSMZ to the proteins. Equilibrium dialysis was conducted for 8 hours.

Post-dialysis measurement of SMZ/AcSMZ in the buffer and serum chambers provided the free and bound fractions based upon equilibrium total serum concentrations. The 'control' data revealed concentration-dependent binding for both drugs while the interaction study clearly indicated the presence of competitive binding. Computer analysis of the observations identified the most appropriate binding model to consist of specific and non-specific binding. The mean (SE) 'control' parameter estimates for Pt_{SMZ}, Pt_{AcSMZ}, Kd_{SMZ}, Kd_{AcSMZ}, NSP_{SMZ}, NSP_{AcSMZ} were: 0.561(0.041), 0.605(0.024), 0.078(0.006), 0.031(0.002) [mM], 0.205(0.053) and 0.229(0.042), respectively. In the interaction study these values were: 0.599(0.022), 0.479(0.019), 0.091(0.004), 0.023(0.001), 0.228(0.020) and 0.434(0.061), respectively. These findings indicate that AcSMZ binds more strongly to serum proteins than SMZ. The metabolite can therefore alter the *in vivo* SMZ binding thereby in turn changing the acetylation rate.

55.12

UPTAKE OF ENALAPRILAT BY THE PERFUSED RAT LIVER IS BARRIER-LIMITED. Andreas J. Schwab, K. Sandy Pang*, and Carl A. Goresky. *Fac. of Pharmacy and Dept of Pharmacology, University of Toronto, Toronto, Ont. M5S 2S2, and University Medical Clinic, The Montreal General Hospital, Montreal, Que H3G 1A4.

Previous experiments with perfused rat liver, using a steady-state protocol, led to the conclusion that enalapril (E) readily enters hepatocytes, where it is deethylated, yielding enalaprilat (EA), which is subsequently predominantly excreted into bile, and that the more polar product EA, when presented to hepatocytes from within the vascular space, is not readily taken up because of a barrier effect at the plasma membrane. We have now been able to support this hypothesis in a more direct fashion with multiple indicator dilution experiments using tracer EA. Tritiated EA was injected into the portal vein of a perfused liver, together with the non-sequestered reference indicators 51-Cr-labelled red blood cells, 125-I-labeled albumin and [14C]sucrose. Timed samples were collected from the hepatic vein and analyzed for the tracers. Extrapolated recovery of EA in venous blood was 95%, with 5% appearing in bile. In a single pass, 87% of the injected EA traveled along the vasculature without entering hepatocytes. Of the remaining 13%, 40% was excreted into bile, and 60% returned to the vascular compartment. The calculated permeability-surface area product ("diffusional clearance") was 0.26 ml/min/g. This corresponds proportionally to 23 % of blood flow. The plasma membrane of the hepatocytes thus provides a substantial resistance to EA transfer, in either direction.

TEMPERATURE REGULATION

56.1

THE EFFECTS OF REPEATED COLD STRESS TESTS ON COLONIC TEMPERATURE LOSS AND HEAT PRODUCTION IN ADULT AND AGED MICE. Hal Tatelman* and Mark Talan
Gerontology Research Center, N.I.A., N.I.H., Baltimore, Md. 21224

Adult and aged C57BL/6J mice (14 and 29 mo) were subjected to 3 consecutive Cold Stress Tests (CSTs) (3 hrs restraint at 6°C) repeated with 2 week intervals between tests. The change in colonic temperature (Tco) (°C/hr) was measured and metabolic Heat Production (mHP) (kcal/body weight$^{2/3}$/hr) was calculated. Aged mice had a much steeper loss in Tco and a much smaller mHP than adults. Only adults exhibited an habituation to repeated CSTs (a significant improvement by test 3 when comparing the three tests) in both Tco and mHP. In a second experiment, adult and aged mice (9-11 and 30 mo) were subjected to three CSTs each preceded by a Baseline Test (BT) (1 hr restraint at 23°C), both repeated with 2 week intervals between tests, during which Tco and mHP were calculated. During the CSTs, aged mice once again had a much steeper loss in Tco and a much smaller mHP. Unlike the previous experiment, neither adult nor aged mice exhibited habituation in either Tco or mHP. Adults produced greater mHP in CSTs than in BTs, while aged mice produced similar amounts of mHP in CSTs and BTs. In both experiments, the increased loss of temperature in the aged mice compared with adults during the CSTs is, probably, related to their inability to compensate by increasing mHP in the cold.

56.2

BEHAVIORAL FEVER IN GUINEA PIGS BEFORE AND AFTER KNIFE CUTS IN THE BRAINSTEM. C.M. Blatteis. Department of Physiology and Biophysics, University of Tennessee College of Medicine, Memphis, Tennessee, 38163.

Several studies have implicated the preoptic area (PO), lateral hypothalamus (LH), posterior hypothalamus (PH), and medulla oblongata (MO) in the behavioral thermoregulation of various species. In guinea pigs, all these regions but the PH also activate autonomic febrigenic responses to locally injected interleukin-1β (IL1). Since behavioral adjustments are an integral part of fever development, the thermopreferenda of guinea pigs (6/group) freely moving in a thermocline (end-to-end gradient 27 to 38°C) were monitored before and after microinjection of IL1 (10 ng/ul, bilaterally) into these sites; body temperature was recorded by radiotelemetry. Behavioral fevers were evoked when IL1 was administered into the PO or LH, but not when it was given into the PH or MO. Possible interactions among the sites were examined by disconnecting them with microknives and injecting IL1 iv or directly into each site. Iv injections induced behavioral fevers in all the animals, but no response was elicited by any intracerebral injection (although autonomic fevers were produced by intraMO IL1 injections). These results suggest that the neural elements mediating autonomic and behavioral fevers may be distinct. (Supported by NIH grants NS-14929 and 22716)

56.3

THE THERMIC EFFECT OF COCAINE DURING STRESS. O.O. Aroyewun (SPON: M. Matsuzaki). NYS Division of Substance Abuse Service Brooklyn, N.Y. 11217

Experiments were performed to determine whether cold stress alters cocaine thermolytic action in the rats in a manner similar to the reported stress-induced alterations of cocaine behavioral functions. Intraperitoneal injection of cocaine (5-30 mg/kg) elicited dose-dependent hypothermia in rats exposed to cold (4°C). It had no significant effect on the rectal temperature of rats maintained at a thermoneutral (22-23 °C) ambient temperature. The significant interaction of the drug with cold stress supports the contention that stress potentiates cocaine effect. The hypothermia induced by cocaine (20 mg/kg) in the cold was attenuated by yohimbine, verapamil or reserpine, enhanced by methysergide, but unaltered by pretreatment with haloperidol, ascorbic acid, naloxone, atropine, nimodipine, DL-propanolol, alpha-methyl-paratyrosine, nialamide or parachlorophenylalanine. Procaine, a local anesthetic, had no effect on the temperature of rat in the cold. It seems that endogenous level of noradrenaline might be of some importance in the hypothermia. Rats treated chronically with cocaine (20 mg/kg/twice per day for 15 days) and exposed to 2-hr cold stress every other day of drug injection developed tolerance to the thermoregulatory effect. The tolerance may not depend entirely on the habituation of the stress component, since rats exposed to the cold stress for the first time after the drug treatment regimen also showed some dimunition in thermic response.

56.4

TYMPANIC TEMPERATURE IS A CORE TEMPERATURE IN HUMANS. Michel Cabanac and Heiner Brinnel*. Dept. Physiol., School of Med. Laval Univ. Quebec, Canada, G1K 7P4 and Hôpital de l'Arbresle BP 116,69210 L'Arbresle, France.

The validity of tympanic temperature as an index of core temperature can be questioned since it has been found to be affected by ambient temperature during exposure to cold. This work has been undertaken to explore the possible influence of head skin temperature on tympanic temperature. In a group of ten subjects at rest, mean temperature on the lower anterior quarter of the tympanic membrane was 0.1°C higher than oesophageal temperature. Tympanic, oesophageal, interdigital, and mastoid skin temperatures were recorded in 6 subjects during 30 min exposure to cold at 0°C and the following 30 min recovery. When the thermocouple was placed on the lower anterior quarter of the tympanic membrane, the influence of skin temperature on tympanic temperature was as low as 0.006°C per 0°C fall in skin temperature, and the influence of air temperature as low as 0.0025°C per °C fall in air temperature. At cold ambient temperature, the changes in tympanic temperature were strikingly parallel to those of oesophageal temperature whereas skin temperatures generally evolved in the opposite direction. These results suggest that tympanic temperature, when measured accurately, is a good index of core temperature and its variations may reflect variations in brain temperature

(Supported by D.R.E.T. and F.R.M.F. France)

56.5

COMPARISON OF TYMPANIC MEMBRANE AND ESOPHAGEAL TEMPERATURE DURING EXERCISE. **Alain Deschamps*, Robert D. Levy*, Manuel G. Cosio*, Errol B. Marliss*, and Sheldon Magder. McGill University and Royal Victoria Hospital, Montreal, Canada.**

We tested the hypothesis that tympanic membrane temperature (T_{ty}) is influenced by skin blood flow. Six healthy males exercised on a cycle ergometer until exhaustion at 73.9±5.1% of their maximal capacity. Two were cooled by fanning of the upper chest during recovery. Four also exercised with a work load which increased at 32.6 watts/ min. Mean skin temperature (T_{sk}), esophageal temperature (T_{es}) and T_{ty} were measured. T_{ty} was lower than T_{es} during both constant and incremental load exercise ($p<0.025$ and $p<0.05$ respectively). Changes in T_{ty} always lagged changes in T_{es} and the magnitude of the increase in T_{ty} was always lower then T_{es}. Similar to T_{sk}, immediately following constant exercise, T_{ty} increased and later sharply decreased whereas T_{es} progressively decreased throughout recovery. Without fanning, the decreases in temperature from the maximum were 0.6±0.2°C for T_{es}, 0.3±0.3°C for T_{ty}, and 0.7±0.2°C for T_{sk}. With fanning, however, the decrease in T_{es} was slightly smaller (0.3±0.1°C) whereas the decreases in T_{ty} and T_{sk} were greater (0.9±0.1 and 2.2±0.6°C respectively). In conclusion, tympanic membrane temperature is influenced by skin temperature and it does not assess changes in core temperature.

56.6

ENERGY EXCHANGE IN DOWNHILL AND UPHILL WALKING: A CALORIMETRIC STUDY. Francis J. Nagle, Paul Webb and Dan M. Wanta*. Biodynamics Laboratory, University of Wisconsin, Madison, WI 53706.

From the principle of conservation of energy, energy balance can be expressed as (1) $\dot{Q}M = \dot{Q}HL + \dot{Q}W + \dot{Q}\Delta Hb$, where $\dot{Q}$ is power (watts), M metabolic rate, HL heat loss, W external work and ΔHb change in body heat storage. When submaximal treadmill exercise continues long enough, body temperature stops changing, ΔHb becomes zero and the heat storage term is dropped from (1). For uphill walking the equation becomes (2) $\dot{Q}M = \dot{Q}HL + \dot{Q}Wvert$, and for downhill walking it becomes (3) $\dot{Q}M = \dot{Q}HL - \dot{Q}Wvert$. The study tested energy balance eqs. (2) and (3) with direct measurements of heat exchange using a suit calorimeter and standard measurements for respiratory gas exchange. Ten healthy men 28.4 ±5.6 yr, 73±4.7 kg walked on a motor driven treadmill at 90 m/min at grades of 0%,5%,10%,-5% and -10% for 70 to 90 minutes, insuring steady state. Values for each term of eqs. (2) & (3) demonstrated that $-\dot{Q}Wvert$ is a power input which appears as heat and that $+\dot{Q}Wvert$ is a power output separate from heat loss. Furthermore, there is a quantity of nonthermal energy, $\dot{Q}Wwlk$, needed to satisfy the energy balance equation that was significant at 0% grade, 5% and 10% but small and not significant at -5% and -10%. Delta efficiency for $+\dot{Q}Wvert$ was 24% and for $\dot{Q}Wwlk$ 26%. These data confirm previous findings of Webb, et al (Fed. Proc. 46:318, 1987) for a nonthermal energy component in level walking and extends them to grade walking. $\dot{Q}Wwlk$ appears to be work done on the walking surface.

56.7

HEAT REGULATION DURING GRADE WALKING: A CALORIMETRIC STUDY. Paul Webb, Francis J. Nagle and Dan M. Wanta*. Biodynamics Laboratory, University of Wisconsin, Madison, WI 53706.

Heat loss, for example by sweating, rises to match the need for heat dissipation, sometimes called "total heat", which is the sum of metabolic rate plus energy received (as in resistive cycling, diathermy and downhill walking) minus power output (as in the external work of cycling and uphill walking). It has been thought that thermoregulatory sweating responds principally to rising internal temperature, modified to some degree by the level of skin temperature. But it is possible to make heat dissipation so easy with a water cooled suit that one can exercise without sweating, yet not affect the usual rise in Tre. The same water cooled suit, properly insulated and instrumented, is a direct calorimeter. We measured heat loss with the suit calorimeter and metabolic rate during grade walking in 10 fit young men. They walked at a constant treadmill speed of 90 m/min (5.4 km/hr) at grades of -10%, -5%, 0%, 5% and 10% for 70 to 90 min to insure steady levels of heat loss and body temperatures. Evaporative water loss was only 80-190 g/hr, with higher rates at higher exercise levels, so that evaporative heat loss was about 15% of total heat loss. Heat loss was more closely correlated to total heat than to metabolic rate and Tre correlated better with heat loss and total heat than with metabolic rate. It appears that heat loss is the independent (regulated) variable, with Tre dependent on it.

56.8

THERMAL RESPONSE AND SKIN BLOOD FLOW OF OLD WOMEN DURING EXERCISE IN HEAT. M.K. Yousef, K. Shiraki, K.A. Reed* and L.A. Colding*. Desert Biol. Res. Ctr., Dept. of Biol. Sci., Univ. of Nev., Las Vegas, Las Vegas, NV 89154

Recent data suggest that increased heat strain in older yet healthy individuals seems to be an impaired cardiovascular and vasomotor functions. The objective of this experiment was to examine some thermal responses and Laser-Doppler measurements of skin blood flow (SKBF) of 6 young (YW: 20-25 y old) and 7 old (OW: 63-72 y old) women during rest and exercise in heat (41C and 40% RH) and in a thermoneutral temperature (27C, 40% RH). Esophageal (Tes), rectal (Tre), O_2 uptake (VO_2), sweat rate (SR), and SKBF on the arm and shoulder were measured. The experimental protocol for all subjects was to rest in a chair for 45 min then exercise for an additional 30 min on a bicycle ergometer at a work load equal to 40% of VO_2 max. During rest at 41C, the OW had increased their VO_2, Tre and Tes similar to the young, however, SKBF increased more in the YW than the OW. During exercise at 41C, SR was about the same in both groups (5.84 and 6.86 $g/m^2 \cdot min$ in OW and YW respectively). However, Tes and not Tre increased significantly more in the OW (Δ Tes: 0.96 and 0.68 in OW and YW respectively). The percentage increase in SKBF was greater in YW. Ventilation equivalent was higher in OW during rest and exercise. The data support the premise that problems encountered by the elderly in hot environments may be primarily a loss of efficiency in the vasomotor responses.

56.9

UTILIZATION OF ENERGY SUBSTRATES DURING COLD EXPOSURE IN MAN. A.L. Vallerand, and I. Jacobs*. Defence and Civil Institute of Environmental Medicine, Toronto Ont. M3M 3B9 Canada.

Although it is well known in animals that shivering thermogenesis markedly increases the oxidation of energy substrates, this phenomenon is poorly understood in man. This study intended to compare substrate utilization in seven healthy young male subjects that were either exposed to the warm (29°C; semi-nude, fasted) or to the cold for 2h (10°C). Mean body temperature (Tb) was assessed by: 0.67 rectal + 0.33 mean skin temperature. Metabolic rate was determined via indirect calorimetry. Substrate utilization was calculated using the nonprotein respiratory quotient, which was derived from the urinary urea nitrogen output. Cold exposure induced a 3.2 ± 0.1°C drop in Tb and a body heat debt of 866 ± 54 kJ ($p<0.01$). These parameters remained essentially unchanged in the warm. Cold exposure also elevated the 2h energy expenditure by 2.5 fold in comparison to the warm ($p<0.01$). This enhanced thermogenesis was accompanied by a 580% increase in carbohydrate oxidation ($p<0.01$), a 63% increment in fat oxidation ($p<0.05$), and an unchanged oxidation of protein. The results demonstrate that cold exposure causes a much greater increase in the utilization of carbohydrate than lipid. It is suggested that these substrates are mainly oxidized in the shivering skeletal muscles.

HYPERTENSION I

57.1

Temperature dependent sensitivity in the SHR aorta after endothelium removal. J.M. Price. University of South Florida, Department of Physiology and Biophysics, Tampa, FL 33612.

The objective of this study is to determine if removal of the endothelium from the SHR aorta eliminates temperature induced changes in its norepinephrine sensitivity. We have found that sensitivity to norepinephrine, maximum active wall tension, and resting wall tension in the intact SHR aorta are significantly increased when bath temperature is increased from 37°C to the SHR's body temperature, 39°C. The endothelium was removed by gently scraping the intima with a wooden stick (1mm dia.). The absence of endothelium was tested by exposure of norepinephrine contracted rings to acetylcholine. In preliminary experiments the vessel rings were examined histologically for the absence of endothelia cells with a scanning electron microscope and with a light microscope. Aortic rings from 16 week old SHRs were examined for the effect of an increase in temperature from 37°C to 39°C on their sensitivity to norepinephrine. Rings without the endothelium produced a greater maximum active wall tension and were more sensitive at 39°C than at 37°C. The ED50 was $1.1\pm0.3\ 10^{-9}$M at 39°C and $2.0\pm0.4\ 10^{-9}$M at 37°C. It may be concluded that the endothelium does not cause a temperature dependent sensitivity in the SHR aorta. Supported by NIH grant HL21103.

57.2

INOTROPIC RESPONSE TO PRENALTEROL IS PRESERVED DESPITE INCREASED WALL STRESS EARLY IN HYPERTENSION. RP Shannon*, L Hittinger*, R Gelpi*, I Mirsky, and SF Vatner. Dept of Medicine, Harvard Med School, New England Regional Primate Research Center, Southboro, MA 01772

To test the hypothesis that the inotropic response to β-adrenergic stimulation is altered early in hypertension (HTN), we studied LV systolic function during infusion of the β_1-specific agonist, prenalterol, in 10 conscious dogs while normotensive (NORM) and 2-3 weeks after the development of perinephritic HTN. There was a significant increase ($p<0.05$) in LV systolic pressure (121±3 mmHg vs. 168±5 mmHg), mean arterial pressure (91±4 mmHg vs. 126±7 mmHg) and LV systolic stress (168±7 g/cm^2 vs. 242±11 g/cm^2) following HTN, associated with a 28% increase in LV mass ($p<0.05$). Both isovolumic and ejection phase indices were increased in response to prenalterol following HTN (see Table).

		Prenalterol (µg/kg/min)	
LV dP/dt (mmHg/sec)	Control	4	8
NORM	2868±217	3983±342	5212±444
HTN	3997±294*	5034±322*	6472±531*
LV dD/dt (mm/sec)			
NORM	68±6	93±5	109±7
HTN	87±7*	111±8*	130±9*

* Different from NORM, $p<0.01$

Thus, the inotropic response to the β_1-specific agonist, prenalterol, is preserved early in the course of perinephritic HTN despite greatly increased systolic wall stress.

57.3

THE EFFECT OF VOLUME DEPLETION ON THE ABILITY OF HIGH-AND-LOW-NORMOTENSIVE SUBJECTS TO MAINTAIN BLOOD PRESSURE DURING LOWER BODY NEGATIVE PRESSURE (LBNP). C. Knapp*, J. Evans*, S. DuPlessis*, M. Berk*, J. Kotchen*, P. Taylor*, C. Ott and I. Kotchen*, University of Kentucky, Lexington, KY.

The cardiovascular responses of 24 normotensive male subjects (12 high and 12 low normotensives) were measured during LBNP (2 min each at control, -10, -20, -30, -40, -50 and -60 mmHg). Each subject was studied following 36 hrs. on a 250 meq NA^+/da diet and following 36 hrs. on a 10 meq NA^+/da diet plus furosemide (40 mg, p.o. x 2). For the whole group, NA^+ deprivation significantly decreased extracellular fluid volume (10.5 ± 0.5 to 9.2 ± 0.5 Lit), cardiac output (CO) (5.75 ± .39 to 4.91 ± .32 L/min), systolic BP (120 ± 3 to 110.5 ± 2 mmHg) and stroke volume (SV) (92 ± 6 to 76 ± 6 ml) while significantly increasing vascular resistance (SVR) (15.9 ± 1.1 to 18.5 ± 1.3 mmHg/(L/min)). There was no change in diastolic BP (64 ± 3 to 64 ± 2 mmHg) or heart rate (HR) (63 ± 2 to 66 ± 3 b/min). During LBNP the increase in HR and SVR were significantly greater in NA^+ deprived subjects but were insufficient to maintain CO and BP due to the significantly smaller SV. Higher levels of LBNP combined with volume depletion resulted in a drop of diastolic pressure in high normotensives from 75 ± 4 to 70 ± 2.5 mmHg. Low normotensives did not have a significant diastolic BP response to LBNP in either state. Supported by NIH Grant #HL 37753 and UK Center for Biomedical Engineering.

57.4

QUANTITATION OF β-ESTRADIOL AND PROGESTERONE IN CYTOSOLIC AND NUCLEAR FRACTIONS OF TERM PLACENTAE OF NORMAL AND HYPERTENSIVE PATIENTS. K.G. Bhansali[1]*, J.P. Buckley[2], and Y.H. Tsai[3]. College of Pharm, TX Southern Univ[1]; Dept of Phamcol, Sch. of Pharm, Univ of Houston[2]; and Dept of Ob/Gyn/Reprod Sci, The Univ of TX Med Sch at Houston Houston, TX. 77030[3].

The correlation between concentrations and production of placental estradiol (E_2) and progesterone (P) with hypertensive or pre-eclamptic pregnancy have been reported. In this study, concentrations of E_2 and P in five NP (placentae of normal patients) and HP (placentae of hypertensive patients) were determined by radioimmunoassay. Concentrations of cytosolic E_2 were significantly greater in NP (2.4 times in average) compared to those in HP, while those of nuclear E_2 were less (0.38 times) in NP than those in HP. The mean value of the ratios of nuclear E_2 to cytosolic E_2 was seven times as much in HP as in NP. Although no significant difference in cytosolic P concentrations was observed for NP and HP, the nuclear P concentrations of NP tended to be less than those of HP. The ratios of nuclear P to cytosolic P in HP was relatively greater than those in NP. The data further demonstrated that greater nuclear concentrations of estradiol and progesterone in placentae are associated with hypertensive pregnancy. Supported by HL07434 and 2 S07 RR-05745-15.

57.5

EFFECT OF INCREASED DIETARY CALCIUM ON THE DEVELOPMENT OF REDUCED RENAL MASS SALINE HYPERTENSION IN RATS. M. Pamnani, H. Bryant, S. Chen*, J. Schooley* and F. Haddy. Department of Physiology, Uniformed Services Univ., Bethesda, MD 20814

Diet-fortified with $CaCo_3$ has been reported to reduce blood pressure(BP) in low renin and salt sensitive hypertensive patients. We have therefore examined the effect of increased dietary Ca on the development of reduced renal mass (RRM)-Saline (S) hypertension (HT) in rats, a classical low renin, volume and Na dependent model of HT. Rats with 70-75% RRM were divided into experimental (E) and control (C) groups. The E rats were fed a Na free diet supplemented with $CaCo_3$ (2.0% Ca) and drank 1% S for 5 wks whereas C rats consumed the Na free diet and drank 1% S for the same period. In C rats as expected BP progressively increased from a control value of 120.0±1.2 to 174.2±1.2 mmHg by the 5th wk. In contrast, in E rats, Ca significantly attenuated the development of HT; the BP only increased from 117.0±1.2 to 134±3.8 mmHg by the 5th wk. In E rats this was associated with a decrease in S intake, which by the 5th wk had declined by 30% with a proportionate decrease in UV and $U_{Na}V$, but not U_KV. No such decreases were seen in C rats. At the end of the treatment period plasma levels of Na, K, Ca, creatine, BUN and protein were not different but plasma Cl and Mg were lower in E rats; vascular muscle cell E_ms were not different. These data show that dietary $CaCo_3$ can attenuate the development of RRM-S HT in the rat, possibly in part by altering Na and water intake.

57.6

NALOXONE (NX) INDUCED BRADYCARDIA IN CONSCIOUS NORMOTENSIVE (NT) AND HYPERTENSIVE (HT) RATS. Subhash Vyas*, Stan Z. Kurowski* and Julianna E. Szilagyi. Dept. of Pharmacology, Univ. of Houston, Houston, TX 77004.

Opiates have been linked to cardiovascular control in the hypertensive state. Chronic administration of NX to HT rats has been shown to reduce blood pressure and heart rate as well as levels of circulating ß-endorphin. NX administration is also known to attenuate the development of hypertension. We investigated the effect of short-term intravenous infusions of NX (1 mg/kg/min for 30 min) on blood pressure and heart rate in conscious NT and 2-kidney Goldblatt HT rats in order to elucidate the mechanism of action of NX on the cardiovascular system. NX was tested alone and after i.v. administration of atropine (0.5 mg/kg), propranolol (1 mg/kg) and clorisondamine (3 mg/kg). Mean arterial blood pressure was not significantly affected by NX infusion in either group of rats. In contrast, NX produced a significant fall in heart rate in both NT and HT rats but the magnitude of the response was greater in the HT rats (-32±5 versus -52±6 bpm respectively). After atropine the NX induced bradycardia was enhanced in both NT (-77±6 bpm) and HT (-90±6 bpm) rats. Neither propranolol nor clorisondamine significantly affected the NX induced bradycardia in NT rats. In HT rats the fall in heart rate due to NX was only slightly, but not significantly, diminished by these two substances. These data indicate that the influence of endogenous opiates on heart rate is greater in HT animals compared to NT rats, however, the mechanism by which NX reduces heart rate has yet to be determined. Interestingly, the data suggest that the NX induced bradycardia is not neuronally mediated. (Support: AHA #85-739)

57.7

EFFECTS OF BUFALIN, A Na^+,K^+-ATPase INHIBITOR, ON CARDIOVASCULAR HEMODYNAMICS AND RENAL WATER AND SODIUM EXCRETION IN DOGS AND RATS. F.J. Haddy, M.B. Pamnani, D.C. Eliades, B.T. Swindall*, J.F. Schooley* and J.R. Johnston*. Department of Physiology, Uniformed Services University, Bethesda, MD 20814

Studies in Lichstein's laboratory suggest that the endogenous digitalis-like substance (DLS) implicated in volume expansion (VE) and low renin hypertension (LRH) might be a steroidal dienolide derivative. If true, the bufodienolides should block K^+ vasodilation, potentiate NE vasoconstriction, increase vascular resistance, increase left ventricular dP/dt, raise arterial blood pressure (BP), and produce natriuresis and diuresis. We have examined these parameters in the anesthetized dog and Wistar rat while infusing bufalin (aglycone), a bufodienolide, intrabrachially (IA) (dog) and IV (dog, rat). In the dog, IA infusion of 5-25 µg/min with brachial arterial blood flow held constant produced a dose-dependent increase in perfusion pressure, decrease in the vasodilator response to IA injection of KCl (but not to Ach) and increase in the vasoconstriction response to IA injection of NE. IV infusion at 5 to 50 µg/min produced dose-dependent increases in dP/dt and BP. In the rat, IV infusion of 0.5-0.16 µg/min produced dose dependent increases in dP/dt, BP, UV, $U_{Na}V$, and U_KV. Thus bufalin does in fact have the physiological characteristics required to be considered a candidate for the DLS found in VE and LRH. The data also show show that a DLS can raise BP despite its diuretic and natriuretic effects.

57.8

CIRCADIAN DETERMINATIONS OF CORE TEMPERATURE IN SHR AND DOCA- SALT HYPERTENSIVE RATS. S. Cardoso, M. Shafi*, E. Songu-Mize*, F. Osuji*, S. Oyugi*, S. Abell* and J. Llamos-Quevedo*. Univ. of Tennessee, Memphis, TN 38163, Rust College, Holly Springs, MS 38635.

Spontaneously hypertensive rats (SHR) are hyperthermic when compared with normotensive WKY. To characterize the hyperthermia of SHR, circadian determinations of rectal temperature were carried out in SHR, WKY and Sprague-Dawley rats (SD). Also, to verify the influence of hypertension per se on core temperature, a group of SD rats were made hypertensive by unilateral nephrectomy followed by treatment with DOCA (25 mg/kg, 1X week) and ad lib intake of 1% NaCl+0.2% KCl solution replacing water. When the blood pressure of these SD rats reached hypertensive levels (> 160 mm Hg), circadian determinations of core temperature were carried out and compared with those of sham operated SD rats on saline, not receiving DOCA. The data are presented below.

Rectal "Core" Temperature °Celsius (Means±SE)

	Time of Day						
	0700	1100	1500	1700	2000	2300	0300
SHR	37.9±.2	37.9±.17	37.5±.2	39.2±.12	39.6±.16	39.7±.10	38.6±.1
WKY	37.2±.1	37.4±.12	37.7±.12	38.4±.14	38.2±.07	37.9±.16	37.5±.16
SD Control	37.0±.1	36.9±.06	37.3±.1	---	38.0±.08	38.7±.13	---
SD DOCA-Salt	36.7±.08	36.8±.04	36.6±.2	---	38.0±.1	38.2±.12	---

Rats (N=15/Group) on light/dark schedule of 12:12; lights: 0600 to 1800 hrs.

The results obtained demonstrate: (1) a remarkable hyperthermia in SHR vs. WKY and DOCA-Salt hypertensive rats; (2) hyperthermia in SHR is present through 24 hr. cycle, but is greater during the active phase of their day (early dark 1900 to 2300 hours); (3) that hypertension is not necessarily casually related to the hyperthermia observed in SHR.

57.9

HEMODYNAMIC ALTERATIONS IN ONE KIDNEY, ONE CLIP GOLDBLATT HYPERTENSIVE RATS WITH EXTRACORPOREAL CIRCULATION AFTER RENAL ARTERY UNCLIPPING. Yunn-Hwa Ma* and Earl W. Dunham. Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455.

Removal of the renal arterial clip (unclipping) in one kidney, one clip Goldblatt hypertensive rats is followed by return of blood pressure (BP, mmHg) to normotensive levels. In order to evaluate unclipping-induced hemodynamic changes, including changes of renal blood flow (RBF, ml/min), the renal venous effluent of inactin-anesthetized (120 mg/kg, i.p.) rats was shunted *via* a vena caval cannula through a flow-through electromagnetic flow probe into a reservoir. The blood collected in the reservoir was returned to the rat *via* the jugular vein using a pump that was servo-controlled based on the volume of blood in the reservoir, thus the rats' blood volume was maintained constant. BP was monitored from a carotid artery. The trachea and urinary bladder were cannulated. BP in rats with extracorporeal circulation was 175±9 (n=5) and 190±9 (n=3) before unclipping and sham unclipping, respectively. Fifty min after unclipping, BP decreased to 128±9 ($p<0.01$) in unclipped rats compared to 204±12 in sham-operated rats. After unclipping, RBF increased 54.6±10.5% (n=5) from a basal level of 7.4±1.1 but did not change in sham-unclipped rats. Heart rate did not change in either group. The stability of this preparation was compared with rats studied without the extracorporeal circulation. BP was 178±6 before and 141±4 fifty min after unclipping, and 183±8 and 180±2 in rats that were sham-unclipped (n=6, $p<0.05$). Supported in part by HL 26111.

57.10

FUNCTIONAL ARTERIAL WALL PROPERTIES IN GENETICALLY OBESE RATS. Dianne C. Kikta and Robert H. Cox. Bockus Research Institute, Graduate Hospital, Philadelphia, PA 19146.

Twelve week old male genetically obese (Zucker fa/fa) rats were studied along with age-matched control lean (Zucker FA/FA) rats. By 12 weeks, body weight was significantly elevated (501 vs 350 g) in the obese rats, while systolic blood pressure measured via the tail-cuff method did not differ between the two groups. Serum electrolytes did not differ between the two groups at 12 weeks, except Cl which was reduced in the obese animals. As expected, serum triglycerides, cholesterol and glucose as well as blood urea nitrogen were elevated in the obese rats. Rings of aortic and caudal arterial smooth muscle were obtained for in vitro measurements of vascular reactivity. No significant differences in passive or active mechanics between lean and obese rats were observed. The aorta from the two groups showed no differences in maximal responses to KCl, norepinephrine (NE) or serotonin (5HT); however, sensitivity to NE and the ED_{50} for NE and 5HT were increased in aorta from obese rats. Meanwhile, caudal artery from obese rats showed an increase in maximal response to KCl, NE and 5HT with no differences in sensitivity or ED_{50} to these agonists. Aortic smooth muscle from the obese rats precontracted with a submaximal concentration of NE displayed greater maximal relaxation to acetylcholine (ACH) with no difference in relaxation to nitroprusside (NP). Caudal arterial smooth muscle from obese rats showed enhanced relaxation to ACH but reduced relaxation to NP. Thus, the observed changes in arterial responsiveness in genetically obese rats may be related to the elevated serum lipids. (Supported by NIH grant HL 39388.)

57.11

NEUROPEPTIDE Y ACTIVITY MAY BE ENHANCED IN HYPERTENSION.
R.N. Daly*, J.P. Hieble, M.I. Roberts*, R.R. Ruffolo, Jr. and M.S. Kreider. Smith Kline and French Laboratories, Philadelphia, PA 19406 and University of Pennsylvania, Philadelphia, PA 19104

Neuropeptide Y (NPY) has been shown to facilitate adrenergic neurotransmission and may be involved in the pathogenesis of hypertension. We compared the contractile effects of field stimulated release of endogenous NPY in superfused segments of caudal arteries from normotensive (Wistar-Kyoto, WKY) and spontaneously hypertensive (SHR) rats. Perfusion of SHR arteries with a polyclonal anti-NPY antibody to block NPY-mediated responses resulted in a significant depression of contractile responses at all frequencies tested (0.5-64 Hz). In contrast, field stimulated responses in arteries from WKY rats were slightly, but not significantly, reduced throughout the frequency response curve. Measurement of levels of endogenous NPY in this tissue by radioimmunoassay revealed no significant differences in concentration between WKY and SHR (46.9 ± 6 vs 49.6 ± 5 pg/mg tissue wet weight). These findings of unaltered tissue levels and enhanced release of NPY in caudal arteries of SHR rats suggest that the functional activity of NPY systems is increased in this model of hypertension.

57.12

NEUROPEPTIDE Y (NPY) POTENTIATES NOREPINEPHRINE (NE) RESPONSE IN PITHED SPONTANEOUSLY HYPERTENSIVE RATS (SHR).
Merrill B. Kardon, Joan M. Hunter* and Barbara L. Pegram*. Alton Ochsner Medical Foundation, New Orleans, LA 70121.

Male SHR (12-13 wks) anesthetized with hexabarbital were pithed and artificially ventilated. While supporting body temperature, catheters for arterial pressure measurement and drug infusion were implanted into the left carotid artery and both jugular veins. Following a short control period, α-adrenergic antagonists (rauwolscine (R); 1.0 mg/kg or prazosin (P); 0.1 mg/kg) were injected. Eleven minutes later either NPY (460 pmol/kg.min^{-1}) or vehicle (5 ul/kg.min^{-1}) infusion was begun. Four minutes hence a cumulative dose-response for diastolic arterial blood pressure (DBP) for NE ($3x10^{-11}$ to $3x10^{-6}$ mol/kg) or tyramine (Ty; $7x10^{-8}$ to $2x10^{-4}$ mol/kg) was begun. NPY infusion raised DBP>17.4±3.4 mmHg after 4 minutes. When compared to vehicle DBP responses, NPY augmented the dose-response to both NE and Ty. Neither α_1-blockade by P nor α_2-blockade by R altered the NPY potentiation of the NE dose-response. In a similar manner, NPY augmentation of the Ty DBP dose-response was not altered. Consequently, in SHR, NPY potentiates both α_1- and α_2-responses to both exogenously infused or endogenously released (Ty) NE. These data are in agreement with those derived from normotensive rats indicating a generalized NPY enhancement of DBP by alpha adrenergic mechanisms.

57.13

NEUROPEPTIDE Y (NPY) POTENTIATION OF ALPHA ADRENERGIC RESPONSES IN PITHED RATS.
Joan M. Hunter*, Bob Wilffert* and Barbara L. Pegram*.(Spon: Merrill B. Kardon). Alton Ochsner Medical Foundation, New Orleans, LA 70121 and Janssen Research Institute, Neuss, FRG.

Male Wistar rats (200g) were anesthetized with hexobarbital, pithed and catheters placed in the carotid artery and jugular veins to monitor arterial pressure and inject drugs, respectively. Prazosin (P; 0.1mg/kg) or rauwolscine (R; 1mg/kg) were injected 15 min prior to agonists. Vehicle (5ul/kg/min) or NPY (460 pmol/kg/min) infusion began 4 min before cumulative agonist response curves. NPY infusion increased diastolic pressure by 23±2 mmHg and was unaltered by adrenergic receptor blockade. Nifedipine (N;1 mg/kg,ia) decreased the direct effect of NPY to 12±1 mmHg. Following P α_2-adrenergic responses induced by norepinephrine (NE;iv) or NE released by tyramine were potentiated by NPY as were responses to the α_2-adrenergic agonist BHT-920. Similarly, blockade of the α_2-adrenergic receptors by R indicated that NPY potentiates the α_1-adrenergic responses of exogenously administered or endogenously released (tyramine) NE. N completely blocked the NPY potentiation of α_1-adrenergic responses to exogenous NE. The data indicate that NPY produces a calcium entry dependent increase in diastolic pressure via adrenergic receptors, while direct vasoconstriction by NPY is only partially calcium entry dependent.

57.14

HEMODYNAMIC EFFECTS OF NEUROPEPTIDE (NPY) IN CONSCIOUS RATS UNALTERED BY ADRENERGIC BLOCKADE WITH PHENTOLAMINE.
Barbara L. Pegram* and Joan M. Hunter*. (Spon: Merrill B. Kardon). Alton Ochsner Medical Foundation, New Orleans, LA 70121.

To determine if phentolamine (Ph;0.5mg/kg) would alter the systemic and regional hemodynamic effects of NPY, catheters were placed in femoral arteries, vein and left ventricle of anesthesized Wistar rats. Four hours later, control mean arterial pressure (MAP), cardiac index (CI, reference sample), heart rate (HR) and regional hemodynamics (microspheres) were determined after injection of vehicle. Measurements were repeated after vehicle, NPY (4ug/kg) or Ph and NPY. No significant changes were seen in vehicle animals. *p<0.05 compared with vehicle.

	VEHICLE	NPY	NPY+PH
Δ MAP (mmHg)	2±1	17± 3*	22±3*
Δ HR (b/min)	-3±3	-36± 5*	-33±4*
Δ CI (ml/min/kg)	-17±18	-52±20	-34±18
Δ TPRI (units)	0.02±0.02	0.14±0.04*	0.08±0.02*

Decreased blood flow (ml/min/g) to kidneys (7.2±0.6 vs 4.6±0.3) and skin (0.21±0.02 vs 0.09±0.01) induced by NPY were similar to those in rats receiving Ph and NPY (8.3±0.4 vs 5.3±0.5 and 0.16±0.02 vs 0.08±0.01, respectively). The MAP increase induced by NPY is associated with an increased TPRI and bradycardia that tends to decrease CI. This marked decrease in renal blood flow suggests a direct effect of NPY on the kidney independent of the adrenergic receptor.

57.15

NEUROPEPTIDE Y (NPY) LEVELS ARE ELEVATED IN PLASMA AND DUODENUM BUT DEPLETED IN ADRENAL GLAND AND VENA CAVA DURING DEVELOPMENT OF ENDOTOXICOSIS IN THE RAT.
Ronald R. Fiscus, Xian Wang*, Chi-De Han*, Zheng-Zheng Zhou*, Jiang Gu*, and Stephen B. Jones. Department of Physiology, Loyola University Medical Center, Maywood, IL 60153 and Deborah Cardiovascular Research Institute, Browns Mills, NJ 08015

Epinephrine (Ep) and norepinephrine (NE) are thought to be involved in compensatory mechanisms of endotoxin (ET) shock. Since NPY is sometimes co-released with Ep and NE and is a potent vasoconstrictor, NPY may also participate. We tested whether plasma NPY levels are elevated and organ levels depleted by ET using RIA and immunohistochemistry. Male Sprague-Dawley rats (300-350g) were anesthetized and arterial and venous cannulae implanted. Following 2 days recovery, ET (16 mg/kg of LPS from salmonella enteritidis) was injected and samples taken after 30 min and 3 hr. We found that plasma NPY levels were elevated at 30 min (saline control [SC]= 905±108 vs ET= 1510±223 pg/ml) and 3 hr (SC= 950±111 vs ET= 1590±153 pg/ml). Levels of NPY in duodenum rose at 3 hr (C= 157±5.2 vs ET= 204±9.9 pg/mg), but not at 30 min. Marked depletion of NPY was observed in adrenal gland (C= 346±35 vs ET at 30 min= 203±22 & 3 hr= 178±19 pg/mg) and vena cava (C= 73.4±7.5 vs ET at 30 min= 53.5±10.5 & 3 hr= 43.6±5.5 pg/mg). We conclude that injection of ET into rats causes release of NPY from adrenal gland and vena cava, thereby elevating plasma NPY levels, which may help to preserve blood pressure during compensatory phase of shock. Support NIH HL331163 and AHA 860962

57.16

MECHANISM OF BRADYKININ STIMULATION OF PROSTANOID SYNTHESIS IN AORTIC SMOOTH MUSCLE CELLS.
H. Zhang, T.S. Gaginella and D.G. Cornwell. Departments of Physiological Chemistry and Internal Medicine, The Ohio State University, Columbus, OH 43210

Bradykinin (BK) releases arachidonic acid (AA) in many cell types. This effect is presumed to be due to stimulation of phospholipase A2 or C activity. We have used cultured smooth muscle cells (SMC) from guinea pig aorta to study further the mechanism of action of BK. SMC were stimulated by BK, in the absence and presence of exogenous (AA). 6-keto-PGF$_{1\alpha}$(PG) was estimated by RIA. Cells also were labeled with ^{14}C-AA to determine by HPLC the pathway responsible for PG synthesis. BK, with or without AA, increased PG levels (*P<0.05).

Amount (n moles/plate) of PG (Mean ± S.E.M., n=experiments)

	No AA	60μM AA	120μM AA
Control	0.45±0.08(17)	1.45±0.30(14)*	1.72±0.16(11)*
Bradykinin			
50 μM	0.85±0.25(12)*	2.39±0.69(9)*	2.89±0.42(6)*
100 μM	1.10±0.21(6)*	2.54±0.24(6)*	2.99±0.22(6)*

BK also enhanced release of labeled AA metabolites, which was abolished by 10μM of indomethacin (IM). The enhanced PG synthesis even in the presence of excess AA and blockade of ^{14}C-AA metabolite release by IM, suggests that in aortic SMC BK stimulates PG synthesis through enhanced cyclooxygenase activity, not via AA release from membrane phospholipids.

57.17

RECEPTORS FOR KININS IN ISOLATED DOG ARTERIES. Nour-Eddine Rhaleb, Stéphane Dion, Guy Drapeau, Noureddine Rouissi and Domenico Regoli. Department of Pharmacology, Medical School, University of Sherbrooke, Sherbrooke J1H 5N4.

Two dog isolated vessels, the carotid and renal artery, with and without endothelium were used to characterize kinin receptors and elucidate the kinin mechanism of action on arterial smooth muscles. Using bradykinin (BK) and other B_2 receptor stimulants and antagonists as well as the B_1 agonist desArg^9-BK and a B_1 receptor antagonist, it was demonstrated that: the dog carotid artery has B_2 receptors in the endothelium, mediating arterial relaxation through the release of an endothelium derived relaxing factor (EDRF); the same was found in the renal artery: however, in this tissue, B_2 and B_1 receptors (presumably on the smooth muscle) were demonstrated in the absence of endothelium. The effects of kinins on muscular B_2 and B_1 receptors were blocked by indomethacin, suggesting that the muscular kinin receptors act through the activation of the arachidonic acid cascade. B_1 receptors were found to be present in the dog renal vessels from the beginning of the incubation and did not appear to be generated **de novo**. These findings may be of considerable importance for interpreting the mechanism of action of anti-hypertensive agents that may act by potentiating the action of kinins **in vivo**.

Supported by the Medical Research Council of Canada.

57.18

BRAINSTEM METABOLIC CORRELATES OF THE DEPRESSOR RESPONSE TO AREA POSTREMA STIMULATION IN RATS. S.W. Shaver*, D.S. Wainman*, A.V. Ferguson and P.M. Gross. Neurosurg. Res. Unit and Dept. Physiol., Queen's Univ. Kingston, Ontario K7L 3N6

Electrical stimulation (ES) of the area postrema (AP) in anesthetized rats produces arterial hypotension and bradycardia. AP neurons project to pontomedullary sites that may regulate cardiovascular depressor reflexes, such as dorsomedial NTS subnuclei, dorsal motor nucleus of the vagus nerve (DMN), and nucleus ambiguus (NA). Using albino rats ventilated with N_2O, O_2 and halothane, we studied the metabolic activity of AP projections by applying the [^{14}C]deoxyglucose method during sustained hypotension (20% decrease in MAP) produced by ES of the dorsocentral AP (120-200 uA, 15 Hz). Rates of glucose metabolism (umol/g/min; control values given) in dorsal strip, commissural and medial subnuclei of NTS were increased twofold (mean = 0.61). DMN (0.56), NA/A1 noradrenergic cell group (0.63), locus coeruleus (0.88), and lateral parabrachial nuclei (0.56) were also activated (avg +47%). The results indicate that excitatory or inhibitory neuronal mechanisms in AP pathways, either of which could increase glucose metabolism, are active and integrated during arterial hypotension resulting from AP stimulation.

57.19

ANTIHYPERTENSIVE RESPONSE TO A SINGLE DOSE OF A NEW ACEI RS-10085. Jan N. Lessem, Walter Flamenbaum and John Koshiver*; Syntex Research, Palo Alto, CA 94303, Beth Israel, New York, N.Y. 10003

RS-10085 is a novel non-sulfhydryl angiotensin converting enzyme inhibitor (ACEI), which is converted to an active diacid RS-10029. 30 patients with mild to moderate hypertension, 95-114 mmHg, age 26-70, mean of 48 years, were enrolled into a single dose, double blind placebo controlled study, to determine duration of effect. Patients were randomized to receive 3.75, 7.5, 15, 30 and 60 mg of RS-10085, or placebo after two weeks washout of previous antihypertensive medication. SDBP and SSBP was measured each 15 minute during the first hour post dose, hourly up to eight hours, and then 12, 24 and 48 hours post dose, using a random zero sphygmomanometer. ACE activity, PRA and plasma concentrations of RS-10085 was measured at the same time points. SDBP significantly decreased with 15, 30 and 60 mg RS-10085 while 7.5 mg was equal to placebo, and 3.75 mg less active than placebo. The peak antihypertensive effect occurred for all dose 2-4 hours post dose, lasted for 24 hours for 15, 30 and 60 mg but returned to baseline 48 hours post dose. HR was not affected by RS-10085. Blocking of the ACE correlated well with antihypertensive effect and for 15, 30 and 60 mg a 100% blocking occurred with a gradual increase over the first 4 hours. Plasma concentration of RS-10029 or 85 did not correlate with the antihypertensive effect. It is concluded that RS-10085 is a novel long acting ACEI.

METABOLISM RELATIONSHIP TO TOXICOLOGY I

58.1

PREVENTION OF CARDIOVASCULAR EFFECTS OF COPPER DEFICIENCY BY TREATMENT WITH DIMETHYL SULFOXIDE (DMSO). Jack T. Saari. USDA-ARS, Human Nutrition Res. Ctr., Grand Forks, ND 58202

Dietary Cu deficiency produces a variety of cardiovascular defects, some of which may result from oxidative damage. DMSO, an antiinflammatory agent and hydroxyl radical scavenger, was used to try to prevent the effects of Cu deficiency. Forty male, weanling, Sprague-Dawley rats were fed a Cu-deficient, Zn-deficient, sucrose-egg white based diet for 4 weeks. Their water contained 10 ppm Zn and either 2 ppm Cu (CuA, n=20) or no Cu (CuD, n=20). Ten of the CuA and ten of the CuD animals received DMSO (5%) in their water. As indicated in the table: CuD animals were significantly Cu deficient and remained so under DMSO (col 2); the enhanced heart weight/body weight ratio (HW/BW) caused by Cu deficiency was significantly inhibited by DMSO (col 3); the anemia occurring in Cu deficiency was significantly inhibited with DMSO (col 4), as was the depression of heart Cu (col 5). These findings support the idea that oxidative damage may play a role in Cu deficiency and that chronic treatment with an antioxidant may partially prevent the damage.

Diet	Liver Cu(μg/g)	HW/BW (mg/g)	Hct(%)	Heart Cu (μg/g)
CuA	6.3 ± 2.3	4.2 ± 0.1	42 ± 2	7.4 ± 2.1
CuA-DMSO	12.0 ± 1.6	4.2 ± 0.2	45 ± 2	18.0 ± 4.0
CuD	0.8 ± 0.4	7.5 ± 1.8	22 ± 10	4.1 ± 0.6
CuD-DMSO	0.9 ± 0.8	5.5 ± 0.5	32 ± 7	6.1 ± 1.2

58.2

POTENTIATION OF CIS-PLATINUM NEPHROTOXICITY BY COPPER DEFICIENCY. B. Noordewier* and J.T. Saari. Dept. of Pharmacol., Univ. of North Dakota and USDA-ARS, Human Nutrition Res. Ctr., Grand Forks, ND 58202

Cellular protection against nephrotoxins may involve Cu-dependent enzymes. This study examined the nephrotoxicity of cis-Pt in Cu-deficient rats. Weanling male Sprague-Dawley rats were fed a purified diet deficient in Cu and either distilled water (Depl) or water with 5 ppm Cu (Repl). After 24 days, rats were given (iv) either cis-Pt (5 mg/kg) or saline in a 2x2 design. Rats from each group were killed on day 0 (preinjection) and 4 day postinjection. Compared to Repl rats, the Depl rats on day 0 showed physiologic evidence of Cu depletion: Lower body weight, lower hematocrit and greater heart weight. The blood urea nitrogen (BUN, mg/dl) of Depl rats given cis-Pt was 50.9 on day 4 versus 19.3 on day 0. Saline administration did not affect BUN of Depl rats (21.4 on day 4). The BUN of the Repl rats given saline fell from 14.4 (day 0) to 11.9 (day 4), while that of cis-Pt rats rose to 26.0. Two-way ANOVA revealed that both diet and cis-Pt effects on BUN were significant ($p<.001$). Further, the Depl rats were more sensitive to cis-Pt than the Repl rats ($p<.05$). Cis-Pt decreased food consumption and increased water consumption in both Depl and Repl rats, though these effects were more marked in the Depl rats. These data support the hypothesis that, in part, cellular protection against cis-Pt involves Cu dependent processes.

58.3

CARBON TETRACHLORIDE TOXICITY IN PRECISION-CUT RAT LIVER SLICES. Shana Azri*, A. Jay Gandolfi, Klaus Brendel, Departments of Anesthesiology and Pharmacology, University of Arizona, Tucson, AZ 85724.

The toxicity of CCl_4 was examined in precision cut rat liver slices from male Sprague-Dawley rats (225-245 g). Liver slices were incubated in a roller culture system in Waymouth's media (10 mg/ml gentamycin) at 37°C for upto 3-9 hr. The slices were exposed to CCl_4 by vaporizing small volumes (1-10 µl) from a suspended paper wick. 2.5 µl of vaporized CCl_4 produced an initial concentration of 0.6 mM in the media. Intracellular K^+ and enzyme release (GPT and ICDH) were used as indicators of toxicity. The toxicity of CCl_4 was found to be dose-dependent. At 0.6 mM CCl4, K^+ leakage (↓ 60%) occurred between 6 and 9 hr. Higher concentrations produced significant losses of K^+ by 3 hr (↓ 70%). Liver slices obtained from rats pretreated with phenobarbital were more rapidly intoxicated even at the lower CCl_4 concentrations. As expected, the CCl_4 toxicity to the liver slices was O_2 dependent. As the O_2 atmospheres became more hypoxic (95 → 2% O_2), the toxicity was increased and appeared faster (↓ 50% K^+ by 3 hr with 0.6 mM CCl_4). This model will allow us to investigate the susceptible target cells for CCl_4 intoxication in a biological system where all the architecture for the liver is retained. (Supported by GM38290).

58.4

CYCLOSPORIN A (CsA) TOXICITY IN PRIMARY RABBIT PROXIMAL TUBULAR CELLS. Paul P. Sokol*, Peter D. Holohan and Charles R. Ross. SUNY-Health Science Center, Syracuse, NY 13210 and Indiana University School of Medicine, Indianapolis, IN 46202

Primary proximal tubular cells were used to study CsA nephrotoxicity. These cells, grown on millicell inserts, retain the functional polarity of the proximal tubule, i.e. generate a transepithelial pH gradient (ΔpH). The brush border compartment acidifies indicative of Na^+/H^+ exchange. The chemicals tested were as follows: Sandimmune, the commercial form of CsA; its vehicle, Cremophor El; and CsA in ethanol. The effect of these compounds on the ΔpH was tested. Sandimmune (25 and 50µM) administration inhibited the generation of the ΔpH in 24 hours. Surprisingly, the cremophor also blocked the ΔpH, albeit to a lesser extent. In contrast, 10µM CsA had no effect on the ΔpH for up to 96 hours. In conclusion, these cells are sensitive to certain forms of CsA and to the vehicle. These findings suggest that the vehicle may contribute to the well established nephrotoxicity of CsA. (Supported by NIH #02835)

58.5

CYTOTOXICITY OF CEPHALORIDINE IN PURIFIED RAT KIDNEY PROXIMAL AND DISTAL TUBULAR CELLS. Lawrence H. Lash* (SPON: P.F. Hollenberg). Wayne State Univ. Sch. Med., Detroit, MI 48201

Proximal (PT) and distal (DT) tubular cells were purified from rat kidney cortex by collagenase digestion and Percoll density-gradient centrifugation. Purity was assessed by activity of marker enzymes and by respiratory characteristics. Four populations of cells were obtained: Fraction 1 (top) contained some PT cells, and cell debris; fraction 2 was the most highly enriched in PT cells, as judged by high alkaline phosphatase (AP) and γ-glutamyl-transferase (GT) activities, and by stimulation of respiration by succinate; fraction 3 contained a mixture of PT and DT cells; fraction 4 (bottom) was the most highly enriched in DT cells, as judged by high hexokinase and low AP and GT activities. Fractions 2 and 4 were employed to study the cell-type specificity of cephaloridine (CPH)-induced nephrotoxicity. CPH was toxic in PT cells but not in DT cells; viability of PT cells incubated with 0.1 or 1 mM CPH for 2 h was 45% or 15%, respectively, compared to 80% for control cells; viability of DT cells incubated with 0.1 or 1 mM CPH for 2 h was 70% or 65%, respectively, compared to 70% for control cells. These results demonstrate the usefulness of isolated PT and DT cells to examine mechanisms of nephron cellular heterogeneity with regards to metabolism and disposition of xenobiotics. (Supported by a PMA Foundation Starter Grant and NIH Grant DK40725.)

58.6

MOLECULAR MECHANISMS OF ALLYL ALCOHOL INDUCED CYTOTOXICITY IN ISOLATED HEPATOCYTES J. Silva*, D.L. Drolet* and P.J. O'Brien* (SPON: P.G. Wells), Faculty of Pharmacy, University of Toronto, Toronto, Canada, M5S 2S2.

Allyl alcohol induced hepatotoxicity, primarily seen in the periportal region, probably occurs following metabolic activation to acrolein by alcohol dehydrogenase. Allyl alcohol and acrolein was found to readily deplete glutathione in isolated hepatocytes before cytotoxicity ensued. The changes induced by allyl alcohol but not acrolein were prevented by pyrazole > > dimethylsulfoxide or allopurinol. Alcohol dehydrogenase was also inhibited. Metyrapone or imidazole had no effect whereas azide enhanced these changes. This suggests that alcohol dehydrogenase and not catalase or mixed function oxidase was responsible for the alcohol activation. Allyl alcohol and acrolein induced cytotoxicity, but not glutathione depletion, was delayed by antioxidants or the ferric chelator desferrioxamine whereas lipid peroxidation was completely prevented. Lipid peroxidation did not occur as a result of glutathione depletion but was attributed to a microsomal mixed function oxidase catalysed autooxidation of acrolein to peroxy radicals and peracids which initiate lipid peroxidation. The location of allyl alcohol induced necrosis could therefore reflect the higher oxygen concentration in Zone I being higher than that in the Zone III region of the liver. (Supported by the Medical Research Council of Canada.)

58.7

IN VITRO EVIDENCE FOR LIPOXYGENASE-CATALYSED BIOACTIVATION OF PHENYTOIN. Stanley Kubow* and Peter G. Wells, Faculty of Pharmacy, University of Toronto, Toronto, Canada.

The anticonvulsant drug phenytoin can be bioactivated by prostaglandin synthetase (PGS) to a reactive free radical intermediate that may be toxicologically relevant (Kubow and Wells, Pharmacologist 28: 195, 1986). Cooxidation of xenobiotics has been shown to occur during PGS catalysed reduction of hydroperoxy PGG_2 to the hydroxy PGH_2. Since arachidonic acid also is a substrate for the lipoxygenase (LPO) pathway, which catalyses a similar reduction of 12-HPETE to 12-HETE, this study evaluated the potential bioactivation of phenytoin by LPO. Unlabelled phenytoin (0.1 mM) and ^3H-phenytoin (4.24 nM, 0.2 µCi) was incubated for 60 min at 37°C with rat liver microsomes (RLM) or equine thyroid microsomes (ETM) in the presence or absence of 6.0 mM arachidonic acid. Bioactivation of phenytoin to a reactive intermediate was quantified by the irreversible (covalent) binding of radiolabelled phenytoin to microsomal protein. Addition of arachidonic acid to RLM or ETM resulted in respective 4.1-fold and 5.7-fold increases in the covalent binding of phenytoin ($p<0.05$). Addition of 0.2 to 2.0 mM nordihydroguaiaretic acid, an inhibitor of LPO that does not inhibit the cyclooxygenase pathway, inhibited the arachidonic acid-dependent covalent binding of phenytoin in RLM by 88%, and in ETM by 77% ($p<0.05$). These studies suggest that LPO may be an alternative pathway for the bioactivation of phenytoin to a reactive intermediate. (Supported by the Medical Research Council of Canada).

58.8

THE EFFECT OF TWO ALKYL XANTHATE TETRASULFIDES ON THE FORMATION OF THIOCYANATE (SCN) FROM THIOSULFATE IN THE CONVERSION OF CYANIDE (CN) BY RHODANESE IN GUINEA PIG LIVER HOMOGENATE. L.S. Pellicore*, S.I. Baskin, M.D. Dulaney, Jr.*, and R.H. Legore*. United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground - Edgewood Area, MD 21010 - 5425.

The conversion of toxic CN to the less toxic SCN by rhodanese is limited by the availability of sulfur for the reaction. The effectiveness of two alkyl xanthate tetrasulfides in potentiating the sulfur donating activity of sodium thiosulfate to rhodanese was examined in the guinea pig liver preparation according to the method of Westley (Adv. Enz. 39:327, 1973). The tetrasulfides AM66105 (S,S'-dithio bis(methylxanthate)) and AM66169 (S,S'-dithio bis(n-propylxanthate)), at a concentration of 1 mM, were examined in the presence and absence of sodium thiosulfate ranging in concentration from 0.01 to 10.0 mM. The guinea pig liver rhodanese assay in the presence of thiosulfate alone behaves according to the following Michaelis-Menten equation: 1/velocity = 2.89 × 1/[substrate] + 1.56 ($r=0.99$, $V_{max}=0.64$ umoles/min/mg protein, $K_m=1.85$ mM). AM66105 decreased the slope from 2.89 to 0.25 ($r=0.87$), increased V_{max} from 0.64 to 0.67 and decreased K_m from 1.85 to 0.16. AM66169 decreased the slope from 2.89 to 0.73 ($r=0.91$), decreased V_{max} from 0.64 to 0.60 and decreased K_m from 1.85 to 0.44. Neither alkyl xanthate tetrasulfide significantly changed V_{max}, indicating that neither AM66105 nor AM66169 was acting alone as sulfur donors. However, as evidenced by the decreased K_m of AM66105 (0.16) and AM66169 (0.44), both alkyl xanthate tetrasulfides were able to potentiate rhodanese activity in the presence of thiosulfate. AM66105 proved 134% more effective as a sulfur source than thiosulfate alone, while AM66169 was 43% more effective as a sulfur source than thiosulfate alone. Both AM66105 and AM66169 increased the rate of formation of SCN from CN and might prove useful in enhancing the sulfur-donating activity of thiosulfate in the conversion of CN.

58.9

COMPARISON OF GUANIDINOSULFIDES WITH THIOSULFATE (S_2O_3) IN THE CONVERSION OF CYANIDE (CN) TO THIOCYANATE (SCN) BY GUINEA PIG LIVER RHODANESE.

S. Baskin, M. Dulaney, Jr.*, L. Pellicore* and R. Legere* USAMRICD, Pharmacol. Div., APG, MD 21010

The rate of formation of the less toxic SCN from CN by rhodanese is dependent on the sulfane-sulfur source and can be stimulated in vitro by the presence of certain polysulfides. 2, 2' guanidinoethane hexasulfide (HS) and 2, 2' guanidinoethane tetrasulfide (TS) (1 mM) were examined for their donor activity to rhodanese in the absence or presence of 0.01-10 mM S_2O_3. Guinea pig liver rhodanese activity was measured by following the formation of SCN at 460 nm (Westley, Adv. Enz. 39:327, 1973). TS and HS showed 38 and 276% greater effect, respectively, than equimolar S_2O_3 alone. In the presence of S_2O_3 (0.01-10 mM), TS and HS showed 351 and 237% greater effect than S_2O_3 alone. The K_m of rhodanese with TS, HS and S_2O_3 alone was 0.57, 0.15 and 0.40 mM, respectively and the Vmax was 0.72, 0.53 and 0.16 umol/min/mg protein, respectively. These data suggest that the length of the polysulfide differentially influences the ability of S_2O_3 to both act as a sulfur donor and to stimulate rhodanese. These studies may provide useful therapy for the conversion of CN to SCN.

58.10

ASCORBIC ACID DEFICIENCY AND HEPATIC UDP-GLUCURONYLTRANSFERASE. C. M. Neumann* and V. C. Zannoni. Univ. of Michigan, Ann Arbor, MI 48109.

The effect of dietary ascorbic acid on hepatic microsomal UDP-glucuronyltransferase (UDPGT) activity towards p-aminophenol (p-AP), p-nitrophenol (p-NP), bilirubin and acetaminophen was investigated. Ascorbate-deficiency produced a 33% reduction towards p-AP and a 46% reduction towards p-NP. There was no difference in ascorbate-deficient and supplemented guinea pigs in the activity towards bilirubin or acetaminophen. Competitive kinetics of p-AP and p-NP suggest the effect of the vitamin is on a specific isoenzyme. No difference was found in the apparent affinity for p-AP or UDP-glucuronic acid (UDPGA). Differences in UDPGT activity towards p-AP occurred between the two groups with sonication, triton X-100 and emulgen 911. These membrane-perturbants increased activity 3.3-fold in the ascorbate-supplemented animals and 1.4-fold in the deficient group. The addition of ascorbate in vitro could protect against the detrimental effects of excess p-AP by maintaining a linear enzymatic rate over a 30 min. period. Glutathione was as effective as ascorbic acid; cysteine and dimethyltetrapterdine were partially effective. Ascorbyl-2-sulfate and α-tocopherol had no effect. Phosphatidylcholine, an essential phospholipid for UDPGT, had no significant effect on UDPGT activity in the ascorbate-supplemented group while it increased the activity by 34% in the ascorbate-deficient group.

(Supported by Hoffmann-LaRoche Grant 23007)

58.11

BIOTRANSFORMATION AND TOXICITY OF ACETAMINOPHEN IN CONGENIC RHA RATS WITH BILIRUBIN GLUCURONYL TRANSFERASE DEFICIENCY. Susanna Y.M. Chow*, Sonia M.F. de Morais* and Peter G. Wells, Faculty of Pharmacy, University of Toronto, Toronto, Canada.

Acetaminophen (APAP) is eliminated primarily by glucuronidation. APAP (750 mg/kg ip) was administered to congenic heterozygous and homozygous RHA rats with normal (++), moderately deficient (+j) and severely deficient (jj) activities of bilirubin glucuronyl transferase (GT). APAP metabolites were measured by high-performance liquid chromatography and production of the APAP-glucuronide (GLUC) conjugate was quantified by the area under the plasma concentration-time curve (AUC) for APAP-GLUC/AUC APAP. Hepatotoxicity and nephrotoxicity were assessed respectively by the peak plasma alanine aminotransferase (ALT) concentration and blood urea nitrogen (BUN). RHA/jj rats showed a 52% reduction in APAP glucuronidation ($p<0.05$), and respective 83-fold ($p<0.05$) and 4-fold increases in ALT and BUN compared to RHA/++ controls (table).

TABLE: Toxicity of APAP, 750 mg/kg ip, in 14 week old rats

Strain	GLUC	ALT (IU/L)	BUN (mg/dL)
RHA/++	0.28±0.02	118±26	17.23±0.74
RHA/j+	0.24±0.02	235±132	19.08±2.21
RHA/jj	0.13±0.04*	9753±4850*	67.43±51.16

Data are mean ± SE. * different from RHA/++ and RHA/j+ ($p<0.05$).

ALT elevations correlated with decreased APAP glucuronidation ($r=-0.80$, $p<0.001$). These results using congenic controls support earlier studies using non-congenic controls (Hepatology 7: 1046, 1987) which suggested that the enhanced susceptibility of GT-deficient rats to APAP toxicity is due to decreased glucuronidation. (Support: Medical Research Council of Canada).

58.12

IN VIVO STUDIES ON THE BIOCHEMICAL MECHANISM OF ACETAMINOPHEN CATARACTOGENICITY USING C57BL/6 and DBA/2 MICE. Barry M. Lubek*, Barry Wilson* and Peter G. Wells, Faculty of Pharmacy and Department of Ophthalmology, University of Toronto, Toronto, Canada.

C57BL/6 and DBA/2 mice are respectively susceptible and resistant to the cataractogenicity of acetaminophen (APAP), which may involve cytochromes P-450-catalysed bioactivation to a toxic reactive intermediate. Following induction of P-450 using beta-naphthoflavone (BNF), the cataractogenicity of APAP (400 mg/kg ip) in C57BL/6 mice was reduced by pretreatment the P-450 inhibitors SKF 525A and metyrapone, the glutathione (GSH) precursor N-acetylcysteine, the antioxidant vitamine E and the free radical spin trapping agent alpha-phenyl-N-t-butylnitrone ($p<0.05$). APAP (200 mg/kg) cataractogenicity was enhanced by pretreatment with the gamma-glutamylcysteine synthetase inhibitor buthionine sulfoximine (BSO) ($p<0.05$). No significant effect on APAP cataractogenicity was observed using the prostaglandin synthetase inhibitor aspirin or the GSH reductase inhibitor 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU). In DBA/2 mice induced with BNF, APAP (750 mg/kg ip) was not cataractogenic, even after pretreatment with DEM, BSO or BCNU. These results suggest that APAP cataractogenicity results from P-450-catalysed bioactivation of APAP to a reactive intermediate, possibly a benzoquinone imine and/or a free radical, the toxicity of which is reduced by GSH-dependent reactions. (Support: Medical Research Council of Canada and the Connaught Fund, University of Toronto)

58.13

IN VIVO MURINE STUDIES ON THE BIOCHEMICAL MECHANISM OF NAPHTHALENE CATARACTOGENESIS. Peter G. Wells, Barry Wilson* and Barry M. Lubek*, Faculty of Pharmacy and Department of Ophthalmology, University of Toronto, Toronto, Canada.

The polycyclic aromatic hydrocarbon naphthalene (N) is bioactivated by cytochromes P-450 to an electrophilic epoxide intermediate, which subsequently is metabolised to naphthoquinone (NQ), and possibly to a free radical intermediate. These reactive intermediates may bind covalently to lenticular tissues, cause oxidant stress and/or lipid peroxidation, thereby initiating cataracts. In C57BL/6 mice, cataracts were caused by N (125-1000 mg/kg ip) in a dose-dependent fashion. The incidence of N-induced cataracts was reduced by pretreatment with the P-450 inhibitors SKF 525A and metyrapone, the antioxidants caffeic acid and vitamin E, the glutathione (GSH) precursor N-acetylcysteine, and the free radical spin trapping agent alpha-phenyl-N-t-butylnitrone ($p<0.05$). N cataractogenicity was enhanced by pretreatment with the GSH depletor diethyl maleate (DEM) ($p<0.05$), and unaffected by pretreatment with the prostaglandin synthetase inhibitor aspirin. Cataracts were initiated by 1,2-NQ and 1,4-NQ (5-125 mg/kg ip) in a dose-dependent fashion, with a molar potency about 10-fold higher than that of N. NQ cataractogenicity was enhanced by pretreatment with DEM ($p<0.05$). These results suggest that N cataractogenesis requires P-450-catalysed bioactivation to a reactive intermediate, which may be the NQ and/or a free radical derivative. (Support: Medical Research Council of Canada and the Connaught Fund, University of Toronto)

58.14

INDUCTIVE PROPERTIES OF ALIPHATIC MONOKETONES ON CYTOCHROME P-450 OXIDASES. Manon Vézina*, Patrick du Souich, Lise Jutras* and Gabriel L. Plaa. Université de Montréal, Montréal, Québec, H3C 3J7, Canada.

Aliphatic monoketones are solvents widely used industrially. Cytochrome P-450 has been postulated as a contributing factor in the potentiation of liver injury by some ketones. The inducing properties of nine (9) monoketones of different chain lengths were evaluated by preparing rat liver and kidney microsomes. Ketones were given by gavage at a dosage of 7.5 mmol/kg to groups of 4 rats for 3 days: 2-propanone (3C), 2-butanone (4C), 2-pentanone (5C), 2-hexanone (6C), 4-methyl-2-pentanone (6C), cyclohexanone (6C), 2-heptanone (7C), 2-methyl cyclohexanone (7C) and 2-octanone (8C). Cytochrome P-450 total liver content was significantly increased in microsomes from rats given 2-propanone, 2-butanone, 4-methyl-2-pentanone and 2-hexanone, whereas the kidney content was not affected by any ketone. Proteins of 47, 49, 50, 51, 53, 56 and 57kD were separated by gel electrophoresis. The 49, 51 and 53kD fractions were significantly increased in liver microsomes of rats given ketones. The 50 and 53 kD fractions were most often increased in the kidney. The inducing potency differed according to the source of microsomes and the ketone used. The 6 most promising ketones in terms of induction are 2-propanone, 2-hexanone, 4-methyl-2-pentanone, cyclohexanone, 2-heptanone and 2-octanone. (Supported by NSERC and MRC).

58.15

METABOLISM OF ACETAMINOPHEN IN ISOLATED HEPATOCYTES. T.A. Smolarek, C.V. Higgins, D.E. Amacher, and S. Cohen (SPON: M.Schach von Wittenau). Pfizer Central Research, Groton, CT, 06340, Univ. of Connecticut, Storrs, CT 06268

To determine the mechanisms of acetaminophen (APAP) induced hepatotoxicity, the metabolism of APAP in isolated hepatocyte cultures from sensitive and resistant species was studied. The amounts of APAP metabolized and the amounts of conjugates formed varied for the sensitive and the resistant species: 20 HOUR EXPOSURE TO 2mM APAP

	%APAP Metabolized	% Metabolite A	B	C	in vitro Toxicity
Rat (resistant)	53	41	54	5	-
Rabbit (resistant)	57	11	85	4	-
Dog (sensitive)	31	16	83	1	++
Monkey (sensitive)	42	1	97	2	+

HPLC analylses demonstrated the presence of APAP-sulfate(A), APAP-glucuronide(B) and APAP-glutathione(C) conjugates in all species. The percentage of APAP metabolized in the resistant species was higher than for the sensitive species. The rat formed the highest percentage of APAP-sulfate conjugate. The glucuronide conjugate was the major metabolite formed for all three species while the glutathione conjugate was a relatively minor metabolite for all three species. Thus these results indicate that the study of APAP metabolism in isolated hepatocyte cultures correlates with the species specific differences in APAP induced hepatotoxicity in vivo.

58.16

COVARIATION IN THE POLYMORPHIC EXPRESSION OF AcCoA-DEPENDENT N-ACETYLTRANSFERASE AND O-ACETYLTRANSFERSE ACTIVITY BY HUMAN BLADDER CYTOSOL. W.G. Kirlin, A. Trinidad, T. Yerokun, F. Ogolla, R.J.Ferguson, A.F. Andrews, P.K. Brady, and D.W. Hein. Department of Pharmacology, Morehouse School of Medicine, Atlanta, Georgia 30310.

The capacity of human bladder to N-acetylate arylamines, via N-acetyltransferase (NAT), may be involved in metabolic pathways leading to bladder cancer. Another possible step is direct O-acetyltransferase (OAT) activation of N-hydroxy arylamines. Human bladder cytosol from 9 fresh autopsy specimens were studied for NAT activity towards p-aminobenzoic acid (PABA), and the arylamine carcinogens 4-aminobiphenyl (ABP), 2-aminofluorene (AF), and beta-naphthylamine (BNA). Apparent Vmax NAT activities towards ABP, AF, and BNA separated the bladders into rapid or slow acetylator phenotypes. Four bladder cytosols had mean activities significantly ($p<.01$) higher (10-fold) than the mean NAT activities of the other five bladder cytosols towards each arylamine carcinogen. However, no difference was detected in their PABA NAT activities. OAT-mediated binding of N-OH-3,2'-dimethyl-4-aminobiphenyl to DNA also was significantly ($p=.0002$) higher (2-fold) in rapid N-acetylator bladder cytosols, and correlated ($r=0.94$) with NAT activity. These results suggest that NAT and OAT activity of the human bladder vary concordantly with N-acetylator phenotype. Polymorphic expression of acetylation activities may be important risk factors in human susceptibility to arylamine-induced bladder cancer.(Supported by USPHS CA-34627, RR-08248).

58.17

KINETIC CHARACTERIZATION OF ACETYLATOR GENOTYPE-DEPENDENT AND -INDEPENDENT ARYLAMINE N-ACETYLTRANSFERASE ISOZYMES IN HOMOZYGOUS RAPID AND SLOW ACETYLATOR HAMSTER LIVER CYTOSOL. **A. Trinidad, W.G. Kirlin, F. Ogolla, A. Andrews, T. Yerokun, R. Ferguson, and D.W. Hein. Dept. of Pharmacology, Morehouse School of Medicine, Atlanta, GA 30310.**

We have characterized the expression of hepatic N-acetyltransferase (NAT) from homozygous rapid (RR) and homozygous slow (rr) acetylator inbred hamsters towards the arylamine carcinogens 2-aminofluorene (AF) and 4-aminobiphenyl (ABP). We previously showed that partial purification of the NAT activity in liver of RR and rr acetylators yields two NAT isozymes in each acetylator genotype. This study characterizes liver cytosol and the two NAT isozymes with respect to their kinetic constants towards AF and ABP. Both crude cytosol and the first eluting NAT isozyme exhibited a significantly greater apparent Vmax (6 to 10-fold) and Km in RR acetylator liver cytosol towards AF and ABP, while the second eluting NAT isozyme did not exhibit Vmax and Km variations towards AF and ABP between acetylator genotypes. This study suggests that the acetylator genotype-dependent expression of arylamine carcinogen NAT activity in hepatic cytosol is due to structural variants of a polymorphic NAT isozyme. (Supported by USPHS CA-34627 RR-08248 and GM-07808.)

58.18

GENETIC CONTROL OF ARYLAMINE N-ACETYLTRANSFERASE ACTIVITY IN LIVER, INTESTINE AND COLON CYTOSOL OF SYRIAN INBRED HAMSTERS. **Fred Ogolla, Ronald J. Ferguson, Ward G. Kirlin, Alma Trinidad, Tokunbo Yerokun, Allen F. Andrews, and David W. Hein. Dept. of Pharmacology, Morehouse School of Medicine, Atlanta, GA 30310.**

N-acetyltransferase (NAT) catalyzes the acetylation of a number of arylamine and hydrazine drugs and carcinogens. AcCoA-dependent NAT activity towards p-aminobenzoic acid (PABA) and the arylamine carcinogen 2-aminofluorene (AF) was measured and compared in liver, intestine and colon cytosols of inbred hamsters of defined acetylator genotypes. A significant acetylator gene-dose response was observed for NAT activity in colon and intestine cytosols towards PABA and AF analogous to that observed in liver cytosol. Determination of colon and intestine cytosolic NAT activities in an F_2 generation yielded a trimodal distribution of progeny consistent with 1:2:1 ratio of homozygous rapid, heterozygous, and homozygous slow acetylator genotypes similar to liver. These results identify NAT activity in gut and colon that is regulated by the acetylator gene locus. Similar expression of high levels of NAT activity in gut and colon may contribute to the predisposition of human rapid acetylators to arylamine-related colorectal cancer. (USPHS grants CA 34627 and RR 08248).

58.19

HUMAN ACETYLATION GENOTYPE DETERMINATION BY URINARY CAFFEINE METABOLITES. AJ Kilbane*, LK Silbart*, M Manis*, IZ Beitins** and WW Weber*. Departments of Pharmacology* and Pediatric Endocrinology,** Univ. of Mich. Sch. of Med., Ann Arbor, MI.

With a modification of a previously published HPLC method (Tang et al. 1987), the human acetylation genotype can now be reliably determined, by measuring urinary caffeine metabolites. The method, which again utilizes the molar ratio: AAMU/(AAMU + 1-U + 1-X) has been modified, to facilitate the chromatographic separation of 1-U from other interfering methylxanthinc and methylurate metabolites. Consequently, the delicate separation of 1-U from 7-X is consistently achieved, by the addition of a phenyl column in tandem with a C-18 reverse phase column, while running a methanol:acetic acid gradient solvent system. An AAMU molar ratio < 38% indicates slow acetylator genotype and an antimode at 65% separates hetero- and homozygous rapids.

Twenty subjects phenotyped with dapsone, exhibited 100% concordance when compared with the caffeine method. The slow acetylator gene frequency(q), estimated from studying 44 unrelated individuals, was 0.69. The genotypic distribution-4(RR), 19(Rr), 21(rr)- satisfied the Hardy-Weinberg law of population genetics(X^2_1=0.014). Definitive pedigree analysis of 18 families(80 subjects) yielded a trimodal distribution pattern and agreed with expected Mendelian segregation.

A non-invasive acetylator genotyping method is described, which potentially has more widespread use, in future investigation of the acetylation polymorphism and clinical disease.

Supported by USPHS Grants GM-27028 and CA-39018..

58.20

OXIDATIVE CYCLIZATION, 1,4-BENZOTHIAZINE FORMATION AND POLYMERIZATION OF 2-BROMO-3-(CYSTEIN-S-YL)HYDROQUINONE Terrence J. Monks, Robert J. Highet and Serrine S. Lau. The Univ. Texas M.D. Anderson Cancer Center, Science Park-Research Division, Smithville, TX 78957, NHLBI, NIH, Bethesda, MD 20892 and Div. of Pharm., The Univ. of Texas at Austin, Austin, TX 78712.

The renal specific toxicity of quinol-linked glutathione (GSH) conjugates is probably a result of their selective accumulation by proximal tubular cells mediated by γ-glutamyl transpeptidase. Transport of the resultant cysteine conjugate followed by oxidation to the quinone may be the mechanism of toxicity. Factors modulating the intracellular concentration of the cysteine conjugate will be important determinants of toxicity. We have now synthesized and purified 2-bromo-3-(cystein-S-yl)hydroquinone. The compound is unstable and undergoes a pH dependent rearrangement that requires initial oxidation to the quinone. UV spectroscopy revealed the formation of a chromophore consistent with 1,4-benzothiazine formation. This product arises via cyclization of the cysteine residue via an intramolecular 1,4-Michael addition. At neutral pH, further reaction results in the precipitation of a pigment which exhibits properties of a pH indicator. The pigment undergoes a marked pH dependent bathochromic shift (~100 nm); it is red in alkali (λ_{max} 480 nm) and violet in acid (λ_{max} 578 nm). These properties are similar to those of the trichochrome polymers which are formed during melanin biosynthesis from cystein-S-yl-DOPA. Since the intramolecular cyclization reaction removes the reactive quinone moeity from the molecule, it might be regarded as a detoxification reaction. However, the ultimate formation and intracellular accumulation of insoluble polymers might contribute to the mechanism of toxicity of quinol-linked GSH/cysteine conjugates. (Supported by ES04662 and GM39338)

58.21

MULTIPLE GLUTATHIONE ADDITION TO 1,4-BENZOQUINONE: CORRELATION OF NEPHROTOXICITY WITH INCREASED GLUTATHIONE SUBSTITUTION AND OXIDATION POTENTIAL Serrine S. Lau, Barbara A. Hill*, Robert J. Highet and Terrence J. Monks Div. of Pharm., The Univ. of Texas at Austin, Austin, TX 78712, NHLBI,NIH, Bethesda MD 20892 and The Univ. Texas M.D. Anderson Cancer Center, Science Park-Research Division, Smithville, TX 78957.

We have previously shown that the oxidation of 2-bromohydroquinone (2-BrHQ) in the presence of glutathione (GSH) gives rise to both mono- and di-substituted GSH adducts. 2-Br-(diGSyl)HQ is a potent and selective nephrotoxicant. We have now studied the reaction of 1,4-benzoquinone (BQ) with GSH and identified 2-(GSyl)HQ, 2,3-(diGSyl)HQ, 2,5-(diGSyl)HQ, 2,6-(diGSyl)HQ , 2,3,5-(triGSyl)HQ and 2,3,5,6-(tetraGSyl)HQ as the products. The initial conjugation of BQ with GSH did not affect the oxidation potential (E_o) of the compound. However, subsequent oxidation and GSH addition resulted in conjugates which, dependent upon the position of addition, became increasingly more difficult to oxidize. Increased GSH substitution which resulted in higher E_o also resulted in enhanced nephrotoxicity. 2,3,5-(Tri-GSyl)HQ was the most potent nephrotoxicant; each of the three di-GSyl conjugates exhibited similar degrees of toxicity. In contrast, 2-(GSyl)HQ was only slightly toxic and 2,3,5,6-(tetraGSyl)HQ was not toxic. Thus, with the exception of fully substituted isomer, the severity of the necrosis correlated with the extent of GSH substitution. Thus, the conjugation of GSH with quinones does not necessarily result in detoxification, even when the resulting conjugates are more stable to oxidation. The lack of toxicity of 2,3,5,6-(tetraGSyl)HQ is probably a consequence of its inability to alkylate tissue macromolecules. (Supported by USPHS awards ES04662 and GM39338)

58.22

APICAL VERSUS BASOLATERAL MEDIATED TOXICITY OF 2,3,5-(TRI-GLUTATHION-S-YL) HYDROQUINONE IN LLC-PK1 CELLS. Barbara A. Hill*, Terrence J. Monks and Serrine S. Lau. Div. of Pharm., The Univ. of Texas at Austin, Austin, TX 78712 and The Univ. Texas M.D. Anderson Cancer Center, Science Park-Research Division, Smithville, TX 78957.

We have previously shown that the conjugation of simple quinones with glutathione (GSH) results in the formation of multi-substituted GSH adducts which are potent and selective nephrotoxicants in rats. The reason(s) for the target organ toxicity of these conjugates are unclear but may be related to their selective uptake into proximal tubular cells mediated by γ-glutamyl transpeptidase (GGT). For example, the *in vivo* inhibition of GGT by AT-125 protects animals from 2,3,5-(tri-GSyl)hydroquinone [HQ-$(GSH)_3$] nephrotoxicity. We have now developed a cell culture model in which LLC-PK1 cells (pig renal epithelial cell line) are grown in Costar Transwell Cell Culture Chambers. The cells can be treated from either the apical (brush border membrane) or basolateral (plasma membrane) cell surface. Diffusion across the monolayer at confluency is minimal as evidenced by the inability of these cells to actively transport para-aminohippurate. Apical treatment of confluent LLC-PK1 cells with HQ-$(GSH)_3$ caused a dose- and time- dependent leakage of LDH. Thus, apical treatment with 0.5 mM HQ-$(GSH)_3$ caused maximal LDH leakage (68.0 ± 5.9%) after 15 hr. In contrast, basolateral treatment with 0.5 mM HQ-$(GSH)_3$ caused only 17.5 ± 0.1% LDH leakage after 15 hrs. These results correlated directly with the GGT activity measured at confluency. The activity of GGT within the basolateral membrane represented only 17% of that present on the apical brush border membrane. The *in vitro* data support previous *in vivo* observations and suggest that GGT-mediated transport plays an important role in concentrating quinol-linked GSH conjugates in proximal tubular cells. (ES 04662 and GM 39338)

58.23

GLUTATHIONE HOMEOSTASIS AND OXYGEN CONCENTRATION MODULATE THE CYTOTOXICITY OF CCl_4 IN RAT HEPATOCYTES. J.C. Veltman* and M.W. Anders. Department of Pharmacology, University of Rochester, Rochester, NY 14642.

An inverse relationship was observed between oxygen concentration and the cytotoxic effects of CCl_4 in rat hepatocytes. With 5% O_2:90% N_2:5% CO_2, CCl_4 (2.5-7.5 mM) elicited a concentration- and time-dependent loss of cell viability, significant increases in lipid peroxidation and in binding of [^{14}C]-CCl_4 metabolites to hepatocyte lipids, and depletion of cellular glutathione concentrations. These effects were not seen in CCl_4-exposed hepatocytes incubated under 95% O_2:5% CO_2. Addition of the antioxidant N,N'-diphenyl-p-phenylenediamine (DPPD, 0.01 mM) inhibited lipid peroxidation but not the bindings of [^{14}C]CCl_4 metabolites to hepatocyte lipids; CCl_4-induced cytotoxicity was delayed, but not prevented, by DPPD, and DPPD did not block the CCl_4-induced loss of glutathione. Addition of precursor amino acids for glutathione biosynthesis (5 mM, L-Met, L-Glut, Gly) blocked the binding of [^{14}C]CCl_4 metabolites to lipids, inhibited lipid peroxidation, and blocked the cytotoxicity of CCl_4. Moreover, precursor amino acids prevented the CCl_4-induced loss of hepatocyte glutathione. Glutathione may protect hypoxic hepatocytes by forming an adduct with CCl_4 metabolites. This hypothesis is based on the observation that only modest increases in glutathione disulfide concentrations are seen in CCl_4-exposed hepatocytes and the possible formation of an adduct of CCl_4 only in hypoxic cells. (Supported by grants ES03127 and HL07475.)

58.24

L-THIOMORPHOLINE-3-CARBOXYLIC ACID (L-TMC)-INDUCED CYTOTOXICITY IN ISOLATED RAT KIDNEY CELLS. Kathy D. Webster* and M. W. Anders, Dept. of Pharmacology, Univ. of Rochester, Rochester, NY 14642.

L-TMC is the cyclized product of S-(2-chloroethyl)-L-cysteine (CEC), which is cytotoxic in vitro and nephrotoxic in vivo. To determine whether L-TMC may play a role in CEC-induced toxicity, the cytotoxicity of L-TMC was studied in isolated rat kidney cells. L-TMC produced time- and concentration-dependent cytotoxicity. Rat kidney cytosol catalyzed the metabolism of L-TMC to a product absorbing at 300 nm. The absorbance at 300 nm was quenched with KCN (5 mM) and $NaBH_4$, which indicated the formation of a cyclic imine. The increase in absorbance at 300 nm was accompanied by an increase in oxygen consumption and was inhibited by increasing concentrations of L-α-hydroxyisocaproic acid. When L-TMC was incubated with rat kidney cytosol and NaB^2H_4 was added at the end of the incubation period, GC/MS analysis of the L-TMC tert-butyldimethylsilyl ester showed the formation of [^{2}H]L-TMC, indicating the intermediate formation of an imine; chemically synthesized L-TMC imine showed similar behavior. The enzyme responsible for the metabolism of L-TMC was purified from rat kidney and was identified as L-AAO. L-Pipecolic acid (piperidine-2-carboxylic acid) was not a substrate for L-AAO and was not cytotoxic. These results show that L-TMC is a substrate for L-AAO and support a role for L-AAO in the bioactivation and cytotoxicity of L-TMC. (Supported by NIH grants ES03127 and ES05380.)

58.25

RATE-LIMITING STEPS FOR ACTIVATION OF S-(1,2-DICHLOROVINYL)-L-CYSTEINE (DCVC) AND N-ACETYL-S-(1,2-DICHLOROVINYL)-L-CYSTEINE (NAC-DCVC) BY PURIFIED RAT KIDNEY PROXIMAL TUBULES. Guo-Hong Zhang and James L. Stevens, (SPON: S. Lau). W. Alton Jones Cell Science Center, Lake Placid, NY 12946

Cysteine and N-acetyl-cysteine conjugates of some halogenated hydrocarbons are known to be nephrotoxic. Specifically the S_3 segment of the proximal tubules is most sensitive. The S_1 and S_2 segments are unaffected at doses which damage the S_3 segment. To clarify this specificity we have identified the rate-limiting steps leading to the activation of DCVC and NAC-DCVC in isolated proximal tubules.

The uptake of DCVC and NAC-DCVC was mediated by Na^+-dependent and Na^+-independent processes. With increasing Na^+ concentration in the medium the uptake increased. The amount of DCVC and NAC-DCVC taken up over a period of 10 minutes is similar. Kinetics study with DCVC suggested the existence of two transport systems. Probenecid, a well-known inhibitor of the renal tubular organic anion transport system, caused a significant inhibition of NAC-DCVC uptake, but not DCVC, suggesting that only NAC-DCVC is transported via the organic anion transporter. The binding of [^{35}S] to trichloroacetic acids insoluble material was much greater from DCVC than NAC-DCVC. We propose that the transport of DCVC across the tubular membrane is the rate-limiting factor for DCVC activation, but deacetylation is the rate-limiting step for NAC-DCVC activation.

58.26

MANIPULATION OF GLUTATHIONE LEVELS IN CULTURED HUMAN MELANOMA CELLS. V. Todorovic and T.M. Guenthner Dept. of Pharmacology, U. of Illinois College of Medicine, Chicago IL 60680

Glutathione (GSH) is a major cellular defense against toxic electrophiles, including antineoplastic alkylating agents. As one means of enhancing the selective toxicity of alkylating agents towards human melanoma, we have investigated the possibility of manipulation of GSH levels in these cells. We have studied the effects of N,N-bis-(2-chloroethyl)-N-nitrosourea, a selective inhibitor of GSH reductase, and Doxorubicin, a promotor of oxidative stress within the cell, on GSH levels in an established human melanoma cell line. High doses of either compound alone will deplete GSH levels to about 20% of control values in these cells. When low doses of the two compounds are given in combination, a depletion of GSH that is both enhanced and prolonged is observed. Initial in vivo perfusion experiments in dogs indicate that doses greater than those used in culture do not deplete GSH in normal tissue. Depletion of GSH by these agents in culture enhances the cytotoxicity of the alkylating agent melphalan to melanoma cells. Supported by PHS CA-01287.

58.27

GLUTATHIONE ESTER PROTECTS AGAINST GLUTATHIONE DEFICIENCIES DUE TO AGING AND ACETAMINOPHEN. Theresa S. Chen, John P. Richie Jr. and Calvin A. Lang. Departments of Pharmacology and Toxicology, and Biochemistry, University of Louisville, Louisville, KY 40292.

Our previous results indicated that a glutathione (GSH) deficiency occurs in many aging tissues and organisms and also after acetaminophen (APAP) administration. To correct these deficiencies, glutathione monoethyl ester (GE) was injected i.p., 10 mmol/kg, into mature (12 mo) and old (30 mo) male C57BL/6 mice. Two hours later, the mice were killed and liver samples were taken, processed and analyzed for GSH using HPLC with dual electrochemical detection. Another series of mice was injected with GE and 30 min later with APAP, 375 mg/kg, i.p. Liver GSH content in 12 mo was 6.45 ± 0.184 and was 30% lower in old mice. This aging decrease was completely prevented by GE administration. Liver GSH content in 30 mo old was 4.53 ± 0.609, which decreased 70% four hours after APAP injection. Similarly, this decrease was completely prevented when GE was given prior to APAP. All changes were statistically significant ($p<0.01$ to 0.005). In conclusion, GE is an effective enhancer of tissue GSH conc. and thus is effective in protecting against biological aging and APAP toxicity. In addition, the results confirm the specificity of GSH in aging and detoxification. (Supported by Kyowa Hakko Kogyo Co., Tokyo, Japan).

58.28

CHLORPROMAZINE (CPZ) INHIBITS THE TOXICITY OF CYSTEINE S-CONJUGATES IN LLC-PK_1 CELL. Qin Chen and James L. Stevens (SPON: S. Lau). W. Alton Jones Cell Science Center, Lake Placid, NY 12946

Nephrotoxic cysteine S-conjugates cause cell death in the pig kidney-derived cell line LLC-PK_1 proceeded by the formation of reactive metabolites via the β-lyase pathway. To study the processes by which covalent binding of the metabolites leads to cell death, a number of cellular functional inhibitors were studied. CPZ, which has been reported to be a phospholipase inhibitor, calcium channel blocker and a calmodulin antagonist, was found to protect LLC-PK_1 cells from S-(1,2-dichlorovinyl)-L-cysteine (DCVC) toxicity as reflected by LDH release and a decrease in protein synthesis. CPZ is effective from 10 μM to 50 μM, and over a wide range of DCVC. The toxicity of S-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine (CTFC), and S-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC) is also inhibited by CPZ (25 μM). This effect is not due to the inactivation of cysteine conjugate β-lyase nor a decrease in the binding of [^{35}S] metabolites from [^{35}S]DCVC to cellular macromolecules. Furthermore, in LLC-PK_1 cells, cell death caused by the alkylating agents iodoacetamide, and peroxidative toxin t-butylhydroperoxide can also be blocked by CPZ. The data suggest that CPZ blocks cell death at a step which is distal to the binding of reactive metabolites from cysteine conjugates.

CHOLINERGIC PHARMACOLOGY

59.1

MUSCARINIC RECEPTOR SUBTYPE SPECIFICITY OF CYCLOHEXYL-APROPHEN ANALOGS H. Leader*, R.M. Smejkal*, J.W. Covington*, F.N. Padilla*, R.K. Gordon* and P.K. Chiang. Walter Reed Army Institute of Research, Washington, DC 20307-5100

In the search for muscarinic receptor subtype specific antagonists, aprophen (2,2-diphenylpropionic acid N,N-diethylaminoethyl ester) analogs were synthesized with alterations in (1) the chain length between the carbonyl and nitrogen groups, (2) the alkyl groups on the amino nitrogen, and (3) a cyclohexyl group replacement for one of the phenyl rings. These analogs were tested for their inhibition of acetylcholine-induced contraction of guinea pig ileum versus carbachol-stimulated release of α-amylase from rat pancreatic acinar cells (each tissue representing a different muscarinic receptor subtype). With the exception of one analog, the introduction of the cyclohexyl group increased the specificity of the analogs for the pancreatic acinar cells over the ileum. In the ileum, the N,N-diethylamino and N,N-dimethylamino analogs were equipotent, but the N,N-dimethylamino analogs were more potent in the acinar cells. Increasing the chain length beyond three methylene groups decreased the potency of the analogs in both assays. In the acinar cells, the cyclohexyl N,N-dimethylaminopropyl ester analog was the most potent and showed the most specificity, being over 100-fold more active in acinar cells than in ileum. Thus, this compound may be a potent discriminator between the muscarinic receptor subtypes of ileum and pancreas.

59.2

MUSCARINIC CHOLINERGIC RECEPTORS IN NORMAL AND NEUROGENIC BLADDER. Ellen Shapiro*, Herbert Lepor*, and Daniel Gup*. (SPON: K.A. Hruska). Washington University School of Medicine, St. Louis, MO 63110.

Bladder dysfunction secondary to neurologic conditions occurs in all age groups and is associated with significant morbidity. The role of neuroreceptors in the development of detrusor dysfunction has not been studied previously. Control bladder tissue specimens were obtained from 8 children with ureterovesical reflux undergoing ureteral reimplantation and 14 adults with bladder carcinoma undergoing cystectomy. Neurogenic bladder specimens were obtained from 10 children with myelomeningocele and 4 adults with neurogenic bladder dysfunction undergoing augmentation cystoplasty. Saturation experiments using ^{3}H-N-methylscopolamine (^{3}H-NMS) were performed in control and neurogenic bladder homogenates. The mean Kd in the neurogenic and control bladders was 0.40 nM and 0.55 nM, respectively. The mean Bmax in the neurogenic and control bladders was 0.35 fmol/mg wet wt and 0.65 fmol/mg wet wt, respectively ($p<0.05$). Competitive binding experiments with ^{3}H-NMS and various MCh antagonists indicated that the pharmacology of MCh binding sites were similar in neurogenic and control bladders. Age was not significantly correlated with MCh receptor density in control and neurogenic bladders. MCh binding sites are homogeneous in neurogenic and control bladders. The lower density of MCh receptors in the neurogenic bladders may represent down regulation of MCh receptors or a replacement of smooth muscle by fibrosis.

59.3

CHOLINERGIC ACTIVITY WITH DMAE ANALOGS

J.R. Flynn*, Bula Bhattacharyya*, T.D. Anderson*, T.K. Chatterjee*, A.M. Nyanda*, T. Lee*, M.D. Sokoll*, J.G. Cannon*, R.K. Bhatnagar, and J.P. Long Departments of Pharmacology and Anesthesia, Division of Medicinal Chemistry, University of Iowa, Iowa City, Iowa 52242

Five analogs of DMAE (4,4'-bis[N-(2,2'-diethoxy ethyl)-N,N-dimethylaminoacetal]-biphenyl dibromide) were tested for anti-nicotinic activity, inhibition of choline transport, inhibition of acetylcholinesterase, interaction on neuromuscular transmission and protection against organo-phosphate (O-P) induced-toxicity (I-Tox). DMAE, TL-402 (β-hydroxyl DMAE), NAM-242 (bicyclohexyl DMAE), NAM-250 (β-hydroxyl NAM-242), and the acetyl ester of hemicholinium-3, TL-404, and its reverse carboxy analog, JGC-110, were equi-potent inhibitors of the +-chronotropic response induced by nicotine in isolated guinea pig atria. All compounds protected mice from O-P I-Tox; TL-402 is the most active. TL-402 and DMAE antagonized O-P in vitro using the voltage clamp technique at the neuromuscular junction of frog sartorius muscle and decreased spontaneous miniature end-plate currents and time constants of decay. DMAE is a potent inhibitor of choline transport in synaptosomes but its analogs are weak. All are very weak inhibitors of bovine erythrocyte acetylcholinesterase. Protection against O-P I-Tox correlates with anti-nicotinic activity and does not depend on inhibition of choline transport. Structurally the β-carbonyl analog of DMAE is a better protective agent than the hydroxy. Changes at the end of the chain do not enhance activity. Army Cont. No. DAMD17-87-C-7113.

59.4

EXPRESSION OF NICOTINIC RECEPTORS BY QUIN 2 FLUORESCENSE IN A HUMAN NEUROBLASTOMA CELL LINE. Jesse Baumgold*, Andrea Patton* and Lalita Noronha-Blob*. (Spon: W.J. Kinnier). NOVA Pharmaceutical Corp., Baltimore, MD 21224 and NINCDS, Lab. of Molecular and Cellular Neurobiology, Bethesda, MD 20892.

Human SK-N-SH neuroblastoma cells treated with carbachol (CBC), or nicotine (NIC) showed a 3-5 fold increase in intracellular calcium ($[Ca^{2+}]_i$) accumulation as detected by quin 2 fluorescence. Resting $[Ca^{2+}]_i$ values rose from 220 ± 12 nM to 1470 ± 243 nM with CBC (1.0 mM). Muscarine and oxotremorine (1.0 mM) produced barely detectable signals. Atropine (10 μM) partially blocked both NIC and CBC-mediated $[Ca^{2+}]_i$ increase (~ 50%). Tubocurarine (10 μM) blocked the NIC response by 100% and CBC-induced $[Ca^{2+}]_i$ accumulation by ~55%. Atropine and tubocurarine together (10 μM each) abolished the CBC-induced response. TTX (3 μM) and TEA (10 μM) had no effect on $[Ca^{2+}]_i$ accumulation induced by either agonist. $[Ca^{2+}]_i$ increases were not observed in a Ca^{2+}-free EGTA containing buffer in response to both CBC and NIC. The data suggest that in addition to (M_3) muscarinic receptors (Fisher and Heacock, J. Neurochem. 50:1988), SK-N-SH cells also express nicotinic receptors that mediate Ca^{2+} influx.

59.5

PHARMACOLOGICAL ACTIVITY OF N-METHYL-CARBAMYLCHOLINE, A NOVEL COMPOUND INTERACTING WITH NICOTINIC RECEPTORS.

P. Boksa*, D. Araujo*, M. Quik, P.A. Lapchak*, B. Collier and R. Quirion, McGill University, Montreal, Canada H3G 1Y6

We have shown that the novel radioligand [^{3}H]N-methylcarbamylcholine (MCC) specifically labels nicotinic (but not muscarinic) acetylcholine (ACh) receptors in brain and is resistant to cholinesterase, providing distinct advantage over previous neuronal nicotinic radioligands. We now report on the pharmacological activity of MCC in several cholinergically innervated tissues. At CNS nicotinic receptors, MCC (10^{-8}-10^{-5} M) was more effective than nicotine in stimulating release of ACh from rat hippocampal slices, an effect blocked by nicotinic but not muscarinic antagonists. At CNS muscarinic receptors, a muscarinic agonist, oxotremorine, inhibited the release of ACh evoked from hippocampal slices by 25 mM K^+, while MCC (10^{-6} M) had no effect. At peripheral nicotinic receptors, MCC stimulated catecholamine release from cultured adrenal medullary cells, induced depolarization of the rat sympathetic ganglion and stimulated contraction of the frog rectus abdominis muscle; all activities were blocked by nicotinic but not by muscarinic antagonists and MCC was about equipotent with carbachol at these sites. At peripheral muscarinic receptors, MCC contracted the rat ileum, but with a potency about 1/40th that of carbachol. Thus MCC has the profile of a cholinergic agonist with greater activity at nicotinic than at muscarinic sites. Supported by MRC of Canada.

59.6

ACTIONS OF NICOTINE AND DMPP ON LIMBIC NEURONS RECORDED *IN VITRO*.

Linda A. Wong and Joel P. Gallagher. Univ. of Texas Medical Branch at Galveston, Texas 77550.

Based on information from behavioral studies, nicotine has been shown to be the effective component of cigarette smoke responsible for improving information processing. Its mechanism of action at the membrane level has not been clearly established. The present study was designed to examine the pharmacology of nicotine on CNS neurons. We used the rat septal brain slice preparation as a model for in vitro drug testing since the rat septum contains nicotinic binding sites and is part of the limbic system involved in learning and memory.

Intracellular recordings were obtained from dorsolateral septal nucleus neurons (DLSN). Nicotine and DMPP (dimethylphenylpiperazinium) were applied by superfusion (1-10 μM) or pressure ejection (10 mM, 10-300 mS, 5-10 psi). Both nicotinic agonists produced membrane hyperpolarization (4-14 mV; n=19) accompanied by a decrease in input resistance (10-40%). This response could be blocked by mecamylamine (50 μM; n=3), a competitive ganglionic blocker, but not α-bungarotoxin (0.5 μM; n=3), a neuromuscular blocker. Neither TTX (0.5 μM) nor low Ca^{2+}/high Mg^{2+} superfusion medium altered the response to nicotinic agonists. Nicotine and DMPP also depressed the inhibitory synaptic potentials evoked by orthodromic stimulation. The findings suggest that nicotinic receptors in the DLSN resemble those on autonomic ganglia. Our results indicate that nicotinic agonists may have direct actions on postsynaptic receptors to produce changes in the intrinsic membrane properties as well as actions on presynaptic receptors to modulate the release of inhibitory neurotransmitters.

(Supported by DAMD-17-86-C-6032).

59.7

MODULATION OF SPONTANEOUS ACETYLCHOLINE RELEASE: DEPENDENCE ON INTRINSIC NEURONAL ACTIVITY.

Vladimir Dolezal* and Lynn Wecker. LSUMC, New Orleans, LA 70112.

The spontaneous release of acetylcholine (ACh) from striatal slices is about 3-fold greater than from hippocampal slices. Since studies have suggested that the regulation of ACh release may differ between these brain regions, we compared the effects of the muscarinic receptor antagonist atropine on the spontaneous release of ACh from striatal and hippocampal slices. Atropine increased ACh release from striatal slices only. This increase was: a) dose-dependent from 1 nM (30% increase) to a maximum at 1 uM (92% increase); b) Ca^+-dependent; and c) abolished by tetrodotoxin (1 uM). When ACh release induced by 4-aminopyridine (4-AP) was measured, a concentration-dependent increase was observed by both brain regions; an effect that was 2-fold greater in striatal slices than in hippocampal slices with 100 uM 4-AP. In contrast, potassium depolarization (35 mM) increased ACh release from striatal slices by 400% and from hippocampal slices by 600%. Results suggest that spontaneous impulse activity in the striatum (containing cholinergic interneurons) and not in hippocampus (containing only cholinergic nerve endings) may be responsible for the differences observed between the effects of atropine and 4-AP and those of potassium. (Supported by NIMH-33443.)

59.8

EFFECTS OF 9-AMINO-1,2,3,4-TETRAHYDROACRIDINE (THA) AND 4-AMINOPYRIDINE(4-AP) ON PHOSPHATIDYL-INOSITOL(PI) TURNOVER AND [^{3}H]NOREPINEPHRINE (NE) RELEASE IN RAT CEREBRAL CORTICAL SLICES.

W. Petko*, C.P. Smith* and F.P. Huger*. (Spon: D.B. Ellis). Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

THA, a potent cholinesterase inhibitor, has been compared structurally with 4-AP, but affects Na^+ channels as well as K^+ channels. The purpose of this study was to compare THA and 4-AP in two functional assays which may reflect differences in ion channel selectivity.

THA and 4-AP were tested for ability to increase PI turnover in pre-labeled cortical slices. We found that 4-AP had no effect at concentrations up to 10 mM, but THA (1-10 mM) did stimulate PI turnover. Stimulation by THA was not blocked by atropine, prazosin, methysergide, pyrilamine, excitatory amino acid antagonists or tetrodotoxin. Calcium-entry blockers also failed to block the effect of THA. Measurement of electrically-stimulated release of [^{3}H]NE from cortical slices also showed that THA and 4-AP increased non-stimulated as well as stimulated release. However, 4-AP had no effect on non-stimulated release in Ca^{2+}-free buffer, but THA did increase non-stimulated NE release in the absence of calcium.

In summary, the THA stimulation of PI turnover appears to be a direct, non-receptor mediated effect. The lack of effect of 4-AP suggests that the stimulation by THA may be mediated by Na^+-channel effects and not by K^+-channel blockade. A second major distinction between THA and 4-AP was revealed by the difference in Ca^{2+}-dependency for release of [^{3}H]NE.

59.9

INDUCTION OF CHOLINE ACETYLTRANSFERASE, TYROSINE HYDROXYLASE, AND ORNITHINE DECARBOXYLASE BY 1,1,3 TRICYANO-2-AMINO-1-PROPENE IN THE PC-12 CELL LINE.

J. West Paul* and John P. DaVanzo Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858

Experiments showed 1,1,3 tricyano-2-amino-1-propene (Triap) to induce neurite outgrowth and potentiate nerve growth factor (NGF) in the PC-12 cell line. This lead us to examine the effect of Triap on several enzyme systems in the PC-12 cell line. Triap alone had no effect on ornithine decarboxylase (ODC) activity at concentrations that induced neurite outgrowth. Combined with NGF, levels of Triap which potentiated NGF increased ODC activity far above the level of NGF alone. At concentrations of Triap that potentiated neurite outgrowth, choline acetyltransferase (ChAT) was increased several fold. Triap at these concentrations also increased tyrosine hydroxylase (TH) activity to the same level as that seen with NGF. Triap combined with NGF showed no increases in TH activity over that of NGF or Triap alone. The induction of enzymes involved in neuronal differentiation and neurotransmitter synthesis may be an important factor in considering compounds that may be useful in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. (Supported in part by Hoechst Roussel, Inc. and thanks to Dr. Gordon Guroff for the PC-12 cell line.)

59.10

EFFECT OF MUSCIMOL ON THE SYNTHESIS OF ACETYLCHOLINE AND ON THE ACTIVITY OF VARIOUS NERVE TERMINAL FRACTIONS OF CHOLINE-O-ACETYLTRANSFERASE (EC 2.3.1.6) IN RAT HIPPOCAMPAL TISSUE.

P.T. Carroll and L.K. Smith*, Texas Tech University Health Sciences Center, Lubbock, TX 79430

Incubation of rat hippocampal tissue with the hyperpolarizing agent muscimol reduced the synthesis of acetylcholine and the activity of detergent soluble choline-O-acetyltransferase (EC 2.3.1.6; ChAT) associated with a crude vesicular fraction. In contrast, this treatment augmented the activity of a water soluble fraction of ChAT in the cytosol without altering the activity of the major nerve terminal ChAT fraction, that which can be solubilized by pH 7.4 salt. When hippocampal tissue was incubated in a normal Krebs solution containing phospholipase C from B. cereus, both lactate dehydrogenase (EC 1.1.1.27) and the water soluble ChAT fraction were liberated from the cytosol, whereas the salt soluble ChAT fraction was not. These results may suggest that muscimol reduces the synthesis of ACh in rat hippocampal tissue by preventing the water soluble cytosolic ChAT fraction from associating with membranes; also, that the water, but not the salt soluble ChAT fraction, may float freely in the cytosol under physiological conditions (Supported in part by NINCDS - 2R01 NS 21289-04).

59.11

DIFFERENCES IN ACUTE CHOLINERGIC AND HISTOCHEMICAL TOXICITY BETWEEN SARIN AND VX. Ramesh C. Gupta, Gary T. Patterson* and Wolf D. Dettbarn. Department of Pharmacology, School of Med. Vanderbilt University, Nashville, TN. 37232

A sublethal sc dose of sarin (110 µg/Kg) or VX (12 µg/Kg) produced onset of toxic signs within 5-15 min, maximal severity by 30-60 min, and lasted for 5-7 hrs. The toxic signs were predominately of central origin. Myonecrotic lesions appeared as early as 1 hr following both agents. Maximum number of lesions when determined after 24 hrs was seen in all muscles, diaphragm was affected the most and EDL the least. Repair of lesions was slow since a reduced number was still present after 7 days. Among skeletal muscles AChE of soleus was markedly reduced to 23% with sarin and 8% with VX. AChE of EDL appeared less sensitive to sarin than to VX (17% and 82% of control, respectively). Activity of AChE molecular forms reflected a similar pattern. Within 1 hr both sarin and VX reduced the AChE activity to 1-6% in brain, with the exception of the striatum after VX (41%). Cortex was maximally affected. A slow but significant recovery of AChE was evident after 24 hrs and more so after 7 days. The inhibition and recovery pattern of BuChE was similar to AChE, however, rate of recovery was more rapid. It is concluded that differences between these inhibitors may be due to differences in their susceptibility to enzymic hydrolysis, storage sites with slow release and unspecific binding sites in plasma, liver and other tissue. (Supported by the US Army DAMD17-83-C-3244)

59.12

EFFICACY OF AZAPROPHEN AND PHYSOSTIGMINE AS A PRETREATMENT FOR SOMAN-INDUCED INCAPACITATION IN GUINEA PIGS BY RESPONSE SURFACE MODELING (RSM). Chris Gennings*, Richard P. Solana*, W.H. Carter, Jr.*, Larrel W. Harris, Richard A. Carchman, E.D. Campbell*, R.M. Boyle*, and Brian G. Talbot* (SPON: L.W. Harris). Med Coll. of VA, Richmond, VA 23298 and USAMRICD, APG, MD 21010-5425

The efficacy of physostigmine/azaprophen (PHY/AZA) pretreatment was evaluated in soman-exposed guinea pigs. The endpoint measured was duration of soman-induced physical incapacitation (SPI). RSM was employed to describe the relationship of the pretreatment combination with SPI. The significance of the combination relative to PHY alone was evaluated; pretreatment combinations that yielded minimal time to recovery from SPI (optimum responses) were also determined. Analysis of the fitted response surface indicated that combination pretreatment with these compounds significanctly reduced the time to recovery from SPI when compared to pretreatment by PHY alone. Hence, a pretreatment combination of carbamate and cholinolytic appears to have potential as a beneficial pretreatment against organophosphate exposure.

59.13

3-CARBAMYL-1-ALLYLQUINUCLIDINIUM BROMIDE: PHARMACOLOGICAL EFFECTS ON CHOLINERGIC ACTIVITY AND PROTECTION AGAINST SOMAN G.H. STERLING, P.H. DOUKAS*, R. LEECH*, C. JACKSON*, K.J. O'NEILL* and J.J. O'NEILL Depts. of Pharmacology, Hahnemann Univ. and Temple Univ. Schools of Medicine, Philadelphia, PA 19102

N-allyl-3-quinuclidinol (NAQ), a potent selective inhibitor of high affinity choline uptake (HAChU) developed in this laboratory, significantly enhances the protection afforded by atropine/pralidoxime against soman intoxication in rat and guinea pig. Previous studies have also demonstrated the effectiveness of carbamate pretreatment in reducing organophosphate toxicity. In these studies, we examined the combined attributes of interference with acetylcholine synthesis and carbamylation of cholinesterase in protection against soman toxicity. The carbamate derivative of NAQ, 3-carbamyl-1-allyl quinuclidinium bromide (CAB) was synthesized and evaluated in several in vitro screens and in vivo protection studies. The R-isomer of CAB proved to be an effective inhibitor of erythrocyte and plasma cholinesterase as well as HAChU. When administered with atropine, the compound prevented deaths against soman for 1-2 hours at doses up to 4 LD50, which normally produces an LD100 within 0.5-1H. The data confirms our previous results and provides insights for the design of protectants against organophosphate intoxication. (This work was supported by USAMRDC-DAMD 17-86-C-6243).

59.14

EFFECT OF SOMAN ON TRACHEAL SMOOTH MUSCLE CONTRACTILITY. M. Adler, D. Moore* and M. Filbert*. Neurotoxicology & Physiology Br., Pathophysiology Div., USAMRICD, APG, MD 21010.

Constriction of airway smooth muscle is one of the life-threatening consequences of nerve-agent intoxication. To determine the mechanisms underlying such constriction, we examined the actions of the irreversible cholinesterase (ChE) inhibitor, soman on contractions evoked by supramaximal electric field stimulation (EFS) in canine trachealis strips. Exposure to 10 nM soman led to a 2.9-fold increase in the amplitude and a 7.8-fold prolongation in the half-relaxation time of contractions evoked by 10 Hz EFS trains. The soman-induced alterations began within 3 min and were maximal after 20 min of incubation. At these times, ChE activities were reduced by 21 and 58%, respectively. The actions of soman on muscle tension were enhanced by factors that increase acetylcholine (ACh) secretion (e.g., increases in the frequency, amplitude and duration of the EFS pulses) and were antagonized by factors that reduce ACh persistence such as addition of purified ChE. These results suggest that there is little excess hydrolytic activity in canine tracheal smooth muscle. This is in marked contrast to the large muscarinic receptor reserve found in the same preparation.

59.15

PROTECTION AGAINST SOMAN BY PRETREATMENT WITH PHYSOSTIGMINE AND AZAPROPHEN. R. P. Solana*, L. W. Harris, W. H. Carter, Jr.*, B. G. Talbot*, R.A. Carchman and C. Gennings*. USAMRICD, Aberdeen Proving Ground, MD 21010-5425

A pretreatment combination of physostigmine and azaprophen (6-methyl-6-azabicyclo-[3.2.1]octan-3-ol-2,2-diphenylpropionate), a novel cholinolytic, was evaluated for its ability to minimize soman-induced incapacitation (SII) and lethality in guinea pigs. This was accomplished by using Response Surface Methodology to model and analyze the combination, varying physostigmine from 0 to 194 ug/kg, azaprophen from 0 to 5 mg/kg, and soman from 30 to 150 ug/kg. 100% survival was achieved against 5 LD50s of soman using as little as 100 ug/kg of physostigmine in the presence of 5 mg/kg of azaprophen. Both survival and SII were similarly affected by this pretreatment combination. For both endpoints, greater efficacy was achieved with the combination than could be achieved with either component alone (therapeutic synergism). This suggests that such a pretreatment combination may prove very efficacious against soman-induced lethality and incapacitation in higher species.

59.16

SOMAN ACTIVATES MUSCARINIC RECEPTORS IN BULLFROG SYMPATHETIC GANGLION CELLS. T.J. Heppner* and J.F. Fiekers. Dept. Anat. and Neurobiol., Univ. of Vermont Coll. Med., Burlington, VT 05405.

The effects of soman in the presence of a muscarinic blocker were examined with intracellular recordings from B cell neurons in the 9th and 10th ganglion isolated from the paravertebral sympathetic chains of the bullfrog Rana catesbeiana. Soman (10uM) addition induced (1) a membrane depolarization of 6.0 mV (2) a decrease in membrane resistance ~49% (3) a shortening of the hyperpolarizing afterpotential (HAP) by ~33% and (4) a decrease in the current amplitude underlying the HAP. Atropine (10uM) addition subsequent to soman treatment reversed only the soman-induced depolarization of the membrane potential (within 5 min) to near pretreatment levels, but did not restore the membrane resistance, duration of the HAP (current clamped near the resting potential), or the amplitude of the current underlying the HAP within at least 30 min. These results demonstrate that some of soman's actions are mediated through muscarinic receptors and are partially reversed with muscarinic receptor blockade subsequent to soman exposure. This work was supported in part by the US Army Medical Research and Development Command, Contract No. DAMD17-86-C-6031.

59.17

EFFICACY OF PHYSOSTIGMINE (PHY) AND ADJUNCT PRETREATMENT AGAINST SOMAN INTOXICATION. Larrel W. Harris, Brian G. Talbot*, Willard J. Lennox*, Dana R. Anderson* and Richard P. Solana*. USAMRICD, Aberdeen Proving Ground, MD 21010-5425.

A pretreatment for soman intoxication should prevent lethality and convulsions (CNV) at 2 LD50s, be behavioral-decrement-free (BDF) when given alone and rapidly reverse debilitation at 5 LD50s. BDF pretreatment regimens (PRGs) for guinea pigs consisted of Phy (0.15 mg/kg, im) and adjunct (ADJ); ADJs [mg/kg, im] tested were atropine (At) [16], azaprophen (AZA) [5], trihexyphenidyl (THP) [2], scopolamine [0.08], ethopropazine [12], dextromethorphan [7.5], benactyzine [1.25] and promethazine [5]. PRGs were given 30 min before soman (60 ug/kg, sc; 2 LD50s); animals were then observed and graded for signs of intoxication, including CNV at 7 time points and at 24-hr. Phy alone reduced CNV and lethality by 50%, whereas PRGs abolished lethality and shortened recovery to < 2-hr. CNVs were observed in animals except those receiving a PRG including AT, AZA or THP, which all possess cholinolytic activity. The data show that several PRGs are effective against soman and may hold promise for higher species.

59.18

IN VIVO DOSE RESPONSE OF PHYSOSTIGMINE AND METABOLITE CONCENTRATIONS VS. CHOLINESTERASE ACTIVITY IN RBC AND TISSUES OF RATS. S.M. Somani and S.N. Dube*, Dept. Pharmacol., SIU School of Medicine, Springfield, IL 62708

In order to study dose response, various dosages (25 to 500 μg kg^{-1}, i.m.) of ^{3}H-Physostigmine (Phy) were administered to rats and 15 min later, blood and tissues were analyzed for cholinesterase (ChE) activity by radiometric method (Johnson and Russell, 1975, Anal. Biochem. 64:229) and Phy and its metabolites by HPLC (Somani and Khalique, 1988, Fund Appl. Tox. 6:327). A comparison of ChE values in different tissues of rats indicated that ChE activity was highest in brain (7.11 μmol $min^{-1}g^{-1}$) and least in diaphragm (0.67 μmol $min^{-1}g^{-1}$). The enzyme activity was eleven times more in brain as compared to diaphragm. Phy produced a dose-dependent inhibition of ChE in RBC (18-42%), brain (23-55%) and diaphragm (25-35%) from 50 to 200 μg kg^{-1}, then ChE inhibition was plateaued from 200 to 500 μg kg^{-1} in these tissues. A dose related ChE inhibition was seen in heart (16-50%) and thigh muscle (8-53%) from 50 to 500 μg kg^{-1}. Phy concentration increased linearly from 50 to 400 μg kg^{-1} in plasma, brain, heart and thigh muscle. Eseroline and two unknown metabolites M_1 and M_2 were present in plasma and tissues, however, another M_3, metabolite (rt 10.5 min) was found predominantly in heart and thigh muscle. These results indicate that ChE inhibition is linear up to 200 μg kg^{-1} in RBC and tissues. (Supported by U.S. Army Contract DAMD 88-C-8024).

59.19

AUTORADIOGRAPHIC ANALYSIS OF PHYSOSTIGMINE DISTRIBUTION IN RAT BRAIN. O.U. Scremin*, A.M.E. Scremin*, S.M. Somani and E. Giacobini. Veterans Administration Med. Ctr. Albuquerque, NM, 87108 and SIU School of Medicine, Springfield, IL 62794-9230.

^{3}H physostigmine (Phy) 50 μg/kg was administered i.v. to rats and its concentration (conc.) in 40 brain regions was assessed by quantitative autoradiography. These values were correlated with cerebral blood flow (rCBF), measured with Iodo-^{14}C-antipyrine autoradiogrpahy, in control rats and in animals injected i.v. with a similar dose of Phy. A high degree of correlation was found between Phy conc. and rCBF (r=.81) at 0.5 min after drug injection. This correlation was progressively lost with increasing intervals between Phy injection and sacrifice of the animals (5 min: r=0.61, 12 min: r=0.09). Regions with a high cholinesterase activity, however, showed greater retention of Phy over time than those with low activity of the enzyme. In the regions with low cholinesterase levels, drug retention was reciprocally related to rCBF levels. The highest initial Phy conc. was found in regions lacking a blood brain barrier (range-10.4-23.8 nCi/mg) and the lowest in white matter (range=1.2-2.6 nCi/mg). Some brain regions equilibrated rapidly with Phy showed the highest conc. initially and then declined gradually up to 12 min. The elimination rate constants of Phy from these regions ranged from 0.058 to 0.18. Phy conc. in regions that showed an increase in rCBF were comparable to regions that did not show rCBF changes. This rules out variations in drug access to brain regions as the mechanism underlying the topographical variations of the cerebrovascular action of Phy. (Supported by the Veterans Administration and U.S. Army Contract DAMD 17-83-C-3195).

59.20

PHENYLMETHYLSULFONYL FLUORIDE (PMSF) INHIBITION OF BRAIN AND MUSCLE ACETYLCHOLINESTERASE. K.A. Skau* & M.T. Shipley* (SPON: D.G. Patel) Div. Pharmacol.Med.Chem. & Dept. Anat.Cell Biol. U.Cincinnati Med. Ctr., Cincinnati, OH 45267.

Sulfonyl fluorides have been proposed as possible drugs to treat symptoms of Alzheimer's disease as these agents are long-lasting inhibitors of acetylcholinesterase (AChE) and appear to selectively inhibit brain vs. peripheral AChE. To further explore this selectivity we have studied inhibition of brain and muscle AChE molecular forms. Injection of PMSF (85 mg/kg) inhibited the brain tetrameric (G_4) AChE peak by 70%; brain monomeric (G_1) AChE was inhibited only 50%. The A_{12}, G_4 and G_1-AChE forms in muscle were inhibited 70, 60 and 30%. In vitro studies showed that the rate of inhibition of solubilized brain AChE was 2-3 times as fast as muscle enzyme at PMSF of 0.5-1.0 mM; at lower concentrations (0.1 mM) this difference was not evident. Similarly, purified G_4-AChE was more sensitive to PMSF than was purified G_1 enzyme. These results suggest that the selectivity of PMSF for brain enzyme may be due to a selective effect on the G_4 form which is the predominant form of brain AChE but a lesser form in muscle. (Supported in part by U.S. Army DAMD 17-86-C-6005.)

59.21

TETRAHYDROAMINOACRIDINE IS CONCENTRATED IN BRAIN FOLLOWING INTRAPERITONEAL ADMINISTRATION. D. Liston*, L. Russo*, E.E. Mena* and J. Williams, Pfizer Central Research, Groton, CT 06340

THA is a cholinesterase inhibitor that is currently undergoing clinical trials for use in Alzheimer's Disease. We have examined some properties of THA *in vitro* and *in vivo* to define the mechanism by which THA produces its therapeutic effects. *In vitro*, THA inhibits acetylcholinesterase (AChE) from rat brain and human erythrocytes with an IC50 of 220 nM. The kinetics of enzyme inhibition are best fitted by a model incorporating mixed competitive and non-competitive inhibition, with a K_{ii}=246 nM a K_{is}= 145 nM. THA is considerably more potent at inhibiting butyrylcholinesterase, with an IC50=9 nM. We examined the ability of THA to displace a number of ligands from rat brain membranes. Binding to cholinergic receptors was weak, with 50% displacement of ^{3}H-QNB (muscarinic) at 5.2 uM, ^{3}H-AFDX-116 (M2) at 1.7 uM, ^{3}H-telenzepine (M1) at 10 uM and ^{3}H-nicotine at >10 uM. THA displaces ^{3}H-prazosin (a_1 adrenergic) with an IC50 of 3.3 uM and ^{3}H-mepyramine (H1 histamine) with an IC50 of 5 uM. Monoamine oxidase from brain or liver was not significantly inhibited by THA below 100 uM. *In vivo*, THA exhibited an unusual distribution. Following 3.2 mg/kg i.p., a dose active in mouse passive avoidance, THA was 10-fold higher in brain than in plasma from 20-120 min, with the highest brain concentration (at 20 min) of 2.4 uM. At 120 min, brain THA was 0.34 uM, well above the IC50 for inhibition of AChE. A monohydroxylated metabolite of THA was observed in brain and plasma; this metabolite was a weak inhibitor of AChE. We conclude that the inhibition of brain AChE by THA is sufficient to explain its therapeutic action in Alzheimer's Disease.

59.22

HPLC VS RIA FOR ASSAY OF PYRIDOSTIGMINE (PYR). Howard G. Meyer*, Brian J. Lukey*, Robin T. Gepp*, and Claire N. Lieske* (SPON: Larrel W. Harris). USAMRICD, APG, MD 21010-5425

The purpose was to show the relative merits of two methods for assaying Pyr in plasma or serum. The HPLC method, currently the standard, employs sample preparation on a C-2 Bond elut cartridge, separation on a Beckman Altex Ultrasphere-Octyl column, and estimation at 208 nm with a UV detector. The immunogen used for the production of the antibody in our radioimmunoassay (RIA) was prepared by covalently binding a Pyr analong [1-(5-carboxypentyl)-3-(N,N-dimethylcarbamyloxy)pyridinium bromide] to Keyhole Limpet Hemocyanin. Assay parameters of sensitivity specificity, accuracy, precision, throughput, sample size, and cost are objectively examined for each assay. Direct measurement by HPLC has superior specificity, but accuracy and precision of each are similar. The lower limits for the HPLC and the RIA are 2.0 ng/ml and 0.5 ng/ml, respectively. The RIA reduces sample size from 0.5 to 0.1 ml and decreases cost per assay from > $20 to < $2. Thus, the RIA is an attractive alternative to the HPLC assay.

60.1

EFFECT OF CYCLOSPORIN A ON VASCULAR PRESSOR RESPONSES.
Alfredo Rego*, Roberto Vargas*, Marie L. Foegh* and Peter W. Ramwell.
Georgetown University Medical Center, Washington, D.C. 20007

Cyclosporin A (CsA) therapy is associated with an increased incidence of hypertension. The mechanism involved is still poorly understood. We examined the mechanism by which CsA causes increment of mean arterial pressure (MAP) in normotensive rats. CsA injected intravenously in a bolus of 10-20 mg/kg to rats under nicotinic blockade induced by chlorisondamine 1.0 mg/kg, significantly raises the MAP (42-60 mmHg) in a dose-dependent, immediate and long lasting (30-45 min) manner. This response is not affected by either alpha-1 adrenoceptor blockade (prazosin, 1.0 mg/kg) or muscarinic blockade (atropine, 1.0 mg/kg). Heart rate was 308±10 beats/min after blockade, and did not change with CsA injection. After CsA injection the calcium (Ca^{2+}) channel blockers nifedipine and verapamil (10 ug/kg; 100 ug/kg respectively) had only a transient hypotensive. Perfusion of the isolated rat mesenteric vascular bed with CsA (0.08-8.3 uM) significantly enhances the response of that preparation to norepinephrine (0.2-3.0 ug) in 25-52% and of KCl (35-500 ug) in 18-45%. This effect is also dose-dependent, almost immediate and would not wash out. The CsA-induced increment of pressor responses is not observed when the preparation is perfused with Ca^{2+} free solution and, is abolished (response to KCl) or attenuated (response to NE) when nifedipine or verapamil are added to the perfusion solution. However, after washing out the Ca^{2+} channel blockers, the CsA-induced increased responses are again observed. These data confirm that CsA directly affects general vascular resistance, and suggest that the effect is greately dependent on extracellular Ca^{2+}. [Supported by NIH grant # HL 31241 & HL 34974]

60.2

STIMULATION OF PROTEIN KINASE C ACTIVITY BY PROLACTIN IN RAT AORTIC SMOOTH MUSCLE.
Marie D. Sauro, Arthur R. Buckley, Paul Crowe, Ronald G. Coffey and David F. Fitzpatrick.
University of South Florida, College of Medicine, Tampa, FL 33612

It has been previously shown that prolactin (PRL) administration causes elevation of blood pressure. Furthermore, it has been suggested that calcium and phospholipid dependent protein kinase, protein kinase C (PKC), may be the intracellular second messenger for prolactin action in a number of tissues. We examined the effects of PRL on PKC activity in aortic smooth muscle from 8 to 10 week old male Sprague Dawley rats. Aortic strips incubated with various physiological concentrations of ovine PRL (PRL-18) (10^{-12}-10^{-8}M) at 37°C for 25 minutes showed a significant stimulation of PKC activity. A lipophilic derivative of phorbol ester, phorbol 12, 13-dibutyrate, a substance known to directly stimulate PKC activity in many tissues by mimicking diacylglycerol, also caused a large increase in PKC activity. We speculate that PRL may cause elevation of blood pressure through an increase in vascular smooth muscle PKC activity, possibly resulting in sustained vascular tone and/or an increased sensitivity to various contractile agonists.

60.3

CONTRACTILE RESPONSES OF ARTERIES FROM DIABETIC RATS TO PHORBOL DIBUTYRATE.
W.Abebe*, K.H.Harris* and K.M.MacLeod
Univ. of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

Previous studies have shown that arteries from male rats with chronic streptozotocin (STZ)-induced diabetes are more responsive to the contractile effects of norepinephrine (NE) than their age-matched controls. In order to examine whether protein kinase C (PKC)-mediated mechanisms are enhanced in diabetic arteries, we investigated the responsiveness of aortic and mesenteric artery rings isolated from 12 week STZ-diabetic rats and age-matched controls to the PKC activator, phorbol dibutyrate (PDB). PDB elicited dose-dependent contractions of arteries in the presence of 2.5mM Ca^{2+}. Maximum contractile responses to PDB (3μM) were greater in diabetic arteries than those in control preparations, although the increase was significant only in diabetic mesenteric arteries. In the absence of extra cellular Ca^{2+}, or in the presence of the Ca^{2+} influx blockers nifedipine or verapamil, contractile responses of aortae and, to a greater extent, mesenteric arteries, to PDB were reduced. Under these conditions, no significant difference in the magnitude of the PDB response could be detected between control and diabetic arteries. These results suggest that PKC-mediated responses are increased in arteries from diabetic rats, and this effect appears to be dependent on increased Ca^{2+} influx through cell membrane Ca^{2+} channels. (Supported by MRC (C) and the B.C. Heart Foundation)

60.4

ENHANCED RESPONSIVENESS TO NOREPINEPHRINE IN THE ABSENCE AND PRESENCE OF CALCIUM IN ARTERIES FROM DIABETIC RATS.
K. M. MacLeod, W. Abebe *and K.H. Harris* Univ. of British Columbia, Vancouver, B.C. V6T 1W5, Canada

Aortae and mesenteric arteries from male rats with chronic streptozotocin-induced diabetes exhibit a selective increase in responsiveness to the contractile effects of norepinephrine (NE). In the present investigation, the dependence of this increased responsiveness on intracellular and extracellular Ca^{2+} was determined. Following incubation of arteries in Ca^{2+}-free medium with 1.0 mM EGTA for 15 min, phasic responses of diabetic arteries to maximal concentrations of NE were significantly increased compared to those of control arteries. However, when expressed as a proportion of the NE response in the presence of Ca^{2+}, the magnitude of the NE response in the absence of Ca^{2+} was similar in control and diabetic arteries. In addition, contractile responses of diabetic arteries in the presence of Ca^{2+} to NE were blocked to a greater extent by maximal concentrations of the Ca^{2+} influx blockers nifedipine and verapamil than were responses of control arteries. These results suggest that both the release of intracellular Ca^{2+} and the influx of extracellular Ca^{2+} in response to NE are enhanced in arteries from diabetic rats. (Supported by the Medical Research Council of Canada).

60.5

Serotonin and calcitonin-gene related peptide induce endothelium-independent vasodilatation in isolated guinea pig pulmonary arteries.
R. Saban*, S. Goelzer, E. M. Harris* N. Li* and J. A. Will.
Departments of Anesthesiology and Veterinary Sciences, University of Wisconsin, Madison, WI 53792

Responses to serotonin, without pre contraction, were examined in the presence and absence of endothelial cells. The responses serotonin consisted of two contractile phases separated by a relaxation phase. Removal of the endothelial cells enhanced both contractile phases without alteration of the relaxation phase. In arteries pre-contracted with phenylephrine, serotonin was unable to further contract the vessels but produced dose-dependent relaxations that also were not altered by the removal of the endothelial layer. In contrast, the relaxant responses to neurokinin A and substance P, in arteries pre-contracted with phenylephrine, were of short duration and endothelium-dependent. Relaxation induced by calcitonin gene-related peptide (CGRP) was independent of the presence of endothelium and about the same magnitude of the responses induced by serotonin (58% and 64 % of papaverine maximum respectively). Since CGRP is found in the vascular wall it might be possible that in the guinea pig pulmonary artery there is a co-mediation between CGRP and serotonin in inducing vasodilatation.

60.6

THE ROLE OF TYPE I PHOSPHODIESTERASES (PDE I) IN REGULATING RABBIT AORTA (A) AND PULMONARY ARTERY (PA) RELAXATION RESPONSES.
R. L. Panek, T. C. Major, D. C. Kobylarz-Singer and R. E. Weishaar. Dept. of Pharmacology, Parke-Davis Res. Div., Warner Lambert Co. Ann Arbor, MI 48105

We have identified three subclasses of PDE I from A and PA (see Table) and have characterized their role in regulating relaxation responses.

subclass	soluble (S)/ particulate (P)	substrate	calmodulin sensitivity	selective inhibitor
IA	S/P	cAMP,cGMP	+++	----
IB	S	cGMP	+	TCV-3B
IC	S	cGMP	-	M&B 22,948

Tissues were contracted with 1 uM phenylephrine (PE) and exposed to increasing concentrations of inhibitor alone or pretreated for 30 min with vehicle or a single concentration of inhibitor. Pretreated tissues were then relaxed by the cumulative addition of sodium nitroprusside (SNP), an activator of soluble guanylate cyclase or atriopeptin III (AP-III), an activator of particulate guanylate cyclase. TCV-3B and M&B 22,948 directly relaxed PE-contracted A and PA rings, with TCV-3B being more potent and producing a greater maximal relaxation in both vessels. TCV-3B and M&B 22,948 also potentiated the SNP and AP-III induced relaxation in A, but only the AP-III induced relaxation in the PA. The data indicate that differences may exist in the involvement of PDE IB and PDE IC in regulating A and PA contractile function.

60.7
STIMULATION OF Na/K ATPase BY NE IN RAT AORTA DOES NOT INVOLVE Na/H or Na/Ca EXCHANGE OR PROTEIN KINASE C. F. Shen and R.C. Deth. College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA 02115

The role of protein kinase C (PKC) or sodium transport pathways during norepinephrine (NE-induced stimulation of Na^+/K^+ ATPase was studied using the method of ouabain-sensitive ^{86}Rb uptake (os-Rb uptake) in rat aorta. NE (10 μM) normally caused a transient increase of 46% in os-Rb uptake which peaked at 2 minutes of agonist exposure. The phorbol ester, phorbol dibutyrate (PDBu; 100 nM) stimulated os-Rb uptake by 46%. Staurosporine (100 nM) reduced os-Rb uptake by 25% and inhibited PDBu stimulation. NE was still able to stimulate uptake in the presence of staurosporine. In tissues displaying high basal levels of os-Rb uptake, NE was only able to cause a stimulation after prior staurosporine treatment. Inhibition of Na^+/H^+ exchange (with methylisobutylamiloride; 100 μM) or Na^+/Ca^{++} exchange (with propylbutyldimethylbenzamil; 4 μM) caused a partial reduction of basal os-Rb uptake (20% and 30% respectively), indicating a role for these pathways in determining basal Na^+/K^+ ATPase activity. NE was still able to stimulate os-Rb uptake in the presence of each or a combination of these Na^+ transport inhibitors. Our results suggest significant roles for PKC, Na^+/H^+ and Na^+/Ca^{++} exchange in the basal Na^+/K^+ ATPase activity in rat aorta. However, NE stimulation of Na^+/K^+ ATPase does not appear to involve these pathways.

60.8
SELECTIVE INHIBITION OF RECEPTOR-OPERATED Ca^{2+}-CHANNELS (ROC) BY QUINACRINE IN RABBIT AORTA. N. Satake*, M. Morikawa* and S. Shibata. Dept. Pharmacol., Sch. Med., Univ. of Hawaii, Honolulu, HI 96822

Quinacrine inhibited the concentration-response curve of norepinephrine (NE) non-competitively. However, quinacrine (10^{-5} M) had no effect on the responses to histamine and KCl. In a Ca^{2+}-free medium containing EGTA and nifedipine, NE induced a contraction and a subsequent addition of Ca^{2+} induced a tonic contraction. Quinacrine inhibited the Ca^{2+}-response in the presence of NE, but did not affect the residual NE-response. Further, the effect of quinacrine on the Ca^{2+}-response in the presence of NE was not affected by indomethacin, nordihydroguaiaretic acid, methylene blue, propranolol, forskolin and tetraethylammonium. In addition, removal of endothelium did not affect the inhibitory action of quinacrine. M&B 22,948 inhibited the Ca^{2+}-response and reduced the inhibitory effect of quinacrine. However, M&B 22,948 also inhibited the residual NE response. Incubation of the aortas with NE caused an increase in the level of inositol monophosphate (IP). Quinacrine had no effect on the IP-level. These results suggest that quinacrine selectively inhibit the responses due to Ca^{2+}-influx through ROC. Further, the inhibitory action of quinacrine is not related to arachidonic acid metabolism, cGMP, cAMP, β-adrenoceptors, K^+-conductance or endothelium. (Supported by a research grant from the University of Hawaii.)

60.9
CHARACTERISTIC VASORELAXING ACTION OF KT2-158, A NEW SYNTHESIZED BENZOTHIAZEPINE DERIVATIVE, IN RABBIT AORTAS: AN INHIBITOR OF RECEPTOR OPERATED CHANNELS (ROC) AND INTRACELLULAR Ca-RELEASE. S. Shibata, N. Satake*, A. Tomiyama* and S. Wakabayashi*. Dept. Pharmacol., Sch. Med., Univ. of Hawaii, Honolulu, Hawaii 96822

In rabbit aortas, KT2-158 shifted the responses of norepinephrine (NE), methoxamine (MO) and clonidine (CL) to the right with reduced maximal responses. KT2-158 also inhibited the responses to 5-hydroxytryptamine (5-HT), but had no effect on the responses to histamine. In a Ca^{2+}-free medium NE, MO and CL induced a phasic response and a subsequent addition of Ca^{2+} induced a tonic contraction (ROC). KT2-158 and nitroglycerin (NG) inhibited both the agonists- and Ca^{2+}-induced contractions. The effect of NG on the responses to NE and CL was greater than that of KT2-158. NG and KT2-158 similarly inhibited the residual responses to MO, but the inhibitory action of KT2-158 on the Ca^{2+}-response in the presence of MO was greater than that of NG. In the aortas contracted by NE, KT2-158 caused relaxation. NG potentiated the relaxation by KT2-158. Further, KT2-158 markedly inhibited the increase in the level of inositol monophosphate (IP) caused by NE. These results suggest that KT2-158 inhibits the α-agonists induced contractions due to ROC or Ca_i release, and IP metabolism. In addition, the inhibitory action of KT2-158 is different from that of NG. Further, KT2-158 inhibits the 5-HT receptors. (Supported by a research grant from the University of Hawaii.)

60.10
AUTORADIOGRAPHIC LOCALIZATION OF MUSCARINIC BINDING SITES IN BLOOD VESSELS. Sue Piper Duckles, Leslie E. Bailey, and Frances M. Leslie.* Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717

Functional and radioligand binding studies have shown that vascular muscarinic receptors are distributed on both smooth muscle and endothelial cells. To explore the distribution and properties of these receptors, autoradiography has been used to visualize binding sites for the ligand, ^{125}I-quinuclidinyl benzilate (QNB), in cross sections of the rabbit carotid artery. Non-specific binding, which was defined using atropine (10^{-6} M), represented 50% of total binding. Comparison of autoradiograms and corresponding sections stained with cresyl-violet suggests that specific QNB binding sites show a uniform density across the entire smooth muscle cell layer, and that the endothelial cell layer also contains binding sites for QNB. Patches of binding are also found in the adventitia, but further studies are necessary to establish the density of specific binding in the adventitia and to determine if the pharmacology of these sites differs from binding sites in smooth muscle or endothelial cells. Saturation isotherms derived by computerized image analysis revealed a Kd for QNB binding in the combined media and adventitia of 0.2 nM. Using this technique we can compare the properties and distribution of muscarinic receptors across the blood vessel wall as well as from vessel to vessel.
Supported by NIH grants #PO1 36289 and NS 22215.

60.11
ALPHA- AND BETA-ADRENORECEPTOR BLOCKING PROPERTIES OF BEVANTOLOL IN VASCULAR SMOOTH MUSCLE. S. Chai*, H.R. Kaplan and R.C. Webb. Univ. of Michigan and Warner-Lambert Pharmaceutical Research, Ann Arbor, MI 48109.

Bevantolol, a cardioselective, beta-adrenoreceptor antagonist, lowers blood pressure in renal hypertensive rats whereas propranolol does not. This study characterizes vascular actions of bevantolol in order to gain information about its pharmacological activity. Helically cut strips of dog femoral veins and rabbit aortae and mesenteric arteries were suspended in organ chambers for measurement of isometric force development. Bevantolol (10^{-7}-10^{-4}M) did not alter resting force in the segments nor did it alter contractile responses to KCl. In veins (n=6), isoproterenol (10^{-8}-10^{-6}M) caused relaxation of contractions to prostaglandin F_{2x} (10^{-6}M). Propranolol (10^{-8}-10^{-6}M) and bevantolol (10^{-7}-10^{-5}M) inhibited relaxation responses to isoproterenol in a dose-related manner; bevantolol was approximately 1/5 as potent as propranolol in this action. Contractions to norepinephrine (10^{-9}-10^{-6}M) in rabbit aortae and mesenteric arteries (n=4-6) were inhibited by bevantolol (10^{-6}-10^{-4}M) and phentolamine (10^{-7}-10^{-6}M). Dose-response curves to norepinephrine were shifted to the right in a non-parallel fashion by bevantolol and the inhibitory effect of bevantolol was approximately 1/25 that of phentolamine. We conclude that bevantolol acts as an antagonist of both alpha- and beta-adrenoreceptors in isolated arteries and veins.

60.12
EVIDENCE FOR ATP AND NOREPINEPHRINE AS CO-NEUROTRANSMITTERS IN THE GUINEA-PIG PORTAL VEIN.
William L. Chau* and David P. Westfall. Department of Pharmacology, University of Nevada School of Medicine, Reno, NV 89557.

The present study was designed to investigate the possibility of cotransmission by ATP and norepinephrine (NE) in the guinea-pig portal vein. Field stimulation of isolated segments of portal vein (16 Hz, 0.5 msec pulses) produced a biphasic contraction. Guanethidine (3 uM) which prevents transmitter release from adrenergic nerves, diminished both phases of the biphasic contraction equally. Prazosin (0.3 uM), an alpha$_1$-adrenoceptor antagonist, decreased the second phase of the contraction more than the first phase. After pretreatment with reserpine (1.0 mg/kg for 24 hrs) the second phase of the contraction was diminished substantially while the first phase was only slightly decreased. Cocaine (3 uM) a catecholamine uptake blocker, enhanced both phases of the contraction. Alpha, beta methylene ATP (10 uM), an analog of ATP that desensitizes P_2 purinergic receptors, reduced only the first phase of the contraction. In the presence of both prazosin (1 uM) and alpha, beta methylene ATP (10 uM) both phases of the contraction were diminished. The results of this study indicate that the first phase of the neurogenic contraction appears to be mediated by both ATP and NE, while the second phase is mediated by NE exclusively. (Supported by NIH grants AM07478 and HL38126).

60.13

NOREPINEPHRINE ACTS ON VASCULAR DOPAMINE RECEPTORS IN RAT PERFUSED MESENTERY:INFLUENCE OF AGE. J.C. Wanstall* and S.R. O'Donnell, Department of Physiology and Pharmacology, University of Queensland, Brisbane, Australia, 4067.

Dilator responses to norepinephrine (NE, 5-75 nmol), isoproterenol (I, 0.1-1.5 nmol) and dopamine (DA, 5-150 nmol) were obtained on isolated perfused mesentery preparations from reserpinised rats of different ages. Preparations were treated with 1 μM phenoxybenzamine and perfused with physiological salt solution containing 10 μM cocaine, 20 mM KCl and 0.1 μM vasopressin. Dilator responses to I were abolished by 0.1 μM propranolol (Prop) and those to DA by 10 nM SCH 23390 (DA_1 receptor antagonist; 8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol maleate), but responses to NE could only be blocked with Prop and SCH 23390 together. On DA_1 receptors (Prop present), the potency ratio DA:(-)NE was 7:1 and the isomeric potency ratio (-)NE:(+)NE was only 5:1 (c.f. >50:1 on β-adrenoceptors). DA_1 receptor-mediated responses were seen in rats aged 1, 2 or 4 months but not 6 or 24 months. β-Adrenoceptor-mediated responses were seen at all ages except 24 months. Responses to isobutylmethylxanthine and nitroprusside were not blocked by either of the blocking drugs and were seen at all ages. It is concluded that dilator responses of rat mesenteric arterioles to NE are age-dependent, and, in young but not mature or aged rats, they involve DA_1 receptors as well as β-adrenoceptors. (Supported by the National Health and Medical Research Council of Australia).

60.14

G-PROTEIN REGULATION OF α-ADRENOCEPTOR COUPLING TO INOSITOL PHOSPHOLIPID BREAKDOWN IN RAT TAIL ARTERY. I. Feltham.* Y.D. Cheung.* P.M. Thompson.* and C.R. Triggle. Faculty of Medicine, Memorial University of Nfld., St. John's, NF, Canada A1B 3V6

It has been suggested that postsynaptic α_1 and α_2 adrenoceptors, both mediating contraction, co-exist in vascular smooth muscle (VSM) and, furthermore, membrane polyphosphoinositide (PIns) metabolism plays a major role in VSM excitation-contraction coupling. In the present study we have investigated and compared the role of PIns breakdown in the actions of a number of adrenoceptor agonists and antagonists on the rat-tail artery (TA). In addition we have studied the effects of prior in vivo and in vitro administration of pertussis toxin (PT) and in vitro N-ethylmaleimide (NEM) on adrenoceptor mediated increases in PIns metabolism. Norepinephrine (NE) and phenylephrine stimulated PIns metabolism 40-60 fold above basal and this effect was antagonized by 10^{-7}-10^{-6}M prazosin, but not by 10^{-6}M rauwolscine. Similarly, the effects of the α_2 selective agonist, UK 14304, were prazosin sensitive and rauwolscine resistant. The actions of NE were not altered by either in vitro (5 μg/ml) or in vivo (1.5 μg/100g rat/i.p. for 48 hrs) PT but were inhibited by in vitro 30μM NEM. These results indicate that increases in PIns metabolism in the TA are mediated by α_1, but not α_2, adrenoceptors coupled to a PT insensitive, NEM sensitive G protein. (Supported by the Canadian Heart Foundation)

60.15

AMBIGUOUS SIGNALLING BY ADENOSINE IN VASCULAR SMOOTH MUSCLE CELL MEMBRANES FROM PORCINE CORONARY ARTERY: EVIDENCE FOR BOTH A1 AND A2 RECEPTOR SUBTYPES. Ira Mills* and Henry Gewirtz. Rhode Island Hospital, Brown University, Providence, RI 02903

We tested the hypothesis that more than minimal coronary arteriolar tone in the setting of a coronary stenosis and reduced myocardial blood flow may reflect ambiguous signalling by adenosine. Stimulation of the A1 adenosine receptor results in inhibition of adenylate cyclase (AC) and could offset in part vasodilation due to A2 receptor agonism which stimulates AC. The ability of phenylisopropyladenosine (PIA; A1 agonist) to inhibit Gpp (NH)p stimulated AC was measured in a crude membrane preparation (8000xg) of cultured porcine coronary artery vascular smooth muscle cells. Basal AC of 2.5±0.5 pmol/min/mg (mean±SEM;N=3) was roughly doubled (4.5±0.1) by the addition of 0.1 μM Gpp (NH)p. PIA (2 x 10^{-9} M) consistently ($p< .05$) inhibited Gpp (NH)p stimulated AC but only weakly (4.1±0.03). This inhibition was reversed by 1 mM theophylline. PIA at higher concentration (2 x 10^{-5} M) was an A2 agonist and enhanced 1.0 μM Gpp (NH)p stimulated AC (26±5 to 33±7;N=7). Finally, in one study cyclopentyl adenosine (A1 agonist) in the presence of the A2 antagonist, 30 nM CGS 15943 (triazoloquinazoline derivative), reduced 0.1 μM Gpp (NH)p stimulated AC from 4.8 to 3.5 (26%). Thus, in addition to A2 receptor activity, porcine vascular smooth muscle displays evidence of A1 receptor activity. However, weak inhibition of AC by PIA suggests that A1 receptor activity may contribute only modestly to the regulation of myocardial blood flow.

60.16

VASCULAR REACTIVITY TO BOVINE α-THROMBIN AND MEIZOTHROMBIN IN RABBIT FEMORAL ARTERIES. L.P. Thompson, M.F. Doyle, K.G. Mann and J.A. Bevan, Dept. of Pharmacology and Biochemistry, University of Vermont, Burlington, VT 05405

Contractile responses of the rabbit femoral artery to bovine α-thrombin and meizothrombin were compared. Meizothrombin is of interest in that it is a catalytic intermediate in the formation of α-thrombin which retains the lipid binding region of its precursor prothrombin. Arteries were cut into ring segments, placed in a tissue bath containing aerated physiological buffer (37°C) and mounted on a myograph apparatus for measurement of force. Contractile responses to cumulative doses (3E-10 - 3E-7M) of α-thrombin and meizothrombin were measured. Maximal contraction to α-thrombin (4.2 ± .6 g; n = 10) and meizothrombin (3.3 ± .6 g; n = 9) was not significantly different and was approximately 60 ± 7% of total tissue maximum. Vascular sensitivity (log ED_{50} value) for meizothrombin (-8.2 ± .2) was significantly greater ($p < .01$) than α-thrombin (-7.5 ± .2). Neither α-thrombin nor meizothrombin caused relaxation although acetylcholine (.3μM) and sodium nitroprusside (10μM) caused maximal relaxation. These results indicate that meizothrombin exhibits potent contractile activity and that the sensitivity of rabbit femoral arteries to meizothrombin is greater than α-thrombin. Supported by HL 35058.

NEUROMUSCULAR PHARMACOLOGY

61.1

ALTERATION OF EVOKED AND SPONTANEOUS RELEASE OF ACETYLCHOLINE DURING PARALYSIS OF RATS WITH 2,4-DITHIOBIURET. William D. Atchison, Dept. of Pharmacol./Toxicol. and Neurosci. Program, Michigan State Univ., E. Lansing, MI 48824.

Daily treatment of rats with 2,4-dithiobiuret (DTB) causes a flaccid neuromuscular weakness in rats first seen in the hindlimbs after 5-6 days of treatment at 1 mg/kg/day, i.p. This weakness is thought to be due to impaired release of acetylcholine (ACh). The purpose of this study was to investigate effects of DTB toxicity on neuromuscular transmission in affected rats using the extensor digitorum longus preparation. Endplate potentials (EPPs) and miniature endplate potentials (MEPPs) were recorded from single junctions of muscles from DTB-treated animals (1 mg/kg/day, 6 days) or pair-fed controls. EPP amplitude and mean quantal content were decreased at the time of observable weakness in DTB-treated rats; depression of quantal content was not more pronounced when stimulus frequency was increased from 0.5-50 Hz, although increasing stimulus frequency was associated with an increased incidence of transmission failures. Neither increasing extracellular Ca^{2+} nor application of 4-aminopyridine could restore quantal content of the DTB-treated group to that of control. Mean MEPP frequency was significantly reduced in the DTB-treated animals. DTB-paralyzed preparations were characterized by the presence of very large MEPPs with prolonged rise and decay times. Moreover, rise and decay times for EPPs were also prolonged in the treated rats. Thus, paresis due to chronic DTB treatment is associated with prejunctional alterations of both evoked and spontaneous ACh release. (Supported by NIH grant NS20683.)

61.2

DETERMINATION OF UNBIASED QUANTAL RELEASE PARAMETERS, INCLUDING SPATIAL VARIANCE IN P, USING MINIATURE ENDPLATE POTENTIAL (MEPP) FREQUENCIES. Michael D. Miyamoto. E. Tenn. State Univ., Johnson City, TN 37614

A method has been developed for handling two problems which have hindered the use of quantal release parameters in analyzing transmitter release. The first, involving biasing in the estimates, is resolved by use of new equations to compute spatial variance in probability of release (var_p). The second, involving inaccuracies due to nerve stimulation, is avoided by use of mepps alone (the number of mepps in a 50 msec "bin" replacing the m of a single nerve-evoked epp). A sample of 500 bins was used to compute each point, as the estimates of p obtained with 400 or more bins did not differ from those obtained with 1000 bins. Temporal variation was minimized by dividing data into subgroups of 100 and discarding data not passing stationarity tests. Increasing $[K^+]_o$ caused an increase in m, n and p with a saturation in the latter response (in accord with prior results obtained with nerve stimulation). Var_p was unchanged by $[K^+]_o$ but the values obtained were slightly negative. This could be explained by the presence of some temporal variance which escaped detection. Calculations of var_p could be used to monitor non-uniformity in $[Ca^{2+}]_i$ (e.g., that produced by Ca^{2+} release from intracellular organelles). The results support the continued use of quantal release parameters for investigating the release process. Supported by RDC of ETSU.

61.3

EVIDENCE FOR COMPARTMENTATION OF CYCLIC AMP EFFECTS IN THE MOTOR NERVE TERMINAL. J.K. Hirsh*, C.S. Solsona*, and E.M. Silinsky. Northwestern University, Chicago, IL 60611

A study was made on the effects of agents which elevate cytoplasmic levels of cyclic adenosine monophosphate (cAMP) on (i) acetylcholine (ACh) release and (ii) the effects of adenosine derivatives in motor nerve terminals of the frog *cutaneous pectoris*. The permeable cAMP analogs dibutyryl cAMP and 8-(4-chlorophenylthio) cAMP were found to increase irreversibly the level of spontaneous ACh release (miniature end-plate potential frequency or m.e.p.p.f) from 2-to 5-fold. These cAMP analogs occasionally produced smaller and reversible increases in evoked ACh secretion (M). The cAMP analogs also decreased the potency of the inhibitory adenosine derivatives. In contrast, cAMP delivered to the cytoplasmic aspect of the presynaptic membrane via phospholipid vesicles reduced both M and m.e.p.p.f, resembling the action of adenosine. The results are consistent with the hypothesis that the adenylate cyclase system is compartmentalized within the motor nerve terminal and may mediate and/or modulate the effects of adenosine derivatives. These results are also consistent with the suggestion that elevation of cAMP levels at the vesicle membrane may increase release while elevation of cAMP levels at plasma membrane sites may decrease release (Moskowitz and Puszkin, J. Theor. Biol., (1985) 112, 513-534). (U.S. Public Health Service NS 12782)

61.4

HYPERPOLARIZATION OF Em BY THEOPHYLLINE IS NOT SEEN IN CHRONICALLY HYPOXIC HAMSTER DIAPHRAGM MUSCLE. S. A. Esau Univ. of Virginia, Charlottesville, VA 22901

The resting membrane potential (Em) of hamster diaphragm muscle in vitro is hyperpolarized by theophylline. The mechanism is unknown. The effect of hypoxia on the hyperpolarization produced by theophylline was examined. Three hamsters (Group A) were maintained in a hypoxic environment (FiO_2=0.10) for 7-10 days prior to study. The diaphragm strips were removed and stored in Krebs solution at 0°C and 21%O_2/5%CO_2 (20-60 minutes). Strips were studied in Krebs solution aerated with 15%O_2/5%CO_2 (pO_2=119) at 36°C. Em was measured using 3M KCl-filled glass microelectrodes (10-20 MΩ tip resistance). After initial measures theophylline 1 mM was added and Em was remeasured. Grp B animals (n=4) were not exposed to hypoxia prior to study, otherwise, an identical protocol was followed. Em before theophylline was -73±4 mV in Grp A and -75±3 mV (mean±S.D.) in Grp B. In muscle strips exposed to chronic hypoxia in vivo, theophylline had no effect on Em (-074±8 mV). With only acute hypoxia (Grp B strips) theophylline did hyperpolarize Em to -80±4 mV, $p<.015$. Thus, chronic hypoxia abolished theophylline induced hyperpolarization. This suggests that theophylline requires a substrate which is decreased by chronic hypoxia for its action on Em.
Supported by NHLBI grant #K08 HL01183-05

61.5

OPIOID DRUGS BLOCK K^+ CONTRACTURES BUT NOT CAFFEINE CONTRACTURES IN FROG'S SKELETAL MUSCLE. Tushar G. Kokate* and George B. Frank. Dept. Pharmacology, University of Alberta, Edmonton, Alberta, Canada. T6G 2H7

K^+ contractures in skeletal muscle are produced by the entrance of extracellular Ca^{++} ions via voltage dependent calcium channels located in the t-tubules. Morphine and several other opioid agonists in concentrations ranging from 10^{-10} M to 10^{-5} M inhibited K^+ contractures when tested in frog's toe muscle. This was a nonstereospecific effect and resistant to antagonism by naloxone. While opioids completely blocked K^+ contractures they did not produce any effect on caffeine contractures showing that opioids do not deplete intracellular Ca^{++} stores nor inhibit the release of Ca^{++} from intracellular stores. Thus morphine (10^{-5} M and 10^{-7} M) and methadone (10^{-5} M and 10^{-7} M) caused 69% to 100% inhibition of K^+ contractures but showed little or no effect on caffeine contractures. It was concluded that several opioid drugs can block K^+ contractures by blocking voltage sensitive slow calcium channels in frog's skeletal muscle without affecting the release of Ca^{++} from intracellular stores, i.e. the sarcoplasmic reticulum.

(Supported by the Medical Research Council of Canada).

61.6

EFFECT OF AN ISOQUINOLINE ANALOG AND HALLUCINOGEN ON NEUROMUSCULAR TRANSMISSION. M. A. Maleque*, M. M. Williams-Johnson*, and A. Brossi* (SPON: D. C. Shockley). Dept. of Pharmacol., Meharry Med. Coll., Nashville, TN 37208, Lab. Med. Chem., NIH, Bethesda, MD. 20205.

Synthetic congeners of the alkaloid from the Mexican cactus Pachycereus weberi have been shown to reversibly block neuromuscular transmission in rats and frogs. These compounds have a structural similarity with the hallucinogen mescaline. However, a comparative study of these agents has not been done. Mescaline (MES) and 2,3,4,5-tetramethoxy- -phenethylamine (#1) were studied for their effects on eletrotonic potentials (APs), end plate and minature end plate potentials (EPPs, MEPPS) in frog sartorius muscle-sciatic nerve preparations. Both compounds (10^{-4} M) slightly decreased the amplitude and overshoot of directly evoked APs and slowed their rate of rise and falling phase. These compounds did not alter MEPP amplitude or frequency. However, MES prolonged the rate of rise of EPP and decreased the amplitude of EPP. MES, but not #1, decreased the quantal content. Inhibition of neuromuscular transmission by these agents may involve different mechanisms on pre- and postsynaptic membranes.
(Supported by NIH (MBRS) #S06-RR08037-A1)

61.7

ANTHELMINTIC-INDUCED BLOCK OF MAMMALIAN NEUROMUSCULAR TRANSMISSION. Elizabeth A. VandeWaa*, Ben Manning* and William D. Atchison, Department of Pharmacology/Toxicology, Michigan State Univ., E. Lansing, MI 48824

Levamisole (L) and Pyrantel (P) are anthelmintic drugs believed to act at the nicotinic cholinergic receptor in parasitic nematodes. L and P are characterized by low therapeutic indices, which severely limit their use. In this study, we examined the effects of L and P on somatic nicotinic cholinergic transmission in the rat isolated hemidiaphragm preparation in order to assess the effects of these drugs on vertebrate cholinergic transmission. Contractile responses to electrical stimulation of the phrenic nerve were measured before, continuously during bath application of L and/or P and during wash with drug-free media. At concentrations of 10-100 μM, P caused a dose-dependent decrease of nerve-evoked twitches. Within the range of 10-50 μM, a time-dependent suppression occurred; a complete block occurred within 1-2 min at 100 μM P. In contrast, 1 μM L caused a 10-30% reduction in twitch tension within 10 min. At 100 μM, L completely blocked nerve-evoked twitches; the onset of block was slower than with P. Twitch depression was reversible by washing with drug-free media; however, physostigmine did not cause a dramatic recovery of twitch tension following L and P. With both drugs, twitch depression was preceded by a brief period of facilitation. For comparative purposes, 1 μM nicotine facilitated twitch responses whereas 10-50 μM significantly reduced twitch tension. These results are consistent with depolarizing block of vertebrate neuromuscular transmission by L and P. (Supported by a grant from The Upjohn Co.)

61.8

A PREJUNCTIONAL EFFECT OF NON-DEPOLARIZING MUSCLE BLOCKING Drugs. Thomas Baker and Anna Stanec*. St. Joseph's Hospital and Medical Center, Paterson, NJ 07503

This study investigated the prejunctional effects of the non-depolarizing neuromuscular blocking drugs, vecuronium, pancuronium, atracurium, and d-tubocurarine, and the non-depolarizing ganglionic blocking agent, hexamethonium (C6). The cat soleus nerve-muscle preparation developed by Standaert and Riker utilizes the capacity of soleus motor nerve endings to generate stimulus-bound repetition (SBR) after high frequency conditioning. This in vivo preparation was employed to determine the incidence of SBR in the absence and presence of these agents. All the non-depolarizing muscle relaxants suppressed SBR in a dose-related manner in a dose range that did not affect single impulse transmission. The dose-response regression slopes were similar. C-6 also suppressed SBR in a dose related manner and its dose-response regression slope was similar to the slopes of the muscle relaxants. The data suggest that the prejunctional nicotinic receptor responsible for SBR supression differs from the postjunctional receptor.

61.9

ARTERIAL AND VENOUS ATRACURIUM LEVELS DURING ONSET OF NEUROMUSCULAR BLOCKADE. F. Varin[1], J. Ducharme[1], J.G. Besner[1], Y. Théorêt[1], D.R. Bevan[2] and F. Donati[2]. [1]Univ. de Montréal, [2] McGill Univ., Montréal, Canada, H3C 3J7.

The pharmacokinetic parameters of non-depolarizing neuromuscular relaxants are normally obtained using either arterial or peripheral venous blood samples. Venous (V) levels are expected to be lower than the arterial (A) ones during the onset of blockade, because of tissular drug uptake. The extent of this phenomenon was studied with atracurium (ATRA). Four ASA I-II patients were given ATRA (0.2 mg/Kg, IV). A and V samples from the antecubital fossa were collected for at least 20 min and additional V samples were drawn for 90 min to characterize the elimination phase. Neuromuscular blockade was monitored by recording the adductor pollicis muscle tension in response to train-of-four stimulation of the ulnar nerve. The maximum block obtained was 90% at 6.9 min. ATRA kinetics could be described by a two compartment model with a $t_{1/2}$ α of 2.4 min and a $t_{1/2}$ β of 17.2 min. Over the first 20 min the AUC_A was 20.8% higher than the AUC_V suggesting that the uptake of ATRA by muscle tissue was sufficient to cause significant differences between A and V levels. **(Supported by MRC grant)**

61.10

THE EFFECTS OF ISOANDROSTERONE, A WEAK ANABOLIC STEROID, ON SURGICALLY INDUCED WOUND HEALING IN ANTERIOR LEG MUSCLES. H.S. Pitkow, A. Keis,* L. Cicchinelli,* M. Kressler,* and M. Bitar*. Penna. Col. of Podiatric Medicine, Phila., PA 19107

In order to determine the effects of Isoandrosterone (IS) on Anterior Leg Muscle (ALM) wound healing in normal as well as diabetic rats, adult male Sprague-Dawley rats (200 gms; 10 animals/group) were injected with streptozotocin (STZ) (65mg/kg) for 30 days prior to the experiment and their blood sugars checked. Four groups of rats (oil control, IS control, oil diabetic, IS diabetic) were either injected subcutaneously daily with either 0.2ml sesame oil or IS (500 μg) two days pre-surgical to 5 days post-surgical wound induction (6mm Baker biopsy punch under anesthesia) at which time all rats were sacrificed. We observed that IS significantly increased the percentage of wound healing in the ALM when compared to normal oil injected controls on day 5 post-surgery. Both the muscle tensil strength (1360.6± 282.0 gms force) and the 6mm plug weight (70.8±8.2 mg) were significantly greater in the IS injected diabetic group when compared to the values (654.2 ±112.3 and 42.7 ±4.1 respectively) of the oil injected diabetic group on day 5. Our data suggests that IS has a significant effect in both normal ALM wound healing as well as in diabetes where it can noticeably improve wound healing as evidenced by the significant increase in the 6mm plug weight and muscle tensil strength.

CALCIUM HOMEOSTASIS/CALCIUM ACTION

62.1

INDOMETHACIN IMPAIRS CONTRACTION/RELAXATION OF AORTAS FROM GENETIC AND STEROID MODELS OF HYPERTENSIVE RATS.
J. Steven Richardson, University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada.

Abnormalities of the sympathetic nervous system in hypertension could reflect defects subsequent to the actual process of neurotransmission in addition to, or even instead of, defective neurotransmission per se. To test this hypothesis, the contraction-relaxation characteristics of vascular smooth muscle taken from 2 animal models of hypertension were examined. 5 mm sections of aorta from genetic hypertensive (SHR) and normotensive (WKY) rats, and from rats made hypertensive by injections of 2.5 mg/kg dexamethasone (Dex H) and vehicle treated normotensive controls (Veh N), were placed in an organ bath at 37 degrees C. Maximal contraction was induced with 20 μM noradrenaline or 80 mM potassium. Relaxation was observed in normal Krebs buffer, in calcium free Krebs and in normal Krebs containing the calcium channel blocker diltiazem 10 μM. Aortas from other rats were tested in similar buffer solutions containing the cyclooxygenase inhibitor indomethacin 1 μM. The maximal contraction of SHR aorta was less than that of the WKY in normal Krebs buffer and was further reduced by indomethacin. There were no differences in the maximal contraction of Dex H and Veh N aortas in Krebs or in 1 μM indomethacin. Following maximal contraction, SHR aortas relaxed faster in Krebs buffer than WKY aortas and this difference was amplified in calcium free and diltiazem containing buffers. Indomethacin impaired spontaneous relaxation in normal Krebs, but potentiated relaxation in calcium free and diltiazem buffers. Dex H aortas relaxed faster than Veh N aortas in normal Krebs. These results suggest that hypertensive and normotensive tissues differ in calicum handling and that prostaglandins may be involved in this difference. The relationship of these findings to the etiology of hypertension remains to be established.
Supported in part by grants from the Saskatchewan Heart Foundation.

62.2

THE EFFECT OF TAURINE ON CALCIUM BINDING TO BRAIN SYNAPTOSOMAL MEMBRANES. Ryan J. Huxtable and Ann Peterson*. Dept. of Pharmacology, College of Medicine, Univ. Arizona, Tucson, Az. 85724

Taurine is a neuroactive amino acid present in high concentrations in the brain. A number of its actions have been postulated to be due to a modulation of calcium movement. We have studied the influence of taurine on Ca^{2+} binding to rat brain synaptosomal membranes (the P_2B fraction). Incubations were performed in a buffer of 20 mM Tris (pH 7.4), 145 mM KCl and 5 mM NaCl, ionic conditions approximating those found intracellularly. Ca^{2+} concentrations in the μM range were buffered with EDTA as described by Fabiato and Fabiato (J. Physiol. Paris 75:463, 1979). Calcium binding was separable into two components: a high affinity binding with a Kd of 30.7 μM and a Bmax of 5.6 $nmol.mg^{-1}$ protein, and a low affinity binding with a Kd of 2.26 mM and a Bmax of 67 $nmol.mg^{-1}$ protein. Taurine concentrations in the intracellular range increased the affinity and decreased the binding capacity of both components. Thus, in the presence of 10 mM taurine, the high affinity system had a Kd of 20.3 μM and a Bmax of 3.22 $nmol.mg^{-1}$ protein, and the low affinity a Kd of 0.97 mM and a Bmax of 40.9 $nmol.mg^{-1}$ protein. In the presence of 25 mM taurine, the high affinity system had a Kd of 6.4 μM and a Bmax of 1.05 $nmol.mg^{-1}$ protein, while the low affinity system had a Kd of 0.43 mM and a Bmax of 31.6 $nmol.mg^{-1}$ protein. These observations are consistent with the hypothesis that certain of the actions of taurine involve modulation of Ca^{2+} availability.

62.3

EFFECT OF (Na+) ON AGONIST-STIMULATED Ca2+ INFLUX IN HUMAN BLOOD PLATELETS

Elaine A. Batka* and Harold Feinberg, Department of Pharmacology, University of Illinois at Chicago, Chicago, IL 60612

Cytosolic (Ca2+) rise, an important component of stimulus/response coupling in platelets, occurs by two mechanisms: mobilization of intracellular bound Ca2+, and Ca2+ influx. The mechanism of influx is not known. Na+/Ca2+ exchange, shown to reside in platelet plasma membrane, could function as a Ca2+ influx mechanism during activation. Thus, agonist-induced Na+ influx, which occurs via Na+/H+ exchange, should stimulate Na^+_{out}/Ca^{2+}_{in} exchange. Conversely, preventing Na+/H+ exchange (by decreasing either extracellular (Na+) or pH) should inhibit Ca2+ influx. We tested this hypothesis using washed, aspirin-treated platelets, and a 45Ca2+ uptake assay. Thrombin (.075 U/mL for 1 min.) induced a significant uptake of 45Ca2+. Reducing extracellular (Na+) from 145 to 80 mM abolished this response. Lowering external pH from 7.40 to 6.70 inhibited 45Ca2+ uptake of both resting and thrombin-stimulated platelets. 45Ca2+ did not increase in response to ADP (10 uM), although platelets became activated as evidenced by loss of discoid shape. Thus Ca2+ influx is dependent upon the agonist used, as well as the extracellular levels of Na+ and H+. These results support the concept of Na+/Ca2+ exchange as a Ca2+ influx mechanism. (Supported by BRSG and HL 29721.)

62.4

ALKALINE PHOSPHATASE AND OSTEOCALCIN IN THE BLOOD OF TWO GENETIC STRAINS OF RATS. S.B. Arnaud, P. Fung*, P. Buckendahl*, M. Vasquez*, and R. Grindeland. NASA Ames Research Center, Moffett Field, CA. 94035, Univ. of California, San Francisco, CA. 94143 and Santa Clara Univ., Santa Clara, CA. 95053.

Serum levels of two products of the osteoblast, an isoenzyme of alkaline phosphatase (B-AP) and a bone protein, osteocalcin (OC), reflect turnover of bone and its mineralization. Effects of age on serum values are well known, but the extent to which diet and housing change concentrations is uncertain. We analyzed B-AP and OC in 300 g male Taconic (T) and Czech Wistar (C) rats (3.5 weeks older than T) from the same source as rats used in American and Soviet flight experiments. B-AP was identified by its heat sensitivity and comprised the major form in blood; OC was measured by a species specific immunoassay. Rats were fed Soviet paste (PD) or Teklad L358 (TD) diet, and housed in group (n=10) (G) or single cages (S). Mean values showed B-AP more affected by diet and age than OC, *=p<.05 compared to G,PD:

	Wt g		B-AP IU/L		OC ng/ml	
Strain-diet	G	S	G	S	G	S
C+PD	308	306	53	74*	265	203*
C+TD	339*	354*	115*	83*	261	255
T+TD	291	291	145	115*	302	266
T+TD	356*	348*	122	126	253	238

In C+S, B-AP increased and OC decreased. Within strains, B-AP and OC were unrelated to body weight or to one another. Assays of both B-AP and OC appear to be of value in understanding the different causes and nature of changes in bone formation.

62.5

α-DIFLUOROMETHYLORNITHINE INHIBITS BONE RESORPTION WITHOUT ALTERING LYSOSOMAL ENZYME ACTIVITY. R.C. Lucas,* J. Seidenfeld and P.H. Stern. Northwestern University, Chicago, IL 60611

We previously reported that α-difluoromethylornithine (DFMO), a mechanism-based irreversible inhibitor of ornithine decarboxylase (ODC), inhibits bone resorption stimulated by PTH and calcitriol in vitro and that DFMO decreases calvarial putrescine and spermidine content. Further in vitro studies with cultured neonatal mouse calvaria suggest that DFMO's effect on stimulated resorption may be independent of ODC inhibition. Exogenous polyamines (putrescine, spermidine, spermine), at 200 or 500 μM, have generally failed to reverse the inhibitory effect of DFMO on calcitriol-stimulated resorption; higher concentrations of the polyamines (>10 mM) were themselves inhibitory. Although ornithine, the normal substrate for ODC, had no effect on resorption at concentrations up to 20 mM, inhibition was observed at 30 mM and higher. To further characterize the mechanism of the inhibitory action of DFMO on bone resorption, effects of the agent on lysosomal enzymes in bone were determined. Surprisingly, concentrations of DFMO (15 and 20 mM) which inhibited calcitriol-stimulated resorption did not inhibit stimulation of β-glucuronidase activity. The failure of DFMO to inhibit lysosomal enzyme activity suggests that inhibition of bone resorption by DFMO, and possibly by the polyamines, may be a result of its effects at a step beyond secretory events in osteoclasts, perhaps one involving the bone matrix.

62.6

DEPLETION OF MITOCHONDRIAL GLUTATHIONE AND Ca^{2+} BY OXIDATIVE STRESS IS ENHANCED BY ANOXIA. Dean P. Jones, Diane L. Tribble* and Tak Yee Aw.* Dept. Biochemistry, Emory Univ., Atlanta, GA 30322

Hepatocytes are more susceptible to oxidative injury under anoxic conditions than under aerobic conditions. Cellular GSH and NADPH are decreased to a greater extent, and GSH synthesis and NADPH supply are limited. To examine the senstivity of mitochondrial function to oxidative stress during anoxia, we incubated hepatocytes under either aerobic or anaerobic conditions with a toxic concentration (0.6 mM) of t-butylhydroperoxide (t-BuOOH). Changes in mitochondrial GSH and NADPH were measured following digitonin fractionation. Mitochondrial Ca^{2+} was measured by selective release with FCCP in the presence of extracellular arsenazo III. Respiration rate was measured polarographically. The results show that GSH is depleted to a much greater extent in the mitochondrial fraction of the anoxic cells by t-BuOOH than in either the cytosol from these cells or the mitochondrial fraction of aerobic cells with t-BuOOH. The decrease in GSH was associated with a decrease in mitochondrial NADPH, a loss of mitochondrial Ca^{2+} and an inhibition of O_2 consumption. For all parameters, the changes induced by t-BuOOH were greater for anoxic than aerobic cells. Of particular importance, recovery of these parameters was dramatically impaired by anoxia. Thus, the enhanced sensitivity of anoxic cells to oxidative injury is associated with the inability of mitochondria to recover normal function. Supported by NIH Grant GM 36538.

62.7

DECREASING EXTRACELLULAR SODIUM (Na_o) INCREASES INOSITOL PHOSPHATES (IP_2 & IP_3) AND RELEASES STORED CALCIUM. Scott D. Dwyer,* Tao Zheng,* and Jeffrey Bingham Smith. Dept. of Pharmacol., Univ. of Alabama at Birmingham, UAB Station, Birmingham, Alabama 35294.

Cytosolic free Ca (Ca_i) was monitored with fura-2 in human skin fibroblasts (FB) and dog coronary endothelial cells grown on coverslips. Replacing Na_o with other cations transiently increased Ca_i by several fold. The half-maximal increase in Ca_i in FB occurred at 20 mM Na_o. Removing Na_o had no effect on 45Ca influx, but strongly stimulated 45Ca efflux either in the presence or absence of extracellular Ca in both cell types. Na_o removal decreased total cell Ca in FB by 40% within a few min. Bradykinin (BK) decreased total cell Ca more rapidly than Na_o removal, but to the same maximal extent. Prior stimulation of FB with BK prevented the rise in Ca_i in response to replacing Na_o and vice versa. IP_3 and IP_2 in FB increased 2 to 4 fold in 30 sec in response to Na_o replacement. Pertussis toxin strongly inhibited the effects of Na_o replacement on Ca_i and 45Ca efflux in FB suggesting that the "Na sensitive receptor" is coupled via a G protein to phospholipase C. The "receptor" probably senses a change in extra-, not intra-, cellular Na because Na-loading had no effect on the stimulation of 45Ca efflux by Na_o removal. Calcium mobilization evoked by Na_o replacement in FB was independent of Na pump, Na/H exchange, or Na/K/Cl cotransport activity. (Supported by grants HL32508 and DK39258 from NIH).

62.8

EFFECTS OF METHYLMERCURY ON RESPIRATORY CONTROL AND $^{45}Ca^{2+}$ UPTAKE BY MITOCHONDRIA ISOLATED FROM RAT BRAIN. Paul C. Levesque* and William D. Atchison. Dept. of Pharmacol./Toxicol. and Ctr. for Environ. Toxicol., Michigan State Univ., E. Lansing, MI 48824.

Previous microelectrode recording studies provided evidence that methylmercury (MeHg) may stimulate spontaneous quantal release of acetylcholine at the rat neuromuscular junction subsequent to an interaction with mitochondria to prevent uptake of Ca^{2+} or to promote its release (Levesque and Atchison, Tox. Appl. Pharm. 87: 315, 1987; 94: 55, 1988). The present study was designed to obtain more detailed information regarding the direct effects of MeHg on mitochondria isolated from rat brain. The ratio of State 3 to State 4 respiration (Respiratory Control Ratio) was measured as a means of assessing the functional integrity of isolated mitochondria in the absence and presence of MeHg. Control ratios of from 3 to 5 were marginally reduced by 2 μM MeHg but were greatly reduced by 10 and 20 μM MeHg. The higher concentrations of MeHg markedly stimulated State 4 respiration while inhibiting State 3 respiration. The effects of MeHg on Ca^{2+} sequestration by mitochondria were assessed by measuring uptake of $^{45}Ca^{2+}$ into mitochondria as a function of time and MeHg concentration. Uptake of $^{45}Ca^{2+}$ was reduced by about 15-20% in the presence of 10 μM MeHg and by over 60% at 100 or 200 μM MeHg. The results of this study indicate that MeHg impairs mitochondrial respiration as well as the ability of this organelle to sequester Ca^{2+}. (Supported by NIEHS grant ES03299.)

CALCIUM ANTAGONISTS I

63.1

BRAIN CALCIUM CHANNEL BINDING SITES ARE ALTERED IN ALZHEIMER'S DISEASE. Richard G. Williams*, Donald T. Eagle*, Joseph A. Oibo*, Gary D. Miner*, Robert A. Colvin* (SPON: J.L. Valentine) Dept. of Pharmacology, ORU School of Medicine and Familial Alzheimer's Disease Research Foundation, Tulsa, Oklahoma 74137.

We studied the binding of [^{3}H]-nitrendipine (NIT) in normal, Alzheimer's disease (AD), and non-Alzheimer's dementia (NAD) age-matched brains. Plasmalemma were incubated at 37°C for 30 min in 132 mM NaCl, 5 mM KCl, 1.3 mM $MgCl_2$, 1.2 mM CaCl, 10 mM glucose and 25 mM TRIS-HCl pH 7.4. Scatchard analysis of saturation isotherms yielded maximal binding capacity (B_{max}) and binding affinity (K_d) with the following results (Mean ± S.D.):

	DIAGNOSIS	(n)	K_d (nM)	B_{max} (pMol/mg)
FRONTAL	Normal	(2)	0.578 ± 0.46	0.134 ± .01
CORTEX	NAD	(3)	0.870 ± 0.26	0.114 ± .003
	AD	(3)	1.52 ± 1.23	0.100 ± .042
TEMPORAL	Normal	(2)	0.613 ± 0.30	0.129 ± .045
CORTEX	NAD	(3)	0.777 ± 0.32	0.102 ± .012
	AD	(3)	1.15 ± 0.63	0.0955± .022

[^{3}H]-NIT binding in the frontal and temporal cortex of AD was of reduced affinity when compared with normal and NAD brains. The changes in binding site density were less striking. The results suggest that derangements in neuronal calcium metabolism may occur in AD and that drugs which modulate calcium channel activity may provide novel therapeutic approaches to the treatment of AD.

63.2

PROTECTIVE ACTIONS OF THE NEW CALCIUM CHANNEL BLOCKER TA-3090 IN CEREBRAL ISCHEMIA. W.Morrone*, R.Zobrist*, T.Mecca and J.Lacz*. Marion Laboratories, Inc., Kansas City, MO 64134

Previous studies have demonstrated beneficial effects of calcium channel blockers in cerebral ischemia. This study investigated the ability of TA-3090 (TA), 8-chloro diltiazem, to reduce the consequences of experimental ischemia in the Mongolian gerbil. Animals were divided into sham control, stroke control (SC), prestroke (PRE) and post-stroke (POST) drug treatment groups. In the (PRE) drug treatment group TA-3090 (TA; 3 mg/kg), diltiazem (DTZ; 20 mg/kg), nimodipine (NIM; 1 mg/kg), and verapamil (VER; 30 mg/kg) were administered BID by oral gavage starting 24 hours prior to unilateral carotid artery occlusion (UCAO). In the (POST) treatment groups, drug administration began 1 hour after UCAO. Drug treatment continued BID for 5 full days after UCAO in all drug treatment groups. In the TA PRE and POST groups, neural damage was decreased by 45% and 25%, respectively, as compared to the SC group. In these same groups ischemia-induced stereotypic behavior was decreased by 77% and 70%, and 5 day survival rates were increased by 197% and 80%, respectively, when compared to SC. NIM produced similar results, whereas DTZ and VER were less effective. These results suggest that calcium channel blockers are beneficial in reducing the negative consequences of cerebral ischemia and that TA is as effective as NIM in this regard.

63.3

INTERACTION OF A NOVEL BISPHOSPHONATE Ca CHANNEL BLOCKER, BMY-21891 (SR-7037), WITH THE CHANNEL ACTIVATING AND INACTIVATING BINDING SITE. J.G. Sarmiento*, C.A. Childs*, R.L. Cavanagh*, G.D. Goggins* and J.P. Buyniski.
Pharmaceutical Research and Development Div., Bristol-Myers Co., Wallingford, CT 06492

BMY-21891, tetrabutyl 2-(2-phenoxyethyl)-1,3-propylidene-diphosphonate, is a novel chemotype discovered by Symphar S.A. in Geneva, Switzerland that interacts at the dihydropyridine (DHP) binding site of Ca channels from brain, cardiac and smooth muscle tissue. BMY-21891 displaces ^{3}H-PN 200-110 from cardiac binding sites (Ki = 22.7 nM) and brain binding sites (Ki = 22.0 nM) in an apparent competitive manner, though less potently than nifedipine (cardiac membranes Ki = 0.5 nM). BMY-21891 partially displaces ^{3}H Bay K 8644 (Ca channel activator) from rat cardiac membranes, 20% of the ^{3}H Bay K 8644 remains bound at 1 μM; BMY-21891 has an IC50 = 320 μM and the slope of the probit analysis is less than 1, indicative of a noncompetitive interaction with the agonist binding site of cardiac membranes. In rat aortic smooth muscle strips BMY-21891 was equipotent to nifedipine in blocking contraction where K_b values for each compound are about 0.5 nM. BMY-21891 reverses the activation of contraction induced by Bay K 8644 in partially depolarized rat aortic strips, in an apparent competitive manner and thus resembles the reversal occurring with certain DHP antagonists. These data suggest that BMY-21891, although structurally unrelated to the DHP's, can interact potently at both the inactivating and activating sites for DHP's as shown by radioligand binding and pharmacological studies.

63.4

CHARACTERIZATION OF HIGH AFFINITY BINDING SITES FOR CHARYBDOTOXIN IN SARCOLEMMAL MEMBRANE VESICLES DERIVED FROM BOVINE AORTIC SMOOTH MUSCLE. Maria L. Garcia*, Jesus Vazquez*, John P. Reuben and Gregory J. Kaczorowski*.
Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ. 07065.

Charybdotoxin (ChTX), a 37 amino acid peptide, purified from venom of the scorpion Leiurus quinquestriatus var. hebraeus blocks with high affinity (K_i = 2 nM) high-conductance Ca^{2+}-activated K^+ ($P_{K,Ca}$) channels in cultured bovine aortic smooth muscle cells. ChTX has been radiolabeled with ^{125}I and the mono-iodo component was separated and characterized. ^{125}I-ChTX binds with high affinity (K_d = 100 pM) to a single class of receptor sites in sarcolemmal membrane vesicles derived from bovine aortic smooth muscle that display a density of ca. 500 fmol/mg protein. Binding of ChTX is modulated by ionic strength of the medium and by a number of mono- and divalent cations which are known to interact potently with $P_{K,Ca}$ channels. In addition, tetraethylammonium ion, which blocks $P_{K,Ca}$ channels with high affinity by binding to the extracellular face of the channel, inhibits ChTX binding by decreasing toxin affinity. All these data, taken together, suggest that the receptor sites identified for ChTX are functionally associated with $P_{K,Ca}$ channels and further illustrate the utility of ChTX for elucidating the pharmacological, biochemical and physiological importance of $P_{K,Ca}$ channels.

63.5

SKELETAL MUSCLE FROM MALIGNANT HYPERTHERMIA-SUSCEPTIBLE SWINE CONTAINS DECREASED LEVELS OF MONOCLONAL ANTIBODY REACTIVE DIHYDROPYRIDINE RECEPTOR. R.J. Chang*[1], M. Dershwitz[2], F.A. Sréter[2], and H. Smilowitz[1]. [1]Dept. of Pharmacology, Univ. of Conn. Health Center, Farmington, CT 06032; and [2]Dept. of Muscle Research, Boston Biomedical Research Inst. and Dept. of Anesthesia, Massachusetts General Hospital, Boston, MA 02114.

The basic biochemical defect in human and swine malignant hyperthermia (MH) is not known; much evidence suggests an abnormality in calcium regulation. Decreased [^{3}H] nitrendipine binding to transverse tubule vesicles from MH swine has been reported [J.M. Ervasti, et al., *FASEB J.*, 2, A394 (1988)]. Our results confirm that membranes prepared from MH swine muscle contain 30-40% less [^{3}H] PN200-110 (a dihydropyridine antagonist) binding sites than control swine muscle membranes. Furthermore, we found that MH muscle membranes contain 60-70% less [^{3}H] D888 (desmethoxyverapamil) binding sites. We prepared a monoclonal antibody to the 170 kDa subunit of the dihydropyridine receptor (DHPR), and found 50% less antibody immunoreactive DHPR in MH muscle as evidenced by ELISA and immunoblotting techniques. Ca^{2+}-ATPase and cholesterol levels were comparable, however, in control and MH muscle membranes. These results suggest that an abnormality in DHPR participation in excitation-contraction coupling may be involved in susceptibility to MH. Studies are now in progress to determine if other transverse tubular components or other DHPR subunits are selectively altered in swine MH muscle.

Supported by NIH Program Project Grant HL33026, Center Grant GM15904, and GM11656.

63.6

Comparative effects of doxorubicin and aclacinomycin on calcium levels, protein kinases and calcium uptake in sarcoplasmic reticulum. O. Akogyeram and W.L. West, Department of Pharmacology, Howard University, Washington, D.C., U.S.A.

Doxorubicin (5 mg/kg) significantly increased the myocardial tissue calcium over control of Sprague-Dawley rats when these animals were injected with the drug two times a week for 4 weeks. Aclacinomycin, an analogue of doxorubicin, which has been shown to be less cardiotoxic, also increased myocardial tissue calcium. However the increase in calcium in the doxorubicin group was significantly greater than that of the aclacinomycin group. In vitro studies of these drugs on phosphorylation processes such as phosphorylation of phospholamban and other proteins known to be substrates of three different types of protein kinases as well as calcium uptake into canine cardiac sarcoplasmic reticulum showed that these drugs did not affect the phosphorylation processes. Doxorubicin up to 100 uM did not influence the calcium uptake of cardiac sarcoplasmic reticulum but aclacinomycin inhibited this calcium uptake. Also aclacinomycin stimulated nitredipine binding to cardiac sarcoplasmic reticulum whereas doxorubicin did not. These different effects on the biochemical processes per se do not explain the difference in their cardiotoxicity.

63.7

GUANOSINE TRIPHOSPHATE-DEPENDENT STIMULATION OF L-TYPE CALCIUM CHANNELS OF VASCULAR SMOOTH MUSCLE CELLS. Y. Ohya* & N. Sperelakis, Dept. of Physiology & Biophysics, Univ. of Cincinnati, Cincinnati, OH 45267-0576

Possible involvement of guanosine triphosphate (GTP) in the regulation of L-type (slow-type, high-threshold) Ca^{2+} channel in vascular smooth muscle was examined using patch-clamp single-channel recording. Single Ca^{2+} channel current (conductance of about 20 pS) was recorded, in cell-attached mode, from freshly isolated single cells of guinea-pig portal vein. The intracellular content was modified by permeabilizing the membrane at one end of the cell. The pipette contained isotonic Ba^{2+} and 1 μ M Bay-K-8644 (Ca^{2+} channel agonist), and the bath contained high-K^+ solution to depolarize the membrane to near 0 mV. Although Ca^{2+} channel activity initially decreased just after permeabilizing the membrane, it usually continued for over 15 min. In about 50% of the examined patches, application of 100 μ M GTP-γ-S to the bath (access to cell interior) enhanced the channel activity or prevented its run-down; 100 μ M GTP had effects similar to GTP-γ-S. Pretreatment with GDP-β-S prevented the effects of GTP-γ-S. These results suggest that GTP may contribute to the regulation of the L-type Ca^{2+} channel in vascular smooth muscle cells, probably via a GTP-binding protein, as reported for cardiac and skeletal muscles (Yatani et al., 1987). The Ca^{2+} channel may be directly activated by a GTP-binding protein, as it is not activated by cAMP in smooth muscle cells (Ohya et al., 1987). (Supported by NIH grant HL-31942.)

63.8

EFFECTS OF AMINOPYRIDINES ON ^{3}H-DOPAMINE RELEASE AND CALCIUM UPTAKE IN RAT STRIATAL SYNAPTOSOMAL PREPARATIONS. H.Scheer* (SPON: M. Sharkawi) Department of Pharmacology, Université de Montréal, Montreal, Canada.

Aminopyridines (APs) enhance release of neurotransmitters in the peripheral nervous system by facilitating Ca^{2+}-influx into the nerve terminal via as yet unidentified means. To investigate effects of APs in the CNS, rat striatal synaptosomal preparations were tested for APs ability to release preloaded ^{3}H-dopamine (^{3}H-DA) and to stimulate $^{45}Ca^{2+}$ uptake. In Krebs-Ringer buffer, ^{3}H-DA release was stimulated to a similar extent by both 4-AP and 3,4-AP in a Ca^{2+}-dependant manner at concentrations up to 200 μM. 2-AP, 3-AP, 2,6-AP, pyridine-4-aldoxime and adenine did not induced release ^{3}H-DA themselves nor altered 4-AP (100 μM) release (25.1 ± 2.1 ^{3}H-DA stores released at 10 min). 4-AP and 3,4-AP stimulated synaptosomal $^{45}Ca^{2+}$ uptake similarly (2.7 ± 0.5 and 2.9 ± 0.3 nmole Ca^{2+}/mg protein). Neither AP depolarized synaptosomes as judged by distribution of ^{3}H-triphenylmethylphosphonium. Of the various compounds tested for their ability to inhibit AP induced ^{3}H-DA release (e.g. nitrendipine, tetrodotoxin) only the inorganic Ca^{2+}-channel blockers Cd^{2+}, Ni^{2+} and Co^{2+} were effective (IC_{50}: 4μM, 500 μM and 650 μM resp.). The relative ability of these ions to inhibit both ^{3}H-DA release and $^{45}Ca^{2+}$-uptake suggests that AP effects in synaptosomes may be linked to activation of Ca^{2+}-channels.

63.9

CALMODULIN-BINDING PROTEINS AND MULTIDRUG RESISTANCE. M. Ido* and J.G. Chafouleas* (Spon: F. Labrie). MRC Group in Molecular Endocrinology, Laval Univ. Med. Center, Quebec G1V 4G2, Canada

The mechanism of multidrug resistance to anticancer drugs such as vinca alkaloids, anthracylins, actinomycin D, and VP-16 is not fully understood. Ca^{2+}-CaM-dependent processes may be linked to multidrug resistance. However, the CaM content is not different between drug-sensitive and -resistant cells. This study was designed to determine whether the calmodulin-binding proteins (CaMBPs) might be different between drug-sensitive and resistant cells. CHO cells were induced to become drug resistant with vincristine. The resistant cell line (CHO/VCR) exhibited the classic multidrug resistance pattern and demonstrated cross-resistance to vinblastine, adriamycin and VP-16. Total cellular proteins of CHO and CHO/VCR cells were analyzed by 2-dimensional gel electrophoresis. As expected, when the gels were stained for total proteins with silver stain, essentially no discernable differences between the protein patterns of the two cell lines could be detected. Replicate gels were also processed by the ^{125}I-CaM overlay procedure in order to distinguish the CaMBP pattern. Most of the CaMBPs were also identical between the sensitive and resistant cell lines. However, distinct changes in the expression of several CaMBPs were indeed observed between these two cell lines. These data suggest that changes in the expression of several CaMBPs occur during induction of drug resistance. Future studies will clarify the relationship between drug resistance and these CaMBPs.

63.10

SELECTIVE INHIBITION OF CALMODULIN (CM) -DEPENDENT ENZYMES BY PHENOTHIAZINE-CM ADDUCTS. **S.-P. Zhang*, W. Prozialeck, and B. Weiss, Department of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129**

Drugs that inhibit calmodulin (CM) activity by binding directly to it are relatively non-selective in that they inhibit all the CM-dependent enzymes. In an attempt to develop more selective CM antagonists we have synthesized a number of drug-CM adducts and examined the influence of these adducts on several CM-dependent enzymes, including phosphodiesterase and Ca^{2+}-ATPase and on the non-CM dependent enzyme, protein kinase C. A chlorpromazine-calmodulin (CPZ-CM) adduct was formed by irradiating CPZ with CM. A fluphenazine-N-mustard (FNM)-CM adduct forms irreversibly without UV irradiation. These two phenothiazines form different types of adducts since the binding of CPZ to CM occurs on the phenothiazine nucleus whereas the binding of FNM to CM occurs on the ethylene chloride side chain. Neither of the adducts activated any of the enzymes studied. However, they differentially blocked the activation of CM-dependent enzymes induced by native CM. Both CPZ-CM and FNM-CM inhibited CM-stimulated phosphodiesterase (IC_{50}=100 nM and 1000 nM, respectively) but neither had any effect on the activation of ATPase by CM. Moreover, unlike the phenothiazines themselves, neither adduct inhibited protein kinase-C activity. These findings support the possibility of developing a new class of selective CM antagonists that are directed at CM binding sites on CM-sensitive enzymes. (Supported by GM34334)

RESPIRATION PHARMACOLOGY

64.1

EFFECT OF THE K^+ CHANNEL ANTAGONIST CHARYBDOTOXIN (ChTx) ON GUINEA PIG AIRWAY SMOOTH MUSCLE. T.R. Jones, L. Charette*, and E. Champion*. Merck Frosst Canada Inc., P.O. Box 1005, Pointe Claire-Dorval, Québec, Canada, H9R 4P8.

ChTx is a 37 amino acid protein purified from scorpion venom (Leiurus quinquestriadus). It has nM affinity for Ca^{2+}-activated K^+ channels and is a potent inhibitor of K^+-conductance in smooth muscle. In the present study we investigated the actions of the purified toxin on isolated guinea pig trachea (GPT). ChTx (5 - 90 nM) induced slowly developing, concentration-dependent contractions of GPT which were tonic and well maintained in the absence of indomethacin but were usually more variable, phasic and not well maintained in the presence of indomethacin. ChTx was not a full agonist but was approximately 100 times more potent than histamine at the EC_{40} level. ChTx-induced contractions were reversed by the Ca^{2+}-entry blocker nifedipine, the K^+-channel agonist, BRL-34915, the beta agonist, isoproterenol, the phosphodiesterase inhibitor, aminophylline and Na^+ nitroprusside but were not reversed by PGE_1 or PGE_2. In another series of experiments, low concentrations of ChTx did not potentiate contractile responses to histamine on indomethacin-treated tissues. The present findings demonstrate that ChTx is a potent spasmogen on guinea pig trachea. This toxin should be very useful to probe the physiological role of Ca^{2+}-activated K^+-channels in the regulation of airway smooth muscle contractility.

64.2

ARACHIDONIC ACID-INDUCED BRONCHOSPASM AS A METHOD FOR EVALUATING DUAL CYCLOOXYGENASE/LIPOXYGENASE INHIBITORS. Thomas Kirchner*, Anita S. Meeks* and David M. Ritchie, Ortho Pharmaceutical Research Laboratories, Raritan, New Jersey 08869.

Currently, evaluation of the in vivo activity of dual cyclooxygenase/lipoxygenase inhibitors relies on several different test systems. Pulmonary models which determine inhibition of a specific agonist or models involving arachidonic acid (AA) products produced endogenously are used along with models of inflammation. An in vivo method to evaluate inhibitors of both metabolic pathways in the same animal has been developed. By challenging a guinea pig with low dose arachidonic acid (1.0 mg/kg, i.v.), a rapid bronchospasm results which can be prevented by cyclooxygenase inhibitors. Indomethacin has an ED_{50} of 0.09 mg/kg, i.v. High doses of AA (10 mg/kg) in the presence of indomethacin (10 mg/kg) and propranolol (0.1 mg/kg) result in a sustained bronchoconstriction associated with elevated LTB_4 blood levels. Indomethacin had no effect on this response while FPL 55712, a leukotriene receptor antagonist, produced inhibition (ED_{50} = 3.2 mg/kg, i.v.). Phenidone, a lipoxygenase inhibitor, not only blocked the high dose AA response (ED_{50} = 5.04 mg/kg, i.v.), but also prevented the elevation of circulating LTB_4 levels (ED_{50} = 14.1 mg/kg, i.v.). This model allows the in vivo evaluation of cyclooxygenase and/or lipoxygenase inhibitors which may be useful anti-allergic or anti-rheumatic therapies.

64.3

PLATELET-ACTIVATING FACTOR (Paf) IN AIRWAY HYPERRESPONSIVENESS IN THE GUINEA PIG. T. Inoue* AND M.S. Kannan*. (SPON: A. Larson). Department of Veterinary Biology, Coll. of Vet. Med., St. Paul, MN 55108.

The role of Paf in airway hyperresponsiveness was investigated in guinea pigs challenged in vivo for 7 days, 10 min each day, with saline aerosol containing 0.2 mg Paf. Control animals received saline aerosols. The isometric tension responses and the membrane potential were measured in vitro in response to histamine (H), acetylcholine, and KCl in isolated central airway smooth muscle (ASM). ASM from Paf-treated animals showed increased sensitivity to all the three agonists, with most pronounced changes in the concentration to elicit 50% of maximum contractions to H (about a 1000-fold shift in the concentration-response curves). The resting membrane potentials (Em) of the cells from ASM of control and Paf-treated animals were similar. However, H-induced depolarizations were greater in the ASM cells from treated animals than in the controls. Contractions to threshold concentrations of H were unaccompanied by any detectable changes in Em. The results suggest non-specific hypersensitivity to agonists in vitro following in vivo Paf challenge in the guinea pig ASM, with characteristic changes in the excitation-contraction coupling to histamine.

Supported by the University of Minnesota School of Graduate Studies and the Canadian Heart Foundation.

64.4

BRONCHOPULMONARY AND CARDIAC EFFECTS OF SEVERAL XANTHINES IN THE ANESTHETIZED GUINEA PIG. J.A. Schenden*, S.L. Macon*, D.E. Wilkins*, B.J. Canning*, W.J. Kinnier and R.E. Howell*. Nova Pharmaceutical Corporation, Baltimore, MD 21224-2788

Theophylline's usefulness in the treatment of asthma, as well as its cardiac side effects, have been attributed to adenosine antagonism and to phosphodiesterase (PDE) inhibition. We examined the ability of several xanthines to reverse histamine-induced bronchoconstriction and to prophylactically inhibit antigen-induced bronchoconstriction, as well as their effects on heart rate, in anesthetized guinea pigs. The potencies in reversing histamine-induced bronchoconstriction were ranked: isobutylmethylxanthine (IBMX)>enprofylline> theophylline>8-phenyltheophylline (8-PT), which follows their potencies as PDE III inhibitors. The prevention of antigen-induced bronchoconstriction at a dose of 10 mg/kg was ranked: IBMX>enprofylline>8-PT. Interestingly, theophylline was not effective in preventing an antigen-induced bronchoconstriction at 10 mg/kg; however, it did significantly prevent the bronchoconstriction at greater doses ($\geq$ 18 mg/kg). Heart rate was increased by the PDE III inhibitors IBMX, enprofylline and theophylline, but not by the adenosine antagonist 8-PT. These results suggest that at least two distinct mechanisms of action are involved in theophylline's effectiveness in the treatment of asthma.

64.5

STUDIES OF NONADRENERGIC, NONCHOLINERGIC RELAXATIONS IN AN ISOLATED, INNERVATED GUINEA-PIG TRACHEAL PREPARATION. **D.F. Biggs*, A.K. Wang*, K.E. Banks* and J.G. Martin. Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada H3A 2B4.**

The guinea-pig trachea is innervated by cholinergic excitatory, and inhibitory sympathetic and nonadrenergic noncholinergic (NANC) nerve fibers. We determined the origin of NANC-mediated relaxations in innervated guinea-pig tracheal tube preparations, in vitro. Tracheal tubes with the vagi and recurrent laryngeal nerves intact were obtained from guinea pigs anesthetized with pentobarbital. They were mounted in Krebs solution gassed with 95%O_2/5%CO_2 in a 25-ml organ bath and stimulated alternately via the vagi (NS) or via field stimulation (FS), while changes in intratracheal pressure were measured. In the presence of guanethidine (10 uM) and propranolol (5uM), FS induced current-dependent relaxations, and NS minimal or no relaxations, before, or after atropine (0.5 uM). Responses to FS and NS of tracheal tubes from guinea pigs given 6-hydroxydopamine (50 mg/kg, ip) 12 h before experiment, were similar to those of control animals. Tetrodotoxin (1 uM) almost totally eliminated contractile and relaxant responses to FS and NS. Thus, in this preparation in vitro, the relaxant responses are dependent on sodium-channel opening, however, parasympathetic afferents and efferents, and sympathetic efferents do not mediate NANC inhibitory responses. (Supported by the Medical Research Council of Canada.)

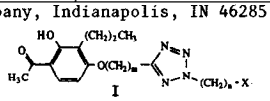
64.6

CI-949 INHIBITS THE DEVELOPMENT OF AIRWAY HYPERREACTIVITY (AH) IN A NEW "IN VIVO" AH MODEL. J.E. Low*, C.D. Schuman*, D.O. Thueson*, and M.C. Conroy, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

An "in vivo" AH assay was developed to examine the abilities of CI-949 (5-methoxy-3-(1-methylethoxy)-1-phenyl-N-1H-tetrazol-5-yl-1H-indole-2-carboxamide, L-arginine salt) and standard compounds to inhibit the development of AH. AH was present in ovalbumin (OVA)-sensitized guinea pigs 24 hr after a 6 min aerosolized OVA (5 mg/ml) challenge under the cover of mepyramine pretreatment. Dose-response (D/R) curves of compliance and resistance changes in challenged animals infused with IV bolus doses of histamine were shifted, in a parallel manner, 2-3 fold to the left of the D/R curves obtained from sensitized unchallenged control animals. The compliance or resistance baselines obtained from control and challenged animals immediately prior to IV histamine infusions were not statistically different. CI-949 (100 mg/kg IP) administered either 20 min before or immediately after OVA challenge completely prevented the development of AH. Pretreatment with hydrocortisone (40 mg/kg IP, BID 3 days) or nedocromil (100 mg/kg IP) was also examined. The rank order of efficacy obtained with the compounds tested was CI-949 > hydrocortisone > nedocromil. When administered after OVA challenge, nedocromil was completely ineffective. These results demonstrate the superior ability of CI-949 to protect in both a prophylactic and non-prophylactic regimen against the development of "in vivo" AH.

64.7

LEUKOTRIENE(LT) RECEPTOR ANTAGONISTS; DEVELOPMENT OF LY203647. C.A. Whitesitt*, S.K. Sigmund*, S.L. Lifer*, R.A. Hahn*, C.R. Roman*, L.E. Rinkema, J.H. Fleisch and W.S. Marshall. Eli Lilly and Company, Indianapolis, IN 46285.



I

A series of acetophenone LT receptor antagonists, I, were prepared and tested for their ability to antagonize LTD_4- and LTE_4-induced contractions of guinea pig ilea and trachea. The compounds were also examined as antagonists of pressor responses to i.v. LTD_4 in pithed rat and to LTD_4- and antigen-induced bronchospasm in anesthetized guinea pigs. LY203647, I, m=4, n=4, x=5-tetrazolyl, proved the best compound in the series. pK_B values of 7.1 and 7.4 were obtained against LTD_4 and LTE_4 on ileum and 6.4 and 6.8 against LTD_4 and LTE_4 on trachea. LY203647 did not alter smooth muscle responses to a variety of other agonists. Pressor responses in rats to LTD_4 were reduced by LY203647 in a dose-dependent manner; ED_{50}=7.5 mg/kg i.v. LY203647, 3 and 10 mg/kg i.v. or 10 and 30 mg/kg p.o., blocked bronchoconstriction caused by LTD_4 in the guinea pig. Antigen-induced bronchospasm was blocked by i.v. doses of 3 and 10 mg/kg. Based on its pharmacologic and toxicologic profiles, LY203647 was chosen for clinical evaluation as a LT receptor antagonist in diseases thought to be associated with an overproduction of LTD_4 and LTE_4.

64.8

DIFFERENTIAL INFLUENCE OF THE EPITHELIUM ON ISOLATED GUINEA-PIG TRACHEAL RESPONSE TO NEUROKININ A AND SUBSTANCE P. C.K. Buckner, S.V. Ghanekar* and R.D. Krell, ICI Pharmaceuticals Group, ICI Americas, Inc., Wilmington, DE 19897 USA.

The airway epithelium has been suggested to be a site of action and/or metabolism of several bronchoactive substances. This study examined the influence of the epithelium using paired ring segments of guinea-pig trachea suspended in isolated tissue baths containing Krebs bicarbonate solution with indomethacin, 5 x 10^{-6} M. Cumulative dose-response curves for contractile effects of neurokinin A or substance P were obtained also in the absence and presence of metabolic inhibitors. Removal of the tracheal epithelium resulted in a leftward shift of the substance P dose-response curve and a smaller alteration of responses to neurokinin A. Phosphoramidon and thiorphan, 1 x 10^{-6} M, potentiated both agonists without altering responses to carbachol. When examined in combination with thiorphan, other enzyme inhibitors captopril, leupeptin and bestatin did not influence dose-response effects of neurokinin A or substance P. After removal of the influence of the airway epithelium and metabolic degradation, neurokinin A was about 3-fold more potent than substance P. These studies illustrate the importance of controlling experimental conditions in the determination of relative potencies of peptides acting at neurokinin receptors to contract the guinea-pig trachea.

64.9

LEUKOTRIENE (LT) E_4 RECEPTOR ANTAGONISM AND PHOSPHODIESTERASE (PDE) INHIBITION OF LY171883, ISOBUTYLMETHYLXANTHINE (IBMX), AND THEOPHYLLINE (THEO) IN VITRO AND IN VIVO. L. E. Rinkema, C. R. Roman*, K. G. Bemis*, W. S. Marshall, and J. H. Fleisch. Eli Lilly and Company, Indianapolis, IN 46285

The pharmacologic consequence of LT receptor antagonism and of PDE inhibition on the activity of LY171883, IBMX, and THEO was assessed. Potentiation of isoproterenol (ISO) was used as an indicator of PDE activity. Carbachol-contracted guinea pig trachea were relaxed with ISO before and after LY171883, IBMX, or THEO. A 2 fold leftward shift in ISO-induced relaxation was produced by 2.5 μM IBMX, 28 μM LY171883, and 140 μM THEO. At these concentrations, IBMX or THEO did not antagonize LTE_4 in trachea whereas LY171883 had marked LTE_4 blocking activity. Dose related bronchospasms to i.v. histamine were obtained in anesthetized guinea pigs. ISO was infused at a constant rate and the histamine dose response curve (DRC) repeated. ISO caused a 3-4 fold rightward shift of the histamine DRC. Drug or vehicle was given i.v. and 20 minutes later histamine DRCs, without and with ISO, determined. LY171883, 30 mg/kg, had no effect on either the histamine DRC or on the shift induced by ISO. IBMX, 1 mg/kg, only reduced histamine responses. IBMX, 3 mg/kg, or THEO, 30 mg/kg, reduced the histamine-induced bronchospasm and potentiated the response to ISO. IBMX or THEO did not antagonize bronchospasm induced by LTE_4; LY171883 was active at 3 mg/kg. Therefore, LY171883 functioned primarily as an LTE_4 receptor antagonist whereas the actions of IBMX and THEO appeared to reflect PDE inhibition.

64.10

REVERSAL OF A23187-INDUCED AIRWAY CONSTRICTION IN THE GUINEA PIG. P.W. Stengel and S.A. Silbaugh. Eli Lilly and Company, Indianapolis, IN 46285

Previously, we examined the initiation of A23187-induced bronchoconstriction (Prostaglandins 33:567, 1987). In this study, we determined mediators maintaining this response. Conscious, male Hartley guinea pigs were exposed to A23187 aerosol (4 mg/ml) until dynamic compliance (Cdyn) decreased by 50% (~5 min). By 10 min, Cdyn was 20-25% of baseline and remained at that level in control animals. At 20, 25, and 30 min, cumulative i.v. doses (mg/kg) of these drugs were given: salbutamol (SA) and LY53857 (5-HT_2 antagonist), .01-.1; pyrilamine (PY), .04-.4; atropine (AT), .07-.7; indomethacin (IN) and dazoxiben (DAZ, thromboxane synthetase inhibitor), 1-10; and aminophylline (AM), phenidone (PH), REV-6866 (5-lipoxygenase inhibitor), SRI 63-072 (PAF antagonist), and LY183001 (LTD_4/E_4 antagonist), 3-30. At 35 min, animals were killed and excised lung gas volumes (ELGV), i.e. pulmonary gas trapping, measured. ELGVs were compared to final Cdyn values. After the final dose, SA, PH, AM, DAZ, LY53857, REV-6866, and LY183001 reversed Cdyn by 61 ± 11, 53 ± 11, 42 ± 10, 38 ± 6, 28 ± 10, 26 ± 5, and 13 ± 5% respectively. The other drugs had little or no effect. Reversal of Cdyn was highly correlated with reduction of pulmonary gas trapping ($r = -.86$, $p < 0.0001$). We conclude that (1) thromboxane A_2, serotonin, and lipoxygenase products may play a role in maintaining A23187-induced smooth muscle constriction and (2) the model is useful to study reversal of airway obstruction.

64.11

LEUKOTRIENE AND PROSTAGLANDIN PRODUCTION FOLLOWING ANTIGEN INHALATION IN PRIMATES. R.H. Gundel*, C.A. Torcellini*, C.C. Clarke*, J. Watrous*, C.A. Homon*, P. Kinkade*, P.R. Farina*, and C.D. Wegner* (SPON: L.G. Letts). Depts. of Pharma. and Biochem., Boehringer Ingelheim Pharma., Inc., Ridgefield, CT.

Seven anesthetized adult male cynomolgus monkeys with a naturally occurring sensitivity to *Ascaris suum* extract were studied. Threshold doses of *Ascaris* extract were administered via IPPB for 2 minutes. Respiratory system resistance (Rrs) was measured by forced oscillations (4-40 Hz) prior to and 10 minutes following antigen challenge, after which bronchoalveolar lavage (BAL) was performed. BAL samples were made 80% ethanolic and concentrated approximately 5 times before quantitation of immunoreactive leukotriene C_4 (i-LTC_4) and prostaglandin D_2 (i-PGD_2) by radioimmunoassay. Selected BAL samples were also analyzed by RP-HPLC. Dexamethasone (1 mg/kg, im -24, -4, and 0.5 mg/kg, im -1 hr prior to antigen, N=7) inhibited i-LTC_4 (71.1 ± 6.3%) and i-PGD_2 (37.3 ± 21.4%) while having no effect on antigen-induced changes in Rrs. Phenidone (30 mg/kg, po -24, -4, -1 hr, N=4) weakly inhibited i-LTC_4 (47.4 ± 26.5%) and did not alter i-PGD_2 (3.2 + 18.6%) or changes in Rrs. Indomethacin (10 mg/kg, ip -24, -4, -1 hr, N=4) had a variable effect on i-LTC_4 levels (6.2 ± 50.5%), strongly inhibited i-PGD_2 (85.3 ± 10.3%) and did not alter changes in Rrs. This study indicates that specific antigen inhalation results in the production and release of i-LTC_4 and i-PGD_2 which are recoverable by BAL and that the production of these mediators can be affected by biosynthesis inhibitors.

64.12

THE RELATIVE EFFECTS OF VARIOUS AGONISTS ON ISOLATED HUMAN AIRWAY OF VARYING DIAMETER. Robert N. Gryfe* and E.A. Zorychta*(SPON: J.B. Richardson). McGill University, Montreal, Quebec, Canada H3A 2B4

Cumulative dose-response curves were determined for contractile agonists on isolated human airway obtained from 9 autopsies within 6 hours of death. Relative potencies were calculated by comparing ED_{50} values of different drugs in the trachea (T), main large bronchus (LB), 4-6 mm diameter medium bronchus (MB) and 1-2 mm diameter small bronchus (SB).

	Relative Potency			
Drug	T	LB	MB	SB
Acetylcholine	1.0	1.0	0.82	1.4
Histamine	0.29	1.2	1.0	2.8
Phenylephrine	0.26	0.05	0	0
Serotonin	0.19	0	0	0
Leukotriene C_4	2500	--	6300	1600
Leukotriene D_4	2100	--	2300	1600

Serotonin increased the cholinergic excitatory response to electrical field stimulation in 69% of tissues tested. The rate of response to leukotrienes, assessed by comparing time to 1/2 maximal contraction, was inversely related to airway diameter.

	Relative Response Rate (0.1 uM)		
Drug	T	MB	SB
Leukotriene C_4	1.0	0.87	0.54
Leukotriene D_4	1.2	0.68	0.51

64.13

ANTAGONISTIC ACTION OF AMILORIDE ON THE MUSCARINIC RECEPTOR OF RAT TRACHEA AND BRAIN. Guido E. Santacana* and Walter Silva. Depts. of Physiology and Pharmacology, Univ. Central del Caribe, Sch. of Medicine, Cayey, P.R. 00634

Amiloride (A) is a well known potassium sparing diuretic. The effects of (A) at the cellular level include blockade of the Na^+/H^+ exchange in several tissues and inhibition of passive sodium flux in epithelia. More recently, antagonistic actions of (A) at the muscarinic receptor level have been describe in rabbit pancreas. Results from our laboratory show that (A) (10^{-4}M-10^{-3}M) non-competitively inhibits acetylcholine(Ach) induced rat tracheal contractions (Ki=478μM). This effect is observed also in a Na^+ free choline medium. Thus, indicating that it is not dependent on Na^+ transport processes inhibited by (A). Moreover, binding studies in rat brain vesicles in which QNB displacement has been studied in the presence of (A) have demonstrated that (A) produces significant displacement of QNB (Ki=12.3μM).

These results indicate that (A) non-competitively inhibits the muscarinic receptor of rat trachea and brain in a dose dependent manner. This effect of (A) is receptor related and additional to its effect in cell membrane Na^+ transport.

64.14

EVIDENCE FOR AN EPITHELIAL DERIVED RELAXANT FACTOR IN SHEEP. Jolanta Jackowski*, Ken Tomioka*, Andrew T. Mariassy*, and William M. Abraham. University of Miami at Mount Sinai Medical Center, Miami Beach, Florida 33140.

We tested whether sheep airway epithelium releases a relaxant factor (EpDRF) by determining if: a) epithelial removal affects tracheal smooth muscle (TSM) contractile responses; b) indomethacin influences this result and; c) the EpDRF could be bioassayed. Sheep TSM strips (n=7 for all protocols) were hung under 2g tension in organ baths containing Krebs-Henseleit solution (39°C) equilibrated with 95% O_2 and 5% CO_2. For studies a and b, TSM strips with (ep+) and without (ep-) epithelium were studied separately, while for the bioassay (study c) two TSM strips (ep+/ep-; ep+/ep+; ep-/ep-) were hung in one organ bath. Isometric force generated in response to increasing concentrations of carbachol (10^{-10} to 10^{-5} M) was measured to determine the concentration required to produce a 50% maximum contraction (ED_{50}). We found that TSM (ep-) was more sensitive than TSM (ep+) (mean ±SE log ED_{50} = -8.0±0.1 vs -7.8±0.1 respectively, P<0.05). Indomethacin (5 μM) did not enhance this difference. When TSM strips were hung together, TSM (ep-/ep-) (log ED_{50} = -7.9±0.2) were more sensitive (p<0.05) than TSM (ep+/ep+) (log ED_{50} = -7.7±0.2). When TSM strips (ep+/ep-) were hung together, however, the increased sensitivity of TSM (ep-) was lost. These findings suggest that sheep airway epithelium produces a non-prostanoid EpDRF. Supp. Dade & FL Lung Assoc.; NIH HL-33897 & NRSA HL-07578.

64.15

MATURATION OF BETA-ADRENORECEPTOR BINDING AND COUPLING IN RABBIT TRACHEAL SMOOTH MUSCLE. Craig M. Schramm and Michael M. Grunstein*. Univ. of Pennsylvania, School of Medicine, Children's Hosp. of Philadelphia, Philadelphia, PA 19104.

To evaluate whether maturational changes in tracheal smooth muscle (TSM) responsiveness to beta-adrenoreceptor (BAR) stimulation are coupled to altered BAR binding and receptor-effector coupling, BAR binding affinity (pKa) and receptor reserve were determined by analysis of the functional antagonism of BAR with muscarinic stimulation. Isometric tension was monitored in TSM isolated from 2-wk old and adult rabbits, and mounted in organ baths containing modified Krebs-Ringer solution aerated with 95% O2 / 5% CO2. Cumulative doses of isoproterenol (ISO: 10^{-9} to 10^{-4} M) were administered during contraction of each TSM with varying concentrations of methacholine. The tissues' pKa and receptor reserve values were then determined by comparing equipotent relaxant responses to ISO, as per Furchgott's technique. Values for pKa, maximum relaxation (Rmax), sensitivity (negative log concentration associated with 50% Rmax: pC50) obtained during half-maximal cholinergic stimulation, and receptor reserve (Rres=pC50-pKa) are shown below.

	Rmax	pC50	pKA	pC50-pKA
2 week (9)	90.7±2.6	7.26±0.25	5.89±0.13	1.31±0.26
Adult (8)	54.5±8.4	6.02±0.14	5.43±0.15	0.65±0.23
	p<0.0005	p<0.005	p<0.05	p<0.05

These data demonstrate that: 1) TSM maximal responsiveness and sensitivity to ISO significantly decrease with age; and 2) the latter are associated with significant diminutions in functional BAR affinity and receptor reserve. Further, the decrease in receptor reserve in adult TSM implies a reduced efficacy of BAR coupling with age.

64.16

MECHANISM OF LTB_4-INDUCED AIRWAY OBSTRUCTION IN THE GUINEA PIG. Steven A. Silbaugh, Peter W. Stengel, Sandra L. Cockerham*, Barbara E. Mallett* and D. Mark Gapinski*. Eli Lilly and Company, Indianapolis, IN 46285

Although leukotriene B_4 (LTB_4) is recognized as a potent chemotactic agent, less is known concerning its airway constrictive effects. Male Hartley guinea pigs were given intravenous LTB_4 or other challenge agents, killed 1.0 min later, and the severity of airway obstruction evaluated by measurement of pulmonary gas trapping. LTB_4 produced dose-related increases in excised lung gas volumes (ELGV) that were highly correlated with dynamic compliance values at the time of death (r=-0.883, p<0.001). Maximum ELGV increases of 219±26% occurred at 3.0 μg/kg. On a molar basis, LTB_4 was approximately 12 times more potent than histamine and 5 times less potent than LTD_4 in producing elevated gas trapping. ELGV values did not increase with 6-trans LTB_4 or 5S,12S DiHETE (all trans) stereoisomers, but 20-OH-LTB_4 was nearly as potent as LTB_4. Indomethacin or atropine pretreatment prevented LTB_4-induced ELGV increases. The LTB_4 antagonist LY203057* inhibited both LTB_4 and 20-OH-LTB_4 effects, but not histamine or LTD_4 effects. These results suggest that LTB_4-induced airway obstruction 1) is a stereospecific response mediated via LTB_4 receptor activation, 2) involves the release of contractile cyclooxygenase products and 3) requires intact cholinergic pathways. *5-(3-Carboxybenzoyl)-2-((6-(4-methylsulfinyl)-phenyl)-5-hexenyl)oxy) benzenepropanoic acid.

64.17

ACIVICIN (AT-125) PREVENTS BIOTRANSFORMATION OF LEUKOTRIENE (LT)C_4 TO LTD_4 BY GUINEA PIG TRACHEA. W Maguire*, N Gerard*, E Israel* and J Drazen. Harvard Med. Sch., Boston, MA 02215.

Bioconversion of LTC_4 to LTD_4 by target tissues can complicate interpretation of physiological data obtained using LTC_4 as an agonist. Although serine-borate complex has been shown to block this biotransformation, the availability of additional agents with a similar action would provide an alternative method to differentiate the effects of LTC_4 and LTD_4. We examined the effects of AT-125, a gamma-glutamyltranspeptidase inhibitor, on guinea pig tracheal spirals. Tissues were suspended in oxygenated Tyrode's solution at 37°C and incubated with 1mM AT-125 for periods of 0-3.5 hrs. Then 10nM LTC_4 containing trace amounts of ^{3}H-LTC_4 was added to the baths for 40 minutes. LTC_4 and LTD_4 were separated by RP-HPLC and quantitated by scintillation counting. Without AT-125 pretreatment, 32% of the ^{3}H-LTC_4 was converted to LTD_4. Pretreatment with AT-125 for 0.5, 1 and 3.5 hrs. reduced this conversion by 0%, 31% and 84%, respectively. Another group of animals was treated with AT-125 in vivo (30mg/kg, intraperitoneally) one day prior to study. In this group, bioconversion of LTC_4 to LTD_4 was reduced by 62%, 66% and 75% following additional treatment with AT-125 in vitro for 0, 0.5 and 1 hr., respectively. These data demonstrate that AT-125 can reduce LTC_4 to LTD_4 conversion by guinea pig trachea.

64.18

VAGAL INNERVATION OF THE GUINEA PIG RIGHT BRONCHUS. B.J. Undem*, D.Weinreich, H. Barthlow* and A. Meyers*, Johns Hopkins University and University of Maryland School of Medicine, Baltimore, MD 21239.

The right main stem bronchus was isolated with the right vagus nerve intact, and the preparation was superfused with buffer solution at 37°C. Parasympathetic ganglia within the right bronchus were consistently identified without staining. Vagal stimulation caudal to the recurrent laryngeal nerve with a single square pulse of subthreshold intensity evoked multiple EPSPs in the ganglion cell. Increasing the intensity consistently evoked action potentials (50-70 mV, n = 10). These events were abolished by hexamethonium. Stimulation of the vagus nerve immediately distal to the nodose ganglion with 10 sec trains of pulses resulted in a biphasic contraction of the bronchus, consisting of a rapid and short-lived atropine sensitive phase and a prolonged capsaicin-sensitive phase. The rapid cholinergic contraction was abolished by hexamethonium. The frequency-dependence of the two types of contractions was similar. However, longer pulse durations were required to elicit the noncholinergic contractions. The 1/2 maximal pulse durations for the noncholinergic and cholinergic contractions were 0.32 ± .09 msec and .07 ±.01 msec, respectively ($p < 0.01$, n = 9). At maximal stimulation the ratio of the amplitude of the noncholinergic contraction to that of the cholinergic contraction was 0.41 ± .08. This ratio was positively correlated with that obtained with field stimulation (slope = .9, $r^2 = .6$, $p < 0.01$, n = 17). These data describe a single preparation in which the physiology and pharmacology of the bronchial ganglion, as well as synapse-dependent cholinergic and synapse-independent noncholinergic contractions can be investigated.

PULMONARY CIRCULATION I

65.1

THE COMPARATIVE EFFECT OF NIFEDIPINE ON RABBIT PULMONARY VEINS CONTRACTED BY NPY, PHENYLEPHRINE AND 40mM KCl. R. H. Kinsey*, S. F. Gugino* and J. A. Russell. State University of New York at Buffalo, Buffalo, NY 14214

Contractions of pulmonary vein rings isolated from rabbit lungs were studied using in vitro tissue bath techniques. Rings contracted by exposure to 40mM KCl in 2.5mM Ca^{++} containing Krebs solution showed a concentration-dependent decrease in tension when exposed to increasing cumulative concentrations of Nifedipine. Tissues pretreated with Nifedipine ($5x10^{-7}$-$5x10^{-5}$ M) in the presence of 2.5mM Ca^{++} exhibited concentration-dependent depressions of the cumulative concentration response curves for both Phenylephrine (PE) and porcine Neuropeptide Y (NPY). The EC_{50}'s for Nifedipine in the presence of 40mM KCl, $3x10^{-6}$ M PE and 10^{-7} M NPY were $7.6x10^{-9}$ M, $1.2x10^{-5}$ M, and $1.5x10^{-5}$ M respectively. The control contractions for the three agonists at the above concentrations were not significantly different. Incubation of tissues in Ca^{++} free Krebs for 15 minutes caused 84±5%, 58±5%, and 30±10% reductions of contractions induced by 40mM KCl, 10^{-7} M NPY, and $3x10^{-6}$ M PE, respectively. In tissues incubated in Ca^{++} free Krebs plus $5x10^{-5}$ M Nifedipine, the response to 40mM KCl was abolished, whereas contractions induced by 10^{-7} M NPY and $3x10^{-6}$ M PE were reduced by 82±8% and 67±5%, respectively. We conclude that the order of dependence on extracellular Ca^{++} for contraction appears to be KCl>NPY>PE. (Supported by HL 34323 from NHLBI.)

65.2

CIRCULATING AGENT CAUSES PULMONARY VASOCONSTRICTION AFTER INTRACISTERNAL VERATRINE ADMINISTRATION IN THE SPLENECTOMIZED DOG. M.B. Maron. Dept. of Physiology, NE Ohio Univ. College of Medicine, Rootstown, Ohio 44272

The administration of veratrine intracisternally to the chloralose-anesthetized dog produces systemic and pulmonary hypertension and neurogenic pulmonary edema. To determine if pulmonary vasoconstriction, mediated by a circulating agent, contributes to the pulmonary hypertension, I isolated the left lower lung lobe (LLL) perfusion of 5 dogs so that the LLL could be perfused at constant flow and outflow pressure with blood pumped from the left pulmonary artery. The LLL was denervated by removing it from the dog. Since hematocrit increases substantially after veratrine administration, the spleen was removed to eliminate any effect of increased blood viscosity. Veratrine (120 ug/kg) increased arterial pressure to 218 ± 19(SE) torr ($p < 0.005$) and LLL vascular resistance (LLLVR) by 50.2 ± 2.5% ($p < 0.01$). LLL arterial and venous resistances (partitioned by the double occlusion technique) rose, respectively, 33.9 ± 9.8 ($p < 0.05$) and 65.3 ± 11.6% ($p < 0.01$). The lobar response was similar to that which we previously observed during the infusion of exogenous catecholamines (JAP 49:73, 1980). Since plasma catecholamine concentrations are elevated after veratrine administration (Fed Proc 45:284, 1986), these substances are likely candidates for mediating the LLL vasoconstriction. HL-31070

65.3

ANTIGEN-INDUCED CONTRACTION OF GUINEA PIG PULMONARY ARTERIES. L.J. Kelly*, B.J. Undem* and G.K. Adams, III. The Johns Hopkins Medical Institutions, Baltimore, MD 21239.

The purpose of this study was to characterize the kinetics of and determine the mediators responsible for antigen-induced vasoconstriction in pulmonary arteries (Pa). We isolated the main Pa, which was divided into proximal and distal halves, as well as the left and right branches from actively sensitized guinea pigs. Ovalbumin (10^{-2} mg/ml) caused greater contraction in the branches than in either half of the main Pa (60 ± 7% of $BaCl_2$ maximum, n = 10 vs. 35 ± 6%, n = 15). The kinetics of the contraction were similar in all segments. The peak of the response was reached by 2 min and then decayed by 50% at 4 min, 6 min, and 5 min in the proximal Pa, distal Pa, and branches, respectively. Pyrilamine (10^{-6}M) delayed the onset of contraction and decreased the peak of the response by more than 50% in all segments. Metiamide(10^{-4} M) partially reversed this effect. The addition of indomethacin (10^{-6}M) to the combination of pyrilamine and metiamide had no significant effect. However, the further addition of the LTD_4/LTE_4 receptor antagonist SKF 104353 (10^{-5}M) essentially abolished the contraction in the branches and distal Pa and reduced the contraction in the proximal Pa by 80%. These results suggest that, like the isolated guinea pig trachea, histamine and leukotriene receptor activation is predominantly responsible for antigen-induced contraction of pulmonary arteries. However, the kinetic characteristics of the response are different from the trachea in that the contraction is not maintained but returns more rapidly to baseline.

65.4

CHANGES IN ALVEOLAR LYSOPHOSPHATIDYLCHOLINE (LPC) AND EXTRA-VASCULAR LUNG WATER AFTER ISCHEMIA/REPERFUSION (I/R). B.D. Butler, I. Davies and R.E. Drake, Dept. of Anesthesiology, Univ. of Texas Medical School, Houston, TX 77030

Bronchoalveolar lavages were collected from 6 anesthetized dogs following 5 hours of left lower lobar (LLL) pulmonary ischemia/1 hour reperfusion for phospholipid analysis using 2-dimensional thin layer chromatography and phosphorous analysis. The contralateral right pulmonary lobes were used for controls. In a second group of 5 dogs the extravascular lung water ratio (EVLW) was measured in the left and right lobes following I/R. The percentages of LPC to total phospholipid comparing the lavages from the right lung to the ischemic LLL increased significantly ($p < 0.05$) from 0.018± .003 to 0.029±.004. Increases in EVLW (3.23±.81 to 5.00±.30, $p < 0.05$) following I/R indicated some edema formation. No significant changes were observed in pulmonary artery or left atrial pressures. Exogenous administration of LPC (20 mM) into the LLL airways of 5 normal dogs resulted in EVLW ratios of 3.79±0.63 in the right lungs and 6.77±1.03 ($p < 0.05$) for the left lungs. LPC is derived from phosphatidylcholine by hydrolysis with phospholipase A_2. Its membrane perturbing characteristics may alter permeability by increasing transport pathways or disruption of component lipid structure. Pulmonary edema formation following I/R may therefore be the result of interacting mechanisms that include the toxic effects of LPC on membrane function.

65.5

FIRST PASS UPTAKE OF SUFENTANYL (Suf) AND ALFENTANYL (Alf) IN THE HUMAN LUNG. D.L. Roerig, K. Kotrly*, S.B. Ahlf*, C.A. Dawson and J.P. Kampine. Depts. of Anesthesiology, Pharmacology/ Toxocology and Physiology, Med. College of Wisconsin and VA Med. Ctr., Milwaukee, WI 53295

The first pass uptake of i.v. administered Suf and Alf were studied in patients undergoing anesthesia prior to surgery using double indicator dilution with indocyanine green dye (ICG). A 2 ml bolus of 10 mg ICG and 25 ug of Suf or 250 ug of Alf was injected via a central venous pressure catheter and 1 ml blood samples (1/sec) withdrawn via a pump from the radial artery for 45 sec. Blood conc. of ICG and Suf or Alf were determined from absorbance at 805 nm and by specific radioimmunoassays respectively. Difference in the area under the normalized conc. vs. time curves for ICG and Suf or Alf are proportional to the fraction of drug taken up by the lung. First pass uptake of Suf was significant with 60.2+3.1% of the injected dose remaining in the lung after 95% of the ICG had passed through the lung. This is compared to 82.6+1.4% uptake for fentanyl determined in previous studies. This results in twice as much of the injected dose of Suf entering the systemic circulation immediately after injection compared to fentanyl. With Alf, preliminary data indicate a first pass pulmonary accumulation of only 9.7+.3% of the injected dose. The lower first pass uptake Suf and the very small pulmonary uptake of Alf in comparison to fentanyl may contribute to some of their pharmacological differences such as time to onset of action and incidence of cardiovascular effects.

65.6

NEUROPEPTIDE Y INHIBITS ADENYLATE CYCLASE IN RABBIT PULMONARY VEIN. James A. Russell, T. L. Moore* and E. C. Giese*. State University of New York at Buffalo, NY 14214

The effect of neuropeptide Y (NPY) on adenylate cyclase activity in isolated pulmonary vein rings was investigated using in vitro tissue bath techniques. The effect of NPY on relaxations induced by various agents that utilize the adenylate cyclase pathway was determined in control versus test veins. Active tone in control pulmonary veins was elicited by addition of U-46619, a putative thromboxane A_2 receptor agonist, to the bathing solution. Test veins were contracted by a combination of a lower concentration of U-46619 plus 3 x 10^{-8}M NPY, a pulmonary venoconstrictor itself. For a given relaxing agent, contractions induced in control versus test veins were not significantly different. Isoproterenol (10^{-7} M), PGI_2 (3 x 10^{-7}M), and forskolin (3 x 10^{-6}M) relaxed control veins by 77 ± 4%, 66 ± 9%, and 84 ± 3%, respectively and test veins by 35 ± 6%, 36 ± 4%, and 26 ± 3%, respectively. Thus, the presence of 3 x 10^{-8}M NPY significantly reduced the relaxation response to all three of these agents. In contrast, 3 x 10^{-8}M NPY had no effect on relaxations induced by 8-bromo-cAMP (10^{-5}-10^{-2}M). We conclude that NPY inhibits adenylate cyclase activity in the smooth muscle of rabbit pulmonary veins. (Supported in part by HL 34323 from the Heart, Lung, and Blood Institute of NIH).

65.7

THE EFFECT OF HISTAMINE (HIS) ON THE CANINE PULMONARY CIRCULATION UNDER CONTROL AND ELEVATED TONE CONDITIONS. S.A. Barman* and A.E. Taylor, Dept. of Physiology, University of South Alabama, Mobile, AL 36688.

The canine pulmonary circulation was divided into large (R_{LA}) and small (R_{SA}) artery and large (R_{LV}) and small (R_{SV}) vein resistances using arterial, venous, and double occlusions in isolated lungs perfused under constant pressure (CP) or constant flow (CF). Under CP (n=5) or CF (n=5), HIS (3mg) greatly increased the venous resistance. When vascular tone (CON-T) was elevated by the thromboxane analog U46619, histamine's effect (HIS-T) on venous resistance increases under CP and CF was attenuated, while under CF, small artery resistance decreased (see Table) which shows that elevated tone alters HIS responses to pulmonary (especially postcapillary) vascular resistance (supported by NIH HL22549).

	Segmental Resistances (cmH_2O/l/min/100g)				
CP	R_{LA}	R_{SA}	R_{SV}	R_{LV}	R_T
CON	4.5±0.6	1.8±0.3	2.8±0.4	4.9±0.5	14.0±1.1
HIS	4.8±1.5	3.9±1.4	58.8±13.1	45.3±10.2	112.8±19.3
CON-T	7.7±2.4	8.3±2.0	18.4±2.7	20.2±1.9	54.6±5.4
HIS-T	6.7±1.9	7.7±2.8	25.5±4.6	33.9±8.3	73.8±6.5
CF					
CON	4.7±0.5	2.5±0.3	2.8±0.3	3.4±0.4	13.4±1.1
HIS	4.4±1.4	4.5±1.5	17.0±5.0	22.6±2.2	48.5±3.5
CON-T	5.5±1.4	4.8±1.4	11.3±1.1	19.7±1.3	41.3±3.9
HIS-T	4.4±1.9	2.5±1.4	12.9±3.2	29.2±2.3	49.0±5.6

65.8

PULMONARY PLATELET SEQUESTRATION IS INCREASED IN MONOCROTALINE PYRROLE-TREATED RATS. Susan M. White* and Robert A. Roth. Dept. of Pharmacology/Toxicology, Michigan State Univ., East Lansing, MI 48824.

^{111}In-labeled platelets were used to study the localization and survival of circulating platelets at various times after a single, intravenous administration of 3.5 mg/kg monocrotaline pyrrole (MCTP) to rats. Lung injury, assessed from elevated lung weight and from lavage fluid total protein and albumin concentrations and lactate dehydrogenase activity, was evident at Days 8 and 14. In addition, right ventricular hypertrophy was manifested by 14 days after MCTP administration. Pulmonary sequestration of ^{111}In-labeled platelets was also elevated by Days 8 and 14, while circulating blood platelet number remained unchanged. Concomitantly, the hemoglobin concentration and total hemoglobin content of the lung homogenate supernatant in MCTP-treated rats on these days was decreased when compared to controls. A decrease in splenic platelet sequestration on day 14 was accompanied by an increase in the radioactivity of both the heart and kidneys. Platelet half-life and mean life span were increased only on Day 14. A higher dose of MCTP (35 mg/kg) caused moderate lung injury at 6 hr. However, this treatment did not result in increased platelet sequestration in the lungs, although a trend was observed. Data from this study support the hypothesis that platelets are involved in the development of the pulmonary hypertensive response following MCTP-induced lung injury. (Supported by NIH grant ES02581.)

65.9

EFFECTS OF HISTAMINE H1 AND H2 BLOCKADE ON HYPOXIC VASOCONSTRICTION IN ISOLATED LAMB LUNGS. J.B. Gordon and T. Hakim, Montreal Children's Hosp, McGill Univ., Mtl., Que.,

Histamine infusion causes pulmonary vasodilation in newborn lambs. This study examined the effects of H1 (chlorpheniramine 2mg/kg) and H2 (cimetidine 100ug/ml perfusate) blockade on hypoxic vasoconstriction (HPV) in isolated, blood perfused lungs of newborn lambs. The distribution of resistances during hypoxia were also assessed by inflow-outflow occlusion which partitions the pulmonary circuit into non-compliant up- (Δ Pa) and downstream (Δ Pv) gradients and a compliant middle gradient (Δ Pm). Results are mean ± SE (mmHg), groups compared by ANOVA. Hypoxia caused an increase in the total pressure gradient (Δ Pt) of all groups, however the gradient in H1 blocked lungs was greater than controls. Occlusion showed:

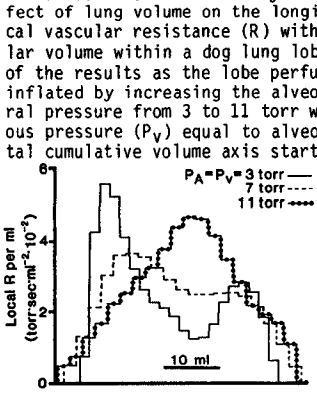
	(n)	ΔPa	ΔPm	ΔPv
control	(5)	6.8±2.3	7.0±4.4	2.8±0.5
H1 block	(5)	12.2±5.5	18.6±1.5*	1.8±0.2
H2 block	(6)	9.5±2.2	8.5±1.1	4.3±0.3**

H1 blockade accentuated HPV primarily by increasing the gradient accross ΔPm (*p<.05). H2 blockade did not significantly increase ΔPt; however it did cause an increase in ΔPv (**p <.05). These results suggest that endogenous histamine modulates HPV in isolated lungs of newborn lambs and H1 and H2 receptors reside in different segments of the pulmonary circuit. Supported by the Quebec Heart Foundation and the M.C.H - McGill Res. Inst.

65.10

EFFECT OF LUNG VOLUME ON THE DISTRIBUTION OF PULMONARY VASCULAR RESISTANCE. C.A. Dawson, D.A. Rickaby, T.A. Bronikowski, and J.H. Linehan. Medical College of Wisconsin, Milwaukee, WI 53226; Zablocki VA Medical Center, Milwaukee, WI 53295; and Marquette University, Milwaukee, WI 53233.

We used the low viscosity bolus method to evaluate the effect of lung volume on the longitudinal distribution of local vascular resistance (R) with respect to cumulative vascular volume within a dog lung lobe. The figure is an example of the results as the lobe perfused with constant flow, was inflated by increasing the alveolar pressure (P_A) minus pleural pressure from 3 to 11 torr while maintaining lobar venous pressure (P_V) equal to alveolar pressure. The horizontal cumulative volume axis starts at the lobar artery on the left and ends at the lobar vein on the right. Inflation in this manner resulted in an increase in total blood volume and a marked change in the R distribution from a bimodal distribution at low lung volume to a unimodal distribution at high lung volume. Supported by Grant HL-19298 and the Veterans Administration.



65.11

OSCILLATORY BEHAVIOR IN ISOLATED SMALL PULMONARY ARTERIES FROM THE CAT. Jane A. Madden. V.A. Medical Center & The Medical College of Wisconsin. 53295.

Small blood vessels, particularly precapillary resistance vessels, from many animal species exhibit oscillatory changes in tension and diameter. We have noted that, under certain experimental conditions, 250-350 μm diameter cat pulmonary arteries will oscillate. Arteries were dissected from cat lungs and mounted on muscle myographs. Eight hundred mg of tension were then applied which increased the resting diameter an average of 2.4 times. The vessels were equilibrated in physiological saline solution (PSS) at PO_2 140 torr, PCO_2 37 torr, and pH 7.37 for at least 1 hr. In 19 arteries which oscillated, 11 began after exposure to $PO_2 < 50$ torr and 6 began spontaneously during the equilibration phase. Characteristics of the oscillatory behavior are a beginning baseline tension of 427 ± 73.5 mg, a peak contraction of 678.9 ± 133.3 mg, and a peak-to-peak period of 21.2 ± 3.5 min. The oscillations could be sustained for as long as 24 hours. These oscillations differ from those reported in other arterial beds in several respects. The magnitude of the contractions is greater and the period is much longer. Additionally, these pulmonary arteries are larger than those which usually exhibit oscillations. Various experimental interventions were tried on the arteries in an effort to stop the oscillations and restore a quiet baseline. Those interventions which attenuated or stopped the oscillations included dropping the temperature to 28-31°C, 0 mM Ca, verapamil, ouabain, or 30 mM KCl. Except for those arteries treated with ouabain, the oscillations resumed after stopping the intervention. Vasodilators such as nitroprusside and acetylcholine had no effect. The magnitude and/or the period could be increased by TEA, and further exposure to hypoxia. The physiological mechanisms and relevance of oscillatory behavior, particularly in the pulmonary arteries, are not fully understood, but may result from both mechanical and humoral factors. Supported by VA Medical Research Funds.

65.12

IBUPROFEN ALTERS THE RESPONSE OF ISOLATED SMALL PULMONARY ARTERIES TO PROSTAGLANDIN F2 ALPHA. Karen J. Wendelberger & Jane A. Madden*. VA Medical Center & Medical College of WI, Milwaukee, WI 53295

Isolated small pulmonary arteries respond to prostaglandin F_2 alpha (PGF_{2a}), and indomethacin appears to blunt this response under hypoxic conditions. Ring segments 2 mm long were cut from small pulmonary arteries (200 - 300 μm diameter) which were harvested from adult mongrel cats. These arteries and threaded with two 22 μm tungsten wires. The wires were fastened over the jaws of open stainless steel rings. One ring was anchored and the other attached to a sensitive load cell (Kulite Semiconductor, Ridgefield, NJ). Under control conditions the vessels were suffused with physiologic saline solution (PSS) in a 100 ml reservoir bath aerated with a gas mixture giving a PO_2 of 140 Torr, PCO_2 of 35 Torr, pH of 7.35 to 7.45, and temperature of 35 to 36°C. Vessels were placed under a standard passive load of 700 to 800 mg and allowed to equilibrate for 60 to 90 min. Solutions of PGF_{2a} were prepared in PSS and cumulative dose response curves were performed under control conditions in the presence of 10^{-4} M ibuprofen. The bath solution was replaced with fresh PSS, ibuprofen added, and the dose response curve for PGF_{2a} repeated under hypoxic conditions ($PO_2 < 50$ Torr). The maximum response to PGF_{2a} occurred at $3x10^{-5}$ M under both control and hypoxic condtions. Under hypoxic conditions the constrictor response to PGF_{2a} appears blunted at lower concentrations. The ED_{50} for control conditions was 10^{-9} M and for hypoxic conditions 10^{-7} M. Although the effects of indomethacin and ibuprofen have been different in other preparations, these differences are not readily apparent in our preparation.
Supported by VA Research Funds and The Medical College of Wisconsin.

65.13

CARBON MONOXIDE UPTAKE AND RELEASE BY WHOLE BLOOD (WB) ARE NOT DESCRIBED BY A FIRST LAW CONDUCTANCE, θ(CO). Edwin Heidelberger* and Robert Blake Reeves. SUNY Schl. of Med., Dept. of Physiol., Buffalo, NY, 14214

Can a First Law conductance, θ(CO), be used to describe WB CO uptake and release? We present here new measurements of the time course of CO uptake and release (P_{CO}: 0 - 6.6 torr) obtained isocapnically (6% CO_2) at 37oC on 14 thin films of WB (thickness: 1.4 ± 0.2 μM, $P_{50}(O_2)$: 26.3 torr) using a naked thin layer technique (Heidelberger and Reeves, Fed. Proc. 46:1427, 1987). O_2 uptake and release (P_{O2}: 0 - 105 torr) kinetics were also measured on each film. Time(msec) required to reach 0.5 S is 7.1 ± 1.7 for O_2 uptake 15.3 ± 5 for O_2 release, 336 ± 18 for CO uptake and 1108 ± 146 for CO release. For each WB film, we calculate both θ(CO) and θ(O_2) as a function of S. $\theta = ([dS/dt]*C)/P_g-P_{eq}$ where C is capacity of WB for gas (ml gas/ml WB), P_g is gas tension external to RBC and P_{eq} is equilibrium partial pressure obtained from measured S and equilibrium binding curve. θ (O_2) vs. S is similar to data reported earlier. θ(CO,uptake) is highest at low S_{CO} (3.4 ml CO/min*torr*ml WB) and falls as S_{CO} increases. θ(CO, release) at S_{CO}= 0.95 is circa 30 ml CO/min*torr*ml WB, rises to a broad maximum at S_{CO}=0.5 (34.4 ml CO/min*torr*ml WB) and gradually falls as S_{CO} decreases. θ is not a property of RBC but a function of experimental conditions. Both θ(CO) and θ(O_2) are strongly saturation and flux direction dependent. There is no true θ for either O_2 or CO. (Supported by NIH grant HL-28542).

72.1

CARDIOTONIC EFFECTS OF A POTENT ADENOSINE ANTAGONIST DURING PENTOBARBITAL-INDUCED HEART FAILURE IN RATS.
L de Garavilla, HL Valentine*, JE Tocker*, KN Jackson* and RC Hanson. NOVA Pharmaceutical Corp., Baltimore, MD 21224

Due to the negative chronotropic and inotropic affects of adenosine (ADO), we postulate that ADO may have detrimental cardiodepressant effects during heart failure (HF). To determine this, we tested two xanthines, NPC 205 (1,3-di-n-propyl-8-(4-hydroxyphenyl) xanthine), a potent ADO antagonist, and theophylline, a weak ADO antagonist, in a model of pentobarbital-induced (PB) HF. Rats were anesthetized with PB (60 mg/kg, ip) and surgically instrumented to monitor left ventricular dP/dt (LV dP/dt), mean arterial blood pressure (MABP) and heart rate (HR). Animals were put into PB-HF using a loading dose of 60-70 mg/kg, iv, of PB and maintained with an i.v. infusion of 0.2 mg/kg/min, resulting in a 50-60% decrease in LV dP/dt. NPC 205 was then administered in a cumulative dose-response manner at 1, 3, 4.5 and 6 mg/kg and percent recovery was calculated. NPC 205 caused a dose dependent recovery in LV dP/dt from 25±5% to 67±13% ($p<.05$) at 1 and 6 mg/kg, respectively. Maximum recoveries in HR and MABP were 53±40% at 3 mg/kg and 36±9% at 6 mg/kg, respectively. Neither of these effects appeared to be dose related. In contrast to NPC 205, theophylline did not improve LV dP/dt or MABP during PB-HF, but did elicit a maximal recovery in HR of 108±20% ($p<.05$) at 50 mg/kg, iv. It is concluded that NPC 205 is a potent cardiotonic agent during drug-induced HF.

72.2

INOTROPIC AGENTS IN EXPERIMENTAL CARDIAC TRANSPLANTATION: LACK OF EFFICACY WITHIN ACUTE REJECTION. Louis Dumont, Anique Ducharme*, Pierre Laflamme*, Eric Dagher*, Christine Lord* and Claude Chartrand*. Département de Pharmacologie, Université de Montréal, Chirurgie Cardiovasculaire, Hôpital Ste-Justine, Montréal, Qc, Canada

Acute or chronic rejection remains a major problem following cardiac transplantation. In this situation, hemodynamic instability requires administration of inotropic agents but cellular dammage may render these interventions ineffective. This hypothesis was tested in a canine cardiac transplantation model. Without immunosuppression, transplanted dogs survive for 8-10 days. Ouabain 20 μg/kg intravenous bolus; amrinone 0.75 mg/kg intravenous bolus + 200 μg/kg/min infusion and calcium 0.05 mEq/kg were studied. All three agents significantly improve cardiac function in the post-op period (40-80%). Two to three days after transplantation the same inotropic challenges still have beneficial but reduce hemodynamic effects. Within acute rejection, 8-10 days post-transplantation all transplants have evidence of myocardial failure which could not be reversed by any of the selected inotropic agents (10-20%). These data suggested that rejection, because of severe myocyte abnormalities such as membrane disruption or intracellular enzyme deficit, complicated the design of effective pharmacological strategies in this critical condition.

Supported by FQMC, FRSQ, ADS and Université de Montréal.

72.3

INDEPENDENCE OF OSCILLATORY AFTERCONTRACTIONS FROM OSCILLATORY AFTERPOTENTIALS IN DIGITALIS TOXICITY. *Tai Akera, Kyosuke Temma and Hiroshi Kondo.* National Children's Hospital Medical Research Center, Setagaya-ku, Tokyo 154 and Kitasato University School of Veterinary Medicine and Animal Sciences, Aomori 034, Japan

The hypothesis that oscillatory aftercontractions (OAC) observed with toxic doses of the cardiac glycosides are caused by oscillatory afterpotentials (OAP) was examined using heart muscle preparations isolated from pentobarbital anesthetized dogs. Transmembrane potentials and the force of contraction were simultaneously recorded in Purkinje fibers, atrial muscle or ventricular muscle. In the presence of 0.1 μM timolol, an exposure of Purkinje fibers to 30 nM ouabain caused a modest positive inotropic effect that reached the peak at about 70 min after the drug addition. Approximately 40 min later, OAC was observed coincident with the onset of OAP. In Purkinje fiber preparations, OAP and OAC were seemingly related. In atrial and ventricular muscle preparations, higher concentrations of ouabain (50 and 100 nM, respectively) were required to produce OAC. No OAP was observed at the time when clear OAC developed. OAC and OAP in Purkinje fibers and OAC in atrial or ventricular muscle preparations were eliminated by ryanodine. These results indicate that the oscillatory Ca^{2+} release from the sarcoplasmic reticulum directly causes OAC, and that OAP is not the necessary condition for the development of OAC.

72.4

SELECTIVE BIOCHEMICAL ACTION OF CK-2130 IN DOG MYOCARDIUM. Elinor H. Cantor, David C. Pang, Debra Natyzak*, Michael A. Walega*, and William R. Ingebretsen. Berlex Laboratories, Inc., Cedar Knolls, NJ 07927

CK-2130 (4-ethyl-1,3-dihydro-5-[4-(2-methyl-1H-imidazol-1-yl)benzoyl]-2H-imidazol-2-one) is a selective cardiotonic agent. It increases left ventricular dp/dt in anesthetized dogs with no significant change in heart rate or blood pressure. This inotropic activity may derive from a variety of biochemical effects. To investigate the mechanism of action of CK-2130 in dog heart, we studied it in several systems that have been shown to be affected by various cardiotonic agents. CK-2130 inhibited canine cardiac particulate cAMP phosphodiesterase (PDE, IC_{50} = 64 ± 15 μM, mean ± SEM, n=6), but was less potent than milrinone (IC_{50} = 3 ± 1 μM, n=12) or 3-isobutyl-1-methylxanthine (IC_{50} = 17 ± 4 μM, n=12). CK-2130 had no effect on the following (highest concentration tested shown in parentheses): adenylate cyclase activity (100 μM), [^{3}H]dihydroalprenolol binding to β-adrenergic receptors (1 mM), [^{3}H]quinuclidinyl benzylate binding to muscarinic receptors (100 μM), sodium/potassium-, calcium-, and myofibrillar-ATPase activity (100 μM), mitochondrial respiration (100 μM), or sodium-calcium exchange (100 μM). Thus, CK-2130 is a selective inhibitor of cAMP PDE. Its relatively low potency, however, suggests that mechanisms other than those investigated in these studies may also contribute to its positive inotropic effect.

72.5

NEGATIVE CHRONOTROPIC AND VASODILATOR EFFECTS OF SUBSTANCE P IN THE ISOLATED PERFUSED GUINEA-PIG HEART. Donald B. Hoover. East Tennessee State Univ., Johnson City, TN 37614

Substance P (SP)-containing afferent nerves are located in the heart and coronary vasculature of several species. The present study was designed to characterize the pharmacological effects of SP on the heart and coronary blood vessels. Hearts from male Hartley guinea-pigs were perfused at a constant rate with buffer. Bolus injections of SP (2.5 to 100 nmoles) caused a dose-dependent bradycardia that was usually followed by a slight tachycardia. The negative chronotropic effect of SP was blocked by 1 μM atropine and potentiated by 0.5 μM neostigmine. A potent vasodilator effect of SP (ED50 = 0.21± 0.05 pmoles) was observed after elevation of perfusion pressure with 1 μM vasopressin. The vasodilation was not blocked by atropine and reached a maximum at doses well below the threshold for eliciting bradycardia. SP also caused vasodilation in hearts in which perfusion pressure was elevated by using buffer that contained 40 mM KCl. The ED50 was similar to that measured in the presence of vasopressin. Infusion of 25 nmoles SP/min caused desensitization to the vasodilator effect of SP. The results indicate that acetylcholine mediates the bradycardia but not the vasodilation produced by SP. These findings are consistent with speculation that SP may have a role in modulation of coronary blood flow and activity of cardiac parasympathetic neurons. (Supported by NIH grant HL38705)

72.6

DIRECT CARDIAC AND PERIPHERAL HEMODYNAMIC EFFECTS OF INTRACORONARY RS-93522. Moysey M. Povzhitkov,* Kirk E. Kanady* and Gregory T. Bates* (Spon. A.P. Roszkowski). Syntex Research, Palo Alto, CA 94304

The hemodynamic effects of a new Ca^{++} entry blocker 2-[4-(2,3-Dihydroxypropoxy)phenyl]ethyl methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylate (RS-93522) were studied in pentobarbital anesthetized closed-chest dogs (n = 14). Under fluoroscopic control the left main coronary artery and coronary sinus were catheterized for drug administration and blood flow (CBF) determination respectively. Intracoronary (IC) administration of RS-93522 at doses of 0.02-62.5 μg/kg produced a significant dose-related increase in CBF, as measured by thermodilution technique. At the same time significant decreases from baseline occurred in diastolic blood pressure and coronary vascular resistance. A statistically significant drop in systolic blood pressure was observed only after 62.5 μg/kg IC dose of RS-93522. No meaningful changes in heart rate, left ventricular (LV) end diastolic pressure, pulmonary capillary wedge pressure, (dP/dt max)/P, LV relaxation time constant, MVO_2 or lactate extraction occurred at any IC dose of RS-93522. These results suggest that IC administration of RS-93522 in the dog produces marked coronary and peripheral vasodilation with no indications of negative inotropicity.

72.7

IN VITRO STUDIES ON THE INOTROPIC MECHANISM OF CK-2130. J. Wiggins, E. Cantor, A. Smart*, M. Carroll*, R. MacNaul* and T. Argentieri*. Berlex Laboratories, Cedar Knolls, NJ 07927

CK-2130 (4-ethyl-1,3-dihydro-5-[4-(2-methyl-1H-imidazol-1-yl)benzoyl]-2H-imidazol-2-one) increases left ventricular dp/dt in anesthetized dogs with no significant change in heart rate or blood pressure. We investigated the mechanism of this inotropic action in isolated ferret papillary muscle, stimulated at 0.2 Hz. At 35°C, CK-2130 (0.1-30 μM) caused a concentration-dependent increase in isometric force (F) of contraction (ED_{50}=3 μM), with a concomitant increase in df/dt but no significant change in relative rate of relaxation (-df/dt/F) or in time to peak force. Nadolol (100 μM) did not affect this response. The negative inotropic effects of carbachol (100μM), but not tetrodotoxin (2 μM) were increased in the presence of CK-2130. CK-2130 had no significant effect on normal cellular electrophysiology, but in K^+-depolarized (22 mM, Em=-49 mV) tissue, 3 μM CK-2130 increased the rate of rise, amplitude, and duration of the slow response action potential. CK-2130 (3 μM) potentiated the inotropic effect of 1 nM isoproterenol. It inhibited ferret heart particulate phosphodiesterase (PDE, IC_{50}=80 μM), but tissue levels of cAMP were not measurably changed by 100 μM CK-2130. The physiologic data are consistent with an involvement of cAMP not mediated by the β-adrenergic system. However, the lack of effect on cAMP and the discrepancy between inotropic potency and PDE inhibition suggest other mechanisms may also be involved.

72.8

CARDIOVASCULAR RESPONSES TO INTRAVENOUSLY ADMINISTERED KAPPA OPIOID RECEPTOR AGONISTS IN THE RAT. **Anil Gulati* and Hemendra N. Bhargava, Dept. of Pharmacodynamics, Univ. of Ill. at Chicago, Chicago, IL 60612.**

The effects of three kappa opioid receptor agonists namely U-50,488H, bremazocine and tifluadom were studied on blood pressure and heart rate in urethane-anesthetized normal and bilateral adrenal demedullated rats. U-50,488H (0.2 to 0.6 mg/kg, i.v.) produced a dose-dependent bradycardia and hypotension in normal rats, and both of these effects were blocked in bilateral adrenal demedullated rats. Tifluadom (0.1 to 0.4 mg/kg, i.v.) produced an initial arrest of heart beat followed by bradycardia which recovered in about 60 min. Except for a very transient hypotension immediately after drug administration no significant effect on blood pressure was observed. In adrenal demedullated rats, tifluadom induced initial arrest of heart was not affected but the subsequent bradycardia was blocked. Bremazocine (0.2 to 0.6 mg/kg, i.v.) produced a dose-dependent bradycardia, while only 0.4 mg/kg dose produced significant hypotension. Bremazocine effects were long lasting. Bilateral adrenal demedullation did not affect bremazocine induced hypotension, however, the bradycardia was blocked. The differential effects of kappa opioid receptor agonists on blood pressure and heart rate suggests either these agents interact differentially with kappa opioid receptors or the subtypes of kappa opioid receptors exist (Supported by grants from NIDA DA-02598 and Chicago Heart Association).

72.9

COMPARISON OF ASPIRIN (A) TO THE SELECTIVE THROMBOXANE RECEPTOR ANTAGONIST, SULOTROBAN (S), ON LYSIS TIME AND REOCCLUSION RATE FOLLOWING tPA-INDUCED CORONARY THROMBOLYSIS IN THE DOG. R.J. Shebuski* and J.M. Smith*, Jr. (Spon: R.R. Ruffolo, Jr.) Smith Kline & French Labs., King of Prussia, PA, 19406.

The potential benefit of A was compared to S (SK&F 95587, BM 13.177) on thrombolysis time and reocclusion in the anesthetized dog. Thrombotic occlusion of the circumflex coronary artery was produced by electrical injury to the intimal surface of the artery in the presence of a critical stenosis. 45 min after spontaneous occlusion, heparin (300 U/kg, i.v.) was administered followed by either vehicle (V), A (5 mg/kg, i.v.), S (10 mg/kg plus 10 mg/kg/hr, i.v.) or A plus S. tPA (Spec. Act. = 540 IU/μg) was administered to all dogs 15 min later at a dose of 10 μg/kg/min, i.v. Animals treated with V (n=15) lysed at 32 ± 6 min with an acute reocclusion rate of 80%. A-treated animals (n=7) lysed at 26 ± 6 min and reoccluded in 66% of cases. S-treated (n=9) however, lysed at 11 ± 2 min ($p < 0.05$ vs. V) and had a reocclusion rate of only 11% ($p < 0.05$). A plus S-treated (n=8) lysed at 24 ± 3 min and reoccluded at 75%. Thus, S decreased lysis time and prevented acute reocclusion whereas A did not exert this effect in this model. Furthermore, A masked the beneficial effect of S, as expected. Therefore selective thromboxane receptor antagonism with S may be a more effective means to improve thrombolytic therapy.

72.10

ROLE OF PROSTAGLANDIN ENDOPEROXIDES IN THE MEDIATION OF ARACHIDONIC ACID-INDUCED CONTRACTIONS OF RABBIT AORTA. P.J. Pagano*, S.F. Roethel*, W.C. Sessa* and A. Nasjletti. Department of Pharmacology, New York Medical College, Valhalla, NY 10595.

Arachidonic acid (AA) can elicit endothelium-dependent contractions of vascular smooth muscle. This effect is mediated by a metabolite of AA by cyclooxygenase, but the precise nature of the mediator is not known. The purpose of this study was to examine the contribution of prostaglandin endoperoxides (PE) and thromboxane A_2 (TxA_2) to the contractile effect of AA in rings of rabbit thoracic aorta bathed in Krebs buffer. AA (10^{-6}-$5x10^{-5}$M) elicited dose-dependent contractions of aortic rings, which were blocked by cyclooxygenase inhibition with aspirin (500 μM) or PE/TxA_2 receptor antagonism with SQ29,548 (1 μM) or BM13.177 (10 μM). In contrast, inhibition of TxA_2 synthesis with CGS-13080 (10 μM) did not affect the AA-induced contraction. In aortic rings in which cyclooxygenase had been inhibited irreversibly with aspirin and then washed, the ability of AA to elicit vascular contraction was restored upon addition of exogenous cyclooxygenase (ram seminal vesicle microsomes) to the bathing media. Again, this effect of AA was blocked by SQ29,548. These data indicate that AA metabolites by cyclooxygenase, including PE, interact with PE/TxA_2 receptors to mediate the contractile effect of AA in rings of rabbit aorta. This work was supported by NIH Grant HL 36670.

72.11

TOPICAL NICOTINE PROVOKES AN INCREASE IN CUTANEOUS ERYTHROCYTE FLUX. John Tegeris* and Jonathan K. Wilkin. Medical College of Virginia, Richmond, VA 23298-0001.

Many studies describe the profound cutaneous vasoconstriction which occurs during systemic administration of nicotine. This vasoconstrictor property is regarded as an important factor in the deleterious effect of nicotine in peripheral vascular disease. We tested the hypothesis that topically applied (-)-nicotine base (NIC) would produce dose-dependent changes in cutaneous erythrocyte flux by laser Doppler velocimetry. In 7 healthy, non-smoking (NS) and 6 smoking (SM) volunteers, the cutaneous erythrocyte flux values after topical application of 25 ul of 0, 0.3, 1.0, 3.0, 10.0, and 30.0% nicotine in aqueous solution, in quadruplicate, on both volar forearms are presented (means ± SEM):

%NIC	0	0.3	1.0	3.0	10.0	30.0
NS	27±3	38±3	48±4	101±14	225±35	347±41
SM	25±3	35±5	43±7	64±8	195±28	247±28

These differences between nonsmokers and smokers were significant ($p<0.05$) for only 3.0 and 30% concentrations of nicotine, but erythrocyte flux was greater in NS for all concentrations tested. These data indicate a local, dose-dependent cutaneous vasodilator effect of nicotine, which may be reduced in chronic nicotinism. At the dosages tested in this study, no systemic, vasoconstrictor effect of nicotine was detected.

72.12

PHARMACOLOGIC CHARACTERIZATION OF CULTURED CHICK AND FROG MYOCARDIAL β-ADRENERGIC RECEPTORS. J.D. Port*, C.C. DeBellis*, M.R. Bristow* (SPON: D.N. FRANZ). Univ. of Utah, Salt Lake City, UT 84132.

The human β-adrenergic receptor (β-AR) pathway is dramatically altered by heart failure. However, the mechanisms of receptor down-regulation and desensitization are not fully understood. Since human myocardial tissue is not amenable to cell culture, we used primary cultures of chick and frog myocardial cells maintained in serum-free media to determine their usefulness as model systems for studying the influences of catecholamines on the β-AR pathway. Chick cells were obtained from 10 day old embryos and frog cells (Xenopus laevis) from stage 64-66 frogs. β-AR densities were determined using the nonselective antagonist ^{125}I-cyanopindolol (ICYP). The chick cell β-AR modeled for a single low affinity site based on competition curves with ICYP vs betaxolol or bisoprolol, both β1-selective, or ICI-118551 (erythro(±)-1-(7methyl-indan-4-yloxy)-3-isopropylamino-butan-2-ol), which is β2-selective, indicating that β_{avian} is neither β1 or β2 with regard to antagonists. However, in agonist competition curves and in terms of the ability of agonists to stimulate adenylate cyclase (AC), the rank order of potency was isoproterenol (ISO) > epinephrine (EPI) = norepinephrine (NE), or β1 like. In contrast, frog cells modeled for approximately 50/50 β1/β2-AR with affinities very similar to mammalian β-AR subtypes based on ICYP-betaxolol and ICI-118551 competition curves. In agonist competition curves and in terms of their ability to stimulate AC, the overall order of potency was ISO > EPI = NE, similar to that of chick cells. We conclude that both chick and frog myocardial cells in primary culture are useful models for studying the effects of catecholamines on the various components of the β-AR pathway. Supported in part by NIH grant #HL13108.

PULMONARY-RESPIRATORY PHARMACOLOGY

73.1

PHARMACOLOGICAL PROFILE OF SK&F S-106203, A NOVEL LEUKOTRIENE (LT) RECEPTOR ANTAGONIST, IN GUINEA-PIG AIRWAYS. D.W.P. Hay*, R.M. Muccitelli*, L.M. Vickery-Clark*, K.A. Wilson*, L. Bailey*, S.S. Tucker*, L.P. Yodis*, R.D. Eckardt*, J.F. Newton, J.M. Smallheer*, M.E. McCarthy*, J.G. Gleason*, M.A. Wasserman and T.J. Torphy. Smith Kline & French Laboratories, King of Prussia, PA 19406-0939.

In guinea-pig isolated trachea SK&F S-106203 3(S)-[2-(carboxyethyl)thio]-3-[2-(8-phenyloctyl)phenyl]-propanoic acid antagonized LTD_4- and LTE_4-induced contractions (pK_B=7.6 and 7.3, respectively) but had little effect on LTC_4-induced contractions (pK_B=5.5). SK&F S-106203 (10 μM) had no effect on contractions produced by histamine, carbachol, KCl, U-44069, $PGF_{2\alpha}$ or PGD_2. In anesthetized, spontaneously-breathing guinea pigs, when given i.v. (bolus) and i.d., the SK&F S-106203 ID_{50}s for inhibition of the increase in resistance, R_L, produced by aerosolized LTD_4 were 1.1 and 2.2 mg/kg, respectively. By i.v. infusion the steady-state plasma concentration for 50% inhibition of LTD_4-induced increase in R_L was 0.79 μg/ml. Oral (i.g.) pretreatment with 49 mg/kg SK&F S-106203 for up to 24 hr essentially abolished LTD_4-induced bronchospasm; this correlated with well-maintained compound plasma levels. Aerosolized SK&F S-106203 (0.25-4.9 μg/animal) produced dose-related inhibition of LTD_4-induced bronchospasm. The data indicate that SK&F S-106203 is a potent and selective LT receptor antagonist that is active via aerosol, oral and i.v. routes of administration.

73.2

INTRAVENOUS AND ORAL PHARMACOKINETICS OF THE LEUKOTRIENE RECEPTOR ANTAGONISTS, SK&F S-106203 AND SK&F 104353, IN GUINEA PIGS. J.F. Newton*, L.P. Yodis*, C.M. Saverino*, J.G. Gleason*, J.M. Smallheer*, M.E. McCarthy*, M.A. Wasserman, T.J. Torphy and D.W.P. Hay*. Smith Kline & French Laboratories, King of Prussia, PA 19406-0939

The pharmacokinetics of SK&F S-106203 [3(S)-(2-carboxyethylthio)-3-[2-(8-phenyloctyl)phenyl]propanoic acid] and SK&F 104353 [2(S)-hydroxy-3(R)-(2-carboxyethylthio)-3-[2-(8-phenyloctyl)phenyl]propanoic acid] were compared in unanesthetized guinea pigs. Plasma concentrations of both compounds were determined by HPLC following methyl formate extraction. Following an i.v. dose (25 mg/kg), SK&F 104353 disappeared from plasma in a biphasic fashion with half-lives of 0.05 (87 % of the area under the plasma concentration time curve (AUC)) and 0.4 hrs (13 % of the AUC). Following an i.v. dose (25 mg/kg), SK&F S-106203 disappeared from plasma in a biphasic fashion with half-lives of 0.1 (50 % of the AUC) and 11 hrs (50 % of the AUC). The AUCs obtained for SK&F S-106203 and 104353 following i.v. administration were 87 ± 7 and 29 ±1 μg-hr/ml, respectively. Following an oral dose of SK&F 104353 (100 mg/kg), the C_{max} and T_{max} were 2.4 ± 0.9 μg/ml and 0.1 hrs, respectively. The AUC was less than 4 μg-hr/ml. In contrast, following an oral dose of SK&F S-106203 (100 mg/kg), the C_{max} and T_{max} were 21.6 ± 2.3 μg/ml and 4 hrs, respectively. The AUC was 235 ± 33 μg-hr/ml. The oral bioavailability in the guinea pig was estimated to be <3% and >65% for SK&F 104353 and S-106203, respectively. These studies indicate that SK&F S-106203 has a superior pharmacokinetic profile, compared to SK&F 104353, following oral administration to guinea pigs.

73.3

CHARACTERIZATION OF SK&F 104353, A POTENT AND SELECTIVE LEUKOTRIENE (LT) RECEPTOR ANTAGONIST: EFFECTS ON PULMONARY FUNCTION IN GUINEA PIGS. T.J. Torphy, M.A. Wasserman, D. Underwood*, L.M. Vickery-Clark*, L.S. Bailey*, R.R. Osborn*, J.F. Newton*, L.P. Yodis* and D.W.P. Hay*. Smith Kline & French Laboratories, King of Prussia, PA 19406.

The ability of SK&F 104353 [2-hydroxy-3-[(2-carboxyethyl) thio]-3-[2-(8-phenyloctyl)phenyl]-propanoic acid] to antagonize LTD_4-induced bronchoconstriction was examined in anesthetized, spontaneously breathing guinea pigs. Changes in airways resistance (R_L) and dynamic lung compliance (C_{dyn}) were monitored over a period of 15 min following the administration of a standard aerosol LTD_4 challenge that produced a 300-600% increase in R_L and a 50-70% decrease in C_{dyn}. Aerosol administration of SK&F 104353 (0.15-62 ug/animal) inhibited LTD_4-induced bronchoconstriction in a dose-dependent manner. The response to LTD_4 was abolished for at least 2 hrs after the aerosol administration of 62 µg SK&F 104353. Pretreatment (10 min) with i.v. SK&F 104353 also reduced LTD_4-induced bronchoconstriction with an ED_{50}=0.275 mg/kg. SK&F 104353 administered intraduodenally 1 hr before challenge antagonized responses to LTD_4 with an ED_{50}=16 mg/kg. Consistent with its poor oral potency relative to its i.v. potency, SK&F 104353 was found to have an oral bioavailability of less than 3%. SK&F 104353 (10 mg/kg, i.v.) failed to antagonize responses to histamine, methacholine or U-44069. Thus, SK&F 104353 is a potent and selective LT antagonist that is active by inhalant, intravenous and oral routes of administration.

73.4

INHIBITION OF ALLERGIC AND NONALLERGIC MEDIATOR RELEASE BY d- AND l-ISOMERS OF AZELASTINE. R. D. Sofia, N. Chand, J. Pillar*, K. Nolan*, and W. Diamantis. Wallace Laboratories, Div. of Carter-Wallace, Inc., Cranbury, NJ 08512.

Azelastine HCl, an orally effective, long acting anti-asthmatic/antiallergic drug, is a racemic mixture of d- and l-isomers. In the present study, the ability of dl-azelastine and its isomers to influence allergic LTC_4 synthesis in actively sensitized guinea pig chopped lung and A23187(0.2 µM)-stimulated LTC_4 formation in rat mixed peritoneal cells (RMPC) was evaluated and compared to specific 5-lipoxygenase inhibitors (NDGA and AA861).

Drug	IC_{50}, 95% Confidence Limits, µM	
	Guinea Pig Lung (2 hr)	RPMC (10 min)
dl-Azelastine	14 (9.3-20.9)	21.2 (11.2-40.1)
d-Azelastine	13 (5.9-28.9)	9.6 (5.2-17.7)
l-Azelastine	50	22.7 (17.1-30.2)
NDGA	9.1 (6.2-13.3)	0.1 (0.02-0.42)
AA861	1.1 (0.9-1.3)	0.034 (0.013-0.092)

The data summarized above demonstrate the relative inhibitory activity of dl-azelastine and its isomers in allergic and nonallergic LTC_4 synthesis in actively sensitized chopped lung and RMPC.

73.5

PLATELET ACTIVATING FACTOR (PAF-ACETHER) INDUCES HISTAMINE SECRETION FROM RABBIT BASOPHILS: EFFECT OF WEB 2086 AND AZELASTINE. N. Chand, J. Pillar*, K. Nolan*, W. Diamantis and R. D. Sofia. Wallace Laboratories, Div. of Carter-Wallace, Inc., Cranbury, NJ 08512.

PAF-acether has been considered as an important mediator of inflammation and airway hyperreactivity. In the present study, PAF-acether (0.01-10 ng/ml) was found to release histamine from rabbit mixed leukocytes with an approximate EC_{50} of 10 ng/ml. The PAF-antagonist, WEB 2086, exerted a concentration-dependent inhibition of PAF-induced histamine secretion with an IC_{50} of 0.002 µM. These observations suggest the existence of specific PAF-receptors on rabbit leukocytes (basophils). dl-Azelastine HCl (10 min. preincubation) exerted nondose-related (bell shaped) inhibition of PAF-induced histamine secretion with a peak inhibition of 27 ± 7% at 0.5 µM. The d-isomer exerted concentration-dependent inhibition of PAF-induced histamine secretion (IC_{30} = 0.9 µM). The l-isomer exhibited extremely weak activity. The addition of dl-azelastine HCl or DSCG (0.05-5.0 µM) immediately before PAF-acether addition did not inhibit histamine secretion. Ketotifen (0.05-5.0 µM, 0 min) exerted significant inhibition of PAF-acether-induced histamine secretion, but its effect was nondose-related.

73.6

INHIBITION OF PHOSPHODIESTERASE (PDE) ISOZYMES IN GUINEA PIG TRACHAEL SMOOTH MUSCLE (TSM). A. Harris#, P. Silver, A. Wallace*, E. Pagani, E. Danis*, D. Hildreth#, M. Connell#, R. Gordon#; Sterling Winthrop Research Institute, Dept. of Pharmacology, Rensselaer, N. Y.

The purpose of this study was to evaluate the effect of inhibiting the rolipram -(R) and/or CI930 -(C) sensitive PDE III on TSM relaxation (Rx). Inhibition of PDE III by R or C produced a biphasic concentration-response (CR) relationship. However, in the presence of a fixed concentration of either R (100 µM) or C (3 µM), a single-phase sigmoidal CR relationship was observed. These results suggest that TSM PDE III contains both R-PDE III and C-PDE III isozymes. An analogous study was performed using carbachol-contracted TSM. A similar biphasic CR relationship to C or R for TSM Rx was observed; in the presence of a fixed concentration of C or R (3 µM), a single-phase sigmoidal CR for TSM Rx was obtained. The EC_{50} value for TSM Rx by C (1 µM) was similar to the IC_{50} value for C-PDE III (0.3 µM), however the EC_{50} value for R (0.02 µM) was less than the IC_{50} value for R-PDE III (4.5 µM). Other compounds were tested for their ability to inhibit TSM PDE III and relax TSM pretreated with C or R. No significant correlation was observed between R-PDE III inhibition and TSM Rx (R=0.21), however a significant correlation was observed for C-PDE III inhibition and TSM Rx (R=0.95, $p<0.01$). These data suggest that R-induced TSM Rx may be differentially linked to R-PDE III inhibition whereas inhibition of C-PDE III is directly linked to TSM Rx.

73.7

cAMP AND cGMP POTENTIATE THE PHASIC ACTION OF TEA. Elliott W. Chideckel, Divesh R. Anireddy, Nadia El-Ayoubi, and Amrit Singh. WV University, Morgantown, WV. 26506

The isolated guinea-pig trachealis can be made phasic by exposure to tetraethylammonium (TEA). We decided to test the effect of cyclic nucleotides in this model. After TEA (3 mM) had induced phasic activity, the addition of 8-Br-cAMP or 8-BR-cGMP resulted in rapid mechanical oscillations. This potentiation of rhythmic frequency in TEA - treated tissue was also induced by NaN_3, forskolin (water-soluble), isoproterenol and aminophylline, all stimulators of cyclic nucleotide accumulation. Tetrodotoxin, atropine, diphenhydramine, and cimetidine were without effect on the rapid oscillations. KCL at 16 mM caused rapid oscillations in TEA treated tissue and increased the frequency of the oscillations in tissue treated with TEA plus 8-Br-cGMP. Ca^{++}-free solution, low Na^+ solution, diltiazem and ouabain abolished all rhythmic activity. In the absence of TEA, diltiazem and ouabain were without effect on cyclic nucleotide-induced relaxation. We conclude, cyclic nucleotides may, in the presence of some forms of phasic activity, play a pacemaker role by stimulating Ca^{++} exit from the cytoplasm.

73.8

COMPARISON OF REACTIVITY OF INTACT GUINEA-PIG TRACHEA (GPT) IN VITRO TO INTRALUMINAL VS. EXTRALUMINAL BRONCHOACTIVE AGENTS. J.S. Fedan and D.G. Frazer. Physiology Section, Div. Resp. Dis. Stud., NIOSH, Morgantown, WV 26505

The airway epithelium modulates reactivity of GPT via an epithelium-derived relaxing factor (EpiDRF). We sought evidence for agonist-induced release of EpiDRF using an intact tracheal perfusion preparation (Munakata et al., J. Appl. Physiol. 64:466, 1988). Pressor responses to EC_{50} concentrations of EX- and IN-applied methacholine (MCh), histamine (Hist) and isoproterenol (Iso) were compared in unstimulated and KCl(30 mM; EX)-contracted preparations. Regardless of the order of application to the luminal and serosal surfaces of the GPT, no relaxations to IN-MCh or IN-Hist were observed. Contractions to EX-MCh, EX-Hist and relaxations to EX-Iso were larger than those after IN-application, regardless of order of administration. As reported by Munakata et al., IN-KCl evoked relaxation. The epithelium is thus a substantial diffusional and/or metabolic barrier to the action of these agents. The inability of IN-MCh and IN-Hist to induce a relaxation suggests 1) that, in contrast to KCl, EpiDRF is not released by these agonists acting on the apical surface of epithelial cells, 2) that the release of EpiDRF could result from an action of the agonists on the basolateral surface of epithelial cells, or 3) that the release of EpiDRF is continuous and unaffected by these agonists.

73.9

PIRENZEPINE ELIMINATES THE DIFFERENTIAL RELAXATION OF CANINE TRACHEAL SMOOTH MUSCLE ELICITED BY FORSKOLIN. R.W. Mitchell, S.M. Koenig*, E. Kelly*, A.R. Leff and K.J. Popovich*. Sect. of Pulm. and Crit. Care Med. and Comm. Clin. Pharmacol., Div. Biol. Sci, Univ. of Chicago, Chicago, IL 60637

It has been demonstrated in canine tracheal smooth muscle (TSM) that forskolin (FSK) relaxes active tension elicited by potassium chloride (KCl) with greater potency than active tension elicited by acetylcholine (ACh). The role of the M_1-muscarinic receptor in differential relaxation to FSK was studied in 42 strips of cervical TSM from 9 dogs. To determine the maximum concentration of pirenzepine (PIR) that blocked M_1 without antagonizing M_2-muscarinic receptors, concentration response curves to ACh were elicited in the presence of 10^{-8} to 10^{-6}M PIR. 10^{-7}M was the maximum concentration of PIR that caused no change in AT_{max} (active tension elicited by 10^{-5}M ACh) or EC_{50} (concentration of ACh eliciting 50% of AT_{max}) of ACh vs controls: AT_{max} = 2991 ± 202 vs 3015 ± 259 g/cm^2; EC_{50} = 2.8 ± 0.9 vs 1.8 ± 0.5 X 10^{-6}M (P = NS, Unpaired T-test). After pretreatment with 10^{-7}M PIR, strips were contracted to target tension (TT = 50% of active tension elicited by 127 mM KCl-substituted Krebs-Henseleit solution) with ACh and KCl and relaxed with FSK (10^{-9} to 10^{-5}M). The concentrations of FSK eliciting 50% relaxation from TT (RC_{50}) were ACh, 2.54 ± 0.26 X 10^{-7}M > ACh + PIR, 1.00 ± 0.14 X 10^{-7}M ≈ KCl, 1.02 ± 0.16 X 10^{-7}M (P < 0.05 ACh vs ACh + PIR, KCl; P = NS ACh + PIR vs KCl; ANOVA). We demonstrate that in the presence of specific M_1-muscarinic receptor blockade with PIR, FSK relaxes active tension elicited by ACh and KCl equipotently. (Supported by the American Lung Association and the Schweppe Foundation)

73.10

PIRENZEPINE AUGMENTS RELAXATION OF ACETYLCHOLINE-ELICITED CONTRACTION BY ISOPROTERENOL IN CANINE AIRWAY SMOOTH MUSCLE IN VITRO. S.M. Koenig*, R.W. Mitchell, E. Kelly*, A.R. Leff and K.J. Popovich*. Sect. of Pulm. and Crit. Care Med. and Comm. Clin. Pharmacol, Div. Biol. Sci., Univ. of Chicago, Chicago IL 60637

Prior investigations have demonstrated that acetylcholine (ACh) antagonizes the relaxation of canine airway smooth muscle by isoproterenol (ISO). To elucidate the mechanism accounting for this inhibition, cervical and thoracic canine tracheal smooth muscle (TSM) strips and 4th generation bronchial rings were pretreated with 10^{-7}M pirenzepine (PIR), a concentration eliciting specific M_1-muscarinic receptor blockade. TSM strips and bronchial rings were contracted to target tension (TT = 50% of active tension elicited by 127 mM KCl-substituted Krebs-Henseleit solution) with ACh and relaxed with ISO. PIR increased the potency of ISO-elicited relaxation vs controls: RC_{50} (concentration of ISO eliciting 50% relaxation from TT) was 2.3 ± 0.4 x 10^{-8}M vs 9.6 ± 2.3 x 10^{-8}M for cervical TSM and 5.3 ± 1.6 x 10^{-8}M vs 13.2 ± 2.0 x 10^{-8}M for 4th generation bronchi (P < 0.05). RC_{30} (concentration of ISO causing 30% relaxation from TT) for thoracic TSM was 2.5 ± 0.8 x 10^{-7}M vs 13.1 ± 4.4 x 10^{-7}M (P < 0.05). RC_{30} was chosen because ISO was less potent in relaxing thoracic TSM, eliciting a maximum relaxation of 45% of TT for the control group. ISO relaxation of cervical TSM exposed to PIR and contracted to TT with KCl were not different. The data demonstrate that 1) M_1-muscarinic receptors exist in cervical and thoracic TSM and 4th generation bronchi of canine airways; 2) blockade of the M_1-muscarinic receptor with PIR augments relaxation of ACh-elicited contractions by ISO. (Supported by: American Lung Association and Schweppe Foundation.)

73.11

INHALED BRADYKININ CAUSES BRONCHOCONSTRICTION IN ALLERGIC SHEEP. William M. Abraham, Marcus Solèr*, Ashfaq Ahmed*. Mount Sinai Medical Center, Miami Beach, Florida 33140.

Inhaled bradykinin causes bronchoconstriction in asthmatic subjects and this response can be partially blocked by cromolyn sodium pretreatment (ARRD 135:176, 1987). In this study we determined if inhalation of bradykinin causes bronchoconstriction in allergic sheep. In four allergic sheep, ~0.07 μmol (20 breaths of 0.5 μmol/ml solution) bradykinin caused a mean ±SE increase in pulmonary airflow resistance (R_L) of 277±93% over baseline (P<0.05); this bronchoconstriction resolved over 20 min. Bradykinin-induced bronchoconstriction showed no tachyphylaxis when challenges were separated by one day. When these same sheep were pretreated with nedocromil sodium (1 mg/kg in 3 ml buffered saline as an aerosol), the immediate bradykinin-induced increase in R_L was 93±51% above baseline and the response resolved by 15 min. Thus, inhaled bradykinin can cause an acute bronchoconstriction in allergic sheep, at a dose that causes bronchoconstriction in asthmatic patients. Nedocromil sodium partially inhibits this bradykinin-induced bronchoconstriction. Supported by NIH-33897.

73.12

THE EFFECT OF THE PAF-ANTAGONIST WEB-2086 IN ANTIGEN-INDUCED AIRWAY HYPERRESPONSIVENESS. Marcus Solèr and William M. Abraham. Mt. Sinai Medical Center, Miami Beach, FL 33140.

Allergic sheep show increased airway responsiveness (AR) soon after the resolution of the acute response to antigen (~2 h after challenge). To determine if platelet activating factor (PAF) has a role in this increased AR, we examined the effects of the PAF-antagonist, WEB-2086, on the acute response to antigen and on the increase in AR. Dose response curves (DRC) to carbachol were performed by measuring specific lung resistance (SR_L) after inhalation of increasing doses of carbachol and using the slope of the DRC as an index of AR. In 8 sheep AR was measured 1-2 days before (baseline) and 2 h after an inhalation challenge with A. suum antigen (when SR_L had returned to baseline). In two other trials, >2 weeks apart, the sheep received WEB-2086, 1 mg/kg i.v., before antigen challenge or immediately before the post challenge DRC. For the three trials, the acute responses to antigen were not significantly different. Slopes of the DRC were increased (P<0.05) after antigen challenge alone (x̄±SD, 4.9±2.7) and when WEB was given before the post challenge DRC (4.8±1.6) when compared to baseline (2.9±2.1). No significant increase in the slope was seen when WEB was given before antigen challenge (3.8±1.4). We conclude that the early release of PAF during airway anaphylaxis may contribute to subsequent increases in AR. Supp. Swiss Nat'l Science Foundation; NIH-33987 & Boehringer Ingelheim.

METABOLISM RELATIONSHIP TO TOXICOLOGY II

74.1

IDENTIFICATION OF DNA MUTATIONS RESPONSIBLE FOR VARIANT FORMS OF HUMAN SERUM CHOLINESTERASE BY GENE AMPLIFICATION. B. LA DU, M.C.McGuire*, C.P.Nogueira*, H.Lightstone* and O.Lockridge*. Dept. of Pharmacology, University of Michigan, Ann Arbor, MI 48109.

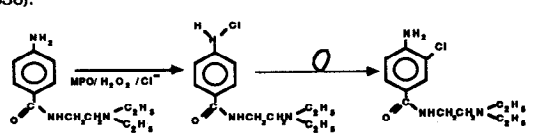
Complete amino acid sequence (Lockridge et al., J. Biol. Chem. 262:549,1987) and cDNA sequence of human serum cholinesterase (McTiernan et al., P.N.A.S.,84:6682,1987), and the location of the 3 introns at positions base -93, base 1433, and base 1600 in our laboratory permits direct DNA sequencing with selected primers after gene amplification of the coded region DNA by the polymerase chain reaction (PCR) technique. DNA isolated from peripheral white blood cells from subjects with known variant forms of serum cholinesterase has been amplified and sequenced for the major exon, which contains about 85% of the entire coded region. In 4 members of 2 unrelated families carrying the atypical (dibucaine-resistant) variant gene, all had a point mutation at position 70 (Aspartate to Glycine), due to one base change at position 209 (GAT→ GGT). One homozygous "silent" woman showed a frame-shift mutation at glycine 117 (GGT→GGAG). Other family members with these alleles, and with the fluoride-resistant variant are now being analyzed.

Further studies on the relationship between variant forms of cholinesterase and the enzyme's stability and catalytic properties will be greatly facilitated by the application of the PCR amplification method. Supported by NIH grant GM-27028.

74.2

PROCAINAMIDE (PA) IS N-CHLORINATED BY MYELOPEROXIDASE (MPO) -IMPLICATIONS FOR PA TOXICITY. Jack Uetrecht and Nasir Zahid*. Faculties of Pharmacy and Medicine, University of Toronto, Toronto, Canada M5S 1A1.

The use of PA is associated with a high incidence of drug-induced lupus. It is also associated with a significant incidence of agranulocytosis. We have proposed that these toxicities are due to a reactive metabolite involving the arylamine group. We demonstrated that reactive hydroxylamine and nitroso metabolites were formed by activated monocytes and neutrophils, apparently due to MPO and H_2O_2. In the presence of Cl^-, MPO is also known to chlorinate substrates and N-chlorination of the arylamine of PA would also lead to a reactive metabolite. We, therefore, sought evidence for the formation of N-chloroPA. PA was incubated with purified MPO, H_2O_2 and Cl^- and formation of products was monitored by HPLC. N-ChloroPA was detected and identified by its spontaneous conversion to o-chloroPA. o-ChloroPA was identified by mass spectroscopy and comparison with synthetic o-chloroPA. The same product was formed when PA was treated with hypochlorite. We propose that the drug-induced lupus and agranulocytosis are due to formation of reactive nitroso and/or N-chloroPA metabolites formed by monocytes and neutrophils respectively. Supported by a grant from the Medical Research Council of Canada (MA9336).



74.3

OXIDATION OF HYDRALAZINE BY MONOCYTES AS A MECHANISM OF HYDRALAZINE INDUCED LUPUS. Angela Hofstra* and Jack Uetrecht. Faculty of Pharmacy, University of Toronto, Toronto, Ontario M5S 1A1.

Therapy with the antihypertensive agent hydralazine is associated with development of a lupus syndrome. The induction of lupus has been linked to increased amounts of the oxidative metabolite of hydralazine, phthalazinone (PO). It has been postulated that reactive intermediates that form PO rather than PO itself are responsible for disease induction. Consistent with this hypothesis hydralazine is oxidized by rat liver microsomes and NADPH to two oxidative metabolites phthalazinone (PO) and phthalazine (PZ). However, reactive intermediates formed during the oxidation of hydralazine would not be expected to live long enough to escape the liver and induce an immune response. We have shown that the myeloperoxidase(MPO)/hydrogen peroxide system in neutrophils and monocytes stimulated to undergo respiratory burst can oxidize drugs with easily oxidizable functional groups to reactive metabolites. Such drugs are associated with idiosyncratic reactions such as lupus or agranulocytosis. When we incubated hydralazine with purified MPO/hydrogen peroxide, two metabolites, PO and PZ, were produced. This reaction was dependent on the amount of enzyme present and the reaction rate was increased in the presence of Cl^- ion. The same two metabolites were produced when we incubated hydralazine with NaOCl. When we incubated hydralazine with stimulated human leukocytes, PO was produced. We speculate that hydralazine oxidation by stimulated monocytes could lead to reactive intermediates that could bind to the surface of the monocyte. Since monocytes are involved in antigen processing and presentation, this binding of reactive intermediates could trigger an immunologic reaction leading to the induction of autoantibodies and a lupus syndrome. Supported by a grant from the Medical Research Council of Canada (MA9336).

74.4

METABOLISM OF ISONIAZID BY ACTIVATED NEUTROPHILS. S.M. Angela Li* and Jack Uetrecht. Faculty of Pharmacy, University of Toronto, Toronto, Ontario M5S 1A1.

Isoniazid (INH) is associated with a high incidence of severe hepatotoxicity. It has been proposed that this is related to the metabolism of INH to reactive intermediates in the liver. INH is also associated with drug-induced lupus. However, these intermediates are unlikely to escape the liver to interact with the leucocytes to elicit an autoimmune response. Recently, we have shown that myeloperoxidase (MPO)/H_2O_2 in activated neutrophils and monocytes can oxidize other lupus-inducing drugs, such as procainamide, to reactive metabolites. When INH was incubated with activated human neutrophils, isonicotinic acid (INA) was formed. INA was also detected with the MPO/H_2O_2 system in vitro. The rate of INA formation was increased by the presence of Cl^-, and was dependent on time, substrate concentration and enzyme concentration. This suggests that reactive metabolites/intermediates are formed in vivo when neutrophils are stimulated to undergo respiratory burst releasing both MPO and H_2O_2. Since INH-specific antibodies towards the isonicotinyl group have been detected in the serum of patients hypersensitive to INH (J. Allergy Clin. Immunol. 1987. 80:582-585), the binding of these reactive metabolites/intermediates to the monocytes likely precedes the development of autoantibodies and a lupus syndrome in INH-treated patients. Supported by a grant from the Medical Research Council of Canada (MA9336).

74.5

DIETHYLDITHIOCARBAMATE (DEDC) DOES NOT ENHANCE OXIDATIVE STRESS CYTOTOXICITY BY INHIBITING SUPEROXIDE DISMUTASE (SOD) V.V.M. Lauriault *, and P.J O'Brien * (SPON: J.P. Uetrecht) University of Toronto, Toronto, Ont., M5S 2S2.

Thiol drugs are widely used to overcome the life threatening effects that may occur following a drug overdose or metal poisoning. DEDC was considered a unique thiol drug in that it inhibits SOD and thereby enhances oxidative stress mediated cytotoxicity eg. erythrocyte haemolysis induced by 1,4-naphthoquinone-2-sulphonate (NQS). We have confirmed that DEDC markedly enhances the cytotoxicity of NQS and 1,4-naphthoquinone (NQ). DEDC, however, protects against oxidative stress mediated cytotoxicity induced by nitrofurantoin, 2,3-dimethoxy-1,4-naphthoquinone and hydrogen peroxide (H_2O_2). In view of the controversy as to whether SOD plays a role in cell function, it is proposed that the enhanced cytotoxicity is probably due to disulfiram (DS) and H_2O_2 formed during the oxidation of DEDC by NQ and NQS. DS rapidly oxidises GSH in hepatocytes. DS cytotoxicity is markedly enhanced by 5-fold if the GSH of the hepatocytes is depleted with DEM. This suggests that GSH reductively detoxifies DS in the hepatocyte. Since no GSH is available to detoxify DS by reduction back to DEDC, DS accumulates and prevents the calcium buffering function of mitochondria thereby increasing cytosolic calcium and causing cytotoxicity. (Supported by National Scientific and Engineering Research Council of Canada.)

74.6

DIFFERENCES IN THE TOXICITY AND METABOLISM OF DIGITOXIN IN RATS AND HAMSTERS. C. Dorian*, M. Halvorson*, R.H. Alper and A. Parkinson. Kansas University Medical Center, Kansas City, KS. 66103

Digitoxin (dt_3) is a highly toxic cardiac glycoside. When administered ip at 10 mg/kg, dt_3 caused 100% lethality in rats, but had no obvious adverse effects on hamsters. Most hamsters survived a dose of 1000 mg dt_3/kg. We have undertaken studies to determine why hamsters are essentially resistant to the toxic effects of dt_3. It has been shown previously that induction of liver microsomal cytochrome P-450 by steroidal agents can protect rats from the toxic effects of digitoxin. Therefore, we determined by HPLC the pathways of dt_3 metabolism catalyzed by liver microsomes from rats and hamsters. Interestingly, rat liver microsomes converted dt_3 primarily to digitoxigenin bisdigitoxoside (dt_2), whereas hamster liver microsomes converted dt_3 primarily to 17α-hydroxy-dt_3. To assess the significance of this difference in digitoxin biotransformation, we attempted to measure the plasma half-life of dt_3 in rats and hamsters. However, this experiment was complicated by the fact that surgical implantation of arterial and venous catheters in hamsters caused a prolonged suppression (7 days) of liver microsomal cytochrome P-450. This suppression of cytochrome P-450 was associated with ~50% decrease in the rate of dt_3 metabolism catalyzed by hamster liver microsomes. The marked and prolonged effect of surgery/catheterization on hamster liver microsomal cytochrome P-450 was an unexpected observation, with important implications for drug metabolism studies. Supported by NIH grant GM-37044.

74.7

THE METABOLISM OF ^{3}H-4,5-DIMETHYLMISONIDAZOLE IN THE ISOLATED PERFUSED HYPOXIC RAT LIVER. Nyla Harper*, Jerry L. Born*, Ali Gargoum*, and Brian R. Smith. The University of New Mexico College of Pharmacy, Albuquerque, NM 87131

Misonidazole (MISO) is a nitroimidazole radiosensitizer which undergoes hypoxia-dependent, reductive biotransformation to form reactive metabolites. The purpose of this study was to determine if methyl substitution on the 4- and 5- positions of the imidazole ring would decrease the biotransformation rate and/or the reactivity of the metabolite(s). Experiments using ^{3}H-dimethylMISO were performed using the isolated perfused rat liver as a hypoxic tissue model. Bile, perfusion medium, and tissue extracts were analyzed for parent drug and metabolites by HPLC; covalent binding and glutathione levels were also determined. The hypoxic rat liver cleared dimethylMISO at 1/4 the rate of MISO, and dimethylMISO formed a six electron reduction product dimethylMISOamine in similar concentrations to the final reductive metabolite of MISO. The extent of dimethylMISO covalent bonding to protein was 85% less than that seen with MISO and dimethylMISO did not change the hypoxic liver glutathione status. These results indicate that dimethylMISO will be a useful tool in studies designed to improve our understanding the metabolism and toxicity of 2-nitroimidazoles.

74.8

The ^{14}C-Dimethylnitrosamine Breath Test: An In Vivo Assay of P-450j. P.B. Watkins* and S.A. Murray* (SPON: W. W. Weber). Dept. of Medicine, Univ. of Michigan Medical Center, Ann Arbor, MI 48109

The acetone-inducible cytochrome P-450j (P-450IIE1) is the low-K_m catalyst of N-dimethylnitrosamine (DMN) demethylation in rat liver. To see if a simple breath test could predict the *in vivo* activity of P-450j, 14 adult S-D rats received acetone (5ml/kg i.p) and, 0.5 to 36 hrs thereafter, tail-vein injections of ^{14}C-DMN. $^{14}CO_2$ production (% dose exhaled/1hr) was inhibited up to 85% compared to controls at 1 hr and then rose to control levels at 10 hrs and up to 2-fold over control at 16-22 hrs. Analysis of liver microsomes prepared from these rats revealed that DMN demethylase activity and immunoreactive P-450j were induced by 3 hrs and reached a maximum at 12 hrs (3.0 fold over control). From 16-36 hrs, breath test results correlated well with both microsomal demethylase activity (R=0.83) and specific content of P-450j protein (R=0.83), and all three parameters had returned to control levels by 30 hrs. In a similar experiment in 8 rats, acetone pretreatment did not at any time significantly influence the production of $^{14}CO_2$ from ^{14}C-N-methyl erythromycin (ERM), a substrate selectively demethylated by P-450p. Likewise, acute administration of P-450j inducers ethanol, ether or pyrazole inhibited production of $^{14}CO_2$ after ^{14}C DMN (>85%) but not after ^{14}C-ERM. We conclude that the DMN breath test may provide a quantitative and specific assay for the *in vivo* activity of P-450j. Furthermore, the acute *in vivo* inhibition of DMN metabolism by P-450j inducers may be a specific effect on P-450j. Supported by RO1 GM38149.

74.9

ENHANCED BIOACTIVATION AND HEPATOTOXICITY OF ACETAMINOPHEN BY PRETREATMENT WITH THE INTERFERON INDUCER POLYINOSINIC-POLYCYTIDYLIC ACID. Grazyna M. Kalabis* and Peter G. Wells. Faculty of Pharmacy, University of Toronto, Toronto, Canada.

Murine acetaminophen (APAP) hepatotoxicity is enhanced when APAP is given 24 days after interferon induction (Kalabis and Wells, Fed. Proc. 46: 1140, 1987), possibly due to induction by interferons of the cytochromes P-450-catalysed bioactivation of APAP to a toxic reactive intermediate. Male C57BL/6 mice were given the interferon inducer polyinosinic-polycytidylic acid (PIC), 10 mg/kg ip, followed 24 days later by APAP, 300 mg/kg ip. Hepatotoxicity was quantified by plasma alanine aminotransferase (ALT), and APAP metabolites by high-performance liquid chromatography. APAP bioactivation was quantified by production of the glutathione-derived metabolites cysteine and mercapturic acid. PIC pretreatment produced a 5-fold increase in the APAP-induced ALT, from 458±223 to 2259±586 IU/L (mean ± SE) ($p<0.05$), which correlated with histological evidence of necrosis. PIC pretreatment produced respective 3-fold and 1.3-fold increases in the production of cysteine and mercapturic acid conjugates ($p<0.05$), which correlated with peak ALT concentrations (cysteine, $r=0.92$, $p<0.001$; mercapturic acid, $r=0.75$, $p=0.006$). These results suggest that interferon induction can enhance the hepatotoxicity of APAP through increased bioactivation by P-450. (Support: Medical Research Council of Canada)

74.10

METHEMOGLOBINEMIA INDUCED BY ACETAMINOPHEN ANALOGUES S. Elguindi* and P.J. O'Brien* (Sponsor: P.G. Wells), Faculty of Pharmacy, University of Toronto, Toronto, Canada, M5S 2S2.

High doses of acetaminophen (APAP) in vivo causes liver damage and, in some cases, kidney damage. In an attempt to find a safe acetaminophen analogue, we have compared the toxicity of various analogues. Surprisingly, several analogues caused rapid and extensive methemoglobinemia in male CD-1 mice injected intraperitoneally. The order of effectiveness in causing methemoglobinemia was ortho-APAP > 3,5-$(CH_3)_2$-APAP > APAP > 2,6-$(CH_3)_2$-APAP. N-methyl-APAP and meta-APAP did not cause methemoglobinemia. The methemoglobinemia probably results from hepatic N-deacetylation as the methemoglobinemia was inhibited by the injection of bis-(p-nitro-phenyl)-phosphate (50 mg/kg), an irreversible deacetylase inhibitor, 30 minutes beforehand. The corresponding aminophenols were also more effective than the acetaminophen analogues at inducing methemoglobinemia. Methemoglobinemia could also be prevented by diethyldithiocarbamate but not cysteamine, N-acetylcysteine or ascorbic acid. Comparing the methemoglobinemia effectiveness of the acetaminophen analogues with the corresponding aminophenols suggests that ortho-APAP undergoes 30% N-deacetylation in vivo whereas N-CH_3-APAP is not deacetylated. The analgesics N-CH_3-APAP or meta-APAP may therefore be safer than APAP. (Support: Medical Research Council of Canada)

74.11

IN VITRO STUDIES ON THE MECHANISM OF ACETAMINOPHEN TOXICITY IN GUNN RATS. Sonia M.F. de Morais*, Zhuohan Hu*, Mary K. Nagai* and Peter G. Wells, Faculty of Pharmacy, Univ. of Toronto, Toronto, Canada.

Acetaminophen (APAP) is primarily glucuronidated, which avoids cytochromes P-450-catalysed bioactivation to a toxic reactive intermediate. Gunn rats genetically deficient in bilirubin UDP-glucuronyl transferase show up to a 72% reduction in APAP glucuronidation and 110-fold higher hepatotoxicity compared to Wistar controls (Hepatology 7: 1046, 1987). To evaluate alternative determinants, enzymes involved in APAP bioactivation and detoxification (glutathione S-transferase, GST) were measured in vitro (table).

Enzyme	Substrate	Wistar[a]	Het. Gunn[a]	Hom. Gunn[a]
P-450 Content[b]		0.88±0.08	0.59±0.04	0.74±0.06
P-450 Activity[c]	ER	2.31±0.53	2.37±0.29	1.87±0.55
	PR	0.56±0.07	0.59±0.04	0.43±0.04
	AN	0.53±0.09	0.57±0.15	0.59±0.06
GST Activity[d]	CDNB	1.04±0.07	1.21±0.13	0.98±0.10
	DCNB	0.04±0.005	0.04±0.003	0.03±0.002
	EA	0.01±0.001	0.02±0.002	0.02±0.001

a. Data are mean ± SE. Het., heterozygous; Hom., homozygous. b. nmole/mg microsomal protein. c. nmole/min/mg protein; ER, ethoxyresorufin; PR, pentoxyresorufin; AN, aniline. d. µmole/min/mg protein; DCNB, dichloronitrobenzene; CDNB, chlorodinitrobenzene; EA, ethacrinic acid.

Gunn rats did not show increased P-450 or decreased GST activities, suggesting that in vivo susceptibility to APAP toxicity is due to reduced glucuronidation and resultant increased bioactivation. (Support: Medical Research Council of Canada)

74.12

MOLECULAR MECHANISMS FOR THE TOXICITY OF THIONO-SULFUR DRUGS IN ISOLATED HEPATOCYTES S.D. Jatoe*, V.V.M. Lauriault* and P.J. O'Brien* (SPON: J.P. Uetrecht), Faculty of Pharmacy, University of Toronto, Toronto, Ontario M5S 1A1.

Disulfide metabolites of thiono-sulfur drugs were found to be much more toxic to hepatocytes than the parent drug. Propylthiouracil sulfonic acid and thiourea sulfinic acid were much less toxic than the corresponding disulfide metabolites. The order of decreasing cytotoxicity was propylthiouracil disulfide, disulfiram > phenylthiourea disulfide > cystamine > formamidine disulfide. All disulfides including cystamine readily depleted hepatocyte glutathione. Cytotoxicity could be partially prevented if the reducing agent, dithiothreitol, was added after the disulfides suggesting that part of the cytotoxicity is due to oxidative stress. However, disulfides readily disrupted Ca^{2+} homeostasis by isolated mitochondria as a result of membrane modification rather than NAD(P)+ hydrolysis. The increased cytosolic Ca^{2+}- that may initiate cytotoxicity may therefore be due to ATP depletion as well as oxidative inactivation of plasma membrane Ca^{2+}-ATPase. A 70-75% depletion of glutathione with diethylmaleate or ethacrynic acid inhibited disulfide detoxification and cytotoxicity occurred at 5-6 fold lower disulfide concentrations. (Supported by the National Research Council of Canada)

RECEPTORS

75.1

CO-CULTURE OF EMBRYONIC CHICK ATRIAL CELLS AND CILIARY GANGLIA ENHANCES PARASYMPATHETIC RESPONSIVENESS. Joey V. Barnett*and Jonas B. Galper* (SPON: B. T. Liang). Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115

We have developed a system for co-culture of embryonic chick heart cells 3 1/2 days in ovo with ciliary ganglia (CG) from chick embryos 7 days in ovo. After 3 days in culture, removal of the CG results in complete degeneration of the neuronal processes leaving the post-innervated heart cell culture devoid of neuronal elements. Embryonic chick heart cells 3 1/2 days in ovo are unresponsive to muscarinic stimulation. Following 3 days of co-culture with CG or with medium conditioned by growth of heart cells and CG cells developed a negative chronotropic response to muscarinic stimulation with a 40% decrease in spontaneous beat rate at 10^{-4} M Carbamylcholine. Comparison of the levels of α_{39} and α_{41} measured by ADP-ribosylation of homogenates of embryonic chick heart cell cultures in the presence of ^{32}P-NAD and pertussis toxin demonstrated a 50% increase in the levels of α_{39} and α_{41} compared to cells cultured without CG. One explanation of these data is that innervation may induce parasympathetic responsiveness by increasing the availability of guanine nucleotide binding proteins which couple the muscarinic receptor to a physiologic response.

75.2

SHORT-TERM REGULATION OF MUSCARINIC RECEPTORS IN PRIMARY CULTURE OF CORTICAL CELLS. C. Eva, S. Kieei-Gamaiero, E. Genazzani and E. Costa. a) Institute of Pharmacology, University of Turin, Italy and b) Fidia-Georgetown Institute for the Neurosciences, Georgetown University, Washington, D.C., 20007

Exposure of rat cortical cells to muscarinic agonists induced a rapid decrease in the density of cell membrane surface muscarinic receptors, as detected by ^{3}H-NMS, (N-methyl-scopolamine) binding to intact cells which was accompanied by a concomitant desensitization of increase in PI (Phosphoinositide) hydrolysis at muscarinic receptors. This decrease may reflect receptor internalization for it was not accompanied by a significant change in the number of total cellular receptors (as measured by 3H-QNB binding to cortical cell homogenate). Agonist incubation lasting longer than 1 hr induced a gradual loss of total cellular receptors. Once the agonist was removed, recovery ensued in 1 hr. Exposure of cortical cells to the protein kinase C, (PKC) activator 12-O-tetradecanoyl phorbol-12,13-acetate (TPA) also induced a rapid and dose dependent decrease of 3H-NMS binding, while the PKA activators forskolin or 8-Br-cAMP were ineffective. Thus, phosphorylation of the receptor by PKC might trigger endocytosis of the receptor. Moreover, experiments will be presented whereby one can exclude PKC translocation in the homologous regulation of muscarinic receptors.

75.3

MUSCARINIC RECEPTORS IN GUINEA PIG BLADDER: CORRELATION AMONG PHOSPHOINOSITIDE BREAKDOWN, ADENYLATE CYCLASE INHIBITION AND DETRUSOR MUSCLE CONTRACTIONS IN VITRO AND IN VIVO. Lalita Noronha-Blob*, Valerie Lowe*, Dianne Costello*, Andrea Patton*, Brendan Canning* and William J. Kinnier. Nova Pharmaceutical Corporation, 6200 Freeport Centre, Baltimore, MD 21224.

Activation of muscarinic cholinergic receptors (MR) in the guinea pig (g.p.) urinary bladder leads to phosphoinositide (PI) breakdown, adenylate cyclase (AC) inhibition and detrusor smooth muscle contraction in vitro (Noronha-Blob et al., Life Sci. 41:1987, Costello et al., Pharmacologist 27:1985). In vivo, MR antagonists decrease peak intravesicular bladder pressure (PvesP) in the slow filling cystometrogram (CMC) and inhibit the micturition reflex (Noronha-Blob et al., FASEB J. 2(4):1988). Significant positive linear correlations were found among the inhibitory potencies of ten muscarinic antagonists to inhibit PI turnover and detrusor muscle contraction in vitro ($r=0.88$, $p<.001$) or PvesP in vivo ($r=0.81$, $p<.001$). In contrast, no significant correlation ($p>0.5$) was seen between the potency of MR antagonists to block the AC inhibitory response and both contractile activities (in vitro and in vivo). The data suggest that PI breakdown which is associated with calcium mobilization may function as the transducing mechanism for cholinergic muscle contraction in the g.p. bladder.

75.4

HOMOLOGOUS DESENSITIZATION OF PHOSPHOLIPASE C-COUPLED NEUROTRANSMITTER RECEPTORS IN CEREBELLAR GRANULE CELLS. D.-M. Chuang and O. Dillon-Carter, Lab. of Preclin. Pharmacol. NIMH Neuroscience Center at St. Elizabeths Hosp. Washington, D.C. 20032

We have found that cerebellar granule cells express phospholipase C-coupled muscarinic cholinergic (mAChR), histaminergic (H_1), adrenergic (alpha$_1$) and serotonergic (5-HT$_2$) receptors. Prestimulation with saturating concentrations of each of these receptor agonists was found to cause a time-dependent desensitization to subsequent stimulation of inositol phosphate formation with the desensitizing agonist. Thus, prestimulation for 0.5, 4 and 18 hr decreased carbachol response to 87, 52 and 40% of the control, respectively, histamine response to 37, 24 and 18% respectively, norepinephrine response to 55, 14 and 10% respectively and 5-HT response to 36, 18 and 9%, respectively. In all cases, the responses mediated by receptors which were not prestimulated remained virtually unchanged, thus indicating homologous desensitization. Loss of mAChR binding sites in intact cells was detected after 2 hr prestimulation and progressively increased up to 18 hr. Biologically active phorbol ester, 4 β-phorbol 12-myristate 13-acetate (PMA) rapidly attenuated basal phospholipase C activity as well as the responses mediated by carbachol, histamine, norepinephrine and 5-HT, suggesting that activation and translocation of protein kinase C might play a role in the desensitization of phospholipase C-coupled receptors.

75.5

THE LIGAND BINDING SITE OF THE HUMAN PLATELET α_2-ADRENOCEPTOR: IDENTIFICATION BY PHOTOAFFINITY LABELING AND PEPTIDE MAPPING. John W. Regan*, Hiroaki Matsui*, Marc G. Caron and Robert J. Lefkowitz. Howard Hughes Medical Institute, Duke University, Durham, NC 27710.

Specific covalent incorporation of two photoaffinity ligands was localized to the fourth transmembrane spanning domain of the α_2-adrenoceptor by peptide mapping. Partially purified human platelet α_2-adrenoceptors were radiolabeled with either the antagonist photoaffinity ligand [^{3}H]SKF 102229 (3-methyl-6-chloro-9-azido-1H-2,3,4,5-tetrahydro-3-benzazepine) or the agonist [^{3}H]PAZC (p-azidoclonidine). Photolabeling of the α_2-adrenoceptor by these ligands could be blocked with appropriate pharmacology: yohimbine> prazosin; p-aminoclonidine>(-)epinephrine; (-)epinephrine> (+)epinephrine. As determined by autoradiography following urea SDS-PAGE, lysylendopeptidase treatment of the [^{3}H]SKF 102229-labeled receptor gave one peptide of M_r 2400 as the product of a complete digest. Using [^{3}H]PAZC-labeled receptor a similar M_r 2400 peptide was obtained after digestion with lysylendopeptidase. From the observed cleavage pattern, and the known amino acid sequence of the α_2-adrenoceptor, this M_r 2400 peptide corresponds to a 23-residue peptide that represents the fourth membrane spanning domain of the receptor. With respect to the structures of the ligands used in this study, the catechol moiety of epinephrine may interact directly with this domain during binding to the α_2-adrenoceptor.

75.6

FUNCTIONAL CHARACTERIZATION OF ALPHA-2 ADRENERGIC RECEPTOR SUBTYPES IN CONTINUOUS CELL LINES. D.B. Bylund, C. Ray-Prenger* and T.J. Murphy*. Dept of Pharmacology, University of Missouri, Columbia, MO 65212.

We have defined subtypes of alpha-2 adrenergic receptors (A2AR) on the basis of their pharmacological specificity in binding studies. In the NG108 cell line (A2AR-B), prazosin, ARC-239 and chlorpromazine are 20- to 60-fold more potent than in the HT29 cells (A2AR-A). By contrast, oxymetazoline is 60-fold more potent at A2AR-A. More recently, we have identified third subtype (A2AR-C) in the OK cell line. In order to confirm the results of these binding studies, we have determined the K_B values by Schild analysis for various antagonist in reversing A2AR inhibition of cyclic AMP production in intact cells using the adenine prelabelling technique.

	K_B Value, nM	
DRUG	HT29 Cell	NG108 Cell
Yohimbine	5.6 ± 0.9	3.4 ± 1.2
Phentolamine	14 ± 1	12 ± 3
ARC-239	950 ± 90	12 ± 2
Prazosin	1700 ± 70	39 ± 4

The correlations between the K_I values from binding studies and K_B values from functional studies were excellent (0.99 for HT29 cells and 0.98 for NG108 cells). These results confirm the radioligand binding studies, and further support the subdivision of A2AR into at least two subtypes. (Supported by Grant HL 32931).

75.7

MODULATION OF THE CYTOPLASMIC LEVELS OF THE α-SUBUNIT OF THE STIMULATORY G-PROTEIN, G_s, BY β-ADRENERGIC RECEPTOR STIMULATION. Lennart A. Ransnäs, Petr Svoboda, and Paul A. Insel. University of California San Diego, La Jolla, CA 92093

We have investigated subcellular distribution of G_s using S49 lymphoma cells. Cells were treated with 1 μM l-isoproterenol for various time periods, subjected to nitrogen cavitation and the homogenate was spun at 900xg for 5 min. The supernatant was then spun at 150,000xg for 1 h and the resulting pellet and supernatant were used in determinations of G_s by a competitive ELISA, based on antipeptide antibodies directed against the α-subunit. This antibody recognizes selectively dissociated α_s and not α_s in its heterotrimeric form; by assaying with or without 20 μM Al^{3+}, 10 mM Mg^{2+}, and 10 mM F^- both forms can be quantitated. We assessed distribution of $\alpha_s+\alpha_s\beta\gamma$ (% of original starting material) by use of the antibody and functional presence of G_s in supernatant and pellet fractions by reconstitution with G_s-deficient membranes and assay of adenylate cyclase activity (pmol/min) after various treatment times with isoproterenol:

	Control	0.5 h	1 h	11 h
Pellet (%)	87	95	68	60
Supernatant (%)	15	13	27	48
Pellet (pmol/min)		663		474
Supernatant (pmol/min)		32		614

The α_s present in the supernatant was of the dissociated form. From these findings we can conclude that β-adrenergic stimulation causes α_s to relocate to the cytoplasm.

75.8

FURTHER EVIDENCE FOR THE DIRECT CATION REGULATION OF HEPATIC V1 RECEPTOR. V. Gopalakrishnan*, JR. McNeill, PV. Sulakhe* and CR. Triggle. Dept. of Pharmacology and Physiology, Univ. of Saskatchewan, Saskatoon, SK. S7N 0W0 ; Div. of Basic Med. Sci., Memorial Univ. Newfoundland, St. John's, NF. A1B 3V6. Canada.

Recently, we reported that Mg2+ (1 mM) enhanced the affinity of 3H AVP binding to the hepatic V1 receptor but decreased the affinity of the 3H V1 antagonist. It was postulated that this differential role of Mg2+ for the agonist/antagonist interaction may occur via activation of a cation binding site located on/or associated with the V1 receptor. In order to assess the regulatory role of this metal ion binding site further, we studied the effects of trivalent cations on the binding of the two radioligands to rat liver plasma membrane. Fe3+ and Al3+ failed to affect the binding of either radioligand whereas La3+ (1 to 200 μM) produced concentration dependent inhibition of both 3H AVP and 3H V1 ant. binding. However, 3H AVP binding was more sensitive to La3+ blockade (Ki 16 μM) than that of the 3H V1 ant. (Ki 41 μM). Inclusion of La3+ in saturation binding assays increased the Kd for 3H-AVP both in the presence (from 0.12 to 1.20 nM by 20 μM La3+) and absence (from 2.40 to 23.70 nM) of Mg2+. La3+ (20 μM) also increased the Kd for the 3H V1 ant., but the changes were less marked (from 0.10 to 0.27 nM in presence and 0.05 to 0.31 nM in the absence of Mg2+). The Bmax of the agonist and antagonist were similar under all conditions (Bmax≈500-900 fmol/mg). Competitive binding studies were in agreement with the saturation data. Finally, in kinetic experiments, addition of La3+ inhibited the rates of association of the radioligands whereas it did not affect the rates of dissociation. The results provide further evidence for the presence of a metal ion binding site on the V1 receptor. While both cations regulate the affinity characteristics, in contrast to Mg2+, La3+ in the μM range induces a low affinity conformation of the receptor (Supported by the MRC, Canada).

75.9

COMPETITIVE REGULATION OF A HORMONE-RESPONSIVE ELEMENT BY MULTIPLE TRANS-ACTING STEROID RECEPTORS. P. Patel* and M.V. Govindan* (Spon: A. Dupont), MRC Group in Molecular Endocrinology, CHUL and Dept. Microbiology and Immunology, Laval University, Quebec G1V 4G2, Canada.

The elucidation of molecules and mechanisms that mediate specific gene regulation in response to exogenous inducers such as hormones or growth factors is a central problem in eukaryotic molecular biology. At the level of transcription, this regulation appears to be resulting through positive and negative effects mediated by trans-acting proteins interacting with cis-acting DNA-promoter elements. Steroid hormones trigger complex developmental and physiological processes. The understanding of i) how the receptor protein is able to recognize its cognate hormone, ii) how this binding results in the interaction of the receptor with target gene promoter elements, iii) how this interaction leads to initiation of specific gene transcription and iv) what is the role of the hormone in these processes, is a prerequisite to the understanding of the molecular mechanism of steroid hormone-regulated gene expression. These receptors are structurally related and share sequence homology with the avian erythroblastosis virus (AEV), V-erbA oncogene. The experiments demonstrating the transcriptional regulation and competition among various steroid-receptors after co-transfection with chimeric plasmids containing the steroid-responsive elements and bacterial chloramphenecol acetyl transferase may give some idea in the target tissue specificity of hormone regulated gene transcription.

75.10

ISOLATION AND SEQUENCE OF THE HUMAN GLUCOCORTICOID RECEPTOR GENE PROMOTER. Claude Lefebvre* and Manjapra V. Govindan* (Spon: A. Dupont), MRC Group in Molecular Endocrinology, Laval University Medical Center, Quebec, Canada G1V 4G2.

We have isolated the promoter region of the human glucocorticoid receptor (HGR) gene from the λEMBL-3 human genomic library using synthetic oligonucleotides corresponding to the 5' end of hGR cDNA. The clone was fine mapped by restriction digestion, by hybridization with hGR cDNA probes and by DNA sequence analysis and found to contain an open reading frame corresponding to the hGR cDNA from amino acids 1-131 separated by a large intron from the 5' non-coding sequences. The promoter region of the HGR has been identified by primer extension, S_1 nuclease mapping using hGR mRNA from human prostatic carcinoma cells (LNCaP) and human breast tumor cells (MCF-7), by in vitro transcription using HGR-DNA fragments as template and by DNA sequence analysis. The sequence of 1600 base pairs of the HGR promoter reveals that it contains a "CAAT" box but no "TATA" box and has an extremely high "G+C" content. Co-transfection of GR expression vector show with HGR-CAT chimeric plasmids into COS-1 and CV-1 cells show that the hormonal regulatory sequences of HGR is contained within a 4.5kb ERI-ERI fragment.

75.11

CLONING OF THE HUMAN ANDROGEN RECEPTOR cDNA. Manjapra V. Govindan, (Spon: A. Dupont), MRC Group in Molecular Endocrinology, Laval University Medical Center, Quebec G1V 4G2, Canada.

In order to define the functional domains of the human androgen receptor (hAR) involved in gene regulation by androgens, complementary DNA (cDNA) clones encoding the human androgen receptor have been isolated from a human testis λgt-11 cDNA library using synthetic oligonucleotides homologous to the human glucocorticoid, estradiol, progesterone and aldosterone receptors as probes. The cDNA clones were characterized after their insertion into a bacterial expression vector in the proper orientation and in vitro transcription using T7-RNA polymerase and in vitro translation of the mRNA in rabbit reticulocyte lysate followed by incubation with tritium-labelled DHT (dihydrotestosterone) in the presence and absence of various steroid hormones. The clones giving rise to proteins which bound [^{3}H]-DHT with high affinity and specificity were chosen for further studies. Northern hybridization results obtained by using the AR cDNA as probe, detected the presence of multiple mRNA species in human MCF-7 cells, human LNCaP cells and rat ventral prostate. Using a similar approach, human mineralocorticoid receptor and human progesterone receptor (hMR) cDNAs from the human testis cDNA library were also isolated.

75.12

GLYCOSYLATION OF THE ESTROGEN RECEPTOR(ER) DURINGBREAST CERCINOMA INFILTRATION INTO THE LYMPH NODES. Anwar A. Hakim &Charle E. Joseph*.Loyola University Medical Center, Maywood. Illinois & University Southern California. Los Angeles, California.

Earlier studies reported on cloning of an estrogen receptor (ER) gene expressed in human breast cancer (Joseph & Hakim FAS EB Journal 2:8718, 1988. Hakim(Europ.J. Cancer Clin.Oncol.23(11)1758, 1987)showed that17β-estradiol mediated glycosylation of human breast carcinoma growth factor receptor. In the following experiments, ERs were prepared from cloned MCF-7ER+. As an acceptor, an aliquot of the ER was glycosylated using standard techniques with specific sialyltransferase, ER was sialylated. Monoclonal antibobies MAbER and MAbSialylER were developed against ER and SialylER. Tumor cells were obtained from lymph node spin-centrifuged cell preparations. The tumor cells were expanded in soft agar, then examined with the MAbER & MAbSialyl. ER by the peroxidase technique. All the nodes (225) from patients with disseminated BCa reacted with MAbSialylER. Twenty five and 223 out of 258 lymph nodes from patients with breast cancer at PS I reacted with MAbER and MAbSialylER. Tumors are of variable receptor, i.e. those containing receptor positive and negative components, always generated metastatic nodes as would be expected if receptor negative cells are indeed the more aggressive. Metastatic spread of BCa to regional lymph node is more common in patients with ER⁻negative primaries than in those with ER⁺positive primaries. After primary surgery, patients at high risk for early recurrence are usually ER⁻negative and reacted with MAbSialylER antibodies.

EXERCISE I

76.1

EFFECTS OF ENDURANCE TRAINING ON VASCULAR SMOOTH MUSCLE. M. Wills*, R. S. Mazzeo* and S. M. Horvath. Univ. of California, Santa Barbara, CA 93106.

Age-related decreases in vascular smooth responsiveness to catecholamines have been well-documented, however, there are few studies on the effects of endurance training on aging in the vasculature. In this study, we evaluated the effects of endurance training on contractility of aortic smooth muscle in rats of different ages. Female Fischer 344 rats (3(Y), 12(M), 24(O) mths of age) were pair-matched based on pre-determined VO_2 max for 10 wks for 1 hour daily. The animals were sacrificed, thoracic aortas removed, cut into helical strips, and mounted in a muscle bath. In vitro dose response curves to norepinephrine (NE) were determined. VO_2max increased 11,18,20% for the Y,M,O groups respectively. In the S group, the maximal contraction (E_{max}) to NE of the O group (3.71 g/mm^2) was significantly reduced as compared with the Y and M groups (7.74g/mm^2, 6.40g/mm^2). In the T group, there were no significant differences between the M and O groups' E_{max} (M, 10.43 g/mm^2, O, 10.43g/mm^2). There was no significant training effect in the Y group (7.74g/mm^2 (S), 5.03g/mm^2 (T)). In summary, we conclude that training attenuated the age related difference in contractility to NE.

76.2

THE EFFECTS OF OPEN VS. CLOSED CIRCULATION ON CONTRACTING RAT MUSCLE ANAEROBIC METABOLISM. L.L. Spriet, School of Human Biology, University of Guelph, Ontario Canada N1G 2W1.

The hindlimbs of anesthetized rats were stimulated to contract maximally at a train rate of 1.0 Hz (200 ms, 80 Hz) with an open or closed circulation. The gastrocnemius (red, RG and white, WG), plantaris (PL) and soleus (SOL) muscles were sampled pre- and post-stimulation. Total ATP production with a closed circulation was 351 ± 7, 245 ± 11, 293 ± 15 and 122 ± 4 umol/g dry muscle in WG, RG, PL and SOL, respectively. Opening the circulation had no effect on ATP production in the fast-twitch muscles, but reduced ATP production in SOL to 84 ± 9 umol/g. Decreases in glycolysis (40%) and phosphocreatine degradation (16%) accounted for the decreased ATP provision. SOL ATP content fell from 19.29 ± 0.37 umol/g at rest to 17.93 ± 0.43 and 15.63 ± 0.42 umol/g in the open and closed conditions, respectively. Isometric tension generation by the triceps surae muscles was significantly higher during open circulation at 45 and 60 s of stimulation. The results suggest that, during short-term stimulation in anesthetized rats, blood flow to the fast-twitch muscles did not increase sufficiently with an open circulation to permit significant aerobic metabolism. Increased blood flow to SOL with an open circulation permitted oxidative ATP production, thereby accounting for the increased tension generation late in the stimulation period.

Funded by the Nat. Sci. and Eng. Res. Council of Canada.

76.3

METABOLIC RATE AND LACTATE UPTAKE BY IN SITU CANINE SKELETAL MUSCLE. L. Bruce Gladden. Exercise Physiology Laboratory, University of Louisville, Louisville, KY 40292

It was the purpose of this study to measure lactate uptake/output by skeletal muscle exposed to an elevated blood lactate concentration at different metabolic rates. The gastrocnemius-plantaris muscle group was isolated in situ in eleven anesthetized dogs. Lactic acid was infused at a pH of 3.5 - 3.6 (Gladden and Yates, J. Appl. Physiol. 54:1254-1260, 1983.) to establish a blood lactate concentration of approximately 9 mM, while maintaining normal blood gas/pH status. During three consecutive 30-min periods, the muscles were 1) allowed to rest, then stimulated to produce twitch contractions at 2) 1.0 Hz, and 3) at 4.0 Hz. Steady state oxygen uptake was 11.9 ml/kg•min at rest, 39.0 at 1.0 Hz, and 124.6 at 4.0 Hz. Steady state lactate uptake by the muscles increased from 113 umol/kg•min at rest to 329 umol/kg•min ($p>.05$) at 1.0 Hz, and to 715 umol/kg•min at 4.0 Hz ($p<.05$ vs. Rest and 1.0 Hz). These results suggest that steady state lactate uptake by skeletal muscle at elevated blood lactate concentration increases as metabolic rate is elevated by contractions. (Supported by NSF Grant #RII- 8610671 through Kentucky EPSCoR Program.)

76.4

INHIBITION OF GROWTH AND METABOLIC RATE IN STRAIN 13 GUINEA PIGS AFTER LONG-TERM DAILY EXERCISE. C. T. Liu. U.S. Army Med. Res. Inst. Infect. Dis., Ft. Detrick, Frederick, MD 21701

Strain 13 guinea pigs are commonly used for physiologic studies, and also have been chosen as an animal model for studying pathogenesis of arenavirus-induced hemorrhagic fevers (Current Topics Microbiol. Immunol. 134: 5, 1987). The purpose of this study was to develop techniques for exercising strain 13 guinea pigs without using electrical shock to drive the animal to the moving drum of a treadmill. This was accomplished by using a tailor-made sling with extended collar. While the body, including the neck was wrapped with the sling, the animal's position on the treadmill was loosely fixed at the back (T2 level) to a stationary metal rod without supporting the body weight. Guinea pigs were exercised at 0.3 M/sec for a maximal 90 min/day in 3 sessions separated by a minimum of 30 min. This exercising program was continued for 40 consecutive days and selected physiological responses to immediate and long-term effects of exercise were measured. Both body weight and metabolic rate were depressed ~15 days post-exercise, while food and water intake were not markedly altered. No significant changes in rectal and body surface (head, forelimb, hindleg, and abdomen) temperatures were observed. In conclusion, special designs have been developed for successfully exercising strain 13 guinea pigs on a drum treadmill. Long-term daily exercise decreased the animal growth and metabolic rates without marked changes in water and food intake, or in rectal and body surface temperatures.

76.5

RELATIONSHIP OF OXYGEN CONSUMPTION TO HEAT PRODUCTION IN RATS DURING AN INCREMENTAL TREADMILL EXERCISE. S.N. Dube*, P. Buckenmeyer*, V. Garcia*, S.M. Somani and R. Knowlton*. Southern Illinois University Sch. Med., Dept. of Pharm., Springfield, IL 62708.

Open circuit indirect calorimentry was used to determine the metabolic variables in rat during exercise on treadmill fabricated in our SIU-SM Research-Services Shop. Twelve male Sprague-Dawley rats (180-230 gm) were exercised on a motor driven treadmill for 35-55 min up to an intensity of 20m/min at a 10% grade. The animals were run in an enclosed 5 l. chamber with a positive flow-rate pressure of 4 l./min. During the incremental exercise protocol, oxygen consumption (VO_2) and heat production were analyzed using a computerized oxyscan analyzer, Omnitech Inc., Ohio. VO_2 increased from 19.63 ± 1.43 to 28.51 ± 1.14 ml/kg/min and also heat production increased from 1260.75 ± 87.77 to 1810.82 ± 69.08 cal/hr. during exercise. A highly significant correlation of 0.99 was observed between VO_2 and heat production at different levels of exercise. Values for cholinesterase in RBC and different tissues (brain, heart, diaphragm and thigh muscle), pyruvate, lactate and hemoglobin were also determined. This data indicated that the heat production did not differ significantly beyond an exercise intensity of 90% VO_2 max and also, there appears to be a specific exercise intensity at which heat production plateaued in the exercising rat. (Supported by U.S. Army Contract No. DAMD 88-C-8024.)

76.6

EFFECTS OF ACUTE EXERCISE AND AMBIENT TEMPERATURES ON SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN DIFFERENT TISSUES. S. Rivest* A. Labrie* and D. Richard. Univ. Laval, Québec, P.Q., Canada, G1K-7P4.

The main and interactive effects of exercise and ambient temperature on oxygen consumption (VO_2) and sympathetic nervous system (SNS) activity in various tissues were studied. Albino mice were exposed to 4°, 14° and 24°C for 1 hour. During the exposure period, the animals were either kept in resting conditions or exercised on a motor-driven treadmill. VO_2 was continuoustly monitored in each group of mice. Accumulation of dopamine (DA) after the blockade of the dopamine beta-hydroxylase activity with 1-cyclohexyl-2-mercapto-imidazole (CHMI) was used to assess SNS activity. Mice were injected with CHMI 30 minutes before being placed in the various ambient temperatures and killed immediately after the exposure time. Heart, interscapular brown adipose tissue (iBAT) and pancreas were then rapidly removed. DA was extracted from the tissues and measured by high performance liquid chromatography. The results show that ambient temperature and exercise interact on VO_2; at 24°C, VO_2 was higher in exercising mice than in resting animals whereas there was no difference between active and non-active mice exposed at 14° and 4°C. DA accumulation in heart, pancreas and iBAT progressively increased while ambient temperature was dropping. Exercise was not shown to markedly affect SNS activity. However, exercise selectively reduced DA accumulation in iBAT. In conclusion, the present results show that BAT thermogenesis, a SNS-mediated process, is substituted by exercise-derived heat in mice living at low ambient temperatures. (Supported by the CRSNG and the FCAR)

76.7

SYMPATHETIC RESPONSES TO EXCITATORY STIMULI IN ENDURANCE ATHLETES (Ath) AND UNTRAINED SUBJECTS (Untr). D.R. Seals, L.A. Clayton-Bare* and M.J. Reiling*. Depts. Exercise & Sport Sciences and Physiology, Univ. of Arizona, Tucson, AZ 85721.

To determine whether the state of physical training influences the sympathetic neural responses to known excitatory stimuli, we measured muscle sympathetic nerve activity (MSNA; peroneal nerve, r. leg), heart rate (HR), and arterial blood pressure (AP) in 10 Ath (aged 24 ± 2 yr, $\bar{X}$ ± SE) and 9 Untr (aged 28 ± 2 yr) before (control) and during the following interventions: a) non-hypotensive lower body negative pressure (LBNP) at -5, -10, -15, and -20 mmHg for 2 min each; b) isometric handgrip exercise at 30% of maximal force for 2.5 min; and c) ice water immersion of one hand for 1.5 min. Control MSNA (23 ± 2 vs 21 ± 3 bursts/min, Ath vs Untr) and mean AP (92 ± 2 vs 93 ± 2 mmHg) were similar in the two groups, but HR was lower in Ath (56 ± 3 vs 69 ± 4 bt/min, $p < 0.05$). During each successive level of LBNP, MSNA tended to increase less in Ath (1 ± 1, 2 ± 1, 7 ± 1, and 9 ± 2 bursts/min) vs Untr (5 ± 2, 7 ± 1, 10 ± 2, and 12 ± 2 bursts/min; $p < 0.05$ at -10 mmHg only). The increases in MSNA, HR, and mean AP above control during exercise (6 ± 3 vs 10 ± 2 bursts/min, 10 ± 2 vs 13 ± 2 bt/min, and 16 ± 2 vs 16 ± 2 mmHg; Ath vs Untr) and during ice water immersion (24 ± 4 vs 17 ± 2 bursts/min, 14 ± 2 vs 12 ± 3 bt/min, and 18 ± 2 vs 17 ± 3 mmHg) were not different in the two groups (all $p > 0.05$). These preliminary results suggest that, in general, the autonomic adjustments to these excitatory stimuli are not markedly influenced by endurance exercise training.

76.8

CONTROL OF ERYTHROCYTE (E) VOLUME DURING AND FOLLOWING HIGH INTENSITY CYCLE ERGOMETRY. R.S. McKelvie*, M.I. Lindinger* and G.J.F. Heigenhauser. McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Previous studies have demonstrated no change in E volume during heavy exercise. The purpose of this study was to examine the ionic exchange between plasma and E that may be responsible for maintaining E volume. Five healthy males performed four 30s bouts of maximal isokinetic cycling with 4-min rest periods between each bout. Arterial blood was sampled during and for 90 min following exercise(EB). During EB, the arterial E [K^+] rose by 11.0 mEq/l intracellular fluid (138.0 - 149.0 mEq/l). Following the fourth EB E [K^+] fell rapidly to below the control and remained there throughout recovery. Arterial E [Cl^-] increased 14 mEq/l (77.0 - 91.0 mEq/l) during EB, decreasing throughout recovery to 80 mEq/l. During EB, E [lactate] increased from 0.2 mEq/l to 9.6 mEq/l. There was a progressive decrease throughout recovery to 0.3 mEq/l at 90 min. No significant difference was observed for arterial E [Na^+]. An increase in the total measured E [ion] occurred during EB (270 - 315 mEq/l) with a decrease during recovery to 260 mEq/l. These changes corresponded to the change in plasma osmolality. There was a loss of fluid from the vascular space during EB, with reabsorption into the vascular space during recovery. Despite a 15% decrease in plasma volume during EB, no difference was observed in E volume. Therefore changes in E volume during EB may be prevented by movement of K^+, La^- and Cl^- into the E.

77.1

CONTINUING STUDIES OF "CELLS" FLIGHT HARDWARE. J.Duke*, J.Moore* and D.Montufar-Solis*. U.Texas Dental Branch, Houston, TX 77225.

The CELLS experiment will test the effect of μg on development in vitro of a cell sensitive in vito to gravitational changes--the mammalian chondrocyte. Drops of cell suspensions, prepared from limb buds of 12 day embryonic mice, are inoculated into the hardware at densities promoting cartilage differentiation. The main unit of the Cells hardware is a culture chamber with two wells, and in each, a bladder of a gas exchanging material (Silastic) which expands or collapses as medium is added or removed through the gasket between the chamber and the bottom plate. A deflector ring in the bottom of the chamber prevents medium injection and withdrawal from dislodging the cells. Initial injections for cell culture are made using a Hamilton syringe inserted through the gasket and through a silicon plug in the deflector ring into the chamber's center. After a 2 or 20 hr attachment period, 1.8ml of medium is added, expanding the Silastic bladder. For preliminary studies, cultures were set up on Thermanox coverslips with or without Cell-Tak in BEX hardware, or as controls in Corning 24-well plates. Four BEX hardware were sealed in a Biorack Type I container, and it and a control plate were placed in a 37°C incubator (humidity, 5% CO_2). Cultures were observed daily for cartilage formation, fixed after 2-5 days of culture, and stained with Alcian blue for detection of cartilage nodules. The chambers were found to support cartilage differentiation, and the amount of cartilage formed was found to be less with longer cell attachment times and also less on Cell-Tak coated coverslips. Supported by NASA NCC2-243.

77.2

SKELETAL MUSCLE ANTIOXIDANT ENZYME LEVELS IN RATS AFTER SIMULATED WEIGHTLESSNESS, EXERCISE AND DOBUTAMINE. B. Girten* C. Oloff*, P. Plato*, E. Eveland*, A.J. Merola* & L. Kazarian* (SPON: D.R. Lamb) Armstrong Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH 45433 and The Ohio State University, Exercise Physiology Laboratory, Columbus, OH 43210

Effects of hypokinesia/hypodynamia(H/H) hindlimb suspension, exercise training (EXER) and intraperitoneal dobutamine (DOB) injections on catalase (CAT) and superoxide dismutase (SOD) levels were examined in the soleus muscle of rats (n=48). Half of the adult male Sprague-Dawleys were exercise trained for 11 weeks and half were not (SED). Animals in both the EXER and SED groups were randomly assigned to one of four additional treatment groups. H/H rats were suspended (~30°∠) for 14 days by tail traction, utilizing the Morey-Holton x-y axis system. Half of the H/H group were injected twice a day with DOB (H/H DOB), while the other half received saline (H/H SAL). Control animals (CON SAL and CON DOB) were treated similarly, but were not suspended. Overall ANOVA and Tukeys pairwise comparisons indicated that antioxidant enzyme levels in the EXER groups were significantly greater ($p<.05$) when compared to their SED counterparts; the only exception was CAT in the H/H SAL group ($p=.06$). The H/H SAL groups had lower ($p<.05$) enzyme levels than the CON SAL groups. These findings indicate that exercise training prior to H/H suspension and DOB treatment during the suspension helps prevent large decreases in skeletal muscle antioxidant enzymes that would otherwise occur with H/H. (Supported in part by AFOSR/AFSC, USAF Project # 2312V6.)

77.3

TEMPERATURE HOMEOSTASIS IN RATS EXPOSED TO 2G. L.M. Ishihama*, D.M. Murakami* and C.A. Fuller. Dept. of Animal Physiology, University of California, Davis, CA 95616.

This study examined the effect of light and dark on the regulation of body temperature in the hyperdynamic environment. Body temperature of 8 male Wistar rats was measured with an implanted biotelemetry system. Rats exposed to a control 24 hr light-dark cycle (LD 12:12) in 1G demonstrated a normal circadian rhythm in body temperature. When the animals were exposed to a high frequency light-dark cycle (LD 3:3) for 24 hours, there was a significant increase in body temperature during the dark periods of the rats subjective day (inactive phase). In contrast, there was a significant depression of body temperature during the light periods of the rats subjective night (active phase). These results demonstrate that light and dark have important influences on the regulation of body temperature. The rats were then exposed to a 2G field for 10 days via centrifugation. The influence of the high frequency light-dark cycle was then reexamined. After exposure to the 2G field, the rats exhibited: 1) a reduced circadian rhythm of body temperature, and 2) a significantly reduced influence of the high frequency light-dark periods on the regulated levels of body temperature. These results demonstrate the long term effects of hyperdynamic fields on the mechanisms of body temperature regulation. [This study was supported in part by NASA grant NAG2-349.]

77.4

EFFECTS OF 7 DAYS OF MICROGRAVITY ON RAT PLASMA HORMONE LEVELS. M. Vasques*, R. Grindeland, M. Martinelli* and R. Furlanetto Santa Clara University, Santa Clara, CA. 95053, NASA/ Ames Research Center, Moffett Field, CA. 94035, and Philadelphia Children's Hospital, Philadelphia, PA. 19104.

To further clarify the effects of microgravity on endocrine function, concentrations of 8 hormones have been measured in plasmas of male rats flown on the 7 day Space Lab 3 mission. Reports from our laboratory have shown decreased GH secretion by flight rat somatotrophs, decreased thymus and testes weights, but no change in adrenal weights. Flight rats, 7 large (400g; LF) and 12 small (250g; SF), were anesthetized with halothane and bled by cardiac puncture 11-17 hrs after landing. Simulation controls (LC;SC) were maintained under similar conditions at 1g. All hormones were measured by established methods. Thyroxine (T4), triiodothyronine (T3), prolactin (P), corticosterone (C), immunoassayable GH (IGH), and somatomedin C (SMC) levels did not differ between F and C rats of either size ($p>0.05$). Testosterone titers (ng/ml) were decreased 75% or more; LC 9.2 ± 2.5 vs LF 0.3 ± 0.1; SC 2.3 ± 0.5 vs SF 0.5 ± 0.3 ($p<0.01$). Bioassable GH (BGH) levels were also significantly lower (~50%) in LF and SF rats. Thus, secretion of T4, T3, P, C, IGH and SMC were either unaffected by space flight or recovered before bleeding. In contrast, exposure to microgravity resulted in sustained decreases in secretion of BGH and testosterone. All studies complied with ARC AHB 7180-1. (Supported by NASA).

77.5

EFFECT OF INCREASED ACCELERATION ON LUNG EXPANSION IN DOGS: PRONE VS. SUPINE BODY POSITIONS. S. J. Lai-Fook, L. V. Brown,* S. Ganesan,* V. S. Maudgalya,* and C. F. Knapp.* Biomedical Engineering Center, University of Kentucky, Lexington, KY 40506.

Under earth's gravitational acceleration (G), vertical gradient in pleural pressure (Ppl) was larger in supine than in prone dogs (J. Appl. Physiol. 59: 597, 1985). In 4 anesthetized, spontaneously breathing dogs, we measured Ppl under conditions of 1G, 2.2G and 3.2G. Animals were oriented in our 25 ft radius centrifuge so that resultant acceleration vector (Accl.) was in direction either dorsal-to-ventral (prone) or ventral-to-dorsal (supine), with a negligible cranial-to-caudal vector. Ppl was measured by 2 rib capsules, one positioned near the ventral surface, the other dorsally. Vertical distance between capsules was ~8 cm. Table summarizes Ppl data at FRC (cmH_2O, mean ± SD):

Accl.	Ppl (prone)		Ppl (supine)	
	ventral	dorsal	ventral	dorsal
1G	-2.5 ± 1.3	-5.5 ± 1.3	-4.9 ± 1.4	+0.7 ± 2.4
2.2G	+4.2 ± 3.2	-3.6 ± 1.3	-8.7 ± 3.9	-0.5 ± 4.6
3.2G	+5.6 ±7.6	-2.3 ± 3.3	-10.8 ±3.2	-2.0 ± 4.3

With increased G, Ppl decreased in supine position but increased in prone; thus lung expanded in supine position but contracted in prone. Lung damage may be less under high acceleration in the prone position. (Suppported by HL 40362 and HL 36597).

77.6

NMR MUSCULAR EXERCICE BEFOR AND AFTER LONG DECUBITUS

Jacques PRADERE, CHU Rangueil
1 Avenue du Pr. Poulhès - 31054 TOULOUSE Cedex - FRANCE
(SPON : C.N.E.S.)

Our aims is to study the effect of one month of antiorthostatism as a method of weightlessness simulation for space flights, on eight volunteers. We use a supraconductive magnet Biospec Bruker 2.35 T, 45 MHz 31-P. Each volunteer do a four minutes exercice and rest three minutes, with the leg in the magnet after one month of decubitus, PCr/Pi (phosphocreatine/inorganic phophorus) does not decrease as well as the first time and mainly PCr/Pi restoration is not so good. In agreement with phosphocreatine shutle theory this imply a fall in the oxydative phosphorylation.

77.7

CHANGES OF MUSCLE FUNCTION AND SIZE WITH BEDREST. G.A. Dudley, P.D. Gollnick, V.A. Convertino and P. Buchanan*. The Bionetics Corporation and Biomedical Operations and Research Office-NASA, Kennedy Space Center, FL 32899 and Washington State University, Pullman, WA 99164

The in vivo torque-velocity relations of the knee extensor (KE) and knee flexor (KF) muscle groups of 7 males were determined pre and post 30 days of 6° headdown bedrest to examine changes in muscle strength during simulated microgravity. Computed tomography scans of the thigh and histochemical analyses of needle biopsy samples of the vastus lateralis provided measures of muscle size. The KE and KF showed an average decrease of angle specific, peak torque across speeds of concentric and eccentric muscle actions of 22% ($P<0.05$) and 6% ($P>0.05$), respectively. These responses were not related to the speed or type of muscle action. Total cross sectional area of the thigh decreased ($P<0.05$) by 8±1 %. Slow- and fast-twitch fiber size decreased ($P<0.05$) by 11% (4124±191 to 3656±207 um^2) and 17% (4456±338 to 3672±177 um^2), respectively. The results suggest that strength loss is greater in extensor than flexor muscle groups of the lower limb with bedrest, that musculature atrophy may not solely account for this response and that this model of simulated microgravity does not cause preferential atrophy of slow-twitch fibers.

Conducted under NASA-KSC Contract (NAS10-10285)

77.8

CAROTID BAROREFLEX RESPONSE FOLLOWING 30 DAYS EXPOSURE TO SIMULATED MICROGRAVITY. V.A. Convertino, D.F. Doerr*, D.L. Eckberg, J.M. Fritsch*, and J. Vernikos-Danellis. NASA, Biomedical Operations and Research Office, Kennedy Space Center, FL 32899; Ames Research Center, Moffett Field, CA 94035; and Medical College of Virginia, Richmond, VA 23249.

To determine if exposure to simulated microgravity altered baroreflex function, response curves for the baroreceptor-cardiac reflex were generated in eleven healthy men (30-45 yr) on day 4 of pre-bedrest control (C4), days 1, 3, 12 and 25 of 6° head-down bedrest (BR1, BR3, BR12, BR25) and on days 2, 5 and 30 of post-bedrest recovery (R2, R5, R30). At the end of BR, the subjects underwent a posture test for determination of orthostatic tolerance. The sigmoid baroreflex response curve shifted downward and to the right with the progression of BR and did not return to baseline by R5. Compared to C4, minimum, maximum, range of, and baseline R-R intervals as well as maximum slope of the response curves were reduced ($P < .05$) from BR12 through R5. The reduction in maximal slope of the response curve for syncopal subjects was greater ($P < .05$) than the reduction in non-syncopal subjects ($-1.8 \pm .7$ vs. $-.4 \pm .4$ msec/mmHg). Long-term exposure to microgravity may induce significant resetting and decreased responsiveness and buffer capacity of the carotid sinus baroreflex. Attenuated carotid baroreflex sensitivity may be a primary mechanism contributing to orthostatic intolerance following spaceflight longer than 12 days.

77.9

SECRETION OF ATRIAL NATRIURETIC FACTOR DURING HEAD-DOWN TILT. C. Gharib, G. Gauquelin, G. Geelen, J.O. Fortrat and M. Cantin* Fac. Méd. Grange-Blanche 69373 Lyon Cédex 08 France *Clin. Res. Inst. Montreéal Q.C. H2W 1R7 Canada.

In man, head-down tilt (HDT) has been currently used during the past 15 years to simulate weightlessness. Although until 1983 only vasopressin (AVP), plasma renin activity (PRA) and aldosterone (Aldo) had been studied, it is now obvious that the atrial natriuretic factor (ANF) is involved in the hormonal responses to HDT. To study the role of ANF in such conditions we used 3 protocols : short-term effects (4 hrs), long-term effects (4 wks) of HDT -6°. During a third protocol we studied the effect of a night HDT, as differences have been observed during night in renal responses to an analogous situation (water immersion). Our results in a 4 hr HDT show that the increase in ANF is very rapid and returns to control values in 3 hrs, while PRA and Aldo remain decreased during a longer time. The same response was observed during night HDT, excluding the possibility that the different renal responsiveness at night could be related to a modified ANF secretion. During the 4 wk HDT, no major modifications were noted for ANF, in contrast to what was observed for PRA. Further experiments are necessary to determine to what extent ANF is involved in plasma volume regulation during HDT (and weightlessness).

Grants from CNES (1986-1987), DRET (n° 87.056) and Université C. Bernard (Physiol. Environ.).

RENAL PHARMACOLOGY I

78.1

SPINAL ACTION OF SUBSTANCE P EFFECTING RENAL FUNCTION IN THE RAT. Réjean Couture and Gustave Denis. Dept. Physiology, University of Montréal, Montréal, Québec, H3C 3J7.

Substance P (SP) may be a transmitter in a bulbospinal pathway that regulates renal activity through sympathetic preganglionic neurons originating from the thoracic and lumbar spinal cord. The aim of this study was to measure the changes in renal function elicited by the intrathecal (i.th.) administration of SP at T-9 vertebral level in the pentobarbital anesthetized Wistar rat. Diuresis was induced by an i.v. infusion (150 µl/min) of a solution containing mannitol 2.5% and NaCl 0.45%. Both ureters were cannulated with a PE-10 catheter for urine collection at 5 min intervals. Urine concentrations of sodium (U_{Na}) and potassium (U_K) were measured by flame photometry with an internal lithium standard. Glomerular filtration rate (GFR) was taken to be the clearance of inulin. Mean arterial pressure (MAP) was monitored from a carotid artery. The i.th. administration of SP (10 ng-10 µg) caused a dose-dependent and short-lasting (5 min) fall of the urinary flow rate (Vu), and of U_{Na} and U_K excretion ($U_{Na}V$ and U_KV). These effects were followed by a long-lasting increase of $U_{Na}V$ while Vu and U_KV had returned to basal levels. MAP was slightly increased by low doses of SP (10 and 100 ng) and briefly (5 min) decreased by higher doses (1 and 10 µg). SP (10 µg) also caused a transient decrease of the GFR. All these parameters remained unaffected by the i.th. administration of vehicle (CSF, 10 µl). These results suggest that bulbospinal SP-containing fibers increase renal excretion of sodium via a mechanism which does not appear to be secondary to hemodynamic changes. (Supported by the Canadian Kidney Foundation).

78.2

SYNTHETIC THROMBOXANE A_2 EFFECTS ON PORCINE RENAL BLOOD FLOW. M. Cirino*, H. Morton*, C. MacDonald*, J. Hadden*, A.W. Ford-Hutchinson. Merck Frosst Canada Inc., P.O. Box 1005, Pointe Claire-Dorval, Québec, H9R 4P8.

Thromboxane A_2 (TxA_2) is an unstable arachidonic acid metabolite resulting from the enzymatic actions of cyclo-oxygenase. TxA_2 has been implicated as a mediator of many cardiovascular and renal diseases. The first synthesis of TxA_2 was reported by Bhagwat et al. (1985). In the present studies this methodology was modified and the effects of synthetic TxA_2 (TxA_2-S) on porcine renal blood flow were compared to those of the endoperoxide analog, U44069. Domestic pigs were anesthetized with sodium pentobarbital and instrumented for measurement of systemic (MAP) and pulmonary (PAP) arterial pressures, heart rate (HR) and renal blood flow (FLOW). Injection into the renal artery of saline or platelet poor plasma decreased FLOW by 5% and 2% respectively. Increasing doses of TxA_2-S injected directly into the renal artery produced dose-related decreases in FLOW which were similar to those produced by U44069. However, TxA_2-S was more potent than U44069 in eliciting a response. There were no effects on MAP, PAP or HR. The maximal effects produced by both U44069 and TxA_2-S on FLOW were reproducible and this response remained unchanged after the animals had been heparinized eliminating the possibility that these effects were secondary to platelet aggregation. Both TxA_2-S and U44069 appear to have a modulatory role in regulating porcine renal blood flow.

78.3

DIETARY Na INDUCED CHANGES IN URINARY DOPAMINE AND ELECTROLYTE EXCRETION IN RATS. A.L. Jadhav and M.F. Lokhandwala, Texas Southern University, and University of Houston, Colleges of Pharmacy, Houston, Tx. 77004.

Effects of dietary Na on plasma, urinary, and renal cortical catecholamines (CAs) and electrolytes were studied in male Sprague-Dawley rats for 4 weeks during which they were divided into three groups, each receiving a diet differing in its Na content i.e., 0.29%, 0.05%, and 3.4% for the control, low, and high Na groups respectively. No significant differences were observed in systolic blood pressure; plasma, or renal cortical CAs; and urinary NE or Epi of any group. Urine volume and urinary elimination of Na & K of the high Na group was significantly greater than that of the control group for all four weeks on the diet. Urinary excretion of DA was significantly greater in the high Na group compared to the control group during the first week (39.8± 2.4 and 21.7 ±1.9 nmol/24 hr respectively, p <(0.05), however, it declined significantly during the weeks 2,3, and 4 (0.27 ± 0.12, 0.12 ±0.09, and 4.95± 2.9 nmol/24 hr respectively). These results indicate that high Na intake increased the urinary elimination of DA during the first week without affecting CAs levels in the plasma or the renal cortex, and that DA may have prompted the increased diuresis and natriuresis. It appears that factors other than renal dopamine may have participated in maintaining Na balance during weeks 2 to 4 of high Na intake. (Supported by NHLBI grant H101707).

78.4

SORBINIL PRODUCES NATRIURESIS AND DIURESIS IN RABBITS AND RATS. R. Fildes*, J. Springate*, J. VanLiew, L. Feld and M. Acara. Depts. Pediatrics, Georgetown Univ. Washington,D.C., Pediatrics, Children's Hospital, Physiology, V.A.M.C. and Pharmacology and Therapeutics, SUNY at Buffalo, NY 14214.

The aldose reductase inhibitor, sorbinil, was administered orally to rabbits (65mg/kg.d) and rats (40mg/kg.d) over a five day period to examine how interference with sorbitol production might affect renal function. Urines were collected during access to water and during the final 18hrs of a 24hr. water deprivation, pre and post drug. Sorbinil increased urine flow rate in rabbits during water access from 22.1 to 27.2ml/kg.d (p<.05) and from 9.9 to 15.7ml/kg.d (p.01) during water deprivation. Sodium excretion increased from 1.44 to 2.92mEq/kg.d (p<.01) during water access and from 1.44 to 3.30mEq/kg.d (p<.01) during water deprivation. Sorbinil increased urine flow rate in rats from 2.82 (control) to 3.4ml/kg.d (p<.01) during water deprivation and sodium excretion increased from 1.3 to 1.9mEq/kg.d (p<.05) during this same period. Rats also showed a significant decrease in urine osmolarity from 1544 to 1280mOsm/kg.H_2O (p<.05) suggesting an inability to optimally concentrate urine. Thus, the aldose reductase inhibitor, Sorbinil, is a diuretic and natriuretic agent and its mechanism of action may be related to its inhibition of the production of the intracellular osmotic solute, sorbitol, in the renal medulla.

78.5

PLASMA ELECTROLYTES AND COMPENSATORY RENAL GROWTH IN RATS IN RESPONSE TO STEROIDS AND CYCLOSPORIN A. G.A. Kinson, R. Layberry*, A. Levine* and K. Sarkar*. Univ. of Ottawa Health Sciences, Ottawa, Ontario, Canada K1H 8M5.

Utilizing the unilaterally nephrectomized rat, compensatory renal growth and electrolyte status was evaluated after two weeks of drug treatment. Groups of rats were injected s.c. twice weekly with 0.75 mg/Kg body of testosterone, corticosterone, prednisone or 10 mg/Kg body of cyclosporin A. Blood samples were taken by cardiac puncture for Na, K, Cl, Ca and Mg determinations by flame photometry and colorimetric analysis. The remaining kidney was examined histologically and growth assessed by protein and nucleic acid determinations. The 35% renal overgrowth arising 3 weeks after nephrectomy was not due to change in water content but did involve a significant increase in tissue DNA, suggestive of hyperplasia. Corticosterone and prednisone sustained compensatory renal growth in terms of mass, protein and nucleic acid contents. Cyclosporin A treatment caused additional increases in kidney DNA and RNA whereas testosterone induced marked declines in protein and RNA suggestive of a catabolic action. Nephrectomy was without effect upon plasma K and Cl, but lea to reduction in Na levels. This effect was unaltered by treatment with steroids and cyclosporin A. Testosterone and cyclosporin A gave rise a 10 and 15% drop in plasma Ca with a more pronounced change in Mg levels. Further investigation with other doses will be necessary to more clearly define actions on kidney function.

78.6

A GLYCEROL-INDUCED MODERATE RENAL FAILURE MODEL IN THE RAT. D.L. McClelland*, M.P. Fulks* and R.P. Rosenkranz. Inst. of Pharmacol., Syntex Research, Palo Alto, CA 94304.

A single i.m. 10 ml/kg injection of 50% w/v glycerol (GLY) induces an established acute renal failure in the rat. 50% w/v GLY produces consistent, although severe renal failure (>2/3 reduction in GFR to <0.49 ml/min) often associated with mortality. We attempted to establish a model for pharmacologic assessment of renotropic agents by producing a moderate level of renal failure (GFR ~1 ml/min; serum creatinine [CR] ~1 mg/dl) utilizing lesser GLY concentrations. 24 hr water-deprived male Sprague-Dawley rats 180-280 g were treated with vehicle, 12.5, 25 or 50% w/v, 10 ml/kg, GLY i.m. Blood samples were taken 0, 4, 24, 48 and 96 hr post-GLY for analysis of creatinine CR and BUN. Dose responsive increases in BUN and CR were observed following i.m. GLY treatment. Mean pre-GLY control levels of CR=0.3 mg/dl; BUN=20 mg/dl. Peak GLY-induced levels were: CR=0.5†, 1.4† and 6.3† mg/dl, and BUN=13†, 68† and 257† mg/dl at the 12.5, 25 and 50% concentrations, respectively. The most severe renal disfunction occured at 24 hr following 12.5 and 25% treatment; 48 hr following 50% GLY treatment. Survival at 96 hr was 93, 100 and 67% at 12.5, 25 and 50% GLY, respectively. These results indicate that it is possible to consistently induce a moderate reversible acute renal failure in the rat utilizing GLY at a 25% concentration w/v. This model may prove useful as a rapid pharmacological screening method for renotropic agents. †p<.05

78.7

EFFECTS OF ENALAPRIL IN GLYCEROL-INDUCED MODERATE RENAL FAILURE IN THE RAT. R.P. Rosenkranz and D.L. McClelland*. Inst. of Pharmacol., Syntex Research, Palo Alto, CA 94304.

Glycerol-induced myohemoglobinuria in the rat is characterized by intense renal vasoconstriction and ischemic pathologic changes. Angiotensin converting enzyme (ACE) inhibitors have proven effective in reducing glomerular capillary hypertension and associated sclerotic damage in progressive renal failure models. Our aim was to determine if ACE inhibitors afforded similar kidney benefits in moderate acute renal failure. 24 hr water-deprived male Sprague-Dawley rats 300-350 g were treated with glycerol 25% w/v, 10 ml/kg, i.m., followed by once daily enalapril administration (0.1, 0.3, 1.0 or 4.0 mg/kg, p.o.) for 48 hr. Blood samples were drawn 4, 24 and 48 hr post-glycerol for serum creatinine (CR) and BUN determinations. Lower doses of enalapril (0.1 & 0.3 mg/kg) elicited no changes in renal function or mortality. 1.0 mg/kg of enalapril significantly improved renal function (↓serum CR & BUN) with no increase in mortality. The highest dose (4 mg/kg) significantly reduced renal function (↑serum CR & BUN) and increased mortality. Our results suggest dosage level is of crucial importance in renal failure treatment. Lower doses of enalapril appeared ineffective, while high doses may have resulted in reduced perfusion, a drop in GFR and further deterioration in the failing kidney; only a narrow midrange of doses provided effective renal functional improvement.

78.8

RENAL EFFECTS OF FENOLDOPAM, IN THE ANESTHETIZED DOG. Michael B. Murphy*, Alan S. Bass*, Leon I. Goldberg. The University of Chicago, Chicago, IL 60637.

The renal vasodilator and natriuretic effects of the dopamine$_1$ (DA_1) receptor agonist fenoldopam (Fen) may be compromised by enhanced sympathetic activity and renin release, due to its hypotensive action following systemic administration. Therefore, we examined the response to direct renal artery infusion of Fen (0.05 or 0.1 ug/kg/min) in anesthetized, volume expanded, mongrel dogs. Experiments were performed with, or without the DA_1 antagonist SCH 23390 (0.5 ug/kg/min) i.v. Fen alone did not alter mean BP (MBP;+2±1%) or heart rate (HR;+3±2%). Fen increased renal blood flow (RBF;+42±8%), urine flow rate (V;+94±27%) and sodium excretion ($U_{Na}V$;+61±15%), all p<0.05, without changing glomerular filtration rate (GFR;+4±3%). Thus, filtration fraction (FF;-12±2%) and fractional excretion of sodium (FE_{Na};+78±27%) changed significantly. All indices had returned to control values 20 mins after discontinuing the infusion. SCH 23390 blocked the effects of Fen: RBF;+7±4%, V;-16±10%, $U_{Na}V$;-17±10%, GFR;-3±4%, FE_{Na};-15±9%, while the combination had no systemic effects: MBP;+2±1% and HR;+0±1%, (all p>0.05). Therefore, Fen exerts diuretic and natriuretic effects, by activating intra-renal vascular or tubular DA_1 receptors in the anesthetized dog. NIH Grant GM-22220

78.9

CISPLATIN NEPHROTOXICITY IN STREPTOZOTOCIN (STZ) INDUCED DIABETIC FISCHER 344 (F344) RATS. Laurie Scott* and Monica Valentovic* (SPON: G.O. Rankin). Marshall University School of Medicine, Dept. Pharmacology, Huntington, WV 25755-9310.

The purpose of the following study was to determine if cisplatin nephrotoxicity was altered by diabetes in the F344 rat. Male F344 (210-310 g) were injected with 27 mg/kg STZ or citrate buffer (pH 4.6). Fourteen days post STZ or vehicle administration, rats were injected with 5 mg/kg cisplatin or vehicle. BUN levels were increased ($P<0.05$) 91% and 50% 4 days after cisplatin treatment in the normoglycemic and diabetic rats, resp. Cisplatin markedly increased proteinuria and glucosuria in the normoglycemic rats within 72 hr. Renal cortical slice accumulation of tetraethylammonium (TEA) as well as basal and lactate stimulated p-aminohippurate (PAH) uptake were decreased ($P<0.05$) in normolgycemic rats 4 days after cisplatin injection. PAH and TEA uptake were not altered 4 days post cisplatin injection in the diabetic rats. In a separate series of expts., normoglycemic F344 rats were permitted free access to 0 or 10% dextrose in the drinking water prior to cisplatin 5 mg/kg (ip) injection. Cisplatin increased BUN levels and kidney wt within 96 hrs in the dextrose treated rats. Renal cortical uptake of TEA and lactate stimulated PAH were diminished ($P<0.05$) in the dextrose treated rats. The mechanism for diabetes protection of cisplatin cannot be attributed to glucose diuresis. (Supported by NIH RR05870).

78.10

THE DIRECT EFFECT OF CEPHALORIDINE ON RENAL CORTICAL GLUCONEOGENESIS IN NORMOGLYCEMIC AND DIABETIC TISSUE. Monica Valentovic* and John Ball* (SPON: G.O. Rankin). Marshall University School of Medicine, Dept. Pharmacology, Huntington, WV 25755-9310.

Previous work in our lab has shown streptozotocin (STZ) induced diabetes modulates cephaloridine nephrotoxicity. The purpose of the following studies was to determine the direct toxicity of cephaloridine on renal cortical slices obtained from normoglycemic or diabetic rats. Male Fischer 344 (F344) rats (250-320 g) were injected with 27 mg/kg streptozotocin (STZ, ip) to induce diabetes. Diabetes was confirmed by glucosuria in excess of 200 mg/dl. Fourteen days post STZ or citrate buffer injection, the rats were ether anesthetized, blood was obtained from the dorsal aorta and the kidneys were excised. Renal cortical slices (75-100 mg) were incubated for 15 or 30 min with 0, 1.25 or 2.5 mM cephaloridine at 37°C under an oxygen atmosphere and constant shaking. Pyruvate stimulated gluconeogenesis was measured 45 min after the addition of 10 mM pyruvate. Upon termination of the incubation, the media was centrifuged at 1,000 xg for 10 min at 4°C. Glucose levels were measured using a glucose oxidase method. Cephaloridine produced a dose dependent inhibition of pyruvate stimulated gluconeogenesis in the normoglycemic tissue. Cephaloridine decrease pyruvate stimulated gluconeogenesis less markedly in the diabetic tissue. These results indicate cephaloridine is directly toxic to renal cortical slices from normoglycemic and diabetic rats.

78.11

SMALL ANIMAL MRI: RENAL STUDIES WITH CONTRAST AGENTS. M. Acara, R. Mazurchuk* and R. Fiel* Depts. Pharmacology and Therapeutics, SUNY/Buffalo, 14214 and Biophysics, Roswell Park Memorial Institute, 14263

Magnetic resonance imaging (MRI) of mice and rats using custom-built or small extremity coils in clinical scanners produced high quality images of kidneys in whole body images. T_2-weighted pulse sequences delineated the cortical-medullary-papillary junction (C/M/P) in normal animals. Three contrast enhancing agents: 1) manganese (II) acetate [Mn(II)Ac] , having nonrenal excretion; 2) manganese (III) tetraphenylporphine sulfonate [$Mn(III)TPPS_4$], having renal excretion; and 3) gadolinium diethylenetriaminepentaacetic acid [GdDTPA], a recognized kidney image enhancer modified the magnetic resonance signal intensity by virtue of their paramagnetic properties. All three agents significantly reduced the relaxation time of excised whole mouse kidneys and dissected kidneys from rats when measured at 10.7 MHz in a relaxometer. An increasing T_1 gradient from cortex to papilla was demonstrated with and without enhancing agents. GdDTPA and Mn(III)TPPS but not Mn(II)Ac delineated the C/M/P/ structure when short TR spin echo pulse sequences failed to achieve sufficient contrast. Similarly, the same agents improved the conspicuity of the boundaries when long TR spin echo pulse sequences were used.

Supported in part by Toxicology Research Center, SUNY/Buffalo.

78.12

ENHANCED NEPHROTOXICITY IN NEWBORN RATS ADMINISTERED GENTAMICIN AND VANCOMYCIN. S. Kacew, W.R. Hewitt and J.B. Hook. Dept. of Pharmacology, Univ. of Ottawa, Ottawa, Ont. Canada K1H 8M5 and Dept. of Investigative Toxicology, Smith Kline and French Labs, Philadelphia, P.A.

In humans the incidence of nephrotoxicity is greatly increased in the presence of gentamicin (GEN)-vancomycin (VAN) combination therapy in comparison to the use of either drug along. Since enhanced nephrotoxicity was also reported in pediatric patients, the objective of this study was to determine the influence of GEN-VAN administration on newborn rat kidney metabolic indicators of tissue damage. GEN (100 mg/kg/day) administered (sc) for 4 days elevated kidney weight and phospholipid content but decreased Na+-K+ ATPase, alkaline phosphatase and pyridoxal-5-phosphate. VAN (500 mg/kg/day) given ip increased kidney weight and phospholipid levels associated with a fall in pyridoxal-5-phosphate content. Simultaneous GEN-VAN treatment resulted in a greater rise in renal phospholipid accompanied by further reduction in alkaline phosphatase and pyridoxal-5-phosphate compared to either drug alone. Surprisingly the renal concentration of both antibiotics was lower in the combined group as compared to VAN alone. These data suggest that combination GEN-VAN produces enhanced nephrotoxicity in newborn rat and that concentration of GEN may play a critical role. (Supported by the Medical Research Council of Canada).

78.13

COMPARATIVE TOXICITY OF CEPHALOSPORIN ANTIBIOTICS IN THE RABBIT AND IN A RABBIT KIDNEY CELL LINE (LLC-RK_1). Buening, M. K., Williams, P. D.*, Gries, C. L.*, Laska, D. A.*, and Heim, R. A.* Lilly Research Laboratories, Toxicology Division, Eli Lilly and Co., Greenfield, IN.

The nephrotoxic potential of 4 oral cephalosporin antibiotics, cephalexin, cefaclor, LY195885 and LY171217, were determined by single P.O. gavage at 500 mg/kg. Histopathological changes, blood chemistry, and ex-vivo renal slice function were evaluated after 48 hr. In addition, the viability of renal cells in culture (RK) was tested with each antibiotic at 0.5-2.0 mg/ml for 48 hr exposure using nigrosin dye exclusion as an endpoint of toxicity. Only LY171217 was significantly nephrotoxic in vivo with prominent lesions observed at 500 mg/kg as well as $\geq$ 7-fold increases in BUN and serum creatinine, and 70% decreases in ex-vivo renal slice gluconeogenesis and PAH and TEA uptake. In vitro toxicity to RK cells correlated well with these results yielding TD_{50} values (TD_{50}=dose producing 50% lethality; mg/ml) of 1.86 (cephalexin), 1.41 (LY195885), 1.21 (cefaclor), and 0.41 (LY171217). These results indicate that the cellular toxicity of the oral cephalosporins tested in renal cells in culture correlates well with their nephrotoxic potential in vivo.

78.14

FUNCTIONAL AND MORPHOLOGIC TOXICITY OF CISPLATINUM IN THE AVIAN KIDNEY. Iris M. Fink*, Klaus Stemmer* and William Cacini. University of Cincinnati College of Pharmacy, Cincinnati, Ohio 45267-0004.

Bird et al. (JPET 231: 752, 1984) have reported high susceptibility of chickens to the nephrotoxic effects of the antineoplastic drug, cisplatinum (CIS). The purpose of the present study was to quantify the functional and structural effects of clinically relevant doses of CIS on the chicken kidney. Inulin clearance (CIN), PAH clearance (CPAH), urine glucose and urine protein were determined in mature Barred Rock chickens before and after administration of CIS at doses ranging from 1.75 to 4.0 mg/kg. Dose related decreases in both CIN and CPAH were evident at all doses within 2 to 4 days. Moderate glucosuria was evident at 3.0 mg/kg but proteinuria was not consistently evident. Body weight losses were seen at all doses. Microscopic examination of H&E stained kidney sections showed parallel dose-related morphologic alterations of both proximal and distal tubules within 2-5 days of dosing with CIS. Tubular necrosis was evident at all doses, with glomerular damage seen at 3.0-4.0 mg/kg. These results indicate that the avian kidney is a sensitive model for CIS toxicity studies. The multifocal pattern of damage produced parallels that seen in humans and suggests that advantage may be taken of the avian portal circulation in relevant mechanistic studies of CIS nephrotoxicity. (Supported by Kidney Foundation of Cincinnati and NIH grant # R15 CA47361-01.)

78.15

ROLE OF LIPID PEROXIDATION IN GENTAMICIN CYTOTOXICITY IN PRIMARY CULTURES OF RAT KIDNEY CORTICAL EPITHELIUM I. Swann* and D. Acosta. College of Pharmacy. University of Texas, Austin, Texas 78712

Several reports in the literature of gentamicin-induced lipid peroxidation in vivo prompted an investigation of this phenomenon in cell culture. Primary cultures of rat renal cortical epithelium were used to determine whether lipid peroxidation occurs prior to or during unmistakable cytotoxicity caused by gentamicin. Primary cultures were prepared from the renal cortex of 7 to 12 day old Sprague-Dawley rats and exposed to 2mM, 3mM and 4 mM concentrations of gentamicin in cell culture medium, when the cultures were 2 to 3 days old. Cellular injury was assesed by leakage of cytosolic lactate dehydrogenase (LDH) into the culture medium after 12, 24, 36 and 48 hours of exposure and by morphologic alterations observed by phase contrast microscopy. Significant LDH release occurred after 24 hours of exposure to 3 mM ($p \leq 0.05$) and 4 mM ($p \leq 0.001$) gentamicin and after 48 hours of exposure to 2 mM gentamicin ($p \leq 0.01$). Morphological derangements caused by gentamicin were time- and concentration-dependent and included vacuole formation and cell rounding. Lipid peroxidation, as estimated by malondialdehyde production, occurred, but was not significant, after 6, 12 and 24 hours of exposure to these same concentrations of gentamicin. We conclude that lipid peroxidation, while it may be caused by gentamicin, did not seem to be a factor in the cytotoxicity of gentamicin in cultured kidney cells.

78.16

RESERPINE BUT NOT DENERVATION REGULATES RENAL β-ADRENERGIC RECEPTORS. Timothy L. Fortin* and Pavur R. Sundaresan* (SPON: M.L. Mangiapane). Univ. of Rochester, Rochester, NY 14642.

The effects of unilateral surgical denervation or reserpine (0.5 mg/kg intraperitoneally daily for 7 days) on renal β-adrenergic receptors (BAR) were examined in rat kidney. ^{125}I-Iodocyanopindolol (ICYP) specific binding was used to quantitate the BAR in the particulate fraction prepared from the renal cortex. Denervation had no effect on BAR concentration seven days post surgery (36.1±1.9 vs. 33.9±3.3 fmol/mg protein, n=7). In contrast, reserpine treatment increased BAR concentration 30% (42.0±1.9 vs. 32.3±1.2 fmol/mg protein (± S.E., n=8, $p < .05$)). Tissue catecholamine levels indicated that both the denervation and the reserpine treatment depleted the norepinephrine (NE) levels to a significant extent. The reserpine effect was investigated further. Reserpine increased both β_1- and β_2-adrenergic receptor subtypes, as determined by computerized curve fitting of betaxolol competition curves, but did not change the relative proportion of these receptors. Isoproterenol stimulated cAMP accumulation was increased over control by 49% (27.1±0.7 vs. 18.2±1.4 pmol/mg protein/min, n=6, $p < .05$) after reserpine treatment, indicating that the elevated receptor number was associated with increased function. Our results suggest that factors other than sympathetic nerve terminal NE may be involved in the regulation of renal BAR. (Supported by NIH grant AM-34539.)

78.17

[^{3}H]-IDAZOXAN IDENTIFIES A NON-ADRENERGIC BINDING SITE IN RAT KIDNEY. M.C. Michel*, H.J. Motulsky, P.A. Insel, UCSD, La Jolla, CA 92093

Recent data have indicated that the α_2-adrenergic receptor radioligand [^{3}H]-idazoxan (RX) might label a different site than [^{3}H]-rauwolscine (RAU). In homogenates of rat kidney, RX bound specifically to an apparently single class of sites with a K_d of 22 nM (non-specific binding defined by tolazoline 100 μM). However, the number of binding sites was twice that of RAU. In competition experiments, only half of the specific binding of RX could be displaced by reasonable concentrations of epinephrine, norepinephrine, prazosin, and yohimbine. Specific RX binding was completely displaced by the guanidine guanabenz and some imidazoline compounds (indanidine, UK 14,304 (5-bromo-N-(4,5-dihydroimidazol-2-yl)-6-quinoxalinamine), tolazoline, idazoxan) but only half displaced by other imidazolines (clonidine, oxymetazoline, moxonidine, phentolamine). In contrast, all compounds tested completely displaced specific RAU binding. When phentolamine prevented RX binding to α_2-adrenergic receptors, the competition of the α_2-adrenergic agonist UK 14,304 for RX binding was not sensitive to Na^+ or GTP. We conclude that some (but not all) imidazolines bind with high affinity to two sites in rat kidney: α_2-adrenergic receptors and an additional non-adrenergic site.

RENAL HEMODYNAMICS

79.1

THE AGE-RELATED DECLINE IN CREATININE CLEARANCE IS NOT DUE TO A DECLINE IN CARDIAC OUTPUT. R. S. Danziger*, E. G. Lakatta, J. Tobin* and J. L. Fleg*. Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

Whether the well documented age-associated decline in the glomerular filtration rate, manifest as a decline in creatinine clearance (CCr), is related to an age-related change in cardiac output is presently unknown. To determine the role of cardiac output in the age-associated decline in CCr, we compared 24 hour CCr indexed to surface area (ml/min/m^2) with cardiac index (CI) in L/min/m^2 measured via gated cardiac blood pool scans (Technetium pyrophosphate imaging) in the sitting position in healthy males (n = 101) and females (n = 46) from the Baltimore Longitudinal Study on Aging (ages 25 to 82 yr), selected for the absence of cardiovascular disease (normal resting and maximal stress EKG, normal stress thallium scan), renal disease and relevant medications. By linear regression analysis CCr of the entire group declined with age (CCr = 89.0-0.31(age), r = .31, $p<.001$); resting CI did not vary with age (r = .03, p = .74) and CCr was not related to CI (r = .04, p = .59). Furthermore, CCr was not related to CI in either sex (r = .13, p = .37 and r = .02, p = .85 for females and males respectively). Thus, CCr declines with age even within a population in which CI does not. This indicates that the age-associated decline in CCr is not cardiac in origin but rather intrinsic to the kidney.

79.2

RENAL HEMODYNAMICS IN $HgCl_2$-INDUCED ACUTE RENAL FAILURE (Hg-ARF): INDEPENDENCE FROM RENAL ADENOSINE (ADO). N. Rossi, T. Kontry, S. Gunther, A. Bidani* and P. Churchill*. Wayne State University, Detroit, Michigan 48201, U.S.A.

ADO-mediated renal hemodynamic changes may be pathogenic in reducing glomerular filtration rate in ARF. ADO receptor antagonists are protective in some models of ARF. We studied the role of ADO in Hg-ARF. Rats were treated with $HgCl_2$ 4.7 mg/kg. Control (C) and experimental (E) rats received vehicle or theophylline (TH) twice daily, respectively. Serum creatinine was higher in E at 48 and 72 h ($p<0.05$, N=10). In other experiments, clearances of inulin (C_{IN}) and PAH (C_{PAH}) and fractional excretion of sodium (FE_{Na}) were measured in sham controls (S) or in three groups of $HgCl_2$-treated animals: vehicle (H+V), TH (H+T), and N^6-cyclohexyladenosine (H+C).

	C_{IN}	C_{PAH}	FE_{Na}
S	9.8 ± 0.6	23.6 ± 1.2	0.87 ± 0.15
H+V	4.8 ± 0.3*	11.3 ± 0.8*	2.32 ± 0.24*
H+T	5.5 ± 0.4*	10.6 ± 1.2*	3.85 ± 0.21* **
H+C	0.7 ± 0.2* +	2.3 ± 0.7* +	2.63 ± 0.42*

*$p<0.0001$ vs S; +$p<0.0001$ vs H+V, H+T; **$p<0.0005$ vs H+V, H+C

Lastly, kidneys were perfused at constant pressure in a non-recirculating system. Infusion of $HgCl_2$ (320 to 1250 μM) produced a dose-dependent decrease in perfusate flow. ADO in venous effluent was elevated at 320 μM (1.28 ± 0.15 nM/min/g) and decreased linearly to less than control at 1250 μM (0.23 ± 0.03 nM/min/g). ADO does not mediate the hemodynamic changes in Hg-ARF, but $HgCl_2$ may interfere with renal ADO metabolism.

79.3

EFFECT OF LOW CALCIUM DOSES ON RENAL HEMODYNAMICS AND SODIUM EXCRETION. V. Lahera*, MJ Fiksen-Olsen*, and JC Romero. Mayo Foundation, Rochester, MN 55905.

We studied the effects of intrarenal infusions of calcium gluconate (Ca^{2+}) (1, 10, 100 and 400 μg/kg/min) on renal blood flow (RBF), glomerular filtration rate (GFR), urine (U) excretion of Na^+, and K^+ in 7 anesthetized dogs (Pentobarbital 25 mg/kg). After 2 clearance periods, each dose was infused at a rate of 1 ml/min for 30 min, followed by 3 recovery periods. Five control dogs were infused with saline. The first 3 doses of Ca^{2+} produced 10, 25, and 94% increases in GFR without affecting RBF, while UNa increased 1.5, 2.5, and 9.5 fold respectively along with UK. Fractional excretion of Na^+ (FeNa) increased 1.4, 2 and 4 fold as well. The highest dose of Ca^{2+} induced a 50% reduction in RBF, a 60% reduction in GFR but UNa and FeNa increased further by 30 fold. U volume increased 1.3, 1.5, 3, and 7.5 fold for each Ca^{2+} dose. No changes were observed in blood pressure, plasma Na^+ and K^+. We concluded that low doses of Ca increase GFR, UNa and FeNa. However, the mechanisms for natriuresis appears to be independent of GFR due to a decrease in tubular reabsorption.

79.4

ROLE OF PROSTAGLANDINS (PG) ON GLOMERULAR FILTRATION RATE (GFR) AND URINARY SODIUM EXCRETION (UNaV) IN RESPONSE TO INCREASED RENAL VENOUS PRESSURE (RVP). Mary J. Fiksen-Olsen*, J.C. Burnett Jr., J.C. Romero. Department of Physiology, Mayo Foundation, Rochester, MN, 55905

While increases in RVP are known to increase UNaV and renal interstitial pressure (RIP) without altering GFR, the role of PG in the response has not been defined. Therefore, the effect of increases in RVP (3, 15 and 30 mm Hg) on mean arterial pressure (MAP), renal blood flow (RBF), GFR, UNaV, and urinary prostaglandin E_2 excretion (PGE_2) was studied in 6 pentobarbital anesthetized dogs before and after blockade of PG synthesis with meclofenamate (Meclo) (5 mg/kg i.v.). RIP was estimated with matrix capsules chronically implanted in the kidney. Elevations in RVP increased RIP (6 ± 1, 11 ± 2, 23 ± 4 mm Hg), UNaV (22 ± 7, 24 ± 4, 56 ± 12 μEq/min) and PGE_2 (1046 ± 230, 1404 ± 355, 6939 ± 3944 pg/min). MAP, RBF and GFR remained unchanged. Following Meclo the increases in RVP produced similar increases in RIP and UNaV. PGE_2 levels were undetectable and RBF (126 ± 14, 108 ± 16, 77 ± 13 ml/min) and GFR (22 ± 4, 22 ± 3, 11 ± 3 ml/min) which were lower than during control decreased further with 30 mm Hg RVP, MAP remained unchanged. In summary, PG are important for the maintenance of GFR and RBF during increased RVP, but not in the natriuresis associated with renal vein constriction.

Supported by NIH grant HL16496

79.5

MAINTAINED STRETCH-DEPENDENT MYOGENIC TONE IN RENAL ARCUATE ARTERIES IN VITRO. R. Bevan and G. Goggins, Dept. of Pharmacology, University of Vermont, Burlington, VT 05405

Renal blood flow measured in anesthetized rabbits is efficiently regulated between 75 - 110mm Hg (Ott and Vari, 1979). We have studied maintained stretch-dependent tone in isolated young adult male white New Zealand rabbit renal arcuate artery segments 2mm in length, cannulated, pressurized and filled with oxygenated physiologic salt solution at 37°C and continuously perfused with an identical solution. They were viewed through a microscope coupled with a TV camera and video dimension analyzing system enabling lumen diameter to be measured (Halpern, Osol and Coy, 1984). At 50mm of Hg, lumen diameter was 282 ± 4μm (n=15). Active and passive reproducible lumen diameter changes in response to stepwise 10mm Hg changes in pressure were obtained. Below a mean of 77.5mm Hg ± 24 (n=15) lumen diameter changes were passive, but above this level maintained Ca^{2+}-dependent constrictor responses developed up to about 110 - 130mm Hg. Within this range increases in pressure caused an immediate small rapid dilatation, followed by constriction, reaching equilibrium within a few minutes. Similar responses were obtained in 4 week old rabbits. We conclude that renal arcuate arteries actively contribute to renal autoregulatory responses.
(Supported by USPHS HL 32985).

79.6

SLIGHT PRESSURE NATRIURESIS DURING WATER DIURESIS IN PHENYEPHRINE-INFUSED DOGS. W.H. Waugh and T.E. Bales*. East Carolina Univ. Sch. of Med., Greenville, N.C. 27858

In contrast to Aperia et al (AJP 220:1205,1971), we now report that pressure natriuresis slopes can be slight during water diuresis with low fractional Na excretions (FENa). Loads of 41 mM NaCl-50 mM glucose to 3% body wt. were given to 7 dogs (80 mg/kg chloralose;1 mg/kg pentobarb). Soln of 7 mM NaCl-3 mM $KHCO_3$-100 mM glucose-0.1 μg/ml aldosterone was infused at 0.3 ml/kg/min. With minimal surgery, clearances were done at initial mean renal arterial pressures (MRAP) & during iv infusion of 1-phenylephrine·HCl (PEP) at low pressor rate (2 μg/kg/min) - before (B), during the last 20 min of 35 min of MRAP reduced by suprarenal aortic balloon inflation (Infl), & during the last 20 min of 35 min of resumed high MRAP (R).

	MRAP	V*	NaE*	%FENa	C,Cr*	ERBF*
PRE-PEP	127±10	3.56±1.0	19±9.5	0.169±0.082	72±5	270±32
PEP-B	153±13	6.15±1.0	22±4.5	0.223±0.046	76±4	286±25
PEP-Infl	101±4	5.20±0.8	7.6±2.5	0.079±0.024	71±3	290±17
PEP-R	149±10	7.64±1.0	21±6.3	0.210±0.064	77±3	297±17

Slope decreases in NaE & FENa were only 2.42±0.46* & 0.023± 0.005 % per 10 mm Hg decrease, resp. ($P<0.01$). Percent changes from mean of PEP-B & -R values were 9.6±3.0% & 8.8±3.3% per 10 mm Hg decrease, resp. ($P<0.05$). Serial hematocrits were 33.3± 1.4, 43.2±2.2, 44.1±2.0, & 45.1±2.0%. Serial plasma renin activities were 3.9±1.2, 3.6±1.2, 5.3±1.3, & 3.8±1.2 ng/ml/hr.
*ml or μEq/min/100 g kidney wt; ERBF=effective renal bl flow

79.7

EFFECT OF ACUTE RENAL DECAPSULATION ON PRESSURE NATRIURESIS IN SHR AND WKY RATS. A. A. Khraibi and F. G. Knox, Mayo Clinic, Rochester, MN 55905.

Recently we have shown that pressure natriuresis and diuresis response is attenuated in the spontaneously hypertensive rat (SHR) and that this attenuation is associated with a blunted increase in renal interstitial hydrostatic pressure (RIHP) in comparison with Wistar-Kyoto (WKY) rats. The objective of these experiments was to study pressure natriuresis in SHR and WKY rats during acute renal decapsulation. Renal decapsulation did not affect the relationships between renal perfusion pressure (RPP), RIHP, and fractional excretion of sodium (FE_{Na}), which were blunted in the first place, in the SHR. At RPP of 134±1.2 and 160±3.6 mmHg (n=5), RIHP was 3.8±0.3 and 4.2±0.3 mmHg, and FE_{Na} was 0.11±0.03 and 0.35±0.10% in the decapsulated SHR. In the WKY, acute renal decapsulation (n=4) prevented most of the increase in RIHP observed when RPP increases in control WKY, and almost abolished pressure natriuresis and diuresis response. When RPP was increased from 106±1.2 to 129±2.5 mmHg in the decapsulated WKY, RIHP and FE_{Na} did not change (3.9±0.7 mmHg and 0.26±0.14% to 4.3±0.6 mmHg and 0.33±0.14%). We conclude that the renal capsule is not essential for the demonstration of the already attenuated pressure natriuresis and diuresis response in the SHR; however, its presence is of critical importance for the increase in RIHP and the brisk pressure natriuresis and diuresis response in the WKY rat.

79.8

ADENOSINE RECEPTOR BLOCKADE ATTENUATES ANGIOTENSIN II INDUCED DECREASES IN RENAL BLOOD FLOW IN VIVO. B.J. Holycross* and E.K. Jackson, Vanderbilt University, Nashville, TN 37232.

Angiotensin II (AngII) elicits a decrease in renal blood flow (RBF) resulting from the peptide's ability to constrict both pre- and post-glomerular arterioles. Adenosine (ADO) also constricts preglomerular vessels. Recent evidence suggests a synergism between AngII and ADO at the afferent arteriole. We have demonstrated that (1) in hypertensive states characterized by high circulating AngII levels, there are concomitantly elevated ADO levels and (2) bolus injections of AngII stimulate release of ADO from perfused rat lungs. The purpose of this study was to test the hypothesis that endogenous ADO, whether released by AngII or derived from other sources, participates in the observed AngII-induced decrease in RBF. The specific ADO receptor blocker, 1,3-dipropyl-8-para-sulfophenylxanthine (DPSPX), was employed to block the actions of endogenous ADO. The in situ blood perfused kidney model was used to measure RBF in anesthetized, captopril-pretreated rats. Baseline RBF was identical in saline and DPSPX treated rats. Thirty minute infusions of AngII into the renal artery decreased RBF in saline treated rats by 46% while only a 26% decrease in RBF was observed in DPSPX treated rats. These data suggest that endogenous ADO may participate in the observed AII-induced decrease in RBF.

80.1

THE ROLE OF ANGIOTENSIN II IN THIRST INDUCED BY THERMAL DEHYDRATION OR WATER DEPRIVATION IN RATS. C. C. BARNEY, J. S. Williams and D. H. Kuiper. Dept. of Biology, Hope College, Holland, MI 49423.

Male Sprague-Dawley rats were used to investigate the role of angiotensin II in the thirst induced by two types of dehydration, thermal dehydration and dehydration due to water deprivation. Thermal dehydration was brought about by exposure of rats to a 40°C environment without water for 0-4 hours. Water deprivation-induced dehydration was brought about by denying rats access to water for 44 hours. Exposure of rats to the heat for 2 or 4 hours did not alter plasma renin activity. Bilateral nephrectomy performed two hours prior to the beginning of heat exposure did not alter the increase in water intake observed following 3 hours of heat exposure. Administration of the angiotensin converting enzyme inhibitor captopril (100 mg/kg, i.p.) was also without effect on thermal dehydration-induced thirst. In contrast, water deprivation caused a four-fold increase in plasma renin activity. Administration of captopril (100 mg/kg, i.p.) one hour prior to allowing the rats access to water significantly reduced the water intake of the water deprived rats. Thus, angiotensin II appears to take part in the development of thirst in rats dehydrated by water deprivation but not in rats dehydrated by exposure to heat. (Supported by NIH grant DK 36857-02 and NSF grant BBS-8712566.)

80.2

HYPOTHALAMIC (Ht) PEPTIDASES INACTIVATING GONADORELIN (GnRH) I.A. Kamberi, R.A. Skaf*, R.M. Jaffe* and D.H. Belsky*. Hendrickson Center for Reprod. Med. CH/UMC and Dept OB/GYN, RW Johnson M. Sch. and SOM UMDNJ, Camden, NJ 08103

Previously we have shown that GnRH and amino acid derivatives of 4-nitroanilides are degraded by peptidase activities (PA). In this study, PA towards specific substrates, the 4-nitroanilides of L-alanine, L-leucine, L-phenylalanine, L-tyrosine, L-cystine, L-glutamic acid, and L-glycine, have been investigated in different regions of the Ht of adult male and castrated female rats. It was found that the PA activities with respect to these substrates decreased in the order listed above, in all tissues examined (cerebral cortex, median eminence and anterior, posterior or lateral Ht). The arylamidase activity toward L-leucine-4-nitroanilide (Leu-4-NA) and L-alanine-4-nitroanilide was elevated in Ht tissues as compared to the cortex. In our enzyme kinetic studies we found that the addition of GnRH inhibited degradation of Leu-4-NA in a dose depended manner. Estradiol and progesterone given to castrated adult rats increased the Ht PA with concomitant decrease in GnRH as measured by RIA. Adult female rats androgenized in the neonatal period, also had increased Ht PA and decreased GnRH content as compared to diestrus rats. Conclusion: PA in the Ht modulate, at least in part, the amount of releasable GnRH which in turn triggers the gonadotropin surge and consequently ovulation.

80.3

LOCAL ADMINISTRATION OF D-ALA^2, d-LEU^5 ENKEPHALIN INCREASES PLASMA RENIN ACTIVITY IN ANESTHETIZED DOGS. R. E. Laskey* and R. L. Tackett. Cardiovascular Pharmacodynamics Lab, College of Pharmacy, University of Georgia.

Several recent studies have implicated opioidergic involvement in cardiovascular regulation. The present study was designed to investigate the possible interaction of opioids with release of renin from the canine kidney as measured by plasma renin activity (PRA). Following laparotomy, left and right renal veins were cannulated for sample collection. Electromagnetic flow probes were placed on both renal arteries. Saline infusion was established in the left renal artery at .5 cc/min. Blood pressure and heart rate were monitored. Injection of the specific delta opioid receptor agonist, DADL (12 µg/kg), into the left renal artery caused a significant increase in left renal vein PRA at 1 and 10 minutes post injection without significantly affecting PRA in contralateral renal vein samples. MAP and RBF were not significantly diminished by DADL. Naloxone infusion (7 µg/kg/min) prevented the increase in PRA. Injection of DAGO, a specific mu opioid receptor agonist, had no effect on the parameters measured. Additionally, studies performed with the isolated perfused rat kidney showed an increase in renin secretion rate with the addition of DADL (10^{-6}M) to the perfusate.

80.4

PROSTAGLANDINS AND ANGIOTENSIN AS REGULATORS OF RENIN RELEASE AND PRORENIN ACTIVATION. P. Ioannou*, R. Ismail* and D.H. Osmond. Univ. of Toronto, Toronto, Canada M5S 1A8.

There is a large pool of blood prorenin, for which an activating mechanism, or "convertase", is required to produce systemic renin. The nature of convertase, and its regulation, need to be established, especially the role of prostaglandins (PGs) and angiotensin II (Ang), which are known to regulate the release of active renal renin. Convertase is released by rat renal cortical slices into their incubation medium and is determined by its capacity to activate the extrarenal prorenin present in post-nephrectomy (2NX) rat plasma, which is renin-free. Slices were incubated for 30 min. at 37°C, pH 7.4, in the presence of Ang, 2µM, or PGs, 5.6 µM. As expected, Ang inhibited, while only PGs I_2 and E_2 stimulated renin release, by about 34% in both directions. Ang inhibited convertase activity while PGE_2 stimulated it in the incubate. PGs I_2, D_2, $F_{2\alpha}$, and 6-keto-$F_{1\alpha}$, did not stimulate convertase. Our data point to the release of a convertase by renal slices and to its down regulation by Ang and up regulation by PGE_2. Thus, in addition to PG and Ang regulated renal secretion, active renin may arise by similarly regulated renal and extrarenal prorenin conversion.

Supported by the Heart and Stroke Foundation of Ontario.

80.5

PRORENIN "CONVERTASE" FROM RAT RENAL CORTICAL SLICES. R. Ismail*, L. Mavrogiannis* and D. H. Osmond. Univ. of Toronto, Toronto, Canada M5S 1A8.

Conversion of prorenin to renin in the systemic circulation is not established, leaving open the role of plasma prorenin. Bilateral nephrectomy (2NX) raises plasma prorenin in rats, indicating an extrarenal source, while active renin disappears, suggesting loss of a renal prorenin "convertase". We incubated renal cortical slices at 37°C for 30 min in Krebs-Henseleit buffer, pH 7.4. The resulting incubate converted essentially all the extrarenal prorenin in 2NX plasma to renin. To characterize this powerful renal convertase we used the protease inhibitors trasylol, soybean trypsin inhibitor, lima bean trypsin inhibitor, benzamidine and N-ethylmaleimide, as well as the specific kallikrein inhibitor, Phe-Phe-Arg chloromethyl ketone. None of these inhibited convertase, thereby ruling out kallikrein, plasmin and other favoured proteases. Cathepsins are unlikely candidates because convertase is very active at pH 8. Activity was recovered in the isoelectric pH range of 4.9 to 5.4. Thus, the powerful prorenin convertase may be a new enzyme, or a known one not yet implicated as a prorenin activator.

Supported by the Heart and Stroke Foundation of Ontario.

80.6

CNS ACTIONS OF ANGIOTENSIN II (AII) DURING PREGNANCY. E.W. Quillen, Jr. and B.S. Nuwayhid. McGill Univ., Montreal.

During pregnancy, diminished direct pressor actions of AII are well known but the indirect effects of AII acting via the CNS are not known. Consequently, the pressor response to i.v. infusions of AII at 5,10,20 and 40 ng/kg/min has been observed under normal intact conditions and during ganglionic blockade with hexamethonium (HEX) to determine the direct vs. indirect effects. Possible alterations in the indirect contributions during pregnancy were studied in nonpregnant (NP; n=5) & 115-125 day pregnant (PG; n=6) sheep prepared with arterial and venous catheters and a pulmonary artery blood flow probe. Basal mean arterial pressure (MAP) and cardiac output (CO) averaged 88±2 mmHg and 3.50±0.20 L/min in NP, and 81±3 mmHg and 6.24±0.25 L/min in PG ewes. The rise in MAP at all AII levels was greater during HEX averaging 149% in NP and 210% in PG ewes when compared to the intact response. CO, under intact conditions, was reduced by 14% and 16% in NP and PG, respectively. With HEX, basal CO in NP ewes was unchanged, but was increased by 17% with AII. With HEX, basal CO in PG ewes was reduced to 4.3±0.5 L/min. AII infusion increased CO by 26%. Thus, the rise in total peripheral resistance (TPR) with HEX in NP ewes averaged 80% of the intact response, but the rise in TPR with HEX in PG ewes averaged only 35% ($p<.01$). This suggests that indirect actions account for 20% of the total response in nonpregnant sheep. During pregnancy, the relative role of the central actions of AII are increased and may contribute 65% of the overall pressor action. MRC9804.

80.7

ROLE OF PERIPHERAL 5-HT_2 RECEPTORS IN THE RENIN RESPONSE TO SEROTONIN AGONISTS. Richard H. Alper. University of Kansas Medical Center, Kansas City, KS 66103

We have shown previously that quipazine, a non-selective serotonin (5-HT) agonist, decreases renal blood flow (RBF) and increases plasma renin activity (PRA) when injected intravenously (iv), but not when injected into the lateral cerebral ventricle; the decrease in RBF was not altered by prazosin or enalapril. The current experiments investigated the actions of quipazine and the selective 5-HT_2 agonist ±DOI [±-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl] administered iv on PRA and RBF in chronically instrumented conscious rats. Both 5-HT agonists **decreased** RBF and **increased** PRA. Two highly selective 5-HT_2 antagonists, LY 53857 (100 μg/kg, iv), a drug that enters the CNS, and xylamidine (100 μg/kg, iv), a **peripheral** 5-HT_2 antagonist, abolished the RBF and renin responses to quipazine and DOI. Also, the β-antagonist ±propranolol (1 or 3 mg/kg, iv) did not alter the increase in PRA produced by quipazine and only slightly attenuated the renin response to DOI. Since LY 53857 and xylamidine have been shown to have a similar affinity for 5-HT_2 receptors *in vitro* but differ only in their CNS effects, and propranolol did not markedly alter the renin response to either 5-HT agonist, it appears that quipazine and DOI act primarily within the renal vasculature to reduce RBF thereby increasing PRA.

(Supported by a grant from the PMAF and NIH Grant # HL-38072)

80.8

STUDIES ON INHIBITION OF ANGIOTENSIN II RECEPTORS IN RABBIT ADRENAL AND AORTA. Robert G. Pendleton, George Gessner* and Eugene Horner*. Rorer Central Research, King of Prussia, PA 19406

Angiotensin II (AII) labelled with ^{125}I binds to rabbit adrenal cortical membranes over a concentration range from 0.5 to 20 nM at a single site with a K_D of 5 nM. This binding was inhibited in a surmountable fashion with respect to AII by the peptide analogues Sar^1,Leu^8 AII and Phe^4,Val^5,Tyr^8 AII when not preincubated with their receptors prior to AII addition. With a 30 minute preincubation, however, the former displayed non-surmountable kinetics while the profile of the latter was unaffected.

In rabbit aortic strips with the same preincubation time, the Sar^1,Leu^8 AII analogue was a non-surmountable antagonist of the contractile effect of AII while the inhibition produced by Phe^4,Val^5,Tyr^8 AII was surmountable by increasing agonist (AII) concentrations. The inhibitory effect of the former was maintained after repeated washing of the tissue while that of the latter was readily reversible. Addition of Phe^4,Val^5,Tyr^8 AII to the bath 5 minutes prior to preincubation protected the tissue from the prolonged AII inhibition by Sar^1,Leu^8 AII.

These findings indicate different kinetic modes of AII inhibition by these two antagonists. Phe^4,Val^5,Tyr^8 AII behaves as a reversible, competitive inhibitor of AII binding, while Sar^1,Leu^8 AII combines with the AII receptor in a slowly dissociable manner and is therefore not readily displaced by AII.

RENAL TRANSPORT AND BODY FLUID REGULATION I

81.1

ROLE OF RENAL CAPSULE IN RENAL INTERSTITIAL HYDROSTATIC PRESSURE AND PRESSURE NATRIURESIS OF WISTAR RATS. J. A. Haas, A. A. Khraibi, and F. G. Knox, Department of Physiology and Biophysics, Mayo Clinic, Rochester, MN 55905

The objective of this study was to establish the relationship between renal perfusion pressure (RPP), renal interstitial hydrostatic pressure (RIHP), and fractional sodium excretion (FE_{Na}) after acute renal decapsulation in the Wistar rat. A polyethylene matrix was implanted chronically in each kidney of Wistar rats (n=10), and was utilized for the measurement of RIHP. A servo-controlled cuff was placed around the abdominal aorta for the manipulation of RPP. Acute renal decapsulation was performed on one kidney and the contralateral kidney was used as control. RIHP measured 3.3±0.4 mmHg in the decapsulated kidney and 4.1±0.4 mmHg in the control kidney ($p<0.05$) at an RPP of 100±1.2 mmHg. At this RPP, glomerular filtration rate and FE_{Na} were similar in both kidneys (1.25±0.10 ml/min/g kidney wt. and 0.57±0.15% for decapsulated, and 1.44±0.09 ml/min/g kidney wt. and 0.86±0.17% for control). When RPP was allowed to increase to 123±1.3 mmHg, RIHP and FE_{Na} increased significantly to 4.3±0.4 mmHg and 1.77±0.41% in decapsulated and 6.9±0.5 mmHg and 2.56±0.38% in control kidneys. We conclude renal decapsulation attenuates the relationships between RPP, RIHP, and FE_{Na}. We suggest that other maneuvers may produce shifts in these relationships.

81.2

ROLE OF PROSTAGLANDINS IN PROXIMAL TUBULE SODIUM REABSORPTION: RESPONSE TO ELEVATED RENAL INTERSTITIAL HYDROSTATIC PRESSURE. Y. Kinoshita* and F. G. Knox, Dept. of Physiology, Mayo Clinic, Rochester, MN. 55905

The objective of the present study was to investigate whether proximal tubules respond to the elevated renal interstitial hydrostatic pressure (RIHP) and whether the inhibition of prostaglandin synthesis would alter the effect of elevated RIHP on proximal sodium reabsorption. Expansion of renal interstitial volume by injecting 100 μl of 2.5% albumin solution through a chronically implanted matrix increased RIHP similarly in control rats (from 3.0±0.3 to 5.7±0.5 mmHg, n=8) and in indomethacin (from 2.6±0.3 to 4.8±0.3 mmHg, n=8) or meclofenamate-treated rats (from 2.0±0.2 to 4.7±0.7 mmHg, n=7) rats. In the absence of prostaglandin synthesis inhibition, renal interstitial volume expansion significantly increased the fractional delivery of sodium (FD_{Na}) at the superficial late proximal tubules from 56.5± 6.1 to 67.0±6.5% ($P<0.01$) with an accompanying increase in the fractional excretion of sodium (FE_{Na}) from 2.1±0.5 to 3.0±0.4% ($p<0.01$). In the presence of indomethacin or meclofenamate, renal interstitial volume expansion failed to augment FD_{Na} and FE_{Na}. In summary, these studies demonstrate that the synthesis of prostaglandins might be crucial for renal interstitial hydrostatic pressure to regulate sodium reabsorption by the proximal tubules.

81.3

ARTERIAL PRESSURE (MAP) - URINARY SODIUM EXCRETION (UNAV) RELATIONSHIPS DURING PREGNANCY. B.Nuwayhid and E.Quillen. McGill Univ., Montreal.

To determine the effects of long-term changes in sodium intake on MAP regulation during pregnancy, nonpregnant (NP; n=8) and 110-140d pregnant (PG; n=6) ewes received daily sodium intakes (NaIN) of 25, 75 and 360 mmol until fluid and electrolyte balance was achieved (5-7 days). Daily water intake (H2OIN) and urine output (UV) were varied directly ($p<.01$) with NaIN in the NP, but not in PG ewes. At NaIN of 25 mmol, H2OIN (5220±734 vs. 2601±321 ml) and UV (3245±480 vs. 1878±195 ml) were greater ($p<.01$) in PG than in NP animals. Plasma angiotensin II (pAII) averaged 13.5±1.8 pg/ml in NP ewes at NaIN of 25 mmol, and was undetectable (<2pg/ml) at NaIN of 360 mmol. In PG ewes, pAII (20.8±5.6 pg/ml) was greater ($p<.01$) than the NP level at 25 mmol. With NaIN of 360 mmol, pAII averaged 19.5±6.2 pg/ml. UNaV and 24h average MAP follow:(* - $p<.05$ cp. nonpregnant data)

NP=>	25	75	360	25	75	360 <= PG
UNaV (mmol)	24±4	77±9	344±38	26±7	81±16	338±12
MAP (mmHg)	80±1	80±1	84±2	78±3*	78±2*	79±1*

During pregnancy, the MAP-UNaV relationship is shifted to a lower level, but the sodium sensitivity of MAP unchanged. Also, the inverse relationship of NaIN and pAII is blunted suggesting a reduced role for AII in the maintenance of renal function during pregnancy. Supported by Kidney Foundation of Canada.

81.4

EFFECTS OF A GROWTH HORMONE-RELEASING FACTOR ANTAGONIST ON THE MAXIMUM RENAL REABSORPTION OF PHOSPHATE IN IMMATURE AND ADULT RATS. Aviad Haramati, Michael D. Lumpkin* and Susan E. Mulroney*, Dept. of Physiology, Georgetown Univ. School of Med., Washington, DC 20007

The maximum renal reabsorption of phosphate (TmPi) per ml GFR is greater in immature rats compared to adults, possibly because of the increased demand of the neonate for Pi during growth. We evaluated the role of growth hormone in this process by comparing the effects of an antagonist to growth hormone-releasing factor (GRF-AN: [N-AC-TYR^1-D-ARG^2]-GRF-(1-29)-NH_2) on growth and TmPi in immature and adult rats. We have recently shown that GRF-AN inhibits the pulsatile release of growth hormone in both immature and adult rats. Silastic catheters were placed in jugular veins of 18 immature (4-5 wks) and 11 adult Wistar rats, and either GRF-AN (100 ug/kg) or vehicle was injected I.V. twice daily for 4 days. The rats were then prepared for clearance experiments to determine the TmPi. During the chronic injection period, growth slowed markedly in GRF-AN treated immature rats (body weight increased 5±2% vs 25±3% in control rats). The rate of growth in control adult rats was less than in neonates (7±2%), but was completely suppressed during GRF-AN treatment (-2±2%). The inhibition of growth by GRF-AN was associated with a decrease in the TmPi in immature rats (4.6±0.3 vs 3.3±0.1 umol/ml, $P<0.01$) but not in adults (3.6+0.1 vs. 3.3+0.2 umol/ml). Thus, the effects of GRF-antagonist to inhibit growth and the TmPi are more prominent in immature rats. These results support the notion that growth hormone, directly or indirectly, contributes to the enhanced renal Pi reabsorption in immature rats.

81.5

RESISTANCE TO THE PHOSPHATURIC EFFECT OF PTH INDUCED BY SHORT TERM LOW PHOSPHATE DIET (LPD) IS BLUNTED BY PROPRANOLOL (PRO) IN RATS. A. Rybczyńska*, A. Hoppe*, and F. G. Knox. Dept. of Physiology, Mayo Clinic, Rochester, MN 55905

The LPD induces resistance to the phosphaturic effect of PTH within hours. The present study evaluated if β-adrenergic blockade by PRO might correct this diminished response to PTH. Animals were fed LPD (0.07%) for 0.5, 1, 2, 3, or 4 days before the experiment. All rats were acutely TPTX, and after 1 hour of infusion with or without PRO (20 μg/kg/min) clearance samples were collected. The infusion was supplemented with PTH (synthetic 1-34, 33 U/kg+1 U/kg/min) and after 1 hour samples were recollected.

LPD (days)		0.5	1	2	3	4 (n=5-2)
1. PTH	FE_p%	26.8*	14.0*	7.9	2.2	0.9
2. PRO+PTH	FE_p%	45.4	34.3	14.7	3.9	0.5
Δ (2.-1.)		18.6	20.3	6.8	1.7	-0.4

FE_p, fractional excretion of phosphate; n, number of animals; *$p<0.05$.

In rats fed normal phosphate diet PRO did not increase response to PTH. No differences in plasma phosphate between groups 1 and 2 were observed. We conclude that immediate, but not long term, resistance to the phosphaturic effect of PTH as a result of LPD seems to be mediated by stimulation of renal β-adrenoceptors.

81.6

MAGNESIUM BALANCES AND ^{28}Mg and ^{47}Ca STUDIES IN MAN. Herta Spencer and Dace Osis*. Metabolic Section, V.A. Hospital, Hines, IL 60141

Magnesium balances were determined in adult males for several weeks during a constant analyzed dietary magnesium intake in a Metabolic Unit. Magnesium analyses of the diet and of urinary and fecal excretions were determined. The intestinal absorption of magnesium was determined as net absorption from magnesium balances and from 6-day cumulative fecal ^{28}Mg excretions following an oral dose of $^{28}MgCl_2$ and by the double tracer technique using ^{28}Mg orally and intravenously. The endogenous fecal magnesium was also determined with intravenous doses of ^{28}Mg. The absorption of magnesium determined by the above described three methods agreed well and averaged 47% of the dose of ^{28}Mg or of the dietary magnesium intake. Calcium intakes ranging from 200 up to 2000 mg/day did not change the magnesium balances nor the absorption of magnesium showing that this high calcium intake did not decrease the absorption of magnesium in humans in contrast to results obtained in animal studies. The exretion of magnesium into the intestine, i.e., the endogenous fecal magnesium was low and averaged 7% of the dose. Comparative ^{28}Mg and ^{47}Ca studies showed differences in both the intestinal absorption and the urinary excretion of the two minerals. In chronic renal failure, the intestinal absorption of magnesium was reduced to one third the normal value.

81.7

IDENTIFICATION OF THE ORGANIC CATION (OC^+/H^+) EXCHANGER IN CANINE RENAL BRUSH BORDER MEMBRANE VESICLES. Peter D. Holohan*, Paul P. Sokol* and Charles R. Ross. SUNY-Health Science Center, Syracuse, NY 13210 and Indiana University School of Medicine, Indianapolis, IN 46202

The renal OC^+/H^+ exchanger was identified on SDS-PAGE by irreversibly labeling sulfhydryl (SH) groups with [^{3}H]N-ethylmaleimide (NEM). This was accomplished because the exchanger has essential SH groups whose reactivity is affected by the substrate, N^1-methylnicotinamide (J. Biol. Chem. 261:3282-3287, 1986). The optimal conditions for labeling were established by following the rate of NEM inactivation of NMN transport in the presence and absence of substrate. The labeled peptide was identified by SDS-PAGE under reducing and nonreducing conditions. The findings suggest that the OC^+/H^+ exchanger is an integral membrane protein consisting of a single polypeptide chain of 110kd. (Supported by NIH #02835)

SYMPATHETIC NERVOUS SYSTEM INTERACTIONS

82.1

HYPOXEMIA INCREASES SYMPATHETIC ACTIVITY BUT NOT CATECHOLAMINES IN HUMANS AT REST. P.B. Chase*, D.R. Seals, D.G. Johnson, K.A. Comess* and L.B. Rowell. University of Arizona, Tucson, AZ 85721 and University of Washington, Seattle, WA 98195

The purpose of this study was to determine whether hypoxemia increases skeletal muscle sympathetic nerve activity (MSNA) in humans at rest. In 9 healthy subjects aged 20-34 yr, we measured MSNA (peroneal nerve), venous plasma norepinephrine (PNE) and epinephrine (PE) levels, arterial blood pressure (AP), heart rate (HR), and end-tidal O_2 and CO_2 before (control) and during breathing of: a) 12% O_2 ($\simeq PaO_2$ 40 mmHg) for 20 min, b) 10% O_2 ($\sim PaO_2$ 34 mmHg) for 20 min, and c) 8% O_2 ($\simeq PaO_2$ 28 mmHg) for 10 min -- in random order. MSNA increased above control in 5, 6, and all 9 subjects during 12, 10, and 8% O_2 breathing, respectively, but only after some delay. On the average, MSNA (total activity) increased 83 $\pm$ 20, 260 $\pm$ 146, and 298 $\pm$ 109% above control by the last min of 12, 10, and 8% O_2 breathing, respectively ($\bar{X} \pm$ SD; all $p < 0.01$). However, neither PNE nor PE increased above control during any level of hypoxemia. HR increased inversely with the level of inspired O_2, whereas AP either decreased slightly (10 and 12% O_2) or not at all (8% O_2) vs control. Individual changes in MSNA during hypoxemia were not related to changes in HR, AP, or end-tidal CO_2. We conclude that, in contrast to other sympathoexcitatory stimuli such as exercise or cold stress, moderate to severe hypoxemia increases MSNA but not plasma catecholamines in humans at rest. (Supported by NHLBI HL 16910 and an Arizona Heart Association Grant-in-Aid).

82.2

TEMPORAL DISSOCIATION OF VASCULAR RESISTANCE AND SYMPATHETIC NEURAL DISCHARGE DURING PROLONGED CARDIOPULMONARY BAROREFLEX INHIBITION IN HUMANS. M.J. Joyner, J.T. Shepherd and D.R. Seals. Mayo Clinic, Rochester, MN 55905 and University of Arizona, Tucson, AZ 85721.

The purpose of this study was to determine if prolonged unloading of the cardiopulmonary baroreceptors (CPBR) elicits constant increases in forearm vascular resistance (FVR) and muscle sympathetic nerve activity (MSNA) in humans. In 8 healthy subjects (21-29 yr) we measured forearm blood flow (FBF, venous occlusion plethysmography), MSNA (microneurography, peroneal nerve), heart rate (HR), and arterial blood pressure (AP) during consecutive 20-min periods of rest (control) and non-hypotensive (-15 mmHg) lower body negative pressure (LBNP). During rest, FVR and MSNA averaged ($\pm$ SE) 22.6 $\pm$ 0.5 units and 23.0 $\pm$ 0.5 bursts/min, respectively, and showed no consistent temporal pattern of variability. During the first min of LBNP, FVR increased to 38.0 $\pm$ 2.9 units. By the fifth min of LBNP, FVR had declined to 32.9 $\pm$ 3.4 units ($p < 0.05$ vs min one) and remained lower during the final 15 min of LBNP ($\bar{X}$ = 30.1 $\pm$ 0.9 units; $p < 0.05$ vs min one). In contrast, MSNA increased to 29.0 $\pm$ 2.9 bursts/min during the first min of LBNP ($p < 0.05$ vs control) and remained constant at this elevated level during the entire 20-min intervention period (range 27.4 $\pm$ 2.4 to 33.8 $\pm$ 2.6 bursts/min). HR and AP remained constant during rest and LBNP. These results demonstrate a dissociation between the reflex increases in FVR and MSNA during prolonged inhibition of cardiopulmonary baroreflexes. (Supported by a grant-in-aid from the AZ Affiliate of the AHA and the Mayo Foundation.)

82.3

AGE-RELATED CHANGES IN SYNAPTIC EFFICACY IN RAT SUPERIOR CERVICAL GANGLIA. Ruilin Wu*, David McKenna*, and Donald McAfee, Dept. of Pharmacology, University of California and Nelson Research, Irvine, CA 92715.

Studies in our laboratory demonstrate that sympathetic ganglia in vitro exhibit several forms of synaptic plasticity. A brief period of preganglionic tetany induces a long term potentiation (LTP) of synaptic efficacy lasting hours. Exposure to substances which raise cyclic AMP concentrations also cause LTP. We hypothesize that LTP is a presynaptic cyclic AMP-dependent process which can be induced by substances released during tetany at cyclase-coupled receptors on the preganglionic terminal. We have compared synaptic responses in ganglia from young and aged rats. The ganglia were isolated from Fisher 344 rats at ages less than 4 months and greater than 24 months. The ganglia were superfused with oxygenated Locke solution (25C) in a chamber fitted with bipolar suction stimulating and recording electrodes. Preganglionic stimulation at 5Hz/5sec, 20Hz/5sec, and 20Hz/20sec induced LTP at progressively greater magnitudes and durations. These enhanced responses were significantly greater in ganglia from young rats rather than old. However LTP induced by forskolin, a cyclase activator, was the same or even larger in ganglia from aged rats. This suggests an age-related deficit in release or receptor mechanisms rather than in second messenger systems. Supported by NS27740 and the USC Alzheimer's Disease Research Consortium.

82.4

MODULATED DELAYED POTENTIATION IN THE CAT SUPERIOR CERVICAL GANGLION IN SITU. M.A. Morales* and F. Alonso-deFlorida. Dpto Biofísica y Biomatemáticas. Inst. Invest. Biomed. U.N.A.M. 04510, D.F. México.

Delayed potentiation (DP), including both posttetanic potentiation (PTP) and long-term potentiation (LTP), was investigated in the cat superior cervical ganglion in situ. The temporal course of DP varied characteristically depending on whether maximal or low-level tetanization was applied and on whether or not partial hexamethonium blockade was performed. Differences were observed in the low-level experiments depending on whether a rostral semitransection of the preganglionic trunk was established or submaximal activation of the entire trunk was attained. Remarkable effects were observed under hexamethonium. The variability of the DP course was ascertained by measuring: (a) the time of summit occurrence; (b) the rate of return of the DP through two stages due to the involvement of the PTP and LTP components; and (c) the extent of these components. The intratrain course of the responses also depended on the mentioned tetanization conditions. These results indicate that, through nicotinic receptors presumably located at axo-axonic synapses, some preganglionic endings could modulate the operation of the main preganglionic terminals at axodendrosomatic synapses, which are known to be mediated by the same kind of receptors. The differences found between the submaximal and semitransection experiments indicate the importance of the kind of preganglionic fibres involved in modulation. These results and conclusions are at variance with those of Briggs et al (J. Physiol, 359: 503, 1985) derived from studies in rat ganglion in vitro. They found no critical differences in LTP attributable to the procedure associated to tetanization thus implying a rigid, not modulated posttrain potentiation.

82.5

FACTORS WHICH MODIFY THE CONTENT OF NEUROTENSIN (NT) IN THE STELLATE (SG) AND SUPERIOR CERVICAL (SCG) GANGLIA OF THE CAT. M.M. Caverson, M. Bachoo*, J. Ciriello and C. Polosa. Depts. of Physiology, McGill Univ., Montreal, Canada and Univ. of Western Ontario, London, Canada.

During cholinergic block, stimulation of the preganglionic (SPN) input to the SG or SCG produces a slow increase in heart rate or contraction of the nictitating membrane, respectively. The response is lost with prolonged stimulation (2h) and recovers slowly (days), coincident with loss and recovery of NT-immunoreactivity in SG and SCG fibers. NT injections into the blood supply of these ganglia evokes similar slow responses. These data suggest a role of NT in non-cholinergic transmission. The present study was done, using a NT radioimmunoassay, to obtain quantitative data on the effect of prolonged SPN stimulation or transection on the NT content in 0.5M acetic acid extracts of SG and SCG. In control animals (n=5) the content of NT in RSG or RSCG was not different from that in LSG or LSCG, respectively. However, the SG had a 10-fold greater amount of NT than the SCG. Prolonged electrical stimulation (2h, 40Hz) or chronic decentralization (7 days) of the SPN input to the ganglia resulted in a 40-50% and 80-90% decrease, respectively, in NT content (n=6). Since sensory and post-ganglionic axons contain little NT the measured NT is contained in SPN axons. The depletion after stimulation suggests that NT is released from SPN axons in the SG and SCG.

(Supported by MRC of Canada and Quebec Heart Foundation).

82.6

DESENSITIZATION TO NOREPINEPHRINE AND ISOPROTERENOL IN CONSCIOUS DOGS. N Uemura*, J Nejima*, TH Hintze, DE Vatner, RM Graham*, CJ Homcy, and SF Vatner. Dept of Med, Harvard Med Sch and New Engl Reg Primate Res Ctr, Southboro MA, 01772

To study the extent to which catecholamine desensitization occurs in the intact animal, mini-osmotic pumps were implanted s.q. in 2 groups of dogs instrumented with left ventricular (LV) pressure gauges and aortic catheters. The pumps delivered norepinephrine (NE, n=6) or isoproterenol (ISO, n=5) over 3-4 weeks. Baseline values of LV dP/dt, an index of LV contractility, tended to increase with NE pumps from 3221±247 to 3984±374 mmHg/sec, and with ISO pumps, from 3463±186 to 3993±417 mmHg/sec. The effects of an acute challenge to NE (0.4 μg/kg/min) and ISO (0.4 μg/kg/min) were examined in the same conscious dogs before and 3-4 weeks after pumps. The increases in LV dP/dt (Δ mmHg/sec) in response to the acute challenges of NE and ISO are shown in the table.

	Before NE Pump	After NE Pump	Before ISO Pump	After ISO Pump
NE	1466±341	1100±196	1607±154	321±122*
ISO	4807±619	1914±377*	6006±939	2635±570*

* Significantly less after pump ($p<0.05$)

In dogs with NE pumps the inotropic response to ISO, but not NE, was depressed significantly. In dogs with ISO pumps responses to both NE and ISO were depressed significantly. Thus, desensitization to ISO is readily demonstrated with either chronically elevated levels of NE or ISO, but desensitization to the neurotransmitter, NE, is only readily demonstrable in the presence of chronically elevated ISO.

82.7

PLASMA NOREPINEPHRINE (NE) RESPONSE TO VASODILATORS IN BARODENERVATED RATS. A. Buchholz, M. Nathan, K. Keeton. Univ. of Texas HSC, San Antonio TX 78284

The role of high (HPB) and low (LPB) pressure baroreceptors in the NE response to nitroprusside (NP) and hydralazine (H) was examined in conscious nucleus tractus solitarii (NTS) lesioned, sinoaortic denervated (SAD) or sham (SO) rats. NTS lesions eliminate both HPB and LPB, while SAD removes only HPB. Baseline MAP was similar between groups (SO=107, NTS=112, SAD=113 mmHg). MAP was lowered 30 mmHg in all groups. Plasma NE values are shown below:

		- NE (pg/ml) -		
(*p<.05 vs pre)		SO (9)	NTS (9)	SAD (9)
H	PRE	125 ± 21	135 ± 19	143 ± 31
	POST	213 ± 44*	135 ± 25	93 ± 15*
NP	PRE	130 ± 12	157 ± 36	146 ± 23
	POST	470 ± 62*	202 ± 17	230 ± 32*

Stimulation of the central end of the cut vagii caused depressor responses in anesthetized SO and SAD rats only. The greater rise in plasma NE after NP vs H in SO rats appears to be due to deactivation of both HPB and LPB since NTS lesions, but not SAD, blocked the response. NTS lesions also blocked the NE response to H, but NE is actually depressed by H after SAD. Thus, H appears to deactivate HPB, but may stimulate LPB via an increase in venous return, blunting the plasma NE response to a fall in MAP.

82.8

EFFECTS OF CLONIDINE ON THE EXERCISE PRESSOR REFLEX IN DOGS. P. Tim Wall*, George A. Ordway, Jere H. Mitchell. UT Southwestern Medical Center, Dallas, TX 75235

Clonidine, an alpha$_2$-adrenergic agonist, recently has been shown to attenuate pressor reflexes. We investigated the influence of this drug on the exercise pressor reflex in chloralose-anesthetized dogs. A lumbar laminectomy was performed to isolate the L_7 ventral roots. The cut ventral roots were stimulated bilaterally at 1-3 X motor threshold before and after the administration of increasing doses of clonidine. Clonidine (2.5, 25, and 250 μg) was dissolved in 100 μl of saline and slowly injected intracerebroventricularly (ICV) into the interpeduncular space. Control injections of the saline vehicle also were performed. Prior to the alpha$_2$-adrenergic blockade with clonidine, there were significant ($p<0.05$) increases in arterial pressure and heart rate when the L_7 ventral roots were stimulated. Although clonidine produced a significant dose-related decrease in pre-stimulation arterial pressure and heart rate, ventral root stimulation still produced marked increases in these variables at the two lower doses of the drug. Following the highest dose of clonidine (250 μg), there was a slight decrease in arterial pressure and an attenuated increase in heart rate when the ventral roots were stimulated. These results indicate that, in anesthetized dogs, ICV clonidine lowers arterial pressure and heart rate but has little effect on the exercise pressor reflex, except at relatively high doses.

82.9

EFFECTS OF PROPRANOLOL AND ETHANOL ON ORNITHINE DECARBOXYLASE, RNA AND PROTEIN IN THE RAT HEART. D.C. King* and M. Hirst. University of Western Ontario, London, Canada N6A 5C1.

Severe, ethanol intoxication for 2 days produces cardiac hypertrophy in the rat, an effect suppressed by propranolol. As a result, the time course of effects of propranolol and ethanol on ornithine decarboxylase (ODC), RNA and protein in rat hearts was examined. Male, Sprague-Dawley rats were given ethanol (10%), or isocaloric maltose-dextrin (17%) in a liquid-diet, by gastric intubation, every 8 hr for up to 48 hr: blood ethanol levels in excess of 300 mg/dL were achieved. Animals were decapitated at 0, 8, 16, 24 and 48 hr and hearts were collected. Hearts used for ODC were frozen in liquid nitrogen and all hearts were stored at -80°C until assayed. The results show that ethanol intoxication induced a significant increase in ODC activity by 8 hr. By 48 hr the activity declined to a level not significantly elevated above control. Total RNA content was significantly elevated by 8 hr and remained so over the 48 hr test period. The protein content was significantly increased by 16 hr and continued to increase through the study. All increases were suppressed by co-treatments with propranolol. It is concluded that the ethanol-induced increase in proportional cardiac weight is, in part, the result of an initiation of de novo synthesis of protein. This increased synthesis appears to be preceded by a beta-adrenoreceptor initiated increase in the level of ODC activity and subsequent RNA synthesis during the period of intoxication.

(Supported by the Heart & Stroke Foundation of Ontario)

82.10

A NEW ANTIDOTE TO COCAINE LETHAL INTOXICATION. R. TROUVE*, G.G NAHAS, M. SITBON* and C. LATOUR*. Columbia University, 630 W168th st., New York 10032 and INSERM U26, Hopital Widal, 75010 Paris.

The antidote effects of calcium channel blockers in cocaine lethal intoxication have been attributed their properties of antagonizing the cocaine induced stimulation of sympathetic system. The present study was designed to investigate the possible role of angiotensin I I in cocaine intoxication, by treating it with Enalaprilat (ENL), a converting enzyme inhibitor. 32 rats fitted with a caudal catheter, were connected to a computerized monitoring system. All received 60 mg/kg of cocaine IP and one of the following treatments.

Group	Nb	Enalaprilat	Diazepam	Survival time
1	8	0	0	9'49" ± 4'56"
2	3	0	1 mg/kg	10'08"±6'05"
3	7	.3 +.3 mg/kg*	0	4:12'27"±2'07", 3:>24h
4	7	.3 +.3 mg/kg*	1 mg/kg	>24h
5	7	.3 mg/kg*	.7 mg/kg	>24 h

* each dose of .3 mg/kg administered 4' before and 1' after intoxication in group and 4, group 5 treated 5' after intoxication.

Diazepam is not an antidote to cocaine lethal intoxication. ENL alone controled blood pressure, but it did not prevent convulsions since it does cross blood brain barrier. Combination of ENL and diazepam was an effective treatment: no convulsions occured and cardiovascular parameters were comparable to those obtained with nitrendipine treatment. As AGII is a potent vasoconstrictor and controls sympathetic activity, it is still unclear if ENL exerts its antidote effects through decrease of sympathetic activity only, or if cocaine effects (including inhibition of gabaergic activity in the brain) are mediated by AGII. Both of these effects are perhaps present.

82.11

ASSESSMENT OF THE ACTION OF LOW AND AND HIGH DOSES OF PRAZOSIN ON SYMPATHETIC NEUROTRANSMISSION IN THE ISOLATED MESENTERIC VASCULATURE OF RATS. R. Yamamoto, W.H. Cline and K. Takasaki, Miyazaki Med. College, Miyazaki 889-16, Japan and Southern IL Univ. Sch. of Med., Springfield, IL 62794-9230

The overflow of endogenous norepinephrine (NE) for the isolated rat mesenteric arterial vasculature was determined along with the perfusion pressure responses to periarterial nerve stimulation (PNS) at 4-12 Hz. The PNS-induced pressor responses were abolished by prazosin at 3 x 10^{-8} M but not at 10^{-6} M. The pressor responses to PNS which remained after prazosin at 10^{-6} M were abolished by alpha, beta-methylene ATP at 3 x 10^{-6} M. The PNS-induced endogenous NE overflow was significantly augmented by prazosin at 10^{-6} M but was unaffected by prazosin at 3 x 10^{-8} M. Pressor responses to exogenous NE were abolished by prazosin at both 3 x 10^{-8} M and 10^{-6} M. These findings suggest that, in the presence of prazosin at 10^{-6} M, ATP is coreleased with NE from sympathetic nerves innervating the mesenteric vasculature and that ATP acts as a vasopressor neurotransmitter in this preparation. Prazosin at 10^{-6} M appears to block prejunctional alpha-2 receptors resulting in an increased PNS-induced overflow of endogenous NE. These mechanisms should be considered when using high doses of prazosin in studies of the transmitters involved in the vasopressor responses to PNS in rat mesenteric vascular preparations. (Supported in part by AHA/IL Affiliate).

DRUG ABUSE I

83.1

ANTAGONISM OF THE DISCRIMINATIVE STIMULUS EFFECTS OF NMDA BY COMPETITIVE AND NON-COMPETITIVE ANTAGONISTS. J. Willetts* and R.L. Balster. Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298.

The competitive N-methyl-D-aspartate (NMDA) antagonist 3-((+/-)-2-carboxy-piperazin-4-yl)propyl-1-phosphonic acid (CPP) and the non-competitive NMDA antagonists phencyclidine (PCP) and MK-801 were assessed for their ability to antagonize effects of NMDA in vivo.

Six rats were trained to discriminate 30 mg/kg i.p. NMDA from saline on a standard two-lever fixed ratio 32 schedule of food reinforcement. NMDA (30mg/kg i.p.) was then tested in combination with CPP (0.03-3.0 mg/kg i.p.), PCP (0.1-3.0 mg/kg i.p.) and MK-801 (0.01-0.173 mg/kg i.p.). NMDA-lever responding was dose-dependently antagonized by CPP. PCP did not antagonize NMDA even at doses of PCP that reduced response rates. Only 55% reduction in NMDA-lever responding was observed in the presence of MK-801 and this was accompanied by substantially reduced response rates.

These results indicate that other behavioral effects of PCP and MK-801 are apparent without antagonism of NMDA, and point to differences between competitive and non-competitive NMDA antagonists. (Supported by NIDA Grant DA-01442)

83.2

EFFECTS OF THE ACUTE AND CHRONIC ADMINISTRATION OF MK-801 ON THE RELEASE OF ACTH AND PROLACTIN IN THE RAT. R.N. Pechnick, R. George and R.E. Poland. Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024-1735

In vitro binding studies indicate that phencyclidine (PCP) interacts at two distinct binding sites, termed the PCP and the sigma receptors. The differential roles of these two receptors in mediating the effects of PCP is not currently known. We have found that the acute administration of PCP stimulates the release of ACTH and inhibits the release of prolactin (PRL). While little tolerance develops to PCP-induced ACTH release, inhibition of PRL release is not observed in chronically-treated subjects. MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate] has been found to have a high degree of selectivity for PCP over sigma receptors. The present study characterized the effects of the acute and chronic administration of MK-801 on the release of ACTH and PRL. For acute studies, male rats were injected with 0.9% saline or MK-801 (0.012, 0.12 or 1.2 mg/kg s.c.) and sacrificed 60 min later. For chronic studies, rats were injected daily for 7 days with 0.9% saline or MK-801 (0.012, 0.12 or 1.2 mg/kg s.c.). On Day 8 the rats were sacrificed 60 min after injection. Plasma levels of ACTH and PRL were measured by radioimmunoassay. As we have previously reported, the acute administration of MK-801 stimulates the release of ACTH, but unlike PCP, does not inhibit the release of PRL. In contrast to the effects of chronically administered PCP, significant tolerance occurrs to MK-801-induced ACTH release. PRL levels were not altered in chronically MK-801-treated subjects. Thus, PCP and sigma receptors may be differentially involved in the development of tolerance to the effects of PCP and MK-801 on ACTH release. (Supported by USPHS grants DA-04113 and MH-00534).

83.3

REINFORCING EFFICACY OF SELF-ADMINISTERED PHENCYCLIDINE (PCP) AND KETAMINE IN RATS. K.L. Marquis, M.G. Webb* and J.E. Moreton. Dept. of Pharmacol. & Toxicol., Univ. of Maryland School of Pharmacy, 20 N. Pine St., Baltimore, MD 21201.

Sprague-Dawley rats self-administered PCP (0.125, 0.25 or 0.5 mg/kg/inj) or ketamine (KET; 2, 4 or 8 mg/kg/inj) on a fixed ratio (FR) schedule of reinforcement under limited access conditions (3 hr) following an initial training with cocaine. Baseline number of injections per session (IPS) were determined at FR10. The ratio was then incremented geometrically (10 to 80) every fifth daily session resulting in a decrease in the IPS for each dose of both drugs. This reduction in IPS was greater for PCP at 0.25 mg/kg than at 0.5 mg/kg while the 0.125 mg/kg dose was not reliably self-administered by all subjects. Similarly, the rate of decrease in IPS for the various doses of KET was different. Using this procedure the relative reinforcing efficacy of the different doses of PCP and KET could be discriminated. Percentage of IPS at each FR did not discriminate the reinforcing efficacy of the different doses of each drug as well. A comparison of this data with progressive ratio tests using a substitution procedure will be presented. (Supported by NIDA Grant DA03173)

83.4

MORPHINE SELF-ADMINISTRATION DURING ADJUVANT-INDUCED ARTHRITIS IN RATS. F.L. Smith*, J.E. Heavner and W.H. Lyness. Departments of Pharmacology and Anesthesiology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Pain-free animals implanted with chronic i.v. cannulae rapidly learn to self-inject morphine (5.0 mg/kg/injection) and maintain a daily drug intake of 92.6 ± 16.2 mg/kg/24 hr despite a gradual increase in Fixed-Ratio schedules up to FR-16. Rats pretreated with Freund's adjuvant were monitored daily for changes in paw diameter, body weight and response to tail pressure. After the onset of paw edema and an increased sensitivity to tail pressure (164.8 ± 11 mm Hg vs. 231.7 ± 9 mm Hg in vehicle controls), arthritic animals were implanted with chronic cannulae and allowed access to the self-injection device. These rats maintain a morphine intake of 32.6 ± 7 mg/kg/24 hr even with increasing FR-schedules. Injection of indomethacin (0.5 mg/kg s.c.) failed to alter morphine self-injection in pain-free rats but markedly reduced self-injection in adjuvant treated arthritic rats. These data suggest that animals in a chronic pain state and allowed access to morphine, self-administer the drug for its analgesic actions and not its euphorigenic properties.

83.5

EFFECTS OF LSD ON FLASH-EVOKED POTENTIALS IN THE AWAKE, RESTRAINED CAT: AN ANALYSIS OF PRINCIPLE COMPONENTS. Douglas M. Wilkison. Medical College of Wisconsin, Milwaukee, WI 53226.

Actions of lysergic acid diethylamide (LSD) have been described on the retina, lateral geniculate, and cortex of the visual pathway. No hypothesis relates these actions to the changes in perception produced by LSD and other hallucinogens. To investigate global effects of LSD on sensory integration, flash-evoked responses at multiple cortical sites, including visual-specific (VIS) and sensory non-specific areas of the posterior suprasylvian gyrus (PSS) were studied in seven unanesthetized cats implanted with electrodes under halothane anesthesia. Single doses of LSD (25-100 µg/kg) were administered iv once a week. Predrug, up to 3 hr postdrug, and 24-hr data samples were collected. A principle component analysis of the combined data (SAS) yielded six independent factors. Factor 3 (100-200 msec latency) was significantly increased by LSD, dose effect $P < 0.01$ at PSS and VIS. Factor 5 (30-60 msec latency) was significantly depressed at all sites. Factor 5 likely represents the primary visual response which is depressed by LSD. Factor 3 is indicative of alterations in long latency integrative function. These effects of LSD appear closely related to visual projection pathways, rather than more global nonspecific areas. Supported by RO1 DA03785.

83.6

THE EFFECTS OF CHRONIC MARIJUANA SMOKE EXPOSURE ON COMPLEX BEHAVIOR IN THE RHESUS MONKEYS. M.G. Paule, D.E. McMillan, J.R. Bailey*, A.C. Scallet*, S. F. Ali* and W. Slikker, Jr. NCTR, Jefferson, AR 72079

A large scale study involving 30 periadolescent male rhesus monkeys was designed to assess the behavioral effects of exposure to marijuana smoke for 1 yr. All monkeys were trained (n=7-8/group) under 5 complex operant schedules, matched between groups for behavioral performance and body weights, and exposed to either the smoke of 1 marijuana cigarette (mask) once a day, 7 days/wk, or 2 days/wk. Two control groups were also exposed 7 days/wk; One received smoke from a cigarette devoid of cannabinoids (placebo) and one was sham-exposed (no smoke). Behavioral assessments occurred 5 days/wk 23 hr after exposures. Chronic marijuana smoke decreased task completion in conditioned-position response (high dose) and incremental repeated acquisition (IRA) tasks (low and high dose). The decreases noted in the IRA task were primarily due to decreased accuracy of responding. When compared to either placebo or sham groups, progressive-ratio response rates and breakpoints were significantly decreased in both the low and the high dose marijuana smoke groups. These data demonstrate that complex behaviors can be routinely assessed in a large number of monkeys. The data also suggest that chronic marijuana exposure whether daily or on weekends only, produces deficits in complex behavior. (Supported in part by NIDA-IAG #224-83-0005).

83.7

EFFECTS OF CHRONIC MARIJUANA SMOKE EXPOSURE ON URINARY CORTISOL (UC) EXCRETION IN THE RHESUS MONKEY. J.R. BAILEY*, M.G. PAULE, S.F. ALI*, A.C. SCALLET* AND W. SLIKKER, JR. DIV. OF REPROD. & DEVELOP. TOX., NCTR, JEFFERSON, AR 72079.

Periadolescent male rhesus monkeys were assessed for 24 hr UC excretion by RIA before and during 1 yr of marijuana smoke exposure. Groups of monkeys (n=15 or 16), matched for body weights were exposed to either the smoke of 1 marijuana cigarette (2.5% delta-9-tetrahydrocannabinol [THC]) by mask 7 days/wk (high dose), or 2 days/wk (low dose). Two control groups, one receiving smoke from an extracted cigarette devoid of cannabinoids (placebo) and one sham exposed (no smoke), were also treated daily. Plasma THC concentrations were 76 ± 22 ng/ml 45 min after smoke exposure in the high dose group and < 2 ng/ml (below assay sensitivity) in the control groups. UC baseline values from all groups were not significantly different ($p > 0.05$). There were no significant differences in UC between the low dose marijuana, placebo and sham groups during the 12 mo of exposure. There was however, a significant overall treatment effect ($p < 0.0001$) with UC excretion in the high dose group higher than all other groups at 1 mo, higher than placebo controls at 4 mo and higher than sham controls at 6 and 8 mo ($p < 0.01$). These data suggest that chronic daily marijuana smoke exposure in the monkey produces a physiological stress response as indicated by elevated UC excretion. (Supported in part by NIDA IAG #224-83-0005).

83.8

TESTICULAR METABOLISM OF GLUCOSE IN RATS EXPOSED TO DELTA-9-TETRAHYDROCANNABINOL AND COCAINE. Syed Husain, Dept. of Pharmacology, School of Medicine, University of North Dakota Grand Forks, ND 58201.

Metabolism of energy substrates like glucose and fructose is vital to the functioning of different tissues in the body. This is particularly true in case of brain and testis. Previously, we reported that delta-9-tetrahydrocannabinol (THC) has an inhibitory effect on the metabolism of these substrates in the testis. The following studies were conducted to compare the THC effects with cocaine (COC) on the metabolism of glucose in the rat testis. Groups of rats were treated acutely with 10 mg/kg,po,THC or 40 mg/kg,ip,COC. Control rats received 2 ml/kg of sesame oil or saline. THC treated rats were sacrificed 2 hr post injection whereas COC animals were killed at 15 and 30 min following drug administration. Their testes were removed and sectioned into small pieces. With these tissues, radio-respirometric studies were conducted using 5.5 mM radiolabled glucose as the substrate. Testes from rats treated with THC showed a 29% inhibition ($p<0.001$) in glucose metabolism as compared to their controls. On the other hand, testicular tissue of rats treated with COC and sacrificed either at 15 or at 30 min had no significant difference in glucose metabolism from their respective controls (3.76±0.59 vs. 3.68±0.64 and 2.89±0.26 vs. 2.84±0.32 µmol CO_2/g dry tissue/100 min). These initial observations suggest different effects of THC and COC on glucose metabolism in rat testis (supported by NIDA grand DA03595).

83.9

COCAINE INDUCES HYPERTENSION AND ANALGESIA BY A CENTRAL NERVOUS SYSTEM (CNS) MECHANISM. S.M. Standish*, J.A. Kiritsy-Roy*, and L.C. Terry. Department of Neurology, Veterans Administration Medical Center and University of Michigan, Ann Arbor, MI 48105.

Cocaine (C) is a potent CNS stimulant that has recently been reported to induce a state of analgesia in several rat models (Lin et al., Brain Res., in press). In addition, systemic administration of C increases blood pressure (BP). These experiments test the hypothesis that C acts in the brain to cause analgesia and hypertension.

Male, Sprague-Dawley rats were surgically implanted with cannulae in the carotid artery for BP and heart rate (HR) recording, and in the right lateral cerebral ventricle for intracerebroventricular (icv) drug administration. BP and HR were recorded continuously in conscious rats and antinociception was assessed using the hot plate test (52.5°C) with a 45 sec cut-off latency. C was injected at doses of 50-250 ug in 10 ul icv. Control animals received 10 ul of saline. Separate groups of rats had systemic injections of C (250 ug intraarterially or 25 mg/kg ip).

Icv injections of C produce dose-related increases in BP. Systolic BP increases from 124±5 to 169±20 and 212±8 mmHg and diastolic BP increases from 84±6 to 113±10 and 145±6 mmHg at 2 min after 200 and 250 ug of C, respectively. HR increases marginally only with the highest dose of C. Lower doses are ineffective. Intraarterial injection of 250 ug of C has no significant effect on BP or HR, indicating that the effect occurs centrally. The higher dose of 25 mg/kg ip increases systolic and diastolic BP by 31±4 and 24±4 mmHg and reduces HR by 60±10 bpm. Hot plate response latency increases from 17±2 sec to 30±6, 41±4 and 45±0 sec at 5 min after 62.5, 125 and 250 ug of C icv. Latencies return to baseline by 20 min.

These results indicate that cardiovascular effects of C are mediated at site(s) within the CNS. These studies also suggest that the antinociceptive properties of C in the rat hot plate test are related to a central rather than a peripheral mechanism. (DK 37669)

83.10

COCAINE: TOLERANCE AND CARDIOVASCULAR CONSEQUENCES. Mario D. Aceto, Susan M. Tucker* and Geoffrey S. Ferguson*, Dept. of Pharmacol. and Toxicol., Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613.

The right jugular vein and descending aorta of male Sprague-Dawley rats in the weight range of 200-250 g were cannulated for purposes of drug administration and measurement of direct blood pressure, respectively (Weeks, J.R., J. Appl. Physiol. 19, 540, 1964). When fully recovered from surgery, unanesthetized rats were given a bolus dose of cocaine (80μg/kg in 50μL saline i.v.). Blood pressure (mmHg; Mean ± S.E.M.) promptly rose from 86±6 to 130±7 and returned to pre-injection levels within 15 sec. When this dose was given at minute intervals complete tolerance developed by the 4th injection. In other experiments, a time-course study conducted at 10, 20 and 40 min indicated that tolerance gradually dissipated over 40 min. This dose regimen of cocaine also produced abnormalities in the ECG which were obvious by the 7th injection and were characterized by altered or missing P waves and a slowed heart rate. These results provide further evidence that dramatic changes in cardiovascular measures in the rat are associated with clinically relevant doses.

Supported by NIDA Grant DA02396.

83.11

BUPRENORPHINE IN COMBINATION WITH COCAINE: EFFECTS ON THE PERFORMANCE OF A DISCRIMINATION IN RATS. C.W. Berthold* and J.M. Moerschbaecher. LSU Medical Center, New Orleans, LA 70112.

Responding in rats was maintained by food presentation under a fixed-ratio discrimination procedure. Drug effects on this discriminative performance were evaluated using a cumulative-dosing technique. When administered alone, cocaine (0.32-18 mg/kg) decreased overall response rate in a dose-dependent manner, but increased errors only at the higher doses. Buprenorphine, when administered alone, had little or no effect on the performance of the discrimination at doses lower than 0.1 mg/kg. When administered in combination, buprenorphine at a dose of 0.018 shifted the cocaine dose-effect curves for both response rate and errors to the left by approximately 1/2-log unit. Similarly, buprenorphine at a dose of 0.032 and 0.1 mg/kg shifted the cocaine dose-effect curves to the left by approximately 1- and 1 1/2-log units respectively. These data replicate and extend to rats, previous results obtained in monkeys (Pharmacologist 29:201, 1987) indicating that the effects of cocaine may be modulated by doses of buprenorphine which have no effect when administered alone. The results further suggest that buprenorphine may substantially increase the behavioral toxicity of cocaine in relation to complex discriminative behaviors. (DA 03573 & DA 04775).

83.12

ATTENUATION OF BEHAVIORAL EFFECTS OF COCAINE AND AMPHETAMINE BY 5'-N-ETHYLCARBOXAMIDE ADENOSINE. R.D. Spealman and V.L. Coffin*. Harvard Medical School and New England Regional Primate Research Center, Southborough, MA 01772

The behavioral effects of cocaine and d-amphetamine were studied alone and after pretreatment with 5'-N-ethylcarboxamide adenosine (NECA) or haloperidol in squirrel monkeys. When administered alone cocaine (.03-1.0 mg/kg) and d-amphetamine (.01-0.3 mg/kg) produced dose-related increases in response rate under fixed-interval schedules involving different consequences and schedule parameters. NECA (.0003-.03 mg/kg) and haloperidol (.0003-.03 mg/kg) had little effect on response rate at lower doses, but decreased responding at higher doses. Pretreatment with NECA (.001-.01 mg/kg) attenuated the rate-increasing effects of both cocaine and d-amphetamine. The degree of attenuation depended on the dose of NECA and usually was greater when NECA was combined with d-amphetamine than when it was combined with cocaine. Haloperidol (.001-.01 mg/kg) also attenuated the rate-increasing effects of cocaine and d-amphetamine. Over the range of doses studied, haloperidol and NECA were comparably effective. NECA might have utility in the pharmacological management of stimulant intoxication. (Supported by USPHS Grants DA02658, DA00088, DA00499 and RR00168).

83.13

INFLUENCE OF MECAMYLAMINE, ATROPINE AND NICOTINE ON THE COCAINE-INDUCED LOCOMOTION IN MICE. Arvind K. Chaturvedi. Dept. of Pharm. Sci./Toxicol., Coll. of Pharmacy, N.D. State Univ., Fargo, ND 58105

Cocaine has been reported to interact with nicotinic, as well as muscarinic, acetylcholine (ACh) receptors. Therefore, to evaluate the possible involvement of the cholinergic receptors during the behavioral effects of cocaine, influence of mecamylamine (ME; 7.5, 14.9, 29.9 & 59.8 umol/kg, ip), atropine (AT; 7.2, 14.4 & 28.8 umol/kg, ip) and nicotine (NI) on the cocaine-induced locomotion in ICR male mice (21-24 g) was investigated. The locomotion was measured using animal activity cages crossed with six photocells. Interruptions of photocells were recorded by electromechanial counters. A dose-response curve indicated that cocaine at 44.1 umol/kg, ip, produced a submaximal locomotion (5909 counts/mouse/hr). This value was about 225% more than the basal locomotion. This cocaine-induced locomotion was decreased (7-64%) by pretreating the animals with ME, while it was enhanced (23-47%) by the AT pretreatment (n=10). NI, at 1.5 & 12.3 umol/kg, ip, doses, was effective in enhancing the locomotion by about 33%. However, at 30.8 umol/kg dose, NI decreased the locomotion by 91%. These observatons indicated that the locomotor actions of cocaine, at least in part, are mediated by cholinergic receptors. It appears that the blockade of nicotinic receptors lowers, while the blockage of muscarinic receptors enhances the cocaine-caused locomotion.

83.14

INHERITANCE OF SUSCEPTIBILITY TO THE LOCOMOTOR STIMULATING ACTION OF COCAINE. Lance Logan*, John M. Carney*, and Thomas W. Seale*. (SPON: D. Christensen). Univ. Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK 73190 and Univ. Kentucky Col. Med., Lexington, KY 40536

Little is known about the genetic determinants influencing inherent susceptibility to the behavioral effects of central stimulants. Two strains of inbred mice, C57BL/6ByJ and BALB/cByJ (8 wk old males obtained from the Jackson Laboratory), differed markedly from one another in the efficacy of (-)cocaine HCl-induced stimulation of locomotor activity (LA). Dose response curves indicated that C57BL/6ByJ mice (n=10 each dose) were stimulated by ip cocaine doses up to 56 mg/kg. Maximal stimulation (324%) occurred at a dose of 32 mg/kg. Maximal stimulation of BALB/cByJ mice by cocaine, only 54% above basal LA values, occurred at a dose of 17.8 mg/kg. Higher doses had no effect or depressed activity in this strain. The 32 mg/kg dose which maximally stimulated LA in C57BL/6ByJ mice had no significant effect on BALB/cByJ mice. This dosage was chosen to evaluate the genetic transmission of the cocaine response trait. F_1 hybrid animals from reciprocal crosses showed an intermediate level of LA stimulation. LA responsiveness of 6 CXB recombinant inbred lines established that 3 lines were phenotypically indistinguishable from their BALB/cByJ progenitor and 3 responded in a manner similar to the C57BL/6ByJ strain. These data suggest that the LA cocaine susceptibility difference between these mouse strains is inherited as an incompletely dominant autosomal trait which may be under the control of a single major gene.

83.15

COCAINE - INDUCED ARTERIAL INJURY IN RABBITS. C.L. Bement*, L. Cohen*, S.W. Nielsen* and R.O. Langner, University of Connecticut, Storrs, CT 06268

The potential for cocaine administration to induce arterial damage in rabbits was evaluated. Male New Zealand rabbits were given daily IV injections of cocaine (5 mg/kg) for 14 days. Control rabbits were injected with saline. Heart rate was monitored prior to, during and following the injections. On day 15 the rabbits were killed, the thoracic aorta was removed, a section was taken for histological evaluation, and the remaining tissue was incubated in ^{14}C-proline. Following incubation the tissue was homogenized and assayed for protein synthetic rates and protein, cholesterol, collagen, cyclic AMP and calcium content. The injection of cocaine resulted in the development of lesions in the aorta that were characterized by severe disruption and fragmentation of the elastic media and minimal intimal changes. Biochemically the aortas exhibited increased collagen and noncollagen protein synthetic rates and elevated cyclic AMP levels. The injection of cocaine did not cause uniform injury since two populations were identified on the basis of biochemical changes in the aorta. The arterial injury was not the result of cocaine-induced elevation in heart rate. These data show that repeated injections of cocaine are capable of inducing arterial damage; therefore, cocaine abuse should be considered as a risk factor in the pathogenesis of arteriosclerosis. (Supported by NIDA Grant DA03846.)

83.16

DIFFERENTIAL ACTION OF HALOPERIDOL ON THE MOTOR ACTIVITY STIMULATING EFFECTS AND REWARDING PROPERTIES OF COCAINE IN C57BL/6J MICE. Melissa Place*, Lance Logan*, John M. Carney* and Thomas W. Seale*. (SPON: D. Christensen). Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190

(-)Cocaine is believed to exert its behavioral and physiological effects through its blockade of catecholamine and dopamine reuptake and its local anesthetic actions. The ability of (-)cocaine HCl to induce stimulation of locomotor activity (LA) and drug-seeking behavior, assessed by the conditioned place preference (CPP) paradigm, and the ability of the dopamine antagoinist, haloperidol, to block these effects were investigated. Low intraperitoneal doses of cocaine (e.g. 1 mg/kg) had no significant effect on LA but induced CPP following 3 days of conditioning. Maximal stimulation of LA and of CPP occurred at a dose of 32 mg/kg ip. Dose response curves were determined for the action of haloperidol on both basal and cocaine-stimulated LA. A 0.3 mg/kg dose of haloperidol completely blocked the stimulation of LA by cocaine (32 mg/kg) but had no impact on the acquisition of CPP induced by this dose. A dose of haloperidol which decreased basal LA by 60% did not impair expression of cocaine-induced CPP. From these data it appears 1) that LA and CPP require different doses of cocaine to stimulate maximal effects, 2) that the rewarding properties of cocaine, as judged by CPP, are not dependent on locomotor activity stimulation and 3) that the two behaviors are not equally susceptible to the blocking effects of haloperidol.

83.17

ACUTE EFFECTS OF AMPHETAMINE (AMP) ON COMPLEX OPERANT PERFORMANCE IN RHESUS MONKEYS. G.E. SCHULZE* AND M.G. PAULE, NCTR, JEFFERSON, AR 72079

The acute effects of iv AMP on performance in a food-reinforced operant test battery (OTB) were examined. The OTB contained incremental repeated acquisition (IRA, n=9), conditioned-position response (CPR, n=7), progressive-ratio (PR, n=8), delayed matching-to-sample (DMTS, n=6), and temporal response differentiation (TRD, n=5) tasks. Performance in these tasks is thought to depend upon specific brain functions such as learning (IRA), color and position discrimination (CPR), motivation to work for food (PR), short-term memory and attention (DMTS), and time perception (TRD). AMP (0.01-1.0 mg/kg), given 15 min presession produced significant dose-dependent decreases in the number of reinforcers obtained in each task. Response accuracy was significantly decreased at doses of 0.3 and 1.0 for TRD and at 1.0 mg/kg for CPR when compared to saline injections. Accuracy was not consistently affected in the DMTS and IRA tests. Response rates decreased or response latencies increased significantly at doses of 0.3 and 1.0 in the PR and DMTS tests and at the 1.0 mg/kg dose in the CPR and IRA tests. A dose of 0.1 mg/kg significantly decreased percent task completed for the IRA and TRD tests, 0.3 mg/kg for DMTS, and 1.0 mg/kg for the CPR tests. Thus, the relative sensitivities of these tests for detecting AMP behavioral effects were IRA = TRD > PR = DMTS > CPR.

PSYCHOTROPICS

84.1

EFFECTS OF ß-CARBOLINE-ETHYL ESTER ON PLASMA CORTICOSTERONE--A COMPARISON WITH DIAZEPAM WITHDRAWAL. Richard M. Eisenberg and Catherine Johnson*. Univ. Minn. Duluth, Sch. of Med., Dept. Pharmacology, Duluth, MN 55812.

Diazepam has been shown to produce physical dependence based on changes of behavioral or hormonal indicators during antagonist-precipitated withdrawal. The behavioral excitation appears similar to that observed following the administration of ß-carboline esters--agents reported to interact with benzodiazepine receptors and termed "inverse agonists." Experiments were done in conscious unrestrained male Sprague-Dawley rats, with chronic i.v. catheters, using sound-attenuated one-way vision boxes. These studies compared the hormonal and behavioral changes induced by ß-carboline ethyl ester (ßCCE) with CGS-8216-precipitated withdrawal in rats treated with diazepam for 8 days. Chronically treated diazepam rats (5 mg/kg) showed a significant increase in plasma corticosterone (CS) following CGS-8216. Behavioral abstinence scores were also significantly elevated. ßCCE (0.5-5.0 mg/kg) showed a significant dose-related increase in plasma CS. Behavioral scores were also increased at doses of 0.5 and 2.0 mg/kg. ßCCE-induced plasma CS increases were antagonized by CGS-8216 at doses of 1.0 and 2.0 mg/kg but not by 0.5 mg/kg. In animals chronically treated with diazepam, ßCCE evoked a more prolonged plasma CS elevation than in vehicle-treated animals suggesting a dual agonist/antagonist effect. These data suggest similarities between the action of ßCCE and diazepam withdrawal. (supported by DA 03845)

84.2

ELECTROPHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF CLONIDINE IN MAUDSLEY MR/Har AND MNRA/Har RATS. R.L. Commissaris*, A. Curtis*, H.J. Altman*, G.M. Harrington* and J. Marwah. (SPON: R.T. Louis-Ferdinand). Sch. of Med. and Coll. Pharmacy, Wayne State Univ., Detroit, MI 48202.

Based upon differences in open field defecation and behavior in the conditioned suppression of drinking (CSD) paradigm, the Maudsley Reactive (MR/Har) and Non-Reactive (MNRA/Har) lines have been proposed as a genetically-based "animal model" for the study of anxiety and/or emotionality. It has been proposed that stress and/or anxiety relates to increased activity of noradrenergic neurons in the nucleus locus coeruleus (LC). The present study examined the possible relationship between LC activity and CSD behavior in MR/Har and MNRA/Har rats. Both basal activity and the responsiveness to the alpha-2-adrenergic agonist clonidine were examined. MNRA/Har rats accepted more shocks in non-drug CSD sessions than did their MR/Har counterparts. There was no strain difference in basal firing of LC neurons in these rat strains. In the CSD, clonidine (5-80 ug/kg, IP) did not exert an anti-conflict effect in either strain. Clonidine (1.25-10 ug/kg, IV) depressed LC activity to a similar extent in the two strains. These data suggest that the MR/Har versus MNRA/Har differences in CSD behavior are not related to differences in LC activity or alpha-2-adrenergic sensitivity. (Supported by MH #42501-01; studies in accordance with NIH Guide).

84.3

ACUTE AND CHRONIC BEHAVIORAL EFFECTS OF LORAZEPAM AND ALPRAZOLAM IN MICE. T. Burke,* L. G. Miller* and J. M. Moerschbaecher. LSU Medical Center, New Orleans, LA 70112.

Two groups of mice were maintained under either a fixed-ratio (FR) 30 schedule or a fixed-interval (FI) 2-minute schedule of food presentation. Both lorazepam and alprazolam produced dose-dependent decreases in response rates under the FR schedule. However, under the FI schedule, alprazolam produced an inverted U-shaped dose-effect curve. That is, at low doses, response rates were generally increased, while at higher doses response rates decreased. In contrast, lorazepam only decreased overall response rates. The FI schedule was found to be ten-fold more sensitive to the rate-decreasing effects of both drugs than was the FR schedule. These same drugs were also administered chronically to mice maintained under a FI 2-minute schedule. Tolerance developed to the rate-decreasing effects of lorazepam in all animals tested while, with alprazolam, tolerance developed either more slowly or not at all. The behavioral results were compared to the results obtained from an in vivo benzodiazepine receptor binding assay in each of these same subjects. The data suggest that the development of tolerance is accompanied by a down-regulation of benzodiazepine receptors. (DA03573, DA04775, DA05258).

84.4

STUDY ON PLASMA NEUROLEPTIC LEVELS BY RADIORECEPTOR ASSAY IN SCHIZOPHRENIC PATIENTS RECEIVING HIGH DOSES. M.-A. Gagné[1,2,3*], T. Di Paolo[1,3*], H.J. Cormier[2*], G. Leblanc[2*], S. Vaillancourt[2*] and D. Lévesque[1,3*]. (SPON: F. Labrie[1]). [1]Molecular Endocrinology Research Unit, [2]Mental Health Research Unit and [3]School of Pharmacy. Laval University, Québec, Québec G1V 4G2, Canada.

Clinical efficacy of neuroleptics (NL) is well established; however, wide individual variations in the response to NL is observed in patients receiving similar doses. In this study, plasma levels of various NL were measured using a radioreceptor assay (RRA) on 32 outpatients of a mental hospital who consented to have their medication reduced. Subjects, suffering from severe mental disorders but whose clinical condition was stable for at least 3 months, had been exposed to high dosages of NL (the equivalent of 18 mg or more of haloperidol per os per day) for at least 6 months. For the first 5 months, dosages of NL were reduced by 50% at the rate of 10% per month and then the reduced dosages of NL were maintained for 5 months. The RRA that we have set up is based on the method of Creese and Snyder (Nature, 270:180, 1977) and uses haloperidol competition for [^{3}H]spiperone binding to dopamine receptors in rat striatum membranes. Plasma levels of neuroleptics were measured 3 times: before and immediately after the 50% reduction and finally 5 months later. A significant decrease of neuroleptics plasma levels following the 50% reduction ($p=0.0027$) and 5 month later ($p=0.001$) is observed (Wilcoxon signed-rank test). A linear correlation is observed between neuroleptics plasma levels obtained by RRA and the administered doses (in "haloperidol" equivalents), before the reduction ($p=0.0011$) and 5 months after the 50% reduction ($p=0.0054$). However, no correlation between plasma levels and doses was observed when assayed immediately after the 50% reduction. These results will be discussed in terms of therapeutic window, symptomatology and clinical usefulness of RRA assays. Supported by the Medical Research Council of Canada..

84.5

EFFECT OF PIMOZIDE AND HALOPERIDOL ON BIOGENIC AMINES METABOLIZING ENZYMES. Nieves Madrid and F.S. Messiha. Texas Tech. Hlth. Sci. Ctr., Lubbock, TX and Dept. of Pharmacology, University of North Dakota School of Medicine, Grand Forks, ND 58201.

Pimozide (PMZ) and haloperidol (HAL) are the drugs of choice in the management of Tourette's disease which may implicate a dopaminergic (DA) dysfunction. The aldehyde metabolite of DA is either reduced by alcohol dehydrogenase (ADH) or oxidized by aldehyde dehydrogenase (ALDH) to the corresponding neutral or acidic metabolite, respectively. The short-term effect of PMZ or HAL, (30 mg/kg, ip/day) given for 2 days, on endogenous liver (L) ADH and L-ALDH was studied in adult male rats. The HAL and PMZ treatment inhibited L-ADH by 42% ($p < 0.01$) and 51% ($p < 0.001$) from controls, respectively. Cytoplasmic (CT), but not mitochondrial ALDH, was inhibited by HAL from controls by 33% ($p < 0.01$) compared to 43% ($p < 0.001$) inhibition determined by PMZ. Conversely, HAL, but not PMZ, induced heart CT lactate dehydrogenase isoenzymes LDH_1 and LDH_5 by 16% ($p < 0.05$) and 31% ($p < 0.02$) from controls, respectively. The results suggest that PMZ has less cardiac toxicity compared to HAL and indicate the sensitivity of ADH and ALDH to these drugs. The inhibition of hepatic enzymes studied could cause a build-up and/or alter metabolic pathway of endogenous monoamine aldehydes. (Laboratory work was performed at the Initial Institution)

84.6

A MODEL OF CENTRAL SIGMA SYSTEMS. Edgar T. Iwamoto. Dept. of Pharmacology, Univ. of Kentucky, Lexington, KY 40536

A major gap in the understanding of the functions of the sigma receptors of the CNS is the lack of information about the effects that are elicited after the endogenous sigma system is activated. We have evidence for a model of central activation of endogenous sigma systems in the rat. The model is the locomotor syndrome that is initiated by a challenge of 1.6 mg/kg SC of d-butaclamol (dB) at time t = -30' and 10 mg/kg SC of l-N-allylnormetazocine (lN) at t = 0' in rats which had received 4 daily injections of 10 mg/kg of lN prior to the dB-lN challenge. The dB-lN challenge caused a bizarre-behavior syndrome of backwards walking, side-to-side head movements, and hindfoot pivoting which peaked at t = +10'; this was followed by intense forward locomotion (LOCO) which peaked at t = +60', range +15' to +110'. The bizarre-behavior/LOCO syndrome was not antagonized by the following SC treatments given at t = -20': 50 mg/kg sulpiride, 0.2 mg/kg spiperone, and 0.2 mg/kg R(+)SCH23390; however, the magnitude of the syndrome was significantly diminished by 30 mg/kg IP of rimcazole (BW234U) and by 0.2 mg/kg SC of haloperidol. The data suggest that the dB-lN-induced bizarre behavior/LOCO syndrome is not mediated by dopamine since it is not blocked by D_1 or D_2 antagonists; the data further suggest that the syndrome may be mediated by central sigma mechanisms since it was blocked by rimcazole, a sigma receptor antagonist, and by haloperidol, which has a high affinity for CNS sigma binding sites. (Supported by KTRB and UKMCRF)

84.7

CORTICAL BETA ADRENOCEPTORS (BAC) WITH HIGH (R_H) AND LOW AGONIST AFFINITY (R_L): EFFECT OF ATYPICAL ANTIDEPRESSANTS AND CHARACTERIZATION OF THE INDUCIBLE (R_L) POPULATION. David D. Gillespie, D. Hal Mānier and Fridolin Sulser. Vanderbilt U. Sch. of Med., Nashville, TN 37232.

The mianserin induced desensitization of the NE BAC coupled adenylate system in the rat cortex has been shown not to be linked to a downregulation of BAC's. Non-linear regression analysis of agonist competition binding curves following mianserin explains this discrepancy. Thus, chronic administration of mianserin significantly decreases R_H (linked to adenylate cyclase) while not changing either R_L or the agonist affinities of isoproterenol for the 2 sites. The chronic administration of fluoxetine had no effect on either R_H or R_L in the cortex of the normal rat or of rats with an increased R_L following 5,7-dihydroxytryptamine. The pharmacological analysis of the inducible R_L sites rules out $5HT_2$ and $5HT_{1A}$ sites but shows that this receptor population has characteristics of a $5HT_{1B}$ site. (Supported by USPHS grant MH29228 and the Tennessee Dept. of Mental Health and Mental Retardation).

84.8

INHIBITORY ACTION OF SOME TRICYCLIC ANTIDEPRESSANTS (TCA) ON THE PRESYNAPTIC MUSCARINIC RECEPTORS OF THE ADRENERGIC NERVE-TERMINALS IN THE RABBIT HEART. G.T.Somogyi* and J.M. Perel. Clinical Pharmacology Program,Western Psychiatric Institute and Clinic,Univ.of Pittsburgh,Pittsburgh, PA 15213.

Strips of the right atrial tissue obtained from male New Zealand rabbits were incubated in Krebs buffer containing 10 uCI/ml ^{3}H-NE at 30°C.After 90 min washing with Krebs solution containing luM cocaine and yohimbine, 2 min effluents were collected for 96 min.The preparations were stimulated 4 times for 3 min S_1,S_2,S_3,S_4(field stimulation 2Hz 1ms 60V) and the tritium content of the samples was measured by scintillation spectrometry. S_1 served as a control and at S_{2-4} oxotremorine dose response curve was taken expressing the dose-related inhibition of the evoked release of ^{3}H-NE as percent of the control. Atropine shifted the dose-response curve to the right producing a pA_2 value of 9.4. The TCAs also displaced the dose-response curve to the right but their inhibitory effect was two orders of magnitude lower and the maximal inhibitory effect was reached at around 5 uM.The order of efficacy was as follows: amitriptyline>imipramine>nortriptyline>desipramine.These results are in good correlation with the isolated muscarinic receptor studies as well as with the cardiotoxic effects of TCAs experienced in the course of clinical applications. The corresponding hydroxy metabolites are being currently evaluated. (Supported in part by NIMH Grant MH-30915 and FFRP #77-612).

84.9

EFFECTS OF AMITRIPTYLINE AND ETHANOL ON SYNAPTIC PLASMA MEMBRANE STRUCTURE. M.A. Carfagna[1*] and B.B. Muhoberac[2*], Department of Pharmacology/Toxicology[1], Indiana University School of Medicine and Department of Chemistry[2], Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46223. (SPON: H.R. Besch Jr.[1])

In vivo studies have indicated greater than additive CNS depression by amitriptyline and ethanol which results in impaired psychomotor abilities. We have previously reported that the in vitro combination of amitriptyline and ethanol synergistically inhibits Na^+/K^+ ATPase (NKA) activity of rat synaptic plasma membranes (SPMs) (The Toxicologist 8(1):37, 1988). In the present study the effects of these drugs on SPM structure were evaluated by examining the binding characteristics of the fluorescent biomembrane probe 1-anilinonaphthalene-8-sulfonic acid (ANS). ANS binds noncovalently to biomembranes near the phospholipid head groups, and its fluorescence is sensitive to changes in its microenvironment. ANS titrations (10-50 μM) with Scatchard analysis gave K_{app} and F_∞ values at various in vitro concentrations of amitriptyline (0-20 μM) and ethanol (0, 100 and 200 mM). Amitriptyline produced a concentration-dependent increase of up to 18 % in F_∞ that correlates ($r=0.99$, $p<0.01$) with the 0-100 % inhibition of NKA activity over the same concentration range, while K_{app} and λ_{max} remained unchanged. The invariant K_{app} indicates no change in ANS binding strength. However, the increase in F_∞ along with the unchanged λ_{max} indicates that amitriptyline perturbs the SPM surface as to increase the number but not the polarity of ANS binding sites. In contrast, ethanol had no effect on F_∞, K_{app} or λ_{max}. Ethanol is known to change biomembrane structure toward the interior of the bilayer. Thus, these simultaneous but distinct structural alterations of SPMs by amitriptyline and ethanol may be responsible for the synergistic inhibition of membrane-bound NKA activity. (Supported by NIAAA 6935)

EPILEPSY: ANTICONVULSANTS, NEUROPHARMACOLOGY AND PATHOPHYSIOLOGY

85.1

AN INTEGRATIVE MODEL OF LONG TERM BRAIN EXCITABILITY FOR SEIZURE REGULATION IN THE EPILEPTIC GERBIL INVOLVING FEEDBACK, FEEDFORWARD, AND ADAPTIVE MECHANISMS, Paul L. Prather* & W. B. Iturrian, Dept. of Pharmacol. & Toxicol., Univ. of Georgia, Athens, GA 30602

Current treatment of epilepsy emphasizes symptomatic as opposed to rectifying interventions. Maintenance of long term homeostasis involves feedback, feedforward, and adaptive control systems. This concept, when applied to control of brain excitability, offers novel insight into epileptogenesis and potential development of curative treatments for epilepsy. We chose the epileptic gerbil, a widely accepted model of tonic-clonic epilepsy, to quantify relevant components in a systems analysis approach to the long term (days) control of seizure susceptibility. We suggest a model in which specific endocoids, such as prostaglandins, opioids, peptides, and benzodiazepines provide sequential feedback information about current brain excitability to an analyzer (or oscillator). By feedforward mechanisms, this activity is then relayed to an integrator. Although many factors affect integration, a critical modifier appears to involve the interval (in days) between seizure testing. The amplitude of the signal is adjusted by adaptive contributions such as habituation, postseizure inhibition and relative degree of sensory kindling. A composite command is then produced, seizure or no seizure. This model, if substantiated, provides various points of intervention not addressed by epileptic research and treatment to date.

85.2

SYNERGISTIC ANTICONVULSANT ACTION OF NIMODIPINE AND MK-801 IN MICE ADMINISTERED PENTYLENETETRAZOL. G.T. Bolger* and S.K. O'Neill* (SPON: C.R. Triggle) Division of Basic Medical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3V6

Both NMDA-receptor antagonists and nimodipine have been shown to possess anticonvulsant activity in mice administered the cortical convulsant pentylenetetrazole(PTZ). We have investigated the effects of i.p. administered nimodipine and the potent NMDA antagonist MK-801, either alone or in combination, on PTZ (85 mg/kg i.p.) convulsions in male CD-1 mice. PTZ produced severe clonic convulsions and posture loss in 85% of the mice (mean onset 71 ± 3 sec; mortality 23% following full tonic-clonic seizures). Nimodipine (20 mg/kg) increased the mortality (30%-50%) from PTZ convulsions. In contrast, 10 and 20 mg/kg nimodipine significantly increased (~ two fold) clonic convulsion onset time. MK-801 (0.1 and 0.5 mg/kg) neither altered the onset time nor the number of animals experiencing clonic convulsions, but did prevent death due to PTZ convulsions. Combinations of nimodipine and MK-801 at doses as low as 2 mg/kg and 0.5 mg/kg respectively, resulted in an increased onset (~ three fold) and a reduction (50%) in both the number of animals experiencing clonic PTZ convulsions and the severity of the convulsions. Furthermore, death due to PTZ convulsions was prevented at all dose combinations of nimodipine and MK-801 investigated. These results suggest that the combination of MK-801 and nimodipine may provide a safe and beneficial adjunct therapy for epilepsy. (MK-801 is (+)-5-methyl-10,11-dihydro-5-H-dibenzo(a,d)cyclohepten-5,10-imine maleate)

85.3

SEIZURE SUSCEPTIBILITY AND OSMOLALITY: EPHAPTIC INTERACTIONS ARE IMPORTANT. R. David Andrew. Department of Anatomy, Queen's University, Kingston, Ontario, K7L 3N6.

Seizure susceptibility generally increases as plasma osmolality is lowered. Conversely, seizure is less frequent when osmolality rises. The evidence comes from a diverse experimental and clinical literature over several decades yet the cause is unknown. We examined the effects of osmolality changes upon epileptiform activity induced by 0 Mg^{2+} saline in hippocampal slices. In -30 mOsm saline, interictal burst amplitude recorded extracellularly from stratum pyramidale increased by 41% ± 21.1% (SE) from control (n = 9 slices). In +30 mOsm, amplitude decreased by 27% ± 9.3%. Intracellular records revealed little change in single cell properties. In 12 slices with CA1 cells displaying repetitive electrographic seizures, + 30 mOsm saline reduced ictal spike amplitude (n=12) and could terminate seizures altogether (n=8). A -30 mOsm change increased spike amplitude (n=12) and reactivated seizures in 4 of 7 slices. The evidence suggests that lowered osmolality reduces the extracellular space which increases field (ephaptic) interactions arising from the larger amplitude population events. The restricted space may also amplify the depolarizing effect of K^+ accumulating extracellularly during seizure onset. Thus, hyposmotic swelling of cortical neurons will tend to synchronize and promote population discharge characteristic of the epileptic state.

85.4

EFFECTS OF ASPARTAME (ASM) AND ITS METABOLITES ON SEIZURE SUSCEPTIBILITY IN MICE. Pauline Chiu* and Dixon M. Woodbury. Univ. of Utah, Dept. Physiol. Salt Lake City, UT 84108.

ASM given orally at 200 mg/kg to C57BL/6J (non-susceptible) and DBA/2J (audiogenic seizure-susceptible, AGS) mice 27 days old significantly increased minimal electroshock seizure threshold (mEST) at 1/2 hr; it stayed elevated in DBA mice but decreased in C57 mice at 4 hrs. ASM-treated DBA mice had significantly higher AGS intensity (I) at 1/2 and 1 hr but lower AGSI at 2, 4 and 8 hrs. At 1/2 hr, 200 mg/kg of ASM did not significantly change the CD_{50} for kainic acid (KA) or bicuculline (BIC) in both strains, but at 4 hrs, KA CD_{50}s were directly and BIC CD_{50}s were inversely correlated with mEST. In C57 mice ASM decreased mEST at 4 hr dose-dependently (10-1000 mg/kg), but not in the DBA strain. In DBA mice given ASM (100-1000 mg/kg), the CD_{50} of BIC for tonic seizures varied inversely with AGSI, whereas the CD_{50} for clonic seizures varied directly with AGSI. The BIC CD_{50} in C57 mice decreased slightly at doses below 100 mg/kg. In cobalt-induced focal epileptogenic model, ASM (200 mg/kg) slightly enhanced cobalt-caused elevation of mEST in 22 day implanted C57 mice but decreased AGSI, mEST and BIC CD_{50} in DBA mice at 4 hr. However, in DBA mice tested 6 days after cobalt implantation, ASM increased susceptibility to both AGS and BIC. In 26 day old Swiss-Webster mice, ASM (200 mg/kg, 4 hr) decreased mEST in fed and to a greater extent in fasted (20 hr) mice. The mEST changes produced by aspartic acid, phenylalanine, alone or in combination, generally paralleled those produced by ASM. These data suggest that ASM possesses both excitatory and inhibitory effects in the brain. The time-, dose-, strain-related biphasic effects of ASM appear to be related to the different actions of its metabolites (Supported by a grant from International Life Sciences Institute-Nutrition Foundation).

85.5

EFFECTS OF ANTITUSSIVES ON EPILEPTIFORM ACTIVITY AND NMDA RESPONSES IN HIPPOCAMPAL SLICES. D.J. Braitman* and J.P. Apland* (SPON: M. Adler). Neurotox. Br., U.S. Army Med. Res. Inst. of Chem. Defense, A.P.G., MD 21010

The centrally acting non-narcotic antitussives dextromethorphan (DM), caramiphen (CM) and carbetapentane (CB) are potent anticonvulsants (Tortella and Musacchio, Fed.Proc., 46:708, 1987). DM blocks epileptiform activity in neocortical slices by blocking NMDA receptors (Wong et al., Neurosci. Ltrs., 85:261, 1988). We have investigated the effects of DM, CM and CB on epileptiform activity and responses to NMDA in hippocampal slices. Transverse slices of guinea pig hippocampus were placed in a total submersion chamber at 32°C in normal oxygenated Ringer's for extracellular recording. DM (50-100 uM) blocked responses to bath-applied NMDA as well as epileptiform activity induced by Mg^{++} free medium, but had little effect on the amplitude of the field potential evoked by stimulation of the Schaffer collaterals. Responses to NMDA were blocked for two hours after exposure to DM. In contrast, CM and CB (100-300 uM) reduced the amplitudes of field potentials, but did not block NMDA responses. However, CM and CB did block epileptiform activity induced by Mg^{++} free medium. These results indicate that these antitussives all block epileptiform activity in hippocampal slices in contrast to their actions in olfactory cortex (Apland et al., Neurosci. Abstr., 1988).

85.6

EFFECTS OF CLONAZEPAM ON REM SLEEP IN THE COBALT-EPILEPTIC RAT. Brenda K. Colasanti and Charles R. Craig. Depts. of Pharmacol./Toxicol. and Ophthalmol., WVU Health Sciences Ctr., Morgantown, WV 26506

Adult female Sprague-Dawley rats rendered epileptic by bilateral cerebral implantation of cobalt wire were simultaneously prepared with permanent cortical and temporalis muscle electrodes for continuous recording of EEG and EMG activities. Clonazepam (4,10 or 40 mg/kg) in gum acacia was administered intraperitoneally once daily for 5 days commencing 9 days after cobalt placement. Cobalt-epileptic rats either not treated or administered the highest volume of vehicle and normal rats administered the highest clonazepam dose served as controls. Results indicated that a dose-related delay in onset of REM sleep occurred in the epileptic rodents during the majority of the treatment days. The delay in REM sleep onset in response to the high dose, however, was not significantly different from that for naive rats. REM sleep time after the high dose, which completely suppressed seizures, likewise did not differ from that of epileptic controls or naive rats on any of the treatment days. On the other hand, these values were significantly higher than those for rats receiving the 10 mg/kg clonazepam dose, which had a lesser effect on seizure frequency. These results suggest that the epileptogenic state of cobalt-treated rats may lead to differential effects of clonazepam on REM sleep.
[Supported by NIH (NINCDS) Grant # NS 20226.]

85.7

ALTERATION OF THE ANTICONVULSANT ACTIVITY OF BENZODIAZEPINES. G.J. Yutrzenka (SPON: A.A. Hagen). Dept. Physiology and Pharmacology. Univ. So. Dak. Sch. Med. Vermillion, SD 57069.

Chronic benzodiazepine (BZ) administration has been linked to the development of tolerance to the anticonvulsant activity of these drugs. The present study evaluated the time-course for the development of tolerance to the anticonvulsant action of diazepam (DZ) and midazolam (MZ). Male, Sprague Dawley rats (200-250g) were prepared with ip cannulas for chronic infusion of DZ (2.5 or 5.0 mg/kg/24hr) or MZ (2.5, 5.0 or 10 mg/kg/24hr). Rats were infused for 8 days with DZ or MZ and then with vehicle for 4 days. Prior to and at 2, 4 and 8 days of BZ infusion and at 2 and 4 days of subsequent vehicle infusion, rats were challenged with pentylenetetrazol (PTZ) administered via tail vein using a timed infusion method. PTZ was infused until onset of a myoclonic convulsion and a Minimal Convulsive Dose (MCD) was determined. Chronic DZ administration resulted in a slight, non-significant, reduction in MCD during the 8 days of DZ infusion. During the following drug-free period, the MCD was noted to be significantly increased to values that were 90% to 120% greater than the initial MCD. The MCD for MZ was noted to be significantly decreased at 4 and 8 days of chronic infusion with a return to initial MCD values by day 4 of the drug-free period. Thus, there was evidence for the induction of, and recovery from, tolerance to the anticonvulsant activity of DZ and MZ.
(Funded by a grant from the General Research Fund, University of South Dakota.)

85.8

SOME CONVULSANTS SHOW HIGH AFFINITY FOR ^{35}S-TBPS BINDING SITES BUT LOW TOXICITY. Richard F. Squires and Else Saederup,* NKI, Orangeburg, NY 10962.

Several polychlorinated convulsant insecticides have been reported to potently block the binding of ^{35}S-TBPS to sites on rat brain membranes (Lawrence and Casida, Life Sci., 35:171, 1984) and we have confirmed these results. Further, we find that these same polychlorinated insecticides, like all "cage" convulsants, potentiate the protective effect of 200 mM NaCl against heat inactivation of ^{3}H-flunitrazepam binding sites, providing additional evidence for direct coupling between picrotoxin, GABA and benzodiazepine binding sites. The rank-orders of potencies in the two systems is similar (α-endosulfan > endrin > dieldrin > toxaphene > lindane). The two most potent polychlorinated insecticides, α-endosulfan and endrin, inhibit ^{35}S-TBPS binding with IC50 values near 10 nM, making these two of the most potent ligands for picrotoxin receptors, although their toxicities in mice are low (LD50 values 76 and 20 mg/kg, respectively). By comparison, TBPO has an LD50 value near 0.053 mg/kg and an IC50 value (^{35}S-TBPS binding) of 73 nM. Thus, TBPO is about 1,000-fold more potent as a convulsant, but 7-fold less potent as a blocker of ^{35}S-TBPS binding. This finding could reflect partial GABA-neutral properties of α-endosulfan and endrin in mammals, not previously reported for ligands binding to picrotoxin receptors, but somewhat analogous to Ro 15-1788, a GABA-neutral ligand for benzodiazepine receptors. Completely GABA-neutral ligands for picrotoxin in (TBPS) receptors might be potentially useful antidotes to both picrotoxin-like convulsants and barbiturate-like anesthetics.

85.9

AN ANALYSIS OF THE ROLE OF QUISQUALATE RECEPTORS IN THE INCREASED SEIZURE SUSCEPTIBILITY OF EPILEPTIC FOWL. Simon C.J. Pedder*, Robert Wilcox*, John M. Tuchek*, and Dennis D. Johnson. Department of Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan S7N OWO.

The high seizure susceptibility seen in epileptic fowl is due to an autosomal recessive mutation. Epileptic birds (homozygotes) have abnormal EEG patterns and spontaneous seizures throughout their lifespan while their carrier (heterozygotes) hatchmates do not. The ability of the potent glutamate-quisqualate receptor agonist, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) to produce seizures was tested in 1-3 day-old epileptic and carrier chicks. AMPA was administered i.p. in 5 different doses (5.0 to 50.0 μmol/Kg) with at least 8 chicks tested at each dose. Characteristic tonic-clonic seizures were produced in epileptics (ED_{50} = 10.8 μmol/Kg) with 100% seizure activity at 25.0 μmol/Kg. No seizures were observed in carrier chicks with doses up to 50.0 μmol/Kg. Seizures produced in the epileptics could be attenuated by prior administration of the quisqualate antagonist 6-cyano-7-nitroquinoxaline - 2,3 - dione (CNQX) but not by the N-methyl-D-aspartate (NMDA) antagonists 2-amino-7-phosphonoheptanoic acid (2AP7) and 5-methyl-10, 11-dihydro-5H-dibenzo [a,d] cyclohepten - 5,10-imine maleate (MK-801). Analysis of the binding sites for [^{3}H]AMPA showed a reduction in binding affinity (K_D) but increases in the number of binding sites (B_{max}) for epileptics when compared to carriers. These results suggest that abnormalities in the quisqualate receptor may influence seizure susceptibility and that quisqualate induced seizure activity appears to be independent of NMDA receptor responses. Supported by MRC.

85.10

THE ANTIEPILEPTIC ACTIVITY OF EIGHT DIHYDROPYRIDINE CALCIUM CHANNEL ANTAGONISTS: MECHANISM OF ACTION. M.A. Morón*, T.L. Yaksh* and C.W. Stevens* (SPON: S.R. Taylor). Neurosurgical Research Laboratory, Mayo Clinic, Rochester, MN 55905.

Antiepileptic and anticonvulsant activity have been reported for the dihydropyridine (DHP) class of calcium channel antagonists (CCA) in various seizure models. Current thought holds that the DHP CCA exert their antiseizure effects via neuronal calcium channel antagonism; however, no evidence substantiating this mechanism has been reported. In order to investigate this possible mechanism we used the awake rat with chronically implanted EEG leads to examine the antiepileptic activity of 8 DHP CCAs utilizing an intracerebroventricular (i.c.v.) route of administration to circumvent differences in brain penetration and peripheral metabolism and distribution. The ordering of i.c.v. DHP antiepileptic activity is compared to reported DHP brain binding affinity. The DHPs examined were nimodipine (NM), nitrendipine (NT), nifedipine (NF), nisoldipine (NS), nicardipine (NC), felodipine (FLP), floridipine (FLR) and PN200-110 (PN). Seizures were induced with metrazol (35 mg/kg i.p.) and quantitated by EEG spike counting. The ED_{50} and 95% CI (μg) were: NM, 136 (109-168); NT, 167 (100-277); FLP, 185 (113-304); NS, 219 (122-392); NF, NC, PN and FLR all >300. This ordering of activity does not correspond to binding affinities for: PN >> NS > NT > NM > NF ≈ NC. This finding suggests that DHP CCA may be exerting their antiepileptic effects by more than just simple neuronal calcium channel antagonism. (Supported by grant NS-24329.)

86.1

ANTICARCINOGENIC ACTIVITY OF AN AYURVEDIC FOOD SUPPLEMENT, MAHARISHI AMRIT KALASH (AK). Chandradhar Dwivedi, Bryan C. Satter*, and Hari M. Sharma*. College of Pharmacy, South Dakota State Univ. Brookings, SD 57007 and Dept. of Pathol., The Ohio State Univ., Columbus, OH 43210.

AK, an Ayurvedic food supplement (provided by MAPI, Stoneham, MA 02180) is tested for anticarcinogenic activity against 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary tumors in rats. Fifty day old Sprague-Dawley rats were divided in 4 groups having 20 rats in each group. Group 1 (C) and group 3 (P) were placed on a rat chow diet. Group 2 (I) and group 4 (I+P) were placed on a rat chow diet supplemented with 0.2% AK. All animals were given DMBA (75 mg/kg in 1 ml of sesame oil) by gavage after being on the diet for one week. One week after the DMBA administration, I group was placed back on normal chow diet and P group was placed on AK supplemented diet. Rats were weighed and examined weekly for the presence of mammary tumors for a period of 18 weeks after DMBA administration. At 18 weeks the tumor incidence was 67, 85, 25 and 31% for C,I,P and I+P groups respectively; the average number of tumors/animals was 1.4, 2, 1 and 0.5 for C,I,P and I+P groups respectively. There was no significant difference in weight gain of animals among all the groups. These results indicate that dietary AK protects against DMBA-induced mammary carcinogenes only during the promotion phase. (Supported in part by Lancaster Foundation)

86.2

ANTICARCINOGENIC ACTIVITY OF AN AYURVEDIC FOOD SUPPLEMENT, M4. Hari M. Sharma*, Bryan C. Satter* and Chandradhar Dwivedi. College of Pharmacy, South Dakota State Univ., Brookings, SD 57007 and Dept. of Pathol., The Ohio State Univ. Columbus, OH 43210

M4, an Ayurvedic food supplement (provided by MAPI, Stoneham, MA 02180) is tested for anticarcinogenic activity against 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary tumors in rats. Fifty day old Sprague-Dawley rats were divided in 4 groups having 20 rats in each group. Group 1 (C) and group 3 (P) were placed on a rat chow diet. Group 2 (I) and group 4 (I+P) were placed on a rat chow diet supplemented with 6% M4. All animals were given DMBA (75 mg/kg in 1 ml sesame oil) by gavage after being on the diet for one week. One week after the DMBA administration, I group was placed back on normal chow diet and P group was placed on M4 supplemented diet. Rats were weighed and examined weekly for the presence of mammary tumors for a period of 18 weeks after DMBA administration. At 18 weeks the tumor incidence was 60, 31, 7 and 14% for C,I, P and I+P groups respectively; the average number of tumors/animal was 1.3, 0.4, 0.1 and 0.1 for C,I,P and I+P groups respectively. There was no significant difference in weight gain of animals among all the groups. These results indicate that dietary M4 protects against DMBA induced carcinogenesis during both the initiation and the promotion phases. However, a higher degree of protection is observed during the promotion phase.

86.3

BIOCHEMICAL EFFECTS OF Ro 15-5458 ON SCHISTOSOMA MANSONI. Feleke Eshete* and James L. Bennett. Dept. of Pharmacol./Toxicol., Michigan State Univ., E. Lansing, MI 48824.

A 15 mg/kg single oral dose of Ro 15-5458, a 9-acridanone hydrazone derivative given to mice infected with S. mansoni will eliminate all parasites within 7 days. We evaluated various biochemical changes in the parasites during the first 4 days after drug administration since no apparent physiological changes were observed during this period. Glycogen was measured by enzymatic hydrolysis to glucose and subsequent assay of the hexose. Protein, lactate and ATP were measured using conventional methods. The relative turnover rates of protein were studied by double isotope labelling method described previously by Arias et al. (J. Biol. Chem. 224; 3303, 1969) and Dehlinger and Schimke (J. Biol. Chem. 246 2574, 1971). While no drug effect could be demonstrated on lactate production, and glycogen, gut pigment or ATP content of the parasite, a significant reduction in parasite biomass and protein content was observed when worms were recovered from mice after 3 days of dosing. Possible drug effects that may contribute to the protein loss have been investigated. With drug-treated worms we noted a reduced incorporation of ^{3}H-leucine into proteins without a change in turnover rates of the proteins. We tentatively conclude that Ro 15-5458 selectively inhibits protein synthesis in the parasite. Mechanisms responsible for the observed phenomena are being investigated. (Supported by Hoffmann-LaRoche Co., Basel, Switzerland.)

86.4

TRANSPORT OF METHYLGLYOXAL BIS(GUANYLHYDRAZONE) AND RESISTANCE IN L1210 MURINE LEUKEMIA CELLS. S. Kanthawatana* and A. H. Neims. Dept. of Pharmacology, Univ. of Florida Coll. of Medicine, Gainesville, FL 32610

Our laboratory has been studying the antiproliferative and cytotoxic actions of MGBG and related polyamine analogs. This work deals with the reversible resistance to MGBG which developed with consistency in L1210 cells within a few days of exposure to sublethal doses. When exponentially-growing L1210 cells were exposed to ca. 1 µM MGBG, the cells accumulated the radiolabelled drug with an apparent K_m of 9.04 (SD, 0.34) µM and a V_{max} of 0.271 (0.019) fmoles/cell/hr. In 24 hr, the apparent intracellular conc of MGBG was > 2000-fold higher than that of the medium. Experiments with isolated mitochondria and with digitonin-permeabilized cells revealed that the high intracellular [MGBG] was not due to mitochondrial accumulation of the drug. Decreased extracellular pH was associated with increased rates of influx of MGBG, most likely due to selective transport of the divalent cationic form of the drug. Cell doubling time increased several fold over the next few days, but then returned to control values despite the continued presence of MGBG in the medium. When the initial MGBG concentration in the medium exceeded 5 µM, the cells lost clonogenicity and viability. But with ca.1 µM MGBG, the apparent intracellular conc of MGBG decreased from > 2mM after two days of exposure to ca. 0.5 mM by day 6, to < 0.1 mM by day 20 of incubation. After 6 days of exposure, the rate of influx of MGBG has decreased significantly [K_m, 24.87 (1.36) µM; V_{max}, 0.142 (0.013)]. The rate of efflux of MGBG was unchanged. The influx of MGBG returned to control values within one month of incubation in drug-free medium. Although L1210 cells that are near plateau growth exhibit decreased accumulation of MGBG, it is important to note that chronic exposure of cells to a sublethal concentration of MGBG caused the reversible adaptation of decreased influx in association with control rates of cell-doubling.

IMMUNOPHARMACOLOGY AND IMMUNOTHERAPY

87.1

DOSE OF INTERLEUKIN-1 (IL-1) AND STIMULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS. A.R. Gwosdow, M.S.A. Kumar, P.A. Meara* & H.H. Bode*. Shriners Burns Inst. & Harvard Med. Sch., Boston, MA 02114.

The effect of varying doses of purified human IL-1 (Cistron) on rectal temperature (Tr), hypothalamic corticotropin releasing hormone (CRH), pituitary adrenocorticotropic hormone (ACTH), plasma ACTH and plasma corticosterone (B) was examined in male rats at 24°C. Tr was measured using thermocouples. CRH, ACTH and B were determined by radioimmunoassay. Intravenous (iv) administration of IL-1 (0.50 to 1.5 ng) resulted in a dose-dependent hyperthermia that began 20 min post-injection and continued for an additional 30 min. The 0.5 ng dose of IL-1 resulted in no change in hypothalamic CRH, pituitary ACTH or plasma ACTH. Plasma B was significantly ($P<0.05$) elevated 30 min after IL-1 administration and returned to control levels within 30 min. IL-1 doses of 0.75 ng or above elevated ($P<0.05$) plasma ACTH and B 30 min post-injection and remained elevated for an additional 90 min. These observations suggest two mechanisms of action for IL-1: low doses may act directly on the adrenal gland while higher doses may stimulate the hypothalamic-pituitary-adrenal axis. (Supported by Shriners Hospitals for Crippled Children Project 15867).

87.2

ANIMAL MODELS OF INTERLEUKIN-2 (IL-2) TOXICITY: EMETIC AND CARDIORESPIRATORY EFFECTS. S.F. Gonsalves, T.L. Ciardelli*, M.L. Goodheart* and H.L. Borison. Dept. of Pharmacol. and Toxicol., and Surgery, Dartmouth Medical School, Hanover, NH 03756.

Toxic effects of IL-2 in cancer therapy include vomiting and physiological collapse. We found that IL-2 (200 µg/kg, im) injected repeatedly at 24 h intervals produced vomiting with avg. onsets of 23 and 16 h in 4 normal and in 3 chronic area postrema (AP)-ablated cats, respectively. Accuracy of the AP lesions was confirmed pharmacologically and histologically. Thus, IL-2 acts independently of the AP to induce vomiting, perhaps utilizing a secondary agent. Measurements in anesthetized cats revealed no notable changes in cardiorespiratory functions after acute iv and subacute im treatment with IL-2. By contrast, rats pretreated with IL-2 im for 3-4 days prior to their acute physiological experiment subsequently responded with a progressive decline in arterial pressure and death from respiratory failure. Autopsy showed hydrothorax and hemorrhagic pulmonary edema. (Supported by HHS Grant ES04063 and American Health Assistance Foundation. Recombinant human IL-2 generously donated by Cetus Corp.)

87.3

VASCULAR LEAK SYNDROME WITH INTERLEUKIN 2 THERAPY. D.W. Mulvin*, C.A. Kruse*, M. Grosso*, and M.R. Johnston* (SPON: I. McMurtry). Univ. of Colorado, Denver, CO 80262

Constant infusion of recombinant Interleukin 2 (IL-2) has been reported to induce tumor regression in patients with advanced malignancies. A side effect of IL-2 therapy is vascular leak syndrome. The mechanism of the vascular leak is unknown. Our previous data shows that IL-2 does not cause injury in salt perfused rat lungs. We hypothesize that IL-2 damages endothelium via a mediator in the blood rather than by direct action on the endothelial cell. We studied the effect of various concentrations of IL-2 in either blood perfused or Krebs Henselite salt perfused rat lungs. Lungs were isolated, ventilated, and perfused for one hour. Vascular leak was measured by increase in lung weight, wet to dry ratio, and pulmonary arterial pressure.

IL-2 Conc.	Weight Gain (g)		Wet/Dry Ratio	
	Blood	Salt	Blood	Salt
Control (0)	*0.70±.1	0.60±.2	*4.72±.1	5.1±.3
333U/ml(50,000U)	1.18±.3	0.40±.3	5.10±.5	5.2±.4
666U/ml(100,000U)	*2.25±.7	0.60±.2	*5.95±.4	5.3±.2

(n=6 all groups) *p<0.01 (mean±SD)

Significant vascular leak was demonstrated in the blood perfused lungs as compared to salt perfused as measured by the above parameters. No change was seen in PA pressures between groups. IL-2 induced vascular leak is caused not by its direct action on endothelial cells but via an intermediatry in the blood.

87.4

PROTEOLYTIC REGULATION OF ANTIGEN PRESENTATION: CLEAVAGE OF I_i to p25 AND OF CLASS II β CHAIN AT Lys_{72}/Arg_{73}, Arg_{93}/Arg_{94}. Lawrence J. Thomas* and Robert E. Humphreys* (SPON: Neal G. Brown). Dept. of Pharmacology, Univ. of Massachusetts Medical School, Worcester, MA 01655

The 25,000 dalton protein (p25), which is seen in immunoprecipitates with antibodies to human class II MHC molecules or I_i, has been shown to be a C-terminal fragment of I_i. Antibodies to a C-terminal peptide of I_i precipitate p25 in mixtures of denatured class II MHC and associated molecules, while antibodies to an N-terminal peptide did not. Digestion by various proteolytic enzymes of native class II complexes containing I_i yielded proteins migrating at or near p25 in 2-D gels.

Human class II β chains contain 2 pairs of well-conserved, basic amino acids, Lys_{72}-Arg_{72} and Arg_{93}-Arg_{94}. Such basic dipeptides have been shown in other systems to be targets of proteolytic cleavage. These 2 pairs of residues bracket the third hypervariable region, which may form part of the class II desetope. In immunoprecipitates from Raji cells, a peptide of about 8,400 daltons has been found to be linked by a disulfide bond to the class II complex. This is the weight of the predicted N-terminal peptide generated by proteolytic cleavage of the beta chain at the basic dipeptides. Such proteolytic cleavages of I_i and class II molecules might regulate the presentation of foreign peptides to the T cell receptor.

87.5

PLATELET-ACTIVATING FACTOR (PAF) ANALOGS INHIBIT MITOGEN-INDUCED HUMAN PERIPHERAL BLOOD T-CELL PROLIFERATION Travers, J.B.* and Fertel, R.H. The Ohio State University, Columbus, OH. 43210

Platelet-activating factor (1-O-hexadecyl/octadecyl-2-acetyl-sn-glycero-3-phosphorylcholine;PAF), is an ether phospholipid that is produced by leukocytes and is a potent mediator of acute allergic and inflammatory reactions. We determined the effect of several analogs of PAF on mitogen-stimulated T-cell proliferation. The addition of the synthetic thiazolium-containing PAF analogs CV3988 and RO193704 to 72-hour cultures of phytohemagglutinin (PHA)-stimulated human peripheral blood mononuclear leukocytes resulted in a dose-dependent inhibition of (^{3}H)-thymidine incorporation into T-cells (IC50: 2 and 0.25 micromolar, respectively). The PAF receptor antagonist BN52021 (Ginkolide B) had no effect (up to 100 micromolar) on T-cell proliferation in this system. The results of this study suggest that PAF may play a role in lymphocyte function.

87.6

ADENOSINE RECEPTORS AND MODULATION OF NATURAL KILLER CELL ACTIVITY (NK) BY NUCLEOSIDE ANALOGS. T. Priebe*, C. D. Platsoucas* and J. A. Nelson. Depts. Exptl. Pediatr. and Immunol., Univ. Tex. M. D. Anderson Cancer Center, Houston, TX. 77030.

The adenosine analog tubercidin (TUB) inhibits whereas the deoxyadenosine analog 2-fluoroadenine arabinoside-5'-phosphate (FAMP) stimulates NK. To test the possibility that adenosine receptors mediate these differential effects, an adenosine A_1 receptor agonist (N^6-S-phenylisopropyladenosine, PIA) and a potent adenosine A_2 receptor agonist (5'-N-ethylcarboxamidoadenosine, NECA) were incubated with B_1 C3H/He mouse spleen cells in vitro in the presence of ^{51}Cr-loaded YAC-1 cells. Release of ^{51}Cr during 4-hr incubation was used to measure NK. PIA (10pM to 10nM) stimulated whereas NECA (1nM to 1μM) inhibited NK. These effects were prevented by the adenosine receptor antagonist, 1,3-dipropylxanthine (XAC, 1μM). The results are consistent with the negative modulation of NK by dibutyryl cyclicAMP. XAC also inhibited the NK stimulatory effect of FAMP (1mM), but failed to influence the NK inhibition by TUB (1 to 100 μM). The nucleoside transport inhibitor, nitrobenzyl thioinosine monophosphate (10 μM), had no effect on NK; however, it prevented nucleotide formation from TUB and inhibition of NK by TUB. These data suggest a possible role for the A_1 receptor in the stimulatory effect of FAMP; however, nucleotide formation may be required for the inhibitory response to TUB.

87.7

CYSTEAMINE PRODUCES DOSE RELATED BIDIRECTIONAL IMMUNOMODULATORY EFFECTS IN MICE. H.U. Bryant*, E.W. Bernton*, T.K. Shakarjian* and J.W. Holaday. Dept. Med. Neurosci., Div. of NP, Walter Reed Army Inst. Res. Washington, D.C. 20307.

Cysteamine (CSH), a sulfhydryl reducing agent known to functionally inactivate prolactin (PRL) and other immunomodulatory neurohormones, was tested for its effects on several immunologic and neuroendocirne parameters. CSH was given to C3H/HeN mice once per day for 3 consecutive days. Low doses of CSH (12.5 mg/kg) stimulated lymphocyte proliferative responses induced by both concanavalin A (Con A) and lipopolysaccharide (LPS). Serum PRL levels were also elevated (67%) at low doses of CSH. By contrast, 300 and 400 mg/kg doses of CSH resulted in significant reductions of both Con A and LPS induced proliferation. This suppression of mitogen induced blastogenesis was accompanied by thymic atrophy. Levels of both PRL and corticosterone were suppressed at the 400 mg/kg dose of CSH (44 and 63% respective reductions relative to control). A strong positive correlation was noted between serum PRL and mitogen induced proliferative responses. Interestingly, a similar pattern of stimulation of mitogen induced proliferation at low doses followed by suppression at higher doses were observed with in vitro addition of CSH (33% elevation with 0.1 mM cysteamine and 44% suppression with 2.0 mM CSH on lymphocyte proliferative responses to 0.5 μg/ml Con A). These studies indicate that, depending upon the dose, CSH has bidirectional effects on immunologic endpoints that are correlated with changes in circulatin PRL, but may also be observed upon direct addition of the drug to stimulated lymphocytes in culture.

87.8

IN VITRO AND IN VIVO PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) INHIBITORY ACTIVITY OF PD 116,124. Mi. K. Dong*, Mark J. Suto*, Jagadish C. Sircar*, and Richard B. Gilbertsen. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

PNP inhibitors are of interest for the treatment of cancer, autoimmune diseases, psoriasis, transplant rejection, malaria, and gout. PD 116,124 (8-amino-2'-nordeoxyguanosine; 2-[(2,8-diamino-6-hydroxy-9H-purin-9-yl)-methoxy]-1,3-propanediol) is a reversible, competitive inhibitor of human PNP (Ki = 0.42 μM) that is highly water-soluble. In the presence of a noninhibitory concentration of 2'-deoxyguanosine (GdR, 10 μM), PD 116,124 inhibited growth (^{3}H-thymidine uptake) of human MOLT-4 (IC_{50} = 3.3 μM) and CEM (IC_{50} = 1.0 μM) T lymphoblasts, but not MGL-8 or NC-37 B lymphoblasts (IC_{50} >500 μM). PD 116,124 alone was nontoxic for T lymphoblasts up to 500 μM. PD 116,124 produced concentration-related elevation of dGTP in T lymphoblasts cultured with GdR. Accumulation of dGTP was significantly greater (49.0-fold control) and more sustained in the presence of PD 116,124 than when only GdR was added to medium. Both the inhibition of growth of T cells and the accumulation of dGTP were prevented by 10 μM 2'-deoxycytidine. When administered p.o. to normal rats, PD 116,124 caused significant and dose-related elevation of plasma inosine (Ino) 1 hr postdosing. The maximal Ino concentration [1.4 μM (17.4-fold vehicle)] was achieved at 500 mg/kg p.o. (highest dose tested). When administered i.v. at 50 mg/kg, PD 116,124 produced a sharp increase in Ino (1.7 μM, 56.3-fold vehicle) 15 min after injection, and Ino levels remained elevated for at least one hr post dosing. Hence, PD 116,124 is a potent PNP inhibitor possessing significant in vitro and in vivo activity.

87.9

SUPPRESSION OF LYMPHOCYTE AND ADRENAL CORTICAL FUNCTION BY CORTICOSTEROIDS: IN VIVO ANTAGONISM BY PROLACTIN. E .W. Bernton*, H.U. Bryant*, J. Woldeyesus* and J.W. Holaday. Dept. Med. Neurosci., Div. of NP, Walter Reed Army Inst. Res. Washington, D.C. 20307.

We recently described immunosuppression resulting from pharmacologic suppression of pituitary prolactin (PRL) secretion in mice and suggested that PRL may serve as an "immunotrophic" hormone (Science 239:401,1988). In further experiments we examined the effects of treatment with exogenous PRL on spleen lymphocyte proliferative responses and adrenal corticosterone (CS) secretion in mice treated sub-chronically with exogenous corticosteroids, which can suppress both these measures. Coincident treatment with ovine PRL (20ug/day by sustained release pellet) or with metaclopromide (MC) (.25 mg/day, a dopamine antagonist which stimulates pituitary secretion of endogenous prolactin) significantly reversed the suppressed proliferation observed in mitogen-stimulated spleen lymphocytes from mice treated with 100 ug/day of hydrocortisone for 4 days. Mitogens used were Con-A, lipopolysacharide, and PHA. In further studies, groups of 5 male C3H/Hen mice were treated 4 days with dexamethasone (Dex) 100 ug/day and either vehicle, MC, or ovine PRL (20ug/day). Serum CS was determined 30 min following ip injection of .5 units of ACTH. CS levels after ACTH were reduced by 89% in Dex-treated mice compared to untreated controls; coincident treatment with either MC or PRL prevented this reduction. These data suggest that both lymphocytes and adrenal cortex may be targets of opposing regulatory effects of corticosteroids and prolactin.

87.10

EFFECT OF CYCLOSPORINE A ON 3-METHYLCHOLANTHRENE-INDUCED TUMORIGENESIS. J.L. Bussiere, J.H. Exon, and G.G. Mather. (SPON: W.L. Hayton) Pharm./Tox. Program, Dept. Vet. Science, Univ. of Idaho, Moscow, ID 83843

The effect of the immunosuppressive agent, cyclosporine A (CsA) on 3-methylcholanthrene (3-MC)-induced tumorigenesis was determined in Sprague-Dawley rats. Natural killer (NK) cell activity, and interleukin 2 (IL-2) and prostaglandin E2 (PGE2) production were measured following tumor formation. Two treatment schedules for CsA were tested: daily doses at 2.5 mg/kg were injected s.c. for 3 days prior and 2 weeks following a single s.c. injection of 5 mg/rat 3-MC; and twice weekly doses of 5 mg/kg CsA were given for 10 weeks following 3-MC exposure. There was no effect on NK activity at day 5 or day 8, however, by week 17 and week 32, NK activity was suppressed in CsA-treated rats over 3-MC controls. CsA caused a decrease in IL-2 and PGE2 production at week 17, but not week 32. Tumor incidence by week 17 was approximately 90% in 3-MC rats. Animals treated with CsA did not develop 3-MC-induced fibrosarcomas. The effect of CsA treatment during the first two weeks of chemical carcinogenesis (i.e., initiation, early promotion) may be due to the immunosuppressive effects during this critical time. Further testing with this 3-MC model may begin to characterize the importance of the immune system in chemical carcinogenesis, which could then be used for testing immunotherapeutic treatments of cancer.

87.11

THE EFFECTS OF NONSPECIFIC CELL MEDIATED IMMUNITY ON CHEMICAL CARCINOGENESIS. **G.G. Mather, J. H. Exon and J.L.Bussiere. (Spon: W.L. Hayton) Pharm./Tox Program, Dept. Vet. Sci., Univ. of Idaho, Moscow, ID 83843.**

Nonspecific cell mediated immunity as demonstrated by natural killer cell (NKC) and macrophage (Mϕ) cytotoxicity is thought to be a critical first line of defense in carcinogenesis. This hypothesis was examined in the Sprague-Dawley rat using an established tumor model system that leads to a 50-60 percent tumor incidence twelve weeks after subcutaneous injection of 3-methylcholanthrene. This was accomplished by depleting NK cell and Mϕ populations in vivo after intraperitoneal injection of specific monoclonal antibodies. Antibody treatments during initiation/early promotion, late promotion (pre-tumor) and early tumor formation were examined and their effects on tumor incidence, size and latency recorded. Secondly, experiments were designed to implement augmentation of nonspecific cell mediated immunity using the naturally occurring cytokines interleukin 2 and interferon αβ. Previously, use of these agents as immunomodulating drugs has been limited due to the severe toxicities associated with high doses. Low doses of IL2 and IFN αβ (20 U and 125 U/10^6 cells respectively) were demonstrated to significantly increase NKC and Mϕ cytotoxicity following an 18 hour incubation with spleen cell suspensions. Furthermore subcutaneous injection of 9 week old S-D rats with either 20,000 U HurIL2 and rat 5000 U IFN αβ also increased NKC and Mϕ cytotoxicity indicating potential for use of low dose combination immunotherapy in future cancer treatments.

87.12

A PRESSURE/INCUBATOR CHAMBER FOR IN VITRO LYMPHOCYTE STUDIES USING HYPERBARIC OXYGEN. J.R. Wilson*, E.F. Harris*, L. Chapman*, P.B. Raven, Texas College of Osteopathic Medicine, Ft. Worth, Tx. 76107 and S.F. Gotlieb, Univ. of S. Alabama, Mobile, Al. 36688

Hyperbaric oxygen (HBO) therapy has been suggested for use clinically in several conditions where the patient's immune response contributes to the disease pathology. Thus, HBO is proposed to act as an immunosuppressive drug. In order to define the dose response curve of this putative immunosuppression on lymphocyte mitogenesis, we have developed a pressure/incubator chamber for growth of lymphocyte cultures under sterile conditions. The chamber utilizes 100% oxygen or air at various pressures between ambient and 6 ATA, at a contolled temperature of 37C ±1C. The pO_2 equilibrates completely in 96 well microculture plates. Control measurements include parallel cultures in 5% CO_2 in air at ambient pressure and air at pressures >1 ATA. Preliminary experiments with murine splenocytes show no change in cell viability (trypan blue exclusion) when HBO treated cells are compared (twice daily) to 1 ATA air and pressure controls over 3 days. Oxygen dose was varied to approximate clinical treatment protocols (doses) of 2 per day for 3 days: 1.5ATA, 60 min BID, 2.0 ATA, 90 min BID and 3.0 ATA, 90 min BID. Superoxide dismutase (SOD), measured by inhibition of NADH oxidation in Con A treated human lymphocytes subjected to 6 hr HBO at 3 ATA, was lower than untreated control cells.

87.13

FUNCTIONAL ALTERATION OF MURINE MONONUCLEAR PHAGOCYTES FOLLOWING IN VIVO EXPOSURE TO BENZENE. M J Klan*, J.G. Lewis* and D.Q. Adams* (SPON: M. Abou Donia). Dept. of Pathology, Duke University, Durham, NC 27710.

These studies were conducted to determine the toxicity of exposure in vivo to benzene on the murine mononuclear phagocyte system. Treated animals received daily subcutaneous injections of benzene (800 mg/kg) for 5 days. Macrophages (MØ) were obtained by lavage from the peritoneal cavity after ip injection of peptone or casein. Enumeration, peroxidase histochemistry and a series of functional assays were performed. Animals exposed to benzene displayed a decreased number of MØ elicited by peptone injection. In contrast, casein injection of treated animals produced an increased number of cells compared to controls. Varied but definite alterations of MØ functions included a 50% decrease of Fc receptor-mediated phagocytosis, and a 20% enhancement of PMA-stimulated H_2O_2 release. Examination of elicited and nonelicited MØ suggested that benzene treatment was affecting developing monocytes and not resident mature cells. Alterations of MØ functions in vehicle controls suggested that determining the primary effect of exposure to benzene is complicated by the inflammation induced by treatment. Continuing studies are investigating the full extent of benzene toxicity on the mononuclear phagocyte system.

87.14

BLASTOGENESIS OF PERIPHERAL BLOOD LYMPHOCYTES IN YOUNG PIGS AFTER EXPOSURE TO MULTI-STRESSORS. Yoshimi Niwano*, B. Ann Becker and Harold D. Johnson. Univ. of Missouri and USDA-ARS, Columbia, MO 65211

A study was conducted to evaluate cell-mediated immune function in young pigs after exposure to multi-stressors. Ten pigs were handled, marketed and transported for 36 hr. Ten pigs (controls) remained in home pens. Blood samples were obtained on d 1, 3, 6, 13 and 21 after relocation. Number of leukocytes and blastogenic responses of whole blood (W) and isolated (I) peripheral lymphocytes to T-cell mitogen phytohemagglutinin (PHA) were determined. No significant differences in responses of I lymphocytes to PHA were found between treatments. In contrast, responses of W lymphocytes to PHA revealed a 60% and 50% reduction on d 1 and 3, respectively. On d 6 and 13 the degree of suppression gradually lessened and by d 21 no differences were found. Changes in leukocyte numbers paralleled changes of W lymphocytes. The data suggest that cell-mediated immune function of young pigs is suppressed for several days after exposure to multi-stressors and that such reduction is attributable to decreased number but not function of circulating lymphocytes.

87.15

Disposition of 2',3'-Dideoxyinosine (ddI) in Mice Dosed Orally or Intravenously. D.L. Hill, K. Tillery and S.M. El Dareer. Southern Research Institute, Birmingham, AL 35255.

Male CD2F1 mice were dosed with ddI, a compound under consideration as an anti-AIDS agent. In preliminary experiments, ddI was determined to be stable in blood, plasma, and urine from mice, dogs, and humans when these samples were maintained at 4°, 25°, or 37° for 3 hr. There was no appreciable binding to plasma proteins. For mice dosed orally with 54.5 mg/kg, ddI was eliminated from plasma with a half-life of 15.8 min. Following an oral dose of 452 mg/kg, which was absorbed and eliminated more slowly, two elimination phases with half-lives of 8.6 and 55.9 min were evident. The contents of ddI in liver and kidney were generally higher than those in plasma. For mice dosed intravenously with 51.2 mg/kg, elimination phases with half-lives of 3.02 and 24.7 min were observed. For the high dose, the corresponding values were 7.80 and 25.8 min. For both doses, 32-33% was excreted in the urine as ddI. Less than 1% appeared in the feces. These studies demonstrate that ddI, in oral and intravenous doses to mice, is rapidly eliminated from plasma, and, to an appreciable extent, excreted unchanged in the urine. A dose-dependent effect is apparent. Supported by Contract NO1-CM-67905, DCT, NCI.

PULMONARY EPITHELIUM I

88.1

EFFECT OF AMINOPHYLLINE ON LUNG LIQUID CLEARANCE IN ANESTHETIZED SHEEP. Y. BERTHIAUME, DEPT. OF MEDICINE, UNIVERSITY OF CALGARY, CANADA, T2N 4N1.

We have reported that terbutaline increases alveolar and lung liquid clearance in sheep (JCI 79, 335-343, 1987). Since cAMP analogs and phosphodiesterase inhibitors can increase sodium transport in isolated Type II cells or isolated rat lung, the increase in lung liquid clearance with the beta adrenergic agonist in sheep could depend on an increase in cAMP. To test this hypothesis, we used a phosphodiesterase inhibitor, aminophylline, to increase cyclic AMP. We instilled 100 ml autologous serum (N=5), serum mixed with aminophylline 10 $^{-}$5M(+I.V. amino, N=3), 10^{-}3M(+I.V. amino, N=3) into one lower lobe of ventilated, anesthetized sheep. There was no significant difference in the pulmonary hemodynamics. After 4H, we removed the lungs and measured excess lung water and the changes in protein concentration of the instilled liquid. The table summarizes the data (mean ±S.D.).

Condition	No.	Excess Lung Water (ml)	Increase in Protein Concentrations (g/dl)
Serum alone	5	75.9 ± 9.1	2.2 ± 0.7
Serum + amino 10^{-}5M	3	76.9 ± 6.2	2.0 ± 0.1
Serum + amino 10^{-}3M	3	62.4 ± 0.8	3.2 ± 2.2

While 10^{-}5M, in a dose similar to the dose of terbutaline used previously did not have any effect on liquid clearance, 10^{-}3M aminophylline increased liquid clearance. The regulation of lung liquid clearance depends in part on cyclic AMP.

88.2

SUBSTANCE P INCREASES LYSOZYME OUTPUT FROM FERRET TRACHEAL GLANDS IN VIVO. H.C. Tseng*, M.A.Haxhiu* and B. Davis. CVRI and Depts of Pediatrics and Medicine, University of California San Francisco, CA 94143

We measured the effect of substance P on lysozyme output of tracheal glands in vivo. In anesthetized ferrets with lungs ventilated via the lower trachea, secretion was collected by perfusing an isolated segment of the upper trachea at a constant rate of 3 ml /5 min via a cannula connected to a syringe infusion pump. Perfusate for the first 20 min was discarded. After three 5 min. baseline collection periods, 0.8 ml. of saline or different concentrations of SP (10^{-7}, 10^{-6}, 10^{-5} M.) in 0.8 ml of saline were injected into a jugular vein over 0.5 min. After each injection, perfusates were collected for five periods of 5 min as control or 'stimulated' secretion. All samples were assayed for lysozyme activity spectrophotometrically by a modification of the method of Shugar (Biochem Biophys Acta. 8:302-309,1952). Following different doses of SP, peak lysozyme output increased from (a) 0.35±0.03 to 0.68±0.10 µg/5 min, (10^{-7}M; mean ±SE) ; (b) 0.39 ±0.08 to 2.7 ± 0.81 µg/5 min, (10^{-6}M); (c) 0.35±0.02 to 6.4±0.96 µg/5 min, (10^{-5}M). The output of lysozyme was increased for 15-45 min following the injection. Pretreatment with an injection of thiorphan (10-5M), an inhibitor of neutral endopeptidase, potentiated the effect of substance P (10-7 M) on lysozyme output (n=3). The data shows that SP causes dose dependent increases in lysozyme output from tracheal submucosal glands. Potentiation of SP induced lysozyme output by thiorphan may be caused by inhibition of neutral endopeptidase which degrades SP. (Supported by NHLBI Grant HL 24136 and Grants from the Cystic Fibrosis Foundation)

88.3

NEUROKININ A (NKA) AND NEUROKININ B (NKB) INCREASE CANINE TRACHEAL GLAND SECRETION *IN VIVO*. B. Davis, H.C. Tseng* and M.A. Haxhiu* CVRI and Depts of Pediatrics and Medicine, University of California San Francisco, CA 94143.

These experiments examined the local effects of two new mammalian tachykinins, NKA and NKB, on tracheal gland secretion. In anesthetized, atropinized (1mg/kg) dogs with lungs ventilated via the lower trachea we counted the hillocks of secretion appearing in the upper trachea from single submucosal glands in an exposed field of tracheal epithelium (1.2cm2) coated with powdered tantalum. Following a 60s baseline period, 1ml of saline, NKA or NKB were infused into a superior thyroid artery over 30s and hillocks appearing in the subsequent 60s were counted; some infusions were preceded by 1 ml of thiorphan 10-5 M (LT). NKA increased the maximum rate of appearance of hillocks/10s as follows: (a) from 1.33 ± 0.33 to 6.33±3.8, 10-8 M (mean ±SE); (b) from 2.1±0.3 to 7.1±1.9, 10-7 M; and (c) from 2.7 ± 0.6 to 9.3 ± 2.54, 10-6 M. In 5 dogs NKB 10-5 M increased the rate of apearance of hillocks. NKA was more potent than NKB in stimulating tracheal gland secretion . Thiorphan (10-5 M) increased the number of hillocks stimulated by NKA and NKB. The data show that NKA and NKB increase fluid secretion from airway submucosal glands and their effect can be modulated by peptidases which cleave neuropeptides such as neutral endopeptidase or enkephalinase.(Supported by NHLBI Grant 24136 and Grants from the Cystic Fibrosis Foundation)

88.4

THE EFFECT OF EPITHELIUM REMOVAL ON AIRWAY CONTRACTILITY IS NOT MEDIATED BY ARACHIDONIC ACID METABOLITES OR NITRIC OXIDE. Robert R. Lorenz,* Yuansheng Gao,* and Paul M. Vanhoutte. Dept. of Physiology and Biophysics, Mayo Clinic and Mayo Foundation, Rochester, MN 55905.

Removal of the epithelium increases airway responsiveness. The present study was designed to determine whether this effect is mediated by arachidonic acid metabolites or nitric oxide. Rings or segments of canine third order bronchi, in some of which the epithelium had been removed mechanically, were mounted in organ baths and isometric tension was recorded. Removal of the epithelium augmented the contraction of the preparations in response to acetylcholine. Indomethacin (10^{-5} M) and Bay G 6575 (10^{-5} M), inhibitors of cyclooxygenase and lipoxygenase, respectively, were without effect on the augmentation. The tissues without epithelium exhibited a dose-dependent relaxation to nitric oxide, which could be blocked by oxyhemoglobin (10^{-5} M); an antagonist of endothelium-derived relaxing factor, or methylene blue (10^{-5} M); an inhibitor or guanylate cyclase. Neither oxyhemoglobin nor methylene blue prevented the effect of epithelium-removal. Sodium oxide dismutase (150 U/ml), which potentiates the effect of nitric oxide, did not augment the effect of epithelium-removal. These observations suggest that metabolites of arachidonic acid or nitric oxide do not play important roles in the effect of epithelium-removal on airway responsivenes. (Supported in part by NIH grant HL21584).

88.5

LUNG PHOSPHOLIPIDOSIS: COMPARING THE ALVEOLAR RESPONSE IN MICE TO AMIODARONE AND CHLORPHENTERMINE. H. Hottlet*, M.G. Côté, Univ. de Montréal, Montréal, Canada H3C 3J7

Amiodarone, a benzofurane currently used as an antiarrythmic, is known to cause an accumulation of phospholipids in various cell types and tissues. Inhibition of phospholipase and/or impaired degradation by binding to phospholipids may explain this phenomenon. Amiodarone interacts with the hydrophobic site of phospholipids while Chlorphentermine (CP) binds with the hydrophilic part and inhibits phospholipase A2. Experimental groups received either 50 mg/kg bw CP or 130 mg/kg bw Amiodarone hydrochloride, three times a week. Differences were previously observed between the pulmonary response seen in animals treated with the clinical preparation of Amiodarone and the response described for CP. For this reason, controls were given the corresponding dose of Amiodarone vehicle (tween 80). Animals were instilled intratracheally with karnovsky' solution after 1,2,4 and 6 weeks of treatment. Optical microscopy showed a similar increase in the number of vacuoles in the type 2 pneumocyte. Theses results were corroborated with electronic microscopy. The major difference was in the increased number of neutrophils seen only in Amiodarone treated animals. This difference could be related to the binding site or to the interaction of CP with phospholipase A2 and the arachidonic acid pathway.

88.6

ULTRASTRUCTURAL ASSESSMENT OF REGIONAL DIFFERENCES IN FETAL LUNG DEVELOPMENT AND MATURITY USING A STRATIFIED BLOCK DESIGN. Y. Lin, D.E. Bender*, and A.J. Lechner. Dept. Physiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Although fetal lung morphology has been widely investigated, there is no information regarding the regional differences in ultrastructural features within the same lung. We have devised a mapping system which divides the entire left fetal lung into specific regions along longitudinal and lateral axes. Groups of 4-6 Hartley-strain guinea pig fetuses were examined at gestational ages of 55, 60, and 65 days; their lungs were sectioned into a parallel array of six planes and 18 blocks within the left cranial and caudal lung lobes. Lung morphology and morphometry of each block were compared within and among animals. Measured parameters included surface (Sv), volume (Vv), and numerical densities (Nv) of endothelial, epithelial, and progenitor cells, with emphasis on epithelial type II cells and lamellar bodies. Harmonic mean thickness and Sv of alveolar type I epithelium were also measured to determine regional diffusing capacities. The Sv, Vv, and Nv of type II cells and lamellar bodies increased significantly with age and body weight. The block locations within the map indicated cranio-caudal gradients as well as planar differences associated with proximity to the central airways. This map technique will be used to monitor other aspects of fetal lung maturation. (Supported by NIH grant HL-37386).

88.7

100% O2 INCREASES ETHANE PRODUCTION FROM LUNGS OF EXSANGUINATED, VENTILATED RATS AND IN VITRO. Habib MP*, Dickerson F*, Katz MA. University of Arizona and VAMC, Tucson, AZ, 85723.

Ethane in alveolar expirate may have its source in organs other than lung and be transported to lung for elimination. To determine if lung is a source of increased ethane production during exposure to 100% O2, we measured ethane in the expirate of 9 exsanguinated, Sprague Dawley rats mechanically ventilated (.75cc/100gm, rate 70 cpm) with hydrocarbon free air (HFA) and then with 100% O2. In all 9 animals, ethane elimination rates on 100% O2 increased (mean:16.2 range:2.17-56.91 pmoles/min/100g) compared with HFA values (mean:2.18 range:0.23-5.05) ($P<.05$, Wilcoxin). In 5 of the 9 rats, HFA ventilation was reinstated after O2. In all 5, ethane elimination fell with HFA ventilation (mean: 2.33 range 0.6-6.65) compared to the value on 100% ($P=.062$, Wilcoxin). Lung slices from 6 other rats were incubated in saline at 37°C with FeCl2 (10mg) added to enhance free radical formation. Paired lung samples from the same rat were incubated with either HFA or 100% O2. Headspace gas was analyzed chromatographically for ethane at 120 min. Mean ($\pm$ SD) ethane in the O2 samples was higher (15.6 $\pm$ 1.65 pmoles/gm) than for HFA (3.98 $\pm$ 1.58) ($P<.005$). Rat lung tissue is a major source of increased ethane production during 100% O2 exposure. Supported by DoD contract 87PP7853, BRSG and the VA.

94.1

UNSURMOUNTABLE ANTAGONISM AS A PROBLEM FOR 5-HYDROXYTRYPTAMINE RECEPTOR CLASSIFICATION: UTILITY OF AGONIST PROBES. R. A. Bond*, D. A. Craig*, A. G. Ornstein* and D. E. Clarke. Dept. of Pharmacology, University of Houston, Houston, TX 77204-5515

The pharmacological definition of receptors rests heavily upon antagonist molecules. However, many 5-hydroxytryptamine (5-HT) antagonists act unsurmountably in test systems. The mechanisms remain ill-defined, but receptor modulation via allosteric regulation may be involved. The isolated perfused rat kidney was used to investigate this problem. The kidney was perfused at a constant rate with Krebs bicarbonate solution containing cocaine (30 µM) and prazosin (0.1 µM). Changes in base-line perfusion pressure were measured to bolus injections of 5-HT, which evoked only vasoconstrictor responses. These responses were antagonized unsurmountably by: metergoline, methiothepin, methysergide, mesulergine, ketanserin, cyproheptadine, spiperone, and ICS 205,930. Only (-)-propranolol acted competitively (pA_2=6.45). Unsurmountable antagonism with metergoline, the most potent antagonist, was inhibited by (-)-propranolol ($3x10^{-5}$M), suggesting an action at the 5-HT receptor, rather than via an allosteric effector. At the present time, a slow off-set rate of metergoline from the receptor, in the face of limited receptor reserve for 5-HT, appears to be the simplest explanation for the unsurmountable antagonism. Receptor classification as a 5-HT_2 receptor was made upon the basis of agonist probes and the equilibrium dissociation constant for 5-HT (K_A=185nM). The results underscore the utility of agonist molecules and the complexity of antagonist molecules for receptor classification. (Supported by NIH Grant NS 24871).

94.2

DIFFERENTIAL EFFECTS OF KNOWN AND NOVEL SEROTONIN-ACTIVE COMPOUNDS IN TWO PUTATIVE 5-HT_2 RECEPTOR CONTAINING VASCULAR TISSUES. A.L. Killam,* S.S. Nikam,* E.W. Taylor,* A.R. Martin,* and D.L. Nelson. Departments of Pharm./Tox. and Pharm. Sci., University of Arizona, Tucson, Arizona, 85721

We have shown previously that several tetrahydropyridylindoles exhibit serotonin (5-HT) agonist activity in the rabbit femoral artery (RFA) and antagonist activity in the rat aorta (RA). Both of these tissues purportedly contain only the 5-HT_2 receptor subtype. We have extended these studies to determine the affinity constants for the partial agonists as well as adding several antagonists to the tissue comparison studies. Affinities for these compounds in the RA show a linear correlation to 5-HT_2 binding affinities in the rat frontal cortex. In contrast, the affinities for the same compounds in the RFA show no correlation to the 5-HT_2 binding. One novel benzyl-substituted tetrahydropyridylindole tested is an antagonist in both preparations with K_B's being significantly different ($p<.025$) between the RA (~51 nM) and the RFA (~190 nM). Whether the differences represent subtypes of the 5-HT_2 receptor, differences in affinity (similar to the suggestion of Bevan et al., TIPS 9(3): 87, 1988), or differences in the excitation contraction coupling, is under study. (Supported by NIH grants NS16605 and NS01009).

94.3

STIMULATION OF $5HT_2$ AND $5HT_1$ RECEPTORS BY M-CHLOROPHENYLPIPERAZINE APPARENTLY CAUSES OPPOSITE CHANGES IN BLOOD PRESSURE, HEART RATE, AND PLASMA CATECHOLAMINES IN THE CONSCIOUS RAT. Gyorgy Bagdy,* Katalin Szemeredi,* Dennis L. Murphy* (SPON: D.M. JACOBOWITZ). LCS, NIMH, and NINCDS, NIH, Bethesda, MD 20892

The serotonin (5HT) agonist, m-chlorophenylpiperazine (m-CPP), has been shown to increase blood pressure (BP), heart rate (HR), and plasma catecholamine (CA) levels in rodents. While the $5HT_1/5HT_2$ antagonist, metergoline, partially blocks these responses, the contributions of different 5HT receptor subtypes and of other neurotransmitter systems to these effects of m-CPP have not yet been evaluated. In the present study, we examined some potential contributions to these effects using different selective 5HT receptor antagonists (ritanserin, xylamidine, MDL 72222, and metergoline), adrenergic receptor antagonists (prazosin, yohimbine, and pindolol), and the opiate antagonist, naloxone, in conscious, freely moving rats. The selective centrally acting $5HT_2$ antagonist, ritanserin, significantly attenuated the plasma norepinephrine and epinephrine elevations produced by m-CPP. Ritanserin pretreatment also converted the pressor and cardioacceleratory responses to m-CPP into a hypotensive response accompanied by a reduction in HR. The other antagonists produced minimal or nonsignificant effects on m-CPP-induced CA changes except naloxone, which attenuated epinephrine responses. BP responses were also only partially affected by the other antagonists, including xylamidine and metergoline. Decreases in HR after m-CPP were found after pretreatment with yohimbine and, initially, pindolol. These results suggest that m-CPP acts via $5HT_2$ receptors to increase plasma CAs, BP, and HR. In the presence of central $5HT_2$ blockade, m-CPP reduces BP and HR, most likely via $5HT_{1B}$ receptor stimulation.

94.4

SEROTONIN INDUCED DEPOLARIZATION OF RAT DORSAL ROOT GANGLION (DRG) CELLS IS BLOCKED BY $5\text{-}HT_2$ ANTAGONISTS. S. Todorovic* and E. G. Anderson, Dept. of Pharmacology, Univ. of Ill. Coll of Med., Chicago, IL 60680.

Serotonin induces a slow depolarization with increased membrane resistance and a fast transient depolarization with decreased membrane resistance in bullfrog DRG cells (Brain Res. 327:71, 1985) and guinea pig enteric neurons (PNAS 83:9799, 1986). Using intracellular recordings from isolated rat DRG cells, we observed 5-HT to depolarize 88% of the 41 neurons studied (30 A type and 11 C type neurons). The input resistance increased in 63% of the cells affected. The 5-HT induced depolarization was dose dependent in concentrations between 0.01 and 10 uM. No desensitization occurred. The 5-HT dose response curve was shifted to the right by 1 uM spiperone. This antagonism did not appear competitive since 100 uM of 5-HT only partially overcame the block. The 5-HT dose-response curve returned to control after 5-10 minutes of wash. The putative $5\text{-}HT_{1A}$ agonist, 8-OH-dipropylaminotetralin did not consistently mimic 5-HT in these cells. Methiothepin (1 uM) blocked the response of 1-10 uM 5-HT, with very slow washout. Ketanserin also blocked the responses to 5-HT. These data suggest a $5\text{-}HT_2$ receptor mediates the 5-HT induced depolarization. (USPH grant NS17834).

94.5

5-HYDROXYTRYPTAMINE-INDUCED RELAXATION OF RAT AND MOUSE OESOPHAGEAL SMOOTH MUSCLE. C.R. Triggle, S.E. Ohia* and D. Bieger*, Faculty of Medicine, Memorial Univ. of Nfld, St. John's, NF, Canada, A1B 3V6.

We have evidence that the 5-hydroxytryptamine (5-HT)-induced relaxation of the rat oesophagus is insensitive to several putative $5\text{-}HT_1$ or $5\text{-}HT_2$ receptor antagonists and persists in cold stored preparations.[1,2] The aim of the present study was two fold: (a) to examine the effects of selective $5\text{-}HT_3$ receptor agonists and antagonists and (b) to investigate the effects of 5-HT in mouse oesophageal smooth muscle. Isometric tension induced by muscarinic receptor activation was measured and the inhibitory effects produced by 5-HT and the selective $5\text{-}HT_3$ receptor agonists were examined on proximal and distal segments of the oesophagus. 2-methyl-5-HT was about 400-fold less potent than 5-HT in relaxing the rat oesophagus whilst phenylbiguanide had no effect. 5-HT elicited a concentration-dependent relaxation of the mouse oesophagus with an EC_{50} in the 5 nM range. ICS 205-930 and MDL 72222 inhibited relaxations induced by 5-HT in both species competitively ($pA_2 \sim 7\text{-}9$); blockade caused by BRL 43694 and metoclopramide was noncompetitive ($pD_2 \sim 5.5$) and quipazine and D-tubocurarine had no effect on the 5-HT-induced relaxations. We conclude that the non-neuronal receptors mediating the relaxation of rat and mouse oesophageal smooth muscle to 5-HT are identical and may share some properties of the neuronal $5\text{-}HT_3$ receptor. (Supported by MRC)

[1] Bieger, D. & Triggle, C. (1985) Br. J. Pharmac. 84, 93-106
[2] Akbarali, H. *et al.* (1987) Can. J. Physiol. Pharmac. 65, 23-29

94.6

ESTROGEN-INDUCED MODULATION OF SEROTONIN $5\text{-}HT_{1A}$ MEDIATED RESPONSES IN THE DORSAL RAPHE NUCLEUS (DRN).
Joan M. Lakoski. University of Texas Medical Branch, Galveston, TX 77550.

We are investigating the role of serotonin (5-HT) neuronal systems in mediating the central regulation of female reproductive function and have hypothesized that exposure to gonadal steroid hormones, including estrogen (E), may directly modulate 5-HT autoreceptor function. Steroid-induced changes in the pharmacology and physiology of $5\text{-}HT_{1A}$ mediated responses were evaluated in young (3-4 mo) female Fischer 344 rats (anesthetized with chloral hydrate) utilizing standard extracellular recording techniques in the DRN. Animals were ovariectomized 7 days prior to s.c. implantation of a silastic capsule delivering physiological proestrus levels of estrogen (E) or sesame oil vehicle (V) for 48 hr prior to recording. E exposure produced a significant shift to the right in the cumulative dose of the selective $5\text{-}HT_{1A}$ agonist 8-OHDPAT (i.v.) required to inhibit spontaneous cell firing; E vs V animals were subsensitive to administration of this 5-HT agonist (ED_{50}, 10 $\mu g/kg$ vs 2.5 $\mu g/kg$). Similarly, evaluation of the iontophoretic sensitivity to 5-HT applied in E vs V treatment groups revealed a significant E-induced subsensitivity (IT_{50}, 848 ± 142 vs 266 ± 26) demonstrated directly at the cellular level. These data provide support for a role for gonadal hormones in modulating central serotonergic function in the young female rat. Supported by AG 06017.

94.7

BIOLOGICAL ACTIVITY OF A PARTIALLY PURIFIED BRAIN ENDOCOID FOR 3H-KETANSERIN (3H-KET) RECOGNITION SITES. J.A. Apud, M.L. Barbaccia, E. Costa (SPON: B. Wise). FGIN, Georgetown University, Washington , D.C. 20007

An endocoid that displaces labeled 3H-KET from specific recognition sites located in crude synaptic membranes has been partially purified from rat brain (Ann. Rev. Pharmacol. Toxicol. 28:451, 1988). This endocoid is not located in 5-HT nerve terminals (Clin. Neuropharmacol. 9 (Suppl. 4):223, 1986). Major steps for its purification include: a)homogenization (1:6;w:v) of brain with 0.1M acetic acid b) heat inactivation (90°C, 10 min) c) gel filtration of the 40,000 x g supernatant on Biogel P-10 column equilibrated with 0.1M acetic acid:20% methanol d) cation exchange CM-Sephadex chromatography (elution with 1M ammonium acetate pH 6 to 8), e) C-18 uBondapack HPLC reverse phase. This endocoid preferentially displaces 3H-KET binding when compared to 3H-Mianserin, 3H-DHA or 3H-Flunitrazepam binding. Displacement of 3H-KET binding is decreased after pronase digestion of the endocoid. At concentrations twice the IC_{50} for displacing 3H-KET binding, this endocoid blocked serotonin-induced platelet aggregation but failed to counteract collagen or ADP-induced aggregation. The present data suggests that this partially purified 3H-KET binding displacing material may represent an endogenous ligand for $5\text{-}HT_2$ receptors.

94.8

EFFECT OF BRL 43694 ON EMESIS IN THE DOG INDUCED BY IONIZING IRRADIATION. R.K. Harding, K.E. Leach* and L. Prud'homme-Lalonde*. Defence Research Establishment Ottawa, Ottawa, Ontario, Canada K1A 0Z4, Department of Physiology, Univ. of Ottawa, Ottawa, Ontario, Canada K1H 8M5.

BRL 43694 is a member of a new class of $5\text{-}HT_3$ receptor blockers. We have evaluated the efficacy of this new antiemetic compound in the irradiated beagle dog. Dogs were used because their radiation-induced vomiting response (sensitivity, latency, severity) is most like that observed in man. Dogs received whole-body, bilateral irradiation (7 Gy), from a ^{60}Co gamma source. Thirty min prior, or 60 min post-irradiation, they received an iv bolus injection of BRL 43694 made-up in saline, or saline alone. 6/6 saline-treated animals vomited, latency, 95 min., mean number of episodes, 5. 2/4 animals in groups receiving 0.1, 0.5 or 1.0 mg/kg BRL 43694, 30 min before irradiation vomited. The latency to the first episode was significantly increased (164 min) and the number of episodes was reduced. 3/8 animals receiving 0.5 mg/kg of drug 60 min after irradiation vomited. The latency to the first episode was significantly increased (152 min), and only 1 episode was seen in each animal. There was no therapeutic advantage in splitting the drug dose (0.2 mg/kg 30 min before and 60 min post-irradiation). BRL 43694 abolishes or reduces radiation-induced vomiting in the dog. No adverse side-effects were observed.

94.9

EFFECTS OF TRIFLUOROMETHYLPHENYLPIPERAZINE (TFMPP) IN THE ELEVATED PLUS-MAZE MOUSE ANXIETY MODEL.

Daniel Benjamin,* Harbans Lal, and Laurence R. Meyerson. American Cyanamid Co, Med Res Div, Ramapo Coll, Mahwah, NJ 07430, and Dept Pharmacol, Texas Coll Osteop Med, Ft Worth, TX 76107

The serotonergic system has been implicated in the modulation of anxiety states, and a number of drugs selective for serotonin receptors have anxiolytic effects. TFMPP binds to 5-HT_1-'like' receptors, and is a preferential agonist at the 5-HT_{1B} receptor. To test for an anxiogenic effect of 5-HT_{1B} receptor stimulation in the mouse, TFMPP was administered before testing in the elevated plus-maze, (Lister RG, Psychopharmacol. 92:180-185, 1987). The elevated plus-maze has two open arms, and two arms with vertical sides, which extend from a central platform. Compared to vehicle controls, anxiolytic agents increase both the number of entries the mice make into the open arms, and the length of time spent on the open arms. These two parameters are consistently decreased by anxiogenic drugs. Pentylenetetrazol, an anxiogenic drug, and diazepam, an anxiolytic drug, produced significant, dose-related decreases and increases, respectively, on the number of entries and the length of time spent on the open arm. TFMPP dose-dependently decreased the percent of total entries made into the open arm, and the percent of time spent on the open arm. General motor activity (total number of arm entries) was not decreased by TFMPP. Pretreatment with diazepam at 1.56 mg/kg completely abolished the effects of TFMPP on plus-maze performance. Collectively, these results suggest that TFMPP is an anxiogenic substance, and demonstrate the utility of the mouse elevated plus-maze as a tool to study anxiety-like behaviors in animals.

94.10

NON-SELECTIVE SEROTONIN (5-HT) ANTAGONIST PROPERTIES OF 1-(1-NAPHTHYL)PIPERAZINE (1-NP) ON OPERANT BEHAVIOR OF SQUIRREL MONKEYS. J. W. McKearney. Worcester Fdn. Exptl. Biology, Shrewsbury, MA 01545.

1-NP has been reported to have 5-HT2 antagonist properties, whereas it has been suggested that it may have 5-HT1 agonist actions. In the present experiments, the effects of 1-NP alone and in combination with a variety of 5-HT agonists were studied. The phenalkylamine hallucinogen 4-bromo-2,5-dimethoxyamphetamine (DOB, 0.003 - 0.3 mg/kg), thought to act predominantly at 5-HT2 sites, reduced responding under FI schedules of food presentation, and these decreases were blocked by 1-NP (0.3 - 1.0 mg/kg) or by the selective 5-HT2 antagonist ketanserin (KET). 1-(m-chlorophenyl)piperazine (CPP) and 1-(m-trifluoromethylphenyl)piperazine (TFMPP), both thought to act primarily at 5-HT1 sites, also decreased responding and this effect was blocked by methysergide (METHY) and by 1-NP, but not by KET. The effects of 1-NP given alone were not like those of CPP or TFMPP. 1-NP produced moderate increases in responding under shock-avoidance schedules whereas only decreases in responding were seen with CPP and TFMPP. All three drugs do produce similar decreases in responding under food schedules, but the effects of CPP and TFMPP are blocked by the nonselective 5-HT antagonist METHY and not by the 5-HT2 antagonist KET whereas the effects of 1-NP are not changed by either antagonist. 1-NP is a non-selective 5-HT antagonist whose 5-HT agonist properties, if any, differ from those of CPP and TFMPP. (Grants: DA-01015 and MH-18421)

NEUROTRANSMITTERS AND NEUROMODULATORS II

95.1

PHORBOL ESTER-INDUCED TRANSLOCATION OF PROTEIN KINASE C (PKC) PRECEDES ITS EFFECTS ON INSULIN RECEPTORS AND GLUCOSE UPTAKE IN ASTROCYTE GLIAL CELLS. Laura M. Mudd* and Mohan K. Raizada. University of Florida, Gainesville, FL 32610.

Previous studies have shown that 12-0-tetradecanoyl phorbol 13-acetate (TPA) stimulates insulin binding and 2-deoxy-D-glucose (2DGlc) uptake in cultured astrocyte glial cells from rat brain. These effects were observed as early as 5 and 30 minutes, respectively, with maximal insulin binding and 2DGlc uptake occurring in 2 and 5 hours, respectively. This study was undertaken to determine whether TPA induces immunoreactive (IR) PKC translocation from the cytosol to the membrane fraction under the same conditions. IR-PKC was present throughout cultured astrocyte glial cells as demonstrated by immunocytochemistry. Western blotting indicated a protein of MW 80kD which was predominatly localized in the cytosolic fraction. Treatment of cells with 100nM TPA caused a significant decrease in IR-PKC in the cytosolic fraction with a concomitant increase in the membrane fraction within five minutes. This translocation was complete within 15 minutes. IR-PKC was absent from both cytosolic and membrane fractions after 24 hours of TPA treatment. These data suggest that TPA-induced translocation of IR-PKC precedes and, thus, may contribute directly or indirectly to TPA's stimulation of insulin receptors and 2DGlc uptake. (Supported by NIH #MH09471-02)

95.2

EVIDENCE FOR THE PRESENCE OF TWO SUBTYPES OF α_1-ADRENERGIC RECEPTORS (α_1-AR) IN NEURONAL CULTURE (NC) FROM THE RAT BRAIN. Mohan K. Raizada, Anthony J. Pacitti* and Colin Sumners. Department of Physiology, University of Florida, Gainesville, Florida, 32610.

Our previous studies have demonstrated that although NC from the brains of hypertensive rats contain 2-3 times the density of α_1-AR compared to controls, the ability of norepinephrine (NE) to stimulate phosphoinositide hydrolysis (PI) is significantly attenuated. We have used clonidine, an α-agonist, to support the hypothesis that NC possess two subtypes of α_1-AR, one coupled to PI hydrolysis and occurring predominately in normotensive neurons and the other not coupled to PI, and occurring predominantly in SH neurons. Incubation of NC with clonidine caused a time- and dose-dependent downregulation of α_1-AR with an ED_{50} of 300 nM and a maximal 40% decrease with 10uM clonidine. This effect was not blocked by rauwalscine but was attenuated by phentolamine, indicating that clonidine's action was mediated directly via its interaction with the α_1-AR. Clonidine also stimulated α_1-AR mediated PI hydrolysis. In contrast, however, this effect was only observed at a concentration of 30uM, a dose well above that required (10uM) to maximally downregulate the receptor. This suggests that neuronal cells may possess two subtypes of α_1-AR, and that the majority of α_1-AR in SH neurons may be of the subtype which has a high affinity for clonidine and is not linked to PI. Supported by AHA FL affiliate and NIH, NS 19441.

95.3

PREPROTACHYKININ GENE EXPRESSION IN SENSORY NEURONS: REGULATORY ROLE OF BLOOD COMPONENTS

M.D. Linnik*, D.E. Sakas*, G.R. Uhl, M.A. Moskowitz
Massachusetts General Hospital, Boston, MA, 02114.

Substance P (SP)-containing C-fibers from the trigeminal ganglia innervate cerebral vessels and may be involved in the transmission of pain information associated with blood in the subarachnoid space. We monitored neuropeptide levels in the cerebral arteries and trigeminal ganglia, and preprotachykinin mRNA levels in trigeminal ganglia, following the intracisternal injection of blood. Marked, 50% decreases in basilar artery SP levels were found within 4 h. The middle cerebral artery and circle of Willis were exposed to less blood and normal SP levels were maintained. In trigeminal ganglia, levels of SP peptide and preprotachykinin mRNA (Northern blot analysis), were also elevated in the trigeminal ganglia at 48 h. To investigate the pathophysiological mechanism in vitro, tachykinin-producing F-11 cells (neuroblastoma x dorsal root ganglion) were cultured and exposed to hemoglobin (Hb). At 24 hrs, Hb caused a dose dependent reduction in SP in these cells. Preliminary results indicate that this reduction is accompanied by an increased expression of preprotachykinin mRNA. These results are consistent with blood-induced alteration of preprotachykinin gene expression in cerebrovascular sensory fibers, which may be partially mediated by Hb. Supported by NS08166, NS10828, The McKnight, Sloan and the American Parkinsons Disease Associations.

95.4

SUBSTANCE B: CONDITIONAL MODULATION OF NEUROTRANSMISSION WITHIN MAMMALIAN BRAIN. L. Bruce Pearce and Theresa A. Buck* (Spon: J.W. Estes). Dept. Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Substance B (SB) is a novel conditional neuromodulator reported to antagonize inhibitory presynaptic agonist effects on peripheral neurotransmission (Pearce et al. PNAS 83:7979,1986). We report here on the effects of SB on presynaptic receptor control of central cholinergic neurotransmission. KCl-evoked release of radiolabeled transmitter was studied using a rapid superfusion system. Rat brain synaptosomes (15 mg/ml, 300 ug) preloaded with 5 µM (150 µCi, 80 Ci/mmol) [^{3}H] choline were superfused with Krebs buffer, pH 7.4 under 40 psi 20% O_2 at 0.5 ml/sec with 4.8 second fractions obtained for 67.2 seconds. Ca^{2+}-dependent KCl (20 mM)-evoked release of radiolabel accounted for 53% of the total release. The muscarinic agonist oxotremorine (100 µM), produced 60.5 % inhibition of Ca^{2+}-dependent KCl (20 mM)-evoked release. Oxotremorine inhibition was time dependent reaching a maximum at 33.6 seconds. The presynaptic muscarinic receptor-mediated inhibition was completely reversed by the introduction of purified SB. However, SB alone did not have a direct stimulatory effect on transmitter release nor did it facilitate KCl (20 mM)-evoked release. These results demonstrate for the first time that SB modulates inhibitory presynaptic receptor function within the CNS. This work was supported by a PMA Foundation Research Starter Grant to L.B.P.

95.5

IN VIVO AND IN VITRO CO-OPERATIVE EFFECTS OF NGF AND GANGLIOSIDES ON CNS CHOLINERGIC NEURONS. A.C. Cuello*, L. Garofalo*, R. Kenigsberg* & D. Maysinger* McGill Univ., Montreal, Que. H3G 1Y6

The intracerebroventricular (i.c.v.) administration of nerve growth factor (NGF) in the rat prevented the retrograde degeneration of forebrain cholinergic neurons of the nucleus basalis magnocellularis (NBM) which occur 30 days after a partial lesion of the neocortex. The monosialoganglioside GM1 (5mg/kg body weight/ day, i.c.v. for 7 days) produced similar protective effects. The combined application of NGF with GM1 resulted in an amplification of the NGF effects on choline acetyl transferase (ChAT) enzymatic activity both in the NBM ipsilateral to the lesion and in the remaining ipsilateral cortex. In an IN VITRO model of dissociated septal cells, NGF was found to increase ChAT activity as well. The application of NGF in combination with 10^{-5}M GM1, which produced a moderate stimulation of ChAT activity or with 10^{-7}M GM1, which is ineffective in these cultures, produced a much greater increase in ChAT activity than NGF alone.

95.6

S11701 FACILITATES NORADRENERGIC AND CHOLINERGIC TRANSMISSION IN THE BRAIN **J. Lepagnol, L. Breton, M. Brocco, and C. Biton (SPON: P.M. Vanhoutte). Institut De Recherches Servier, 92150 Suresnes, France**

S11701 [(morpholinyl-2)methoxy]-8tetrahydro-1,2,3,4 quinoleine] protects the brain against hypoxia and enhances learning and memory retention. Experiments were designed to determine the effects of S11701 on cerebral noradrenergic and cholinergic transmission. In synaptosomal preparations from rat brain, S11701 inhibited the uptake of ^{3}H-norepinephrine in a concentration-dependent manner; unlike imipramine or viloxazine, pretreatment of the donor animals with the compound did not alter the uptake. Brain slices of the rat were superfused. S11701, whether given to the donor animals or administered directly, enhanced the stimulated release of norepinephrine; no additive effects were obtained with S11701 and cocaine. The facilitation of norepinephrine-release was prevented by imipramine. At concentrations facilitating the evoked release of norepinephrine, S11701 also augmented the stimulated release of acetylcholine. These results suggest that S11701: (a) facilitates noradrenergic neurotransmission possibly by interfering with the imipramine-sensitive neuronal uptake; and (b) enhances cholinergic neurotransmission. These actions may help to explain the protective effect of the compound against cerebral hypoxia as well as the improvement of memory that it causes.

95.7

NON-COMPETITIVE N-METHYL-D-ASPARTIC ACID (NMDA) RECEPTOR INHIBITORS PROTECT AGAINST DYNORPHIN A (DYN)-INDUCED SPINAL CORD INJURY IN RATS. J.B. Long*, A. Martinez-Arizala*, D.D. Rigamonti*, and J.W. Holaday. Dept. of Med. Neurosci., WRAIR, Washington, D.C. 20307

Lumbar spinal subarachnoid injection of DYN causes vasospasm, decreased blood flow, ischemia, neuronal degeneration, and persistent hindlimb paralysis in rats. NMDA receptors have been implicated as mediators in the pathophysiology of ischemic CNS injury due to the neuroprotective effects of selective NMDA receptor antagonists. We therefore evaluated DYN-induced hindlimb (HL) motor dysfunction following treatment with: 1) the competitive NMDA antagonists DL-2-amino-5-phosphonovaleric acid (APV) and DL-2-amino-7-phosphonoheptanoic acid (APH), and 2) the phencyclidine/σ non-competitive NMDA anatagonists ketamine (KET), dextromethorphan (DEX), and MK-801. HL motor function was graded following L4-L5 spinal subarachnoid injections of 20 nmoles of DYN in male SD rats (N≥8/group). Thirty minute s.c. pretreatment with KET, DEX, or MK-801 (100, 35, and 5 mg/kg, respectively) failed to alter DYN paralytic effects. Immediate i.t. preinjections of APV and APH (3 µg) also failed to improve recovery, and at higher doses caused HL flaccidity themselves. KET, DEX, and MK-801 (1000, 350, and 50 µg, i.t., respectively) failed to block DYN-induced loss of HL motor function; however, rats treated with these compounds had significantly improved HL motor scores by 24 hours postinjection. Thus, NMDA receptors appear to be involved in the mechanisms by which DYN causes spinal cord injury leading to persistent HL paralysis.

95.8

CHARACTERIZATION OF KYNURENIC ACID AS A NEGATIVE MODULATOR OF NMDA-SENSITIVE GLUTAMATE RECEPTORS. W. Danysz*, E. Fadda*, J.T. Wroblewski and E. Costa. FGIN, Georgetown Univ. Sch. of Med., Washington, D.C. 20007.

Kynurenic acid (KYN) inhibited the binding of [^{3}H]glycine to rat synaptic membranes (IC_{50} = 56 µM). Among two reportedly selective NMDA antagonists, 2-amino-5-phosphonovaleric acid (APV) inhibited [^{3}H]glycine binding (IC_{50} = 250 µM), while 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) reduced this binding by 30%. The binding of [^{3}H]glutamate was inhibited by CPP and APV with K_i= 0.39 and 2.1 µM, respectively. Since KYN reduced [^{3}H]glutamate binding by only 80%, this displacement, unlike that by CPP and APV, may not be competitive. [^{3}H]MK-801 binding, which is activated by glutamate, was inhibited by CPP, APV and KYN with potencies similar to the inhibition of glutamate binding. Glycine antagonized preferentially the effect of KYN, while glutamate reversed the actions of all three antagonists. It seems that both APV and KYN but not CPP may interact with glycine binding sites. In behavioral studies KYN (0.1 µmol, icv) disrupted passive avoidance learning in rats, similarly to CPP and APV. The negative modulation of NMDA-sensitive glutamate receptors by KYN may result from a preferential interaction with glycine recognition sites but also an interaction with glutamate recognition sites appears to be operative.

95.9

INTERACTION BETWEEN N-METHYL-D-ASPARTATE (NMDA) AND GLYCINE SITES OF THE NMDA RECEPTOR COMPLEX AS REVEALED BY SELECTIVE ANTAGONISTS. Lawrence D. Snell*, Susan M. Jones* and Kenneth M. Johnson. Univ. Texas Medical Branch, Galveston, TX 77550

We have previously reported that glycine indirectly enhances radioligand binding to the PCP receptor located within the N-methyl-D-aspartate (NMDA) receptor channel via a strychnine-insensitive site that is located near the NMDA recognition site. The present study compared the effects of the selective glycine antagonist cycloleucine (CYCLO) on ^{3}H-glutamate (GLU) binding to the NMDA recognition site and conversely the effects of NMDA receptor antagonists on ^{3}H-glycine (GLY) binding to rat cortical membranes.

CYCLO produced a maximal 40% inhibition of GLU binding at 10mM that was completely reversed by addition of 10µM glycine. In contrast, the 60% displacement of GLU binding by 3µM 3-((±)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), a selective NMDA antagonist, was not altered by addition of glycine. 100µM CPP produced a maximal 50% displacement of GLY binding that was completely reversed by the NMDA agonists L-Glu and L-homocysteate (10µM and 100µM, respectively). In contrast, 1mM CYCLO produced an approximate 50% displacement of GLY binding that was unaltered by addition of 10µM L-Glu. Kynurenate (KYN), a non-selective glutamate receptor antagonist, completely displaced GLU binding (IC_{50}=100µM) and was reversed by glycine. Further, KYN completely displaced GLY binding (IC_{50}=30µM) and was not reversed by NMDA agonists. Supported by DA-02073.

95.10

L-ARGININE-L-ASPARTATE (AA) AND ELECTROCONVULSIVE TREATMENT (ECT): FACILITATION OF RECOVERY AND LACK OF MEMORY DEFICIT. G. Cehovic*, R.B. Chronister* and M. Velek* (SPON: R.J. Matthews), Pharmakon Laboratories, Waverly, PA 18471. Departments of Anatomy and Psychiatry, The University of South Alabama, College of Medicine, Mobile, Alabama 36688 U.S.A.

Thirty-two Sprague-Dawley male rats (150-200 grams body weight) were separated randomly into 2 groups of equal number. The AA group received 6 daily doses of AA (500 mg/kg). All animals received either the drug or water at 9 A.M. by esophageal intubation for 6 days. On the 7th day, the animals were placed in a modified standard BRS shuttle box: a dark portion with a floor grid, and a well-illuminated portion with a plexiglas floor. When an animal entered the dark portion, a 2 mA constant current was applied to the grid. The animal was removed immediately from the apparatus and given ECT (100 V, 0.4 second duration, Medcraft B24) using ear clip electrodes within 2 minutes. Twenty-four hours after training the animals were placed into the avoidance chamber on the plexiglas platform. The animals were scored on whether or not they entered the dark zone and the total amount of time spent in the dark zone. Analysis of the entry data revealed that the AA group did not enter the dark zone (i.e.; remembered the task) as much as did the control animals ($p<0.01$) and accordingly spent less time in the dark zone ($T=3.69, p<0.01, df=20$). The AA treatment and water treatment in conjunction with the ECT was continued for the next 6 days and recovery times measured on days 5, 6 and 7. An analysis of variance showed a significant difference in the rate of recovery between the AA and water treated groups ($F=28.9, p<0.0001, df=1.17$) with no difference in the repeated measures ($F=1.66, p>0.20, df=2.2$). These combined data indicate that the AA had a protective effect on ECT induced memory disruption and clearly facilitated the recovery from ECT induced seizures.

96.1

EXERCISE OF OLD RATS IMPROVES MYOCARDIAL PERFORMANCE AND SARCOPLASMIC RETICULUM (SR) Ca^{++} UPTAKE. Michael, L.H., Taffet, G.E.*, and Tate, C.A. Sects. of Cardiovascular Sciences and Geriatrics, Baylor College of Medicine, Houston Tx. 77030

Aging deleteriously affects the function of isolated rat papillary muscle and SR Ca^{++} uptake. Studies by other investigators indicated an improvement of relaxation with exercise training and suggested the involvement of the SR. In the present study we tested this hypothesis of SR involvement and exercise trained 24 mo. male retired breeder Fischer 344 rats for 8 weeks. Following the training program, contractile parameters and SR Ca^{++} uptake by the whole homogenate technique were examined with the following results:

		TPT (ms)	RT 1/2 (ms)	Ca^{++} Transport (nmol $^{45}Ca^{++}$/mg prot/min)
Sedentary	14-mo.	129 ± 2	113 ± 1	5.97 ± .79
Sedentary	26-mo.	150 ± 3[a]	134 ± 6[a]	3.13[a] ± .23
Exercised	26-mo.	136 ± 2	116 ± 3	5.32 ± .54

TPT, time-to-peak tension; RT 1/2, one-half relaxation time; N = 12-16

[a] different from the other two groups, $p < 0.01$

These data directly demonstrate that the SR is involved in the improved relaxation of the myocardium with exercise in old rats. Supported by AG 06221.

96.2

AGING: DIGOXIN TOXICITY IN ANESTHETIZED F-344 RATS. S. Ruch*, E. Seifen and R. H. Kennedy. University of Arkansas for Medical Sciences, Little Rock, AR. 72205.

Age-dependent alterations in the tolerance to cardiotoxic actions of digoxin were monitored using a constant i.v. infusion of the steroid (880 ug/kg/min) in urethane-anesthetized 4, 14 and 25 mo-old Fischer- (F-) 344 rats. Doses of urethane were adjusted relative to the age-related changes in anesthetic requirements for surgery (950, 815 and 725 mg/kg i.p. in 4, 14 and 25 mo-old, respectively). Rats were breathing room air spontaneously throughout the experiment. Blood pressure was monitored from a carotid artery, and an ECG was obtained by limb electrodes. Serum digoxin levels were determined at death using [^{3}H]digoxin as a tracer in the infusion solution. There were aging-related reductions in both the time to initial AV-dissociation and the time to continuous irregular ventricular rate. However, there were no significant differences in lethal doses or serum levels of digoxin at death. The positive chronotropic action of digoxin was significantly reduced in aged rats. In conclusion, these results indicate that there is an aging-related increase in the sensitivity to digoxin-induced AV-block and dysrhythmic activity in urethane-anesthetized F-344 rats which may be mediated, at least in part, by alterations in the autonomic nervous system.

96.3

AGING: EFFECTS ON STIMULATION-INDUCED CHANGES IN INTRACELLULAR Na^+ ACTIVITY IN RAT ATRIAL MUSCLE. R.H. Kennedy, S. Ruch*, W-B. Im* and E. Seifen. University of Arkansas for Medical Sciences, Little Rock, AR. 72205

Previous reports indicate that sarcolemmal Na,K-ATPase activity and Na^+-pump reserve capacity of the myocardium decrease during aging in F344 rats. This suggests that senescence may enhance the sensitivity to conditions promoting Na^+ influx such as high stimulation frequency. To test this hypothesis, mean intracellular Na^+ activity was measured with Na^+-selective microelectrodes in left atrial muscle isolated from 4, 15, and 25 month-old F344 rats. These preparations were bathed in a low-Ca^{++}, 1.0 mM Mn^{++} containing Krebs-Henseleit buffer (37°C) in order to prevent contractile movement and allow for stable impalements during stimulation at increasing frequencies between 0 and 12 Hz. Action potential characteristics were also monitored. There were no age-associated differences in intracellular Na^+ activity over the stimulation frequencies tested, suggesting that Na^+ influx may decline concomitantly with the Na^+-pump in aged preparations. This possibility is supported by the decrease in maximum upstroke velocity which was also observed in senescent tissue.

96.4

DEVELOPMENT OF UPREGULATION OF BETA RECEPTOR SUBTYPES IN THE SENESCENT RAT HEART. N. Tumer,* W.T. Houck* J. Roberts J. Med. Coll. of PA. Dept. Pharmacol., Phila., PA 19129

Beta receptor mechanisms in older hearts respond to procedures which cause upregulation of the beta adrenergic receptors (Tumer et al, 1987). To determine which beta receptor subtypes are responsible for the development of upregulation as a function of age, we studied the ratio of $beta_1$- to $beta_2$-adrenergic receptors in the membrane preparations from the ventricles of Fischer-344 hearts at 6 mo and 24 mo of age. The animals were injected with 6-hydroxydopamine hydrobromide (6-OHDA) (2x50 mg/kg, i.v.) on days one and eight and they were decapitated on day fifteen. The depletion of norepinephrine in the heart was about 86% in each age group. ^{125}I-iodopindolol (IPIN) was used as the radioligand at the final concentration of 25 uM. Inhibition of specific IPIN binding was studied by adding ICI 89,406 ($Beta_1$ selective antagonist) and ICI 118,551 ($Beta_2$ selective antagonist) at 25 pM to 40 uM. The relative proportions of the beta receptor subtypes were determined using a competition radioligand selective binding and computer modeling technique. The ventricles contained about 67% $beta_1$ and 33% $beta_2$ adrenergic receptors at 6 and 24 mo of age; the ratio remained the same in sympathectomized animals. These data suggest that both subtypes of cardiac adrenergic receptors participate in the response to chemical denervation by 6-OHDA with age. (Supported by NIA grant AG03326).

96.5

BETA BLOCKADE ALTERS THE EXERCISE-INDUCED INCREASE IN DIASTOLIC FILLING RATE IN YOUNG, BUT NOT OLD, HEALTHY MEN. Steven P. Schulman,* Edward G. Lakatta, Jerome L. Fleg,* Lewis C. Becker, Myron L. Weisfeldt, Gary Gerstenblith*. Johns Hopkins Hosp, Gerontology Res. Ctr., NIA, Baltimore, MD 21205.

Aging alters some cardiovascular parameters during exercise (Ex) so as to suggest a diminished responsiveness to beta adrenergic stimulation. Catecholamines enhance relaxation in animal models and may mediate, in part, the increased filling necessitated by the higher heart rate and stroke volume during Ex in man. Therefore, the effect of beta blockade (BB) with propranolol,.15 mg/kg i.v. on peak filling rate (PFR,EDV/sec) was determined from gated blood pool scans during upright Ex in young (Y) (mean age = 36) and old (O) (mean age = 64) healthy men in the presence and absence (C) of BB. Ex data below are expressed as the change in PFR from rest to 100 watt (DPFR-100) and maximum (DPFR-MAX) workloads. Data = mean ±S.D.

	Y-C(n=10)	Y-BB(n=10)	O-C(n=8)	O-BB(n=8)
PFR-Rest	2.93±.9*	2.85±.7	2.11±.7	2.51±1.3
DPFR-100	2.82±1.2+	1.99±.8	3.23±1.9	3.27±1.4
DPFR-MAX	5.42±.8**	4.03±1.1	4.15±1.5	4.25±1.7

*p < 0.05 vs O-C, +p < 0.08 vs Y-BB, and **p < 0.01 vs Y-BB. BB diminished the Ex augmentation in PFR in Y but not O men. Thus, catecholamines mediate, in part, the increase in PFR during Ex in Y but not O, and the age difference in response to BB is another example of the age associated diminished cardiovascular responsiveness to beta adrenergic stimulation.

96.6

REDUCED SUSCEPTABILITY TO VAGALLY-MEDIATED SYNCOPE (VS) IN ADVANCED AGE. **L.A. Lipsitz*, E.R. Clagett*, J. Koestner*, and J.Y. Wei.* (SPON: D. Elahi) Hebrew Rehab. Ctr. for Aged, Beth Israel and Brigham & Women's Hosps., Harvard Med. Sch., Boston, MA 02115**

Although the elderly have blunted heart rate (HR) responses to orthostasis, our recent studies of cardiovascular responses to head-up tilt (HUT) in elderly subjects (E) with and w/o unexplained syncope and in young controls (Y) were complicated by VS in 4 of 9 Y and only 1 of 22 E (P=.02). We hypothesized that decreased beta-adrenergic responsiveness in old age protected the E during HUT via unopposed alpha-mediated vasoconstriction or decreased vagal reflex activation. To examine the potential protective effect of beta-blockade, blood pressure (BP), HR, and forearm blood flow (FBF) responses to 60o HUT in 10 healthy E (81+/-8 yrs) were compared to those of 11 Y (21+/-4 yrs) randomized to IV propanolol (P;0.1mg/kg) or saline, 1/2 hr. prior to HUT. Supine HR in Y and E was the same (65 BPM) and increased more in Y (+14+/-8) than E (6+/-5, P=.008) during 1 min HUT. In Y given P, HR response to HUT was blunted the same as old w/out P. BP and FBF responses were the same in Y & O, and Y with & w/o P. Sudden onset of bradycardia, characteristic of VS, occurred by 15 min of HUT in no E and 4 Y, independent of P. Thus, VS during HUT is less common in old age, despite blunted cardioacceleration and unenhanced vasoconstriction. Propanolol does not prevent VS.

96.7

AGE-RELATED DIFFERENCES IN REACTIVITY OF THE CENTRAL CIRCULATION TO HEAD-UP TILT. Katherine W. Tawney,* Emily C. Johnson,* Ernest R. Greene* (SPON: G.O. Ballam). Lovelace Medical Foundation, Albuquerque, NM 87108

Declines in tolerance to orthostatic stress with age are well known. To examine age-related differences in central circulatory responses to orthostatic stress in normal subjects, we measured the immediate changes in cardiac output (CO), stroke volume (SV), heart rate (HR), and mean blood pressure (BP) in response to passive 70° head-up tilt (HU) in orthostatically tolerant young and older subjects. Pulsed Doppler ultrasound was used to measure CO non-invasively from the suprasternal notch of fasting, healthy, echogenic subjects aged 30-39 years (G1;n=8) and 58-74 years (G2;n=8) during supine rest (T0), and 20 (T1), 40 (T2), and 60 (T3) sec after HU. Statistical analyses were performed by ANOVA and Neuman-Keuls tests. X ± SEM (% change from T0):

	T1		T2		T3	
	G1	G2	G1	G2	G1	G2
CO	-27±6	-13±9*	-29±6	-13±7*	-31±5	- 9±8*
SV	-38±5	-24±7*	-43±5	-29±5*	-47±5	-28±6*
HR	+17±3	+15±3	+27±4	+23±4	+31±4	+27±4
BP	.	.	.	.	+ 5±3	+0±0*

* significant (p<.05) difference between G1 and G2

We conclude that the rapid response of the central circulation to caudal translocation of blood is blunted in the aged, which may reflect a compensatory reaction to changes in vascular compliance.

96.8

AGING: EFFECTS OF CHRONIC STRESS ON MYOCARDIAL BETA-ADRENERGIC RECEPTOR BINDING. L.M. Plunkett*, R.H. Kennedy and E. Seifen. UAMS, Dept. of Pharmacology, Little Rock, AR. 72205

Aging is associated with changes in cardiovascular function as well as a decreased capacity to respond to stress. This study examined the effects of chronic stress on beta adrenergic receptor binding in hearts removed from male F344 rats, 4, 14 and 24 months of age. Groups of rats of each age swam for 30 min two times a day for 5 days in 25 °C water. On the sixth day, both stressed (S) and control (C) rats were sacrificed by decapitation, and their hearts removed and perfused with Krebs-Henseleit buffer. Ventricular tissue was separated and frozen at -70 °C until assay. Beta adrenergic binding was determined in crude ventricular membrane homogenates incubated in 75 mM Tris-HCl, 25 mM $MgCl_2$, and increasing concentrations of [^{3}H]dihydroalprenolol (DHA; s.a. 95 Ci/mmol) a beta receptor antagonist. Non-specific binding was determined in the presence of 10^{-5} nadolol. The results showed that aging itself is associated with a significant decrease in the K_d for DHA and a trend towards a decrease in B_{max}. Chronic swim stress led to: 1) a significant reduction in B_{max} in 14 mo old rats, 2) a trend towards a decreased B_{max} in 4 mo old rats, 3) no change in B_{max} in 24 mo old rats, 4) a significant increase in K_d in 24 mo old rats, and 5) no change in K_d in the 4 or 14 mo old groups. These data suggest that aging itself is associated with changes in myocardial beta-adrenergic receptors and that chronic stress in the aged rat does not produce changes in myocardial beta receptor number.

96.9

AGING: EFFECTS ON ANESTHETIC POTENCY AND CARDIAC ACTIONS OF HALOTHANE. G.E. Loss*, R.H. Kennedy, E. Seifen and A.B. Seifen*. U. Arkansas for Med. Sci., Little Rock, AR. 72205

Anesthetic requirements of inhalational agents are decreased in geriatric patients, and the cardiovascular depression elicited by these anesthetics is more severe in the elderly. This study was designed to 1) determine if MAC values for halothane are reduced with age in F344 rats and to 2) monitor direct effects of this volatile agent in myocardial preparations isolated from 4, 14 and 25 mo-old rats. MAC values were determined by tail- and paw-clamping. Isolated cardiac preparations were perfused with Krebs-Henseleit buffer (37°C) saturated with 95% O_2/5% CO_2 with and without various levels of halothane. Chronotropic actions were monitored in spontaneously beating right atria, while inotropic effects were measured in electrically paced cardiac muscle. Results indicated that MAC values for halothane are reduced approximately 15% in senescent rats. However, there were no significant differences in the negative chronotropic actions or in the negative inotropic effects observed in atrial or ventricular muscle when compared on a vol% basis. Langendorff preparations from young adults were more sensitive to the halothane-induced decline in contractility; however, there was no difference between 14 and 25 mo-olds. These results suggest 1) that the anesthetic potency of halothane increases with senescence in F344 rats and 2) that direct cardiac effects of volatile agents are not affected during aging.

96.10

HEART DISEASE AND ATRIAL FIBRILLATION SEX DISTRIBUTION AND INCIDENCE: DESCRIPTION AS A FUNCTION OF AGE, AND EARLY MALE INITIATION. Richard P. Spencer. Dept. Nuclear Medicine. Univ. Connecticut Health Center, Farmington, CT 06032.

Certain disorders occur in both sexes but are usually considered "male diseases" such as atrial fibrillation (AF) and death from coronary artery disease (CAD). Using the observation of Burch, that the death rate from malignancies was a power function of age, we have plotted log of incidence (I) of the cardiovascular disorders as a function of the log of age. For the death rate from heart disease in Scotland (Kennedy, 1985), in terms of cases/100,000 population:

Males log I CAD = 5.89 log (Age) - 7.75, Corr. coeff. 0.99
Females log I CAD = 6.52 log (Age) - 8.98, Corr. coeff. 0.99

Slope for the females is higher than for males, but the male line is displaced to the left (earlier male initiation). The same type of analysis was applied to the 2 year incidence of chronic atrial fibrillation in the Framingham study (Wolf, Abbott, Kannel, 1987).

Males log I(AF)= 5.63 log (Age) - 9.28, Corr. coeff. 0.98
Females log I(AF)= 7.29 log (Age) -12.57, Corr. coeff. 0.99

Again the slope for females was higher, but the male line was displaced to the left (earlier male initiation or delayed female initiation). The 2 lines cross at age 96 years. The ratios of the equations gave a good description of the male/female ratio from age 60 years up. Thus while not elucidating etiology, the equations describe incidence quite well.

CARDIAC ELECTROPHYSIOLOGY

97.1

TRANSITIONAL STATES BETWEEN AUTOMATICITY AND QUIESCENCE IN MODELS OF CARDIAC CELLS. M. Landau,* D.C. Michaels,* P. Lorente* and J. Jalife. SUNY Health Science Center, Dept of Pharmacology, Syracuse, NY 13210, U.S.A.

Simulations of single cardiac cells based on either the Van Capelle and Durrer (VCD) model or the Noma and Irisawa (NI) model were used to study the dynamics of transitions between pacemaker and non pacemaker domains. For the simpler two state VCD model, continuation-bifurcation (CB) techniques were used to classify behavior patterns observed when the parameter q, which sets equilibrium potential and depolarization threshold level, was varied. Within the q parameter space 5 zones of specific behaviors were observed, including: (1) a single stationary stable state, (2) coexisting stable stationary and stable pacemaker states, (3) a single stable pacemaker state, (4) 2 stable periodic (high and low amplitude) states and 2 unstable (periodic and stationary) states, and (5) a single stationary stable state. In zone (2), unstable orbits behaved as separatrices between attractor basins corresponding to steady state and periodic solutions in the membrane potential vs activation phase plane. Under these conditions, small depolarizing pulses could initiate sustained periodicity from a stable state, perturb but not alter periodic activity, or annihilate periodic activity depending on the phase, amplitude and sign of the pulse. Predictions made from the CB analysis were confirmed in simulations where single depolarizing pulses were applied at various phases in the more physiologically accurate NI model. In the normal model, pulses led to only transient perturbations. However, when bias hyperpolarizing current was applied (analogous to changing the q parameter), single current pulses were able to annihilate spontaneous activity. We conclude that the pacemaker annihilation phenomenon may be related to a pacemaker/non pacemaker phase transition and that the computation of attractor basins is able to predict when and how pacemaker rhythmic activity can be suppressed.

97.2

BISTABILITY AND HYSTERESIS IN EXCITABLE CARDIAC CELLS. P. Lorente,* M. Landau* and J. Jalife. SUNY Health Science Center. Dept of Pharmacology, Syracuse, NY 13210, U.S.A.

Multiple stable states and hysteresis phenomena in response to repetitive electrical stimuli have not been described previously in isolated cardiac tissues. Yet, experimental evidence in nerve and a recently described theoretical model of the heart cell suggest that these phenomena may indeed occur in excitable cardiac tissue preparations. We investigated this possibility in 14 quiescent sheep Purkinje fibers driven by depolarizing current pulses of constant duration and cycle length, applied through a suction pipette. Stepwise variation in the current strength was used as the input parameter. Starting always from a stable 1:1 pattern, the zone of threshold current for excitation was scanned by decreasing and then increasing the input parameter value, and noting the values at which the 1:1 to 1:0 and 1:0 to 1:1 transitions occurred. Reproducibility and stability of patterns were the required criteria to validate the results. All but three preparations showed hysteresis. In fact, bistability between 1:1 and 1:0 was observed in 61 runs in which either pattern was demonstrable at the same value of current strength, depending on previous history. Moreover, the transition from 1:1 to 1:0 always occurred at lower levels of current strength than the reverse transition (i.e., from 1:0 to 1:1). The area of the loop was used as an index of the degree of hysteresis under various experimental conditions. Loop area decreased gradually, and eventually disappeared, as pulse duration was increased from 2 to 50 msec. On the other hand, loop area increased significantly as the temperature of the bathing medium was decreased from 37 to 25 degrees Centigrade. We conclude that the excitability of cardiac Purkinje fibers depends on past history, which may have important implications in cardiac rhythm and conduction disturbances.

97.3

ELECTRICAL AND MECHANICAL EFFECTS OF STRONTIUM IN SHEEP CARDIAC PURKINJE FIBERS. Mario D. Gonzalez* and Mario Vassalle. SUNY, Health Science Center, Brooklyn, NY 11203.

The effects of strontium (Sr) in the absence of calcium were studied in sheep cardiac Purkinje fibers perfused in vitro. In a Ca-free solution, Sr (1.35-10.8 mM): 1) causes a time-, rate- and concentration-dependent shift of the plateau to more positive potentials, prolongs the action potential and decreases the maximum diastolic potential; 2) increases the time to peak and amplitude of twitch and causes a tonic force which relaxes only on repolarization; 3) is rapidly overcome in its effects by calcium administration (1.35-2.7 mM); 4) is antagonized by Mn (1 mM) and Cd (0.1-0.2 mM); 5) is potentiated by norepinephrine administration (0.1 uM); 6) can induce action potentials in 27 mM $[K]_o$; 7) induces a tail following the action potential in prescence of Cs; 8) can induce a tail in 8 mM $[K]_o$ which sustains force development and is reduced by calcium antagonists; 9) if applied to a quiescent fiber, induces a prolongation of the first resumed action potential and tonic force but a small twitch and these effects are antagonized by Ca and Mn; and 10) induces a strong twitch after quiescence in low $[Na]_o$. Thus, that an increased Sr influx (due to a slow inactivation of I_{si}) and a decreased Sr extrusion through an electrogenic Na-Sr exchange (due to a short diastole) accounts for the pronounced and progressive electrico-mechanical effects of Sr in cardiac Purkinje fibers. Supported by NIH grant HL 17451.

97.4

CONTROL OF Ca CURRENT STAIRCASE AFTER REST IN RABBIT VENTRICULAR MYOCYTES. Larry V. Hryshko* and Donald M. Bers. University of California, Riverside, CA 92521.

The post-rest recovery of Ca current (I_{Ca}) was characterized in rabbit ventricular myocytes at 30°C. Patch pipettes contained (in mM): 135 CsCl, 5 Mg-ATP, 5 HEPES, and 10 EGTA. Bath solution contained: 140 TEA-Cl, 2 $CaCl_2$, 5 HEPES and 10 glucose at pH 7.40. From physiological holding potentials (-80 to -90mV), I_{Ca} at the first post-rest pulse was smaller than at steady state (200 ms clamp pulses to 0 mV at 0.5 Hz), apparently due to accelerated inactivation. I_{Ca} recovery occurred within 4-10 pulses. The magnitude of I_{Ca} depression was relatively constant over a range of rest intervals (15 sec to 5 min). These positive staircases could be abolished by replacement of extracellular Ca with Ba (or less so with Sr). Either of these cations decreased inactivation rates and seemed to prevent the inactivation responsible for the post-rest staircase. Conversely, the staircase was augmented by elevated Ca_o (6mM) whereas lowering Ca_o (0.5mM) had the opposite effect. The staircase was not altered by ryanodine (1 μM) suggesting that transiently unbuffered Ca_i release was not involved. 3,4-diaminopyridine (50μM) was also without effect on currents or staircase supporting the lack of involvement of potassium currents. These data suggest that Ca entry at the first few post-rest pulses has a facilatory effect on I_{Ca} at subsequent pulses (perhaps by accomodation of Ca_i-dependent inactivation). This contrasts with results obtained at less negative holding potentials (-50 to -40mV) where negative staircases are observed which are likely to be due to the slow recovery from inactivation of Ca channels at these potentials.
(Supported by grants from Am. Heart Assn., Calif. Affil. and USPHS)

97.5

QUINIDINE BLOCK OF INWARD RECTIFIER K CHANNELS IN HEART Jeffrey R. Balser*, Dan M. Roden*, Paul B. Bennett* (SPON: Luc M. Hondeghem). Vanderbilt University School of Medicine, Nashville, TN 37232

We have investigated the effects of quinidine on single inward rectifier potassium (K) channels using cell attached patch recording techniques in enzymatically dispersed guinea pig ventricular myocytes. To eliminate current through non-K channels while maintaining physiologic K gradients, we used patch electrodes containing (in mM): N-methyl-d-glucamine 150; HEPES 20; $CaCl_2$ 0.1; $CdCl_2$ 0.1; $LaCl_3$ 0.1; glucose 10; and KCl 4.5. Voltage clamp steps to potentials negative to E_K (-80 mV) revealed inward rectifier unitary currents with a main conductance level of 2.7 pS and, as described by Sakmann and Trube (1984), a prominent subconductance level of 1.4 pS. The existence of substates, rather than multiple channel types, was verified in patches with only one K channel: both the main and subconductance states were observed, but never simultaneously. Single channel conductance was unaltered by 10-50 μM quinidine; however, the probability of channel opening was reduced. Moreover, the probability of substate openings was reduced from (mean ± SD, n=4) .04 ± .02 to .006 ± .007 (↓ 80%), while the probability of opening to the main conductance level was only reduced from .14 ± .13 to .06 ± .03 (↓ 59%) These data suggest that block of the inward rectifier K channel results from drug-induced inhibition of channel opening; the greater reduction of substate openings relative to main state openings suggests that quinidine preferentially inhibits particular conformational transitions of this K channel.

97.6

PHORBOL ESTER HAS TIME-DEPENDENT AND VOLTAGE-DEPENDENT EFFECTS ON CARDIAC CA^{2+} CHANNELS. Antonio E. Lacerda*, David Rampe* and Arthur M. Brown. Baylor College of Medicine, Houston, TX 77030.

Phorbol esters produce inhibitory and stimulatory effects on voltage-dependent cardiac Ca^{2+} channels. In both cases activation of protein kinase C (PKC) was implicated. To examine the discrepancy, we measured $^{45}Ca^{2+}$ uptake and single cardiac Ca^{2+} channel currents in response to phorbol ester treatment. The phorbol ester 12-0-tetradecanoylphorbol-13-acetate (TPA) stimulated dihydropyridine sensitive $^{45}Ca^{2+}$ influx in primary cultures of neonatal rat ventricular myocytes within 5 seconds. However after a 20 minute pre-incubation period, TPA markedly inhibited $^{45}Ca^{2+}$ influx. The sequence of stimulation followed by inhibition was confirmed in cell-attached patch clamp recordings of single Ca^{2+} channel currents. The probability of opening of Ca^{2+} channels was initially increased by TPA and later was reduced which explains the opposite effects on cardiac Ca^{2+} channels that have been reported. The Na^+/H^+ antiporter was inoperative in the 0 Na^+ solutions we used. The stimulatory effect of TPA was typically an order of magnitude faster at 0 mV than it was at -40 mV. This leads to the novel conclusion that the rate of PKC activation is modulated by the state of the Ca^{2+} channel. Supported by HL36930, HL37044, HL07651 and HL07348.

97.7

EFFECTS OF CALCIUM MODULATORS AND ETHANOL ON ATRIAL MEMBRANE POTENTIALS. R. G. Carpentier, A. Gallardo-Carpentier, R. P. Salvatigi* and R.L. Isaacson. Howard U. Washington, DC 20059. and SUNY, Binghamton, N.Y. 13901.

The acute effects of E and calcium modulators (CMs) on atrial membrane potentials (MP) were studied. Rat atrial strips superfused with Tyrode's solution (36 °C) were driven at 5 Hz while recording MP with intracellular microelectrodes. E 2.4 g/l reduced the amplitude of the action potential (AAP) without affecting the membrane resting potential (MRP) or the Vmax of phase 0 (Vmax 0). The velocity of polarization of phase 2 (dV/dt 2) was increased, while the velocity of phase 3 (dV/dt 3) was not modified, which caused a decrease in the action potential duration (APD). Nimodipine 1.75 mg/l (N) had the same effects. Similar results were obtained with N + E. Bay K 8644 250 ug/l (B) increased the AAP without changing MRP or Vmax 0. The dV/dt 2 was decreased, while the dV/dt 3 was not changed, which resulted in an increase in the APD. The MP remained unchanged when the preparations were exposed to B + E. In summary, E and N exerted similar actions on the MP while B had opposite actions. The effects of E and B cancelled each other. Supported by NIH/MBRS and ECFMG grants.

97.8

DEMONSTRATION OF THE FATIGUE AND SLOW RECOVERY IN THE HUMAN ATRIOVENTRICULAR NODE (AVN). A. Mokrane*, M. Shenasa, D. Lacroix*, M. Dubuc*, A.-R. LeBlanc*, Sacré-Coeur Hosp. Univ. Montreal, Canada.

The "fatigue phenomenon" already described in the rabbit AVN is defined as a time-dependent prolongation in AVN conduction time during fast pacing rate over several minutes. The characteristics of AVN fatigue and recovery in man were less defined. In 10 patients, a beat-to-beat analysis of AVN conduction (AH interval) was done during atrial pacing over periods of 30 seconds to 2 minutes. Computer-assisted analysis of digitized signals (sampling rate: 2 KHz) from tape recordings was used. The basic cycle lengths were 800 or 700 ms. Fatigue was determined during fast atrial pacing cycle lengths of 500 or 400 ms and the recovery was determined during return to basic cycle length. In all cases, an instantaneous prolongation in the AVN conduction was observed at the onset of the fast rate which stabilized with some degree of beat-to-beat variation in AVN conduction (5-20 ms). Upon return to the basic drive (recovery), a beat-to-beat decrease in AVN conduction was observed for several beats depending on the cycle length and duration of the fast rate. These observations suggest that fatigue and slow recovery do occur in intact human AVN and can be manifested only with fast pacing rate for long periods.

97.9

UNCOUPLED TIME COURSE CHANGES IN SINOATRIAL AND ATRIOVENTRICULAR NODAL RESPONSE TO ATROPINE IN MAN. M. Shenasa, M. Dubuc*, T. Kus*, D. Lacroix*, R. Nadeau, Sacré-Coeur Hospital, Univ. of Montreal, Montreal, Canada.

To test the hypothesis that the sinoatrial (SA) and atrioventricular (AV) nodal response demonstrate different phase response curves to vagal inhibition induced by atropine (Atr), we studied 17 subjects with normal SA and AV nodal function. The sinus cycle length (SCL), AV nodal conduction time (AH interval), and atrial pacing CL that induced Wenckeback phenomenon (WPCL) were measured before and after I.V. Atr. Atr was given as 0.4 mg/kg body weight in divided doses. Changes in SCL, AH interval, ratio of AH/SCL and WPCL were measured at 0.5, 1, 2, 5 and 10 minutes after Atr injection. SA node responded faster to Atr than the AV node by 45-190 seconds (mean 115 ± 40 seconds). Dose response curves to Atr were obtained by calculating the maximum percentage changes (% Δ) from baseline values:

Atropine IV:	0.4 mg	0.8 mg	1.2 mg	1.6 mg	2.0 mg
% Δ SCL	- 8%	-12%	-22%	-29%	-35%
% Δ AH	- 6%	-13%	-20%	-49%	-50%
Δ AH/ Δ SCL	13%	17%	15%	20%	24%
% Δ WPCL	- 9%	-16%	-25%	-29%	-31%

The data suggest that the SA and AV node demonstrate a different time course change and a non-linear dose response curves to vagal inhibition induced by atropine. This dissimilar pattern may be due to differential responsiveness of the two nodes to vagal modulation.

97.10

ATRIOVENTRICULAR NODAL FUNCTIONAL PROPERTIES AND ASSESSMENT OF NODAL RECOVERY TIME IN RABBIT HEART. J. Zhao, J. Billette and R. Métayer. Dept. of Physiology, Univ. of Montreal, Montreal, Canada, H3C 3J7.

The origin of apparent differences in nodal functional properties according to the variable used to assess nodal recovery time was studied in 6 isolated rabbit heart preparations. Premature conduction times (A2H2 intervals) were obtained at control, during a facilitation, during a steady-state fatigue and during a combined facilitation and steady-state fatigue all of which were induced with specifically designed periodic premature stimulation protocols applied to the atrium. These A2H2 intervals were plotted against both the corresponding preceding atrial (A1A2) and His-atrial (H1A2) intervals, yielding for each preparation 4 AA- and 4 HA-recovery curves, respectively. The AA- and HA-recovery curve associated to any given protocol had an identical form and differed only in the position on abcissa by a constant equal to nodal conduction time of last basic beat. Moreover, when the AA-recovery curves were corrected for recovery-dependent changes in conduction time of last basic beat, the relative changes in A2H2 intervals caused by the last 3 protocols as compared to the control one were identical on the AA- and the HA-recovery curve. In conclusion, the same intrinsic nodal functional properties can be identified on the AA- and HA-recovery curves. (Supported by Medical Research Council of Canada and Quebec Heart Foundation.)

SKELETAL MUSCLE PHYSIOLOGY II

98.1

HISTOCHEMICAL PROFILES OF SOLEUS MUSCLE GRAFTS FOLLOWING TRAINING. Kathryn I. Clark*, Pedro G. Morales*, and Timothy P. White. Department of Kinesiology, The University of Michigan, Ann Arbor, MI. 48109-2214.

The objective was to characterize the effect of endurance training on fiber size and fiber type of soleus muscle grafts in rats. We tested the hypothesis that run-training initiated 28 days following grafting would increase fiber cross-sectional area concomitant with an increase in muscle mass. Nerve-reimplant orthotopic grafting surgery was performed on 6 week old rats anesthetized with pentobarbital sodium. A cohort of the animals began running 28 days later. Grafted muscles from non-run and run-trained animals were compared to unoperated control muscles from age-matched, non-run rats. Run training increased graft mass 34% over the non-run graft value of 82 ± 7 mg at 56 days post-grafting (i.e. after 28 days of running). Mass continued to increase through 112 days, but the magnitude of change was smaller (129 ± 9 mg in run grafts vs. 93 ± 5 mg in non-run grafts). Running had no effect on fiber cross-sectional area of grafts through 56 days. Continued running through 112 days increased fiber area 21% over non-run graft values (1193 ± 115 μm^2). Running had the greatest effect on the cross-sectional area of type I fibers, a transient effect on type IIB fibers and no effect on IIA fibers. By 112 days muscle fiber composition was not different between control muscles, grafts, or grafts from run-trained animals. We conclude that the effect of run-training on muscle mass and fiber cross-sectional area differs with respect to the time course and magnitude of the increase.

Supported by NIH DE-07687.

98.2

PLASTICITY OF SOLEUS AND PLANTARIS MUSCLES IN 15- AND 28-MO F344 RATS. Timothy P. White, Christopher K. Daw*, Kathryn I. Clark*, and Susan C. Kandarian*. Department of Kinesiology and Institute of Gerontology, The University of Michigan, Ann Arbor, MI 48109-2214.

The objective was to study the plasticity of soleus and plantaris muscles in 15- and 28-mo rats as evidenced by the adaptive response to ablation of synergist muscle. Hypotheses were tested regarding mass, muscle and fiber cross-sectional areas (CSA), and maximum isometric force (P_0). Rats were anesthetized with pentobarbital sodium before unilateral ablation of the gastrocnemius (GTN) muscle. Soleus and plantaris muscles were studied 28 days thereafter; contralateral muscles served as controls. Contractile properties were measured *in vitro* followed by histological preparation. Fiber CSA was measured by image analysis. Following ablation in 15-mo rats, soleus muscles mass was 106% of the control value (81 ± 3 mg) while P_0 was not different from 1.22 ± 0.02 N (X ± SE). In 28-mo rats, mass and P_0 were 133 and 150% of the respective control values of 66 ± 8 mg and 0.79 ± 0.19 N ($p < 0.05$). In 15-mo rats, plantaris muscle mass and P_0 were 119% and 108% of the control values of 192 ± 9 mg and 4.02 ± 0.30 N, respectively. In 28-mo rats, mass and P_0 were 175 and 213% of the control values of 116 ± 16 mg and 1.74 ± 0.56 N, respectively ($p < 0.05$). While there was a trend toward increased fiber CSA due to ablation, the high variability obviated significant differences. Adaptation to GTN removal was greater in soleus and plantaris muscles from 28- compared to 15-mo rats.

Supported by AG06130 and AG00114.

98.3

HYPERTROPHIC RESPONSE OF SKELETAL MUSCLES TO SYNERGIST ABLATION IN 28-MO RATS FOLLOWING 4 MO OF RUN-TRAINING. Christopher K. Daw*, Susan C. Kandarian*, Kathryn I. Clark* and Timothy P. White. Department of Kinesiology and Institute of Gerontology, The University of Michigan, Ann Arbor, MI 48109-2214.

The aim was to determine the influence of prior run-training on the adaptive response to synergist removal in skeletal muscles from aged rats. Following completion of 4 mo of run-training (5 d/wk, 16 m/min, 45 min/d, 15% grade), 27-mo female F344 rats (n = 13) underwent unilateral ablation of the gastrocnemius (GTN) muscle. Further comparisons were made with age-matched sedentary rats (n = 8) following unilateral ablation. Twenty-eight days after surgery, contractile properties of soleus and plantaris muscles were studied *in vitro* followed by histological preparation. Fiber cross-sectional area (CSA) was measured by image analysis. After ablation, soleus muscle mass increased 33 and 20% compared to the control values of 66 ± 8 mg (X ± SE) and 78 ± 6 for sedentary and run-trained rats, respectively. Maximum isometric force (P_0), specific P_0, and fiber CSA increased due to ablation independent of run-training ($p < 0.05$). The run-trained animals exhibited an increase ($p < 0.05$) in soleus muscle P_0, specific P_0, and fiber CSA but not muscle mass ($p = 0.12$). Changes in plantaris muscle were qualitatively similar for most variables. Although most variables from run-trained animals were greater than sedentary rats, prior run-training did not alter the relative magnitude of compensatory growth in muscles of aged rats.

Supported by NIH AG-06130 and AG-00114.

98.4

ENHANCEMENT OF RECOVERY FROM CONTRACTION-INDUCED INJURY OF SKELETAL MUSCLE IN MICE BY OXYGEN-FREE RADICAL SCAVENGERS. Eileen Zerba* and John A. Faulkner. Department of Physiology and Institute of Gerontology, The University of Michigan, Ann Arbor, MI 48109.

Lengthening contractions cause histological evidence of injury and decreases in the maximum force developed by muscles. We tested the hypothesis that the recovery of muscles after contraction-induced injury are enhanced by oxygen-free radical scavengers. Extensor digitorum longus (EDL) muscles of young and aged mice were injured in situ by a 5 minute protocol of repeated lengthening contractions. Experimental animals were injected ip with 1000 units/kg of polyethyleneglycol superoxide dismutase (SOD), an oxygen-free radical scavenger, 24 hrs pre-injury and 24 and 48 hrs post-injury. Contractile properties were measured in situ before and after the injury protocol was performed. Compared to control values, 3 days after contraction-induced injury maximum force was 36% and 25% for muscles in young and aged mice respectively. In the presence of SOD, injured EDL muscles from young mice recovered 100% of maximum force by day 3, whereas those of aged mice recovered 60%. The data support our hypothesis that recovery from contraction-induced injury is enhanced by oxygen-free radical scavengers, but muscles from aged mice are protected less than muscles from young mice. Supported by AG 06157.

98.5

POWER OUTPUT OF EDL MUSCLES FROM YOUNG AND OLD MICE. Susan V. Brooks* and John A. Faulkner, Dept. of Physiology and Bioengineering Program, University of Michigan, Ann Arbor, Michigan 48109

With aging, the extensor digitorum longus (EDL) muscle of the mouse shows a decrease in force of 20-30% and no change in velocity of shortening. We hypothesized that EDL muscles from young (2-3 months) mice develop and sustain higher power (force x velocity) than EDL muscles from old (26-27 months) mice. A frequency-power relation for maximum power during a single contraction was determined for stimulation frequencies from 80-350 Hz. With fiber length (L_f) optimized for force development, a Cambridge ergometer produced constant velocity displacements from 105% of L_f to 95% of L_f. At each frequency, the velocity was adjusted for maximum power. The maximum power was 308±21 W/kg and 264±18 W/kg for EDL muscles from young and old mice respectively. The power sustained over time was determined with muscles stimulated at 150 Hz. Repetitive contractions began at a train rate of 1 Hz with increments of 1 Hz every 5 minutes for young muscles and 0.5 Hz every 10 minutes for old muscles. The power sustained by the muscles from young mice plateaued at 8.6±0.5 W/kg and a train rate of 7 Hz and those from old mice at 4.4±0.4 W/kg and a train rate of 1.5 Hz. Muscles from the old mice could not function at train rates greater than 3-4 Hz. Supported by AG 06157.

98.6

BIOENERGETICS OF SKELETAL MUSCLE POWER, FORCE AND FATIGUE: NEW INSIGHTS USING 31-P NMR. C.R. Bridges*, B.J. Clark*, R.L. Hammond*, W.A. Anderson*, F.D. DiMeo*, B. Chance, L.W. Stephenson. Univ. of PA, Philadelphia, PA 19104.

A model is presented which allows measurement of the power output of latissimus dorsi muscle with simultaneous aquisition of 31-P NMR spectra. Skeletal muscle ventricles (SMVs) were constructed from mobilized canine latissimus dorsi muscle (N=4). A device was inserted into the SMV cavity which allows for independent control of preload and afterload. The SMV was stimulated via the thoracodorsal nerve at 25 and 85 Hz burst frequencies both during isovolumetric and isotonic contractions. Using a surface coil placed directly over the SMV, spectra were obtained during an 11 minute exercise period for each protocol. During the first three minutes Pi/PCr correlated with power output both at 25 Hz (R=0.9) and at 85 Hz (R=0.7). The ratio (Pi/PCr)/(wall stress) $[mmHg]^{-1}$ was .025 ± .003 for isovolumetric, and .036 ± .005 for isotonic exercise ($p<.03$). During isovolumetric stimulation at 25 Hz, SMV wall stress decreased by 32%, while the (Pi/PCr)/(wall stress) ratio increased from .027 ± .004 to .035 ± .003 ($p<.03$). In contrast, at 85 Hz this ratio remained constant despite a 72% decrease in wall stress. These data demonstrate a higher energy cost of isotonic vs. isovolumetric contraction at equal levels of stress and support the hypothesis that high frequency fatigue is not metabolic in origin.

98.7

IN-VIVO C-11 ALPHA AMINOISOBUTYRIC ACID (AIB) SKELETAL MUSCLE TRANSPORT IN STARVATION. M.T. Corbally, J.R. Bading, J. Fissekis, G.R. DiResta, K.C. Conlon and M.F. Brennan. (SPON: L. Freedman). Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021.

To determine the role of A-system amino acid transport in the metabolic adaptation to starvation, the non-metabolized amino acid C-11 AIB was used to measure hindlimb A-system transport in the intact dog (n=6) before (B) and after fasting (S) for 14 days. Capillary (Ecap) and cellular (Ecell) extraction of AIB were determined following bolus femoral artery injection and external residue detection. A mathematical model was used to determine cellular transport (PScell). Regional plasma flow (F) was determined from the kinetics of co-injected Tc-99m labelled human serum albumin HSA by the height/area method. Capillary clearance (FxEcap) of AIB was also computed.

	Flow	PScell (ml/min/100gm)	Clearance
B.	23 ±8	0.51±0.1	3.9±2.7
S.	*1.4±0.3	0.81±0.2	*1.3±0.6

Data are mean ± SEM. *$p<0.05$ by repeat measures ANOVA. A-system transport is low in skeletal muscle and does not change in response to starvation. The finding of decreased regional plasma flow and clearance suggests that capillary flow may regulate peripheral amino acid exchange.

98.8

IN VIVO SKELETAL MUSCLE GLUTAMINE TRANSPORT IN CANCER. K.C.Conlon*, J.R.Bading*, A.S.Gelbard*, M.T.Corbally*, G.R. DiResta*, N.A.Vydelingum*, M.F.Brennan*. (SPON: G.W.Pasternak) Memorial Sloan-Kettering Cancer Center, New York, NY 10021

To determine the role of transport in the glutamine (GLN) efflux that accompanies acute starvation and other catabolic states, we examined GLN transport in the hind limbs of normal (N, n=12) and VX2 tumor bearing, cachectic (TB, n=5, >15% weight loss) rabbits, using an in vivo residue detection technique following bolus femoral artery injection of L-(amide-N-13)GLN and Tc-99m Human serum albumin (HSA). Graphical analysis and a two barrier Renkin-Crone model were used to compute transport parameters for cellular entry (PScell) and intracellular fractional washout rate (k_3) for GLN. Regional plasma flow (PF) was computed from the HSA kinetics and transmission measurements of tissue mass. Hindlimb GLN flux was also measured.

	PF (ml/min/100g)	PScell (ml/min/100g)	k_3 (%/min)	GLN flux (µmol/min/100g)
N	5.8±0.7	3.5±0.5	0.77±0.07	-0.17±0.13
TB	6.8±1.4	3.1±1.0	0.71±0.14	-0.51±0.05*

Data are mean ± sem; * $P<0.05$. Results suggest the increased GLN efflux in tumor induced cachexia is not associated with altered GLN transport, but reflects increased net intracellular production of GLN.

98.9

CELL INJECTION TREATMENT FOR MUSCLE DEGENERATION. P.K. Law, T.G. Goodwin*, H.J. Li* and M. Chen*. Depts. of Neurol and Physiol/Biophy, Univ. of Tennessee, Memphis, TN 38163

A treatment has been developed to prevent hindlimb and intercostal muscle weakness in murine dystrophy. Injection of histoincompatible normal myoblasts into dystrophic intercostal and leg muscles improved the structure and function of the muscles to almost normal. Immunosuppression of the C57BL/6J-dy^{2J}/dy^{2J} hosts was by way of daily subcutaneous injection of cyclosporin-A. Injected dystrophic muscles exhibited greater cross-sectional area, total fiber number, wet weight, and twitch and tetanus tensions six months postoperatively. Fiber typing was more defined and they contained more normal-appearing and less abnormal-appearing fibers than non-injected controls. Eleven out of nineteen mice that received myoblast injections on both sides showed such behavioral improvement that their locomotive patterns were indistinguishable from normal. Using dimeric isozymes as genotype markers for host and donor cells, the demonstration of parental and hybrid isozymes inside the injected muscles substantiated the survival and development of donor myoblasts into normal myofibers, and the fusion of normal myoblasts with dystrophic satellite cells to form genetically mosaic myofibers. Since the treatment design is based on muscle developmental processes universal to all mammals, it has potential for clinical application. (Supported by USPHS NS-20251 and NS-26185).

98.10

THYROID HORMONE REGULATION OF NA-CHANNELS IN CULTURED RAT MYOTUBES. S.R. Sampson AND Chaya Brodie. Bar-Ilan University, Ramat-Gan, Israel.

The number of Na-channels in mammalian skeletal muscle increases with age and is influenced by several factors including innervation and possible trophic factors. The role of hormones has not yet been studied. We have examined the effects of thyroid hormones (TH) on the number and activity of Na-channels in cultured rat skeletal myotubes, as measured by [^{3}H]Saxitoxin (STX) in myotubes of age 7-9 days in vitro. Cultured myotubes display parallel increases in the number of Na-channels and the rate of rise and frequency of spontaneously-occurring action potentials with age. TH caused dose-dependent increases in [^{3}H]STX binding and frequency of spontaneous action potentials. The increase in number of channels was prevented by treatment with cyclohexemide. Scatchard analysis of [^{3}H]STX binding showed that hormone-treated cells have channels with lower affinity than control. Blockade of Cs-influx potentiated the effect of TH. Action potentials in TH-treated cells had faster rates of rise and fall than those in control myotubes. We conclude that TH play an important role in regulation of Na-channels of skeletal muscle. (Supported by The Ben and Effie Raber Neuroscience Research Fund and The Spingold Foundation.)

99.1

MUSCARINIC CONTROL OF EPITHELIAL DERIVED RELAXANT FACTOR (EpDRF). A. Lev, C. Christensen, J. Ryan, M. Wang and S.G. Kelsen. Temple Univ. Sch. of Med., Phila. PA 19140

Mechanical removal of the epithelium augments the tracheal smooth muscle tension responses to acetylcholine, presumably by eliminating the EpDRF. The present study tested the hypotheses that the release of EpDRF is: 1) under muscarinic receptor control; and 2) inhibited by M1 and augmented by M2/M3 muscarinic receptors. Experiments were performed in vitro at 37°C on 164 rabbit tracheal strips. Blockade of M2 receptors with gallamine (10^{-7}M) abolished the epithelial effect. On the other hand, pretreatment with pirenzepine, an M1 receptor blocker (10^{-5}M), augmented the epithelial effect during ACh stimulus response curves. Conversely, an M1 receptor agonist (McN-A-343) abolished the epithelial effect. Since we have previously shown that cooling tracheal strips abolishes the effect of epithelial removal on the ACh dose response relationship, we also examined the response to pirenzepine and bethanechol an M2/M3 agonist, at 23°C. Removal of the epithelium was associated with leftward shift of the bethanechol and pirenzepine dose response curves. The present study supports the hypothesis that EpDRF production and/or release is modulated by distinct populations of muscarinic receptors located on the respiratory epithelial cells.

99.2

INTERLEUKIN 2 (IL-2) INCREASES ALBUMIN PERMEABILITY (P) OF CULTURED PULMONARY ENDOTHELIAL CELL (PEC) MONOLAYERS. Gordon H. Downie*, Megan M. Hennessy* and Mitchell Friedman. University of North Carolina, Chapel Hill N.C. 27599.

IL-2 infusion results in a "vascular leak" syndrome. The mechanism(s) for this increase in vascular permeability are unknown. To study the direct effects of IL-2 on P, bovine PEC were grown to confluence on gelatin-coated polycarbonate filters and incubated either with recombinant IL-2 (500 or 5000 units, Cetus), vehicle only (as a negative control), or with 2000 units of tumor necrosis factor (TNF, as a positive control) in serum-free media for 4H at 37°C. The PEC were washed, fresh serum-free media added and the filters mounted in a flux chamber. Steady-state P was computed as the average rate of ^{125}I-albumin clearance (%/hour) during the 90-240 minute period after addition of tracer to the upper well (J. Cell. Physiol. 129:237,1986). Each experimental point represents the average P of paired filters. The data are shown below as mean ± SEM [*significant ($p<.01$) difference from vehicle; +significant difference ($p<.01$) from 500 unit values]:

VEHICLE (n=4)	IL-2 (500 UNITS) (n=4)	IL-2 (5000 UNITS) (n=4)
3.3 ± 0.4	5.7 ± 0.2*	7.8 ± 0.3*+

TNF increased P 1.7±0.3-fold above control values (n=3). Using an enzyme-linked immunosorbent assay to measure IL-2 in the media, 39±4% of the added IL-2 was present at 4 hours. These data demonstrate that IL-2 is toxic to cultured PEC, causing increased P of PEC monolayers in the absence of serum components or other cell types. This suggests a role for direct IL-2-mediated PEC injury in the pathogenesis of the "vascular leak" syndrome (Supported by NIH grant HL-39720).

99.3

BACTERIAL PROTEASES INCREASE THE CLEARANCE OF ^{99m}Tc-ALBUMIN FROM THE AIR SPACES OF GUINEA PIG LUNGS. A.O. Azghani*, J. Connelly, A.R. Johnson, and B.T. Peterson. The Univ. of Texas Health Center at Tyler, Tyler, Texas 75710.

We tested the hypothesis that elastase from Pseudomonas aeruginosa contributes to the pathogenicity of this bacteria by increasing the permeability of the lung epithelium to macromolecules. Elastase (400μg/0.5 ml) was instilled intratracheally into 5 anesthetized, ventilated guinea pigs. After 15-20 minutes the animals were ventilated for 6 minutes with an aerosol of ^{99m}Tc-human serum albumin (HSA). A gamma camera, which monitored the fall in the HSA concentration in the chest during the next 2 hours, allowed us to calculate the clearance rate of the HSA (K). The postmortem measurement of the lung weight (gm/kg body wt.) showed that 3 guinea pigs developed moderate edema (lung wt.< 2x control) and 2 developed severe edema.

Group	Number	K (%/min)	Lung wt. (gm/kg)
Control	4	0.12 ± 0.02	5.0 ± 0.4
Elastase: Moderate	3	0.26 ± 0.07	5.9 ± 1.6
Severe	2	0.77 ± 0.06	11.9 ± 0.4

The increase in the HSA clearance rate (K) correlated well with the degree of lung injury assessed by the lung weight ($r=0.93$, $p<0.01$). The increase in the HSA clearance supports the hypothesis that elastase of P. aeruginosa contributes to the pathogenicity of the bacteria by increasing lung epithelial permeability to macromolecules.

99.4

EFFECTS OF AMILORIDE AND PHLORIDZIN ON TRANS-EPITHELIAL Na AND GLUCOSE FLUXES IN RAT LUNGS. B.E. Goodman, J.L. Anderson*, and J.K. Stemsrud*. Dept. of Physiology and Pharmacology, Univ. of South Dakota, Vermillion, SD 57069.

Both active sodium transport and active glucose transport have been implicated in the clearance of solutes and water from the airspaces of the lungs. Amiloride is known to inhibit conductive Na^+ entry into cells. Phloridzin inhibits Na^+-coupled D-glucose entry into intestinal epithelial cells. We investigated the separate and combined effects of amiloride (A) and phloridzin (P) on the fluxes of ^{22}Na, ^{14}C-D-glucose, and FITC-dextran (FITCD20) from airspace to vascular space in isolated perfused rat lungs. Airspaces were instilled with KRB solution containing the three tracers. In some experiments, P (3×10^{-3} M) was included in the instillate. After a 30 min sampling period, A (10^{-3} M) was added to the perfusate. Based on a single-pass technique and Fick's First Law of Diffusion, the permeability-surface area products (PS) for each tracer were calculated before and after A. Significant changes in PS are shown below.

	A	P	P + A
Na^+	↓ 40%	↓ 24%	↓ 44%
D-glucose	↑ 39%	↓ 89%	N.C.
FITCD20	↓ 34%	N.C.	↓ 29%

Column 1 compares A to KRB by paired t-test. Column 2 compares P to KRB by 1-way ANOVA. Column 3 compares P + A to P by paired t-test. Both active transepithelial sodium transport and Na^+-coupled D-glucose transport are present in rat lungs. (Supported by AHA-Dakota Affiliate & Parson's Fund-USDSM).

99.5

DOSE-RESPONSE RELATIONSHIP FOR AMILORIDE IN ISOLATED PERFUSED LUNGS OF GOLDEN SYRIAN HAMSTERS. William F. Waltz and Barbara E. Goodman. Dept. of Physiology and Pharmacology, University of South Dakota School of Medicine, Vermillion, SD 57069.

We examined the response of isolated perfused lungs of Golden Syrian hamsters to amiloride and determined the dose-response relationship. The degassed lungs of male Golden Syrian hamsters were perfused with Krebs-Ringers-bicarbonate (KRB) equilibrated with 95% O_2/ 5% CO_2 and hung in a 37° C chamber. The lungs were lavaged with KRB containing ^{22}Na, ^{14}C-sucrose, and fluorescein isothiocyanate dextran 20 (FITCD20). After collecting samples for a 30 minute control period, amiloride was added and samples were collected for a 30 minute test period. The permeability surface area products (PS) were calculated for each labeled solute based on the appearance of these markers in the perfusate passing out of the lungs and Fick's First Law of Diffusion. The percent PS change (%PSΔ) was used to determine the decrease in ^{22}Na PS and was calculated by dividing the difference in ^{22}Na PS from control period to test period by the control value. Amiloride decreased the PS for ^{22}Na in a dose-dependent manner over a concentration range of 10^{-6}M to 3×10^{-3}M. No changes in the PS for ^{14}C-sucrose or FITCD20 were seen. Amiloride showed a maximal %PSΔ of 50% at 2×10^{-3}M, an ED_{50} of 1.75×10^{-4}M, and a log dose-response slope of 5.29 between 15% and 85% maximal effect. The correlation coefficient was 0.67. Amiloride decreases net sodium flux, probably by decreasing transepithelial sodium transport and sodium conductance. This is the first demonstration of active sodium transport in hamster lungs. (Supp. by AHA-Dakota and Parson's Grant-USDSM)

99.6

BRONCHIAL MUCUS IN CANINE SINGLE LUNG TRANSPLANTATON. M. King, J.G. Zayas, A. Paul, D. Marelli, J.A.S. Wilson, D.S. Mulder. Pulmonary Defense Group, Univ. of Alberta, Edmonton; Dept. of Surgery, Montreal General Hospital, Montreal, CANADA.

We studied the bronchial mucus from dogs undergoing left lung transplantation. Four groups were studied: A) Autotransplants -- removal and re-implantation of the native left lung, implying total denervation and bronchial devascularization. B) Allotransplants -- removal of the left lung from a donor dog and its implantation in a size-matched recipient; these dogs received cyclosporin 20 mg/kg/day p.o. to prevent organ rejection. C) Left sleeve upper lobectomy, a quasi-control, i.e. partial denervation and devascularization. A, B, and C dogs were studied 3 weeks after surgery. D) Rejected allotransplants, one week after withdrawal of cyclosporin. Mucus was collected under xylazine/valium anesthesia by resting a cytology brush for 10 min on either mainstem bronchus below the anastomosis. The mucus was stored at -80° until analyzed by magnetic microrheometry. Mechanical impedance, G* ("viscosity + elasticity") and loss tangent, tan δ ("viscosity/elasticity") were determined at 1 and 100 rad/s. Log G* at 1 rad/s (mean ± SE) varied as follows:

	N	L-lung	R-lung	p
Group A	6	2.54±.09	2.33±.11	.004
Group B	6	2.65±.22	2.39±.09	.17
Group C	5	2.88±.11	2.75±.04	.52
Group D	3	2.27±.14	2.42±.14	.50

The variations in log G* at 100 rad/s were similar. Variations in tan δ were not significant except Group A at 100 rad/s, where L lung values were lower than R lung ($p<.05$). The increase in log G* in autotransplant lung is consistent with observations for atropine administration, and suggests the mucus is less easily clearable by ciliary action. The additional decrease in tan δ at high frequency (augmented elasticity) indicates an even greater reduction in clearability by air flow. The changes in non-rejected allotransplants are qualitatively similar; these alterations in mucus are most likely related to denervation. In group C dogs, with only partial denervation, the differences were minimal. The post-rejection data indicate that native lung mucus remains "normal", while mucus in the rejected lung becomes less rigid than control, perhaps indicating transudation.

99.7

ACTIVE ION TRANSPORT ACROSS PRIMARY CULTURES OF FETAL TYPE II EPITHELIAL CELLS. B.A. Orser*, L. Fedorko*, M. Post*, H. O'Brodovich. Hosp. Sick Children, Res. Inst. Toronto, Canada, M5G 1X8.

To determine if the alveolar epithelium actively transports electrolytes and is therefore involved in fetal lung liquid secretion we studied alveolar like structures (ALS) isolated in primary culture from 20 day gestation fetal rats. ALS are spheres of type II epithelium polarized with cell apices directed inward towards the lumen. ALS were punctured with micropipettes and potential difference (PD) was recorded with a high impedence amplifier at room temperature. Under baseline conditions the recorded PD relative to bath (n = 74) showed a bimodal distribution at -10mV and -22 mV. The average PD was reduced ($p < 0.01$) by 57% with 10^{-3}M ouabain and increased by 32% ($p < 0.05$) with 10^{-4}M terbutaline. This study demonstrates that fetal type II epithelium actively transports electrolytes and could participate in fetal lung liquid movement. This ion transport is Na-K ATPase dependent and can be augmented by beta adrenergic stimulation.

Supported by MRC (Canada) Grant #MA7486

99.8

COUGH ENHANCES CLEARANCE OF MUCUS FROM THE NORMAL LUNG.

W.D. Bennett and W.M. Foster. SUNY at StonyBrook, N.Y. 11794.

Previous studies have concluded that cough is effective at clearing mucus from the lung only when the mucociliary system is compromised by disease. Using monodisperse aerosols radiolabelled with Tc99m, we studied the effectiveness of cough for clearing mucus in ten (10) nonsmoking subjects with normal lung function. On two separate study days, each subject breathed 6um (MMAD) Tc99m-iron oxide particles under controlled breathing conditions while seated in front of a gamma camera. Retention (R) of lung activity (measured as % of initial activity) was measured over the initial 2 hours and again at 24 hours following particle inhalation. On the control day the subject sat quietly in front of the camera, while on the cough day each subject performed sixty (60) controlled coughs during the first hour of retention measurements. Because breathing patterns were controlled for particle inhalation, initial lung deposition patterns (measured as a central to peripheral airway ratio of lung activity, C/P) were matched on control and cough study days (control C/P = 2.20 +/-1.25 and cough C/P = 2.19 +/- 0.97). By paired analysis, retentions at both 1 and 2 hours (R1 and R2) for the cough measurements were significantly less than control (mean control R1 = 85% vs. mean cough R1 = 71%, $p<.005$, mean control R2 = 76% vs. mean cough R2 = 65%, $p<.02$). Retention at 24 hrs (R24) was not significantly different between cough and control measurements (mean cough R24 = 35% and mean control R24 = 36%). In other words, the rate of mucus clearance was increased by coughing in these normal subjects. This enhancement of clearance by cough may be due to air-mucus interaction (i.e. two-phase gas-liquid flow) or to stimulation of the mucociliary apparatus (i.e. increased secretions or ciliary activity) by increased shearing of the airway surface. Supported by NIH New Investigator Award HL36107 and NIH HL31429.

99.9

MATURATION OF MUCOCILIARY AND COUGH CLEARANCE IN YOUNG CHRONIC BRONCHITIS PATIENTS WITH AND WITHOUT CYSTIC FIBROSIS. E.M. App, D. Köhler, H. Matthys (SPON: M. King). Div. of Pulmonary Diseases, Dept. of Internal Medicine, University of Freiburg, FRG

Several lines of evidence indicate that mucociliary clearance (MC) undergoes age related changes. Our group has previously demonstrated a strong inverse relationship between MC and age in healthy adults 20 to 80 years old (Eur J Respir Dis 1985; 66: 93). However, Whaley et al (J Appl Physiol 1987; 62: 1331) found slower tracheal clearance in immature beagle dogs compared with young adults of the same species, showing that MC also exhibits a maturation effect. These observations led us to investigate whether a similar maturation effect in MC exists in human subjects. We also wanted to study the relationship between cough clearance (CC) and MC in younger patients, since our previous studies in chronic bronchitis (CBR) had demonstrated an inverse relationship between the two. We therefore studied a total of 42 young patients of both sexes -- 23 with cystic fibrosis (CF) and 19 with CBR. Each patient was studied twice. MC was measured by using a gamma camera to detect movement of ^{99m}Tc labeled erythrocytes during one hour following inhalation. CC was determined by the amount of radioactivity clearable by forced expiration maneuvers. In patients with CF, MC appeared to peak in early adulthood [$r=0.41$, $p<0.01$, 2nd-order polynomial], while CC was directly related to age [$r=+0.36$, $p<0.01$]. There were no significant age correlations in patients with CBR, but the relationships were consistent with those observed in CF. In line with previous studies, there was an inverse relationship between MC and CC in CBR patients [$r=-0.64$, $p<0.003$] at all ages, but not in CF. The age related changes in MC fit well with previously described age effects in other pulmonary functions. Presumably with increasing severity of illness, there is a progressive loss of mucociliary function and a shift to a dependence upon cough clearance, which seems to be insufficient in CF.

LIVER/PANCREAS

100.1

TRACER UPTAKE AND BULK SECRETION OF NOREPINEPHRINE BY THE DOG LIVER. C.A.Goresky, G.G. Bach, D. Cousineau, A.J. Schwab, C. Rose, S. Lee, and S.Goresky. University Medical Clinic, Montreal General Hospital, Montreal, Quebec, H3G 1A4

In vivo norepinephrine handling by the liver was appraised in the dog by carrying out tracer transient multiple indicator dilution studies under various steady state conditions: in a basal situation; during norepinephrine infusion; and after desipramine administration. In the basal situation, tracer norepinephrine extraction was 60%, on average, whereas the steady state extraction of bulk norepinephrine was only 25%; intrahepatic secretion of unlabeled norepinephrine accounted for the difference. Infusion of norepinephrine constricted the hepatic vascular space but did not change proportional tracer extraction; norepinephrine secretion remained essentially unchanged and, as a result, bulk extraction converged on tracer extraction. Analysis of tracer uptake provided values for influx, efflux, and sequestration coefficients; and simultaneous analysis of bulk levels provided an estimate of the concomitant rate of secretion of unlabeled norepinephrine. Tracer estimates of permeability surface products for influx and efflux, expressed per g liver, remained unchanged with norepinephrine infusion, and desipramine was found not to affect uptake kinetics, indicating that uptake was virtually completely non-neurogenic (it was presumably hepatocytic). This study demonstrated that norepinephrine secretion coexists with uptake, that the major uptake process is hepatocytic, and that this does not saturate.

100.2

IMPAIRED UPTAKE IN CIRRHOSIS(C):ITS EFFECT ON DRUG ELIMINATION. L.Gariepy*,D.Fenyves*,J.P.Villeneuve*(SPON: P. duSouich).Dpt. of pharmacology and of medicine.Univ. of Montreal, Quebec.

The aim of this study was to evaluate the respective influence of the metabolic activity and the uptake on the elimination of 3 model-drugs in rats with CCl4-induced cirrhosis:antipyrine(A)(an index of hepatic functionnal mass), propranolol (P)(lipid soluble with high extraction)and taurocholate(T)(water soluble with high extraction).The livers were perfused at a flow rate(Q)of 20ml/min in a closed circuit, and the clearance and extraction(E)of all 3 substrates was measured in each animal. Using the sinusoidal perfusion model,the intrinsic clearance(Cli)was derived from the measurement of E and Q as Cli=-Qln(1-E).The Cli(ml/min)of the 3 substances was reduced in the cirrohtic livers in comparison to controls;1.18±0.14 to 0.85±0.32 for A(-28%),78.9±15.9 to 28.4±12.5 for P(-65%)and 65.9± 3.4 to 9.9±6.2 for T(-85%).The decrease in functionnal mass as assessed by antipyrine cannot account completely for the results obtained for P and T suggesting the contribution an impaired uptake due to shunts or capillarization.The uptake of P,a very lipophilic drug,should not be impeded by capillarization of hepatic sinusoids,suggesting that shunts are the responsible factor, whereas both capillarization and shunts can affect T uptake, explaining why the Cli of T is more altered than that of P by cirrhosis.

100.3

RAT LIVER REGENERATION AFTER PARTIAL HEPATECTOMY: INFLUENCE OF THE CALCIUM AND VITAMIN D STATUS. C. Ethier*, F. Lambert*, C. Dubé*, M. Gascon-Barré*, (SPON: P. DuSouich) Hôp. St-Luc, and Dept Pharmacologie, Université de Montréal, Montréal, Québec, Canada.

Vitamin D (D) and calcium are known to influence cell proliferation and/or differentiation. To study their effect on liver regeneration, hypocalcemic D-depleted rats were treated with D_3, or calcium alone while controls received vehicule only. They were then subjected to 2/3 hepatectomy (HPX) or sham operations. Liver weight, total DNA, RNA, ^{3}H-thymidine incorporation into DNA, and ornithine decarboxylase activity (ODC) were determined 4,16,24,30,48,72hr after HPX. In all groups, maximum ODC was found 16hr post-HPX with, however, a 2nd peak of activity at 35 and 48hr in D_3 and Ca-treated rats respectively. Maximum ^{3}H-thymidine incorporation occured 24hr after HPX in D_3 and Ca-supplemented rats with a 2nd phase of activity occuring at ~ 50hr while in D-depleted rats, a single peak was apparent 33hr post-HPX. 72hr after HPX, liver weight was 3.3± 0.2, 2.8±0.1, and 2.3±0.1g/100g b.w. representing 75%, 71% and 62% of sham operated animals in the D_3, Ca-treated, and controls respectively (D_3 vs D-depleted, $p<0.02$; Ca-treated vs D-depleted, $p<0.01$; D_3 vs Ca-treated, N.S.). These data indicate that, following partial HPX, D-depletion perturbes the normal pattern of liver regeneration as well as of liver weight recuperation which seems, however, to be restored by calcium and/or D_3 supplementation.

100.4

STIMULATION OF HEPATIC MITOCHONDRIAL AND CYTOSOLIC ACETOACETYL-COA THIOLASE ACTIVITY BY LOVASTATIN. W. Salam,* B. Khan,* L. Cagen, H. Wilcox and M. Heimberg. Dept. of Pharmacology, Univ. of Tennessee, Memphis, TN 38163

Lovastatin feeding (0.1% supplement to normal chow diet for one week) to normal male rats increased the specific activity of liver mitochondrial AcAc-CoA thiolase (from 0.41±0.04 to 0.83±0.07 μmol/min/mg). This stimulation was not reversed by perfusion of the livers for 4 hours in vitro in the absence of lovastatin (0.73±0.09 vs. 1.59±0.17 μmol/min/mg for control and lovastatin fed animals, respectively). Lovastatin feeding also elevated the activities of cytosolic AcAc-CoA thiolase (from 0.20±0.01 to 0.61±0.04 μmol/min/mg) and microsomal HMG-CoA reductase (from 302±88 to 834±78 pmol/min/mg), estimated after 4 hours of perfusion. The stimulatory effect of lovastatin on the activities of hepatic mitochondrial and cytosolic AcAc-CoA thiolase and microsomal HMG-CoA reductase was prevented by inclusion of cholesterol (0.1%) in the diet. Inclusion of cholesterol (as human low density lipoprotein) in the perfusion medium also reduced the activities of these three enzymes in livers isolated from lovastatin-fed animals. The activity of cytosolic AcAc-CoA thiolase in livers from normal chow-fed animals was not affected after 4 hours of perfusion by inclusion of lovastatin (10 or 100 μg/ml) in the medium. These data suggest that the activity of mitochondrial, as well as cytosolic AcAc-CoA thiolase, is regulated in part by a putative metabolic pool of cholesterol.

100.5

PHENOBARBITAL (Pb) IMPAIRED BILIARY EXCRETION OF ACETAMINOPHEN METABOLITES IN RATS. K.L.R. Brouwer and J. Jones [SPON: T.S. Miya]. School of Pharmacy and Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC 27599.

The effects of chronic Pb pretreatment (58 mg/kg/12 hr x 5 d) on acetaminophen (AA) and metabolite disposition were examined following AA (100 mg/kg IV bolus) administration to rats. AA, acetaminophen glucuronide (AG) and acetaminophen sulfate (AS) concentrations in serum, bile and urine were quantitated by HPLC. Mean ± SEM recovery of AA and metabolites in bile and urine is listed below (n=4/group; expressed as mg equivalents of AA; *p<0.05, **p<0.001):

	AA	AG	AS
Bile:			
Intact Kidney:			
Pb	0.24±0.21	0.27±0.12*	0.33±0.13*
Vehicle	0.09±0.01	1.96±0.52	1.01±0.21
Renal Ligated:			
Pb	0.11±0.07	0.64±0.07*	1.42±0.31
Vehicle	0.09±0.02	2.25±0.35	2.16±0.26
Urine:			
Pb	0.74±0.17*	7.57±0.23**	10.00±0.37**
Vehicle	0.18±0.11	3.45±0.56	13.79±0.38

Pb pretreatment significantly decreased the total amount of AG and AS excreted in bile. Biliary excretion of AG was impaired to a greater extent than AS. Pb and/or Pb-glucuronide appear to inhibit the biliary excretion of acetaminophen metabolites. (Supported by a PMA Foundation Research Starter Grant)

100.6

EXCRETION OF DRUGS IN RAT PERFUSED INTESTINE. Jin-ding Huang* (SPON: H.I. Chen). National Cheng Kung University, Department of Pharmacology, Tainan, Taiwan, Republic of China.

Intestine of anesthetized rats was perfused with Tyrode's solution. Drug solutions were infused via the jugular vein and the blood samples were take through the carotid artery. The rate of drug appearance in the intestinal perfusate was divided by serum concentration of the drug to determine the gastrointestinal clearance (CLg). For most drugs studied, e.g. thiobarbiturates, barbiturates, dextran, and other molecular weight markers, CLg is limited by the flow of intestinal perfusion. Lipophilicity of drugs did not significantly affect CLg of the drug, but CLg were proportional to the unbound fraction of drugs (fu). CLg of thiobarbital, thiopental, and thioseconal varied as fu of the drug varied; the unbound CLg of the drugs were nevertheless the same. Among polar molecular weight markers, increased molecular weight decreased CLg. Dextran of N. W. 77,000 showed a significant CLg (0.53±0.19 ml/h). A few lipophilic basic drugs, e.g. disopyramide, quinidine, showed a CLg larger than the perfusate flow, indicating a non-diffusional pathway of gastrointestinal excretion. A physiological model was constructed to explain the observations. (The work was supported by grants NSC77-0412-B006-02Z and NSC77-0412-B006-05 of National Science Council, Republic of China.)

100.7

MIREX (M) BUT NOT KEPONE (K) DECREASES THE UPTAKE OF ^{3}H-ESTRADIOL-17β-D-GLUCURONIDE ($E_2$17G) and ^{3}H-TAUROCHOLATE (TC) INTO ISOLATED RAT HEPATOCYTES (IRH). Steve Teo and Mary Vore, Dept. Pharmacol., Grad. Ctr. Toxicol., Univ. Kentucky, Lexington, KY.

M is an insecticide that decreases the biliary excretion of drug metabolites in vivo, and the uptake of the organic anions, $E_2$17G and TC in IRH. In the present studies, female Sprague-Dawley rats were treated with M (50mg/kg/day p.o.) or K (6.25, 12.5 or 18.75mg/kg/day p.o.) for 3 days and IRH prepared (viability 88-95% by trypan blue exclusion). K, a metabolite of M, had no effect on the uptake of 0.5 or 10μM $E_2$17G or 10μM TC. The effect of M on kinetic parameters for $E_2$17G uptake were determined using 15 concentrations ranging from 0.1 to 100μM. The data fit best to a 2 site model using a Least Squares Modelling Program ($1/y^2$ weighting factor): C=control, corn oil; M=mirex; N=4; M±SEM; *p<0.01,

$Vmax_1$		Km_1		$Vmax_2$		Km_2	
C	M	C	M	C	M	C	M
0.21 ±0.04	0.06 ±0.02*	3.80 ±1.0	1.48 ±0.58	3.51 ±0.40	1.08 ±0.09*	237 ±27	43 ±11*

M decreased Vmax (nmol/min/mg protein) for both low and high affinity uptake sites and decreased the Km (μM) for the low affinity site. In addition, the inhibitory action of M on organic anion uptake by IRH is not mediated by its metabolism. (Supported by a grant from E. I. Dupont de Nemours #532481)

100.8

PHARMACOKINETICS OF THE LHRH AGONIST [D-Trp^6, des-Gly-NH_2^{10}]-LHRH ETHYLAMIDE IN MEN. B. Candas*, D. Lacoste*, F. Labrie, and M. Normand, Laval University, Quebec, Canada G1K 7P4.

The kinetics of immunoreactive [D-Trp^6, des-Gly-NH_2^{10}]LHRH ethylamide in plasma were studied in 9 adult men for 24h following a rapid sc injection of 1 or 10 μg/kg BW of the peptide. If the apparent volume of distribution is set proportional to the log of the concentration, both time courses are statistically well represented by a 2-compartment model coupled to a source compartment representing the sc input. The sc diffusion to plasma is 5 times faster than any other fractional transport rate (5%/min). No exchange between the 1st, which includes plasma, and the 2nd compartment exceeds 1% of the compartmental content/min. Elimination occurs at maximum rates of 0.50 and 0.25%/min, for the 1st and 2nd compartments, respectively. According to simulation, 90% of the steady-state is reached 13.75 h after the onset of a sc continuous infusion, whereas it is reached approximately 30 min earlier following an i.v. infusion. A molecule spends on average 6h to 9.75h in the body, 30-50% of this period being accounted for by the 1st compartment. The apparent volume of distribution reaches 208 ml/kg BW and the MCR is 1.18 $ml.min^{-1}.kg\ BW^{-1}$ at a steady-state of 1 ng/ml plasma. Plasma dynamics of the LHRH agonist in men is at least 10 times slower than that of LHRH, thus demonstrating the relevance of its clinical use for the suppressive therapy of gonadotropic hormone secretion. (Supported by MRC).

100.9

HEPATOBILIARY ELIMINATION OF THE LEUKOTRIENE (LT) ANTAGONIST, Ro 23-3544, IS INHIBITED BY DIBROMSULFOPHTHALEIN (DBSP) IN THE RAT. A. Takacs*, C. Laurencot* and J. Carbone* (SPON: F.-J. Leinweber) Dept. of Drug Metabolism, Hoffmann-La Roche, Inc., Nutley, NJ 07110.

Ro 23-3544, chroman carboxylic acid, is being developed as a leukotriene antagonist in the treatment of inflammation. The LTs and other organic anions (bile acids and DBSP) undergo shared carrier mediated hepatobiliary transport. The aims of this study were to determine if the biliary excretion of Ro 23-3544 is carrier mediated and inhibited by DBSP. Ro 23-3544 was administered to anesthesized rats at iv doses of 0.1, 0.5, 1, 5, 10 and 20 mg/kg (n=3-4). Bile was collected via cannulas at various intervals up to 4 hours after dose and Ro 23-3544 concentrations were determined by HPLC assay. The maximum biliary excretion rate (30 min) of Ro 23-3544 increased in proportion to dose at the three lower doses, but was non-linear at higher doses. The total µg of Ro 23-3544 excreted in 4 hrs were 154.3 ± 5.4 (X ± S.E.) after Ro 23-3544 (1 mg/kg) administration alone and decreased significantly (P<.05) to 75.0 ± 15.1 (X ± S.E.) when the inhibitor DBSP (10 mg/kg) was administered 30 sec after Ro 23-3544. The maximum biliary excretion rate of Ro 23-3544 was also less after DBSP administration. These data suggest that hepatobiliary transport of Ro 23-3544 occurs via a carrier mediated process shared by DBSP.

100.10

Hepatic Intermediary Metabolism During Hyperplastic Liver Growth. M. Badr and J. Yarbrough. Divisions of Pharmacology and Structural and Systems Biology, Univ. Missouri-Kansas City, Kansas City, MO 64108.

In intact (INT) rats, mirex (MX) induces adaptive liver growth that involves hyperplasia and hypertrophy. However, in adrenalectomized (ADX) rats, its effect is predominantly hyperplastic. The purpose of this study was to investigate whether these different responses to MX are accompanied by changes in hepatic intermediary metabolism. Glycogenolysis, gluconeogenesis and oxygen uptake were studied in perfused livers from INT and ADX rats 48 hrs after an oral dose of MX (100 mg/kg). Rates of glucose production in control INT rats were 121±23 umole/g/hr which were diminished by 94% in MX-dosed rats. Similarly, MX-dosed ADX rats showed a 93% decrease in glucose production compared to control ADX rats. However, infusion of fructose (5 mM) into livers from both MX-dosed INT and ADX rats, increased glucose production significantly to 60±5 and 78±11 umole/g/hr, respectively. Livers from MX-dosed INT rats had significantly higher O_2 uptake than control INT rats. Conversely, livers from ADX rats showed no change in O_2 uptake after MX. Thus, while carbohydrate metabolism was similarly altered during hyperplastic and hypertrophic liver growth, hepatic oxygen uptake increased only under conditions of hypertrophy suggesting a link between oxidative phosphorylation and mechanisms involved in liver hypertrophic growth (ES 04778).

100.11

Hepatic Carbohydrate and Oxygen Metabolism During Hypertrophic Liver Growth. J. Yarbrough and M. Badr. Div. of Structural and Systems Biology and Pharmacology, Univ. Missouri-Kansas City, Kansas City, MO 64108.

In intact (INT) rats, mirex (MX) induces adaptive liver growth that involves hyperplasia and hypertrophy. However, in thyroidectomized (THX) rats, its effect is exclusively hypertrophic. The purpose of this study was to investigate whether these different responses to MX are accompanied by changes in hepatic carbohydrate and oxygen metabolism using perfused livers from INT and THX rats 48 hrs after an oral dose of MX (100 mg/kg). Rates of glucose production in livers of control INT rats were 121±23 umole/g/hr. These rates were diminished by 94% in livers from MX-dosed rats. However, in livers from MX-dosed THX rats, rates of glucose production were decreased only by 66% compared to control THX rats. In both INT and THX rats, decreases in glucose production were accompanied by corresponding decreases in production of lactate and pyruvate. However, in both MX-dosed INT and THX rats, rates of glucose and lactate production were increased significantly upon the infusion of fructose (5 mM). Livers from both INT and THX MX-dosed rats showed significant and similar increases in O_2 uptake over corresponding control values. These data suggest that hepatic carbohydrate metabolism plays an important role in determining the type of liver cell growth induced by MX (ES 04778).

100.12

STIMULATION OF THE EXOCRINE PANCREAS PROMOTES SELECTIVE SECRETION OF DIGESTIVE ENZYMES. R. Clarizio*, P.E. Miller* and J.W. Adelson* (SPON: J.G. Forte) Div. of GI, Dept. of Physiol., Montreal Children's Hospital - McGill U. Montreal, Quebec, Canada, H3H 1P3.

In 1987, Miller and Adelson proposed a secretory model to explain nonparallel pancreatic secretion, in which, the sources of digestive enzymes are heterogeneous and, that, CCK-stimulable enzymes pools are subsets of a more broader and multiple cholinergic (MCh)-stimulable pool. We further investigated this model by analyzing the nonparallel secretory patterns of digestive enzymes. Pancreatic secretion was collected at 5 min intervals for 3 hrs from the in-situ cannulated pancreas of the anesthetized male New Zealand rabbit under basal or stimulatory conditions. Proteins were separated by SDS-PAGE gels and the proportions, enzyme %, were measured by densitometric analysis. Enzyme secretion was found to vary dramatically and significantly in both enzyme% and enzyme ratio and, that, the variability decreased significantly under stimulated conditions. The range of both enzyme% and ratios under CCK stimulation was found to overlap the range obtained under MCh stimulation. Our findings clearly show nonparallel secretion and, that, the CCK-stimulable pools are more unique or homogeneous and appears to be a specific subset of the MCh-stimulable pools, which are multiple, variable, and hetrogeneous. Supported by the Canadian Cystic Fibrosis Foundation.

SMOOTH MUSCLE PHYSIOLOGY

101.1

ACETYLCHOLINE, SEROTONIN, AND FMRFAMIDE ENHANCE DEPOLARIZA- OF MOLLUSCAN MUSCLE BY POTASSIUM-FREE SOLUTION. Robert B. Hill. University of Rhode Island, Kingston, RI 02881

The radular protractor muscle of Busycon canaliculatum has a complex response to K-free solution, in which the depolarizing component is relatively slow and slight, and accompanied by a slight slow contraction. This response is susceptible to a concentration-dependent modification by acetylcholine (ACh), serotonin (5HT), or phenylalanyl-methionyl-arginyl-phenylalanine amide (Fa). The modification takes the form of an increase in speed of onset and amplitude of the depolarization and the appearance of superimposed rhythmicity. Contractile force parallels the depolarization. Modification increases with the concentration of ACh from 10^{-7} M to 10^{-3} M, of 5HT from 10^{-8} M to 10^{-4} M, and of Fa from 10^{-8} to 10^{-7} M. The depolarizing component of the response to K-free solution is abolished in Na-free solution and is blocked by 10^{-5} M ouabain or strophanthidin. Na-free solution or 10^{-5} M strophanthidin also block the modification induced by ACh or 5HT. Na-free solution blocks the modification induced by Fa.

101.2

BETA-ADRENERGIC SENSITIVITY IN TRACHEAL SMOOTH MUSCLE IS DEPENDENT ON THE RECEPTOR-RESERVE OF THE CONTRACTILE AGONIST. S.J. Gunst, J.Q. Stropp and N.A. Flavahan. Department of Physiology, Mayo Clinic and Foundation, Rochester, MN 55905.

Beta-adrenergic stimulation is less effective at inhibiting the contractile responses evoked by acetylcholine (ACh) than those evoked by 5-hydroxytryptamine (5-Ht) in tracheal smooth muscle. The present experiments were performed to analyze the possibility that this difference in sensitivity may result in part from the large difference in receptor-reserves available for these contractile agonists (Gunst et al., J. Appl. Physiol. 62:1755, 1987). Strips of canine trachealis muscle were suspended for isometric tension recording in organ chambers which were filled with physiological salt solution and were gassed with 95% O_2/5% CO_2 at 37° C. Muscles were contracted with equipotent concentrations of ACh, 5-Ht, and McN 343 (a muscarinic agonist of low efficacy) and then relaxed with cumulatively increasing concentrations of isoproterenol. The sensitivity to isoproterenol relaxation was McN 343 > 5-Ht >> ACh. The potency of pirenzipine at inhibiting contractions to ACh and McN 343 was similar. The results suggest that the relative resistance of ACh-induced contractions to relaxation by isoproterenol may result from the large receptor-reserve available for this agonist and that it is not an inherent quality of muscarinic receptor stimulation. The large receptor-reserve may in turn enable ACh to initiate subcellular mechanisms that are unavailable to agonists of lower efficacy. Supported by PHS-HL 29289.

101.3

ALTERATIONS IN MEMBRANE PHOSPHOLIPID BILAYER COMPOSITION WITH AGE IN THE FISHER 344 RAT AORTA. T.N. Tulenko, D. Lopatofsky, R.H. Cox. The Medical College of Penna., Philadelphia, PA 19129

The object of this study was to determine whether arterial cell membrane lipid composition is affected by the aging process. Segments of thoracic aorta were obtained from anesthetized Fisher 344 rats of ages 1, 2, 6, 12, 24 and 30 months. The adventitia was carefully removed in Krebs solution, and the vessel lipids extracted using the Folsch procedure. The organic phase was subjected to GLC chromatography and 2-D TLC to measure unesterified cholesterol (FC) and the individual phospholipid (PL) classes. Total PL content decreased with age while FC increased only in the 24 and 30 mo animals. The FC/PL ratio, an index of membrane viscosity, increased with senescence (24-30 mo). Reorganization of membrane PL with age was also apparent. For the most part, all PL classes remained constant over the 6 to 24 month ages. Senescence was associated with reduced PI, lysoPC and DPG content and increased PA content. Developmental changes (1-6 mo) were also observed with PC and PE contents falling and PS rising. These results clearly indicate that cell membranes of the arterial wall undergo significant reorganization with respect to cholesterol and phospholipid profiles during the developmental and senescent periods, but are relatively constant throughout the middle adult life period in the Fisher 344 rat. (Suppported by PHS Grants AG04908 & HL30496)

101.4

EFFECTS OF K^+ ON CALCIUM INFLUX AND INOSITOL TRISPHOSPHATE (IP_3) GENERATION IN THE RAT VAS DEFERENS. M.A. Khoyi*, M.A. Smith*, I.L.O. Buxton and D.P. Westfall. Dept. of Pharmacology, University of Nevada School of Medicine, Reno, NV 89557

A large number of studies indicate that K^+ induced contractions of smooth muscle depend on extracellular calcium. If these contractions depended exclusively on extracellular calcium then contractile resposes to 140 mM K^+, which are larger than the responses to 35 mM K^+, should be associated with a larger influx of ^{45}Ca. This was not the case in the vas deferens from reserpine pretreated rats. During a 2 min interval, ^{45}Ca influx induced by 35 mM K^+ was 147.5 ± 22.4 umoles/kg wet weight and 140 mM K^+ produced an identical influx (147.4 ± 1.94 umoles/kg wet weight). This suggests a second mechanism may be involved in responses to the higher K^+ concentration. Indeed, 140 mM K^+ caused a 320% increase above control in the formation of IP_3 in tissues pre-labelled with ^3H-myoinositol whereas 35 mM K^+ did not increase IP_3. IP_3 is thought to cause the release of calcium from internal stores. If so then it appears that in the rat vas deferens high K^+ promotes tension in smooth muscle by a dual mechanism: influx of extracellular calcium and release of calcium from internal stores via an IP_3 mechanism. (Supported by NIH grants HL38126 and HL 35416).

101.5

EFFECT OF EXTRACELLULAR Ca^{2+} AND Na^+ ON CYTOSOLIC FREE Ca^{2+} AND Ca^{2+} INFLUX OF CULTURED VASCULAR SMOOTH MUSCLE CELLS. Raouf A. Khalil and Cornelis van Breemen. Department of Pharmacology, Miami, Florida 33101

The effect of extracellular Ca^{2+} and Na^+ on agonist-induced fluctuations in cytosolic free Ca^{2+} ($[Ca^{2+}]_c$) and Ca^{2+} influx of cultured SHR aortic smooth muscle cells was investigated by parallel measurements of the changes in fura-2 fluorescence and ^{45}Ca influx; respectively. In the presence of external Ca^{2+} and Na^+, angiotensin II (100 nM), vasopressin (1 μM) and ATP (10 μM) induced an initial rapid rise followed by a maintained increase in $[Ca^{2+}]_c$ without stimulation of ^{45}Ca influx. The initial rise in $[Ca^{2+}]_c$ was reduced in Ca^{2+}-free solution but not in Na^+-free solution. On the other hand, the maintained steady-state increase in $[Ca^{2+}]_c$ was not inhibited by diltiazem but was completely abolished by La^{3+} or in Ca^{2+}-free solution. In contrast, the steady-state increase in $[Ca^{2+}]_c$ was augmented in Na^+-free solution without a concomitant increase in ^{45}Ca influx. These results indicate that this cell line lacks receptor-operated Ca^{2+} channels and therefore the Ca^{2+} leak becomes a potential source of activator Ca^{2+} when Ca^{2+} uptake by the sarcoplasmic reticulum is compromised by the continuous presence of an agonist. These results also suggest that the Na^+-Ca^{2+} exchange is an important Ca^{2+} extrusion mechanism during agonist activation of these vascular smooth muscle cells.

101.6

Effect of Vanadate on Skinned Smooth Muscle Contraction and Myofibrillar ATPase activity. Siow-Kee Kong* and Newman L. Stephens. Univ. of Manitoba, Dept. of Physiology, Winnipeg MB, Canada, R3E 0W3.

Some recent studies have indicated that vanadate (Vi) can inhibit active tension development in the skinned smooth muscle preparation. In the current study we wished to determine whether the inhibitory effect of Vi was due to the formation of an inactive complex: M.ADP.Vi. Canine tracheal smooth muscle (TSM) was skinned for at least 2 hr in buffer solution containing 1% Triton X-100, at 25°C. The skinned TSM developed 786 g/cm² ± 59.9 (SE) active isometric tension in a contraction solution containing 2mM ATP and 10^{-5}M Ca^{++}; showed tension could be maintained for at least 1 hr. Assays showed that 200-300 uM ATP was hydrolyzed during the contraction. Skinned TSM which was preincubated with 200 uM Vi with or without 400 uM ADP prior to the addition of 2mM MgATP and 10^{-5}M Ca^{++}, developed only 184 g/cm² ° 20.5 (SE) and 187 g/cm² ± 30.6 (SE) maximal isometric tension, respectively. However, the maximal tension was not maintained for more than a minute and thereafter fell back to resting tension level. Contrary to this, the contraction of skinned TSM could almost completely be abolished by adding 200 uM Vi at the plateau of a maximal isometric contraction induced by 2mM MgATP and Ca^{++} with or without 400 uM ADP. Biochemical studies also indicated that 200 uM Vi with or without 400 uM ADP could significantly inhibit TSM myofibrillar ATPase activity. These results suggest that Vi may act directly on the actomyosin complex and dissociate actin and myosin, rather than by forming the inactive complex: M.ADP.Vi. Vi may prove to be a useful tool for study of smooth muscle contraction.

101.7

EFFECTS OF AGING ON THE FUNCTIONAL PROPERTIES OF THE ARTERIAL Na-PUMP IN FISCHER 344 RATS. Robert H. Cox and Thomas N. Tulenko. Graduate Hospital, University of Pennsylvania, and Medical College of Pennsylvania, Phila., PA. 19146.

Segments of thoracic aorta, carotid and tail artery (TA) were obtained from male Fischer 344 rats at ages of 1, 2, 6, 12, 24 and 30 months. Na-pump activity was determined using ouabain-sensitive ^{86}Rb uptake and cell Na^+ was determined using ^{22}Na efflux. Following removal from the animals, segments were cleaned of surrounding tissue and place in normal Krebs solution at 37°C for 2 hrs. One half of the tissues were then placed in K^+-free Krebs for 4 more hours to Na^+ load the tissues. Total, ouabain insensitive and ouabain-sensitive ^{86}Rb uptake showed an age-related decline at all three arterial sites under both normal and Na^+ loaded conditions. The largest of these changes occurred over the early ages from 1-6 months but a significant aging change (6-30 months) occurred in TA. There were no significant differences in levels of cell Na^+ with age under either normal or Na^+ loaded conditions. The effects of varying ouabain levels on Na-pump activity was also determined. The IC_{50} value of ouabain (50% inhibition) was not significantly different among the various ages or between different arteries. The effects of 1 - 10 mM external K^+ was also determined. There were no significant age-related changes in the apparent equilibrium constant (K_m) while the maximum Na-pump activity (V_{max}) decreased with age. The results of these studies suggest that changes in the Na-pump activity of arterial smooth muscle with age occurs as a result of a decrease in the number of kinetically similar pump units. (Supported by NIH AG 04908).

101.8

SOMAN EFFECTS ON CALCIUM UPTAKE IN MICROSOMES AND MITOCHONDRIA FROM RABBIT AORTA. Chao-Yu Hu, Chia-Hsuh Hsu and Casey P. Robinson. College of Pharmacy, University of Oklahoma, Health Sciences Center, Oklahoma City, OK 73190

Effects of soman on ^{45}Ca uptake by microsomes and mitochondria prepared from rabbit deadventitiated aorta were determined. Some aortae were obtained from rabbits given 5 µg/kg of soman s.c. per day for 7 days. The effects of acutely-added soman on calcium uptake were determined in aortae from rabbits receiving no soman. Soman, added acutely in concentrations from 1 to 100 µM, did not alter calcium uptake by either microsomes or mitochondria. Seven-day soman administration, however, reduced ^{45}Ca uptake at 0.5, 2, 4, 6 and 8 minutes with both preparations. At eight minutes, calcium uptake was reduced in microsomes from 152 ± 18 to 88± 28 µmols/g protein (different at $P < 0.05$), and in mitochondria from 135 ± 3 to 51 ± 9 µmols/g protein (different at $P < 0.005$). This impairment of calcium uptake may alter contractions or relaxations of vascular smooth muscle. Supported by DOD Contract DAMD17-85-C-5114.

101.9

SODIUM-CALCIUM EXCHANGE IN CANINE AIRWAY SMOOTH MUSCLE

Yuansheng Gao* and Paul M. Vanhoutte. Dept. of Physiology and Biophysics, Mayo Clinic, Rochester, MN 55905

A sodium-calcium exchange mechanism exists in respiratory smooth muscle of guinea-pig, bovine and humans. The present study was designed to determine whether it also operates in canine airway smooth muscle. Rectangular strips of canine trachealis muscle were mounted in organ chambers filled with modified Kreb-Ringer bicarbonate solution maintained at 37°C and aerated with 95% O_2-5% CO_2 (pH-7.4). Isometric tension was recorded. All experiments were performed in the presence of atropine (10^{-5}M), phentolamine (10^{-6}M), propranolol (5 x 10^{-6}M), and nimodipine (5 x 10^{-6}M), to antagonize cholinergic, alpha and beta-adrenergic receptors and calcium channels, respectively. Low-sodium solutions (1.2 to 60 mM; sodium replaced by equimolar choline) induced concentration-dependent contractions which were blocked by calcium-free solution. The relaxation after contractions due to exposure to low-sodium in calcium-free media, depended upon the extracellular sodium concentration as it was slowed down in low-sodium solution. Potassium-free solution (potassium replaced by equimolar sodium) or ouabain (10^{-5}M and 10^{-4}M), which increase intracellular sodium by inhibiting the sodium potassium pump, also caused contractions which depended on the external calcium concentration. These observations suggest that a sodium-calcium exchange is operative in canine airway smooth muscle. **(Supported in part by NIH grant HL21584).**

101.10

THE INFLUENCE OF VASCULAR SMOOTH MUSCLE CONTRACTION ON THE VISCOELASTIC PROPERTIES OF THE ARTERIAL WALL. N. R. Bandick, W. Williams*, K J. Clark*, W. P. McCall*,and D. E. Roberts. Western Oregon State College, Monmouth, OR 97361 and US Army Research Institute of Environmental Medicine, Natick, MA 01760

Elastic and viscous constants of rat femoral arteries and abdominal aortae were measured to determine the extent of influence that active contractility has on the viscoelastic properties of arterial walls. Helical strips from these arteries were observed while the strips were in maximal contraction from a dose of 10^{-6}g/ml of norepinephrine and after the strips could no longer contract due to superfusion in a physiological salt solution free of added Ca^{++}. We found, for both types of arteries, that all elastic and viscous constants increased significantly ($p<0.05$) during vascular smooth muscle contraction. The Young's modulus for the femoral arteries increased from 0.76 to 1.05 X 10^6 dyn/cm^2, whereas the abdominal aortae went from 0.90 to 1.1 X 10^6 dyn/cm^2. The viscosity of the femoral walls showed the greatest changes going from 0.40 to 0.85 X 10^5poise/cm. By comparison, the aortic viscosity climbed from 0.34 to 0.55 X 10^5 poise/cm. In answer to the question of what % of the total wall stiffness and viscosity is due to muscular contraction, the folowing was observed. Abdominal Aortae: viscosity = 28%, Young's modulus = 18%. Femorals: viscosity = 60%, Young's modulus = 28%.

GASTROINTESTINAL MOTILITY

102.1

A RADIOTELEMETRY CAPSULE EMPTIES FROM THE STOMACH DURING THE INTESTINAL HOUSEKEEPER IN MAN: A NONINVASIVE METHOD OF DETECTING PHASE III OF THE MIGRATING MOTOR COMPLEX (MMC). P Mojaverian, JC Reynolds*, PH Vlasses, F Wirth*, A Ouyang*. Division of Clinical Pharmacology Jefferson Medical College and GI section, Department of Medicine, University of Pennsylvania, Philadelphia, PA.

The purpose of the present investigation was to determine the phase (P) of the MMC when a pH sensitive radiotelemetry Heidelberg capsule (HC) empties from the stomach, and if the HC alters the character or timing of the MMC. 77 MMCs were recorded over 94.3 h in 6 normal male subjects in 12 studies using 8 catheter-mounted strain gauges (Millar Instr). The catheter (2.6 mm) was passed transnasally and positioned fluoroscopically in the duodenum and jejunum. The signal of a HC, 7mm x 20mm, was recorded after ingestion until the pH rose > 3 units. The duration of P's in the control recordings and with the HC present were similar, $p>0.1$ (P I 35.3±3.4 vs 34.6±1.6, P II 55.1±7 vs 40.9±11.6, P III 5.1 vs 5.2±0.5 min). No effect was seen on the motility index during each P. The HC had no effect on the pattern of contractions. In 4 subjects in the right lateral position, the HC emptied with, and never before, the first gastric MMC. Only in 2 subjects in the left lateral position, the capsule emptied with the first MMC. In summary, character and duration of MMC are unchanged by the presence of a nondigestible capsule in the stomach. Nondigestible solids are shown for the first time in man to empty from the fasted stomach during P III. The HC may be used as a noninvasive method to examine the physiology of MMC, the presence of Phase III, and the emptying of enteric coated and solid controlled release formulations.

102.2

THE EFFECT OF GABA ON CYSTEAMINE INDUCED DUODENAL ULCER. M. Nicholson* and A. Krantis. Digestive Diseases Research Group, Dept. of Physiology, Univ. of Ottawa, Canada K1H 8M5.

Various neurotransmitters are affected during the development of duodenal ulcer (DU), including GABA. GABA agonists (icv) aggravate cysteamine-induced DU in the rat, while GABA antagonists are protective. GABA is a putative enteric transmitter in the rat, and enteric GABA modulates gastrin and somatostatin release from mucosal cells. Recently, we showed cysteamine to interact/interfere with enteric neurones. Therefore enteric GABAergic sites may be involved in the development of DU. We present here evidence for systemically applied GABA to augment cysteamine incuded ulcer. Sprague Dawley rats were given Cysteamine-HCL 28 mg/100 g p.o., 1-3 times on day 1 and maintained on H_2O-0.1% cysteamine for 24-48 hr. Drug pretreatments used include aminooxyacetic acid (AOAA) 2.5 mg/100 g sc. with GABA 10 mg/100 g, sc. or AOAA+GABA and/or bicuculline, 30 µg/100 g sc. Cysteamine (3 doses) caused DU by 24 hr. By 48 hrs, penetrating ulcers were evident. Cysteamine (2 doses) caused less ulceration at 48 hr, and none at 24 hr. AOAA+GABA caused an increase (sig $p<0.05$) in DU. This effect of AOAA+GABA was reduced by the antagonist bicuculline. Bicuculline alone also reduced (sig $p<0.05$) ulceration. These results suggest peripheral GABA-receptors can modulate cysteamine-induced DU.

102.3

PRESENCE OF NEURAL UPTAKE SITES FOR [^{3}H]GABA IN RAT MYENTERIC PLEXUS AND ANTRAL MUCOSA. A. Krantis* and T. Webb. Digestive Diseases Research Group, Dept. of Physiology, University of Ottawa, Canada K1H 8M5.

γ- Aminobutyrate (GABA) is a myenteric neurotransmitter in the guinea-pig, and putative enteric transmitter in the rat, cat and human intestine (Tanaka 1985, Life Sciences, 37: 2221-2235). Recently, Harty and Franklin (1986, Gastroenterology 91: 1221-1226) showed GABA to modulate nerve-mediated release of gastrin and somatostatin from rat antrum in vitro. The disposition of GABAergic neurones in the rat antral mucosa is unknown. Therefore we sought to determine the disposition of high affinity uptake sites for [^{3}H]GABA in the rat stomach. Autoradiography (Krantis et al. 1986, Neuroscience 17(4): 1243-1255) was performed on segments of the rat antrum incubated with 50 Ci/mmole (Amersham) 2,3 [^{3}]GABA, 5.10^{-9} to 10^{-8}M, in the absence/presence of specific inhibitors of high affinity GABA uptake, and GABA degradation. Radiolabelled GABA was accumulated to myenteric ganglia, and to a subpopulation of mucosal cells. In some instances, labelled processes could be seen around the base of the gastric pits. These results show that GABA is transported into neural elements of the myenteric plexus and mucosa of the antrum by a high affinity system.

102.4

THE ACTIONS OF ETHYLENEDIAMINE IN THE RAT INTESTINE IN VITRO. A.E. McKay and A. Krantis*. Digestive Diseases Research Group, Dept. Physiology, Univ. of Ottawa, Ottawa K1H 8M5.

Ethylenediamine (EDA), a non-toxic agent, exerts direct actions in the rat central and peripheral nervous systems through GABA receptors. However, it has been recently shown in the guinea-pig small intestine that EDA does not have direct GABA-mimetic actions at enteric GABA receptors. Rather, EDA stimulates the release of endogenous GABA. There is now considerable evidence for GABA and its receptors to be present in the rat intestine. Therefore we sought to examine the effects of EDA in isolated gut preparations of the rat duodenum and ileum. EDA induced concentration-dependent relaxations of the rat small intestine which were determined to be predominantly "myogenic". In a small proportion of the responses, tetrodotoxin (TTX) partially (36±10%) antagonized (sig. $p<0.05$) the induced relaxations, therefore indicating a small neurogenic component to the actions of EDA. Whether this neurogenic action of EDA is due to GABA-mimetic action(s) of this agent or to EDA-evoked release of endogenous GABA is unclear. EDA "myogenic" actions were not antagonized by adrenergic blockade (phentoalmine + propranolol), adenosine triphosphate (ATP) tachyphylaxis, or purinergic blockade (cibacron blue). Furthermore, EDA was comparable to the smooth muscle relaxant papaverine in maximally relaxing the intestinal segments. This suggests that EDA may be a useful agent in examining gastrointestinal function.

102.5

FUNCTION OF SMOOTH MUSCLE AND NERVE IN RAT SYNGENEIC SMALL INTESTINAL TRANSPLANTATION. E. Zorychta*, T. Taguchi*, R. Sonnino*, F. Guttman*(SPON: J.B. Richardson). McGill University, Montreal, Quebec, Canada H3A 2B4

The effect of transplantation on motility and innervation of the small intestine was studied in the Lewis rat. Animals received heterotopic isografts of jejunum using microvascular anastomosis. The graft (G) and an identical segment of native bowel (C) were removed from each of 2 animals at 4, 6, 8, and 10 days after surgery. Circular rings and longitudinal strips of jejunum were mounted in isolated tissue baths, bubbled with 95% O_2/5% CO_2, and maintained at 37°C. Transmitter release from nerve endings was evoked by electrical field stimulation. Receptor activity on the smooth muscle was determined by adding graded concentrations of drugs to the bath. Spontaneous rhythmic activity in both G and C was about 30/min. All preparations contracted in a dose dependent manner to cholinergic agonists, serotonin, and Substance P, and were relaxed by noradrenaline; potency of each drug was similar in all C and G. Stimulus-response characteristics of innervation were determined. Excitatory innervation was similar in C and G, but the inhibitory response was altered by transplantation. Maximal inhibition of G occurred at 5 Hz and 0.5 ms compared to 30 Hz and 2 ms in C, reflecting absence of extrinsic adrenergic inhibitory nerves in the graft. Smooth muscles, receptors, and intrinsic nerves were intact and functional in the transplanted intestine.

102.6

ACUTE EFFECTS OF IONIZING IRRADIATION ON INTESTINAL MOTOR ACTIVITY. K.Z. Rana*, R.K. Harding and A. Krantis. Digestive Diseases Research Group, Dept. of Physiology, Univ. of Ottawa and Defence Research Establishment Ottawa, Ottawa, CANADA.

Many unpleasant and potentially dangerous symptoms, such as vomiting and diarrhea, characterize the early stages of radiation sickness and these symptoms are suggestive of a disruption in intestinal motility. Therefore, we investigated the acute effects of irradiation on intestinal motor activity stimulated by specific myogenic, neurogenic, and humoral agents. Experiments were performed on isolated gut-bath preparations of rat duodenum and jejunum harvested from both unirradiated and irradiated animals. Controlled irradiations were performed using a shielded bilateral ^{137}Cs drawer source which delivered a uniform, whole-body dose of 10 Gy. Irradiated rats were sacrificed at 1, 12, 18 and 24 hours post-irradiation. Effects were statistically significant at 18h post-irradiation. The concentration-response effects of carbachol (cholinergic muscle stimulant) in the duodenum indicated an increased sensitivity ($p<0.01$) to this agent. In the jejunum, the concentration-response effects of DMPP iodide (ganglionic nicotinic agonist) also showed an increased sensitivity ($p<0.05$). Responses elicited by histamine, bradykinin and electrical stimulation in both regions of the intestine were unaffected by irradiation at 18 h post-exposure. These results suggest that exposure to irradiation leads to a hypersensitive cholinergic system.

102.7

STANDARDIZING THE IN VITRO MEASUREMENT OF SMALL INTESTINAL SMOOTH MUSCLE CONTRACTIONS. M.S. Werley*, J.S. Martin, F.M. Kendall and M.F. Tansy. Temple Univ., Philadelphia, PA 19140

The intercontractile interval (ICI) has been used as a means of assessing small intestinal motility (Bull. Environ. Contam. Toxicol. 21: 496, 1978). Duodena were studied immediately after removal from sacrificed male rats. Segments were mounted in chambers containing Tyrodes (pH 7.4) gassed with 95% O_2 and 5% CO_2 at 37°C. Spontaneous isometric force data were recorded on magnetic tape and later input to a PDP 11/40 computer which determined elapsed time between zero axis crossings by means of a quartz-crystal clock operating at 1 KHz. These data were used to construct ICI histograms. Sample sizes ranged from 500 to 2000 events which represented observational periods from one-half to two hours. Etherized rats had significantly shorter mean ICIs than those sacrificed by decapitation or pentobarbital. ICIs for Long-Evans and Spontaneously Hypertensive rats were significantly shorter than those of Sprague-Dawley and Wistar rats. Fasting regimen had little effect upon mean ICIs. Increasing temperature and Ringers solution greatly decreases and increases mean ICIs respectively. Mean ICI is dependent upon the intestinal segment employed. Preloading of the duodenal segment from 1-3 g did not significantly alter the mean ICI. In sum, isolated motility studies should employ standard protocols which ensure adequate definition of biological and physical parameters.

102.8

DIFFERENT EFFECTS OF CERULEIN AND CCK-8 ON GUINEA PIG JEJUNAL PROPULSION. M.G. Ulrich-Baker, Z.C. Lian* L.R. Johnson, and W.A. Weems. Univ. of Texas Health Science Center at Houston, TX, 77225

Experiments were conducted in vitro to determine whether arterial perfusion of cerulein or CCK-8 into isolated segments of terminal jejunum from guinea pigs could affect their propulsive behavior. Segments (17 cm in length) were attached to a propulsion evaluation system that required hydrostatic work to affect fluid movement. In all segments (n=8), cerulein (10^{-8} M) decreased the time interval between propulsive complexes an average of 44% as compared to control values (range of 20-63% of control, $P<.01$), but did not alter complex duration. In contrast, CCK-8 (10^{-8} M) did not alter average interval or duration (n=6). However, the propulsive behavior of individual segments was altered in one of two ways: interval was decreased (33-68% of control, $P<.01$, n=3) or duration was increased (128-270% of control, $P<.05$, n=3). At a higher dose (10^{-7} M), CCK-8 perfusion (n=4) both decreased complex interval (n=4) and increased complex duration (n=3). Additionally, CCK-8 at both doses, but not cerulein, increased the maximum rate of fluid ejected per complex, but did not affect amount of fluid ejected. These data indicate that both cerulein and CCK-8 can decrease complex interval but that CCK-8 can under certain conditions also increase complex duration as well as rate of fluid ejection. (Supported by NIH grant DK 32760).

102.9

NICOTINE GUM DOES NOT EFFECT GASTRIC EMPTYING. Sidney Fink* and Tapan K. Chaudhuri. V.A. Medical Center, Hampton, Va. 23667

We studied the effect of nicotine (N) gum (NG) upon gastric emptying (GE) in ten habitual cigarette smokers (ages 38-76, mean 56 years) who had negative gastrointestinal histories.

GE of a Tc-99m-sulfur colloid labelled semisolid meal was measured by dynamically recording scintiphotos of the stomach every three minutes for two hours on two occasions. The subjects fasted and refrained from smoking for 10 hours, then chewed gum containing two mg of N during the first 15 minutes of the study in one test and identical gum devoid of N in the other. NG caused minor local symptoms in every patient but no tests had to be discontinued.

The difference in GE between NG and placebo gum was not statistically significant. There was no order versus medication effect, and no correlation between GE and patient age, high or low N cigarette use, or years of cigarette use.

We conclude that NG does not delay GE or thereby influence the absorption of other drugs.

102.10

KAPPA OPIOID AGONISTS INHIBIT GASTROINTESTINAL TRANSIT IN MICE.
K. Ramabadran, M. Bansinath, H. Turndorf and M. M. Puig, Dept Anesthesiology, NYU Medical Center, 550 First Avenue, New York, NY 10016.

The effect of selective kappa agonists, viz., U-69593 and U-50488H as well as the (+) isomer of the latter, U-53455E, on gastrointestinal propulsion was studied using charcoal meal test. Prior to the test meal, mice (SW 20-25 G) were injected i.p. with either vehicle, 3, 10 or 30 mg/kg of the agonist alone, or along with the kappa antagonist, Mr 2266 (3 mg/kg). The animals were sacrificed 45 min after the meal to measure % of the total length of the intestine traversed by charcoal. U-69593 (ANOVA: $F_{3,29} = 11.2$, $p < 0.0001$) as well as U-50488H ($F_{3,28} = 5.6$, $p < 0.004$) inhibited the gut transit. When compared with controls, both drugs significantly (Newman-Keuls: $p < 0.05$) inhibited the transit at all doses. In contrast, the (+) isomer, U-53455E, did not modify the gut transit. Mr 2266 completely antagonized the effects of U-69593 ($F_{1,52} = 11.7$, $p < 0.001$) as well as U-50488H ($F_{1,51} = 10.5$, $p < 0.002$). The results demonstrate that stereospecific kappa opiate receptors are involved in gut inhibition.

102.11

ANALGESIC AND GASTROINTESTINAL OPIOID EFFECTS OF THE PEPTIDE E FRAGMENT 15-25: FURTHER EVIDENCE FOR DELTA SELECTIVITY. T.P. Davis, T.F. Burks, T.H. Kramer*. Dept. of Pharmacology, College of Medicine, Univ. of Arizona, Tucson, AZ 85724.

Peptide E, a 25 amino acid, mu-selective opioid peptide derived from proenkephalin A, is known to be metabolically converted to the fragment 15-25 (PE15-25), which lacks an amino terminal tyrosine but is a potent delta-selective opioid in vitro. To evaluate potential endogenous activity, we have administered PE15-25 intracerebroventricularly (icv) to mice, and measured analgesic (mu and delta) and intestinal transit inhibition (mu only) responses. Analgesia was assessed by the hotplate test. Inhibition of gastrointestinal transit was measured by the geometric center method, with radiochromium as a non-absorbable marker. PE15-25 (125 μg) produced potent analgesia of short (<20 min) duration, which was completely antagonized by naloxone (2 mg/kg SC) pretreatment. PE15-25 did not cause significant inhibition of intestinal transit at doses up to 125 μg icv. These results are consistent with the in vitro delta selectivity of PE15-25, as delta selective agents given icv are analgesic but do not inhibit intestinal transit. The data further support the role of PE15-25 as an endogenous delta-selective opioid. Supported by USPHS grants DK36289, MH42600, and CA44869.

GASTROINTESTINAL MUCOSA

103.1

THE δ-OPIATE RECEPTOR ALTERS ELECTROGENIC ION TRANSPORT IN PORCINE DISTAL JEJUNUM. Francis L. Quito and David R. Brown* (SPON: Scott O'Grady). Dept. of Vet. Biology, Univ. of Minnesota, St. Paul, MN 55108

Opiates are widely used in the treatment of diarrhea. In vitro studies in rodent and pig gut suggest that opiates interact with mucosal receptors to inhibit active anion secretion and decrease short-circuit current (Isc), a measure of ion transport. We evaluated the potencies of receptor-selective opiate agonists in decreasing Isc and the mechanisms by which they act. Distal jejunal (DJ) mucosal segments were excised from weaned pigs, stripped of smooth muscle and mounted in Ussing chambers. Serosal [D-Pen^2,D-Pen^5]-enkephalin (DPEN; δ-selective), [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin (DAGO; μ-selective), and U50,488H (U50; κ-selective) maximally decreased 8-bromo-cAMP-induced elevations in Isc by 26 ± 8, 12 ± 3 and 31 ± 4 μA/cm^2, respectively. The agonists displayed a rank order of potency DPEN > DAGO >> U50 (1:3:895) with DPEN ED_{50} = 1.9 nM. Agonist effects were reversed by naloxone. Under basal conditions, DPEN maximally decreased Isc by 24 ± 7 μA/cm^2 with ED_{50} = 5.3 nM. Elimination of buffer Cl^- or HCO_3^- or treatment with the cyclooxygenase/lipoxygenase inhibitor ETYA (0.1 mM) reduced basal Isc responses to DPEN; atropine, phentolamine or hexamethonium (1 μM) had no effect. Thus, δ-opiate receptors mediate basal transport in DJ through anion- and eicosanoid-dependent mechanisms.

103.2

THE INFLUENCE OF CALMODULIN ANTAGONISM ON CALCIUM TRANSPORT IN RAT INTESTINE. J.T. Pento and G.K. Mousissian*. College of Pharmacy, Division of Pharmacodynamics and Toxicology, University of Oklahoma, Health Sciences Center, Oklahoma City, OK 73190.

Calmodulin is a ubiquitous calcium-binding protein that mediates the intracellular activity of several important calcium-dependent enzymes. The calmodulin antagonists, trifluoperazine (TFP) and chloropromazine (CPZ) were used in this study to evaluate the influence of calmodulin inhibition on calcium transport and uptake in duodenal segments of rat intestine. The everted gut-sac method was employed to measure calcium translocation in young male Sprague-Dawley rats. The addition of either TFP of CPZ to both the mucosal and serosal surfaces of the duodenal segments decrease calcium transport and tissue uptake in a dose related fashion over a concentration range of 0.1 to 1 mM. The calmodulin antagonists produced a greater reduction in calcium transport than in tissue retention. Further, TFP and CPZ produced a dose-related decrease in the intracellular calcium space in pieces of duodenal tissue. When TFP (1mM) was placed on the mucosal side alone or on both sides of the intestine, calcium transport and tissue uptake were decreased. However, when TFP (1mM) was placed on the serosal side alone there was no change in calcium transport or tissue uptake. The results of this study suggest that calmodulin is involved in the regulation of calcium translocation in duodenal tissue and that this process occurs within the mucosal layer of the intestine.

103.3

COMPARATIVE EFFECTS OF ATRIAL AND ILEAL SELECTIVE MUSCARINIC ANTAGONISTS ON GUINEA PIG ILEAL ELECTROLYTE TRANSPORT AND MUSCLE CONTRACTILITY IN VITRO. B.L. Sturm *, T.S. Gaginella, L. Noronha-Blob* and J.F. Kachur*. NOVA Pharmaceutical Corp., Baltimore, MD and Dept. of Medicine, Ohio State Univ., Columbus, OH.

There is increasing evidence to support the concept of distinct ileal and atrial muscarinic M_2-receptor subtypes [Br.J. Pharmac. (1987) 90, 701]. Well known cardioselective (AF-DX 116, methoctramine) and ileal-selective muscarinic antagonists (4-DAMP, HHSiD) were used to investigate muscarinic receptor selectivity in guinea pig ileal mucosa. The antagonists behaved competitively both against carbachol-induced contractions of longitudinal muscle strips and against carbachol-induced Cl^-secretion in segments of ileal mucosa (Ussing chambers).

Antagonist	Muscle K_b (nM)	Mucosa K_b (nM)
4-DAMP	2.9 ± 0.6	2.7 ± 0.2
HHSiD	18.3 ± 3.0	23 ± 8.5
AF-DX 116	369 ± 22	844 ± 145
methoctramine	287 ± 48	342 ± 108

Thus, these agents do not discriminate between muscarinic receptors mediating Cl^-secretion and muscle contractility suggesting a pharmacological similarity between the two muscarinic receptors.

103.4

ADR-847 and ADR-851: NEW ANTAGONISTS OF SEROTONIN-INDUCED COLONIC SECRETION. **T.S. Gaginella and C. Arrigoni. Ohio State University College of Medicine and Adria Laboratories, Columbus, OH 43210**

ADR-847[(S)-5-chloro-N-(2-pyrrolidinyl-methyl)-2,3-dihydro-benzo[6]furan-7-carboxamide] and its 4-amino congener ADR-851 are novel antiemetics whose mechanism of action is incompletely understood. We report that these agents are antagonists of serotonin (5-HT)-induced colonic secretion. Rat distal colon was stripped of muscularis, mounted in an Ussing chamber and bathed with physiologic buffer (pH 7.4, 37°C) gassed with 95% O_2/5% CO_2. The effects on short- circuit current (ΔSCC) of serosal addition (1-100 μM) of ADR-847 or ADR-851 against the approximate EC50 of 5-HT (1μM) were determined; antagonists were added 15 min. before to 5-HT.

	Change (μA/cm^2) in SCC (Means + S.E., n=5 or more)			
		with Antagonist (μM)		
	5-HT Only	1	10	100
ADR-847	143.4+21.9	49.6+15.3*	20.2+ 8.3*	1.1+1.0**
ADR-851	118.4+17.2	71.9+21.4	42.3+11.7*	1.2+1.1**

*P<.05; **P<.001 compared to 5-HT.

ADR-851 also had agonist activity; SCC increased by 50.4+12μA/cm^2 at 1μM. ADR-847 was approximately equiactive with the 5-HT_3 antagonist MDL-72222 in our system. ADR-847 and ADR-851 are 5-HT antagonists on rat colonic mucosal secretion. The apparent difference in potency may be due to the dual agonist/antagonist activity of ADR-851.

103.5

EFFECT OF GOLD SALTS ON THE CANINE COLONIC MUCOSA - DIRECT AND INDIRECT EFFECTS. A. deBeaux*, C.M. Keenan*, K. Tytgat* and P.K. Rangachari. McMaster University, Hamilton, Ontario, Canada. L8N 3Z5

Oral gold salts used to treat rheumatoid arthritis produce diarrhea in 40% of patients. The underlying mechanisms were studied using two preparations from the canine colon - a "mucosa" with intact muscularis mucosa and attendant submucosal plexuses and a functionally "nerve free" epithelium. Auranofin, a lipophilic compound, increased short circuit current (Isc) across both preparations on serosal and luminal addition. However, serosal responses were seen at concentrations 100 fold lower. TTX,which attenuated serosal responses on the mucosa,had no effect either on luminal responses nor on the epithelium. Cl^- removal reduced responses. Sodium aurothiosulphate, a hydrophilic compound, elicited responses on serosal addition alone;TTX inhibited these . Ouabain decreased the Isc across the epithelium. Thus gold salts stimulate Cl^- secretion across the colon, a significant neural component is present and luminal permeation is essential. Na^+ pump inhibition cannot explain the results obtained. (supported by MRC, Canada)

103.6

DIFFERENTIAL EFFECTS OF VERATRINE ON ION TRANSPORT IN INTACT AND STRIPPED MOUSE JEJUNUM. Russell J. Sheldon*, Margaret E. Malarchik*, Thomas F. Burks and Frank Porreca. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724

The ion transport effects of neural stimulation were studied using veratrine (VER), a mixture of neuroactive plant alkaloids, to evoke neurotransmitter release in intact (all mucosal, neural and muscular components present) and stripped (mucosa, lamina propria and muscularis mucosa) preparations of mouse jejunum. Short-circuit current (Isc) was measured as an indicator of net ion transport using standard Ussing chamber techniques. In the intact jejunum, VER (75 μM) and tetrodotoxin (TTX) (0.1 μM) produced a tonic, decrease of basal Isc (35 $\mu A/cm^2$) when applied to the serosal medium, suggestive of a net proabsorptive and/or antisecretory effect with regard to sodium and chloride transport. The response to VER, but not to TTX, was antagonized by serosal application of yohimbine (YOH, 3 μM), an α_2-adrenoceptor antagonist; naloxone (NLX, 2 μM), an opioid antagonist, failed to antagonize the effect of either of these agents. In stripped preparations, VER elicited a tonic increase of Isc (220 $\mu A/cm^2$ maximal increase), a response that is characteristic of an increase in anion secretion. In contrast, TTX was without effect. Serosal application of YOH or NLX neither affected the response of VER, nor revealed a response of TTX in stripped preparations. These findings suggest that neurons in the mouse jejunum, intimately associated with the intestinal mucosa, may serve a predominant prosecretory role on mucosal transport processes, while neurons more distant to the mucosa, which are partly adrenergic, may serve to limit net intestinal secretion, via antisecretory and/or proabsorptive mechanisms.

103.7

LONGITUDINAL VARIATION OF ELECTROGENIC D-GLUCOSE AND L-ALANINE TRANSPORT IN NECTURUS SMALL INTESTINE: A MICRO-ELECTRODE STUDY. Thierry Chinet*, S.Randall Thomas and Fakis Anagnostopoulos*. INSERM U.192,Hôpital Necker,F-75015 Paris.

We determined the longitudinal distribution of electrogenic sodium-dependent D-glucose and L-alanine cotransport in isolated mucosa from the proximal (n=30) and distal (n=25) Necturus small intestine. The glucose- and alanine-induced changes in the short-circuit current (ΔI_{sc}) and apical membrane potential difference (ΔV_{mc}) were taken as epithelial and cellular indexes of transport. Varying apical concentrations (0.5 to 20mM) of glucose or alanine led to linear Lineweaver-Burk and Eadie plots both in the proximal and distal intestine, arguing against the presence of multiple transporters for either substrate. Kinetic studies based on ΔV_{mc} showed a proximo-distal decrease in maximal effects of both substrates (Alanine: 47.9 $\pm$ 1.5 vs. 24.9 $\pm$ 6.9mV; glucose: 21.3 $\pm$ 0.7 vs. 10.0 $\pm$ 0.6mV), but no apparent proximo-distal change in Kt (Alanine: 1.4 $\pm$ 0.2 vs. 1.4 $\pm$ 1.4mM; glucose: 1.3 $\pm$ 0.1 vs. 1.1 $\pm$ 0.2 mM). No difference was found in sodium dependence or pH sensitivity of these transports along the gut. These results suggest that in Necturus, for the range of concentration studied, there is only one type of D-glucose Na^+ cotransporter and one type of L-alanine Na^+ cotransporter along the gut, and that their number per absorbing cell is reduced in the distal compared to the proximal intestine.

103.8

TEMPERATURE EFFECTS ON INTESTINAL D-GLUCOSE (GLC) AND D-ASPARTATE (ASP) TRANSPORT AS MEASURED USING A FAST SAMPLING, RAPID FILTRATION APPARATUS (FSRFA). S. Breton* and A. Berteloot, Membrane Transport Research Group, University of Montreal, H3C 3J7.

Uptake time courses of Asp and Glc have been evaluated under Na-gradient conditions over the temperature range 5-35°C using rabbit intestinal brush border membrane vesicles and our recently designed FSRFA. With both substrates, the time (T_M) at which maximum overshoot values (O_M) were recorded was shifted towards earlier time points with initial linearity corresponding to the first 10-15% of T_M. Increased O_M were also recorded over the whole temperature range for Asp but only up to 25°C for Glc while decreasing thereafter. In the presence of Na^+-(out > in) and K^+-(in > out) gradients, both T_M and O_M for Asp uptake followed the pattern observed with Na^+ alone but absence of initial linearity was the major finding. Arrhenius plots of these data were linear for Asp with similar activation energies in the presence or absence of K^+ while deviation from linearity above 25°C was noted with Glc. It is concluded: i) that the membrane lipid physical state may not affect transport activities; ii) that K^+-activation of Asp transport may not involve an acceleration of free carrier recycling; iii) that presteady states in Asp uptake may be observed under appropriate conditions (supported by MRC grant MA-7607).

103.9

pH-DEPENDENT HETEROGENEITY OF GLUTAMIC ACID TRANSPORT IN RABBIT INTESTINAL BRUSH BORDER MEMBRANE VESICLES (BBMV). David D. Maenz*, Sylvie Breton* and Alfred Berteloot. University of Montreal, Quebec, Canada H3C 3J7.

Na^+-dependent carrier-mediated transport of glutamic acid by rabbit jejunal BBMV is known to be affected by changes in pH and we now report kinetic and inhibition studies aimed at characterizing this pH-dependence. Eadie-Hofstee plots of initial rates of L-glutamic and D-aspartic acids uptake at pH 8 and D-aspartic acid uptake at pH 6 were found to be linear and thus consistant with the presence of one transport system. However, a curvilinear plot was obtained for L-glutamic acid uptake at pH 6 indicative of flux through both a high and low affinity transport system. Amino acids known to be transported by the high affinity acidic amino acid carrier showed complete inhibition of D-aspartic acid uptake at pH 6 and 8 and L-glutamic acid uptake at pH 8 but only partial inhibition of L-glutamic acid uptake at pH 6. Moreover, neutral amino acids were found to inhibit L-glutamic but not D-aspartic acid uptake with a maximum effect at pH 6. pH activation curves showed an increase in the activity of the low affinity system with decreasing pH from 8 to 5 while the high affinity system showed a bell-shaped profile with maximum transport at pH 7.0-7.5. We conclude that systems X_{AG}^- and X_A^- are present in the rabbit jejunal brush border and that L-glutamic acid is transported by both systems at acidic pH. (Supported by MRC grant MA-7607).

103.10

Na^+-H^+ EXCHANGE IN RAT COLONIC BASOLATERAL MEMBRANE (BLM) VESICLES. P.K. Dudeja*, E.S. Foster* and T.A. Brasitus* (Spon: D.A. Bushinsky) Univ. of Chicago Hospitals and Clinics and Michael Reese Hosp. and Med. Ctr., Pritzker School of Med., Univ. of Chicago, Chicago, IL 60637

An acridine orange fluorescence technique and ^{22}Na uptake studies were used to demonstrate the presence of a neutral Na^+-H^+ exchange process in rat colonic BLM. An outwardly directed Na^+ gradient stimulated H^+ influx and an inwardly directed Na^+ gradient stimulated H^+ efflux. Uphill ^{22}Na uptake was induced by an outwardly directed H^+ gradient exhibiting an "overshoot phenomenon." Under Na^+ efflux conditions an acidification of intravesicular space occurred: 1) due to electroneutral exchange of Na^+ for H^+; 2) due to electrical coupling of Na^+ and H^+ fluxes. The former accounted for more than 75% of the total proton influx. Na^+ stimulated H^+ efflux and ^{22}Na uptake exhibited saturation kinetics with an apparent K_m for Na^+ of approx. 6.50 mM. Amiloride (1 mM) significantly inhibited (60-80%) Na^+ stimulated H^+ efflux and influx, and ^{22}Na uptake. Inwardly directly Li^+ and NH_4^+ gradients also stimulated H^+ efflux with an apparent K_m for Li^+ of approx. 12.5mM. Conclusion: An electroneutral carrier mediated Na^+-H^+ exchange process is present in colonic BLM and may be an important mechanism of Na^+ transport across contraluminal membrane of colonocytes.

103.11

cAMP REGULATION OF Cl^- SECRETION IN PORCINE GALLBLADDER. K.R. Hildebrand*, P.J. Wolters*, D.R. Brown, and S.M. O'Grady. Univ of Minnesota, Vet Biol, St Paul, MN 55108

The neural regulation of ion transport in mammalian gallbladder is not well understood. We examined the actions of two cholecystic neurotransmitters, norepinephrine(NE) and vasoactive intestinal peptide(VIP) on ion transport in porcine gallbladder mucosa in vitro. Gallbladder stripped of serosal musculature was mounted in Ussing chambers and bathed in a Ringer-HCO_3 solution. The mucosa generates a serosa positive transepithelial potential of 4.7 mV and a short circuit current(Isc) of 90-120 $\mu A/cm^2$. Serosal VIP increases Isc and stimulates net Cl^- secretion. Serosal NE causes net NaCl absorption and nullifies Isc. The selective adrenergic antagonists, yohimbine (α_2), but not prazosin (α_1) inhibits NE action. The effects of VIP and NE are unaltered by tetrodotoxin. 8-Br-cAMP mimics the effects of VIP and prevents the Isc inhibition by NE. To assess the involvement of cAMP as a second messenger of VIP-induced Cl^- secretion, cAMP levels were measured in control epithelia, VIP-exposed, and VIP/NE-exposed tissue. Cyclic AMP levels of VIP-exposed epithelia is 2.5-fold higher than controls whereas the VIP/NE-exposed epithelia does not differ significantly from controls. These results suggest (i) VIP and NE affect epithelial transport directly, (ii) cAMP acts as a second messenger of VIP stimulation, (iii) NE inhibits VIP-induced secretion by decreasing cAMP levels.

103.12

F-MET-LEU-PHE-INDUCED CHANGES IN INTESTINAL MUCOSAL PERMEABILITY IN VIVO ARE NOT CAUSED BY NEUTROPHIL-MIGRATION ACROSS THE MUCOSA. C. von Ritter*, D.N. Granger. LSU Medical Center, Shreveport, LA 71130.

The chemotactic peptide FMLP increases permeability in epithelial cell cultures by inducing neutrophil-migration across the epithelial monolayer. Lumenal perfusion of the terminal ileum of rats with FMLP also increases mucosal permeability, an effect that is largely prevented by neutrophil depletion. In this study we addressed the question of whether the FMLP-induced changes in mucosal permeability in rats are caused by migration of neutrophils across the epithelial barrier. Myeloperoxidase (MPO) activity was used as a marker to assess the number of neutrophils present in the perfusate. Despite a fourfold increase in mucosal permeability, two hours of FMLP-perfusion did not increase the levels of MPO in the intestinal lumen. To exclude the possibility that transmigrated neutrophils were trapped underneath the adherent mucus layer, we added the mucolytic agent, N-acetyl-cysteine (10mM), to the perfusate. Mucolysis also failed to increase lumenal MPO. Our data suggest that FMLP-induced increases in mucosal permeability in the terminal ileum of the rat are not caused by migration of neutrophils across the epithelium. Tissue damage mediated by neutrophil-derived toxins is a more likely mechanism to explain the effect of FMLP on mucosal permeability. Preliminary data indicate that neutrophil derived oxidants, i.e., superoxide, hydrogen peroxide and hydroxyl radicals, are important in this respect. Supported by DK 33594.

103.13

DEVELOPMENTAL BIOLOGY OF INTESTINAL OXIDANT PRODUCTION AND PROTECTION. Karen D. Crissinger*, Matthew B. Grisham, and D. Neil Granger. LSU Medical Center, Shreveport, LA 71130

The pathogenesis of neonatal necrotizing enterocolitis is unknown, but a possible role for reactive oxygen metabolites has been postulated. We evaluated whether developmental differences exist in the activities of (1) free radical-generating enzymes (xanthine dehydrogenase/xanthine oxidase (XD/XO) and myeloperoxidase (MPO)), (2) free radical-scavenging enzymes (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH Px)), and (3) endogenous antioxidants (glutathione (GSH)) in the intestinal mucosa. RESULTS indicate that in the ileum and colon of 1 day, 3 day, 2 week, and 1 month old piglets, no XD/XO activity exists, although this enzyme is present in adult pigs. MPO activity is significantly lower in one day than in one month old piglets. SOD activity is significantly higher, CAT no different, and GSH Px and GSH significantly lower in ileum and colon of one day vs one month old animals. CONCLUSIONS: Based on the lack of free radical-generating enzyme activity, the intestine of one day old piglets appears to be less susceptible to mucosal damage by generation of reactive oxygen metabolites than that of one month old animals. (Supported by DK33594 and DK08056).

103.14

Polyamine-dependent growth and transport in a duodenal crypt cell line. D.D. Ginty, D.L. Osborne and E.R. Seidel Dept. of Physiol., East Carolina Univ. Greenville, NC 27858.

The IEC-6 cell is a normal, undifferentiated duodenal crypt epithelial cell line derived from the fetal rat. Cells were grown in DMEM containing 5% fetal calf serum. Reverse phase HPLC analysis revealed that serum contains (uM) 28.4 ± 4.9 putrescine, 77.4 ± 12.5 spermidine and 136.78 ± 23.8 spermine. Analysis of serum after 48 h of dialysis showed all polyamines to be completely removed by this procedure, thus dialyzed serum was used in all subsequent experiments. Addition of 5 mM difluoromethylornithine, a specific inhibitor of polyamine biosynthesis, completely inhibited growth of IEC-6 cells. This effect was reversed by addition of 10 uM putrescine to the media demonstrating the absolute requirement for polyamines during IEC-6 cell proliferation. To determine whether these cells could transport polyamines, cells were incubated in Tyrode's solution containing (^{14}C)putrescine. Michaelis-Menton analysis of transport data indicated a K_t of 1.3 uM and a V_{max} of 5.2 nmoles putrescine/mg prot.-hr. Specific inhibitors of polyamine transport such as MGBG, and other polyamines blocked putrescine transport by approximately 95%. These data demonstrate that addition of putrescine to polyamine-deficient cells will support normal growth through a specific membrane polyamine transport system.
This work supported by AGA and Sigma Xi.

103.15

INFLUENCE OF VILLOUS MOVEMENT ON UNSTIRRED WATER LAYERS (USWL) IN CANINE INTESTINE. D. Mailman, W. Womack*, P. Kvietys and D.N Granger. Univ. Houston, Houston, TX 77004 and Univ. S. Alab. Med. Schl., Mobile, Al 36688.

The movements of villi in canine jejunum and ileum were observed videomicroscopically and compared to the transport of labelled H_2O or fatty acids measured simultaneously in vivo in a perfusion chamber. The aim was to determine if increased villous movement increased the stirring of the USWL as reflected by increased mucosal to serosal absorptive fluxes. Net and absorptive H_2O fluxes were significantly correlated with increased perfusion rate in jejunal segments with no change in villous contraction rate. There was no correlation between perfusion rate and lauric acid absorption. Volume expansion with saline increased villous motility but decreased lauric acid absorption only at lower perfusion rates. Villous motility was greater in the jejunum than in the ileum . Butyric acid absorption was correlated with perfusion rate in the jejunum but not in the ileum but there was only one perfusion rate at which the difference between jejunal and ileal absorption was significantly different. It was concluded that villous motility does not stir the USWL. Supported by NIH AM32139, AM33594 and NRSA AM 07792.

104.1

BICARBONATE PERMEABILITY AND pH DEPENDENCE OF THE OUTWARDLY-RECTIFYING ANION CHANNEL. J.A. Tabcharani[1], T.J. Jensen[2], J.R. Riordan[2] and J.W. Hanrahan[1]. [1]Dept. of Physiol., McGill University, McIntyre Bldg., 3655 Drummond St., Montreal, P.Q., H3G 1Y6 Canada, and [2] Hosp. for Sick Children and Univ. of Toronto, Toronto, Ont., M5G 1X8 Canada

Epithelial bicarbonate transport is reduced in cystic fibrosis (CF) but the HCO_3 pathway and its lesion have not been identified. We studied HCO_3 permeation through the outwardly-rectifying Cl channel, which is abnormally regulated in CF, to determine whether it could also provide a route for HCO_3. Inside-out membrane patches from cultured sweat duct and T_{84} cells were used. Control pH experiments were carried out with weakly buffered, CO_2,HCO_3-free solutions. Lowering cytoplasmic pH (pH_i) stepwise to 6.0 increased channel conductance by ~22%, induced flickering, and eventually caused reversible deactivation; elevating pH_i to 9.7 reduced conductance by ~10%. Extracellular pH had qualitatively similar effects on conductance but did not alter gating. Bicarbonate permeation was studied from both ends of the channel under biionic conditions. Substituting HCO_3 for extracellular Cl caused the reversal potential to shift +9.2 mV, consistent with a HCO_3:Cl permeability ratio of 0.70. However, comparison of HCO_3 and Cl currents at positive membrane potentials suggested a permeability ratio of 1.28 for anion influx; i.e. external HCO_3 appears less permeant than Cl according to reversal potentials but more permeant according to conductances. This discrepancy was not detectable from the cytoplasmic side: Replacing intracellular Cl by HCO_3 shifted the reversal potential to -13.4 mV, consistent with a permeability ratio of 0.59. This value is similar to the HCO_3:Cl conductance ratio of 0.53 obtained for anion efflux. Finally, when only 8-12 mM Cl was added to a pure HCO_3 solution at the cytoplasmic surface, conductance increased to near that measured with 150 mM Cl, and the reversal potential shifted toward 0 mV. We conclude that activation of the outwardly-rectifying Cl channel could increase epithelial bicarbonate conductance and transiently lower cell pH. Defective modulation of this channel might partially account for reduced HCO_3 transport in CF. Support: U.S. and Canadian CF Foundation.

104.2

INWARDLY RECTIFYING K^+ CURRENT IN RAT OSTEOCLASTS. Stephen M. Sims and S. Jeffrey Dixon. Dept. of Physiology and Division of Oral Biology, Univ. of Western Ontario, London, Ontario, Canada. N6A 5C1

Whole-cell patch-clamp recording methods were used to study membrane currents of osteoclasts, the multinucleated cells responsible for bone resorption. Freshly isolated from longbones of newborn rats, osteoclasts ranged in size from 40 to 70 μm in diameter, and had total cell capacitance of 26 to 165 pF (mean 68 ± 34 pF, SD n=31). Under voltage clamp, hyperpolarizing commands from -70 mV induced inward currents while depolarization elicited only linear leakage currents. Current-voltage (I-V) curves showed inward rectification. Raising external K^+ concentration to 140 mM shifted the I-V curve positive along the voltage axis, consistent with the whole-cell currents being carried by K^+ ions. Both cesium and barium, applied extracellularly, blocked the inward rectification. We conclude that the dominant current exhibited by rat osteoclasts is an inwardly rectifying K^+ current, resembling that seen in several other cell types, including macrophages, cardiac and skeletal muscle and tunicate eggs. In current clamp configuration, cells had two stable membrane potentials, -70 mV and -20 mV. Transition between these two levels could be induced by injection of current, but also occurred spontaneously. This is the first description of ionic currents and "excitable" behaviour in osteoclasts. (Supported by MRC Canada.)

104.3

PENETRATION OF CYANIDE INTO NEURONAL CELLS THROUGH CHLORIDE CHANNELS. J.L. Borowitz, A. Rathinavelu and G.E. Isom. Dept. of Pharmacology & Toxicology, School of Pharmacy & Pharmacal Sci., Purdue University, W. Lafayette, IN 47907.

The possibility that cyanide anion may penetrate into neuronal cells by chloride channels was examined in rat pheochromocytoma (PC12) cells, a cultured neuronal model. Cells (0.5 to 5 x 10^6/ml) were exposed to ^{14}C labeled KCN (0.1 mM) for 1 hr at 37°C with or without agents known to influence chloride flux into cells. DIDS (4,4' diisothiocyano-2,2'-stilbene disulfonic acid) (0.1 to 0.8 mM), a specific, irreversible inhibitor of anion transport, decreased the ^{14}CN content of PC12 cells in a dose related manner to a peak of 50% of control. By contrast GABA (0.01 to 1 mM) enhanced cyanide accumulation in PC12 cells by a maximum of 40%. The cyanide antidote sodium thiosulfate (1-4 mM) inhibited cyanide penetration into PC12 cells by a maximum of 20 percent whereas 4 mM sodium sulfate had no effect. Thus a substantial part of cyanide entry into PC12 cells occurs by way of chloride channels, and both DIDS sensitive and GABA activated chloride channels appear to allow penetration of cyanide into these cells. Blockade of cyanide influx into neuronal cells through chloride channels may be an effective means of treating cyanide intoxication. (Supported by PHS grants 532ES07039, ES04140 and SO75505586).

104.4

CHLORIDE CHANNELS IN THE APICAL MEMBRANE OF HUMAN NASAL EPITHELIAL CELLS. **M. Duszyk*. A.S. French and S.F.P. Man. Departments of Physiology and Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2H7**

The single channel patch-clamp technique was used to characterize chloride channels in apical membranes of human nasal epithelial cells maintained in primary culture. Seals were obtained on isolated cells or on cells at the edges of confluent sheets. Single channels were studied in the cell-attached and excised inside-out configurations. At least 4 different sized chloride channels were seen, with the most common channel having a conductance of 18-23 pS at physiological chloride concentrations. This channel was seen in ~6% of total patches (n=316). It did not rectify in symmetric chloride solutions. The dependence of channel conductance on chloride concentration was compared with the predictions of two models of channel permeation. The effects of isoproterenol, and other cAMP mediated agonists, on channel activity was studied. Isoproterenol changed the kinetics and permeation characteristics of chloride channels, increasing their probability of opening and inducing rectification in symmetric chloride solutions.

Supported by the Canadian Medical Research Council and the Alberta Heritage Foundation for Medical Research.

104.5

STRETCH-ACTIVATED ION CHANNELS IN ISOLATED MECHANORECEPTOR CELLS FROM THE COCKROACH. **Andrew S. French and Lisa L. Stockbridge*. Department of Physiology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7**

The second antennal segment (pedicel) of the cockroach, contains several mechanosensory structures, including the connective chordotonal organ and Johnston's Organ. These two structures each contain more than 100 mechanosensory neurons. We have developed a procedure for isolating single mechanosensory cells from this preparation. Dissected pedicels were digested with papain at 37°C for 15 min., followed by mild centrifugation to separate single cells from undigested tissue. This procedure was repeated several times and the separated cells plated onto tissue-culture dishes and maintained in insect medium at 29°C. Bipolar neurons were obtained throughout the digestion with cell body diameters in two clear groups of ~30 μM and ~10 μM. The larger size agrees well with published descriptions of connective chordotonal organ neurons, while the smaller cells may originate from Johnston's organ. Single channel recordings using the excised inside-out patch-clamp technique have shown stretch-activated ion channels of several sizes, including a channel of approximately 100 pS permeant to potassium and sodium ions.

Supported by the Canadian Medical Research Council and the Alberta Heritage Foundation for Medical Research.

104.6

DIMETHTYL SULFOXIDE POTENTIATES ELECTROPHYSIOLOGICAL RESPONSE KINETICS OF BALANUS PHOTORECEPTORS. H. Mack Brown, Dept. Physiol., Univ. of Utah, Salt Lake City, Utah 84108

Increased stimulus strength increases receptor potential amplitude and decreases stimulus-response latency in receptors of many modalities. DMSO added to the saline bath of isolated Balanus eburneus photoreceptors exerts similar effects. A 1% (v/v) solution of DMSO a) increased receptor potential amplitude 40-50% and b) shortened time to peak and latency 20-25%. The light sensitive membrane current of voltage clamped cells was increased systematically as DMSO concentration was increased from 1% to 10%. This potentiation of light-induced current in large part accounts for the increase in receptor potential amplitude; a smaller contribution is made by a decreased dark conductance. The null potential of the light-sensitive current was unaffected by DMSO with short pulses of light, whereas sustained illumination shifted the null potential in the negative direction by 15 mV. This indicates that DMSO has no direct effect on ion selectivity of the light sensitive channel, but that DMSO increases conductance of the light-sensitive channel so effectively that the driving force for the ions involved is run-down during sustained illumination. Due to the similarity of the effects between DMSO and increased light intensity, it is suggested that DMSO acts by potentiating a critical catalytic stage in the phototransduction process.

104.7

APPARENT DITHIOERYTHRITOL INHIBITION OF Rb+ FLUX IN SARCOPLASMIC RETICULUM VESICLES: DUE TO INCREASED MEMBRANE LEAKINESS? L. Gailis and A.J. Williams*, Cardiothoracic Inst., London, U.K.

The uptake of ^{86}Rb+ was measured in rabbit skeletal muscle sarcoplasmic reticulum (SR) vesicles loaded with 100 mM Rb glutamate (glu) and preincubated for 10 min with dithioerythritol (DTE). Rb+ uptake was expressed as % of total ^{86}Rb in the medium. At 37°C, ^{86}Rb+ uptake peaked at 20 min and then declined linearly by 26% over 40 min. DTE (7 mM) decreased the initial 10 min ^{86}Rb+ uptake from 0.19±0.01% (n=4) to 0.08±0.01% ($p<0.001$). At 60 min, the vesicle ^{86}Rb+ content declined to 0.005±0.006%, compared to 0.168±0.010% for the controls ($p<0.001$). A similar effect was seen with 2 mM DTE. In control vesicles loaded with glu-^{3}H, glu-^{3}H loss was only seen during the first 20 min of incubation. DTE accentuated the early decline, but did not cause a continued loss; after 60 min, glu-^{3}H content of the treated vesicles was still 52% of control ($p<0.02$). The results show that under our conditions, DTE renders SR vesicles more permeable to glu and Rb+; however, the loss of glu may not entirely account for the Rb+ loss. (Supported by the Schering Travelling Fellowship and Nuffield Foundation).

104.8

MONENSIN-INDUCED CATION MOVEMENTS IN BOVINE ERYTHROCYTES. Earl Dixon. Department of Physiology, Sch. Vet. Med., Tuskegee University, Tuskegee Institute, AL 36088

Monensin is an ionophore that increases cation movements across the cell membrane. The objectives of these experiments were to determine the ability of this ionophore to stimulate sodium and potassium movements across bovine erythrocyte membranes and study the effects of anion channel inhibitors in altering the action of this compound. Erythrocytes were isolated from cattle ranging in age from 2 weeks to 10 years. Erythrocyte ion content analysis revealed that all of the animals used in this study were low potassium(LK). The cells were washed, incubated in synthetic media and exposed to the ionophore for various time periods. It was found that monensin facilitated the movement of sodium and potassium down their respective concentration gradients. Blockade of the anion channel with DIDS or substitution of chloride with gluconate, acetate or sulfate reduced the cation stimulation by monensin. These studies suggest that monensin can be used to change the internal cation and water content of cells and the action of the ionophore may be modified by manipulation of the anion channel.

104.9

THE IN VITRO INSERTION OF MONOAMINE OXIDASE B INTO MITOCHONDRIAL OUTER MEMBRANES.* Z. Zhuang, M. Hogan and R. McCauley,* Department of Pharmacology, Wayne State University, Detroit, MI 48201

Bovine MAO B has been synthesized in vitro using a reticulocyte lysate translation system directed by bovine liver poly A+ RNA. The newly synthesized enzyme apparently lacks a cleavable N-terminal extension, but MAO B is readily incorporated into mitochondria or isolated mitochondrial outer membranes prepared from rat liver. ATP is not required for the binding of the newly synthesized enzyme to the outer membranes, but it is necessary for the insertion of MAO B into these membrane vesicles. The ATP is not required to generate a mitochondrial membrane potential as assembly occurs under conditions that preclude either the formation or the maintainance of the potential. MAO B will bind to but not become incorporated into outer membrane vesicles which have been treated with trypsin suggesting that the insertion of MAO B also depends on protein factors present on the outer membranes. This work was supported by the American Heart Association of Michigan and the Wayne State Center for Molecular Biology.

ALTITUDE

105.1

CHRONIC CO INHALATION DOES NOT AFFECT CAROTID BODY CATECHOLAMINE CONTENT IN THE RAT. D. Penney, S. Lahiri, K. Albertine, and A. Mokashi*. Univ. of Penna., Sch. of Med., Phila., PA 19104-6085 and the Wayne State Univ., Detroit, MI 48201.

The hypothesis that erythropoietic stimulus would stimulate carotid body cellular metabolism, structure and function was tested. As a part of the study the effects of chronic CO (0.05%-0.07% in air) inhalation for 22 days on catecholamine contents of carotid body (CB) along with blood hematocrits were measured in the rats. The control rats breathed room air. Rats were anesthetized, tail-blood was sampled for hematocrit and CB was collected in cold 0.1M perchloric acid for the measurement of dopamine (DA) norepinephrine (NE) epinephrine (E) and protein. The hematocrit increased from 48% to 75% but DA, NE and E in CB did not change significantly. Since a similar hemotocrit increase due to chronic hypoxia strikingly increases catecholamine contents of CB we conclude that CO did not significantly lower CB tissue PO_2 for the chemosensory stimulation and for enhanced catecholamine metabolism. (Supported in part by grant HL-19737).

105.2

CHRONIC CARBON MONOXIDE (CO) INHALATION STIMULATES ERYTHROPOIESIS BUT NOT CAROTID BODY (CB) GROWTH. A.K. Sherpa*, K.H. Albertine, D.G. Penney, B. Thompkins*, and S. Lahiri. University of Pennsylvania and Jefferson Medical College, Phila., PA, and Wayne State University, Detroit, MI.

The hypothesis that CB structure is altered by the stimulus which enhances erythropoiesis unless a special local feature distinguishes the CB from erythropoietic tissues was tested by using chronic CO inhalation (0.05-0.07% in room air for 21 days) in young male rats. Age-matched control rats were maintained in room air. Venous blood was sampled for hematocrit and the CBs were fixed *in situ*. Calibrated electron micrographs were used to determine changes in Type I and Type II cell volume density (Vv), numerical density (Nv), and average cell volume ($\bar{v}$) by standard stereologic methods. The table summarizes the results.

Group	Type I Cell			Type II Cell		
(n)	Vv	Nv (cells/cm^3)	$\bar{v}$ (μm^3)	Vv	Nv (cells/cm^3)	$\bar{v}$ (μm^3)
Control	0.53	12.9×10^8	424	0.03	3.3×10^8	90
(5)	± 0.08	± 2.3×10^8	± 121	± 0.01	± 0.8×10^8	± 21
CO	0.54	12.5×10^8	441	0.04	2.8×10^8	146
(5)	± 0.03	± 2.2×10^8	± 74	± 0.01	± 1.3×10^8	± 59

Chronic CO inhalation stimulated erythropoiesis (hematocrit=49% for control and 72% for CO) whereas CB growth was not stimulated (no change in Vv, Nv, or $\bar{v}$). We conclude that the moderate carboxyhemoglobinemia did not compromise tissue PO_2 and hence did not stimulate CB growth. This conclusion is consistent with the fact that acute carboxyhemoglobinemia does not stimulate CB chemoreceptors (J.A.P. 50:580, 1981). [Supported by grants HL 07027 and HL 19737]

105.3

O_2 DEPENDENCE OF CYTOCHROME C OXIDATION IN HEPATOCYTES ISOLATED FROM HYPOXIC RATS. A. H. Sillau, T. Y. Aw and D. P. Jones, Dept. of Physiology, Univ. Puerto Rico, San Juan, PR 00936 and Dept. of Biochem., Emory University, Atlanta, GA 30322.

The O_2 concentration needed to oxidize cytochrome c by 50% (P50) is 6-7 μM in freshly isolated hepatocytes and only 0.6 μM in isolated mitochondria. It has been postulated that this difference is due to the resistance of the cytosol to the diffusion of O_2 and to the uneven distribution of mitochondria in clusters (Jones, A.J.P. 247:C83, 1984). The clustering of mitochondria increases their effective radius and their O_2 dependence. To explore the possibility that exposure to chronic hypoxia changes the O_2 dependence of mitochondrial function in cells we have studied the oxidation of cytochrome c in isolated hepatocytes, digitonin-permeabilized hepatocytes and isolated mitochondria from livers of normoxic (N) (P_{IO_2}-140 torr) and hypoxic (H) rats (PIO_2-80 torr for 8-9 days). P50 was smaller in hepatocytes isolated from H rats (5.8 $\pm$ 0.3 vs 7.2 $\pm$ 0.2 μM) and in digitonin permeabilized hepatocytes (2.9 $\pm$ 0.1 vs 3.9 $\pm$ 0.3 μM). However, P50 of isolated mitochondria was not changed by hypoxia. Using the model of Boag (Curr. Top. Radiat. Res. 5:141, 1969) the calculated effective mitochondrial radius of the digitonin permeabilized cells was 20% smaller in the H than in the N rats. These results indicate that chronic hypoxia results in a decrease in the O_2 dependence of mitochondrial function and that this could be due in part to a redistribution of the mitochondria. Supported by NIH grants GM 36538 and GM 11900.

105.4

ALTERED PULMONARY VASCULAR REACTIVITY IN CHRONICALLY HYPOXIC RATS EXPOSED TO 10,000 FT AT 2 OR 30 DAYS OF AGE. A. Tucker, S.D. Babyak*, and W.L. Wilke*. Colo. State Univ., Ft Collins, CO 80523

Studies of pulmonary vascular reactivity (PVR) have demonstrated reduced responses to acute hypoxia and enhanced responses to angiotensin II (AII) in chronically hypoxic rats exposed as adults. However, little is known about changes in PVR in animals exposed to hypoxia at a young age. We exposed litters of 2-day-old (NEO) male rats to either 5,000 ft (5K; resident altitude) or 10,000 ft (10K; in a hypobaric chamber) and studied animals at 30, 60 and 90 days of age. Another group of male rats (YNG) was exposed to the same altitudes at 30 days of age and studied at 60 and 90 days of age. Rats were anesthetized and the lungs were isolated and perfused with a salt solution (Earles + $NaHCO_3$ + Ficoll, 4 g%) at 3.5 ml/min/100 g BW and ventilated with 20% O_2, 5% CO_2. Lungs were challenged with hypoxia (3% O_2, 5% CO_2 for 3 min), AII (0.05 to 0.40 ug), and PGF_{2a} (20 ug). 10K NEO rats exhibited more polycythemia and much greater right ventricular hypertrophy than did the YNG rats at all ages. Baseline pulmonary artery pressure was elevated in the 10K NEO rats compared to controls, but not in the YNG rats. Pulmonary pressor responses to acute hypoxia increased with age in 5K NEO rats, but were much greater at 30 days in 10K NEO rats and then decreased with age. No significant changes in the hypoxic pressor responses were seen in the 5K or 10K YNG rats. Pulmonary pressor responses to AII and PGF_{2a} were increased in all 10K rats, but the responses were markedly increased in the NEO rats at all three ages. Thus, exposure to 10,000 ft in young rats induces polycythemia, right ventricular hypertrophy, and enhanced PVR, and these responses are more marked the younger the age at initial exposure to altitude. (Supported by EPA #R-812243010)

105.5

COMPENSATION OF HYPOCAPNIC ALKALOSIS INDUCED AFTER ACCLIMATION TO SIMULATED ALTITUDE IN RATS. Norberto C. Gonzalez, Richard L. Clancy and Thomas Albrecht*. Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66103

We have observed that rats acclimated to simulated altitude for 3 weeks (3WHA, PB 370-380 torr) regulate plasma pH better than normoxic rats (Nx) when made hypercapnic. The objective of these experiments was to study the opposite end of the spectrum, hypocapnic alkalosis. Alkalosis was induced in conscious 3WHA and Nx by lowering FIO_2 from 0.10 to 0.075 (3WHA) and from 0.209 to 0.10 (Nx) for 3h. Control plasma pH was 7.47±0.004 and 7.46±0.005 in 3WHA and Nx, respectively (NS). At 15 min of decreased FIO_2 plasma pH had increased by 0.096±0.004 in 3WHA and by 0.146±0.009 in Nx ($p<0.01$). As hypocapnia was maintained, non-respiratory compensation was more evident in Nx than in 3WHA: the ratio plasma $dHCO_3^-/dpH$ (mmol/pH x L) increased, in the Nx group, from 15.2±2.5 at 15 min, to 37.6±3.6 at 180 min of hypocapnia ($p<0.01$) while it did not change significantly in 3WHA. In a separate group, hypocapnia resulted in an increase in the net renal excretion of base that was 2.5 times larger in Nx than in 3WHA. This difference was due in part to a larger increase in the excretion of HCO_3^- by Nx. These results show that correction of respiratory alkalosis is less complete in rats acclimated to altitude, and suggest that a less effective renal compensation may be one of the mechanisms. Supported by NIH grant HL39443.

105.6

ALVEOLAR AND MIXED VENOUS OXYGEN TENSIONS DURING RAPID LOSS OF AIRCRAFT CABIN PRESSURE. Albert J. Olszowka and Hermann Rahn. Dept. Physiol. State Univ. of New York at Buffalo, Buffalo, NY

A computer simulation was performed to analyze the changes in alveolar, arterial, and venous blood composition during sudden decompression from a cabin pressure of 6500 ft. (P_{IO_2} = 115 Torr) to an altitude of 34,000 ft. (P_{IO_2} = 29 Torr). Predicted changes in arterial oxygen tension at rest and exercise are consistent with "time of useful consciousness" measurements obtained by Busby et al. (Aviat Space Environ. Med., 1976) in simulated decompression. In general, alveolar oxygen falls rapidly in parallel with changes in inspired oxygen until mixed venous tensions are reached. Thereafter, changes in tension are slowed by reversal of the oxygen gradient between venous blood and the alveoli. The latter changes depend on the size of effective blood volume and the metabolic rate. A similar reversal in the oxygen flux between venous blood and the alveoli has been described in breath-hold divers as a result of rapid expansion of the lungs during ascent (Olszowka and Rahn, In: Hypoxia and Cold, Praeger Press, 1987).

105.7

MUSCLE FIBER SIZE IN LOWLAND RESIDENTS BEFORE AND AFTER ALTITUDE ACCLIMATIZATION. A.J. Young, P.D. Neufer, R.L. Hesslink and J.T. Reeves. US Army Research Institute of Environmental Medicine, Natick, MA 01760 and University of Colorado Health Science Center, Denver, CO 80262

A reduction in muscle fiber area was previously observed in subjects participating in a 40 day hypobaric exposure which simulated the ascent of Mt. Everest. A decreased fiber area might be beneficial at high altitude (HA) since diffusion distance for O_2 would be shortened. However, it is unknown whether or not acclimatization to moderate altitudes would produce this adaptation. In the present study, resting vastus lateralis samples were obtained from 10 lowland natives, once at sea level (SL) and again on day 20 of continuous residence at 4300 m. Type I and II muscle fibers were differentiated and fiber cross-sectional areas were determined. Type II fiber areas were larger than Type I ($P<0.01$). Type I fiber areas ($\bar{x}$±SE) were the same ($P=0.20$) at SL (4395 ±393 um²) as on day 20 at HA (4949 ±545 um²). There were no differences in area of Type II fibers at SL (4829 ±328 um²) compared to HA (6138 ±697 um²). Maximum O_2 uptake ($\dot{V}O_{2\ max}$) was 27 ±3% lower ($P<0.01$) at HA than SL, but no relationship was observed between the decrement in $\dot{V}O_{2\ max}$ at HA and the percent fiber type distribution or fiber area. Thus, acclimatization to moderate altitude does not result in reduced muscle fiber area as observed during sojourn to extreme altitude.

106.1

GLUCOSE-INDUCED INTESTINAL HYPEREMIA AND AUTOREGULATION IN DEVELOPING SWINE. Nancy M.Buckley and Isaac D.Frasier*. Albert Einstein College of Medicine, New York, NY 10461, U.S.A.

Gavage feeding with 2% and 5% glucose (G, 10 ml/kg) was carried out in 28 swine aged 1 day, 1 week, 2 weeks or 1 month. All were fasting and anesthetized (20-30 mg pentobarbital/kg). Abdominal aortic and intestinal venous pressures and regional blood flows (intestinal, renal, femoral)were recorded. Arterial and venous O_2 contents were determined by LexO_2Con or Hemoximeter. Recordings and blood samples were taken before a feeding, at 15 and 30 minutes after a feeding,and at end of experiments. Blood flow autoregulation before and after each feeding was tested by one-step compression of the supraceliac abdominal aorta to 80-90% control pressure. Intestinal vascular resistance (R) was calculated as mean pressure difference/mean flow, and O_2 consumption ($\dot{V}O_2$) as arteriovenous O_2 difference X mean flow. Data were normalized to initial control values and grouped according to age to compare mean values by ANOVA and t-tests.Significant effects of G feedings were: decreased R and increased $\dot{V}O_2$ at all ages; widened arteriovenous O_2 difference only after 2%G in 1-day-olds; decreased femoral but unchanged renal blood flow at all ages; autoregulation of intestinal blood flow after 5%G in 2-week-olds. Thus, G in the intestinal lumen can lead to redistribution of blood from hindlimb to small intestine in swine at birth. However, further maturation is required before this metabolic stimulus can induce intestinal blood flow autoregulation. (Supported by PHS Grant HL-21865).

106.2

THE CRITICAL OXYGEN DELIVERY IN CANINE LIVER. R.W. Samsel*, D. Chergui,* A. Pietrabissa,* W.M. Sanders,* M. Roncella,* J.C. Emond,* and P.T. Schumacker. Univ. of Chicago, Chicago, IL 60637.

As O_2 delivery ($\dot{Q}O_2$, = blood flow X arterial O_2 content) falls, tissues must extract increasing amounts of O_2 from blood, in order to maintain a normal O_2 consumption ($\dot{V}O_2$). Below a critical delivery ($\dot{Q}O_{2c}$), increases in the O_2 extraction ratio (arteriovenous content difference/arterial content) cannot compensate for the falling delivery, and $\dot{V}O_2$ falls in a supply dependent fashion. While studies have identified a systemic $\dot{Q}O_{2c}$ in whole animals, the regional contributions are not fully understood. In the present study, we explored the limits of O_2 extraction in 8 normal, *ex vivo* pump-perfused canine livers, using anesthetized support dogs as a source for oxygenated blood. By lowering the blood flow, the relationship between $\dot{V}O_2$ and $\dot{Q}O_2$ was explored over the entire physiologic range of $\dot{Q}O_2$. The critical $\dot{Q}O_2$ was 28±5 (SD) ml/kg/min; the livers extracted 66±7% of the delivered O_2 prior to reaching supply dependence, suggesting the hepatic O_2 extraction capacity to be similar to the rest of the body, and to other isolated tissues. At high blood flows, the livers extracted approximately 10% of the lactate delivered by the blood, but the arteriovenous lactate differences were small. At low blood flows, however, the livers changed from lactate consumption to production. The O_2 delivery coinciding with the fall in lactate consumption (27±8 ml/kg/min) did not differ significantly from the $\dot{Q}O_{2c}$. Laser doppler measurements suggested that as blood flow was reduced, both derecruitment of capillaries and a fall in blood velocity contributed to the drop in blood flow. We conclude that lactate extraction by the liver remains constant until the $\dot{Q}O_2$ has fallen to the critical point, where O_2 consumption also becomes O_2 supply dependent.

Supported by HL01857, HL01682, and HL32646.

106.3

VASOACTIVE INTESTINAL PEPTIDE(VIP)RELEASE AND MESENTERIC (Mes)FLOW(F)RESPONSE TO FEEDING IN THE NEWBORN. AC Yao, CV Coren*, #BJ Buckley*, M Blank*, #NA Kaplan*, #N Gootman, BM Jaffe. PM Gootman. SUNY, Health Science Ctr. Brooklyn, NY 11203, #The Schneider Children's Hosp., New Hyde Park, NY.

The effects of feeding on MesF and release of VIP into the systemic circulation were examined to determine their possible relationship. Six term Yorkshire piglets 1-3 days old weighing 1.5-2 Kg were lightly anesthetized (halothane). Phasic MesF, abdominal aortic pressure(AoP), ECG were recorded continuously for 2 hr. After 1 hr stabilization, the animals were gavage fed 26 ml/kg of modified cow's milk. Plasma samples were taken pre-and postprandially at 0, 15, 60 and 120 min for radioimmunoassay of VIP concentration. RESULTS: (Mean ± S.E.)

Change from Control	Minutes Postprandial 0	15	60	120	
ΔMesF (ml/min)	12.2** ±3.1	31.6** ±7.9	28.2 ±11.7	13.1 ±10.4	*p<0.035 **p<0.011
ΔVIP (pmol/l)	6.8* ±2.2	6.4 ±2.8	3.0 ±3.0	2.0 ±3.2	vs control

Mes vascular resistance (ΔoP/MesF) decreased as the MesF increased. There was a temporal association between postprandial VIP release and increased MesF. The data suggest that the transient VIP release after feeding may modulate the MesF response in newborn piglets.

Supported by NHLBI Grant HL-20864.

106.4

ASSOCIATION OF BAND-3 PROTEIN WITH ANOXIA-INDUCED RELEASE OF ATP FROM HUMAN ERYTHROCYTES. G.R. Bergfeld* and T. Forrester. Department of Physiology, St. Louis University Medical Center, 1402 S. Grand Blvd., St. Louis, MO 63104.

ATP levels increase in plasma effluent from exercising muscle (Forrester, J. Physiol. 224: 611), but the source of this ATP is unclear. Washed human RBCs were suspended in Krebs-Henseleit solution at cell counts of ~5000.mm^{-3}. ATP was quantified in the suspension fluid using firefly extract. At 37°C background levels of ATP average 5 x 10^5molecules.$cell^{-1}$ and correspond to a 1% hemolysis as assessed by measurement of free hemoglobin in the suspension fluid. Incubation of RBCs at 40°C produced a time-dependent increase in ATP release which peaked after 2hrs at 2.5 x 10^6molecules.$cell^{-1}$. When the RBCs were exposed to a pulse (< 1min) of anoxia, ATP levels also rose from background to 2.5 x 10^6molecules.$cell^{-1}$. Hemoglobin levels in the suspensions did not increase in response to temperature or anoxia, indicating hemolysis was not the cause of the increase in extracellular ATP. Exposure of the RBCs to 50μM niflumic acid, a band-3 anion channel inhibitor, abolished the increase in ATP in response to temperature and anoxia but did not affect the background level of ATP. It is concluded that RBCs slowly release ATP in response to temperature, rapidly release ATP in response to anoxia, and that, as indicated by niflumic acid inhibition, band-3 protein may be the site of ATP release from the RBC.

106.5

METABOLIC AND CIRCULATORY RESPONSES OF NORMOXIC SKELETAL MUSCLE TO WHOLE BODY HYPOXIA AND α-BLOCKADE. D.L. Bredle, C.K. Chapler, and S.M. Cain. U. of Ala. at Birmingham, Birmingham, AL 35294 and Queen's U., Kingston, Ont. K7L 3N6

We previously reported that global hypoxia increased peripheral O_2 demand due to catecholamine calorigenesis which required β_2-adrenoceptors (FASEB J 2:A1311, 1988). If sympathetic vasoconstriction were also present, α-adrenoceptor blockade might improve muscle blood flow in hypoxia and further increase $\dot{V}O_2$. Innervated hindlimbs of 6 dogs pretreated with phenoxybenzamine (α-block) were pump-perfused at constant flow with autologous blood kept normoxic by a membrane oxygenator. The animals were ventilated with room air or 9% O_2 in N_2. α-block prevented the increase in normoxic limb $\dot{V}O_2$ previously observed in unblocked dogs during severe hypoxia. Limb resistance increased in hypoxia in spite of α-block, demonstrating non-adrenergic vasoconstriction. We repeated the protocol using constant-pressure perfusion (n=3). Regardless of perfusion method, results with α-block were surprisingly similar to those with β_2-block. We conclude that both α- and β_2-receptors are necessary for the hypoxia-stimulated increase in muscle O_2 demand. Since flow and resistance were similarly affected in all groups, adrenergic effects on muscle O_2 uptake were mediated by microcirculatory distribution of blood flow and/or by some direct action on muscle cell metabolism. Support: HL #26927, 5T32HL0755305 and MRC.

106.6

NEURAL REGULATION OF SKELETAL MUSCLE BLOOD FLOW DURING HYPOXIC HYPOXIA. P. Kubes*, S. M. Cain and C. K. Chapler. Depts. of Physiology, Queen's Univ., Kingston, Ontario, K7L 3N6 and Univ. of Alabama at Birmingham 35294.

The role of sympathetic innervation in the regulation of hindlimb skeletal muscle blood flow ($\dot{Q}_L$) was studied prior to and during hypoxic hypoxia (HH) in anesthetized, paralyzed and ventilated dogs (n=9). Neural activity in the sciatic nerve was reversibly cold blocked while the animals were ventilated on room air, and again at 15, 30 and 60 min of 9.1% O2 (PaO2-24±2 mmHg). Mean arterial blood pressure (MAP) and $\dot{Q}_L$ were measured continuously; values for cardiac output and whole body and limb oxygen uptake ($\dot{V}O2$) were calculated. Both limb and whole body $\dot{V}O2$ were decreased ($p<0.01$) during HH. Cardiac output was increased ($p<0.01$) during HH, but $\dot{Q}_L$ and MAP remained at prehypoxic levels. With nerve cold block prior to HH, $\dot{Q}_L$ increased to a mean peak value of 111 ml/kg/min and at 15, 30 and 60 min of HH the peak values were 137, 151 and 160 ml/kg/min, respectively. These peak $\dot{Q}_L$ values during HH were greater ($p<0.05$) than that observed during nerve block prior to HH, indicating that sympathetic tone to the hindlimb was increased at this time. Further, the observation that the peak $\dot{Q}_L$ at 60 min was significantly greater than at 15 min of HH demonstrated that sympathetic activity progressively increased during HH, with the result that $\dot{Q}_L$ was maintained at the control level. (Supported by MRC of Canada and NHLBI 14693)

106.7

EFFECT OF INSULIN ON FORELIMB VASCULAR RESISTANCE AND K^+ VASODILATION IN THE NORMAL DOG. D. Eliades, B. Swindall*, M. Pamnani, and F. Haddy. USUHS, Bethesda, MD 20814-4799.

Insulin stimulates Na K-ATPase in skeletal muscle but its effect in vascular smooth muscle is not known. If locally administered insulin stimulates vascular smooth muscle Na K-ATPase, we would expect local vasodilation and increased K^+ vasodilation. We have examined these parameters in the forelimb of the anesthetized dog perfused at constant flow with arterial blood while infusing purified Porcine Insulin (Lily) intrabrachially (IA). Infusion of insulin (0.6 mU/kg/min) for 20 min slightly increased the A-V Glu difference across the limb without changing arterial Glu concentration. However brachial artery perfusion pressure (PP) and K^+ vasodilation (KCl injected IA) were unchanged from control during and after the insulin infusion. When the Na K-ATPase was partially inhibited by IA infusion of ouabain (4 µg/min), addition of insulin did not reverse the inhibited K^+ vasodilation and PP did not change. With a 10-fold increase in insulin and euglycemic Glu clamp, PP was still unchanged. To determine if alpha-mediated vasoconstriction were masking the vasodilator potency of insulin, another series of animals had limb nerves cut and phentolamine (100-150 µg/min) administered IA. However, PP and K^+ dilation were unchanged, even when the Na K-ATPase was partially inhibited with ouabain. Thus insulin does not appear to be a direct vasodilator when administered locally in the forelimb of the anesthetized dog.

106.8

BLOOD FLOW DISTRIBUTION WITH ADRENERGIC AND HISTAMINERGIC ANTAGONISTS. Carleton H. Baker, Darrell L. Davis, E. Truitt Sutton*. Dept. of Physiol. & Biophysics, Coll. of Med., Univ. of So. Fl., Tampa, FL 33612

Stimulation of the superficial fibular nerve (SFNS) causes increased pre- and post-capillary resistances as well as increased capillary permeability in the dog hindpaw. These responses indicate possible adrenergic and histaminergic interactions. The distribution of blood flow between capillaries and arteriovenous anastomoses (AVA) may depend on the relative effects of these neural inputs. Right hindpaws of anesthetized heparinized dogs were vascularly and neurally isolated and perfused with controlled pressure. Blood flow distribution was calculated from the venous recovery of Sr^{85}-microspheres (15µm). The mean transit times of I^{131}-albumin and Sr^{85}-microspheres were calculated. The effects of adrenergic and histaminergic antagonists with and without SFNS were determined. Phentolamine blocked the entire response to SFNS. Prazosin attenuated increases in total and AVA resistance. Yohimbine prevented increased total resistance, attenuated the AVA resistance increase and revealed a decrease in capillary circuit resistance. Pyrilamine attenuated total resistance increase while SFNS increased capillary and AVA resistances. Metiamide had no effect on blood flow distribution with SFNS. The increase in AVA resistance with SFNS apparently resulted from a combination of α_1 and α_2 receptor stimulation but not histaminergic effects.

106.9

ATRIAL NATRIURETIC FACTOR (ANF) INFUSION INTO BLOOD-PERFUSED DOG GRACILIS MUSCLE DOES NOT PRODUCE VASODILATION. Richard E. Klabunde and Mary C. Helgren*. Abbott Laboratories, Abbott Park, IL 60064.

Precontracted blood vessels in vitro relax when exposed to ANF; however, blood vessels in vivo have been shown to either constrict or dilate when ANF is administered systemically. This study evaluated the effects of rat ANF(5-28) infusion into the blood-perfused dog gracilis muscle at concentrations ranging from 30 to 10,000 pg/ml. The vasculature of gracilis muscles from anesthetized beagle dogs was isolated and pump-perfused at constant flow with blood utilizing an extracorporeal circuit. Flow rate was adjusted to give a control perfusion pressure of 110 mmHg. Maximal vasodilatory capacity was determined by adenosine injection. ANF, in 0.1% BSA saline solution, was infused into the arterial circuit to produce increasing arterial blood concentrations. Each infusion lasted 10 min. Systemic arterial pressure, central venous pressure, cardiac output and heart rate did not change during ANF infusion into the gracilis vasculature. ANF at arterial blood concentrations up to 10,000 pg/ml did not produce significant vasodilation although the vasculature showed pronounced vasodilation in response to adenosine. Therefore, rat ANF(5-28) at concentrations within and well above physiological and pharmacological ranges does not produce vasodilation in dog skeletal muscle.

106.10

Atrial natriuretic peptide reverses angiotensin venoconstriction. R.W. Lee, R. Gay and S. Goldman. Tucson VAMC and U of Arizona, Tucson AZ. To determine if atrial natriuretic peptide (ANP) can reverse angiotensin induced venoconstriction, ANP was infused (0.3 µg/kg/min) in the presence of angiotensin hypertension in 6 splenectomized and ganglion blocked dogs. Angiotensin was administered to increase MAP 50% above control. Angiotensin did not change HR, and LV dP/dt but increased total peripheral vascular resistance (TPVR), and LVEDP. CO fell from 190±12 to 167±12 ml/kg/min ($p<0.01$). Mean circulatory filling pressure (MCFP) increased, 6.2±0.3 to 9.9±0.3 mmHg ($p<0.001$) and venous compliance (VC) decreased, 2.1±0.1 to 1.4±0.1 ml/mmHg/kg ($p<0.01$). Unstressed volume did not change but central blood volume (CBV) increased ($p<0.001$). ANP infusion during angiotensin hypertension decreased MAP from 124±9 mmHg to 99±9 mmHg ($p<0.05$) but TPVR did not change. There were no changes in HR or LV dP/dt. ANP decreased CO further from 167±12 to 145±7 ml/kg/min ($p<0.01$). LVEDP returned to baseline with ANP ($p<0.001$). ANP also decreased MCFP from 9.9±0.3 to 6.4±0.3 mmHg ($p<0.001$) and normalized VC from 1.4±0.1 to 1.9±0.5 ml/mmHg/kg ($p<0.05$). There was no change in total blood volume but CBV decreased. In summary ANP can reverse the arterial and venoconstriction produced by angiotensin. The increase in VC causes volume mobilization and results in a decrease in CO and arterial pressure.

106.11

HEART RATE BAROREFLEX CONTROL AND VASCULAR RESPONSIVENESS IN CONSCIOUS, UNRESTRAINED RATS WITH PRE-HEPATIC PORTAL HYPERTENSION. Harold D. Battarbee, Glenn E. Farrar, and Robert P. Spears. Louisiana State University Medical Center, Shreveport, LA 71130.

Heart rate, blood pressure (MAP), and vascular resistance responses to phenylephrine (PE) and sodium nitroprusside (NP) were compared in 13 portal vein-stenosed (PS) and 11 sham-operated rats (SO) chronically instrumented for pulsed Doppler flowmetry. Ten days after stenosis, MAP was lower in PS than in SO (93 ± 3 vs 103 ± 3 mmHg; $p<.05$), and PS heart rates (HR) were higher (330 ± 9 vs 301 ± 8 BPM; $p<.02$). PE pressor responses were attenuated in PS (ED50 = 12.9 ± 0.6 vs 9.5 ± 0.9 ug/kg/min; $p < .01$), whereas NP depressor responses were not different (ED50 = 30 ± 4 and 35 ± 6 ug/kg/min for PS and SO, respectively). HR baroreflex gain with PE-induced increases in MAP was greater in PS rats than in SO (-2.29 ± 0.21 vs -1.51 ± 0.10 BPM/mmHg; $p < .025$), but HR gain did not differ with NP-induced decreases in MAP (-1.97 ± 0.32 and -2.68 ± 0.31 BPM/mmHg for PS and SO groups, respectively; $p<.1$). After ganglionic blockade, differences between PS and SO pressor responses were no longer apparent (ED50 = 7.5 ± 0.7 vs 6.6 ± 0.6 ug/kg/min for PS and SO groups, respectively); NP depressor responses also did not differ (ED50 = 14 ± 2 vs 15 ± 2 ug/kg/min for PS and SO, respectively). Basal HR of PS remained greater than SO (318 ± 12 vs 289 ± 8 BPM; $p < .05$). When renal artery, abdominal aorta, and superior mesenteric artery vascular resistance responses to PE and NP were compared after ganglionic block, PS and PO group responses did not significantly differ. These findings suggest that the reduced PE pressor response of conscious, unrestrained PS rats was due to increased HR baroreflex gain and not decreased resistance vessel responsiveness in renal, skeletal muscle, and visceral vascular beds.

106.12

BACTEREMIA IN THE SHEEP: SITE OF INJECTION DICTATES SITE OF UPTAKE AND INJURY. M.M. DeCamp, A.E. Warner, R.M. Molina, J.D. Brain. Harvard School of Public Health, Boston MA 02115

We have previously shown that inert particles injected into the systemic circulation of sheep are taken up by pulmonary intravascular macrophages (PIMs), and that similar uptake of endotoxin is associated with rapid development of inflammatory lung injury. Bacteremia may be caused by translocation of enteric bacteria from the gut lumen to portal blood. We asked whether the route of bacterial injection in sheep would determine the site of uptake and of consequent tissue injury. We injected live Psuedomonas aeruginosa ($3x10^7$ cfu/kg) into a mesenteric vein or into the jugular vein of sheep (n=4/group). Clearance from the blood was rapid in both groups ($T_{1/2} \leq 1.0$ min). Quantitative cultures of organ homogenates showed predominantly hepatic uptake after portal injection (95.6 ± 1.7% recovered dose) and pulmonary uptake after jugular injection (93.2 ± 2.4%). Electron microscopy localized bacteria to phagosomes of Kupffer cells in the liver and PIMs in the lungs. Site of uptake at one hour correlated well with morphologic evidence of early tissue injury. After jugular injection, lung capillaries were congested with neutrophils, red cells, platelets, and fibrin, and similar, though less severe, inflammatory changes were seen in liver after portal bacteremia. We thus can target lung or liver macrophages in sheep by route of injection of bacteria, and localization within either organ initiates inflammation and local injury. (Supported by HL01670 and HL07118).

106.13

INCREASED SUSCEPTIBILITY TO E. COLI ENDOTOXIN SHOCK IN CHRONIC ANEMIC PIGLETS. John C. Lee, Charles D. McGrath~, Robert Martin~ and Margaret H. Wilson~. VA-MD Regional College of Veterinary Medicine, Blacksburg, VA 24061.

The purpose of this study was to assess the effect of chronic anemia on the sensitivity to E. coli endotoxin shock in neonatal pigs. Piglets of 1 to 5 weeks of age were anesthetized and prepared for hemodynamic and metabolic studies. E. coli (E, 1 mg/kg) was given intravenously over 1 min to each piglet. In piglets with normal Hct (N, n = 5, Hct = 30.5 ± 1.6%), there was no significant reduction of MABP after 60 min exposure to E. In contrast, piglets with spontaneous anemia (A, n = 5, Hct = 15.9 ± 0.6%) showed a significant decline in MABP 45 min after E ($P < 0.025$). By 60 min the MABP had slipped to severe hypotension (30.5 ± 5 mmHg), while the HR was significantly increased in N but not in A. The plasma NE and Epi increased sharply approx 7-fold from control in A. However, there was no significant change in N. Normal piglets with acute hemodilution with dextran exchange (H, n = 5) Hct reduced from 28.3 ± 0.5% to 10.9 ± 0.9% exhibited no increased sensitivity of hemodynamic stress to E when compared to N. The changes in plasma NE and Epi were more than N but markedly less than A. It appears that factors other than reduced oxygen carrying capacity may contribute to the increased sensitivity to endotoxic shock in chronic anemia.

106.14

DETERMINATION OF THE SYNTHESIS RATE OF PLASMA FIBRONECTIN IN INJURED AND SEPTIC HUMANS. C. Thompson*, F.A. Blumenstock, T.M. Saba, P. Feustel, J.E. Kaplan, L. Hough, J. Hasselbarth*, and V. Gray*. Albany Medical College, Albany, NY 12208.

Plasma fibronectin (pFn) is important to reticuloendothelial phagocytic function and host defense. pFn concentrations decrease in humans after trauma and burn injury. Sustained depressions have been noted in some patients manifesting sepsis following injury. This sustained depression may be due to increased consumption or decreased synthesis. Previous studies from this laboratory have shown that the fractional synthesis rate (FSR) in normal volunteers by the methods used in this study is 39.37±1.0%/ day (FASEB J. 2:A1512, 1988) with a synthesis rate (Js/V) of 5.07±0.14 ug/ml/hr. The present study measured the synthesis of pFn in injured humans using stable isotope (15-N glycine) infusion. This group consisted of 4 studies of injury without sepsis and 3 studies of injury with sepsis. The results of this study revealed that the mean Js/V of the entire group was 3.32±0.17 ug/ml/hr. The FSR in the whole group was 27.27±1.07%/day. These preliminary studies suggest that injured patients may have decreased fibronectin synthesis compared to normals. The influence of sepsis on this pattern is being investigated. (GM-15426)

106.15

INCREASED FIBRONECTIN SYNTHETIC RATE FOLLOWING ACUTE PARTICULATE-INDUCED PLASMA FIBRONECTIN DEPLETION. P. Vincent*, E. Cho*, T.M. Saba. Albany Medical College, Albany, NY 12208

Injection (i.v.) of gelatinized particles into rats elicits acute depletion of plasma fibronectin (pFn), followed by restoration to normal levels in 7-8 hrs and hyperfibronectinemia by 24 hrs. Depletion of pFn reflects its opsonic consumption during particle clearance by the RES. We determined if increased pFn synthesis helps restore pFn. We measured the incorporation of ^{75}Selenomethionine (^{75}Se-Met) into pFn after its acute depletion and the effect of blocking protein synthesis on the response. Net incorporation of ^{75}Se-Met over 3-8 hrs equaled total pFn times pFn specific activity. Rats injected with particles had a greater ($p<0.001$) incorporation (cpm±SEM x 10^{-4}) of ^{75}Se-Met into pFn than controls (3 hrs-13.61±1.59 vs 9.70±0.61; 4.5 hrs-12.89±1.66 vs 8.14±0.92; 6 hrs-13.37±2.64 vs 7.58±0.46; 8 hrs-12.05±1.47 vs 7.84± 0.72). Experimentals also showed less ($p<0.05$) incorporation (cpm±SEM x 10^{-7}) of ^{75}Se-Met into total plasma proteins than controls (3 hrs-1.67±0.07 vs 1.89±0.08; 4.5 hrs-1.63±0.06 vs 1.79±0.08; 6 hrs-1.55±0.05 vs 1.74±0.05; 8 hrs-1.51± 0.06 vs 1.71± 0.08). Inhibition of protein synthesis by cycloheximide inhibited about 50% of the total pFn recovery. Thus, there is a specific increase in pFn synthetic rate following acute pFn depletion. Moreover, (a) release from a preformed storage pool; (b) release from the tissue pool; and/or (c) re-utilization coupled with increased synthesis normalize pFn levels. (GM-24117; HL-07194)

106.16

PERIPHERAL AND CIRCULATORY CORRELATES OF BRAIN MISSILE WOUNDING IN ANESTHETIZED PARALYZED CATS. Dan Torbati, Michael E. Carey* and June F. Davidson* Deptartment of Neurosurgery Louisiana State University, Medical Center 1542 Tulane Ave. New Orleans, LA 70112.

Cardiac output (CO), arterial blood pressure (BP) and blood flow in brain, heart, kidney, spleen, adrenals, skeletal muscle and spinal cord were measured (microspheres method) before and after Brain Missile Wounding (BMW) both during normotensive and hemorrhagic hypotensive conditions. EEG and ECG were recorded and blood was periodically sampled for gas and pH analysis. Cats were wounded in the right hemisphere by a 2mm, 31 mg steel sphere penetrating an intact cranium (280 m/s, 1.4 J). Normotensive injured cats showed significant reductions in blood flow in most organs at variable times during the 90 min post-BMW period, associated with metabolic acidosis. However, heart blood flow increased transiently, accompanied by severe cardiac arrythmias. These changes preceeded significant reduction in brain blood flow. Post-BMW hemorrhagic hypotension (BP of 106, 63 and 44 Torr), further reduced organ blood flows to less than 40% of the controls'. CO and EEG were severely depressed and metabolic acidosis was intensified. Blood reinfusion did not restore CO, and organ blood flows to normal levels, except in kidney and adrenals. These data indicate that the peripheral pathophysiological correlates of BMW, particularly following hemorrhagic hypotension may aggravate BMW-induced pathology. Supported by contract No. DAMD17-86-C-6098 LAIR, USMRDC.

CARDIOVASCULAR PHARMACOLOGY II

107.1

HYPOXIC PULMONARY HYPERTENSION IS PREVENTED BY PHENYLISOPROPYL ADENOSINE(PIA). KC Sekar*, PL Toubas*, RE Sheldon*. Univ.Okla.,Dept.Pediatr.,Okla. City,OK.

Adenosine analogs are potent vasodilators. In newborn lambs we tested the hypothesis that PIA would prevent the increase in pulmonary artery pressure (PaP) and resistance (PaR) without a significant decrease in aortic pressure (AoP) during hypoxia. Under anesthesia, catheters were placed in the pulmonary artery (PA), aorta, inferior vena cava, and flow probe around PA. After recovery 7 animals (3-14 days old) were studied in the following sequence:1)control period, 2)hypoxia (9 FiO2, PO2, 25±5 torr), 3)PIA (10 or 20 mg/kg IV bolus), 4)PIA+hypoxia. Heart rate (HR), respirations (RR), PaP, PaR and flow, AoP and arterial blood gases were measured. Results are:

	Cont.	Hypoxia	PIA	PIA+Hypoxia
HR (rate/min)	163±28	261±77*	101±34*	131±29
RR (rate/min)	44±14	58±16*	38±4	66±26*
PaP (torr)	12±5	20±6*	9±3*	15±4**
PaF (ml/kg/min)	285±132	324±151*	202±86	241±117
PaR (units)	9±2	14±10	13±7	12±5
AoP (torr)	68±21	70±15	44±21*	58±14

(* $p<.05$ vs. controls; ** $p<.05$ vs. hypoxia)

The results suggest that PIA prevents increase in PaP and PaR during hypoxia with moderate decrease in AoP.(Individual experiment control values not shown)

107.2

Effect of Pinacidil on Coronary Flow and Function in Isolated Perfused Rat or Guinea Pig Hearts During Ischemic or Nonischemic Conditions

S.F. Flaim, P.G. Sleph*, M.T. Stranieri* and G.J. Grover. Squibb Institute for Medical Research, Princeton N.J. 08543-4000.

In order to determine the relative coronary dilator and cardiodepressant effects of the potassium channel activator pinacidil, isolated buffer perfused guinea pig hearts were used. Pinacidil increased flow and decreased contractile force in a dose dependent fashion. Flow was increased approximately 40, 50, and 75% at the 0.01, 1.0, and 100 µM doses respectively while force was decreased 20, 30, and >90% for these doses. To determine if pinacidil may possess some direct cytoprotective effects, rat hearts pretreated with 1, 10, or 100 µM pinacidil were subjected to 25 min of global ischemia and 30 min of reperfusion. Post-ischemic function (left ventricular developed pressure X HR/1000) was significantly improved only with the 10 µM dose compared with vehicle controls (23±2 vs 9±1 for pinacidil and vehicle groups respectively). End diastolic pressure during reperfusion was significantly increased in vehicle hearts (50±4 mm Hg) and this was reduced with 10 µM pinacidil to 26±2 mm Hg. LDH release was slightly, but not significantly reduced with 10 µM pinacidil (21±2 and 16±2 U/g for vehicle and pinacidil). Flow during reperfusion was reduced in controls and this no-reflow was not altered with pinacidil. No beneficial effects were noted at the 1 and 100 µM dose of pinacidil. Treatment with the potassium channel inactivator, glyburide (10 µM) resulted in a complete reversal of the beneficial effects of 10 µM pinacidil. Thus, pinacidil is both a coronary vasodilator and a cardiodepressant agent. Pinacidil also improves post-ischemic contractile function as well as cardiac compliance.

107.3

Coronary Vasodilator and Cardiac Functional Effects of the Potassium Channel Activator Cromakalim in Ischemic and Nonischemic Rat and Guinea Pig Hearts. GJ Grover, PG Sleph*, M Stranieri* and SF Flaim. Squibb Institute for Medical Research, Princeton, N.J. 08543-4000

To determine the relative coronary dilator and cardiodepressant effects of the potassium channel activator cromakalim (CRO), isolated guinea pig hearts were used. Coronary flow increased and cardiac function decreased in a dose dependent fashion (cumulative) with coronary flow increasing 30% (n.s.) and 75% (p>0.05) at the 0.3 μM and 10μM doses respectively. Function decreased 15% (n.s.) and 40% (p>0.05) respectively at these doses. We also examined the effect of CRO on post-ischemic recovery of function and LDH release following global ischemia in isolated, rat hearts. Rat hearts were subjected to vehicle or 1 and 7 μM CRO (noncumulative) 10 min prior to ischemia. The ischemia was maintained for 25 min at which time the hearts were reperfused with non-drug treated buffer for 30 min. Pre-ischemic function (HR X left ventricular developed pressure/1000) was not affected by either dose of CRO (33.9±1.2, 30.9±0.7, and 33.8±1.3 for vehicle, 1, and 7 μM CRO respectively). Pre-ischemic flow was significantly increased with CRO compared to control. At 30 min post-ischemia, function in vehicle hearts was depressed (10.6±2.8) and this was significantly improved only with the high dose of CRO (22.5±1.8). Reperfusion end diastolic pressure was high in vehicle hearts (53±6 mmHg) and this was reduced with 7 μM CRO (23±4 mmHg). LDH release during reperfusion was high in the vehicle group (20±1.9 U/g) and this was significantly reduced with 7 μM CRO (11±1 U/g). A no-reflow phenomenon was observed during reperfusion and CRO did not significantly improve reflow. Thus, CRO is a coronary vasodilator and to a lesser extent a cardiodepressant. At 7 μM, CRO significantly improves post-ischemic function and compliance, while reducing necrosis as measured by LDH release.

107.4

PROBUCOL REDUCES SEVERITY OF ISOPROTERENOL-AND Mg-DEFICIENCY INDUCED MYOCARDIAL LESIONS
Aisar H. Atrakchi*, Benjamin F. Dickens*, William B. Weglicki and Sherman Bloom, Depts. of Pathology and Medicine, The George Washington University, Washington, D.C. 20037

Probucol (Pro), or Lorelco®, an antihyperlipidemic agent, is used clinically to reduce blood cholesterol. The mechanism(s) of action of Pro at the cellular level is still undefined. Recent studies suggest that Pro possesses antioxidant properties. Since Mg-deficiency (MD) and isoproterenol (ISO) induced myocardial necrosis (MN) may be linked to free radicals, treatment with an antioxidant, in this case Pro, should provide protection. Hamsters were kept for 14 days on MD diet (0.5mM Mg) with or without Pro. On day 14 specific groups received ISO i.p. Hamster hearts were excised 48 hr later, and H&E stained sections analysed for MD and ISO lesions. MD-MN was morphologically distinct from ISO-MN. However, MD was shown to potentiate the severity and abundance of ISO-MN. Treatment with Pro significantly decreased the number of ISO-induced lesions in MD hamsters and MD-MN in animals not given ISO as shown in the following table.

	without Pro	+0.25%Pro	P ≤
	(# lesions as % of normal myocardium)		
MD	*5.8*	*1.2*	*0.04*
MD + ISO	*45*	*12*	*0.001*

The observed protection with Pro against lesions developed in ISO and MD is consistant with its anti-radical properties, and suggest that both ISO- and MD-induced lesions involve a free radical mechanism. Supported by NIH PO1-HL38079

107.5

BUCINDOLOL IS A POTENT β-ADRENERGIC RECEPTOR ANTAGONIST BUT LACKS MYOCARDIAL DEPRESSANT ACTIVITY IN VIVO. M.J. Panzenbeck, R.L. Cavanagh*, A.P. Florczyk*, J.P. Buyniski and M.J. Antonaccio. Pharmaceutical Research and Development Div., Bristol-Myers Co., Wallingford, CT 06492

Bucindolol (B) is a non-specific β-adrenergic antagonist with vasodilator activity. The present experiments were performed to determine the effects of B in isolated electrically stimulated guinea-pig left atria and in anesthetized, vagotomized ferrets. In left atria, B antagonized the developed tension to isoproterenolol with an affinity constant (K_b) at the β-adrenergic receptor of 0.32 nM. Propranolol (P) exhibited a K_b of 1.8 nM. B or P given alone induced direct negative inotropic effects with EC50 values of 48 μM and 27 μM, respectively. In reserpine pretreated atria, these values increased significantly to 120 μM and 43 μM, respectively. In ferrets, i.v. injection of cumulative doses of B (0.01 - 1.0 mg/kg) resulted in dose related decreases in mean arterial pressure (MABP) but no change in heart rate (HR) or right ventricular contractile force (RVCF). In contrast, propranolol causes reductions in HR and RVCF. In ferrets pretreated with reserpine (4 mg/kg, i.p.), B significantly increased HR and RVCF at these doses. Thus B is a potent β-adrenergic antagonist that causes vasodilation without myocardial depression in vivo; B would be useful in cardiovascular therapy where β-blockade is indicated and cardiac depression is to be avoided.

107.6

BETA BLOCKADE DOES NOT PREVENT INSULIN RESISTANCE DURING ENDOTOXIN SHOCK W.R. Law, M.P. McLane*, and R.M. Raymond Loyola University, Stritch School of Medicine, Maywood, IL. 60153, and the V.A. hospital, Hines, IL, 60141.

Catecholamines can antagonize the glucose uptake (GU) stimulating capability of insulin (IN). Elevation of plasma catecholamines is a recognized event associated with endotoxin shock (ES), and may be responsible for the observed IN-resistant state in the heart. Thus, we sought to determine if β-adrenergic blockade (BAB) during ES would restore myocardial IN responsiveness with respect to GU. Mongrel dogs of either sex (20-25 kg) were anesthetized with pentobarbital sodium (30 mg/kg i.v.) and ventilated. Aortic (A) blood and blood pressure were obtained through a catheter inserted via a femoral artery. Cannulas were placed in a femoral vein for infusion of IN (Lilly) and 20% D-glucose. The heart was exposed through a left side laparotomy and instrumented to obtain coronary sinus (CS) blood samples and circumflex artery blood flow (Q; electromagnetic). GU was calculated as the product of Q and the A-CS concentration difference. After baseline measurements ES was caused by iv. S. typhimurium endotoxin (1 mg/kg; Difco). Control dogs received no ES. Basal shock measurements were made 60 minutes post-ES. BAB was achieved with propranolol (150 μg/kg; 5 μg/kg/min). The response to IN was determined under hyperinsulinemic (4U/min), euglycemic clamp conditions during continuous BAB. BAB did not affect IN stimulated GU in control dogs, but did not restore the response to IN during ES. Thus, increased beta-adrenergic activity cannot explain myocardial IN-resistance during ES. Supported by NIH Grant#HL31163 and Vet. Adm.

107.7

SUPEROXIDE ANION (O_2^-) PRODUCTION FROM ZAP (ZYMOSAN ACTIVATED PLASMA)- ACTIVATED GRANULOCYTES IN CANINE WHOLE BLOOD. Geetha Ghai and Kevin Mullane*, Research Dept, CIBA-GEIGY, Summit, NJ 07901

The role of oxygen-derived free radicals (FR) in cardiovascular disease has been inferred primarily from the effects of either FR scavengers or xanthine oxidase inhibitors, given at arbitrary doses and producing variable results. Determining the efficacy of scavengers in vivo is difficult without complicated electron paramagnetic resonance (EPR) spectroscopy. Consequently, we have modified an ex-vivo technique for measuring granulocyte O_2^- production in whole blood (Bellavite et al Eur J Clin Inves, 1983, 13, 363). Canine blood (0.1 ml) anticoagulated with heparin (10 IU/ml) was incubated with ZAP (25 to 200 ul) and the O_2^- formation was measured by the superoxide dismutase inhibitable reduction of cytochrome C. ZAP was prepared by incubating canine plasma (obtained from blood containing 1 IU of heparin/ml) with hydrolysed zymosan (5 mg/ml) for 40 min at 37 + 1°C. ZAP induced a concentration- dependent O_2^- generation which was linear over a 30 min incubation period. For example, 100 ul of ZAP stimulated 5.4 + 1.6 nmol O_2^- /10^6 leukocytes / 30 min (n=5). Thus, FR can be measured ex-vivo in whole blood. This method may provide a simple means to assess the activity of FR scavengers in vivo.

107.8

OPPOSITE CARDIOVASCULAR EFFECTS OF TWO ADENOSINE ANALOGS IN THE CONSCIOUS LAMB. Paul L. Toubas*, Kris Sekar*, and Thomas W. Seale*. (SPON: D. Christensen). Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190

Few studies describe the cardiovascular effects of adenosine agonists on unanesthetized animals. The chronically instrumented sheep is a valuable clinical model for the characterization of changes in cardiopulmonary regulation associated with the transition from fetal to neonatal life. Lambs (n=5, 5-20 days old) were instrumented with a catheter implanted in the pulmonary artery with a 10 mm magnetic flowprobe around the vessel, into the inferior vena cava for IV drug delivery and in the abdominal aorta. Basal values following vehicle administration were stable 3 days after recovery from surgery. Heart rate (HR), aortic blood pressure (AOP), pulmonary artery flow (VAP) and pressure, stroke volume (SV), respiratory rate and 0_2 saturation were determined as a function of acute doses of N^6-(2-phenylisopropyl)adenosine (RPIA) or 2-phenylaminoadenosine (CV1808). HR was depressed by RPIA and elevated significantly by CV1808 (basal value 154±28; RPIA 81±10, CV1808 233±29 bpm). AOP decreased with RPIA (75±12 vs 41±9 mmHg) while no significant change occurred at the highest CV1808 dose. VPA decreased significantly after RPIA administration but increased after CV1808. SV increased following RPIA. These data indicate that prototype adenosine A-1 receptor agonist RPIA and the prototype adenosine A-2 selective agonist CV1808 have distinctly different cardiovascular effects on the conscious lamb.

107.9

ALTERATIONS IN CARDIOVASCULAR RESPONSES AND ADENYLATE CYCLASE ACTIVITY IN CARDIOMYOPATHIC HAMSTER HEARTS. P.G. Conway*, J.W.Hubbard, A.P.Angelac*, J.E. Roehr*, L.L.Ricker* and H.B. Hartman*. Dept. of Biological Research, Hoechst Roussel Pharmaceuticals Inc., Somerville, NJ 08876

The cardiac effects of rate and force as well as the biochemical functions of the cardiac adenylate cyclase system were studied in 200-250 day old genetic cardiomyopathic hamsters (CHF-146). Langendorff studies indicated that the increase in cardiac contractile force, but not rate, produced by dobutamine (0.03-3.0 nM) was significantly less in CHF-146 (CHF) hamsters compared to age matched normals (NOR). In an attempt to explain this effect biochemically, the functional activity of the cardiac adenylate cyclase (AC) system was studied in vitro. Both basal and forskolin stimulated AC activity were found to be significantly reduced in the CHF hamster heart. NOR hamsters exhibited basal AC activity of 13.3±2.6 pmol/mg protein/min. and a Vmax for forskolin stimulation of 315±18 pmol/mg protein/min. whereas these values for CHF hamsters were 6.4±.64 and 228±13 pmol/mg protein/min., respectively. Additionally the potential for an alteration in the activity of the guanine nucleotide stimulatory subunit (Ns)was also determined by studying cardiac AC activity in the presence or absence of NaF. The Ns subunit of CHF hamsters appears, however, to function normally since NaF enhanced both basal and forskolin stimulated AC activity equally in NOR and CHF hamsters. These studies demonstrate that the reduced inotropic response to dobutamine in congestive heart failure may be explained in part by diminished cardiac AC levels as well as responsiveness.

107.10

INHIBITORY ACTION OF (+)3PPP IN THE RAT TAIL ARTERY AND RABBIT EAR ARTERY MAY BE MEDIATED BY PREJUNCTIONAL DOPAMINE RECEPTORS. T. Massamiri* and S.P. Duckles. Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

While sigma agonists have been shown to potentiate the electrically evoked contractile response in both guinea pig and mouse vas deferens, we have previously demonstrated that (+)3PPP (3-[3-hydroxyphenyl]-N-(1-propyl)-piperidine) inhibits TNS induced contraction in the perfused rat tail artery. Perfused rabbit ear arteries and rat tail arteries were stimulated transmurally (TNS: 2-4 Hz, 40V, 2 ms pulse duration, 2 s train duration). In the rat tail artery, the inhibitory response to (+)3PPP (1-100 µM) was reversed to a marked potentiation in presence of the dopamine antagonist sulpiride (3 µM). In the rabbit ear artery, however, (+)3PPP shows a dual effect. (+)3PPP (0.1-5 µM) potentiated the contractile response to TNS in 4 experiments and inhibited in 5. In the latter case, haloperidol (3 µM), a dopamine and putative sigma antagonist, reversed the inhibitory effect of (+)3PPP to a potentiation. Furthermore, in the rabbit ear artery, the contractile response to TNS was potentiated by the sigma agonist, (+)pentazocine, contrary to the rat tail artery in which (+)pentazocine caused little or no effect. Further studies will test the hypothesis that by blocking the dopaminergic effect of (+)3PPP, a prejunctional potentiating action on sigma receptors may then be unmasked.

Supported by NIH grant # DK36289.

107.11

CARDIOVASCULAR ACTION AND DOPAMINERGIC ACTIVITY OF DERIVATIVES OF 4-HYDROXYL, 5-METHYL AMINOINDAN. S.X. Ma, J.P. Long, J.R. Flynn and J.G. Cannon. Depts. of Pharmacology and Medicinal Chemistry, Univ. of Iowa, Iowa City, IA 52242

The ± isomer 4-hydroxyl, 5-methyl-di-n-propylaminoindan (RD-211) (0.03, 0.09, 0.3, 0.9 µmol/kg) produced dose-dependent decrease heart rate, mean arterial blood pressure and inhibition of tachycardia and hypertension induced by stimulation of cardioaccelerator nerve in anesthetized cats. The duration of action was 1-2 hours following i.v. administration. Concentration (0.03, 0.09, 0.3, 0.9 µM) dependent inhibition of tachycardia and force increase induced by transmural stimulation was observed using isolated right cat atria. All of the above pharmacological responses were significaltly inhibited by the dopamine-receptor antagonist sulpiride (0.29 µmol/kg, µM), and not by the α_2-adrenoceptor antagonist yohimbine (2.56 µmol/kg, µM) using both in vivo and in vitro preparations.

Results suggest that RD-211 preferentially stimulates presynaptic dopamine receptors thus decreasing sympathetic neuronal transmission and does not effect α_2-adrenoceptors. Dopamine receptor agonist properties are apparently not due to metabolic activition of the compound. Other neuronal sites of action may also produce the hypotensive and bradycardic activity of the chemical. The compound is stereospecific. Lower n-alkyl analogs are much less active as DA2-receptor agonists. (Supported in part by NIH Grant HL-38136).

107.12

INHIBITORY EFFECTS OF ANTIHYPERTENSIVE AGENTS ON CARDIOVASCULAR REFLEXES. K.H. Park*, J.P. Long and J.G. Cannon*. Depts. of Pharmacology and Medicinal Chemistry, Univ. of Iowa, Iowa City, IA 52242

Since clonidine and DA_2 receptor agonists inhibit sympathetic neurotransmission only at low frequencies of stimulation, perhaps these frequency specific agents might have, compared with a nonspecific inhibitor guanethidine, no or weak inhibitory action on cardiovascular reflexes associated with high frequencies of sympathetic outflow. In anesthetized rats, two equi-hypotensive doses (32-47 mm Hg fall, i.v.) were chosen for comparison. Guanethidine (1 and 3 mg/kg) depressed markedly the responses to 45° head-up tilt, bilateral carotid occlusion (BCO) and sciatic nerve stimulation (SNS). In contrast, clonidine (2 and 6 µg/kg) or two DA_2 receptor agonists, apomorphine (100 and 300 µg/kg) and VICO 81 (trans-6,9-dimethoxy-1-n-propyl-1,2,3,4,4a,5,10,10a-octahydrobenzo[g]quinoline HCl,60 and 200 µg/kg) depressed moderately the tilt reflex delaying the compensation time. Apomorphine inhibited BCO slightly, not affecting SNS. However neither the BCO nor the SNS response was influenced by clonidine or VICO 81. On the other hand, $5\text{-}HT_{1A}$ receptor agonists, 8-OH-DPAT (8-hydroxy-2(di-n-propylamino)tetralin HBr, 100 and 300 µg/kg) and PM 1000 (10-methyl,11-hydroxy aporphine HCl, 1 and 3 mg/kg), did not inhibit the tilt response, instead the arterial pressure level was slightly higher than controls during tilt. Although BCO and SNS were not influenced by 8-OH-DPAT, both responses were slightly depressed by a higher dose of PM 1000 (3 mg/kg). It was concluded that these reflex responses reveal the frequency specific inhibition by antihypertensive agents. (Supported in part by NIH Grant HL-38136).

107.13

AUTORADIOGRAPHIC LOCALIZATION OF DOPAMINE RECEPTORS IN THE CARDIOVASCULAR SYSTEM. Francesco Amenta* (SPON:M.F.Lokhandwala). Univ. Tor Vergata,Dip. Sanità Pubblica & Biologia Cell.,00173 Roma (Italy)

Combined radioreceptor binding and autoradiographic techniques were used to characterize pharmacologically and to visualize anatomically dopamine DA1 and DA2 receptor sites in the rat cardiovascular system.

DA1 receptors were labelled using (3H)-SCH 23390 as a ligand. DA2 receptor sites were labelled with (3H)-spiperone plus Ketanserin.

In the heart prejunctional DA2 receptor sites located primarily in the right atrium predominate. Ventricular myocites bind both (3H)-SCH 23390 and (3H)-spiperone.

In the blood vessels DA1 receptors were found postjunctionally,within smooth muscle cells of the medial layer. DA2 receptors are located prejunctionally as well as within the endothelium of the intimal layer. The density of both DA1 and prejunctional DA2 receptor sites shows an inverse relation with the size of blood vessels.

To know where cardiovascular dopamine receptors are located may contribute to better understand their role in health and disease.

107.14

AUTORADIOGRAPHIC LOCALIZATION OF α_2-ADRENOCEPTORS IN THE RAT MEDULLA BEFORE AND AFTER VAGOTOMY AND NODOSE GANGLIONECTOMY. John C. Hancock, Mark W. Cloud* and Donald B. Hoover. Dept. of Pharmacology, Quillen-Dishner College of Medicine, East Tennessee State University, Johnson City, Tennessee 37614

Light microscopic autoradiography of [^{3}H]clonidine binding was used to determine the effect of efferent denervation by vagotomy and afferent denervation by nodose ganglionectomy (NG) on the localization of α_2-adrenoceptors in the rat medulla oblongata. High density [^{3}H]clonidine binding was localized in the dorsal motor nucleus of the vagus (DNV) and the nucleus of the solitary tract (NTS). Neither vagotomy nor NG affected binding in these nuclei. Specific binding was inhibited by unlabeled phentolamine (10 µM). Loss of AChE in vagal efferent neurons in DNV as a result of axotomy was verified by staining of alternate sections for acetylcholinesterase (AChE). No changes in AChE staining were seen in the medulla of sham operated rats. Failure to see changes in the binding of [^{3}H] clonidine after vagotomy indicates that binding was not associated with efferent cell bodies in the DNV. Failure to see changes in the NTS after NG indicates that clonidine binding was not associated with central terminals of afferent neurons in the nodose ganglion. The results indicate that the α_2-receptors which mediate the hypotensive and bradycardic effects of clonidine are not associated with efferent cell bodies of the DNV or vagal afferent nerve terminals within the NTS.

107.15

EFFECT OF MYO-INOSITOL (INOSITOL) AND/OR TRIIODOTHYRONINE (T_3) TREATMENT ON MYOCARDIAL LIPID LEVELS AND CARDIAC FUNCTION IN STZ-DIABETIC RATS. Hong Xiang*, C.E. Heyliger* and J.H. McNeill. Fac. Pharm. Sci., U.B.C., Vancouver, V6T 1W5.

The beneficial effects of inositol (a lipotropic agent) and T_3 were studied in streptozotocin(55 mg/kg, i.v.)-diabetic rats. Inositol (2.5 g/kg/day, in the drinking water) and T_3 (30 ug/kg/day, s.c.) were given for a 8-week period three days after diabetes induction. Untreated diabetic rats were characterized by a decreased body weight, hyperglycemia and hypoinsulinemia, which were not altered after either treatment. Thyroid status of diabetic animals was normalized by T_3 alone or in combination with inositol, but not by inositol alone. The elevations in plasma and myocardial lipids associated with the diabetic state were prevented by inositol. However, the plasma lipid and myocardial cholesterol levels in diabetic rats remained elevated or were further increased with T_3 or inositol plus T_3 treatment. Inositol partially improved cardiac performance in STZ-diabetic rats. T_3 alone did not prevent cardiac dysfunction in diabetic rats. There was, however, some improvement in heart function in the groups treated with both, associated with a significant decrease in the myocardial triacylglycerol level. The data indicate that a possible correlation may exist between elevated myocardial triacylglycerol levels and cardiac dysfunction in diabetic rats.

(Supported by the B.C. Heart Fdn.)

107.16

STUDIES ON THE VERATRAMINE-INDUCED BURSTING RHYTHM IN THE SINOATRIAL NODE OF THE GUINEA PIG. C. D. Thron and F. V. McCann. Dartmouth Medical School, Hanover, NH 03756

Veratramine (>1 μM) often induces a periodic rhythm (similar to neuronal bursting) in spontaneously-beating isolated guinea pig sinus-atria (Hawkins, J. Pharmacol. 137:306-12, 1962). We have studied this phenomenon with atrial electrograms, atrial electrical stimulation and pacing, and transmembrane potential recordings from the guinea pig sinoatrial node maintained in Krebs-Henseleit solution at 37.5 °C. Periodic rhythm was extremely sensitive to mechanical deformation of the tissue, and was rarely seen in preparations mounted flat. In undeformed preparations, periodic rhythm was often produced within 30-90 min by 2.4 μM veratramine. If periodic rhythm did not appear (or just before it appeared), brief overdrive pacing induced a period of asystole lasting several seconds, followed by a transient tachycardia. If periodic rhythm did not appear with 2.4 μM veratramine, it could be induced by increasing $[Ca^{++}]_o$ from 2.5 mM to 5 mM. Conversely, bursting could be converted to regular rhythm by reducing $[Ca^{++}]_o$. During the bursts the frequency of the action potentials increased and then decreased gradually, as did also the rate of spontaneous diastolic depolarization (pacemaker potential), while the maximum diastolic potential usually decreased slightly and then increased. The last beat in the burst was usually followed by a small oscillatory afterdepolarization. Membrane polarization usually increased during the first few seconds of asystole following a burst, then slowly decreased until the start of the next burst. These observations suggest underlying mechanisms similar to those in bursting neurons. (This work was supported by a Grant-in-Aid from the American Heart Association with funds contributed in part by the New Hampshire Affiliate.)

107.17

BLOOD PRESSURE RESPONSES OF AWAKE RATS EXPOSED CHRONICALLY TO NICOTINE AND COTININE. John D. Connor, Dept.Pharmacol., Penn State Univ. College of Medicine, Hershey, PA 17033.

Cardiovascular effects of nicotine have been reported mainly after acute treatment, often in anesthetized animals. The contribution of cotinine, a major and persistent metabolite, to the pressor effects of nicotine has received only limited attention. Pellets containing 0.5, 2.5, 10 or 25 mg of nicotine or cotinine were implanted subcutaneously into young male Sprague-Dawley rats. Placebo pellets were implanted in one group, another was left untreated (5 or 6 rats per group). No mortalities or overt signs of toxicity were observed. Blood pressures were monitored for 21 days by a tail cuff method. Systolic pressures in placebo-treated and untreated controls rose about 30 mm Hg during the course of the study. Cotinine had little effect on blood pressure at any of the "doses" utilized. Higher concentrations of nicotine significantly (ANOVA) depressed pressures by the end of the study. For example, mean systolic pressure in the nicotine-25 mg group was 17 mm Hg lower than placebo by day 21. At earlier times, the blood pressures were not different from controls. Rats given low dose pellets of nicotine tended to have elevated pressures at later times. These results suggest that in awake rats pressor and depressor responses to nicotine are functions of dose and duration. Cotinine may not be involved in these responses. (Supported by a research grant from RJR Nabisco).

107.18

EFFECT OF KETANSERIN (K) AND METHYSERGIDE (M) ON HYPOXIA AND SEROTONIN INDUCED PULMONARY VASOCONSTRICTION. A.S. Tadepalli. Department of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709

Effects of K & M on pulmonary hemodynamic responses induced by systemic hypoxia (HYP, 10% O_2 ventilation) or serotonin (5HT) infusion (30 μg/kg/min) were examined in anesthetized open chest cats. Both challenges were performed for 10 min. and repeated after cumulative doses of 1 and 3 mg/kg i.v. of K or M. During control period HYP caused significant increases in pulmonary arterial pressure (PAP) and vascular resistance (PVR) and increased cardiac index (CI). Systemic arterial pressure (SMAP) and vascular resistance (SVR) were slightly reduced. Pulmonary venous pressure (PVP) was little affected. 5HT infusion increased PAP, PVR and PVP but reduced CI, SMAP and SVR. Tachyphylaxis to 5HT or HYP did not develop as indicated from time control experiments. Neither K nor M affected the HYP responses indicating a lack for 5HT involvement in hypoxic pulmonary vasoconstriction. M, a $5HT_1$&$_2$ receptor antagonist reduced 5HT induced increment in PAP. In the presence of K, a $5HT_2$ receptor antagonist, 5HT caused a decrease in PAP. Thus, the results suggest that 5HT causes constriction of pulmonary arteries via $5HT_2$ receptor activation, while vasodilation occurs with $5HT_1$ receptor stimulation.

107.19

EFFECT OF NIFEDIPINE AND INHALATIONAL ANESTHETICS ON HEART RATE, CONDUCTION AND CONTRACTILITY IN ISOLATED GUINEA PIG HEARTS. L.A. Gallenberg, E.Y. Cheng*, Z.J. Bosnjak and J.P. Kampine. Dept. of Anesthesiology, Med. College of Wisconsin and VA Med. Ctr., Milwaukee, WI 53295

Effects of the nifedipine (NIF) plus halothane (HAL,0.2+.01 mM), enflurane (ENF,0.4+.04 mM) or isoflurane (ISO,0.2+.01 mM) on atrial interval (AI), atrial-septal conduction time (AST) and left ventricular pressure (LVP) were examined. Hearts were perfused via the aorta at constant pressure (55 mmHg). Electrodes were placed in the right atrium, septal and right ventricular walls. LVP was significantly decreased by 0.7 MAC ISO, HAL and ENF vs. control and NIF (NIF 1.5 & $3x10^{-8}$ M; 77 + 4% & 69 + 5% of control) (Table 1). NIF plus anesthetic significantly decreased LVP vs. anesthetic alone. AI was significantly prolonged with NIF ($1.5x10^{-8}$ = 317 + 5 msec; $3x10^{-8}$ = 329 + 4.3 msec) and also with ISO, HAL or ENF vs. control (276 + 3 msec) (Table 2). NIF plus ISO, HAL or ENF significantly prolonged AI vs. anesthetic alone. No significant changes in AST were caused by NIF plus ISO, HAL or ENF.

Table 1.

NIF	LVP (% CONTROL $\bar{x}$ ± SEM)		
10^{-8} M	ISO	HAL (0.7 MAC)	ENF
0	90.1±1.5*	75.6±2.7*	73.7±2.9*
1.5	56.8±9.7+	48.5±6.5+	47.7±8.6+
3.0	53.1±6.8+	48.9±6.9+	44.8±9.3+

Table 2.

	ATRIAL INTERVAL (msec $\bar{x}$ ± SEM)		
	ISO	HAL (0.7 MAC)	ENF
	300.4±8.5*	309.3±6.5*	330.0±5.8*
	351.4±13.4+	350.4±11.8+	356.4±4.4+
	343.3±11.2+	348.1±18.3+	370.7±8.6+

*P ≤ 0.05 vs. control; +P ≤ 0.05 vs. anesthetic alone and control

107.20

COMPARATIVE ANTIHYPERTENSIVE EVALUATION OF K^+ CHANNEL EFFLUX STIMULATORS IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS (SHR). EC Giardino*, LB Katz, TM Casey*, EM Osifchin*, DA Shriver, R Falotico and AJ Tobia. Research Labs, Ortho Pharmaceutical Corp., Raritan, N.J. 08869

Studies were conducted to evaluate the oral and iv blood pressure lowering effects of a novel class of vasodilators that reportedly stimulate efflux of potassium ions from smooth muscle cells. The carotid artery was catheterized for direct measurement of systolic and diastolic blood pressure and rats were restrained in Bollman cages during the 4 hr test. The doses that decreased mean arterial blood pressure (MABP) by 30% (ED_{30}) for chromakalim (C), pinacidil (P), nicorandil (N) and minoxidil sulfate (M) are shown below. Maximum decrease in MABP in response to C, P and N occurred 15-20 min post-drug, whereas M lowered MABP gradually with the maximum decrease occurring 3-4 hr post-drug. In a different group of conscious SHR, duration of action was determined by infusing each drug into a jugular vein for about 5 min until a 35-40% drop in MABP was observed. Drug infusion was then stopped and the time required for MABP to return to 95% of pre-drug values was determined.

	Oral ED_{30}(95% F.L.)	IV ED_{30} ±S.E.	IV duration
C	0.18 (0.13-0.25) mg/kg	0.05 ±0.002mg/kg	45 ±4 min
P	0.90 (0.19-3.40) mg/kg	0.21 ±0.01 mg/kg	54 ±3 min
N	3.11 (0.82-11.8) mg/kg	1.27 ±0.11 mg/kg	97 ±19 min
M	0.90 (0.16-5.28) mg/kg	0.51 ±0.04 mg/kg	>5.0 hr

The data indicate that C is 5-17x (oral) and 4-25x (iv) more potent than P, N or M, however, M has a significantly longer duration of action.

107.21

HEMODYNAMIC PROFILE IN DOGS OF K+ EFFLUX STIMULATORS, A NOVEL CLASS OF VASODILATORS. LB Katz, EC Giardino*, EM Osifchin*, TM Casey*, DA Shriver, R Falotico and AJ Tobia. Research Labs, Ortho Pharmaceutical Corp., Raritan, N.J. 08869

Studies were conducted in pentobarbital anesthetized open chest dogs to evaluate the hemodynamic effects of chromakalim (C), pinacidil (P), nicorandil (N) and minoxidil sulfate (M). Cardiac output (CO), coronary artery blood flow (CBF) and femoral artery blood flow (FBF) were measured using electromagnetic flowmetry. Total peripheral resistance (TPR), coronary vascular resistance (CVR) and femoral vascular resistance (FVR) were calculated as the ratio of mean arterial blood pressure (MABP) to blood flow. At iv doses that significantly lowered MABP, C, P, N and M significantly decreased TPR, CVR and stroke work, with no change in CO, heart rate, dP/dt, stroke volume or FVR.

	Dose*	MABP	TPR	CBF	CVR	Dose*	RBF	RVR
C	0.043	-28±4	-35±6	163±46	-69±6	0.1	31±11	-46±5
P	0.43	-27±2	-33±5	167±34	-72±3	0.6	-25±14	-34±7
N	0.43	-25±5	-29±4	132±28	-32±7	1.2	64±15	-59±8
M	0.43	-42±7	-45±7	180±69	-78±3	0.6	19±5	-36±5

Data=Mean % change ±SE from pre-drug baseline; *mg/kg i.v.

Maximum effects occurred during infusion (N), at the end of infusion (C, P), or 60 min post-infusion (M). In a different group of closed chest anesthetized dogs, antihypertensive doses of each drug significantly decreased renal vascular resistance (RVR) with variable, non-significant increases in renal blood flow (RBF), suggesting that these compounds dilate regional circulations other than the renal bed. In summary, these novel vasodilators have similar hemodynamic profiles, differing in their potency and duration of action.

ANALGESICS AND ANTAGONISTS I

108.1

SIMULTANEOUS PHARMACOKINETIC/PHARMACODYNAMIC MODELING OF FENTANYL IN THE RAT: APPLICATION TO ANALGESIC STUDIES. T.H. Kramer*, P.K. Lemcke*, and T.F. Burks. Dept. Pharmacology, College of Medicine, Univ. of Arizona, Tucson, AZ 85724.

In vivo concentration/response relationships for fentanyl (FEN) analgesia were developed in the rat. Jugular venous cannulae were implanted in male Sprague-Dawley rats under general anesthesia. 3 days later, FEN (30-100 μg/kg) was injected SC, IP, or IV. Multiple blood samples and hotplate analgesia tests were performed through 5 hours after injection. Plasma FEN concentrations were determined by RIA. Pharmacokinetic parameters of FEN were calculated using the trapezoidal rule and standard equations. The best-fit pharmacokinetic model for each rat was linked to a model of pharmacologic effect (Hill equation) and the two equations solved simultaneously by a computerized nonlinear least-squares method (PCNONLIN) to estimate the EC50ss (EC50 at steady-state) of FEN in vivo. Mean pharmacokinetic parameters (±St. Dev.) were: Clearance = 209.7 (81.1) ml/kg/min; half-life = 119.8 (38.5) min; volume of distribution = 41.34 (21.34) l/kg. Values of fentanyl EC50ss ranged from 3.95 to 8.58 ng/ml, and appear to be independent of route of administration. This method appears to produce precise estimates of potency using fewer animal subjects, and may have advantages over traditional dose-response studies of analgesics. Supported by USPHS grant DK36289.

108.2

SPINAL BETA-ENDORPHIN DIFFERENTIALLY INTERACTS WITH SPINAL MONOAMINE SYSTEMS TO ALLEVIATE DIFFERENT TYPES OF PAIN. T. Crisp *, J.L. Stefinsky * and M. Uram *(Spon: M.D. Schechter). Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272

Male Sprague-Dawley rats (350-400 g) were chronically implanted with indwelling PE-10 spinal catheters, and the analgesic efficacy of beta-endorphin (B-EP) administered intrathecally (i.t.) was assessed using the tail-flick and hot plate tests. B-EP dose-dependently elevated tail-flick latencies (TFL) and hot plate latencies (HPL) in a naltrexone-reversible manner. In experiments designed to evaluate the local spinal involvement of serotonin (5-HT) in the antinociceptive action of B-EP, it was observed that opioid-induced elevations in TFL and HPL were blocked by the 5-HT antagonists spiroxatrine, ritanserin and ICS 205-930. When experiments were done to determine the local spinal noradrenergic (NE) mediation of B-EP, it was noted that the alpha 1 antagonist WB-4101 failed to significantly alter the analgesic actions of the opioid on both the tail flick and hot plate tests. The alpha 2 antagonist yohimbine completely blocked B-EP-induced elevations in TFL, but was without effect against the antinociceptive effects of the opioid on the hot plate test. These data indicate that B-EP differentially interacts with spinal monoaminergic systems to diminish different types of noxious stimuli.

108.3

CHARACTERIZATION OF THE MU AGONIST, DAMPGO, IN SPINAL ANTINOCICEPTION. L. J. Spanos* and T. Crisp.* (SPON: E.C. Krimmer). Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272

The monoaminergic contribution to mu receptor mediated spinal antinociception was assessed in male Sprague Dawley rats. Rats (340±40g) were cannulated with indwelling PE-10 catheters under ketamine anesthesia. After a one week recovery period, the animals were injected intrathecally, (i.t.) with the mu agonist, DAMPGO, and monitored for changes in tail flick latency (TFL). Analysis of dose response data indicated that DAMPGO (0.1 nmol/10μl) significantly elevated TFL. Naltrexone (0.3 μg/10μl) effectively blocked the analgesic response to the opioid. To determine the possible involvement of adrenergic systems in mu-mediated spinal anti-nociception, either WB-4101, an alpha$_1$ antagonist, or yohimbine, an alpha$_2$ antagonist, was administered i.t. 10 minutes prior to the opioid and tested for the ability to alter DAMPGO-induced analgesia. Both noradrenergic antagonists effectively attenuated opioid-induced elevations in TFL. A serotonergic component was also indicated by the ability of serotonin antagonists (e.g., pindolol and ritanserin) to block the response to DAMPGO. None of the antagonists administered alone produced significant changes in TFL.

108.4

CLONIDINE ANTINOCICEPTION INVOLVES DIFFERENT RECEPTOR SUBTYPES IN DIFFERENT TYPES OF PAIN. R.A.R. Tasker[1], B.J. Connell[1] and R. Melzack[2] (SPON: J.F. Burka). [1]AVC, UPEI, Charlottetown, PEI, C1A 4P3 and [2]McGill University, Montreal, Que., H3A 1B1.

Many studies using the rodent tail-flick test have concluded that clonidine antinociception is mediated predominantly by the α2 receptor subtype in this test. However, there is considerable evidence that different mechanisms mediate antinociception in different pain tests. We chose to investigate the relative role of both α1 and α2 adrenoceptors in the mechanism(s) of clonidine antinociception in different types of pain. Dose-response curves (DRC) for i.p. clonidine (male LE rats) were constructed using the tail-flick test (TF) and the formalin test (FORM). Clonidine was 2.7 times more potent in FORM than in TF. Prior injection of tolazoline (20 mg/kg) antagonized clonidine (ED50) analgesia in both tests. Prior injection of yohimbine (2 mg/kg) antagonized clonidine (ED50) analgesia in TF but not in FORM, whereas prazosin (0.15 mg/kg) completely antagonized clonidine (ED50) analgesia in FORM but was without effect in TF. We are currently investigating the actions of various antagonists on the full clonidine DRCs in both tests. Results obtained to date indicate that the role of different α receptor subtypes in antinociception is a function of both the dose of clonidine used and the pain test employed. Supported in part by NSERC Grant A7891 to RM.

108.5

FURTHER CHARACTERIZATION OF THE BEHAVIORAL AND HYPOGLYCEMIC EFFECTS OF INTRATHECAL MORPHINE IN MICE. U. Estrada,* F. Lux,* D. A. Brase and W. L. Dewey. Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

The i.t. injection of morphine in mice was reported to cause a dose-dependent, naloxone-antagonizable hypoglycemia, and caused scratching, biting behaviors and seizures (JPET 245: 187, 1988). Further studies showed that (+)-morphine (50 ug) and (-)-morphine-3-glucuronide (1-2 ug) also produced a behavioral syndrome, but not hypoglycemia. Several other opioids tested at a dose of 50 ug, i.t., produced either a saline-like hyperglycemia [methadone, (-)-ketocyclazocine, U-50,488H, (-)- and (+)-N-allyl-normetazocine, levomethorphan, DAGO, (-)- and (+)-pentazocine, (-)- and (+)-naloxone] or no significant change from basal blood glucose levels [heroin, ethylmorphine, levorphanol, (-)- and (+)-codeine]. Leu- and met-enkephalin (10 ug each) and DPDPE (5 ug) failed to mimic morphine-induced hypoglycemia or behavior. Only i.t. normorphine mimicked all of these effects. Parts of the behavioral syndrome were also produced by the glycine antagonist strychnine (5 ug) or the histamine releaser, compound 48/80 (20 ug), which were not hypoglycemic; but the effects of i.t. morphine (40 ug) were not blocked by i.p. pretreatment with glycine (5 g/kg) or diphenhydramine (10-20 mg/kg). Thus, the hypoglycemic effect appears to be quite selective for (-)- morphine and normorphine, and does not occur simply as a result of behavioral activation. (Supported in part by USPHS grants DA-01647 and DA-00490.)

108.6

INHIBITION OF MORPHINE SULFATE (MS) ANALGESIA BY INTRACEREBROVENTRICULAR (ICV) CLONIDINE IN MICE. James M. Fujimoto and Kathleen Schaus Arts. VA Medical Center, Medical College of Wisconsin, Milwaukee, WI 53295.

Even though a synergistic interaction might be expected between MS and clonidine, we find that clonidine hydrochloride (CL) given SC antagonized the antinociceptive effect of morphine in the ICR Sasco male mice in the tail flick (TF) test. ED50 values (95% CI), mg/kg, were for SC CL 0.55 (0.26-1.13) at 15 min and MS 7.1 (4.4-11.4) at 20 min. At a normalized relative dose ratio of 1/1, the combination had an ED50 greater than the theoretical additive ED50 by the Litchfield Wilcoxon potency ratio comparison. Similar antagonism was shown for SC CL on ICV MS. This antagonistic effect was due to the supraspinal action of CL because ICV CL given 10 min before TF test (but not intrathecal, IT CL) was shown to antagonize IT, ICV or SC MS. Thus, CL ICV, 0.6 µg, followed in 5 min by IT MS shifted the MS ED50 to the right by 4 fold (.83/.21, µg/µg). For ICV MS, ED50 value of MS shifted 12 fold (28.3/2.3). For SC MS ED50 (MS given 10 min before CL) shift was 2 fold (15.1/7.1). These results indicate that supraspinally, an alpha adrenergic system exists which can produce an antagonistic effect on morphine analgesia. (Supported by VA and DA00451).

108.7

A SELECTIVE KAPPA OPIOID AGONIST DOES NOT PROVOKE FELINE RAGE. W. Butt*, B. Krevsky*, B.J. Chase* and A. Cowan. Depts. of Pharmacology and Medicine, Temple U., Philadelphia, PA 19140

It is not clear whether the opioid-induced rage reaction in cats is mediated solely by mu binding sites. In the present work, we compared autonomic/behavioral changes in cats resulting from the i.m. injection of saline (S), morphine (M) or U-50,488H (3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide) (U), a selective kappa agonist. Adult female cats (n=5) were given S (0.1 ml/kg), M (0.5, 1 and 5 mg/kg) or U (0.25, 0.5 and 1 mg/kg) and scored under blind conditions for 1 hr for the following: agitation, body tremor, ataxia, circling, convulsion, defecation, dozing, emesis, hunchback posture, licking, micturition, piloerection, pupillary and respiratory changes, salivation, sedation, staring and vocalization. M caused emesis at all doses and provoked psychomotor excitation (rage) at 5 mg/kg. U did not induce emesis. U caused behavioral depression at all doses. Miosis after U and mydriasis after M were consistently noted. A weighted cumulative autonomic/behavioral score was developed and the results are shown below.

	S	M(0.5)	M(1)	M(5)	S	U(0.25)	U(0.5)	U(1)
Mean	5.5	14.4	16.0	16.9	7.5	12.1	15.7	10.2
SEM	2.1	1.2	1.5	1.8	2.6	1.5	2.0	1.4

It is concluded that (a) morphine and U-50,488H may be distinguished in the cat on the basis of behavioral and autonomic changes, and (b) feline rage is probably mediated by mu, rather than kappa, binding sites.

108.8

ANTINOCICEPTION AND SUBSTANCE P ANTAGONIST ACTIVITY IN A SERIES OF ANALOGS OF SUBSTANCE P6-11. S. J. Ward, E.R. Baizman*, and B.A. Morgan*. Dept. of Pharmacology and Medicinal Chemistry. Sterling-Winthrop Res. Inst., Rensselaer, NY 12144.

Antagonists of Substance P (SP), a putative primary afferent transmitter, may be antinociceptive. Analogs of SP6-11, including some with novel azole dipeptide-mimetic fragments, were tested in vitro for antagonist potency at SP-P (neurokinin-1) receptors in the atropinized guinea pig ileum (GPI). In vivo SP antagonist potency was assessed by attenuation of SP-induced scratching in mice after intrathecal (i.t.) co-administration with SP. The mouse ACh-induced writhing test assessed antinociception, and a mouse rotarod assay revealed motor impairment. SP6-11 analogs showed both in vitro antagonist activity vs SP (pA_2 = 7.3-4.0) and i.t. antagonist potency in vivo (SP scratching ED_{50} = 0.07 - >10 µg/kg, i.t.). A clear correlation was found between antinociception (ACh ED_{50} = 0.7 - >10 µg/kg, i.t.) and in vitro SP antagonist potency ($p < 0.0001$). Correlations were less clear between SP scratching ED_{50} and either in vitro pA_2 ($p = 0.17$) or in vivo ACh ED_{50} ($p = 0.11$). Though most analogs impaired rotarod performance in antinociceptive doses, 3 analogs, including an azole peptide-mimetic (pA_2 = 7.3) produced antinociception but were inactive in the rotarod at doses ~ 30 times the antinociceptive ED_{50}. We conclude that some SP antagonists can produce antinociception in vivo that is dissociated from impairment of motor performance. The specific tachykinin receptor with which these antagonists may interact in vivo is not known.

108.9

Butorphanol Exhibits Multiple Actions On The Opioid Receptor System. P.J. Horan* and I.K. Ho Dept. Pharmacol. and Toxicol., Univ. Ms. Med. Cntr. Jackson, Ms 39216

Butorphanol is a potent, synthetic narcotic analgesic presently under non-controlled status. However it's actions relative to morphine are largely unknown. Therefore studies were undertaken exploring both the pharmacologic and biochemical properties of butorphanol and morphine. Male Sprague-Dawley rats received equimolar icv infusions of morphine and butorphanol (52.3 nmols/hr) for 3 days.The addictive liabilities of these compounds were then evaluated via precipitated withdrawal with naloxone. Naloxone challenge induced a wide variety of withdrawal behaviors, with the ED_{50} of naloxone for the production of wet shakes and escape behavior being equivalent in animals receiving either morphine or butorphanol. In a separate study, animals were administered equimolar doses of morphine and butorphanol (36.4 and 22.9 mg/kg,respectively) in addition to a dose of butorphanol approximating it's ED_{50} in the rat tail flick test (5.7 mg/kg). Urine volume was then evaluated at 1,2,4,6, and 8 hours, in addition to urine osmolality at 8 hours post-dosing. Butorphanol produced a marked increase in urine volume in contrast with morphine, which produced an antidiuresis. However, by 8 hours after dosing, urine osmolality was reduced compared to control in both morphine and butorphanol treated animals. In in vitro radioligand displacement studies, morphine and butorphanol were potent displacers of ^{3}H-DAGO binding. In contrast, butorphanol was approximately 10 and 40 times more potent than morphine in inhibiting ^{3}H-DPDPE and ^{3}H-(-)EKC binding , respectively. Both ligands were weak displacers of ^{3}H-(+)SKF10047 binding, yielding IC_{50} values> 1 µM. These results demonstrate that butorphanol posesses a substantial addiction liability. In addition, butorphanol appears to have agonistic properties at mu and kappa opioid receptors, and also interacts potently with the delta opioid receptor type.(Supported by NIEHS grant ES-07045)

108.10

THE NMDA RECEPTOR: CENTRAL ROLE IN MEDIATING PAIN INHIBITION IN RAT PERIAQUEDUCTAL GRAY. Yasuko Jacquet. Behavioral Neuropharmacology Lab., Nathan Kline Institute, Orangeburg, NY 10962.

The role of the periaqueductal gray (PAG) in the mediation of opiate analgesia is well established (Jacquet & Lajtha, Science 1974). An injection of morphine in the PAG resulted in a naloxone-reversible analgesia, indicating an opiate receptor mediated action. The present study investigated what role, if any, the excitatory amino acids (EAA) may also play in pain inhibition at this CNS site. An injection of the EAA analogue, N-methyl-D-aspartate (NMDA) (10 nmol) in the rat PAG resulted in potent analgesia in 2 analgesia tests (Tail Flick & Noxious Pinch). A prior injection of the NMDA antagonist, (-)-2-amino-7-phosphonoheptanoate (D-AP7)(3 nmol) antagonized this action, indicating a receptor-mediated action. D-AP7 also completely blocked morphine analgesia. NMDA (10 nmol) given with morphine (25 nmol) potentiated morphine analgesia, while the opiate antagonist, naloxone (5 nmol), only partially reversed this analgesic action. These results are consistent with the view that opiate-mediated analgesia in the PAG may be due to a disinhibitory action on an excitatory descending pain inhibitory pathway (Basbaum & Fields, Ann Rev Neurosci., 1984). The present findings delineate for the first time a functional role for the NMDA receptor in the control of pain in the mammalian central nervous system.

108.11

Analgesic Profile of the Sodium Salt of Pemedolac.
T.T. Chau and B.M. Weichman. Wyeth-Ayerst Research Princeton, NJ 08543-8000

Pemedolac sodium, cis-1-ethyl-1,3,4,9-tetrahydro-4-(phenyl methyl)-pyrano [3,4-b]-indole-1-acetic acid sodium salt, showed potent analgesic activity in different writhing models in mice. Similar analgesic potency was observed regardless of the routes of administration (po, im, iv), with ED_{50} values ranging from 0.3 mg/kg (vs acetylcholine) to 4.3 mg/kg (vs PBQ). In the rat Randall-Selitto assay, using Freund's complete adjuvant as the hyperalgesic stimulus, the median analgesic dose (derived from a flat dose-response curve) was 1 µg/kg for all 3 routes of administration. Thus, the compound appears to be well absorbed. It had a fast onset (15 min po, 5 min im, 2 min iv) and a long duration of action (7-9 hr po, 12-18 hr im, 5 hr iv). Intracerebroventricular (icv) injection of large doses of pemedolac Na produced a naloxone-insensitive antinociceptive effect in the mouse PBQ writhing test (ED_{50}= 43.5 µg/mouse). Pemedolac Na had a low iv/icv ratio of 2 compared to 20 for morphine, and further, was inactive in the hot plate and tail flick assays at 100 µg/mouse icv. These results suggest that pemedolac Na, a potent and long acting analgesic, exerts its effect via a peripheral, non-opiate mechanism.

108.12

CHANGES IN THE CHARACTERISTICS OF κ-OPIOID AND 5-HT_2 BINDING SITES IN U50,488H TOLERANT MICE. Begonia Y. Ho* and A.E. Takemori. Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

Tolerance to the analgesic effect of U50,488H (±trans-3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl]cyclohexyl)-benzeneacetamide), a selective κ-opioid agonist, was induced in mice using a slow release method. Tolerance appeared 30 hr after treatment and was maintained up to 60 hr. The ED_{50} of subcutaneous U50,488H increased from 6.7µmol/kg to 57µmol/kg. A smaller degree of cross tolerance to the analgesic effect of serotonin (5-HT) was developed also. The ED_{50} of intrathecal serotonin increased from 66.5nmol/mouse to 111nmol/mouse. Cortical, striatal and spinal tissues of control and tolerant mice were used for κ-opioid and 5-HT_2 receptor binding studies. In the cortex, striatum and spinal cord, the K_D's of [^{3}H]ethylketazocine (EK) were found to be 1.9nM, 1.2nM and 1.3nM, and those of [^{3}H]ketanserin were found to be 0.8nM, 2.0nM and 2.2nM, respectively. An approximately twofold increase in the KD's of both ligands was found in most of the regions. There was no change in the B_{max}'s of these two ligands. Competitive binding studies using U50,488H and nor-binaltorphimine (nor-BNI), a selective κ-opioid antagonist, showed a similar increase in the K_i's. These findings suggest that a slight decrease in the affinities of κ-opioid and 5-HT_2 receptors occurred with the development of tolerance to U50,488H. The densities of these receptors appear not to be altered. (Supported by USPH Service Grant DA00289.)

108.13

PHENYL-P-QUINONE WRITHING IN MICE: CAN IT PREDICT ANALGESIC EFFICACY? D. Luttinger*, D. Koonz*,and S. Chippari* (SPON: S.J. Ward). Dept. of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, NY 12144.

Phenyl-p-quinone (PPQ) has been used routinely as a writhing stimulus to assess antinociceptive effects of compounds. This is due in part to the fact that both opiate and NSAID analgesics inhibit PPQ-induced writhing. However, NSAIDs are generally believed to be less efficacious at alleviating pain than opiates. The present studies investigated whether the clinically-observed differences in efficacy between opiates and NSAIDs could be detected by varying the dose of PPQ. Mice were dosed orally with the antinociceptive compound twenty minutes before i.p. administration of PPQ (1 or 3 mg/kg), then observed for the presence of writhes between 5 and 20 minutes post-PPQ injection by an observer blinded to the treatments. The opiates, morphine, and codeine and the mixed agonist/antagonist, pentazocine, were effective and equipotent at inhibiting writhing induced by the two doses of PPQ. The NSAIDs, aspirin, indomethacin, ibuprofen and zomepirac effectively inhibited writhing induced by 1 mg/kg PPQ in a dose-related fashion. However, writhing induced by 3 mg/kg of PPQ was not significantly inhibited by NSAID at doses that were 3 to 30 fold higher than those necessary to inhibit PPQ (1 mg/kg)-induced writhing. Naproxen was unique among the NSAIDs in that its profile resembled that of the opiates. These results suggest that by varying the dose of PPQ, this model is better able to predict the potential antinociceptive efficacy of compounds that act by different mechanisms.

108.14

CHANGE IN MORPHINE/CLONIDINE INTERACTION INDUCED BY OPIOID BUT NOT NORADRENERGIC ANTAGONIST TREATMENT. S.C. Roerig, K.F. Kitto* and G.L. Wilcox, Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455

Interactions between opioid and noradrenergic agonists for production of antinociception were studied using morphine sulfate (MS) and clonidine (Cl) as inhibitors of Substance P (SP)-induced behavior in male, ICR mice. MS and/or Cl was coadministered intrathecally (i.t.) with SP and biting and scratching episodes were counted for 1 min. ED50 (95% CI) values were calculated and MS/Cl interactions were evaluated isobolographically. A multiplicative interaction between MS and Cl was found. Addition of i.t. naloxone (Nx) or idazoxan (Id) in the tests gave these ED50 values:

Drugs	MS (nmol)	Cl (nmol)	Interaction
Control			
Drugs alone	5.5 (4.8-6.5)	0.06 (0.05-0.07)	
MS+Cl (100:1)	0.5 (0.3-0.7)	0.007 (0.005-0.01)	Multiplicative
Nx (0.1 ug)			
Drugs alone	27.2 (23.4-32.2)	0.14 (0.12-0.16)	
MS+Cl (200:1)	11.3 (10.0-12.7)	0.08 (0.07-0.09)	Additive
Id (0.01 ug)			
Drugs alone	21.1 (17.4-26.2)	0.18 (0.15-0.23)	
MS+Cl (200:1)	1.1 (0.8-1.4)	0.008 (0.006-0.01)	Multiplicative

The opioid antagonist (Nx) changed the MS+Cl interaction from multiplicative to additive, while the α_2 antagonist (Id) did not, even though both antagonists had similar effects on MS or Cl given alone. Thus, the opiate/noradrenergic interaction is more susceptible to opioid than noradrenergic antagonism. (supported by DA01933 and DA04274).

108.15

Change in the Multiplicative Interaction of Antinociceptive Effects of Intrathecal (i.t.) Norepinephrine and Morphine in Morphine Tolerant Mice S. Lei*, S. C. Roerig and G. L. Wilcox, Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455

Morphine sulfate (MS) and norepinephrine (NE) given i.t. produce antinociception in the substance P (SP) biting and scratching behavior test. When MS and NE are given concurrently i.t., a multiplicative interaction for the antinociceptive response occurs (Lei and Wilcox, Neurosci. Abst., 1987). The present studies investigated the interaction between i.t. MS and NE in morphine pellet (MP) implanted mice which have been shown to show a decrease in the antinociceptive multiplicative interaction between intracerebroventricular (i.c.v.) and i.t. morphine. Male, ICR mice (25-30 g) were implanted subcutaneously with either placebo (PP) or MP (75 mg) for 72 hr. MS and/or NE were coadministered i.t. with SP and behaviors were scored for 1 min. ED50 values (95% CI) were as follows (ng):

	Separate Administration		Coadministration (1:10, NE:MS)	
	PP	MP	PP	MP
MS	183 (128-237)	1392 (975-2141)	17.2 (11.3 -24.3)	42.4 (30.2-60.4)
NE	9.4 (7.1-12.3)	5.4 (4.0-7.1)	1.9 (1.4-2.4)	4.2 (3.2-5.5)

Interactions between coadministered MS and NE were analyzed isobolographically and showed that the multiplicative interaction between MS and NE in PP mice changed to an additive interaction in MP animals. This changed interaction is similar to that found for i.c.v. plus i.t. morphine in the tail flick test and supports the role of a descending noradrenergic system in development of tolerance to morphine. (Supported by NIDA grants: DA01933 and DA04274).

108.16

CHARACTERIZATION OF SPINAL KAINIC ACID (KA) RECEPTORS SUPPORTS POSSIBLE INVOLVEMENT IN NOCICEPTIVE SYSTEMS. G.E. DeLander and J.J. Wahl, Oregon State University, College of Pharmacy, Corvallis, OR 97331.

Investigations in our laboratory and others have centered upon similarities among agonists selective for NMDA excitatory amino acid (EAA) receptors and other putative pain neurotransmitters, such as substance P (SP). In the present study, we examined the pharmacology of spinal KA EAA receptors. Mice exhibited a characteristic biting and scratching behavior after 60 pmol KA injected i.t. Behavior was similar to that seen after i.t. injections of SP or NMDA. KA-induced behavior was inhibited by 5 nmol kyurenate (i.t.), a non-selective EAA antagonist that also inhibits NMDA-induced behavior, but unaffected by 2-amino-7-phosphonoheptanoate, a selective NMDA receptor antagonist. As previously reported for NMDA and SP, KA-induced behavior could be inhibited by N-ethyl-carboxamido-adenosine (i.t.) or by pretreatment with 1 ug morphine i.c.v. PCP receptor selective agonists PCP and MK801 were partially effective as inhibitors of KA-induced behavior. KA-induced behavior was also inhibited by D-pro^2-D-tryp^{7,9}-substance P (i.t.), a substance P receptor antagonist. The results suggest interactions at KA receptors may be involved at spinal sites mediating nociception. It is not clear whether interactions at these receptors might directly mediate transmission of nociceptive stimuli or whether they serve to modulate the actions of other putative pain neurotransmitters.

108.17

MU-OPIOID COMPONENT OF THE ETHYLKETOCYCLAZOCINE (EKC) DISCRIMINATIVE CUE.
T. A. Wettlaufer*, K. W. Locke*, B. Gorney*, M. Cornfeldt*, and S. Fielding. Dept. Biological Research, Hoechst-Roussel Pharmaceuticals, Inc.,

The opioid receptor selectivity of the EKC discriminative stimulus was characterized in Fischer rats trained to discriminate 0.3 mg/kg sc of EKC from saline in a two-choice discrete-trial avoidance paradigm. The putative *kappa*-opioid receptor agonists EKC and U50,488H completely generalized with the EKC cue at doses of 0.3 and 10 mg/kg sc, respectively. In contrast to other reports (Shearman, G. T. and Herz, A., J. Pharmacol. Exp. Ther. 221:735, 1982), the putative *mu*-opioid receptor agonists morphine and fentanyl also dose-dependently generalized with the EKC stimulus. This generalization pattern may reflect a lack of opioid receptor selectivity of the EKC stimulus. However, distinct *mu*- and *kappa*- opioid components of the EKC cue could be identified using a relatively low dose of naloxone (0.1 mg/kg sc). The discriminative effects of morphine and fentanyl were blocked by this dose of naloxone, whereas the discriminative effects of EKC and U50,488H were not. These results suggest that EKC produces a complex discriminative stimulus with *mu*- and *kappa*- opioid components that can be separated using narcotic antagonists such as naloxone.

108.18

TAIL PINCH LATENCY TEST: CONDITIONED VOCALIZATION IMPROVES RELIABILITY WITHOUT PRODUCING OPIOID MEDIATED STRESS ANALGESIA
J.W. Kemp*, P.F. Osgood, A. Kazianis*, N.E. Atchison*, D.B. Carr*, & S.K. Szyfelbein*, Shriners Burns Institute, Harvard Medical School, Boston, MA 02114

Training (TRN) rats to squeak in response to a tail pinch improves reliability of the tail pinch latency (TPL) test of analgesia, but may be stressful and could thus produce a stress induced analgesia. Since the endogenous opioid beta-endorphin (B-EP) may be sensitive to stress analgesia, we examined the effect of TRN on baseline TPL and B-EP in order to determine whether TRN improves reliability of the tail pinch test without producing opioid mediated stress analgesia or confounding the study of B-EP. Ten male S.D. rats were implanted with i.v. cannulae on day one, sampled for B-EP before and after TRN on day two, and sampled for B-EP before and after determination of baseline tail pinch latency on day three. An additional group of 10 rats was sampled in a parallel fashion but did not receive TRN. Termination of a tail pinch served as negative reinforcement and was used to condition the squeak response. TRN did not significantly effect B-EP within or between groups at any point ($F_w = 0.9$ & $F_b = 1.0$, $p > 0.05$, 2-way ANOVA). However, TRN did significantly effect baseline tail pinch latency ($t = 2.6$, $p < 0.05$), reducing the coefficient of variation ($SD/\bar{X}$) by 67%. These findings suggest that TRN rats improves the reliability of baseline TPL without producing stress analgesia or confounding the study of endogenous pain inhibitory systems that may be sensitive to stress. (Supported by SBI grant)

CLINICAL PHARMACOLOGY

109.1

THE PHARMACOKINETICS AND PHARMACODYNAMICS OF SQ 29,852, A NEW METABOLICALLY-STABLE PHOSPHORUS-CONTAINING ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITOR.
J.Foley*, R. Morrison*, S. Singhvi, J. Manning*, and D. Willard*, Squibb Institute for Medical Res. and the Princeton Medical Center, Princeton, NJ

SQ 29,852-^{14}C [SQ; (S)-1-[6-amino-2[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-L-proline] was given to eight healthy male subjects as single 10-mg i.v. and oral doses 1 week apart in random order using a balanced crossover design. SQ was analyzed by thin-layer radiochromatography in plasma and urine up to 96 hours after dosing. Serum ACE activity was measured up to 24 hours after each dose. The mean (±SEM) absolute bioavailability of oral SQ was 67 ± 3%; no metabolites were detected in plasma or urine. Tmax and mean absorption time averaged 3.9 ± 0.1 and 5.6 ± 0.5 hours, respectively. Elimination half-life and mean residence time were 14.8 ± 0.9 and 6.2 ± 0.3 hours, respectively, for the i.v. dose; the steady-state volume of distribution averaged 16.5 ± 0.8 L/kg. Total clearance (57 ± 3 mL/min) was essentially renal, with 1% of the i.v. dose of radioactivity recovered in the 0 to 96-hour feces. The extent of ACE inhibition was correlated with plasma levels of SQ ($r = 0.89$, $p < 0.001$) and was 94% 1 hour after the i.v. dose, decreasing to 44% at 24 hours. For the oral dose, maximal inhibition (92%) occurred at 2 to 4 hours, declining to 56% at 24 hours.

109.2

PHARMACOKINETICS OF AZIDOTHYMIDINE IN PATIENTS WITH AIDS RELATED KAPOSI'S SARCOMA.
R.P. Agarwal, D. Kolonias*, A. Krishan*, R. Uttamchandani*, and M.A. Fischl*. Univ. of Miami Sch. of Med., Depts. of Oncology and Medicine, Miami, FL 33101.

3'-Azido-3'-Deoxythymidine (AZT) is a thymidine analog that inhibits the infectivity and the cytopathic effects of human immunodeficiency virus (HIV) both in vitro and in vivo. Pharmacokinetics of AZT and its 5'-glucuronide (GAZT) were studied in 6 male patients with AIDS-associated Kaposi's sarcoma who were also treated with 18 million units of α-interferon (INF; 1 q d x 8). One hundred mg AZT was given orally q 4 hrs and blood samples were collected at 0, 5, 15, 25, 35, and 45 min and 1, 1.25, 1.5, 1.75, 2, 2.5, 3.0, 3.5, and 4 hr after the ingestion of an AZT dose. Following solid phase extraction of AZT and GAZT from serum, the drug and the metabolite were quantitated by high performance liquid chromatography. The peak AZT and GAZT conc. 2.02 ± 1.5 μM and 4.7 ± 1.9 μM were achieved at 69 ± 29 min and 80.6 ± 23.4 min, respectively, following ingestion of the drug. The AUC's from 0-4 hr for AZT and GAZT were 2.5 ± 0.9 and 6.0 ± 1.9 μM x hr, respectively. Cumulative half-lives of serum AZT and GAZT were estimated to be about 80 and 52 min, respectively. These parameters were consistent with those obtained without INF (Laskin et al, Clin. Res., 36, 366A, 1988) except that the ratio of GAZT/AZT in the present study was much lower (2.3) than the ratio of 3.6 observed in the absence of INF. To fully assess the toxicity of AZT chemotherapy in combination with INF, the knowledge of intracellular metabolism of AZT is necessary. (Supported by NIH-N01-A1-62536).

109.3

SIMULTANEOUS DETERMINATION OF SIX CAROTENOIDS IN HUMAN SERUM.
Louis R. Cantilena* and David W. Nierenberg. Dartmouth Medical School, Hanover, NH 03756.

A method for the simultaneous determination of serum concentrations of six carotenoids (lutein, zeaxanthin, cryptoxanthin, lycopene, alpha-carotene, and beta-carotene) was developed utilizing reverse-phase high-pressure liquid chromatography, ultraviolet detection, and an isocratic mobile phase (acetonitrile: tetrahydrofuran: methanol: 1% ammonium acetate, 65:25:6:4). Triple extraction of ethanol-denatured serum with hexane was followed by evaporation of the hexane layer to dryness under nitrogen at 40°C and resuspension of the residue in 50 ul ethanol (with 0.1% ascorbic acid as preservative), with further dilution into 150 ul mobile phase prior to injection. Quantitation of serum carotenoids was accomplished with a standard curve prepared from a solution of pure carotenoid compounds. The precision of the analysis of carotenoids in control human serum (within-day coefficients of variation, N=5) was determined for lutein/zeaxanthin (3.6%); cryptoxanthin (11.2%); lycopene (3.7%); alpha-carotene (5.9%); and beta-carotene (4.0 %). Sensitivity of the assay was 10-30 ng/ml for the group of carotenoids. Specificity of the assay was confirmed by measuring the ratio of absorbance at 450 nm to 436 nm of pure carotenoid solutions and patient specimens. Accuracy was determined for beta-carotene by demonstrating close agreement between determinations of beta-carotene using this method and an established method known to be in agreement with National Bureau of Standards reference values.

109.4

RAPID ANALYSIS OF IFOSFAMIDE (IFO) IN HUMAN PLASMA BY THERMOSPRAY-LC/MS/MS.
Robert A. Newman and Daniel A. Gartiez*. Dept. of Medical Oncology Univ. Texas M.D. Anderson Cancer Center, Houston, TX 77030 and TEXms Inc., Houston, TX 77030

IFO is a widely used anticancer drug. There are, at present, only two methods to quantitate IFO in plasma. A GC procedure (Pantarotto et al, J. Pharm. Sci. 63, 1554, 1974) and an HPLC method (Margison et al, Biomed. Chrom., 1, 101, 1986). The GC procedure involves extensive sample preparation and a derivatization step. During the assay, however, decomposition is likely to occur. The HPLC procedure reduces the sample preparation time, but lacks the use of an internal standard. When cyclophosphamide (CYC) is used as the internal standard, its retention time is ca 16 minutes. The lengthy run time limits the usefulness of the analysis for large numbers of samples. We now report a rapid thermospray LC/MS/MS analysis method for IFO, using CYC as the internal standard. The MS/MS spectrum of IFO displays a daughter at m/z 92 which contains the chloroethyl substituent on the ring nitrogen. This daughter is not present in the spectrum of CYC which displays a daughter at m/z 140 arising from the dichloroethyl substituents on the phosphoramido nitrogen. These daughters were used to quantitate IFO in human plasma. Because complete separation was not necessary, it was possible to reduce analysis time to less than 3 minutes. Results will be presented and discussed.

109.5

A METHOD FOR SAMPLING HUMAN BLOOD FOR MEASUREMENT OF PLASMA ADENOSINE. J. Shryock*, M.T. Boykin*, L. Belardinelli. Univ. of Florida College of Med., Gainesville, FL 32610

The half-life of adenosine in human blood is estimated to be 1-2 sec at 37°C. In order to measure plasma adenosine concentration, it may be necessary to inhibit the metabolism of adenosine during transit of blood through the sampling catheter. We have tested a double-lumen catheter for this purpose. "Stopping solution" (dipyridamole, 200μM; AOPCP, 79μM; EHNA, 5 μM; EDTA, 4.2mM; heparin, 25U/ml; pH 4.5) and 14C-adenosine were delivered via an inner lumen, mixed with blood at the catheter tip, and withdrawn to a collection syringe in an outer lumen (transit time 7 sec). Recovery of 14C-adenosine was 84±7% (mean±S.D.,n=8) at 1μM [Ado], and was a linear function of the content of label in the sample over the range of adenosine concentrations 0.3-10μM. In contrast, when "stopping solution" was mixed with blood in the collecting syringe rather than at the catheter tip (single lumen catheter model), recovery of 14C-Ado was 8±2% (n=8) at 1μM [Ado]. The importance of inhibition of pathways of uptake/degradation of adenosine and formation of adenosine during transit through the catheter was further demonstrated. The results suggest that accurate estimation of human plasma adenosine concentration necessitates the inhibition of adenosine metabolism during blood withdrawal. A double-lumen catheter is suitable for this purpose.

Supported by a grant from NIH (HL35272)

109.6

USE OF A NEW OPTICAL ASSESSMENT DEVICE TO MEASURE OPIOID-INDUCED CHANGES IN PUPILS. P. Welch,* A. Radzius,* J. Henningfield* and E.J. Cone. NIDA Addiction Research Center, Baltimore, MD 21224

Acute administration of opioid agonists to man reliably constricts pupils. Methods for pupil measures have changed little since the early 1960's when pupil diameter was measured photographically. We compared a new computer-controlled pupilometry system (Pupilscan™) with photographic measures. Two doses of each of 6 opioids were tested under placebo-controlled double-blind conditions. Pupil measures were made prior to and periodically after administration. Measures of absolute pupil diameter and change from baseline were similar by both techniques and were significantly correlated. The Pupilscan system also measured rate of constriction in response to light stimuli. At the time of maximum opioid effect, the rate of constriction and re-dilation was decreased in comparison to placebo. It is likely that the drug-induced constriction in baseline diameter prior to light stimulus challenge accounted for the effect. Overall, the Pupilscan optical unit appears to provide an accurate means of measuring drug-induced pupillary effects in man.

109.7

DEVICE WITH IMMOBILIZED CHELATOR FOR EXTRACORPOREAL REMOVAL OF EXCESS cis-DIAMMINEDICHLOROPLATINUM (CDDP) FROM BLOOD. CM Ambrus, K Karakousis,* S Anthone,* C Horvath,* K Kalghatgi,* F Rieders,* Children's Hospital, SUNY Buffalo, NY 14222, Roswell Park Mem. Inst. Buffalo NY 14263, Yale Univ. New Haven, CT, 06520, National Med. Services, Willow Grove, PA 19090.

The dose of CDDP applied to patients with malignancies is limited by toxicity of platinum. CDDP canbe delivered directly to the tumor when the organ or extremity with the tumor has a single well defined artery. The bulk of CDDP leaves through the venous drainage into the systemic circulation. A hollow fiber device was developed with immobilized chelator (Amberlite-IRC-718 (AMB)) for the extracorporeal removal of CDDP from blood. As blood flows through the fibers the chelator behind the fiber membrane retains platinum that crosses the pores (cut-off value 10K dalton). Thus, CDDP removal can be accomplished without the chelator entering the blood. Blood samples of 150 mL, collected from melanoma patients during a 6 hrs iv infusion of 40 mg/m^2 CDDP were recirculated through AMB devices for 60 min, at 100 mL/min speed, at 37°C. Within 15 min, 40-60% of blood CDDP was removed. AMB devices applied to dogs for 2 hrs in an extracorporeal circuit did not produce physiological side effects, nor biochemical or hematologic changes. Clinical trials are planned for regional CDDP therapy with AMB devices applied to the venous drainage of the tumor. The goal is to chelate CDDP when most of it is still in the ultrafiltrable form, to reduce systemic toxicity of CDDP.

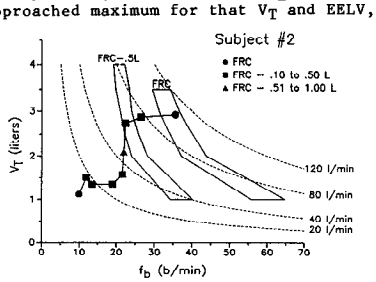
EXERCISE II

110.1

MAXIMAL VENTILATION, BREATHING PATTERN, AND EELV. T. Babb*, J. Rodarte, and J. Young*. Mayo Clinic & Mayo Foundation, Rochester, MN 55905.

Given the maximum flow-volume (FV) envelope, maximal ventilation (V_E max) during exercise is determined by tidal volume (V_T), breathing frequency (f_b), duty cycle (Ti/T_{tot}), and end-expiratory lung volume (EELV). Using individual FV curves, V_E max was computed for various V_Ts and EELVs, assuming Ti/T_{tot} of .42-.50. On plots of V_T vs f_b these curves are steeper than constant ventilation isopleths. Two fit subjects with borderline and mild airflow limitation underwent progressive cycle ergometry. At low V_E EELV decreased. As V_E approached maximum for that V_T and EELV, they increased EELV rather than increasing V_T or decreasing Ti/T_{tot} (figure). This EELV pattern differs from that observed in normals.

(Supported by grants HL 21584 and 07222).



110.2

CARDIAC VAGAL TONE DURING EXERCISE IN HUMANS Jean-Pierre L. Dujardin, Dorothy A. Jackson* and George E. Billman. The Ohio State University, Columbus, Ohio 43210.

Although it is well known that cardiac vagal tone is gradually withdrawn with increasing levels of dynamic exercise, until recently no method was available to study this process in detail. In this study respiratory sinus arrhythmia (RSA), a well-accepted index of cardiac vagal tone was determined continuously using a signal processing technique developed by Porges (1986). In this technique spectral analysis is applied on the fluctuating heart period function after removal of non-periodic trends using a moving cubic polynomial filter. RSA is determined as the spectral density in the respiratory frequency band. In 14 college age students of both sexes, ECG, respiratory rate and VO_2 were determined at rest and during a maximal exercise stress test on a bicycle ergometer. The workload was increased in 150 kpm/min. steps. HR and cardiac vagal tone were plotted as a function of VO_2 and $\%VO_2MAX$. It was concluded that this technique is well suited to study dynamic changes in cardiac vagal tone during exercise.

(Supported by NIH grant HL36336)

Porges, S.W. (1986). Respiratory sinus arrhythmia: Physiological basis, quantitative methods and clinical implications. In P. Grossman, K. Janssen and D. Vaitl (eds.) Cardiac Respiratory and Somatic Psychophysiology (101-115) New York: Plenum Press.

110.3

SKIN VASCULAR RESISTANCE IN THE FOREARM AND CALF DURING ONE-LEG CYCLING. J.A. Taylor*, M.J. Joyner, P.B. Chase* and D.R. Seals. Depts. of Exercise & Sport Sciences and Physiology, Univ. of Arizona, Tucson, AZ 85721.

We have recently reported (**FASEB J** 2:A520, 1988) that during brief (3 min ea), graded (40, 55, and 70% peak O_2 uptake) one-leg cycling exercise (EX): 1) forearm vascular resistance (FVR) decreased during the initial 60-90 s and then increased progressively to or above baseline levels, and 2) contralateral calf vascular resistance (CVR) was unchanged initially and then increased progressively, exceeding baseline levels. The purpose of the present study was to determine the contribution of changes in skin vascular resistance in the forearm (FSVR) and contralateral calf (CSVR) to the respective whole limb responses during EX. In 5 subjects, arterial blood pressure (AP) was recorded and skin blood flow was measured in the forearm and contralateral calf using laser-Doppler velocimetry before (control) and during EX. FSVR and CSVR were calculated from AP and skin flows. FSVR was unchanged from control during the initial 30-60 s of EX and then increased progressively to end EX (115 ± 5, 126 ± 13, and 143 ± 20% of control at 40, 55, and 70% peak O_2 uptake). CSVR was also unchanged from control initially but decreased thereafter (64-83% of control at end EX). Thus, after the initial min of EX, forearm skin vasoconstricts whereas calf skin vasodilates. We conclude: 1) the decrease in FVR at the onset of EX is mediated solely by skeletal muscle vasodilation, 2) skin vasoconstriction contributes to the time-dependent increase in FVR during EX, and 3) the increase in CVR during EX is mediated solely by skeletal muscle vasoconstriction.

110.4

EFFECTS OF LOW-LEVEL EXERCISE TRAINING (ET) ON RESTING AND AMBULATORY BLOOD PRESSURE (BP) IN OLDER PERSONS WITH HYPERTENSION. M.J. Reiling*, L.A. Clayton-Bare*, P.B. Chase* and D.R. Seals. Depts. of Exercise & Sport Sciences and Physiology, Univ. of Arizona, Tucson, AZ 85721.

To determine the influence of ET on BP in older men and women with mild essential hypertension, we measured systemic hemodynamics at rest in the laboratory (L) and 24-hr ambulatory (A) BP in 8 subjects aged 62.1 ± 2.6 yr ($\bar{X}$ ± SE; range 52-70) before and after 6 mo of ET (walking 3-4 d/wk for 30-40 min/d at 40-50% max O_2 uptake). ET increased max O_2 uptake slightly (6-7%) but significantly (26.7 ± 2.0 vs 28.6 ± 2.3 ml/kg/min; 2.10 ± 0.15 vs 2.23 ± 0.16 ℓ/min; both $p < 0.05$). Systolic (146 ± 4 vs 138 ± 5 mmHg, ns), diastolic (94 ± 1 vs 88 ± 2 mmHg, $p < 0.05$), and mean (111 ± 2 vs 104 ± 3 mmHg, $p < 0.05$) L-BP were lower after ET. This lower L-BP after ET was associated with decreases in cardiac output and stroke volume; heart rate and vascular resistance were unchanged. In contrast to the training-induced reduction in L-BP observed, systolic (138 ± 5 vs 138 ± 5 mmHg), diastolic (88 ± 2 vs 92 ± 3 mmHg), and mean (104 ± 2 vs 104 ± 3 mmHg) A-BP were unchanged ($p > 0.05$) after ET. Neither body weight (79.6 ± 3.0 vs 78.8 ± 3.0 kg) nor % body fat (25.9 ± 2.6 vs 24.8 ± 2.4) were changed after ET (both $p > 0.05$). These preliminary findings suggest that prolonged, low-intensity, aerobic exercise training lowers casually-determined BP at rest but not 24-hr ambulatory BP in older men and women with mild hypertension. (Supported by NIA AG06537).

110.5

HYDRAULIC RESISTANCE EXERCISE INCREASES FAT-FREE MASS OF OLDER MALES M.D. Becque* and V.L. Katch* (Spon: T.P. White). The University of Michigan, Ann Arbor, MI, 48109-2214.

Previous data are equivocal whether muscular hypertrophy and growth of fat-free mass occur in response to conventional resistance weight training in older males. The purpose of this experiment was to determine the effects of hydraulic resistance weight training on fat-free mass (FFM) and muscular strength of older males. 30 males between 60 and 78 yrs (X = 65 yrs) participated in an 18 wk control/exercise program. Subjects were tested before (T_1) and after (T_2) a 6 wk control period, and after a 12 wk exercise program (T_3). Subjects exercised for 42 min, 3 times per wk for the first 6 wks and 4 times per wk for the last 6 wks (42 sessions). Hydraulic resistance exercise equipment designed to exercise the major muscle groups of the body with only shortening contractions was used. Body composition was determined by underwater weighing at residual lung volume. Peak torque, total power, and total work were assessed during 6 standardized work tests. Data analysis included repeated measures ANOVA with multiple post hoc analyses.

	T_1	T_2	T_3
Body Mass	75.4 ± 1.8	76.0 ± 1.9	75.0 ± 1.8
FFM	53.6 ± 1.3	53.5 ± 1.2	54.9 ± 1.3*
Fat Mass	21.8 ± 0.9	22.5 ± 1.0	20.1 ± 0.9*

No change ($p > .01$) was noted for body mass, while a significant ($p < .01$) 2.6% increase in FFM was found along with a significant ($p < .01$) 9.5% decrease in fat mass. Increases in peak torque ranged from 0 to 6%; total power and total work increased between 0 to 14%. These data demonstrate that older males increase both FFM and strength as a result of whole body hydraulic resistance training.

Supported by Hydra Fitness Industries.

110.6

CENTRAL VERSUS PERIPHERAL ADAPTATIONS IN HIGHLY TRAINED SENIORS. J. L. Fleg*, S. Schulman*, G. Gerstenblith*, A. Goldberg*, C. Tankersley*, L. Becker*, J. Clulow*, D. Drinkwater*, L. Lakatta* and E. G. Lakatta. Gerontology Research Center, NIA, NIH, and Johns Hopkins Medical Institutions, Baltimore, MD 21224

Whether the well documented marked increase in maximal oxygen consumption (VO_2max) of older endurance trained men relative to that of age-matched peers is mediated primarily by central versus peripheral adaptations is unclear. We performed radionuclide ventriculography and respiratory gas exchange measurements during maximal upright bicycle ergometry in 8 endurance trained (mean age = 65 ± 5 yr, treadmill VO_2max = 51 ± 4 ml/kg/min) and 6 age-matched sedentary (treadmill VO_2max = 31 ± 4 ml/kg/min) men. The maximum workload (MWL), the measured VO_2 at MWL (peak VO_2) and cardiac output (CO) at MWL and derived average arteriovenous oxygen difference $(A\text{-}V)O_2$ at MWL are shown below (mean ± S.D.).

	Endurance Trained	Sedentary	p
MWL (watts)	172 ± 31	129 ± 19	.02
Peak VO_2 (L/min)	2.27 ± 0.33	1.52 ± 0.30	.003
CO (L/min)	17.6 ± 4.2	15.9 ± 3.4	NS
$(A\text{-}V)O_2$ (vol. %)	13.4 ± 2.9	9.9 ± 2.7	.05

Thus, the augmented aerobic capacity of endurance trained seniors at exhaustion during upright bicycle exercise is due primarily to peripheral rather than central adaptations.

110.7

VENTILATORY RESPONSES TO PHYSICAL CONDITIONING DURING PREGNANCY. P.J. Ohtake*, L.A. Wolfe*, P. Hall* and M.McGrath* (SPON: C.E. King). Queen's University, Kingston, Ontario, Canada K7L 3N6.

Pulmonary responses to aerobic conditioning during pregnancy were studied in 27 healthy pregnant women (EG) who underwent cycle ergometer conditioning during the 2nd and 3rd trimesters (Tm's). Results were compared with a sedentary pregnant control group (CG, n=20). Data were obtained at 3 levels of steady state, submaximal cycle ergometer exercise at the end of the 1st, 2nd and 3rd Tm's and 3 months postpartum (PP). In both groups, minute ventilation (V_E), alveolar ventilation and the ventilatory equivalent for oxygen were greater throughout pregnancy at all work rates relative to PP. Significantly greater tidal volumes (V_T) contributed to the increase in V_E. V_D/V_T decreased with increasing exercise intensity and was unaltered by pregnancy or conditioning. $P_{ET}CO_2$ remained constant during exercise and was lower during pregnancy than PP. The magnitude and time course of pulmonary adaptations to submaximal exercise in pregnancy were not altered significantly by physical conditioning. Funded by ARC (Queen's Univ.), Ont. Min. of Health, Can. Fit. Lifestyle Res. Inst., Can. Heart Foundation.

110.8

HORMONAL CHANGES AND EXERCISE-INDUCED AMENORRHEA IN HIGHLY-TRAINED FEMALE ATHLETES

M. Regimbal*, G.A. Kinson and M. Jette*. Univ. of Ottawa Health Sciences, Ottawa, Ontario, Canada K1H 8M5

The study was carried out to evaluate levels of sex steroids in amenorrheic body-builders. Androstenedione, testosterone (free), progesterone and estradiol-17B were measured by RIA in serum samples collected every 7th day through two monthly cycles. Amenorrhea developed during intensive weight-training in all 7 body-builders. Mean androstenedione levels were 17.6% higher in athletes and free testosterene was elevated by 27.25%. Estradiol levels were unchanged in comparison to control values. This was surprizing since all subjects were amenorrheric and the literature indicates marked decreases in estrogens under comparable conditions. Most dramatic changes occurred in serum progesterone with a 93% reduction in the athletic subjects. Coupled with changes in basal body temperature, the low progesterone is strongly indicative of anovulation. Current clinical concerns over endometrial hyperplasia and the suggested use of progesterone supplementation are likely associated with lack of progesterone and unopposed estrogen action, as substantiated in the present study. Elevations in androgen titer in response to severe exercise may reflect some influence on aromatase systems and peripheral steroid metabolism with changes in female fat: lean body distribution.

110.9

O2 DELIVERY AND CARDIAC OUTPUT DURING EXERCISE FOLLOWING ORAL PHOSPHATE-GLUCOSE. ET Mannix†, JM Stager, MO Farber†, and A Harris* Human Perf Lab & Sch of Med (VAMC), Indiana Univ, Bltgn & Indpls IN 47401

Phosphate has been proposed as an ergogenic aid since it may enhance O2 delivery and cardiac work efficiency by increasing erythrocyte(RBC) phosphate(Pi), 2,3-diphosphoglycerate(DPG), RBC adenosine triphosphate (ATP), and P50. In 10 normal males 12hrs post-prandial we measured cardiac output(Q) by CO2 rebreathing, stroke volume(SV), heart rate(HR), O2 deficit(O2Def), aerobic metabolism(VO2), and systemic arteriovenous O2 difference(A-VO2) during constant load cycle ergometer exercise(60% of peak VO2). After a basal venous blood sample for RBC Pi, DPG, RBC ATP, and P50 (3hrs before exercise) a single dose of dicalcium phosphate(129mmol) and glucose(500ml/10%sol)(PHOS), or inert placebo, was administered in a random, crossover, double blind fashion. Blood sampling was repeated immediately before and after the 2hr exercise period to monitor the hematological variables. With PHOS there were increases($p<.05$) in RBC Pi(3.9 to 5.2mg/dl); DPG(11.8 to 13.1umol/gHb); RBC ATP(4.2 to 4.4umol/gHb); and P50(26.8 to 28.0mmHg), but P50 was not different across conditions at exercise onset (PHOS P50=28.0, Placebo P50=28.3mmHg). No differences in Q, SV, HR, O2DEF, VO2, or A-VO2 were noted across treatments. Thus, the acute increases in RBC high energy phosphates resulting from PHOS do not affect O2 delivery or cardiac output during exercise at this intensity. Supported in part by a Doctoral Grant in Aid. Indiana University.

110.10

STUDY OF THE ENERGY BALANCE IN ELITE FEMALE SWIMMERS. F. Vallières*, A. Tremblay*, and L. St-Jean* (Spon: D. Richard). Physical Activity Sciences Laboratory, Laval University, Quebec, Canada, G1K 7P4.

Despite the high energy cost of their exercise-training, female endurance athletes frequently report low to moderate energy intake, suggesting that their intake could be inadequate to equilibrate their daily energy expenditure. In the present study, we reevaluated this concept in six elite female swimmers performing about 15 hrs of training/week. Their daily energy needs were assessed during a 30 day cycle of training being representative of their annual training schedule. Three-day dietary and activity records as well as body composition measurements were performed at the beginning and at the end of the study. A mean daily energy intake of 2472±120 kcal was observed during the testing period. This intake corresponded to 108% of the recommended dietary allowance (RDA) for sedentary female subjects of the same body weight. The mean energy expenditure (2676±87 kcal/day) did not differ significantly from the mean energy intake ($p>0.05$). The body composition measurements showed a slight increase in fat-free mass during the study (Δ=1.0±0.4 kg, $p<0.05$), while body weight and fat mass remained unchanged. The comparison of the blood profile with the composition of the diet suggested that some subjects were at risk to develop iron deficiency. Results of this study show that energy intake was adequate to satisfy the increase in energy expenditure associated with training, suggesting that their energy needs were not as high as they would have been predicted on the basis of the extra caloric cost of exercise.

110.11

COMPONENTS OF ENERGY EXPENDITURE AND BODY COMPOSITION OF MALES CONSIDERED TO BE SMALL EATERS OR LARGE EATERS. V.A. George*, A. Tremblay*, G. Fournier*, J.P. Després*, C. Leblanc*, and C. Bouchard*, (SPON: J. Leblanc). Physical Activity Sci. Lab., Laval University, Ste-Foy, Québec G1K 7P4.

Young males (N= 30) were categorized as small eaters (SE) or large eaters (LE) according to their energy intake (EI), following 14 days in a controlled environment. SE were individuals with an EI of ≤ 170 kJ/kg body weight (N = 8) and LE those with an EI ≥ 230 kJ (N = 7). SE had an average EI 65% of that of LE. Yet, SE were significantly ($p < 0.01$) heavier, fatter and had a greater fat-free mass. Resting metabolic rate and the thermic response to a 4.18 MJ meal were greater for LE than for SE, but not significantly so. There was no difference between the groups in thermic response to submaximal exercise or VO_2 max. When the daily estimates for energy expenditure (EE) were considered, LE expended 15% more energy for activity. Thus, for the sedentary LE who were in energy balance, there may have been additional EE during regular daily tasks, spontaneous bodily movement or fidgeting. This type of EE may be important in explaining EI variations among individuals. Supported by Health and Welfare Canada and FCAR, Quebec.

116.1

POSTURAL EFFECTS IN THE BABOON ON THE WINDKESSEL MODEL OF CIRCULATORY DYNAMICS. Ricky D. Latham,* Bernard J. Rubal, Robert S. Schwartz,* Cardiology Service, Brooke Army Medical Center, Fort Sam Houston, Tx. 78234

We evaluated the hemodynamic response to passive upright 70° tilt in 6 baboons to assess the effects of gravity on systemic compliance (C), characteristic aortic input impedance (Zc) and peripheral resistance (R). High-fidelity catheters were used to record aortic root pressure and flow velocity which were digitized at 200 Hz. Thermodilution cardiac outputs were obtained. Pressure and flow data were fitted to a computer model (CM) of a 3-element Windkessel to estimate Zc, C, R. These were compared to conventional calculations (CC) of SVR, Fourier analysis for Zc, and the time constant of pressure decay for C.

	Zc ($d \cdot s \cdot cm^{-5}$)	R ($d \cdot s \cdot cm^{-5}$)	C (cc/mmHg)
CM (supine)	140±16	3678±296	.37±.07
CC (supine)	151±38	3648±241	.36±.02
CM (tilt)	88±18	3931±396	.57±.10
CC (tilt)	113±21	3567±450	.42±.03

†SE *$p < .05$

These data show that the CM fit of pressure and flow to determine Zc, C, and R produces similar results to independent calculations of these parameters. Finally, gravitational stress to passive upright tilt has its most prominent effect on C and little effect on Zc and R.

116.2

REGIONAL AORTIC PRESSURE APPARENT PHASE VELOCITIES IN THE BABOON DURING PASSIVE 70 DEGREE TILT. Barclay Butler*, Bernard J. Rubal, Ricky D. Latham* and Robert S. Schwartz*. Brooke Army Medical Center, Fort Sam Houston, Tx 78234

This study examines the effect of passive tilt on mean apparent phase velocities (C_{app}) at seven contiguous segments from the ascending aorta (Asc-Ao) to the iliac bifurcation (IB) in Papio anubis (n=4). High-fidelity pressures were simultaneously recorded at 5 cm intervals using a multisensor micromanometric catheter. After pressure waveforms were computer digitized (200Hz), a discrete Fourier analysis was performed. Differences in phase angle between pressure waveforms at adjacent sites were used to calculate regional C_{app}. C_{app} (± SD) (cm/sec) derived from averaging harmonics > 3Hz were:

	C_{app} (cm/sec)					
	Asc-Ao	Descending Aorta				IB
Supine:	568	706	615	540	761	1435
(±SD)	±123	±202	±158	±103	±258	±1375
Upright:	478	659	588	654	754	1665
(±SD)	±125	±146	±142	±151	±186	±1407

C_{app} were similar supine and upright. This suggest that vascular characteristic relating to pulse transmission are similar supine and upright in the sedated baboon model.

116.3

PERFORMANCE RECOVERY FOLLOWING $+G_z$-INDUCED LOSS OF CONSCIOUSNESS (GLOC). John W. Burns and Paul M. Werchan.* USAF Sch. of Aerospace Med., Brooks AFB, TX 78235

Tactical maneuvering of high performance aircraft can impose severe head-to-foot inertial stress ($+G_z$) on pilots, with the potential for GLOC and possibly loss of aircraft and life. Efforts are underway to both protect the pilot against GLOC, as well as shorten the recovery period following GLOC. The unanesthetized baboon has been developed as an animal model for GLOC research. Seven male baboons (avg wt=20.6 kg) have been successfully trained with a simple shock-avoidance performance task on the USAFSAM human/animal centrifuge. A red light is presented to the animal every two seconds. One second is allowed for a trigger pull response to turn off the light. If no response, a one second shock is presented to the lower legs. The shock can be abbreviated (escape) by a late trigger pull. Thus, the animal can avoid, escape, or accept the full shock. Transcranial EEG is obtained on all animals for correlation with GLOC. LOC is induced by exposure of the unprotected (no G-suit) animal to 8 $+G_z$ at 6 G/s, sustained until LOC. LOC is determined to occur with the appearance of an isoelectric EEG, which is observed at 8-10 s after onset of $+G_z$. Performance recovery is measured from the time of return to 1 $+G_z$ to the restoration of task performance. This technique is excellent for the investigation of methods to shorten performance recovery time after GLOC, and/or to extend the latency period before LOC occurs during $+G_z$.

116.4

HEMODYNAMICS OF LEG VEINS DURING A 30 DAYS BED REST - EFFECT OF LBNP. F.Louisy- and C.Y. Guezennec- (SPON : A. GUELL) - CERMA - LCBA 5 bis avenue de la porte de Sèvres 75731 PARIS CEDEX 15 - FRANCE -

Six healthy young subjects were exposed to one month of bed-rest (-6°) (CNES Cooperation) in order to study the behavior of capacitating vessels of the lowers limbs and the effect of LBNP countermeasure during simulated microgravity. The leg vein distensibility was measured by means of mercury strain gauge plethysmography with veinous occlusion prior to the decubitus period (Dc) on D6 and D20 of decubitus (D6, D20) and on the fifth day of recovery (D+5). Three subjects were exposed to regular LBNP sessions (LBNP group), the other three (no LBNP) were used as control group.

In the control group, veinous compliance significantly increased during the bed rest exposure, while in the LBNP group ,there was no significant difference in the veinous compliance values Dc,D6, D20 and D+5. These results demonstrate a progressive increase in the distensibility of the veins of the lower limbs during an experiment of microgravity simulated means of bedrest with a LBNP "protecting effect". They arise the question of mecanisms involved in veinous dilatation during exposure to microgravity.

116.5

TEN WEEK AEROBIC TRAINING DOES NOT ALTER LOWER BODY NEGATIVE PRESSURE RESPONSES. JT Lightfoot, RP Claytor, DJ Torok, TW Journell, SM Fortney* Johns Hopkins University, Baltimore, MD 21205 and Miami University, Oxford, OH 45056.

It has been speculated that aerobic training may alter the gain of both low-pressure and high-pressure mechanoreceptors which would then change LBNP tolerance. An experimental group (EXP) of 8 male subjects (22.1±1.4 yrs; mean±SD) underwent a 10 week treadmill and cycle training program which resulted in an average 20% increase in $\dot{V}O_2$max (3.46±.09 l/min vs. 4.16±.13 l/min; means±SE, $p<0.05$). A control group, (CON; n=3; 26.0±5.2 yrs), which did not undergo training had no significant changes in $\dot{V}O_2$max (3.94±.30 l/min vs. 4.09±.22 l/min). PRE- and POST-training, the EXP and CON groups, participated in LBNP tolerance tests (terminated at presyncope) and neck pressure-suction testing (to describe the carotid sinus-heart rate baroreflex; BR). LBNP tolerance, as defined by three different indices, and carotid sinus-heart rate gain (GAIN) were not altered in either group (see table). Furthermore, there were no changes in LBNP heart rate, blood pressure, leg circumference, and forearm blood flow (FBF) responses at any level of LBNP challenge.

Measure	EXP-PRE	EXP-POST	CON-PRE	CON-POST
Stress Index (min*mm Hg)	94±67	738±98	1038±146	903±182
Duration of exposure (min)	20.57±1.14	19.2±1.4	23.29±1.72	21.54±2.4
Pressure tolerated (-mm Hg)	-73±4	-69±5	-83±8	77±9
BR Gain (msec/mm Hg)	8.0±.15	10.4±.14	28.7±2.22	11.5±.47

In conclusion, ten weeks of aerobic training did not affect LBNP tolerance, LBNP responses, low-pressure mechanoreceptors (as indicated by FBF), or high-pressure mechanoreceptor functioning (as indicated by BR GAIN). Supported by NIH HL07534 and HL10342.

116.6

DIFFERENCE IN CARDIOVASCULAR RESPONSES TO BLOOD POOLING PATTERNS BETWEEN LBNP AND HEAD-UP TILTING STIMULATED AFTER MILD SUPINE CYCLING IN WOMAN. S.Torikoshi, K.Yokozawa, J.Nagano, Y.Suzuki, Lab.of Human Physiol.Tokyo Woman's Christian Univ. Suginami-ku Tokyo Japan

In the present study, 5 female students were investigated cardiovascular responses to gravitational stresses given after mild supine cycling by 40° Head-Up Tilting (HUT) and LBNP,which was closely estimated as a LBNP developed by the HUT in each subject. Increasing in calf volume after the cycling got two times in HUT more than in LBNP. Fore-arm blood flow (FBF) and arterial blood pressures after exercise slowly decreased in HUT,following transient increases with changing body position,more than in LBNP, while recovering HR, VO_2, and cardiac output (CO) had a little higher levels in HUT,respectively. HUT would be make a bigger blood pool in the calf but a less total leg.volume more than these in LBNP,and this fact may bring about the difference in cardiovascular responses between the two gravitational stimulations after mild supine exercise.

116.7

CENTRAL HEMODYNAMIC EFFECTS OF TRANSITION FROM SUPINE TO UPRIGHT POSITIONS IN PATIENTS WITH CHEST PAIN SYNDROME. Bernard J. Rubal, David S. Gantt*, Julio J. Bird*, and Ricky D. Latham*. Brooke Army Medical Center, Fort Sam Houston, Tx 78234

This study compares supine and upright hemodynamics in 6 patients undergoing cardiac catheterization for chest pain (ages: 40+6 years). High-fidelity left and right heart hemodynamic data were obtained with multisensor catheters. These catheters were hydrostatically referenced to a fluid-filled catheter while patients were supine. Angiographic studies revealed 3 of the 6 patients had atherosclerotic coronary disease. Transition from supine to a sitting or standing position was associated with a significant decrease ($p<0.05$) in left ventricular (LV) end-diastolic (50%), pulmonary artery (33%), and right ventricular (RV) end-diastolic pressures (33%). Changes in RV ejection time (16%), RV pre-ejection period (21%), LV pre-ejection period (11%), LV ejection time (14%) and aortic pressure were not significant. In addition, the high-fidelity recordings permitted the recognition of the following: disappearance of LV a-wave, reduced amplitude of pulmonary artery wave reflections and reduced rate of RV and LV filling during transition to upright postures. These data suggest that transition from zero Gz to +1Gz induces subtle changes in cardiac loading important in the ambulation of patients recovering from cardiac events or deconditioned states.

116.8

INCREASING CENTRAL BLOOD VOLUME WITH HEAD-DOWN TILTING WOULD INHIBITE WATER INTAKE DURING MILD PEDALING AT 25°C AND 35°C ROOM TEMPERATURES IN WOMAN. K.Yokozawa,S.Torikoshi,J.Nagano andY.Suzuki Tokyo Woman's Christian Univ.,2-6-1,Zenpukuji, Suginami-ku,Tokyo,Japan

The present study has investigated the effect of alterating central blood volume(CBV) estimated by stroke volume(SV) with changing in tilting position on water intake(WI) during a mild pedalling at 25°C and 35°C room temp. in 5 female students.WI was determined as a water volume drunk at 50 min.during exercise by each subjects. 10° head-up tilting(HUT) had a excess of the WI over 10° head-down tilting(HDT), while skin temperature (Tsk)and forearm blood flow were higher than these in HDT,and while SV increased in HDT but decreased in HUT from the supine resting levels. Despite of changing body temp., WI would be affected by CBV(with respect to SV) alterated in exercise position,because WI might be inhibited by increasing in atrium fulling pressure as CBV increased in HDT.

116.9

COMPUTER SIMULATION ANALYSIS OF THE EFFECTS OF COUNTERMEASURES FOR RE-ENTRY ORTHOSTATIC INTOLERANCE. R. Srinivasan*, John B. Charles*, Joel I. Leonard*, (SPON: C. L. Huntoon). NASA Johnson Space Center, Houston, Texas, 77058.

Replacement of fluids and electrolytes (fluid-loading) and application of lower-body positive pressure (anti-g suit inflation) are two commonly-used countermeasures to combat the problem of orthostatic intolerance experienced by Shuttle astronauts as they return to Earth following a spaceflight. Although the beneficial effects of these remedial measures have long been recognized, the physiological responses resulting from their use have not been fully evaluated. Knowledge of the response patterns is essential for the development of effective countermeasures to improve orthostatic tolerance. The requisite data cannot readily be obtained experimentally in an operationally-oriented environment. This study uses computer simulation to provide theoretical answers to questions related to the use of fluid-loading and anti-g suit during Shuttle re-entry. The results suggest a hypertonic fluid-load just prior to re-entry for maximum effectiveness. The simulations pertaining to application of lower-body positive pressure indicate that, for g-forces of 2 G and less, inflation of anti-g suit is warranted only if the blood volume loss exceeds 10 percent. (Supported by NASA Contract NAS9-17720).

116.10

PILOT PERFORMANCE IS INCREASED AFTER ALTERNATING HYPO- AND HYPERGRAVITY STATES

J. Sýkora*, J. Dvořák* and I. Šolcová* (SPON: H. Bjurstedt)
Institute of Physiology, Prague, Czechoslovakia

A method of testing the reactions during alternating hypo- and hypergravity states repeated 10-times during one flight in a small transport plane was elaborated. In 10 experiments with 4 experienced pilots the inflight performance during a standardized acrobatics programme was tested before and after the exposition to the mentioned alternating gravity states. It was found that the tested pilot performance was markedly better after alternating gravity flights. It is supposed that at least in some subjects adaptation to the mentioned states is possible. This seems to be of importance to the problem of artificial gravity during the space flights.

116.11

SIGNIFICANCE OF LIGHT AND SOCIAL CUES IN THE MAINTENANCE OF TEMPORAL ORGANIZATION IN MAN. C.M. Winget, C.W. DeRoshia, K.H. Ogawa and D.C. Holley: Space Life Sciences Payloads Office, National Aeronautics and Space Administration, Ames Research Center, Moffett Field, California 94035, and Department of Biological Sciences, San Jose State University, San Jose, California 95192.

This study was designed to simulate several environmental conditions which will be encountered in future space missions, such as long-term confinement and isolation, exclusion of periodic environmental synchronizers, altered work/rest schedules, and the change in group composition following exchange of crew members. We focused on the relative importance of two environmental synchronizers (e.g., light and social interaction) in the regulation of many biological rhythms, including heart rate (HR) rectal temperature (RT), rest/activity (RA), feed/fast, and selected urinary and plasma parameters. Two 20-24 year old male subject triads (NR and SR) were confined and isolated in 3.4 x 5.2 meter rooms for 105 days (d). A third triad (ER) lived at the test center during the study and served as non-isolated but confined controls. The NR and SR group subjects were exposed to 16L:8D (LD) and constant light (LL) photoperiods which alternated at approximately three week intervals, while the ER group was maintained in LD. At the start of the last experimental regimen (regimen 5, d84), subject S3 from the SR group was exchanged with subject N5 from the NR group. The importance of social interaction between subject triads as a factor in the regulation of physiological processes was shown by; (1) parallel changes in Circadian Rhythms (CR) phase and period during LL within the SR and NR groups; (2) distinct differences in CR period lengths between the NR (24.8 hr) and SR (24.3 hr) group subjects in LL; (3) the lack of consistent differences between CR parameters in LL and LD (which indicated that mutual social CR synchronization probably prevented the changes in CR amplitude, phase and waveshape normally observed in singly isolated subjects in LL); (4) the occurrence of CR desynchronization in subject N5, which occurred after the subject transfer on d84, although N5 was exposed to an unchanged LD environment during this transition; and (5) the dissociation of the CR of subject S3 from the CR of the other NR group subjects in LL on d96. The RA and RT CR's of S3 subsequently internally desynchronized on D102. These results are statistically significant by a non parametric approach ($p<0.05$). In conclusion, the results of this study indicate that small groups adapt well to long-term confinement and isolation, that some aspect of social interaction can affect physiological processes in a positive manner (e.g., mutual CR synchronization) or negative manner (CR desynchronization associated with evidence of a stress response) and that more investigation is needed to identify individual physiological and psychological characteristics which identify individuals who may have problems in adapting to future space station missions. Useful information was obtained relative to crew size, composition, selection and psychological support and interaction between crew and ground support personnel. Our results also suggest the utility of several countermeasures to maintain circadian rhythm entrainment and optimize adaptability to required work-rest schedule changes in future space station missions.

ANALGESICS AND ANTAGONISTS II

117.1

THE EFFECTS OF INTRATHECALLY-ADMINISTERED CALCITONIN GENE-RELATED PEPTIDE (CGRP), ALONE AND IN COMBINATION WITH VARIOUS OPIATE RECEPTOR-SPECIFIC LIGANDS, IN MOUSE ANTINOCICEPTIVE TESTS. S.P. Welch, A. Singha, W.L. Dewey. Dept. of Pharmacology/Toxicology, Medical Coll. of VA, VA Commonwealth Univ., Richmond, VA 23298.

CGRP has been shown to produce small antinociceptive effects following intracerebroventricular (icv.) administration. High binding of CGRP in the spinal cord led to testing for the antinociceptive effects of CGRP following intrathecal administration (i.t.) using the tail-flick (TF) and hot-plate (HP) tests. CGRP (0.01 µg through 10 µg/mouse, i.t.) produced no antinociception. CGRP (1 µg i.t.) did block the antinociceptive effects of morphine (MOR), Ca^{++}, and ionophore A23187 (all i.t.), as well as s.c. MOR, in both TF and HP tests, but blocked MOR (icv.) in only the TF test. In contrast, CGRP (1 µg icv.) blocked MOR (s.c.), but not MOR (i.t.) in the TF test. CGRP (icv., 1µg) did block Ca^{++} (i.t.) in the TF test. CGRP (1 µg i.t.) did not block i.t. U50,488 (kappa agonist), but did alter the antinociceptive effects of DAGO i.t. (mu agonist) and DPDPE i.t. (delta agonist). CGRP appears to produce predominantly spinal modulation of antinociception via alteration of neuronal calcium. This work was supported by NIDA grants #DA01647-09 and DA05340-01.

117.2

INTERACTION OF A MU AND A KAPPA OPIOID RECEPTOR AGONIST IN THE RAT P.F. Osgood, J.W. Kemp*, A. Kazianis*, N.E. Atchison*, D.B. Carr*, & S.K. Szyfelbein*, Shriners Burns Institute, Boston, MA 02114

Since the receptor specific attributes of the predominantly opioid kappa agonist butorphanol (BT) suggest that it may provide greater pain relief in the burn victim than the mu agonist morphine (MS), we assessed the analgesic effects of BT and MS alone and in combination in the rat burn model. Male S.D. rats were anesthetized and subjected to a scald or sham burn. At 2 days post-burn MS, BT or BT with a fixed dose of MS were administered i.v. Analgesia was assessed by tail flick latency (TFL) for 4 hrs. While MS had equipotent effects in sham (SH) and burn (BR) rats, BT had greater potency in the BR than in SH. When BT was combined with MS, in SH there was an immediate rise in TFL to higher levels than with BT alone, but then TFL fell rapidly so that analgesia was markedly shorter in duration. In BR, on the other hand, dose-effects for TFL were reversed (the slope of the dose-effect curve was negative). Thus when given alone BT had greater analgesic potency in BR. When combined with MS, however, responses in SH and BR are similar to the results of a study by Wallenstein et al, 1986, who found that when given to non-tolerant human subjects, a fixed dose of MS with increasing doses of the kappa agonist pentazocine augmented analgesia, but in patients tolerant to MS analgesia was reversed just as in the TFL response in BR. Possibly the marked increase in beta-endorphin that follows burn injury in the rat (Osgood et al, 1987) may have led to a relatively "tolerant" state at 2 days post burn.

(Supported by a grant from the Shriners Hospitals for Crippled Children).

117.3

STRESS-INDUCED POTENTIATION OF MORPHINE ANALGESIA IN MORPHINE TOLERANT RATS. Sharon W. Fleetwood* and Stephen G. Holtzman. Emory University School of Medicine, Atlanta, GA 30322

This investigation was designed to determine whether rats that were tolerant to the analgesic actions of morphine (M) would be tolerant to the stress-induced potentiation(SIP) of M-induced analgesia. Male Sprague-Dawley-derived rats (210-235g) were given scheduled access (10 min every 6 hrs) to either M solutions(0.5mg/ml) or drug-free tap water. Daily M intake averaged 46 mg/kg. Nontolerant and M tolerant rats were tested for M analgesia (tail-flick method) while either unstressed or stressed (i.e., immobilized in Plexiglas cylinders). M (1.0-8.0 mg/kg,s.c.) produced dose- and time-dependent increases in tail-flick latencies. Stressed rats consuming drug-free tap water were more sensitive to the analgesic actions of M than their unstressed counterparts: ED_{50}=2.39 vs. 4.14 mg/kg ($p<0.05$). Increased sensitivity to M analgesia was also evident for stressed rats consuming M chronically when compared to their unstressed counterparts: ED_{50}=4.05 vs. 6.00 mg/kg ($p<0.05$). Stress potentiated the analgesic effect of M comparably in nontolerant (1.8 fold) and tolerant (1.5 fold) rats. Differential tolerance to M analgesia and to SIP of M analgesia suggests that different mechanisms mediate these two effects. Supported by Grants DA00541 and K05 DA00008.

117.4

DOPAMINE D-1 AND D-2 ANTAGONISTS POTENTIATE ANALGESIC AND MOTOR EFFECTS OF MORPHINE. J.A. Kiritsy-Roy*, S.M. Standish* and L.C. Terry. Department of Neurology, Veterans Administration Medical Center and University of Michigan, Ann Arbor, MI 48105.

Brain dopaminergic systems have been implicated in many of the pharmacologic effects of morphine (MOR), but the role of dopamine receptor subtypes is not clear. These studies compare analgesic and cataleptic responses to MOR in the absence and presence of SCH 23390 (SCH) or eticlopride (ETC), highly selective D-1 and D-2 receptor antagonists, respectively.

Male, Sprague-Dawley rats were pretreated with either saline, SCH (50-100 ug/kg ip) or ETC (20-150 ug/kg ip) 10 min prior to MOR (12 mg/kg ip). Antinociception was assessed at 5 min intervals for 15 min before and for 40 min after treatment using the hot plate test (52.5^{o}C). Hindpaw lick or escape attempt were used as endpoints (maximum latency=45 sec). In additional experiments using the same drug treatments, catalepsy was assessed by placing a hindlimb on a 3.5 cm cork and recording the latency to stepdown (maximum latency=45 sec).

MOR increased analgesic response latency to 44.5±7.9% of the maximum possible response within 30 min, but did not prolong stepdown latency in the catalepsy test. Pretreatment with either SCH or ETC potently enhanced the analgesic effect of MOR and elicited a state of catalepsy. Peak analgesic responses to MOR increased to 91.9±7.5% and 100% of maximum with the highest doses of SCH and ETC, respectively. Neither antagonist alone produced significant analgesia at doses that potentiated MOR analgesia, although both drugs were mildly cataleptigenic. However, when administered in combination with MOR there was a pronounced cataleptic response. These results indicate that the inhibitory relationship between dopamine and opioids involves both D-1 and D-2 dopamine receptors and provide support for the thesis of a functional interaction between D-1 and D-2 receptors in some brain regions. (DK 37669)

117.5

MORPHINE-INDUCED TOLERANCE AND DEPENDENCE IN THE RAT IS ASSOCIATED WITH ALTERATIONS IN BRAIN REGIONS AND SPINAL CORD ^{3}H-SCH 23390 RECOGNITION SITES. **Hemendra N. Bhargava and Anil Gulati*, Dept. of Pharmacodynamics, Univ. of Ill. at Chicago, Chicago, IL 60612.**

Male Sprague-Dawley rats were rendered tolerant to and dependent on morphine by s.c. implantation of 6 morphine pellets each containing 75 mg of morphine free base during a 7-day period. Rats implanted similarly with placebo pellets served as controls. The binding of ^{3}H-SCH 23390 to various brain regions and spinal cord was determined in rats from which pellets were either removed (abstinent for 16 hrs) or left intact (nonabstinent rats). The binding of ^{3}H-SCH 23390 to membranes of brain regions and spinal cord of nonabstinent morphine dependent and their placebo control rats did not differ. However, the binding of ^{3}H-SCH 23390 to striatal and hypothalamic membranes of abstinent rats was much greater than in the control rats. The increase in binding was due to a combination of increases in Bmax value and decreases in K_d value of ^{3}H-SCH 23390. Similar effects were observed in spinal cord. The behavioral responses to dopamine D_1 receptor agonist, SKF 38393 were also enhanced in morphine abstinent rats when compared to placebo controls. The results indicate that in morphine tolerant-dependent rats undergoing withdrawal, dopamine D_1 receptors are proliferated but in nonwithdrawing rats dopamine D_1 receptors are unaffected (Supported by a grant DA-02598 from the National Institute on Drug Abuse).

117.6

EFFECT OF U-50,488H, A KAPPA OPIOID RECEPTOR AGONIST ON TOLERANCE TO ITS PHARMACOLOGICAL EFFECTS AND BRAIN AND SPINAL CORD KAPPA OPIOID RECEPTORS. **Poduri Ramarao*, Anil Gulati* and Hemendra N. Bhargava, Dept. of Pharmacodynamics, University of Illinois at Chicago, Chicago, IL 60612.**

Intraperitoneal administration of U-50,488H (U) produced dose-dependent analgesia, hypothermia and diuresis in male Sprague-Dawley rats. Chronic administration of U (25 mg/kg), twice a day for 4 days resulted in the development of tolerance to its analgesic and hypothermic effects but not to the diuretic effect. Chronic administration of U (10, 20 and 40 mg/kg) once a day for 5 days did not produce tolerance to its pharmacological effects, however, the same doses of U given twice a day resulted in the development of tolerance to both analgesic and hypothermic effects. The development of tolerance to U was associated with decreased binding of ^{3}H-ethylketocyclazocine (^{3}H-EKC) to selected brain regions (pons + medulla, midbrain, cortex) and spinal cord membranes but the binding to hypothalamic membranes did not differ in chronic U and its vehicle treated rats. The results suggest that once a day injection of U upto 40 mg/kg does not produce tolerance but twice a day injections are required to induce tolerance to the pharmacological effects of U, and that it is associated with down regulation of brain and spinal cord kappa opioid receptors (Supported by a grant DA-02598 from the National Institute on Drug Abuse and a grant from the Chicago Heart Association).

117.7

Opioid and Opioid Factors in Starvation-Induced Analgesia. H. Akunne* and K.F.A. Soliman. College of Pharmacy, Florida A&M University, Tallahassee, FL 32307.

The effect of opioid and non-opioid factors in starvation-induced analgesia were investigated in male Sprague-Dawley rats. Animals were starved for 12 hours and were divided into 8 groups. Four groups were fed for 2 hours and were treated intraperitoneally with tryptophan (200mg/kg), naloxone (5mg/kg), methysergide (3mg/kg) and methysergide/naloxone. The remaining four groups were not fed but received the same drug treatments. All animals were tested for pain sensitivity before starvation, after starvation, after feeding and at 30 minutes and 2 hours following the various drug treatments. Starvation resulted in significant ($p<.01$) increased pain threshold which was not reversible by institution of feeding. Consequent administration of tryptophan resulted in increased pain threshold while naloxone caused a decrease in pain threshold. Also after starvation, treatment with naloxone or methysergide resulted in a significant ($p<.05$) decrease in pain threshold though the combined treatments did not enhance this effect. These results indicate that starvation-induced analgesia was not reversible by 2 hour feeding but was enhanced by tryptophan and reversible by methysergide or naloxone. (Supported by grants NASA NAG 2-411; NIH RR0811; NIH RR03020).

117.8

Attenuation of Morphine-Dependent Precipitated Withdrawal Signs by Oral and Intraperitoneal Administration of Glucose. L. Hardy*, C. Akunne* and K.F.A. Soliman. College of Pharmacy, Florida A&M University, Tallahassee, FL 32307.

Male Sprague-Dawley rats maintained under controlled lighting and temperature conditions were used in this experiment. Morphine dependency was induced by giving increasing doses of morphine by intraperitoneal injection. Animals were injected with 10,20,30 and 50 mg/kg of morphine sulfate at days 1,2,3 and 4 respectively. After being divided into two groups the morphine dependent animals were given naloxone by the intraperitoneal route to precipitate withdrawal. Glucose (5g/kg, 8g/kg or 10g/kg) was given orally 1 hour prior to the administration of naloxone to one group and glucose (5g/kg or 8g/kg) was given intraperitoneally 20 minutes before naloxone to the second group. All drug treatments had their proper controls. Withdrawal signs were assessed by observing the presence of diarrhea, erection, salivation, piloerection, hunchbacked posture, body tremor, teeth chattering, escape attempts, territorial exploring, restless activity, irritability to handling, vocalization, and jumping. Results obtained indicate that oral glucose administration at 10g/kg and intraperitoneal glucose at 8g/kg abolished most of the withdrawal signs. It was concluded from this study that hyperglycemia could suppress morphine withdrawal signs. (Supported by grants NASA, NAG 2-411; NIH RR0811; NIH RR03020).

117.9

MECHANISM OF OPIOID EFFECTS ON BASAL FLUID ABSORPTION IN THE MOUSE SMALL INTESTINE. Qi Jiang*, Russell J. Sheldon* and Frank Porreca. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724, U.S.A.

Central and peripheral sites of opioid action on basal fluid absorption were studied *in vivo*. Male, ICR mice (20-30 g) previously fasted for 15 hr were anesthetized with intraperitoneal (*i.p.*) urethane (1.2 g/kg) and a loop of small intestine (upper jejunum) isolated. Each end of the intestinal segment (15-20 cm) was ligated and filled with an isotonic solution (0.7-1.0 ml). Compounds were given intracerebroventricularly (*i.c.v.*) or *i.p.*, 15 or 20 min, respectively, prior to filling the intestinal loop. The loop was excised 20 min after filling, weighed, the contents expressed and reweighed. The volume of fluid absorbed was calculated as the difference between the filling volume and the residual intestinal volume. When given *i.c.v.*, [D-Ala^2, NMPhe^4, Gly-ol]enkephalin (DAGO), morphine, [D-Pen^2, D-Pen^5]enkephalin (DPDPE) and U50,488H increased fluid absorption in a dose-related mannner - DAGO was 3.7, 573 and 2579 times more potent than morphine, DPDPE and U50,488H, respectively. All compounds except DPDP were antagonized by naloxone (1 - 10 μg, *i.c.v.*, concurrently with the agonist); DPDPE was also unaffected by the δ antagonist, ICI 174,864 (10 μg, *i.c.v.*). With the exception of morphine, none of these agonists altered fluid absorption when given *i.p.* The effects of *i.p.* or *i.c.v.* morphine were antagonized by *i.c.v.*, but not *i.p.*, N-methyl levallorphan (SR 58002C), a quaternary opioid antagonist. Pretreatment with *i.c.v.* β-funaltrexamine (β-FNA)(20 nmol, 4 hr prior to testing) blocked the effects of *i.c.v.* DAGO, but not U50,488H. These results suggest that opioids enhance basal fluid absorption exclusively through central sites in this species. Furthermore, both central μ and κ opioid receptors are involved in this mechanism.

117.10

ANTAGONIST ACTIONS OF THE OPIOID ANALGESICS NALBUPHINE AND BUTORPHANOL IN HUMANS. George E. Bigelow* and Kenzie L. Preston. The Johns Hopkins University School of Medicine, Baltimore, MD 21224.

The extent to which the opioid mixed agonist-antagonist analgesics nalbuphine and butorphanol would display agonist actions versus antagonist actions when administered to opioid-dependent human volunteers was assessed on a variety of subjective, behavioral and physiological measures. Hydromorphone (4 and 8 mg) and naloxone (0.1 and 0.2 mg) served as profiles of a protoypic mu agonist and opioid antagonist and were compared to saline, nalbuphine 0.375, 0.75, 1.5, 3 and 6 mg and butorphanol 0.375, 0.75, 1.5, 3 and 6 mg in two groups of 5 subjects. Subjects were adult, male opioid abusers enrolled in methadone maintenance treatment (30 mg/day, p.o.). Drug conditions were given IM under double-blind conditions in 2.5 hr experimental sessions. Naloxone precipitated opioid abstinence, as indicated by increased subject- and observer-rated withdrawal symptoms and signs. Nalbuphine and butorphanol precipitated withdrawal as indicated on subjective, behavioral and physiological measures. Nalbuphine 3 mg and butorphanol 1.5 mg produced effects approximately equivalent in magnitude to naloxone 0.2 mg. The profile of nalbuphine-induced withdrawal effects was qualitatively similar to, and indistinguishable from, that of naloxone; in contrast, butorphanol precipitated a withdrawal syndrome that differed somewhat from that of naloxone. Neither nalbuphine nor butorphanol produced any measurable hydromorphone-like agonist effects in these opioid-dependent subjects. (Supported by USPHS Grant DA-04089).

117.11

STUDIES ON THE PHARMACOLOGICAL CHARACTERISTICS OF ACUPUNCTURE POINT INJECTED DRUGS. Zu-Shun Liu* and Zhen-Yi Shoa* Nantong Medical College, Nantong, Jiangsu 226001 PRC

"Nei-Guan" acupuncture point(Acu-P) of mouse was used for drug injecting point run parallel with s.c. or/and iv. inj. Three characteristics were revealed. Firstly, the onset and potencies of main parameter of Tetrazol, Demefline, morphine, insulin and NE are much faster and stronger than that given by s.c. inj and nearly equal to that of iv. inj. Secondly, the potency after Acu-P inj does not strictly relate to the rate of absorption, for example, the radioactivity of 125I-insulin in plasma and liver 5 min. after Acu-P inj was 5.86 and 9 folds less than that of iv. inj at same dose basis, but with same hypoglycemic potency. Thirdly, there may be additional unique pharmacological mechanism(s) in case of Acu-P inj. Analgesic potency of morphine and hypoglycemic activity of insulin were not as effectively antagonized by naloxone and glucagon correspondingly as that by s.c. or iv. inj. Rotation induced nystagmograph of rabbit was equally inhibited by "Yi-Feng" Acu-P or iv.inj, but not inhibited by s.c. inj of Scopolamine. Some of the results are shown as follows: LD50 of Tetrazol Acu-P 42.47 mg/kg(28.17-64.04), iv. 43.65(38.35-49.61), s.c. 102.80(91.46-155.55); of Demefline Acu-P 3.12 (3.03-3.32)mg/kg, s.c.4.07(3.81-4.34). Analgesia ED50 of morphine Acu-P 15.03 mg/kg(13.36-16.91) s.c. 21.07(17.60-26.47). Hypoglycemic % of insulin in STZ induced diabetic mice Acu-P 25.47±13.92, iv. 25.54±9.61, s.c. -5.95±12.43 5 min. after injection.

HYPERTENSION II

118.1

ENDOGENOUS ANGIOTENSIN AUGMENTS EPINEPHRINE RELEASE IN RATS ON A LOW SODIUM DIET. Regis R. Vollmer, Sharon P. Corey* and Steven J. Fluharty*. University of Pittsburgh, Pittsburgh, PA 15261 and University of Pennsylvania, Philadelphia, PA 19104

Experiments were conducted to investigate the relationship between adrenal medullary epinephrine release and endogenously formed angiotensin II in male Sprague-Dawley rats that were maintained on a low or normal sodium intake for one month. During stimulation of the thoracolumbar cord in pithed low sodium rats (.01% sodium by weight of diet) the amount of epinephrine released into blood was significantly elevated above the levels observed in normal sodium rats (.3% sodium by weight of diet). Blockade of the renin-angiotensin system with either the converting enzyme inhibitor, captopril, or the angiotensin II receptor antagonist, saralasin, resulted in a decrease in the amount of epinephrine released in the low sodium rats but did not alter release in the normal sodium animals. Thus, the net result was to equalize the release in low and basal sodium animals. In addition, significant increases were found in the density of adrenal medullary angiotensin II receptors and tyrosine hydroxylase activity in low sodium animals. The results of the present study support the hypothesis that endogenous angiotensin II is capable of providing a positive modulatory influence on the neurally mediated release of adrenal catecholamines. (Supported by NIH HL26212 and NSF BNS85-18035.)

118.2

STUDY ON THE MECHANISM OF CLONIDINE REDUCED DIURESIS. Jolanta Gutkowska‡ Linghe Pan* (SPON: M.B. Anand-Srivastava). Clinical Research Institute of Montreal, Montreal, Canada. H2W 1R7

The ICV administration of clonidine in conscious rats evoked a significant, dose-dependent increase in urine output. With a dose of 0.5 μg of clonidine, urinary sodium excretion was enhanced from 5.43±5.43 to 18.5±5.3 mEq/l ($p < 0.001$) and plasma IR-ANF rose from 30.7±8.8 to 113.3±32.3 pg/ml ($p < 0.05$). Urinary cGMP was augmented from 8.49±4.29 to 27.7±5.0 pmoles/min ($p < 0.05$). This effect was accompanied by an apparent decrease of blood pressure. Pretreatment with peripherally-administered anti-ANF (400 μl) abolished the diuretic effect of clonidine ICV; urine output decreased from 1.49±0.41 to 0.42±0.21 ml/hr. The urinary cGMP level after anti-ANF serum fell from 25.0±7.56 to 7.1±3.5 pmoles/min ($p < 0.05$). Peripheral pretreatment with the α_2-adrenergic antagonist yohimbine, or with the opioid antagonist naloxone partially abolished clonidine's diuretic impact: urine output dropped from 1.91±0.55 to 0.42±0.18 ml/hr and to 0.46±0.18 ml/hr ($p < 0.05$), respectively. At the same time, plasma IR-ANF decreased from 113.3±32.2 to 30.3±11.4 after yohimbine and to 24.6±12.1 pg/ml after naloxone treatment ($p < 0.05$). We conclude that central stimulation of opioid and α_2-adrenergic receptors enhanced release of ANF.

118.3

ALTERED INHIBITORY EFFECT OF B-HT 920 ON PERIARTERIAL NERVE STIMULATION-INDUCED RELEASE OF ENDOGENOUS NOREPINEPHRINE FROM THE MESENTERIC VASCULATURE OF ADULT SHR. William H. Cline and Linda L. Stephenson. Southern IL. Univ. Sch. of Med., Springfield, IL 62794.

The effects of infusion of the selective alpha-2 adrenergic agonist, B-HT 920 (10^{-7}M final concentration), on periarterial nerve stimulation-induced (PNS) release of endogenous norepinephrine (NE) from isolated mesenteric vascular-intestinal loop preparations of 13-16 week old Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were determined. B-HT 920 infusion produced a frequency-dependent inhibition of PNS-induced release of endogenous NE in WKY preparations. Significant inhibition of NE release was observed at 4, 6, 8, 10 and 12 Hz but not at 14 Hz in WKY preparations. In contrast, B-HT 920 infusion did not alter PNS-induced endogenous NE release in SHR preparations at any of the PNS frequencies from 4-14 Hz. Mesenteric vascular perfusion pressure responses to PNS of 4-14 Hz were not altered during B-HT 920 infusion in WKY preparations but were significantly reduced in SHR preparations at higher PNS frequencies. Similarly, perfusion pressure responses to exogenous NE were not altered in WKY preparations but were significantly reduced in SHR preparations at higher NE concentrations. These findings indicate that significant changes in both the pre- and postjunctional actions of B-HT 920 occur in the SHR mesenteric vasculature. (Supported by AHA/IL Affiliate).

118.4

DIFFERENCES IN AGONIST-INDUCED ^{3}H-myo-INOSITOL LABELLING, CONTRACTILE RESPONSE, AND Na^+/H^+ EXCHANGE IN SHR VS. WKY ARTERIES. T.P. Ek, S. Gupta & R.C. Deth. College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA 02115

Recent observations have suggested a possible elevation of protein kinase C (PKC) activity in arteries of spontaneously Hypertensive (SHR) rats. We conducted studies to determine whether elevated PKC might lead to altered α-adrenergic responses (contraction and PI turnover) or altered basal Na^+/H^+ exchange (a PKC-activated process). Basal incorporation of ^{3}H-myo-inositol into inositol monophosphate (IP) was similar for SHR and WKY groups. However, after a 30 min exposure to NE (10 μM), SHR arteries failed to show an increase in ^{3}H-IP levels while labelling was increased 219% in WKY arteries. SHR contractile responses (NE; 10 μM) were smaller than WKY with a reduced tonic phase. Treatment of WKY tissues with the phorbol ester TPA induced a pattern of reduced NE response similar to that observed in SHR. We measured Na^+/H^+ exchange in aorta of SHR and WKY rats using ethylisopropylamiloride (EIPA), a selective inhibitor of this process. In SHR tissues, the EIPA-sensitive component of $^{22}Na^+$ uptake was 6.38± compared to 3.89± nmoles/min/mg for WKY representing a 65% increase. Since PKC regulates Na^+/H^+ exchange, these observations are compatible with an elevation of basal PKC which may also be responsible for reduced α_1 receptor responses in the SHR.

118.5

COMPLETE VASODILATOR DOSE RESPONSES ON A SINGLE HUMAN ARTERIOLE. A.M.M. Shepherd, R.W. Yee*, Y. Yang*, L. Rico-Penny*, C. Brodie*, P. Comeau*. Depts. Pharmacol./Ophthalmol., Univ. of TX Health Science Center, San Antonio, Texas 78284.

Pilocarpine has a direct vasoconstrictive effect on vascular smooth muscle. However, its net effect on many arteriolar beds is vasodilation, probably by releasing endothelium derived relaxation factor (nitric oxide) from the vessel endothelium. We describe a method of obtaining complete vasodilator dose response curves to topical pilocarpine in a single human conjunctival arteriole as a means of studying local vascular reactivity without activating systemic reflexes. Sixteen μl of ophthalmic pilocarpine solution were applied to the conjunctival sac of six healthy male volunteers followed by occlusion of the puncta. A Zeiss photo slit lamp biomicroscope was used to take slides of the conjunctival arteriole. Maximum vasodilation was determined to be at 4 min. followed by a reflex vasoconstriction in arterioles of 15-25 microns luminal diameter. Pilocarpine of concentrations ranging from 0.0001% to 4%, was then administered at 4 min. intervals to 5 healthy male volunteers. Triplicate photographs were taken and analyzed to determine the change in luminal diameter. A well defined log dose of pilocarpine versus degree of vasodilation response curve was obtained. Threshold effective dose of pilocarpine was 0.1% and maximal vasodilation was seen at a concentration of 2%. Conjunctival arterioles may be used as a model for the study of arteriolar responses in human cardiovascular research.

118.6

ATTENUATED BAROREFLEX CONTROL OF RENAL SYMPATHETIC NERVE ACTIVITY DURING EARLY DOC HYPERTENSION. N. Kawazoe*, D.B. Averill, and C.M. Ferrario. Cleveland Clinic Foundation. Cleveland, OH 44195.

In a preliminary study we found that efferent renal sympathetic nerve activity (ERSNA) was suppressed one week after onset of desoxycorticosterone (DOC) hypertension in dogs. This observation prompted us to study the baroreflex control of ERSNA in dogs given a single dose of DOC (20 mg/kg, i.m.) or equal volume of saline (sham). One week later dogs were anesthetized with morphine (2 mg/kg) and pentobarbital sodium (15 mg/kg). To examine reflex control of ERSNA, baseline mean arterial pressure (MAP) was decreased by 40 mmHg and increased by 20 mmHg by subsequent infusion of nitroprusside (100 μg/kg/min) and phenylephrine (20 μg/kg/min). For each dog the relation between MAP and ERSNA was fit to a logistic function. Baseline ERSNA of DOC treated dogs was significantly suppressed (0.85±0.13 μv*sec/5 sec, n=11) compared with sham treated dogs (2.61±0.44, n=11, p<0.001). Comparison of the relation of MAP vs ERSNA between DOC and sham groups revealed two significant effects of DOC treatment. 1) Over the same range of MAP, the change in ERSNA of the DOC group was smaller than that of the sham group (3.65±1.35 μv*s/5 sec and 6.84±0.87 μv*s/5 sec, respectively; p<0.01). 2) The maximum sensitivity for reflex control of ERSNA of the DOC group was approximately one-third that of the sham group (-0.10±0.01 μv*s/5 sec/mmHg and -0.29±0.07 μv*s/5 sec/mmHg, respectively; p<0.01). These results demonstrate that the early phase of DOC hypertension in dogs is accompanied by diminution of tonic sympathetic traffic to the kidney and reduced baroreflex control of sympathetic outflow to the kidney. (Supported in part by HL-6835 and HL-33713)

118.7

PERSISTENCE OF ELEVATED MYOCARDIAL CONTRACTILITY IN CHRONIC 1K1C HYPERTENSIVE DOGS. Marilys G. Randolph*, Getachew Mekasha* and Eleanor L. Ison-Franklin. Howard Univ., Washington, DC 20059.

In 6 chronically instrumented male dogs, weighing 17-22 Kg, changes in left ventricular (LV) volumes, pressures and indices of myocardial contractility were measured during a normotensive control period and for four weeks following the production of 1K1C Goldblatt hypertension. Simultaneous recordings of mean arterial pressure (MAP), LV pressure (LVP), maximum rise of ventricular pressure (dP/dt max) and sonometric measurements of LV dimensions were used to evaluate myocardial adjustments and changes in LV mass during the development and stabilization of the experimental hypertension. These primary variables were used to calculate cardiac output (CO), stroke volume (SV), end diastolic volume (EDV), end systolic volume (ESV), ejection fraction (EF), total peripheral resistance (TPR), wall tension (WT), mean velocity of circumferential fiber shortening (MVCF), heart rate (HR) and LV mass (LVM). Despite a significant increase in afterload, CO, EDV, ESV, and MVCF remained unchanged from control. However, dP/dt max normalized to the LV-to-body weight ratio remained significantly elevated ($p<.05$) indicating an increase in contractility. These results suggest that the noradrenergic system may be involved in protecting cardiac function during the development and maintenance of 1K1C hypertension in dogs.

118.8

INCREASE IN CARDIAC OUTPUT AT THE ONSET OF ALDOSTERONE HYPERTENSION. J-P. Montani*, H.L. Mizelle*, T.H. Adair and A.C. Guyton. Dept. of Physiology & Biophysics, Univ. Miss. Med. Center, Jackson, MS 39216.

The classical hemodynamic transients of volume-loading hypertension (HT) have been difficult to demonstrate in aldosterone HT. One of the reasons is that aldosterone HT develops slowly and the expected initial increase in cardiac output (CO) may be lost in the background noise. In the present studies we monitored the CO continuously for days because our measurements indicate that this is superior to short-term recordings, especially in demonstrating small changes in CO. In 4 chronically instrumented dogs housed in metabolic cages and maintained on a fixed sodium intake of 150 mEq/day, we infused aldosterone (12 μg/kg/day, iv) for 10 days while monitoring arterial pressure (AP) and CO (electromagnetic flow probe) continuously 20 hours each day. Aldosterone caused the mean AP to slowly increase from a control value of 87 ± 1 (SE) mmHg to 108 ± 2 mmHg over a period of 10 days. CO increased transiently from a control of 2.19 ± 0.22 l/min to a peak value of 2.42 ± 0.21 (days 3-4), and remained slightly elevated throughout the infusion period (2.35 ± 0.19: days 8-10). When the infusion was stopped, mean AP and CO returned to control (90 ± 2 mmHg, and 2.10 ± 0.18 l/min). Therefore, the hemodynamic changes associated with aldosterone HT are consistent with those of classical volume-loading HT. (Supported by HL 11678)

118.9

ACUTE STRESS AUGMENTS THE RENIN-RESPONSE TO AORTIC OCCLUSION IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). James P. Porter. Dept. of Physiol., Univ. of Louisville, Louis., KY 40292

Activation of the central nervous system in Sprague-Dawley rats can enhance the renin-response to reductions in renal perfusion pressure (RPP). In the present study, the following questions were addressed. 1) Is the renin-response to reductions in RPP greater under basal conditions in the SHR, which exhibit increased basal sympathetic outflow, than in control Wistar Kyoto (WKY) rats? 2) Will acute stress increase the sensitivity of this response more in SHR than in WKY rats? Adult SHR or WKY rats were instrumented 2 days ahead of time with a catheter in the lower abdominal aorta and an occluding cuff around the aorta proximal to both renal arteries. Plasma renin activity (PRA) was determined before and at the end of three 5-min periods of aortic occlusion to reduce RPP to 100, 75, and 50 mmHg. One hour later a stress in the form of a jet of air to the face was applied and the occlusion protocol was repeated. Linear regression was used with each individual rat to determine the relationship between PRA and RPP. Comparison of the slopes of these relationships showed that in the control state, SHR and WKY rats exhibited the same responsiveness. However, the application of the air significantly increased the slope of this relationship in the SHR but not the WKY rats. SHR have a normal renin-response to reduced RPP under basal conditions, but in the presence of a stress these animals exhibit an enhanced responsiveness. Supported by HL38993

118.10

ENDOTHELIAL DISRUPTION STIMULATES THE STEADY-STATE SODIUM PUMP ACTIVITY OF RAT THORACIC AORTA. David D. Ku, Dept. of Pharmacol., Univ. of Alabama at Birmingham, AL 35294

The importance of endothelium-derived relaxing factor (EDRF) in the regulation of vascular smooth muscle cell (VSM) reactivity has recently been established. More recently, it has been suggested that EDRF-induced relaxation is associated with hyperpolarization of the VSM. To determine whether EDRF may also modulate the vascular sodium pump activity, ouabain-sensitive ^{86}Rb uptake was measured in rat thoracic aorta in the presence and absence of endothelial cells (EC). The steady-state ouabain-sensitive ^{86}Rb uptake (4.7±0.4 nmoles/mg dry wt/10-min, M±SEM of 6 rats), but not the non-specific ouabain-insensitive ^{86}Rb uptake (2.9±0.2), was significantly increased to 6.9±0.5 (M±SEM of 11 rats) following mechanical EC disruption. The maximum capacity of the sodium pump (17.7 ±0.2) estimated in sodium-loaded aorta, on the other hand, was not altered. Pretreatment of EC-intact aorta with 10 μM hemoglobin, a known inhibitor of EDRF, did not produce any significant effect on the sodium pump activity. In isolated rings of aorta, the rate of development of sodium pump inhibition-induced contractile force was also significantly increased in the EC-disrupted aorta, while the maximum developed force was not altered. Thus, these results demonstrate that EC also exerts a potent inhibitory effect on the VSM sodium pump and that this latter EC inhibitory effect appears to be independent of the EDRF. (Supported by grant from NIH DK-39258)

118.11

NONPEPTIDE ANGIOTENSIN II (AII) RECEPTOR ANTAGONISTS: STRUCTURE-FUNCTION STUDIES. A.T. Chiu*, J.V. Duncia*, D.E. McCall*, P.C. Wong*, W.A. Price, Jr.*, M.J.M.C. Thoolen* A.L. Johnson* and P.B.M.W.M. Timmermans. E.I. du Pont de Nemours & Company, Inc., Medical Products Department, E400/4241, Wilmington, DE 19898.

2-Butyl-4-chloro-1-(2-nitrobenzyl)imidazole-5-acetate, sodium (S-8308), was found to be a weak but specific antagonist of AII mediated responses (Chiu et al., Eur. J. Pharmacol., in press, 1988). To improve on its affinity for the AII receptor, a series of imidazole-5-acetate derivatives defining the critical substituents on the phenyl ring was synthesized and evaluated. Three analogs substituted with either 4-carboxy-benzyl (EXP6155), 4-(2-carboxybenzamido) benzyl (EXP6159) or 5-methylacetate of EXP6159 (EXP6803) were found to inhibit the binding of ^{3}H-AII to AII receptors in rat adrenal cortical microsomes with 12-, 35- and 125-fold higher affinity, respectively than S-8308 (IC_{50} 15 uM). Scatchard analysis of ^{3}H-AII revealed that in the presence of EXP6155 (10^{-6} M), the dissociation constant for AII was increased from 1.2 to 3.9 nM whereas the total number of binding sites remained unchanged suggesting a competitive nature of antagonism. A similar order of affinity or potency (saralasin >> EXP6803 > EXP6159 > EXP6155 > S-8308) was observed in displacing ^{125}I-AII from binding to rat smooth muscle cells, blocking $^{45}Ca^{2+}$ influx induced by AII in rat aortic rings, and shifting the concentration-response curve of AII in a parallel manner in isolated rabbit aorta. Responses ($^{45}Ca^{2+}$ and contraction) elicited by norepinephrine (10^{-7} M) or by KCl (55 mM) were unaltered by these agents at concentrations of up to 10^{-4} M. These data demonstrate that imidazole-5-acetate derivatives are specific, competitive receptor antagonists of AII.

118.12

FENOLDOPAM ENHANCES URINARY DOPAMINE EXCRETION IN MAN. William J. Elliott*, Michael B. Murphy* (Spon. Leon I.Goldberg). Committee on Clinical Pharmacology, The University of Chicago, Chicago, IL 60637.

Some studies have suggested that renal dopamine (DA) mobilization may be a prerequisite for dietary or drug induced natriuresis. Since DA induced natriuresis is mediated through the DA_1 receptor we hypothesized that the response to the selective DA_1 receptor agonist fenoldopam (Fen) should not require enhanced DA excretion. Therefore, sodium ($U_{Na}V$) and dopamine ($U_{DA}V$) excretion were measured prior to and during the i.v. administration of Fen in 19 hypertensive patients (18 black, 8 male, aged 47±3 years) at 0.05-0.5 ug/kg/min. Similar measurements were made in 6 control patients (6 black, 4 male, aged 43±2 years) treated with nitroprusside (0.25-1.5 ug/kg/min). Fen increased $U_{Na}V$ from 210±40 to 428±65 uEq/min ($p<0.001$) and $U_{DA}V$ from 231±40 to 349±58 ng/min ($p<0.01$), while nitroprusside increased $U_{Na}V$ from 147±34 to 182±60 uEq/min and $U_{DA}V$ from 228±51 to 263±53 ug/kg/min ($p<0.02$). The increment in urinary DA excretion per uEq Na was 0.76 ng for Fen and 1.00 for nitroprusside (p=n.s.). The data indicate that a DA_1 agonist causes similar increments in the ratio of urinary dopamine to sodium excretion as other vasodilators.

CALCIUM ANTAGONISTS II

119.1

INTERACTION OF VERAPAMIL WITH CNS α_2 RECEPTORS IN THE CAT. Joseph R. Holtman, Jr.* and Michael T. Piascik. Dept. of Pharmacology, U. of Kentucky, Lexington, KY 40536.

Radioligand binding and physiological techniques were used to study the interaction of the Ca^{++} channel antagonist, verapamil, with CNS α_2 receptors in the cat. [^{3}H]yohimbine, an α_2 receptor antagonist, bound to sites in the medulla. The affinity (K_D) and density (B_{max}) were 1.62 nM and 0.075 pmol/mg. [^{3}H]yohimbine (1 nM) was displaced by (-)desmethoxyverapamil and by (+) and (-)verapamil. The K_I's were 150nM for (-)desmethoxyverapamil, 150nM for (-)verapamil and 480nM for (+)verapamil. Specific binding of [^{3}H]desmethoxyverapamil could not be detected in the medulla but was observed in the cortex. To assess the possible role of verapamil binding at the CNS α_2 receptor in blood pressure control, the drug was administered intracisternally in four chloralose-urethane anesthetized cats. Verapamil (100-1000 nmol) produced hypotension in 2 of 4 animals tested. Pretreatment with yohimbine did not attenuate verapamil-induced hypotension. Similar doses of verapamil consistently produced hypotension when administered peripherally. In conclusion, verapamil binds to CNS α_2 receptors. However, this interaction does not appear to be important in the control of blood pressure. Furthermore, the lack of [^{3}H]desmethoxyverapamil binding in the medulla argues against the hypotensive actions of verapamil being due to interaction at calcium channel sites. (Supported by the AHA-KY affiliate and HL 38120).

119.2

ISOLATION AND PARTIAL CHARACTERIZATION OF FRACTIONS FROM STOMACH AND BRAIN THAT INHIBIT 1,4-DIHYDROPYRIDINE BINDING AND CALCIUM CHANNEL CURRENT (CCC). D.E. Johnson*, A.V. Shrikhande*, R.T. McCarthy*, H.K. Fry*, and R.A. Janis. Miles Institute for Preclinical Pharmacology, 400 Morgan Lane, West Haven, CT 06516.

Lamb stomach was subjected to acid extraction and several purification steps to determine if a 1,4-dihydropyridine (DHP)-displacing substance that modulates calcium channels could be isolated. A fraction that eluted from C18 reverse phase columns at 45-50% acetonitrile inhibited [^{3}H]DHP binding to cardiac membranes. Further purification yielded an 8 kDa peptide and a low molecular weight (<1 kDa) fraction (SSI), both of which inhibited DHP binding. Electrophysiological studies were carried out on rat anterior pituitary cells (GH_3) using the whole cell variation of the patch clamp.[5] SSI produced a time- and voltage-dependent effect, such that block of slowly-inactivating CCC is maximized by prolonged depolarization. This inhibition is similar to that produced by DHPs. A fraction that inhibited DHP binding and L-type CCC was also isolated from hexane extracts of lyophilized brain. Partial purification was achieved using solid phase extraction techniques as well as normal and reverse phase HPLC. The results suggest that these fractions contain endogenous substances that may regulate slowly-inactivating calcium channels.

119.3

Nimodipine as an antidote in amphetamine lethal intoxication. Gabriel G. NAHAS and Renaud TROUVE*. Columbia University, 630 W 168th st., NewYork 10032.

Since some calcium channel blockers are efficient antidotes against acute cocaine intoxication, they were also evaluated in amphetamine lethal intoxication. Flunarizine, nimodipine (NM) and nicardipine (NC) did not influence lethality which was preceded by convulsions and symptoms of parasympathetic stimulation resulting in cardiac and respiratory arrest. 27 rats(260-310g) were fitted with a caudal catheter and connected to a computerized monitoring system. All received 120 mg/kg of amphetamine sulfate I.P., a lethal dose. Diltiazem (DL), Atropine (AT), Diazepam (DZ).

Gp	NM	NC	DL	AT	DZ	Survival time
1	0	0	0	0	0	19'51"± 10'03"
2	40	0	0	0	0.5	3:32'50"±18'47", 3:>24h
3	30	0	240	0.32	1	>24h
4	0	50	0	0.32	1	3:16'50± 11'24", 4>24h

Calcium channel blockers in µg. Atropine and Diazepam in mg.

Group 2 did not present any convulsion but 3 animals died after respiratory arrest. In group 3, NM and DZ were associated with AT, to control parasympathetic stimulation, and with DL to prevent the myocardial lesions observed in groups 1 and 2. In group 4, treatment with NCdid not always prevent respiratory arrest, no signs of infarction were present. Association of NM, AT, DZ and DL is an effective treatment against amphetamine lethal intoxication. NM prevents the respiratory arrest through a central mechanism, which remains to be clarified. In contrast with cocaine lethal intoxication, succesfully treated by selected calcium channel blockers given alone, amphetamine requires a combination of drugs which indicates differences in the mecahnisms of action of these two psychostimulants.

119.4

DILTIAZEM REDUCES LIPID PEROXIDATION IN REPERFUSED RABBIT HEARTS. Patrick T. Koller*, David R. Marshall*, and Steven R. Bergmann. Washington University, St. Louis, MO 63110

Reperfusion of ischemic myocardium is thought to result in formation of oxygen centered free radicals (OFR) which may induce injury by peroxidation of membrane lipids. We previously demonstrated in intact dogs that diltiazem enhances salvage after reperfusion without altering myocardial blood flow or work. Using a colorimetric thiobarbituric acid (TBA) assay we showed that diltiazem prevented increased effluent malondialdehyde (MDA, a product of lipid peroxidation) levels during reperfusion of isolated hearts. However, the TBA assay is nonspecific because of non-MDA but TBA reactive substances. Accordingly, we developed a specific HPLC assay and measured MDA tissue content in 5 nonperfused hearts and in 6 isolated hearts after 60 min control perfusion, or 60 min of low flow ischemia (n = 6), or after 60 min low flow ischemia and 3 min reperfusion alone (n = 7) or with 5 x 10^{-7} M diltiazem (n = 7) in the perfusate. MDA content averaged 0.39 ± 0.29 (SD), 0.83 ± 0.76, 0.77 ± 0.90, 3.64 ± 1.59 and 1.64 ± 1.03 nmol/g wet weight in nonperfused, control, and ischemic hearts; and in hearts reperfused without and with diltiazem, respectively ($p < 0.02$ between reperfused groups). The results demonstrate that increased lipid peroxidation in reperfused myocardium can be attenuated with diltiazem, a mechanism which may be responsible for its salutary effects in vivo.

119.5

EFFECT OF VERAPAMIL, AMIODARONE AND DESETHYLAMIODARONE ON LACTATE DEHYDROGENASE (LDH) RELEASE AND INTRACELLULAR INCLUSIONS IN ISOLATED RAT HEPATOCYTES. Pitambar Somani, Subhankar Bandyopadhyay*, and James E. Klaunig*, Med. Coll. of Ohio, Toledo, OH 43699

We showed earlier that release of LDH (cytotoxicity) and intracellular inclusions were induced by amiodarone (AM), its major metabolite, desethylamiodarone (DA) and propranolol in the primary rat hepatocyte culture model. In the present study, we investigated the effect of verapamil (V), a Ca^{2+} channel blocker with cationic amphiphilic structure, on LDH release and electron microscopic changes in this preparation. After 24 hr incubation of the hepatocytes with various conc (5-400 µM) of V, AM or DA, the LD_{50}'s were found to be 225 µM, 60 µM and 25 µM respectively. Incubation of the hepatocytes with subtoxic conc of V (100 µM) and DA (8 µM), but not AM (7.6 µM) resulted in a significantly ($P<.05$) greater release of LDH than with either drug alone:

	AM	DA	V	V+AM	V+DA
% LDH release	28.8	29.5	37.6	45.1	62.3*
± SEM	.9	.9	3.7	1.1	4.1

As with AM or DA, electron microscopic examination showed that numerous multilamellar inclusions were found in hepatocytes incubated with V (50 or 100 µM). These data show that (1) V can induce intracellular inclusions in the isolated rat hepatocytes without showing cytotoxicity; (2) the combined effect of V+DA on cytotoxicity is significantly greater than either drug alone.

119.6

DIHYDROPYRIDINE RECEPTOR AND CALCIUM ANTAGONISTS INTERACTION IN CARDIAC TRANSPLANTATION. Christine Lord*, Louis Dumont, and Claude Chartrand*. Dépt. de Pharmacologie, Université de Montréal; Chirurgie Cardiaque, Hôp. Ste-Justine, Montréal

Cell membrane abnormalities have been identified within acute rejection after transplantation. We hypothetized that dihydropyridine receptors might also be altered. Heterotopic heart implantation was carried out in dogs. Both native and implanted hearts were harvested after rejection. Purified sarcolemmal membranes were isolated. Ligand binding experiments were performed using ^{3}H-nitrendipine (78.3 Ci/mmol), 40 µg of sarcolemmal proteins in 1 mL buffer (NaCl 150 mM, Tris-HCl 50 mM, CaCl, 1 mM, pH 7.4) at 37°C. Competition studies were done using nitrendipine (NTD), nifedipine (NIF), verapamil (VER) or diltiazem (DIL). The following results were obtained with Hofstee computer transformation:

KD	NTD (nM)	NIF (nM)	VER (µM)
Control	1.2 ± 0.7	1.8 ± 0.5	[4.0 - 30]
Rejection	1.0 ± 0.4	2.9 ± 2.0	[1.5 - 52]

In low concentration diltiazem increases binding sites population of nitrendipine receptor by 29% (control) and 44% (rejection). For all experiments, we observed a significant decrease in total binding sites within rejection; computed Bmax were: 2.0±0.5 (control) and 1.4±0.6 (rejection) pMol/mg protein. These results suggested an altered in vivo response to calcium antagonist within rejection.

Supported by FRSQ, FQMC and Université de Montréal.

119.7

LOSS OF CELL ATP DUE TO INCREASED UPTAKE OF CALCIUM AS A POSSIBLE MECHANISM OF MAITOTOXIN-INDUCED CELL DEATH IN CARDIOMYOCYTES. G. Santostasi*, R.K. Kutty* and G. Krishna. NHLBI, NIH, Bethesda, MD 20892

Maitotoxin (MTX), a water soluble toxin (MW 3424) isolated from the dinoflagellate, Gambierdiscus Toxicus, is one of the most potent marine toxins known. In isolated heart, MTX induced calcium-dependent tonic contracture, arrhythmias and ultrastructural derangement. The main aim of the present study is to investigate the mechanism of MTX cardiotoxicity. In cultured neonatal rat heart cells, MTX induced time and dose dependent cell death (as measured by LDH leakage), with a TD_{50} of 90 pM (0.3 ng/ml) at 24 h. Time course of MTX induced LDH leakage into culture medium paralleled leakage of ^{14}C labeled adenine nucleotides from the cells. These effects of MTX were completely abolished by reduction of calcium concentration in the medium from 1.36 mM to 0.1 mM. MTX also induced a rapid uptake of ^{45}Ca from the medium containing 1.36 mM calcium. The uptake was linear up to 30 min, at 1 ng/ml of MTX. Thereafter the ^{45}Ca declined in parallel with the release of LDH from the cells. Verapamil, a calcium channel blocker, at 100 µM concentration completely blocked the MTX induced uptake of ^{45}Ca as well as LDH leakage. The increase in calcium is associated with a decrease in cell ATP followed by leakage of LDH suggesting that increased intracellular Ca^{++} may inhibit mitochondrial oxidative phosphorylation and thereby cause cell death.

119.8

PLATELET ACTIVATION ALTERS THE LYTIC EFFECT OF DETERGENTS MONITORED BY LACTATE DEHYDROGENASE RELEASE. David C. B. Mills and Laurence J. DiStefano*. Temple Univ., Philadelphia Pa. 19140.

Lactate Dehydrogenase (LDh) was measured in suspensions of washed human platelets by a phenazine - tetrazolium technique in 96 well microtiter plates, using INT (p-iodonitrophenyl tetrazolium violet) at 490 nm (17 mAU/nmole) or MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) at 562 nm (34 mAU/nmole) in a final volume of 200 µl. Enzyme activities measured in suspensions of resting platelets were 2 - 5% of the levels obtained after lysis with 0.5% Triton X-100. Treatment of platelet suspensions with different detergents caused lysis and the appearance of increased LDh activity, in the potency order:- Saponin - BAC (benzyldimethyl-n-hexadecylammonium chloride) - CTAB (cetyltrimethylammonium bromide) - Triton X-100 - SDS (sodium dodecyl sulfate) - DOC (sodium deoxycholate) - CHAPS (3-(3-cholamidopropyl)-dimethylammonio)-1-propanesulfonate. Prior activation of the platelets with thrombin (0.1 - 5 u/ml) or the ionophore A23187 (0.2 - 3 µM) caused some increase in LDh activity in the absence of detergents, and a dramatic increase in the lytic effect of the zwitterionic detergent, CHAPS. Pretreatment of platelets with prostaglandin E_1, to raise the level of intracellular cyclic AMP, had the opposite effect.

119.9

HUMAN ALPHA AND GAMMA THROMBINS STIMULATE BONE RESORPTION AND INOSITOL PHOSPHOLIPID METABOLISM IN BONE. P.H. Stern, V.M. Stathopoulos* and J.W. Fenton, II*. Northwestern University, Chicago, IL 60611 and New York State Department of Health, Albany, NY 12201

Human alpha-thrombin (α-Thr), in addition to procoagulant function, has a number of other actions, including mitogenic activity and stimulation of bone resorption. Gamma-thrombin (γ-Thr), an enzymatic cleavage product of α-Thr, lacks procoagulant activity but retains mitogenic potential. To determine the specificity of the effects of thrombin on bone and the possible second messenger for these effects, we have cultured 19-day fetal rat limb bones with human α-Thr or γ-Thr and measured resorption, inositol phosphate turnover and cyclic AMP (cAMP) production. Resorption was determined after 72 hr of thrombin treatment and assessed by release of previously-incorporated ^{45}Ca. cAMP was measured in the medium at the end of this incubation. Inositol phosphates were determined after 5 min of thrombin treatment of bones pre-labelled for 48 hr in vitro with ^{3}H-inositol. Both α-Thr and γ-Thr, at 1 μM, stimulated bone resorption and production of inositol phosphates. Only α-Thr increased cAMP production at this concentration. The results indicate that the structural requirements for stimulation of bone resorption by thrombin may be similar to those for mitogenic activity. Further, they are consistent with a second messenger role of inositol phosphates in the action of thrombin on bone.

119.10

DRUG AND HORMONE BINDING TO CALMODULIN: A SPECTROFLUOROMETRIC APPROACH. M. Pouliot*, J. Vocelle*, P.H. Naccache* and J.G. Chafouleas* (Spon: F. Labrie), MRC Group in Molecular Endocrinology, Laval University Medical Center, Quebec, Canada, G1V 4G2.

Calmodulin (CaM) is the main intracellular Ca^{2+} receptor in eukaryotic cells. Upon binding to Ca^{2+}, the fluorescence of the two CaM tyrosine (tyr) residues increases. Conversely, anti-CaM drugs, upon binding to CaM, cause a specific quenching of this Ca^{2+}-dependent tyr fluorescence, which is dose- and affinity-dependent. Therefore, drug-binding to CaM can be measured by evaluating the quenching of the CaM tyr fluorescence. It is accepted that CaM has a key role in hormone metabolism. The purpose of this study was to evaluate hormone and antihormone binding to CaM using the spectrofluorometric analysis of tyr fluorescence. A variety of naturally occurring steroids were tested for their ability to bind CaM. Some of them, more specifically the estrogens, appear to interact with CaM, altering significantly the tyr fluorescence. More interestingly, antiestrogens, such as Tamoxifen, demonstrate an even greater capacity to quench tyr fluorescence than that observed for anti-CaM drugs. Future studies will fully characterize the hormone and antihormone binding to CaM which should result in a better understanding for the role of CaM in hormone action. Supported by CRM and FRSQ.

119.11

MODIFICATION OF CALCIUM CHANNELS IN AGING RAT HEART S. Navaratnam* and Jagdish C. Khatter (Spon: D. Bose)

Our earlier studies have demonstrated an enhanced inotropic response to dihydropyridine calcium agonist BAY K 8644, with aging in rats. In the present study, ^{3}H BAY K 8644 was used to investigate the state of calcium channels in these aging rats. Sarcolemmal vesicles were isolated from 2 and 12 month old rat ventricles by the method of Pitts (1979). Specific binding of ^{3}H BAY K 8644 was determined with the concentration, ranging from 1 nM to 10 nM. The non-specific binding was defined as the amount of radioligand bound in presence of 1 uM Nitrendipine. Specific binding was saturable and the Scatchard analysis showed a single binding site. Specific binding was reduced to half with the addition of 1 mM EDTA and was restored to control values with 2 mM Ca^{2+}. Maximum number of binding sites was significantly higher in 12 month old rats (2.3 pmoles mg prot $^{-1}$)as compared to 2 month old rats (1.6 pmoles mg prot $^{-1}$). The affinity of the receptor to bind ^{3}H BAY K 8644 was however significantly reduced in older rats than young ones (Kd 14.5 nM vs 4.8 nM at $15^{0}C$). These results indicate that the myocardial calcium channels may be modified in the process of aging.
(Supported by Manitoba Heart Foundation.)

EXERCISE III

120.1

CAPILLARY ADAPTATIONS TO EXERCISE TRAINING AND CHRONIC ALTITUDE EXPOSURE. D.C. Poole* and O. Mathieu-Costello. Dept. of Medicine, UCSD, La Jolla, CA 92093

There is evidence that skeletal muscle capillaries proliferate in response to exercise training and, in some instances, during chronic exposure to altitude. One putative common stimulus for such adaptations is a reduced intramuscular PO_2. To investigate this issue separate groups of rats underwent 1) 4 wks training 2) 5 months at 3800 m (P_IO_2=91 Torr), or 3) were caged at sea level. Capillary tortuosity and capillary length/ fiber volume were estimated in perfusion-fixed M. Soleus by comparison of transverse and longitudinal sections. Capillary diameter was estimated by image analysis (>150 capillaries per muscle) on transverse sections. Compared to control values (i.e., 1886±73 mm^{-2}) capillary length/ fiber volume was significantly increased by exercise training (2496±180 mm^{-2}) and slightly decreased by altitude exposure (1510±70 mm^{-2}). As previously reported, capillary tortuosity was a direct function of sarcomere length under all conditions. Capillary diameter was not changed (control=4.66±0.21, trained=4.51±0.13, altitude=4.26±0.17 μm). Consequently, capillary surface area changed in proportion to capillary length/ fiber volume. In conclusion, the demonstrated capillary proliferation after exercise training but not after chronic altitude exposure does not support the hypothesis that altered intramuscular PO_2 stimulates capillary growth under these conditions. Supported by grants from NIH (HL-17731 & 07212) and The American Lung Association of California.

120.2

CAPILLARY SUPPLY OF HARBOR SEAL AND DOG SKELETAL MUSCLES. O. Mathieu-Costello. Dept. of Medicine, UCSD, La Jolla, CA 92093.

This study examined capillarity in various muscles of animals tolerant to severe hypoxia (Harbor seal; body weight, 18-40 Kg), compared to dogs (14-30 Kg). A total of 20 muscles were fixed by vascular perfusion in situ at sarcomere lengths ranging from 1.5μm to 3.1μm. Because of the effect of fiber shortening on muscle fiber cross-sectional area, capillary tortuosity (Fed. Proc., 46: 352 & 1534, 1987) and capillary-to-fiber perimeter ratio (FASEB J., 2: A1864, 1988), morphometric estimates of fiber size and capillary-to-fiber perimeter ratio were normalized to the same sarcomere length, 2μm, in order to take into account differences in fiber length among samples. Over the broad range of capillary length/ muscle fiber volume found in both species (seal, 1200-2100 mm^{-2}; dog, 1500-2600 mm^{-2}), seal muscles showed:

- smaller diffusion distances (fiber size: seal, 32±2 μm; dog,47±2μm)
- smaller capillary to fiber ratio (seal, 0.7-1.5; dog, 2.0-3.3)
- similar capillary diameter (ca 5 μm)
- smaller capillary-to-fiber perimeter ratio (seal, 0.14-0.29; dog, 0.30-0.42).

Despite the large myoglobin content of seal muscles and its role for O_2 diffusion facilitation, it is notable that in the seal, it is diffusion distance rather than capillary circumferential distribution around the muscle fibers which is optimized compared to the dog.
Supported by NIH grants 5P01 HL-17731 and HL-01534.

120.3

THE INFLUENCE OF ANESTHESIA AND EXERCISE ON PLASMA ATRIAL NATRIURETIC PEPTIDE (ANP) IN TRAINED AND NON-TRAINED SPONTANEOUSLY HYPERTENSIVE RATS (SHR). C.S. Stump, S.M. Beaulieu*, J.M. Overton, L.A. Sebastian*, Z. Rahman*, and C.M. Tipton, Department of Exercise & Sport Sciences, University of Arizona, Tucson, AZ 85721.

To determine the interrelationships between hypertension and various experimental interventions, ANP plasma concentrations were measured (RIA kit, Peninsula Laboratories, Belmont, CA) in non-trained (NT) and trained (T) rats under conditions of ketamine/acepromazine anesthesia (A), conscious resting (C), and treadmill running at 60% of VO_2 max after 10 minutes (E). Attempts were made to secure data for each condition in all animals. Rats were trained for 14-17 weeks on a motor-driven treadmill at 40-65% of their VO_2 max (NT=107.4±1.9; T=118.8±1.1 ml · min^{-1} · kg^{-1}). Blood samples were drawn from and donor blood reinfused into indwelling carotid and jugular catheters respectively. 48 hours separated multiple sampling. Results (X±SE, * $p \leq 0.05$ from C, number in parentheses = N) are as follows:

	ANP CONCENTRATIONS ($pg \cdot ml^{-1}$)		
	Anesthetized	Conscious	
Group	Resting	Resting	Exercise
NT	80.8± 9.3 (10)	191.7±26.9 (13)	340.5±65.5* (8)
T	84.2±11.2*(10)	236.3±40.4 (15)	358.4±42.3*(13)

These data show that ANP concentrations are lower during A and significantly elevated during E. There were no significant differences in ANP concentrations between NT and T. Supported by Arizona Heart Association (G-2-23-87) and NASA (NAG 2-392).

120.4

RESPONSES OF THE QT INTERVAL OF THE ELECTROCARDIOGRAM TO PHYSICAL AND EMOTIONAL STIMULI. Stewart Wolf, Ming H. Huang* and Jacob Ebey*. Totts Gap Medical Research Labs., Bangor, PA 18013

Continuous recording of the electrocardiogram, arterial pressure, respiration, heart rate and QT interval were made by a highly precise, on-line computerized recorder newly devised by J.E. The subjects were studied during resting, stationary bicycle exercise while maintaining 25 watts of power, stress interviews, and attempts to both shorten and prolong the QT interval by biofeedback. RESULTS: 1. Changes in heart rate and QT did not correlate during resting. During the first minute of exercise QT remained fixed while heart rate accelerated an average of 30 beats, although as exercise continued, QT shortened progressively as heart rate accelerated further. 2. During emotional stress characterized by resentment and anger QT shortened consistently in a range of 4 to 51 msec. while mean heart rate increased only 6 beats and mean systolic pressure rose 22 and diastolic 10 mm Hg. 3. Shortening of QT by as much as 34 msec and lengthening by as much as 18 msec was achieved by biofeedback without corresponding heart rate changes. CONCLUSION: The potential independance of QT from heart rate, observed in earlier work, was confirmed. The data suggest that QT is linked to cardiac contractility rather than to heart rate. The subjects were 13 men and 11 women ranging in age from 26 to 74.

120.5

POSTURE AND STROKE INDEX DURING EXERCISE RECOVERY. Emily C. Johnson,* Tracee L. Hudson,* Ernest R. Greene* (SPON: G.O. Ballam). Lovelace Medical Foundation, Albuquerque, NM 87108

Immediately after exercise testing, the orthostatic status of patients and thus cardiac loading and performance may vary. Accordingly, to determine the effects of posture on post-exercise stroke index (SI), we noninvasively measured beat-to-beat SI using pulsed Doppler ultrasound during supine (SU) and seated (SE) recovery from graded, sitting, cycle ergometer exercise to 70% of age-predicted maximum heart rate in 13 normal subjects aged 23-32 years. SI was determined during peak exercise and throughout 10 min of recovery. Results of SI (in ml/m^2) and heart rate (HR in bpm) were:

		Rest	Peak Exercise	Peak Recovery
HR	SE	65±12*+	153±6	139±12*+
	SU	58±8+	153±5	129±13+
SI	SE	65±15*+	84±14	86±17*
	SU	90±18	85±14	110±20+

*p<.01 versus SU +p<.01 versus exercise

SE and SU peak values of SI occurred at 30 s and 180 s, respectively, while SE and SU peak HR occurred at 20 s. SI during SE remained constant for 2 min at peak exercise values before decreasing. Compared to exercise, SI during SU increased for 3 min (p<.01), then decreased. We conclude that posture significantly affects recovery SI and that SI during SU exceeds peak SI attained during seated, submaximal exercise.

(Supported by the American Heart Association, New Mexico Affiliate)

120.6

Atrial Natriuretic Peptide Response to Graded Supine and Upright Cycle Exercise. C.A. Ray*, M.D. Delp, D.K. Hartle*, and H.P. DuVal*. Exercise Physiology Laboratory and College of Pharmacy, The University of Georgia, Athens, GA 30602.

The purpose of this investigation was to test the hypothesis that supine exercise elicits a greater atrial natriuretic peptide (ANP) response than upright exercise due to higher right atrial filling pressure obtained in the supine posture. Venous ANP concentration, corrected for plasma volume shifts, was measured during continuous graded supine and upright exercise in 8 healthy men at rest, following 4 min of cycling exercise at 31, 51, and 79% of VO_{2peak}, following 2 min of cycling exercise at VO_{2peak}, and 5 and 15 min post-exercise. ANP concentration was signicantly increased ($p<.05$) above rest by 52, 103, and 157% during supine exercise at 51, 79%, and VO_{2peak}, respectively. During upright exercise, ANP concentration was significantly increased only at VO_{2peak} (85%). Following 15 min of recovery, ANP remained elevated ($p<.05$) only in the supine posture. ANP concentration was 59, 69, and 67% higher ($p<.05$) in the supine position during exercise at 51, 79%, and VO_{2peak}. Systolic, diastolic, and mean blood pressures were not significantly different ($p>.05$) between postures. Heart rate was lower ($p<.05$) in the supine posture compared to upright posture. In conclusion, these results suggest that supine exercise elicits greater ANP release, presumably due to a greater central blood volume, venous return, and concomitant right atrial filling pressure and stretch. (Supported by AHA-Georgia Affiliate)

120.7

BAROREFLEX CONTROL OF SKIN BLOOD FLOW DURING EXERCISE IN THE HEAT. C.W. Mack, H. Nose* and E.R. Nadel. J.B. Pierce Fndn. and Yale Univ. School of Medicine, New Haven, CT 06519.

To determine whether baroreflex mediated cutaneous vasoconstriction could be abolished by an opposing vasodilator drive associated with dynamic exercise in the heat, we measured mean arterial pressure (MAP) and forearm blood flow to calculate forearm vascular resistance (FVR) during rest and supine cycle ergometer exercise (40% $\dot{V}O_2max$) in six volunteers with and without lower body negative pressure (-40 mmHg LBNP) at ambient temperatures of 25 and 35°C. The FVR and MAP values with LBNP were:

		25°C		35°C	
LBNP		0	-40	0	-40
REST	FVR	29 ± 4	57 ± 4*	13 ± 2	23 ± 4*
	MAP	88 ± 3	83 ± 3	84 ± 2	81 ± 2
EXER.	FVR	19 ± 2	31 ± 4*	8 ± 1	11 ± 3
	MAP	105 ± 2	93 ± 3*	99 ± 1	92 ± 2*

* -40 vs 0 LBNP, $p<0.05$; MAP, mmHg.

During passive heating or mild dynamic supine exercise in a cool environment, the skin retained its ability to vasoconstrict in response to baroreceptor unloading. However, there was no baroreflex-mediated vasoconstriction during mild, supine exercise in the heat. We conclude that cutaneous vasodilation in response to increasing body core temperature during mild, supine exercise in the heat overrides the cutaneous vasoconstrictor response to LBNP.

120.8

EXERCISE, VAGAL-CARDIAC ACTIVITY AND THE CHRONOBIOLOGY OF BLOOD PRESSURE. Ronald E. De Meersman. Teachers College - Columbia University, New York, NY 10027

The possibility that a progressive decline in vagal-cardiac activity and the age-dependent rise in blood pressure could be attenuated through regular aerobic exercise has not been tested. In the current investigation 72 physically active males (PA), ages 15 to 81, were tested for maximal aerobic capacity ($\dot{V}O_2max$), vagal-cardiac activity (RSA), and resting blood pressure (BP). All data were compared to an age and weight matched sedentary control group (S). Average BP's per decade revealed significant differences between the PA and S group ($p < 0.01$). Significant differences were found between groups in vagal-cardiac activity as measured by the amplitude of the respiratory sinus arrhythmia ($p<0.05$). Maintenance of a normotensive blood pressure was seen for all decades of life for the physically active group, whereas a borderline to near hypertensive state was found for the sedentary group. The possible mechanism responsible for this observation could be the presence of augmented vagal-cardiac activity following aerobic training in the physically active group as compared to the sedentary control group.

120.9

FREE FATTY ACIDS, LACTATE AND AMMONIA DURING STEADY-STATE EXERCISE. T. Graham, B. Kiens*, M. Hargreaves*, and E. A. Richter*. August Krogh Institute, Univ. Copenhagen, Denmark.

Previous studies associated muscle ammonium (NH4) production with anaerobic processes rather than amino acid (AA) metabolism. We determined the NH4 and lactate (La) release during steady-state exercise and the impact of elevation of free fatty acids (FFA) on these measures since FFAs may inhibit AA metabolism. Subjects (n=10) performed 1 h of knee extensor exercise (80% of peak performance capacity) in a control condition with one leg and then with the other leg during infusion of intralipid (20%, 1.7 ml/min). Leg blood flow (thermal dilution) and femoral arterial-venous La and NH4 were measured every 10 min. In the control condition La release peaked at 10 min (2.8±0.5 mmol/min) and was only 35% of this value by 60 min. In contrast, NH4 release rose, doubling its value from 10 to 60 min (43±17 to 81±10 µmol/min). The FFA infusion resulted in approximately a 400 µM increase in arterial FFA throughout the exercise. La release was not different from control, but the total NH4 release for the hour was only 65% of the control (4.4±0.6 vs 2.4±0.5 mmoles) with the effect being greatest in the last 30 min. Thus, La and NH4 release are not temporally related in steady-state exercise. The increase in NH4 release with exercise duration and the inhibition associated with elevated FFA suggest that AA metabolism could play a major role in NH4 formation.

Supported in part by NSERC of Canada.

120.10

THE DISTRIBUTION OF AMMONIA AND OF LACTATE IN BLOOD IS ALTERED WITH EXERCISE. R.T. Harris* and G.A. Dudley. Ohio University, Athens, OH 45701 and The Bionetics Corporation, Kennedy Space Center, FL 32899

Subjects (3 M, 3 F) worked (4 min) on a cycle ergometer at 115% of $\dot{V}O_2$ max. Venous samples were drawn pre, directly after (POST) and 15 min after (REC) exercise and analyzed for ammonia (NH_3) and lactate [contents] of plasma, whole blood and red blood cell (RBC) to examine the effect of exercise on blood NH_3 and lactate distribution. Exercise increased ($P<0.05$) the [NH_3] of plasma and RBC with the larger change ($P<0.05$) in plasma than RBC (1.8- vs 0.7-fold). This reduced ($P<0.05$) the RBC to plasma [NH_3] ratio of 2.1 at rest to 1.3. The plasma to RBC [lactate] gradient ($P<0.05$) at rest (0.5 $mmol.l^{-1}$) increased ($P<0.05$) 16-fold POST exercise (8.7 $mmol.l^{-1}$), reflecting the greater increase ($P<0.05$) in plasma than in RBC lactate (15.5 vs 7.5 $mmol.l^{-1}$). [Lactate] and [NH_3] did not decrease ($P>0.05$) POST exercise to recovery. Plasma and whole blood [NH_3] or [lactate] were correlated (r's > 0.93, $P<0.01$) at all sample times, but the slopes of the relations for NH_3 (POST vs REC) or for lactate (pre and POST vs REC) differed ($P<0.05$). The results indicate that exercise alters the distribution of NH_3 and of lactate between plasma and RBC, thus changing the relation between plasma and whole blood contents of these metabolites.

120.11

ALTERED METABOLIC RESPONSES DURING GRADED EXERCISE IN IRON DEFICIENT WOMEN. HC Lukaski, WA Siders* and CB Hall*. USDA, ARS, Human Nutrition Research Center, Grand Forks, ND 58202

To determine the influence of Fe deficiency (FeD) without anemia on metabolic responses to graded exercise, we studied 11 women aged 28 ± 2 ($\bar{x}$ ± SE) yr who resided on a metabolic unit and consumed FeD (4 $mg \cdot d^{-1}$) and Fe repletion (FeR; 16.2 $mg \cdot d^{-1}$) diets made of conventional foods for 80 and 100 d. For the last 14 d of FeR, an Fe supplement of 50 $mg \cdot d^{-1}$ was also fed. Maximal, graded exercise on a cycle ergometer was performed at admission and at the end of each diet period. FeD resulted in a negative cumulative Fe balance, a reduction in serum ferritin (Ftn), an index of body Fe stores, and reductions in hematocrit (Hct) and hemoglobin (Hb) which were within the range of normal values. FeR resulted in an accretion of body Fe and increases in Ftn, Hct, and Hb.

	Hct	Hb	Ftn	$\dot{V}O_2$	$k\dot{O}_2$	RER	LAC
	%	$g \cdot dl^{-1}$	$ng \cdot ml^{-1}$	$L \cdot min^{-1}$	$ml \cdot min^{-1}$		mM
Entry	38.5	13.4	26.3	1.81	112.4	1.06	6.8
FeD	35.5*	12.1*	5.5*	1.78	96.9*	1.23*	9.8*
FeR	36.4**	12.7**	11.8**	1.89	110.7	1.11	7.1

*p <0.05 from entry; **p <0.05 from FeD

Peak oxygen uptake ($\dot{V}O_2$) was unchanged. FeD depressed O_2 kinetics ($k\dot{O}_2$), increased respiratory exchange ratio (RER) and postexercise blood lactate (LAC) levels. Thus reduction of body Fe stores without anemia affects the metabolic response to progressive work by reducing rate of oxygen uptake and increasing carbohydrate use as an energy substrate.

120.12

Effect of exercise on the adaptation in energy intake to a high-fat diet. A. Tremblay, G. Plourde, J.P. Després and C. Bouchard (Spon: D. Richard). Physical Activity Sciences Laboratory, Laval University, Quebec, Canada.

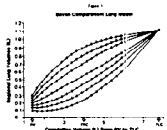
This study was performed to investigate the effect of exercise on short-term regulation of energy intake when consuming a high-fat diet. Six young adult males were tested during two 2-day sessions during which all foods available had a FQ ≥0.85. They were inactive for at least two days before each session except for the fact that a 90-min exercise was performed at 3h PM the day preceding one session. The caloric content of the post-exercise dinner, was adjusted to compensate the surplus in energy expenditure induced by this exercise. The mean increase in daily energy intake noted during the session following exercise was not statistically significant, but considerable interindividual variations were observed (-540 to 537 kcal/day). These variations in energy intake were highly correlated with exercise-induced changes in mean RQ or RQ/FQ ratio (r=0.89 and 0.95, respectively). These results show that exercise may attenuate or amplify the high-fat diet-induced hyperphagia, depending on the magnitude of the exercise-induced increase in fat oxidation.

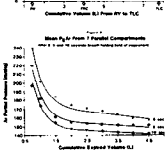
PULMONARY VENTILATION

121.1

COMBINED PARALLEL AND SERIES LUNG COMPARTMENTS TO EXPLAIN THE EXPIRED ALVEOLAR GAS. Julio C. Cruz. Departments of Anesthesiology and Physiology. Medical College of Ohio, Toledo, OH 43699.

The parallel **compartment model (CM)** (JAP 21:749, 1966) and the series **CM** (JAP 21:1331, 1966) were modified here and combined to explain the changes in the phase III and IV of the expired alveolar gas. For a subject with a measured **residual volume (RV)** of 1.4 L, **functional residual capacity (FRC)** of 4 L and **total lung capacity (TLC)** of 7.8 L, seven 4th order polynomial equations have been derived. Each one describes the filling and/or emptying pattern from its corresponding **RV** (9 to 27% of regional **TLC**) to **TLC** where all alveoli are assumed equally expanded (FIG 1). When he inhales a breath of 1.9 L of 79% **argon (Ar)** in O_2 inhaled from **FRC** and exhaled to **RV**, his breath is distributed according to the parallel **CM**. Since Ar is not fully mixed in each parallel compartment with its regional **FRC**, 7 exponential curves were used to describe the decay of **Ar** fraction from .79 at the trachea as a function of 18 aliquots of gas volume for each parallel compartment. The results are shown (FIG 2) for 0, 5 and 10 seconds breath holding time.





121.2

DOES POLYCYTHEMIA IMPAIR PULMONARY GAS EXCHANGE? J. B. West, A.B. Balgos, D. C. Willford. Section of Physiology, University of California San Diego, La Jolla, CA, 92037.

There is considerable disagreement in the literature on whether polycythemia causes hypoxemia. Several studies have claimed that it does, and in some studies phlebotomy has been shown to increase arterial PO_2. However contradictory results have been reported in other studies. We produced acute normovolemic polycythemia in anesthetized dogs by exchange transfusions of packed red cells and measured ventilation-perfusion (VA/Q) relationships using the multiple inert gas elimination technique. The mean baseline hematocrit was 43 ± 5% and this was increased to 57 ± 4% and 68 ± 8% respectively by two transfusions of packed cells. Subsequent plasma transfusions returned the mean hematocrit to 44 ± 4%. Polycythemia caused no significant arterial hypoxemia; indeed there was a slight improvement in the alveolar-arterial PO_2 difference. The multiple inert gas measurements showed no increase in VA/Q inhomogeneity although there was a shift of the distributions to higher VA/Q values due to a decrease in cardiac output caused by increased blood viscosity. We conclude that acute polycythemia has no deleterious effects on pulmonary gas exchange in dog within the hematocrit range of 36 to 76%. Supported by NIH HL 17731.

121.3

EXERCISE CAPACITY FOLLOWING ACUTE EXPOSURE TO HIGH ALTITUDE. N.K. Burki, R. Liles*, J.W. McConnell*. Pul Div/Dept. of Med., University of Kentucky , Lexington, KY 40536

The increase in resting ventilation ($\dot{V}_E$) following acute exposure to high altitude (HA) is mediated primarily by an increase in respiratory frequency (f) with little tidal volume (V_T) change and this is not affected by acute administration of 100% O_2 (Burki, J. Appl Physiol 1984; 56:1027). We examined the effects of HA exposure on exercise capacity in terms of ventilatory pattern and oxygenation in 11 healthy subjects (age range 19-46 yrs) at low altitude (LA; elevation 550 m above sea level) and after rapid transport to HA (elevation 4480 m) where they were studied for 4 days. Subjects exercised according to a standard protocol on a calibrated bicycle ergometer at 0, 50, 100 and 150 watts for 2 minutes at each workload. At HA, mean resting arterial O_2 saturation was 81±4% (mean barometric pressure 444.8mmHg). At HA, $\dot{V}_E$ for any given exercise level was not significantly different ($p>0.05$) from LA at any level of O_2 uptake ($\dot{V}O_2$); however, f was significantly ($p<0.01$) greater and V_T significantly lower ($p<0.01$) for any given workload or $\dot{V}O_2$. Heart rate was significantly ($p<0.01$) higher at all workloads. Thus, the O_2 pulse ($\dot{V}O_2$/HR) was lower for any given workload at HA. At LA, acute administration of 100% O_2 did not alter any of the ventilatory parameters on exercise, nor was there any effect of 100% O_2 on these parameters at HA. We conclude that the changes in ventilatory pattern at rest induced by exposure to HA hypoxia are maintained during exercise, and are not affected by the acute administration of 100% oxygen.

121.4

INSPIRATORY FLOW RATE AND DEAD SPACE IN DOGS. G Berdine*, J Johnson*, D Dale*, and J Lehr* (SPON: R King). Univ Tex Hlth Sci Ctr and Audie Murphy VA Hosp, San Antonio, TX 78284

The stationary front model of gas transport predicts that increased inspiratory flow rate increases dead space (V_D). We tested this hypothesis by measuring physiologic V_D and the kinetics of multiple breath washouts of helium (He) and sulfur hexafluoride (SF_6) from 4 anesthetized, paralyzed dogs (14 - 22 kg). Tidal volume was 20 ml/kg and rate was 12 min^{-1}. pCO_2 was measured from femoral arterial blood after 20 min of ventilation using inspiratory times of 280, 500, 1000, and 2000 msec applied in random order. CO_2 production was calculated from expired gas collections, lung volume was measured by He dilution, and washouts of 10% He and 2% SF_6 were measured by mass spectrometry. V_D for He and SF_6 were calculated from end expiratory concentrations using breath 1 and a pool of breaths 2 - 10. Increased inspiratory flow rate did not increase V_D for CO_2, He, or SF_6. This is inconsistent with the stationary front hypothesis. For each washout, V_D for both He and SF_6 was less for breath 1 than for subsequent breaths and V_D for He (breaths 2 - 10) was less than that for SF_6. Breath 1 V_D for He was not significantly different than that for SF_6. The difference between breath 1 and subsequent breaths is more consistent with axial stratification than parallel inhomogeneity.

121.5

INFLUENCE OF THE PARTICULATE NATURE OF BLOOD ON PULMONARY OXYGEN EXCHANGE. William J. Federspiel. Boston University, Boston, MA 02215

The classical view of oxygen (O_2) uptake in pulmonary capillaries assumes implicitly that capillary blood can be regarded as a continuous hemoglobin solution. In this study a theoretical model was used to examine the role played by the particulate (two-phase) nature of blood on pulmonary oxygen exchange. Red cells were modelled as discrete hemoglobin (Hb) containing spheres flowing in single file through a cylindrical capillary surrounded by a uniform annulus of alveolar tissue. The model accounted for the free diffusion of O_2 from alveolar air space through tissue and plasma, free and Hb facilitated diffusion of O_2 inside red cells, and the intracellular kinetics of O_2-Hb binding. Oxygen uptake was driven by a specified O_2 tension at the air tissue interface. Calculated membrane diffusing capacities (D_m) and hence pulmonary diffusing capacities (D_L) decreased with increasing separation distance between red cells. The reduction in D_m with cell spacing was dependent on the thickness of the tissue and plasma layer relative to red cell dimensions. The results support the hypothesis that the functional area of the alveolar capillary membrane depends on the red cell content of capillaries. Thus D_m cannot be considered solely as a morphological parameter, but has a functional component as well. (Supported by NIH Grant HL-37106.)

121.6

ALTERING THE DISTRIBUTION OF VENTILATION AND PERFUSION IN UNILATERAL LUNG INJURY. Lluis Blanch*, S. Brotherton*, Ch. Roussos, R. Michel and M.R. Angle*. Critical Care Division, Royal Victoria Hospital and Meakins-Christie Laboratories, Montreal, Canada, H3A 1A1.

We studied whether body position could be used in concert with positive end-expiratory pressure (PEEP) or high tidal volumes (V_T) to improve matching of ventilation ($\dot{V}$) with perfusion ($\dot{Q}$) in asymetrical lung disease. Ten anaesthetized ventilated (F_{IO_2} 1.0) dogs were studied in 2 groups: supine (S) or right decubitus (D_R), during PEEP 5 and 12 cmH_2O, at V_T 12 or 24 cc/kg, before and after induction of right lung oedema using ethchlorvynol. We directly measured $\dot{V}$ and $\dot{Q}$ to each lung and expressed than proportionately ($\dot{V}R/\dot{V}TOT$ and $\dot{Q}L/\dot{Q}T$) and derived compliance (C_R,C_L), shunt ($\dot{Q}S/\dot{Q}T$) and alveolar-arterial oxygen gradient (A-a).

			$A\text{-}aO_2$	$\dot{V}R/\dot{V}TOT$	$\dot{Q}L/\dot{Q}T$	C_L	C_R	$\dot{Q}S/\dot{Q}T$
control	S	PEEP			+	-	-	-
		V_T	-		+			
	D_R	PEEP			-	-		
		V_T	+					
oedema	S	PEEP	-	+				-
		V_T						-
	D_R	PEEP	-	+	-	-	+	-
		V_T		+	-	-	+	

P.<05 by 4 way ANOVA

PaO_2 varied from 125 to 451 mmHg with interventions. Supine, PEEP improved gas exchange by improving $\dot{V}$ to the damaged lung. Decubitus, while PEEP increased both $\dot{V}$ and $\dot{Q}$ to the damaged lung, the beneficial effects of $\dot{V}$ redistribution predominated. The effects of V_T were small but additive to those of PEEP. In this model, $\dot{V}$ distribution best explained improved gas exchange during PEEP.

121.7

OVINE RESPONSE TO TRACHEAL TOXIC SMOKE INJURY. SE Morris*, RE Barrow, DN Herndon*. Shriners Burns Institute, Galveston, Texas 77550

Lymph, venous blood flow and permeability changes were measured in the cervical trachea of sheep exposed to cotton smoke for 20 minutes using a double-balloon, double lumen endotracheal tube to limit exposure to a 12 cm segment of trachea. The injured cervical trachea showed a significant increase in blood and lymph flow with no significant change in lymph to plasma protein concentration ratios. With evidence of injury at 4 hours, tracheal blood and lymph flow indicate a maximum injury at 24 hours.

Post Injury (hrs)	Blood Fl. (ml/10cm/hr)	Lymph Flow	Protein Cl/Cp	Wet Wgt (% of Sham)
0	13 ± 4	2.0 ± 1.5	0.46 ± .07	100
4	15 ± 9	7.0 ± 2.2*	0.65 ± .04*	181*
8	25 ± 5*	7.2 ± 1.6*	0.52 ± .09	163*
24	29 ± 5*	9.9 ± 0.5*	0.58 ± .13	172*
48	14 ± 4	7.0 ± 5.5	0.56 ± .14	144*

*Significant difference at $P < 0.05$. Flow rates expressed as ml per 10 cm of tracheal length. Cl/Cp is the ratio of concentrations in lymph to plasma.

Conclusions: The mechanisms which mediate smoke induced hyperemia and tracheobronchial microvascular permeability are at a maximum 24 hours post-injury. Strategies to define and modulate this response must be focused on the immediate post-injury time period.

121.8

FORMATION AND MATHEMATICAL MODELLING OF RESONANT AMPLIFICATION DURING HIGH-FREQUENCY OSCILLATION (HFO). E.H. Bush*, D.R. Spahn*, P.F. Niederer*, E.R. Schmid. Institute of Biomedical Engineering and Medical Informatics, University of Zurich and Swiss Federal Institute of Technology, 8092 Zurich and Institute of Anesthesiology, University Hospital, 8091 Zurich, Switzerland.

In artificial ventilation on the basis of HFO, the oscillatory volume effectively delivered to the lungs is frequency dependent and shows distinct resonance phenomena. With a linear mathematical model, which takes into account the wave propagation characteristics of oscillatory flow, parameters were identified, which determine these resonance characteristics. HFO-circuit properties were found to be essential for the ratio of the oscillatory volume delivered to the lungs in relation to the piston displacement, whereas lung surrogate properties were insignificant. The resonance frequencies were mainly determined by the length of the tube connecting the piston pump with the lung surrogate, and the resonant amplification by the damping factor of the tubes. In addition, the dead space volume (V_d) of the piston pump (volume between the piston at a mean position and the entrance to the tube) showed a striking influence upon the resonance characteristics. At V_d = 0ml, a first resonance frequency (f_{res}) of 40Hz, at V_d = 200ml a f_{res} of 22Hz resulted. These theoretical findings have been substantiated by means of experiments with different lung surrogates. With large piston displacements, additional nonlinear mechanisms influencing the resonance characteristics were found. It is concluded that resonance phenomena during HFO are caused by the wave characteristics of the oscillatory flow and the resulting superposition of primary and reflected waves.

122.1

SPECTRAL ANALYSIS OF PHASIC INSPIRATORY AND EXPIRATORY GENIOGLOSSAL EMG (EMGgg) ACTIVITY IN KITTENS. J.F. Watchko,* K.W. Klesh,* T.L. O'Day,* B.S. Brozanski,* and R.D. Guthrie. University of Pittsburgh Medical School, Pittsburgh, PA 15213

Phasic expiratory (E) EMGgg activity is frequently observed in association with chemically stimulated inspiratory (I) EMGgg recruitment in the kitten (Am Rev Respir Dis 137:379, 1988). We analyzed the power spectral density (PSD) of the EMGgg during I and E to determine the similarities and differences between the neuromuscular electrical activities of the genioglossus muscle during these two distinct phases of the respiratory cycle. EMGgg activity was recorded from bipolar electrodes embedded in the genioglossus muscle in four anesthetized (1.25-1.50% halothane) one-month-old kittens during exposure to a hypoxic gas mixture (FiO_2=0.13). A fast Fourier transformation of EKG free EMGgg was used to compute the PSD of the EMGgg. Greater than 90% of the power within the EMGgg PSD was between 30-500 Hz during both I and E. The mean centroid frequency (±SD) was 278±29 Hz during I and 276±26 Hz during E (p=.71). Moreover, the distribution of power within the EMGgg PSD was comparable between I and E. We conclude that I and E EMGgg PSD are similar. Since there are EMG spectral components that originate in the central nervous system, we speculate that I and E EMGgg activities share common neural input.

122.2

RESPIRATORY ADAPTATION TO HYPERCAPNIA IN NEWBORN RATS AND ITS LONG TERM EFFECTS. R. Rezzonico* nd J.P. Mortola, Dept. of Physiology, McGill Univ., Montreal, Quebec, Canada H3G 1Y6.

We asked which effects chronic hypercapnia in the neonatal period may have on lung growth and the regulation of breathing. Newborn rats were exposed to 7% CO_2-21% O_2 from day 1 to day 7 after birth (Exp) and compared to control litters growing in normocapnia-normoxia (C). At day 7, Exp had similar body weight as C. Ventilation ($\dot{V}E$), measured by flow plethysmography, was higher in Exp since the first day of exposure with an almost double tidal volume. Lung and heart masses were slightly decreased in Exp, with similar total DNA. At day 7 some Exp rats were returned to normocapnia-normoxia for measurements at 45-50 days, i.e. postpuberty. At this age, Exp had a lower $\dot{V}E$ (-13%, P < 0.005, measured by the barometric method) because of a smaller tidal volume (-17%, P < 0.001), with similar oxygen consumption (measured by a manometric technique). In addition, the acute $\dot{V}E$ response to hypercapnia (10% CO_2) was less than in C rats, while that to hypoxia (10% O_2) was the same. We conclude that a) newborn rats respond to chronic hypercapnia with a sustained hyperventilation not associated to an increase in lung mass, differently therefore to the response known to occur during neonatal chronic hypoxia, and b) long term effects on the regulation of $\dot{V}E$ persist at least until postpuberty. (MRC Canada and Migliarina's Foundation).

122.3

EFFECTS OF INHALATION ROUTE ON RESPIRATORY PATTERN AND PULMONARY FUNCTIONS OF HEALTHY ADULTS EXPOSED TO 2 PPM SULFUR DIOXIDE John F. Bedi* and Steven M. Horyath. Institute of Environmental Stress, University of California, Santa Barbara, CA 93106

We have previousily reported of the pulmonary effects of sulfur dioxide (SO2) exposure in normal subjects at concentrations above 1 ppm. We investigated changes in the pulmonary response and respiratory patterns during incremental work of normal subjects following a half hour of continuous exercise in 2 ppm SO2 with either unencumbered breathing or with forced oral or forced nasal breathing. Fourteen healthy subjects (7 men and 7 women), non-smokers, between the ages of 20 and 44 years, participated in three exposures to 2 ppm SO2 and one to filtered air. Three forced expiratory maneuvers were recorded before and after the exposure. Following exposure there was a significant (p<0.0125) pre-post decrease in peak flow independent of the exposure condition. There was a significant interaction effect (p<0.03) between pre-post exposure for Forced Expiratory Flow 25-75%, probably a consequence of differences in pre-values observed between forced oral and unencumbered SO2 trials. There were no differences in the breathing pattern until crossover with the exception of nasal volume and workload (p<.001). The subjects had substantial oral breathing at a lower workload during oral SO2 exposure. (EPRI)

122.4

POSTNATAL DEVELOPMENT OF CAT PHRENIC MOTONEURONS AS REVEALED BY INTRACELLULAR RECORDING. W.E. Cameron, J.S. Jodkowski*, He F.* and R.D. Guthrie. Departments of Neurobiology and Pediatrics, Univ. of Pittsburgh and Magee-Womens Hospital, Pittsburgh, PA 15213

The electrophysiological properties of developing phrenic motoneurons were examined at two postnatal ages (30-42 and 59-66 days) and compared to those of adult cats. Phrenic motoneurons located in the C5 and C6 segments of the spinal cord were identified by antidromic stimulation. This stimulus was delivered by a monopolar electrode placed on the thoracic phrenic nerve close to its muscle entry. The criteria for a successful intracellular impalement was a membrane potential of at least -60 mV and a positive overshoot of the action potential. Axonal conduction velocity (CV) was calculated from the conduction time and the distance between stimulating and recording electrodes: (values are means ± S.D.)

Age (days)	30-43	59-66	Adult
Cond. time (msec)	2.3±0.4	2.2±0.5	2.3±0.4
Axonal CV (m/sec)	33.2±5.5	44.0±7.7	61.8±8.3

Significant differences (p=.0001) were found for mean axonal CV between all groups while no significant differences were found for mean conduction time. Therefore, the increase in axonal CV with age offsets the two-fold increase in length of the phrenic nerve and provides a constant conduction time at all ages. Supported by a grant from NIH (HD22703).

122.5

STIMULATION OF RAPIDLY ADAPTING RECEPTORS (RARs) IN THE CANINE LUNGS BY A SINGLE BREATH OF CIGARETTE SMOKE. Y. R. Kou* and L.-Y. Lee. Dept. of Physiology, Univ. of Kentucky, Lexington, KY 40536.

We previously suggested that the augmented inspiration evoked upon the first breath (b) of cigarette smoke inhalation in dogs is caused by a smoke-induced stimulation of RARs in the lungs (JAP 54:562, 1983). To study the effects of cigarette smoke on RARs, we recorded their activity from afferent filaments of the vagus nerve and delivered approximately 120 ml of high- (HNCS) and low-nicotine cigarette smoke (LNCS) separately in a single ventilatory cycle in 8 anesthetized, open-chest and artificially ventilated dogs. Upon the first breath of smoke delivery, HNCS caused an intense and short burst (lasting 1-2 b) of activity in 15 of 18 RARs; their activity increased from a baseline of 3.1±0.7 imp/b to a peak of 12.4±1.9 imp/b (mean±SEM, n=18). After 10-18 sec when the receptor's discharge returned toward the baseline, a delayed increase in the phasic activity emerged (peak activity=8.6±2.8imp/b, n=18) in 8 of these 18 RARs and lasted for 3-6 b. LNCS evoked a similar immediate stimulation in only 2 of the 15 receptors stimulated by HNCS; the same 2 receptors were also stimulated by the gas phase of LNCS. A mild delayed stimulation (4.1±0.9 imp/b, n=18) induced by LNCS was also noticed. Based upon these preliminary results, we conclude that RARs are stimulated by nicotine delivered in the cigarette smoke. (KTRB grant 41066)

122.6

COMPETITIVE INHIBITION OF CAROTID CHEMOSENSORY RESPONSES TO CO_2 BY O_2. R. Bajaj*, W. Huang*, C. Di-Giulio*, A. Mokashi* and S. Lahiri. Dept. of Physiol., Univ. of Penna., Sch. of Med., Phila., PA 19104-6085.

To further test whether O_2 and CO_2 effects on carotid chemosensory afferents is of competitive type, we studied the responses to CO_2 saturation dose during hypoxia (mean + sem, 48 + 3.8 torr) and hyperoxia (354 + 19 torr) in anesthetized, paralysed and artificially ventilated cats. In one series, steady-state graded levels of CO_2 inhalation showed linear response until a plateau of 18.6 + 1.7 imp/sec was reached at 178 + 18 torr $PaCO_2$ during hyperoxia and 20.1 + 2.3 imp/sec at 109 + 11 torr $PaCO_2$ during hypoxia. In another series, a mixture of saline and blood (3 ml) at PCO_2 of 270 torr was delivered to the carotid body by close intraarterial injection over 8-10 sec. The injectate PO_2 and arterial PO_2 were matched. The injections were promptly followed by a peak chemosensory activity and subsequent adaptation. The peak responses were 63.6 + 8.5 imp/sec and 62.3 + 6.6 imp/sec during hypoxia and hyperoxia respectively. A competitive stimulus interaction between O_2 and CO_2 was confirmed by these studies of CO_2 saturation kinetics. (Supported by NIH grant HL-19737).

122.7

ATRIAL NATRIURETIC FACTOR (ANF) STIMULATES CAROTID BODY CHEMORECEPTORS. V.V. Waters*, W. Huang*, A. Mokashi*, C. Di-Giulio* and S. Lahiri. Dept. of Physiol., Univ. of Penna. Sch. of Med., Phila., PA 19104-6085.

It is known that ANF is released from cardiac granule stores during hypoxia and is involved in cardiovascular reflexes. Since carotid body initiates hypoxic chemoreflexes it was of interest to study the role of ANF in the hypoxic stimulation of carotid body chemoreceptor afferents. We studied the effect of close intraarterial injection of ANF on carotid body chemoreceptor activity in the anesthetized male cats which were paralyzed and artificially ventilated. Graded doses of ANF (10-40 ug) were administered by slow bolus injections at four steady-state levels of arterial PO_2 at a constant PCO_2. ANF increased the chemoreceptor activity over 5 to 10 sec following the injection. These responses were dose dependent, and increased with the decrease of arterial PO_2: the average Δ imp/sec being 0.6, 1.3, 2.9 and 3.9 respectively at arterial PO_2 of > 400, 80, 40 and 30 torr. Since ANF is vasodilatory, the stimulatory effect may be attributed to other than vascular responses. (Supported in part by grants HL-19737 and 5T32 HL-07027).

122.8

EFFECTS OF HISTAMINE (H) INFUSION ON RAPIDLY ADAPTING RECEPTOR (RAR) ACTIVITY AND PULMONARY LYMPH FLOW (PLF). C.T. Kappagoda, K. Ravi* and K.K. Teo*. Dept. of Med., Univ of Alberta, Edmonton, Alta. Canada T6G 2R7.

Experiments were done on anesthetized dogs to examine whether: 1) there is a sustained stimulation of RAR during H infusion and 2) the stimulation occurs through mechanisms other than airway smooth muscle contraction. H was infused through a catheter placed at the right atrium. RAR activity was recorded from the right cervical vagus. Pulmonary lymph was collected by catheterizing the right lymph duct at the neck. During infusion of a threshold dose of H (0.4 ug/kg/min) for 10 min, there was a sustained stimulation of RAR. The neural activity increased significantly from 108±47 imp/min to 140±61 imp/min ($P<0.05$, n=6). There was no significant change in the airway pressure. In five other dogs, infusion of a higher dose of H (4 ug/kg/min) for 10 min also stimulated the RAR in a sustained manner. The RAR activity increased from 40±30 imp/min to 151±41 imp/min ($P<0.001$, n=5). At this dose, the peak airway pressure increased significantly from 6.2±0.3 mmHg to 7.3±0.2 mmHg ($P<0.05$). During H infusion at the rate of 4 ug/kg/min for 30 min, the PLF increased significantly from 1.04±0.34 ml/30 min to 2.93±0.51 ml/30 min ($P<0.05$, n=4). Thus small doses of H infusion produce a sustained stimulation of RAR, independent of airway smooth muscle contraction. It is suggested that an increase in the permeability of the bronchial vasculature may stimulate RAR.

122.9

EVIDENCE FOR O_2-SENSITIVE IONIC CHANNELS IN CAROTID BODY TYPE-I CELLS. Marco A. Delpiano*, [1]Jürgen Hescheler* and Helmut Acker*. (SPON: R. Kinne) MPI für Systemphysiol. Dortmund, F.R.G., [1]Univ. des Saarlandes, Homburg/Saar, F.R.G.

Type-I cells from rabbit carotid bodies in primary culture were studied in voltage-clamp experiments using the whole cell arrangement of the patch-clamp technique. With a pipette containing 140 mM K^+ and 3 mM Mg-ATP as intracellular medium and a modified Locke's solution as extracellular medium, large outward currents were seen positive to a threshold of -30 mV (-50 mV holding potential). Negative to -30 mV, the slope conductance was low (outward rectification). The currents were blocked by K^+ channel blocker (Cs^+ and tetraethylamonium (TEA)) and Co^{2+} (1 mM). Slowly inactivating inward currents were recorded after replacing K^+ by Cs^+ in the intra- and extracellular solution. They showed an U-shaped I-V curve with maximal amplitude of about 300 pA at 10 mV, which was reduced by Ca^{2+} channel blockers (Co^{2+} 1 mM, D600 3/uM, and dihydropyridine (PN 200-110) 10/uM). Lowering pO_2 from control of about 150 Torr (air-gassed medium) to 28 Torr (hypoxia) did not affect inward currents but reduced reversibly outward currents. These data suggest the presence of 1) Ca^{2+}-activated K^+ channels, 2) voltage-activated Ca^{2+} channels, and 3) a linkage between pO_2 changes and the K^+ conductivity of voltage-gated K^+ channels, which could be responsible for cell depolarization and chemosensory response in the carotid body. The nature of this linkage remains to be determined.

122.10

RESPONSES OF RAPIDLY ADAPTING RECEPTORS (RAR) AND SLOWLY ADAPTING RECEPTORS (SAR) TO PULMONARY VENOUS CONGESTION (PVC) AFTER REDUCING THE CONCENTRATION OF PLASMA PROTEINS (PPC). K. Ravi* and C.T. Kappagoda. Dept. of Med., Univ of Alberta, Edmonton, Alta. Canada T6G 2R7.

Experiments were undertaken in anesthetized dogs to examine whether the responses of RAR to PVC could be enhanced by reducing the PPC. Action potentials (AP) were recorded from RAR and SAR in right cervical vagus. PVC was produced in a graded manner by distending a balloon in the left atrium to increase the left atrial pressure (LAP) by 5, 10 and 15 mmHg above control. The PPC was reduced by plasmaphoresis. After identifying a receptor, the effect of graded PVC on it was examined (5 min at each level) both before and after plasmaphoresis. A reduction in PPC from 4.5±0.2 g% to 3.9±0.2 g% enhanced the responses of RAR (n=11) to PVC significantly ($P<0.05$). There was no significant effect upon the activity of SAR (n=5). These results are summarized below:

	LAP (mm Hg)			
RAR activity (AP /min)	Control	+5	+10	+15
Before plasmaphoresis	40±10	95±30	165±66	189±53
After plasmaphoresis	54±16	136±40	267±83	352±108
SAR activity (AP /min)				
Before plasmaphoresis	1627±207	1753±212	1916±211	2166±243
After plasmaphoresis	1679±262	1743±258	1813±261	2022±264

It is suggested that the stimulus to RAR could be fluid transfer in the bronchial vasculature.

122.11

EFFECT OF CAPSAICIN ADMINISTERED INTO THE LOWER AIRWAYS. F. Palecek*, G. Sant'Ambrogio, F.B. Sant'Ambrogio and O.P. Mathew. Dept. Physiology & Biophysics and Dept. Pediatrics, The Univ. of Texas Medical Branch, Galveston, TX 77550.

The cardiopulmonary effects of intravenous capsaicin have been extensively studied. However, it is not clear whether these effects can be elicited by administering capsaicin as an aerosol or topically into the lower airways. We compared the effects of capsaicin injected intravenously, administered topically to the intrathoracic trachea or as an aerosol in 5 anesthetized dogs and 7 rats breathing through a tracheostomy. We measured systemic arterial blood pressure, esophageal pressure and/or airflow. Capsaicin aerosol (100 μg/ml solution for 10-20 s) or topical administration of the same solution (0.05 ml in rats and 0.2 ml in dogs) produced a prolongation of expiration or apnea and transient hypotension similar to those of i.v. injections (5-40 μg/kg). At variance with intravenous injections, the apnea after capsaicin into the airways was not generally followed by tachypnea. Topical instillation of capsaicin was at least as effective as aerosol administration in eliciting these reflexes. We conclude that: 1) capsaicin sensitive receptors are accessible from both the airway lumen and the pulmonary circulation; 2) extrapulmonary afferents play a major role in the reflex responses to intraluminal capsaicin. Supported by NIH Grants HL-20122 and HL-32921.

122.12

HEXAMETHONIUM PREVENTS CIGARETTE SMOKE INDUCED STIMULATION OF VAGAL PULMONARY C-FIBERS IN DOGS. L.-Y. Lee, Y.R. Kou*, C.E. Woolfolk* and D.T. Frazier. Dept. of Physiology, Univ. of Kentucky, Lexington, KY 40536.

We previously suggested that vagal pulmonary C-fibers are stimulated by nicotine delivered in the cigarette smoke (FASEB J. 2:A1297,1988). To test this hypothesis, we studied the responses of these receptors to cigarette smoke before and after pretreatment with hexamethonium bromide (0.8-1.2 mg/kg, i.v.). We recorded the activity of pulmonary C-fibers from afferent filaments of the vagus nerve and delivered 200 ml cigarette smoke in a single respirator cycle in anesthetized, open-chest and artificially ventilated dogs. Cigarette smoke stimulated 8 out of 10 receptors: the activity increased from a base-line of 0.5 ± 0.2 imp/s to a peak of 11.0 ± 3.3 imp/s (mean ± SEM) within 1-2 sec after the smoke delivery. After hexamethonium pretreatment, the same amount of smoke did not activate any of these receptors (peak activity = 0.4 ± 0.2 imp/s). In comparison, responses of the same receptors to right-atrial injection of capsaicin (5 μg/kg) were not abolished by hexamethonium pretreatment: their peak responses to capsaicin were 36.1 ± 3.9 imp/s before and 29.9 ± 4.3 imp/s after hexamethonium, respectively. These results further support the role of nicotine in the cigarette smoke-induced stimulation of pulmonary C-fibers. (Supported by KTRB grant 41066).

122.13

CHARACTERIZATION OF MEDULLARY E NEURONAL RESPONSES TO LUNG INFLATION PATTERNS. J. Bajic*, F.A. Hopp and E.J. Zuperku. Med. Col. Wisconsin and VA Med. Ctr., Milwaukee, WI 53295

The responses of E-neurons in the caudal ventral medulla to various inflation patterns were analyzed in sodium-thiopental anesthetized, paralyzed dogs. A constant level of minute ventilation was obtained from ramp inflations delivered during the I-phase of control breaths by a cycled-triggered solenoid ventilator. Test patterns, measured as transpulmonary pressure (P_T), consisted of graded ramps, reverse ramps, and steps, and were delivered during the E-phase following 6-8 control breaths. The laterality of the responses was assessed using weak electrical stimulation of the intact, desheathed, vagus nerves. Neuronal discharge patterns were quantified using cycle-triggered-histograms. Based on inflation responses, most of the neurons were of two types: 1) P_T inhibited neurons and 2) bidirectional P_T sensitive neurons, i.e. excited by P_T<3-5 mmHg and inhibited by P_T>5-7 mmHg. Responses of both types appear to be mainly P_T dependent with a small degree of time-dependence. Neuronal responses to electrically evoked input patterns were qualitatively similar to those of inflation and indicated that the responses are graded with respect to stimulus strength and frequency. For Type II neurons, the excitatory component appears to be bilaterally mediated by the largest slowly-adapting pulmonary stretch receptor (PSR) afferents, while the inhibition is mainly mediated by ipsilateral PSR afferents with smaller diameters, suggesting the involvement of two types of PSR afferents.

122.14

EFFECTS OF PROGRESSIVE MEDULLARY RESECTIONS ON RHYTHM GENERATION AND CO2 RESPONSIVENESS OF THE IN VITRO BRAINSTEM-SPINALCORD OF THE NEONATAL RAT. H.McLean & J.Remmers, Depts. Medicine & Physiology, U.Calgary, Calgary CANADA T2N 4N1.

The isolated brainstem-spinalcord preparation provides a novel experimental model for the study of neural substrates underlying respiratory rhythm generation and central chemosensitivity. However, two principle concerns of this preparation need to be addressed. One relates to the adequacy of O2 diffusion to the tissue. One approach to reduce hypoxia in this preparation is to reduce the diffusion distance for O2 by resecting medullary tissue not essential to rhythmogenesis or central chemoreception. This approach introduces the other concern, the possibility that surgical removal and sectioning may fundamentally alter these respiratory properties. We have tested the effect of medullary resection on the rhythm generation and CO2 responsiveness of the preparation. Three progressive resections were made:1) removal of the pons 2)a unilateral resection of the rostral medulla 3)a coronal section, removing the dorsal half of the medulla. Respiratory activity was recorded from C3 roots. Removal of the pons increased respiratory frequency while subsequent sections reduced it slightly. The responsiveness of the preparation to 0,3,5, and 10% CO2 were greater after tissue resection than in the intact medulla. The data show that the most rostral aspect and the dorsal half of the medulla are not critical for respiratory rhythmogenesis or central chemoreception. Supported by MRC #MA-9719 & CFSID

122.15

THE DYNAMICS OF CHANGES IN RESPIRATION AND BRAINSTEM BLOOD FLOW DURING HYPOXIA IN THE RAT. Robert A. Darnall*, (SPON: D. Rochester).University of Virginia, Charlottesville, VA 22908.

Changes in brainstem blood flow (BSBF) may contribute to the changes in ventilatory drive occuring during hypoxia. Real time dynamics of these changes have not been described. We recorded changes in phrenic nerve discharge (PND), blood pressure (BP), endtidal CO2, and heart rate during 20 episodes of hypoxic hypoxia (FiO2=0.10) in 9 halothane (0.8%) anesthesized, paralyzed, ventilated rats, and during an additional 3 episodes of hypoxia in 2 carotid body denervated animals (CBD). In three intact and one CBD, laser doppler techniques were used to simultaneously estimate real time changes in perfusion on the dorsal surface of the brainstem. During hypoxia BSBF decreased in both intact and CBD. PND was biphasic (see table) in the intact animals and decreased in the CBD group. BP fell during hypoxia. PND decreased while BP and BSBF were both decreasing. BSBF exhibited a reperfusion peak in both intact and CBD groups (115 ± 31 and 174 ± 38%, respectively) after BP returned to baseline. In the intact animals, an infusion of phenylephrine which maintained BP during hypoxia resulted in a sustained increase in both BSBF and PND. We conclude that BSBF is pressure passive during hypoxia and that the decrease in PND is not due to an alkalosis induced by an increase in BSBF. (R01HL3984)

(% change from baseline)	BSBF	PND peak	PND low
intact	-53 ± 11	157 ± 22	48 ± 25
denervated	-37 ± 17	---	-11 ± 3

122.16

ADENOSINE INHIBITS FETAL BREATHING AND EYE MOVEMENTS IN SHEEP. Brian J. Koos and Kazuhiro Matsuda*. Loma Linda University, Loma Linda, CA 92350.

Hypoxia may inhibit fetal breathing and eye movements by decreasing the cellular phosphate potential (J. Dev. Physiol. 8:67, 1986). A fall in the phosphate potential would increase cellular production of adenosine, which would likely depress breathing. This possibility was tested by infusing intravenously adenosine (0.18 ± 0.04 (SE) mg/kg/min) to 5 chronically catheterized fetal sheep at 125-130 days gestation (0.8 term). Mean fetal P_aO_2 (24.4 ± 1.0 Torr), P_aCO_2 (45.4 ± 2.6 Torr) and pH (7.278 ± 0.021) were not significantly affected by adenosine. During the 4 h control period, the incidences of low voltage electrocortical activity, rapid-eye-movements, and breathing activity were 36 ± 1.8 min/h, 34 ± 2.5 min/h, and 29 ± 1.9 min/h, respectively. During the experimental hour, adenosine reduced the incidence of low voltage electrocortical activity by 20% (N.S.), rapid-eye-movements by 61% ($P < 0.05$), and breathing movements by 77% ($P < 0.05$). These effects on breathing and eye movements are virtually identical to those observed during severe fetal hypoxemia (ΔP_aO_2 = -10 Torr), and they suggest that a hypoxia-induced rise in circulating adenosine concentrations might contribute to hypoxic inhibition in the fetus. (Supported by NIH Grant HD-18478)

122.17

EFFECTS OF PERIAQUEDUCTAL GRAY (PAG) STIMULATION ON VENTILATION IN THE AWAKE GUINEA PIG. Agnes J. Thomas* and E. Chandler Deal, Jr. Case Western Reserve University and the VA Medical Center, Cleveland, OH. 44106

One of us (AT) has shown that electrical stimulation of the PAG in awake animals produces a strong tranquilizing effect, decreasing heart rate, EEG frequency, and galvanic skin potential for several hours (Physiol. Psychol., 12:285, 1982). In the present studies, electrodes were implanted in the PAG (coordinates AP 0.0, lat 0.5, depth 5 mm) in 7 guinea pigs (GP). Two weeks after surgery, breathing was assessed in a body plethysmograph while GP's were awake and breathing room air. Electrical stimulation (0.5 msec pulses, 50 Hz, 0.1 mA for 10 sec) decreased mean minute ventilation (VE) from 419.1±16.8 (x±SEM) to 280.9±27.2 cc/min ($p<0.002$). A second experiment assessed the interaction of these responses with chemical drive to breathe. While GP's breathed 100% O_2, stimulation decreased VE from 316.4±52.0 to 188.5± 28.2 cc/min 10 min post stimulation ($p<0.01$). Slopes of steady state CO_2 curves were not significantly changed, but the post stimulation curve was shifted down and to the right. Twenty-four hrs after stimulation, VE on 100% O_2 was increased toward baseline, but was still significantly reduced. Rectal temperature was unchanged by PAG stimulation. These data indicate that PAG stimulation decreases ventilation while animals breathe either room air or 100% O_2, and does not affect CO_2 responsiveness, suggesting that PAG stimulation may lower O_2 consumption by an unknown mechanism which is unrelated to hypothermia. Supported by: HL-25830 and VA Merit Review

122.18

ACTIVATION OF SUBFORNICAL ORGAN NEURONS INFLUENCES RESPIRATION IN THE RAT. J.T.Fisher, L.M.Beckmann*, and A.V.Ferguson. Dept. Physiology, Queen's University, Kingston, Ont, Canada K7L 3N6.

Despite it's inability to cross the normal blood brain barrier angiotensin II (AII) has been reported to act within the CNS to influence respiration. The subfornical organ (SFO) is a potential CNS site of action for the respiratory effects of AII since it contains a high density of AII receptors, and lacks a normal blood brain barrier. The SFO also sends efferent polysynaptic connections to the medullary respiratory centres, an important CNS site in the control of respiration. The present studies were designed to examine whether activation of SFO neurons influences respiration. Sprague Dawley rats were anaesthetised with urethane (1.4 g/kg). A tracheal cannula / pneumograph was inserted to measure flow and respiratory timing. A stimulating electrode was advanced into the region of the SFO using standard stereotaxic procedures and the effects of electrical stimulation (200uA, 10Hz, 10s) on respiration was evaluated. Augmented breaths were observed in 91% of animals tested with SFO stimulation either during or immediately following the stimulation period while stimulation in adjacent anatomical regions was without effect. No significant changes in respiratory timing, mean inspiratory flow, tidal volume or ventilation were observed during SFO stimulation (ANOVA $p>0.05$). The demonstration of augmented breaths as a result of SFO stimulation supports a role for this circumventricular structure in the neural control of respiration. Supported by the MRC of Canada.

122.19

SIZE-DURATION PRINCIPLE IN PHRENIC ACTIVITY. W. Huang*, C. Liu*, A. Mokashi*, A.K. Sherpa*, C. Di-Giulio* and S. Lahiri. Dept. of Physiol., Univ. of Penna., Sch. of Med., Phila., PA 19104-6085, USA and People's Republic of China.

We studied burst duration and firing threshold of single phrenic units in anesthetized, vagotomised, paralyzed and artificially ventilated cats. The activities of 3-6 units in 3 filaments were compared. The duration of smaller units was 2.2 ± 0.9 sec (mean ± S.D., n=42), and of larger ones 1.3 ± 0.6 sec (n=42). The difference was significant (P 0.01). Hypocapnia gradually diminished the unit activity. The larger units were silenced first. Hypoxia restored the activities, and the smaller units with longer duration reappeared first. Carotid sinus nerve (CSN) section diminished the phrenic activities, the larger units being affected first. Hypercapnia restored the activities with the same relative order of excitation. Hypoxia extinguished the larger units first which, after withdrawal of hypoxia, reappeared last. We conclude that the phrenic burst duration and excitability are an inverse function of unit size. The size-duration principle holds for the hypoxic effects on phrenic unit activities with and without intact CSN. (Supported in part by grants NS 21068 and HL-19737).

122.20

BREATH-TO-BREATH VARIABILITY IN DIFFERENT PHASES OF THE BREATHING CYCLE IN ANESTHETIZED DOGS AT REST. **W. de Vries* and J.H.T. Bates. Meakins-Christie Laboratories, McGill University, Montreal, Quebec, H3A 2B4 Canada.**

Under steady-state metabolic conditions, an animal usually breaths very regularly. There are, however, always slight breath-to-breath variations in the breathing pattern. We have developed a computer program that automatically analyses breathing patterns by dividing a flow signal, recorded from a regularly breathing animal, into individual breaths. Instead of considering only the inspiratory and expiratory parts of each breath, as is usually done, we subdivide each breath into three portions: an inspiratory part, an expiratory part, and a third part labelled the pause. The pause is defined as that part of the breath during which the volume of the lungs (above functional residual capacity) is less than 5% of the tidal volume. We analyzed 10 records of flow containing 41 to 105 consecutive breaths, obtained at 5 minute intervals during regular breathing in each of two normal, anesthetized (pentobarbital sodium), intubated, mongrel dogs. We found in every case that by far the greatest percent and absolute variability occurred in the duration of the pause (standard deviation of pause duration for the two dogs 0.211 s and 0.106 s). The inspiratory durations (s.d's 0.052 s and 0.037 s) and expiratory durations (s.d.'s 0.025 s and 0.036 s) were much more regular. This suggests that, in these animals, once inspiration started, its course was relatively fixed and that subtle breath-to-breath adjustments in ventilation were achieved by varying the time at which each inspiration was initiated. (Supported by MRC Canada.)

122.21

PATTERN OF CYCLIC OXYGEN DE- & RE-SATURATION PREDICTS CENTRAL VS OBSTRUCTIVE APNEA AT 1400 M ALTITUDE. Michael D. Goldman and Kenneth R.Casey.* Univ.of Utah, Salt Lake City, UT 84132.

Continuous monitoring of arterial oxygen saturation (SaO2) during nocturnal sleep is used to detect episodic desaturation occurring with sleep apnea. Oscillographic records of SaO2 provide a useful overview of the pattern of arterial oxygenation over the course of the night, but comparable levels of hypoxemia occur with central and obstructive apnea; and conventional oscillographic records do not permit distinction between the two. We have developed a high resolution computer-assisted oximeter data recording and analysis method which samples SaO2 and heart rate once per second, records these values on disc and simultaneously displays them on the monitor. Obstructive apnea is characterized by a progressive fall in SaO2 followed by an abrupt rise after ventilation resumes, because resumption of ventilation occurs in a "square wave" manner. In contrast, following a central apneic/hypoventilatory episode, ventilation resumes in a gradual "sine wave" manner, and the time course of resaturation is more closely similar to that of the preceding desaturation. Changes in heart rate are much larger in obstructive than central apnea. In a series of 71 consecutive studies heart rate variability was typically 20-50% during obstructive apnea, and 3-10% during central. The "sawtooth" wave of SaO2 correctly predicted obstructive apnea in all but one of 66 studies while the "sine wave" pattern correctly predicted central apnea/hypopnea in each of 11 studies.

122.22

Immediate Diaphragmatic EMG Responses to Imperceptible Mechanical Loads in Conscious Humans. Erik J. Kobylarz*, Joan F. McGovern*, and J. A. Daubenspeck, Thayer School of Engineering, Dartmouth College, and Department of Physiology, Dartmouth Medical School, Hanover, NH 03756.

An esophageal electrode was used to measure the amplitude (EPK) and inspiratory (ETI) and expiratory (ETE) timing responses of the electrical activity of the diaphragm in response to flow resistive (R) and elastic (E) loads at or below the threshold for conscious detection and applied to the external airway of two normal subjects. The pseudorandom loading technique of Daubenspeck and Bennett (J.Appl.Physiol. 55:1160-1166,1983) was used to estimate the immediate electrical responses to loads that are not contaminated (a) by conscious behavioral reactions, (b) by humoral reflexes, nor (c) by any modulating influences of the complex interactions of intrinsic muscle properties and mechanical configuration that would be involved in coupling neural drive to the respiratory mechanics to provide mechanical airflow. Thus these electrical responses are a closer estimate of neural respiratory drive than has been previously available under these loading conditions. There is clear evidence in these results that (1) a rapid, first breath neural reflex exists to modify respiratory timing such that ETE is prolonged significantly in response to R in one subject and shortened in response to E loading in both, and (2) one subject significantly and immediately augmented EPK in response to both R and E loading, while the other did not. These timing responses are consistent in direction with optimally adaptive pattern regulation governed by neural mechanisms. (Supported in part by NIH grants HL29068 and HL07449).

122.23

ARTERIAL BLOOD GASES AND ACID-BASE BALANCE IN BEAGLE DOGS WITH HYPERSENSITIVITY PNEUMONITIS. B. Bota*, P.S. Clifford and R.L. Coon. Depts. of Anesthesiology and Physiology, Medical College of Wisconsin and VA Medical Ctr., Milwaukee, WI 53295

Following sensitization to pigeon serum, acute exposure to this antigen produces an inflammatory condition known as hypersensitivity pneumonitis. Commonly observed sequelae to the development of this condition include a marked tachypnea. To investigate the mechanism of the tachypnea, we studied five dogs which were sensitized and subsequently insufflated with aerosolized pigeon serum. Arterial blood samples were obtained from exteriorized carotid artery loops and blood gases were measured under control conditions and at hourly intervals after insufflation. Prior to insufflation, blood gases were within normal limits (PO_2=89.8 mmHg, PCO_2=36.4 mmHg, pH=7.413). By the second hour, hypoxemia, hypercapnia and acidosis were evident. The largest changes in PO_2 (67.6 mmHg), PCO_2 (47.8 mmHg) and pH (7.292) were observed at the third hour. Breathing frequencies were also maximal at the third hour. Arterial blood gases had returned to control levels by 6 hours and remained stable through the end of the experiment (8th hour). We conclude that these data are consistent with mediation of the observed tachypnea via traditional chemoreceptor mechanisms, although, on the basis of these experiments, we cannot exclude the possible involvement of lung receptors, chest wall receptors or other humoral agents. (Supported by VA Medical Research Service)

122.24

EFFECT OF HEAD-UP TILT ON HUMAN ABDOMINAL EXPIRATORY REFLEX (AER) TO GRADED EXPIRATORY THRESHOLD LOADS (ETLs). J.A. Hirsch, B. Bishop, and F. Cerny. Depts. of Physiology, Physical Therapy and Exercise Science. SUNY/Buffalo, Buffalo, NY 14214.

Human abdominal muscles are recruited during ETLs of 3-25 cm H2O. The ETL required to activate these muscles depends on many factors that modulate abdominal motoneuron (MN) excitability. Our goal is to determine how gravitational loading alters the AER. We change MN excitability by tilting the subject (S) from supine to 60°, thereby shifting the viscera and stretching the abdominal wall. From digitized surface EMGs, airflow recordings, and changes in gastric and pleural pressures, obtained from esophageal balloons, we determine the end-expiratory lung volumes and ETLs at which the AER 1) is just detectable and 2) generates sufficient gastric pressure to aid exhalation. In supine Ss, end-expiratory lung volume (EELV) is reduced; abdominal muscles are silent during ETLs below 15 cm H20, thus EELV increases. On ETLS of 15 cm H20 or greater phasic abdominal activity appears concurrent with expiratory increases in Pga and partially reverses the increase in EELV. With 60° tilt abdominal muscles are tonically active with or without ETL; phasic expiratory activity and Pga oscillations appear at an ETL of 9 cm H20; and EELV is not increased until ETL is 20 cm H20. Thus, the enhancement of AER by head-up tilt facilitates expiration and helps maintain EELV close to control FRC.

123.1

REPLACEMENT VS SUPPLEMENTATION OF DIETARY LIPID WITH FISH OIL: EFFECTS ON CARDIAC INOTROPIC RESPONSIVENESS TO PHENYLEPHRINE. Diane K. Reibel, Michael D. Brown and Carl E. Hock. Thomas Jefferson University, Phila., PA 19107 and University of Medicine and Dentistry of New Jersey-SOM, Camden, NJ, 08103.

Weanling rats were fed either purified diets in which the lipid was replaced with corn oil (CO) or menhaden oil (MO) (REP diet) or diets in which the CO or MO was added to a standard rat chow (SUP diet). Following four weeks of feeding, hearts were isolated and perfused at 60 mmHg aortic pressure. The alpha-adrenoceptor agonist, phenylephrine, was infused into the aorta in graded doses and inotropic responsiveness was monitored by changes in the rate of left ventricular pressure development (Δ+dP/dt). At each dose of phenylephrine employed Δ+dP/dt in hearts of rats fed the MO REP diet was 50% lower than that in hearts of rats fed the CO REP diet. The MO SUP diet resulted in a 25% lower Δ+dP/dt in hearts when compared to the CO SUP diet. The n-3/n-6 fatty acid ratio in myocardial phospholipids was 10-fold higher with MO REP and 3-fold higher with MO SUP when compared to the respective CO diets. The data suggest a correlation between the change in the n-3/n-6 fatty acid ratio in myocardial phospholipids and the reduction in inotropic responsiveness to phenylephrine with fish oil feeding.

123.2

EFFECTS OF POLAR AND NEUTRAL SUGAR DIGITALIS AGENTS ON CARDIAC SYMPATHETIC NERVE ACTIVITY (SNA). Robert W. Caldwell, Nona R. Rebagay*, and Clinton B. Nash. The Medical College of Georgia, Augusta, GA 30912.

Sympathetic nerve activity can be reduced by digitalis through sensitization of certain reflexogenic areas or increased by activation of CNS or peripheral sites. Our purpose was to determine the actions of continuous, progressive i.v. administration of either ASI-222, a polar aminosugar digitalis agent, or digoxin, a neutral cardenolide, on cardiac SNA. Digoxin or ASI-222 were infused into anesthetized dogs at dose-rates which caused cardiac arrhythmias in about 100 mins; these were 1.2 and 0.7 µ/kg/min, respectively. Cardiac SNA was monitored and recorded by a system of differential amplifiers and a digital storage oscilloscope. Infusion of ASI-222 progressively reduced cardiac SNA with increasing doses to a maximum depression of about 38%; the SNA remained depressed even through the onset of cardiac arrhythmias. Digoxin also depressed cardiac SNA (~30% at nadir) at low and intermediate dose levels; but when 70% of the toxic dose had been delivered, cardiac SNA began to rise progressively through the time at which cardiac arrhythmias occurred. Our data indicate that cardiac sympathetic nervous activation is not a component of the cardiotoxicity of ASI-222 as it is for digoxin. Further, it appears that the polar aminocardenolide, ASI-222, does not interact with the same array of reflexogenic areas as digoxin.

123.3

EFFECT OF PHARMACOLOGICAL BLOCKADE OF THE RENIN-ANGIOTENSIN SYSTEM UPON THE HEART RATE POWER SPECTRUM. D.C. Randall, J.D. Yingling* and D.R. Brown*. Depts. Physiol. & Biophys. and Biomed. Engin., Univ. Kentucky, Lexington, KY 40536.

Previous reports indicate that the renin-angiotensin (R-A) system modulates the amplitude of the heart rate spectral peak located at ~0.03 Hz., thereby potentially participating in the moment by moment regulation of the cardiovascular system. This was further tested by recording heart rate (HR) for one hour (cf: The Physiologist, 30: 193, 1987) from each of seven awake, mongrel dogs in the control state and fifteen minutes after administering an angiotensin converting enzyme inhibitor (Enalaprilat, 300 g/kg; supplied by Merck Sharp & Dohme, West Point, PA). The HR power spectrum was calculated by a Fast Fourier Transform; the average amplitude and frequency of the spectral peak between 0.003 Hz. and 0.077 Hz. were determined. We did not observe consistent changes in either variable pre- vs. post-treatment: The average (±SD) amplitude and frequency in the control state were 2.54 ±.55 (relative units) and 0.029 ±.003 Hz, respectively; the corresponding data after enaliprilat administration were 2.84 ±1.44 and .030 ±.002 Hz. We conclude that the renin-angiotensin system does not participate in the short-term regulation of HR. (Supported by NIH grant HL-19343 and KTRB 41066)

123.4

DEPENDENCE OF INTRINSIC HEART RATE ON AUTONOMIC INNERVATION. J.M. Evans*, C.F. Knapp*, J.N. Funk*, J.D. Yingling*, W.C. Randall, and D.C. Randall, Depts. of Biomedical Engineering and Physiology, University of Kentucky, Lexington, KY 40506.

Previous studies indicate that surgical cardiac denervation results in a lower rate of depolarization of the heart's pacemaker cells than does autonomic effector or ganglionic blockade (Brunsting et al., Am. J. Physiol. 245: H592, 1983). Our study used pharmacologic autonomic blockade to unmask the intrinsic heart rate (IHR) in 29 normally innervated and 20 surgically denervated dog hearts. Effector blockade (1-3 mg/kg propanolol, 0.1-0.3 mg/kg atropine and 20-30 mg/kg dibenzylene) in 14 normally innervated dogs resulted in an IHR of 140 ± 8 b/min while ganglionic blockade (hexamethonium 20 mg/kg and atropine 0.1 mg/kg) in 15 normally innervated dogs resulted in an IHR of 128 ± 6 b/min. In dogs which had undergone surgical denervation 3 weeks earlier, effector blockade resulted in an IHR of 98.5 ± 4 b/min in 14 dogs while ganglionic blockade produced an IHR of 90 ± 9 in 6 dogs. The decreased IHR due to surgical denervation was significant in both effector and ganglionically blocked animals. The interaction between cardiac sympathetic and parasympathetic innervation of the pacemaker to determine IHR is being explored using selective surgical parasympathectomy. Preliminary results (N=3) yield an IHR of 110 ± 6 b/min. Supported by AFOSR Grant #80-0039 and NIH HL 19343.

123.5

EFFECT OF RENIN-ANGIOTENSIN SYSTEM SUPPRESSION ON HEART RATE POWER SPECTRUM IN CONSCIOUS RABBIT. J.D. Yingling*, J.B. Charles, C.E.Ott, D.R.Brown*, D.C.Randall. Dept. of Physiology and Biophysics, Univ. of Kentucky, Lexington, KY 40536.

Previous studies indicate that the renin-angiotensin (R-A) system modulates the heart rate spectral peak at 0.04 Hz., and is thus capable of cardiovascular regulation on the order of seconds to minutes. To test this hypothesis, a renin-deplete model was developed in rabbit (n=11). Heart rate was recorded for 30 minutes in the control state, and again after at least seven days treatment with DOCA (1 mg/kg/day) and saline ad lib. Data are from two groups. In the first group (n=5), control data were taken, followed by DOCA/salt treatment. The protocol of the second group (n=6) was reversed, with a minimum of 14 days recovery between DOCA/salt and control. A power spectrum was calculated using a Fast Fourier Transform to determine average amplitude and frequency of the peak between 0.003 Hz. and 0.1 Hz.. There was no significant difference in the amplitude of this peak after suppression of the R-A system (Control = 1.22 ± 0.80; DOCA/salt = 1.74 ± 1.58). However, there was a modest decrease ($p<0.05$) in the frequency of this spectral peak (Control = 0.031 ± 0.006 Hz.; DOCA/salt = 0.025 ± 0.006 Hz.). These data suggest that the R-A system does not participate in short term regulation of heart rate. (Supported by NIH grant HL 19343)

123.6

CARDIAC RECEPTORS DO NOT PLAY A ROLE IN MEDIATING ENHANCED PLASMA RENIN ACTIVITY DURING HEMORRHAGE IN CONSCIOUS DOGS Y-T Shen*, DR Knight*, JX Thomas, Jr, and SF Vatner. Dept of Medicine, Harvard Medical School, New England Regional Primate Research Center, Southboro, MA 01772

To study the role of cardiac receptors in mediating increases in plasma renin activity (PRA) in response to hemorrhage, 3 groups of conscious dogs; 8 intact, 6 with chronic surgical cardiac denervation (CD) and 4 with acute CD (intrapericardial lidocaine) were examined. CD was confirmed by absence of reflex heart rate and depressor responses to veratrine alkaloids. Baseline PRA levels were similar in the 3 groups (intact 0.7±0.2, acute CD 0.5±0.1 and chronic CD 0.6 ±0.2 ng AI/ml/h), suggesting that cardiac receptors were unimportant in the tonic inhibition of PRA. Baseline values of mean arterial and right atrial pressures were also similar in the 3 groups. Hemorrhage (25 ml/kg) reduced mean arterial pressure similarly in the 3 groups (intact 64±5, acute CD 59±7, and chronic CD 69±5 mmHg), and reduced right atrial pressure similarly in the 3 groups (intact -2.1±0.5, acute CD -2.5±0.5, and chronic CD -3.0±0.5 mmHg). The increase in PRA in response to hemorrhage (25 ml/kg) was not significantly different among the 3 groups (intact 6.4±1.8, acute CD 7.8± 1.9 and chronic CD 10.4± 2.1 ng AI/ml/h). Thus, after either acute or chronic CD, both hemodynamics and PRA responses to hemorrhage were not different, indicating that cardiac receptors do not play an important role in mediating enhanced PRA during hemorrhage in conscious dogs.

123.7

EFFECT OF SELECTIVE SURGICAL SA-NODAL PARASYMPATHECTOMY UPON HEART RATE POWER SPECTRUM IN AWAKE DOG. W.C. Randall, D.R. Brown*, D.C. Randall & J.D. Yingling*. Dept. Physiol. & Biophys., Univ. Kentucky, Lexington, KY 40536.

Heart rate (HR) was recorded for one hour in four awake, normal dogs. The SA node (SAN) of each animal was then parasympathectomized (PSx), leaving the sympathetic innervation of the pacemaker intact (cf: Am. J. Physiol., 248:H61, 1985). HR was recorded again, with and without ß-adrenergic blockade (propranolol, 1 mg/kg), after the dogs recuperated. A HR power spectrum was computed using a Fast Fourier Transform (cf: The Physiologist, 30:193, 1987). The table shows the average (± SD) amplitudes (relative units) of the spectral peaks centered around ~0.025 Hz (low frequency, LF) and ~0.32 Hz (high frequency, HF):

	Normal		SAN-PSx	
	LF	HF	LF	HF
control	2.4±1.5	2.0±1.3	1.5±1.1	.04±.05
ß-block			0.6±0.6	.02±.02

The PSx essentially eliminated the HF spectral peak associated with respiratory sinus arrhythmia, confirming the vagal mediation of this rhythm. The PSx decreased the average amplitude of the LF peak in three of four dogs; addition of ß-blockade to the PSx further decreased this amplitude, supporting the dual role of the ß-adrenergic and parasympathetic components of the autonomic nervous system in controlling this rhythm. (Supported by NIH Grant HL 19343)

123.8

SINO-AORTIC BARO/CHEMO-RECEPTOR DENERVATION IN PREGNANT SHEEP. G.W. Aberdeen*, D. Archambault*, J-C. Delifer*, B.S. Nuwayhid and E.W. Quillen, Jr. McGill Univ., Montreal.

Ongoing investigations in our lab require studies in the absence of reflex compensation from arterial baroreceptors. Todate, 6 nonpregnant(NP) and 4 pregnant(PG) ewes have been operated with the following methods and results. Anesthesia was induced with pentothal and maintained under halothane, N_2O and O_2. Left thoracotomy allowed placement of atrial and aortic catheters, a pulmonary artery blood flow probe, and aortic arch denervation via surgical stripping. Recovery was routine for both PG and NP ewes. After 7-10 days, a second surgery permitted bilateral carotid denervation. Mean arterial pressure (MAP) and blood gases (PO_2 and PCO_2) were monitored during ventilation before (VI) and after (VX) denervation, and in the unanesthetized state 0-3hr (POST) following surgery.

	NP=> VI	VX	POST	VI	VX	POST <=PG
MAP (mmHg)	79±4	134±17	113±10	72±2	112±7	30±5
PO_2 (mmHg)	234±50	249±31	62±4	262±40	299±40	44±3
PCO_2 (mmHg)	49±4	48±3	48±3	49±4	51±9	60±4

POST data are worst case only during attempts to remove the ewes from the respirator. In NP ewes, normal levels were achieved with 1-2hr of passive O_2 after which blood gases could be maintained with room air. Fully conscious PG ewes required respiratory assist with O_2 for 2-3hr and nasal O_2 for 12-18hr to avoid a shock state. These data suggest an increased role for the chemoreceptors during pregnancy. Supported by MRC-9804.

123.9

CAROTID BARORECEPTOR FIRING CHARACTERISTICS EVOKED BY DYNAMIC PRESSURE STIMULI. J.L.Seagard, C.A.Salter, F.A.Hopp, C.Dean, and J.P.Kampine. VA Medical Center and Med. Col. Wisconsin, Milwaukee, WI. 53295.

Previous studies which recorded single-fiber carotid baroreceptor activity during slow pressure ramps (1-2 mmHg/sec) have identified two types of receptors, based on baroreceptor activity/carotid sinus pressure (CSP) function curves: Type I, with discontinuous, hyperbolic discharge patterns, and Type II, with continuous, sigmoidal discharge patterns. Type I baroreceptors were found to have larger afferent fibers and greater threshold and saturation frequencies, pressure thresholds and sensitivities (slopes) than Type II baroreceptors. This study was performed to examine whether either type of baroreceptor altered their firing characteristics during more dynamic changes in CSP. Single fiber carotid baroreceptor activity was recorded from the dissected carotid sinus nerve from a vascularly isolated carotid sinus in anesthetized dogs (sodium thiopental, 25 mg/kg + 10 mg/kg/hr). Afferent activity was recorded from the same baroreceptor during carotid sinus pressure ramps of 1-30 mmHg/sec. Pressure ramps of 15-30 mmHg/ sec were found to significantly increase the sensitivity and pressure threshold of Type I baroreceptors, with the greatest changes seen for baroreceptors with the largest afferent fibers. Type II baroreceptor firing characteristics were not altered by different ramp speeds. Results suggest that Type I baroreceptors have more dynamic sensitivity than Type II baroreceptors. (Supported VA Merit Review 7793).

ARRHYTHMIAS II

124.1

IN SITU CARDIAC ELECTROPHYSIOLOGY AND ANTI-REENTRY EFFECTS OF A NEW ANTIARRHYTHMIC AGENT--AHR-12234C. Kuei-Meng Wu,* Anthony Proakis, Thomas Hunter* and John Satterfield,* A. H. Robins Research Labs, Richmond, VA 23220.

We have evaluated the effects of AHR-12234C on cardiac electrophysiology (EP) in normal anesthetized dogs and on a reentry model for atrial tachycardia (RAT) in conscious dogs. Three cumulative doses of AHR-12234C (5, 10 and 10 mg/kg, IV) were employed in open-chest dogs (n = 6) instrumented with atrial and ventricular epicardial electrodes and intraarterial His bundle catheter electrode locating near the aortic valves. AHR-12234C at 5 mg/kg, an effective dose for ablating ouabain-induced arrhythmia in dogs, increased atrial ERP and AH, HV intervals without affecting other EP parameters during pacing at 300 ms cycle length (CL). At higher cumulative doses (15-25 mg/kg), increased AERP, ventricular ERP and AH, HV, PR, QRS intervals while QTc (sinus rhythm) remained unchanged. In the RAT model, as created by contiguous T-shaped incisions situated along intercaval region and right atrium front free wall parallel to AV groove, AHR-12234C (6.5-16.5 mg/kg, IV) prolonged RAT CL by 83 ± 36% from 134 ± 17 to 248 ± 72 msec ($P<0.05$) and terminated the circus movement in 4/4 dogs. From the extent of RAT CL prolongation and EP effects in normal heart, AHR-12234C may be considered as a Class I antiarrhythmic agent without significant toxic depressant effects on conduction and pacemaker systems in the heart.

124.2

ANTIARRHYTHMIC PROPERTIES OF AHR-12234C. Anthony Proakis, Kuei-Meng Wu,* James Shanklin, Jr.,* John Satterfield,* and Thomas Hunter.* A. H. Robins Research Labs, Richmond, VA 23220.

AHR-12234C, N'-[2-(diethylamino)ethyl]-N-[3-(diethylamino)propyl]-N-[2-(phenylsulfonyl)ethyl]urea, 2-hydroxy-1,2,3-propanetricarboxylate hydrate (2:4:1), was evaluated for antiarrhythmic effects in several canine arrhythmia models. A dose of 5 mg/kg, IV, infused over 5 min, of AHR-12234C terminated ouabain-induced ventricular arrhythmias in 6 of 7 dogs whereas lidocaine was effective in 3 of 4 dogs after an infused cumulative dose of 10 mg/kg, IV. In conscious dogs with ventricular arrhythmias (PVC) induced by prior (24 hr) left anterior coronary artery occlusion, AHR-12234C, infused at a rate of 0.5 mg/kg/min, produced complete suppression of PVC in 4 of 5 dogs at a mean ± S.E. dose of 3.8 ± 0.9 mg/kg, IV, with no adverse effects within this therapeutic range. Quinidine was effective in only 4 of 7 dogs at a significantly ($p<0.05$) higher dose of 11.5 ± 3.5 mg/kg, IV. Complete arrhythmia suppression (4 of 5 dogs) with 20 mg/kg, PO, of AHR-12234C was similar (4 of 7 dogs) to that produced by the same dose of disopyramide. The antiarrhythmic action of AHR-12234C persisted beyond 3 hr vs 2.3 hr for disopyramide. AHR-12234C terminated reentrant atrial tachycardia in 2 chronically instrumented dogs after 20 mg/kg, PO, and in 2 other dogs after an additional 40 mg/kg. Termination of the tachycardia was associated with prolongation of cycle length. In the same dogs, a sustained tachyarrhythmia was inducible at 48 hr but not at 24 hr after 60 mg/kg, PO, of AHR-12234C. These results indicate that AHR-12234C is an orally effective antiarrhythmic agent.

124.3

VENTRICULAR FIBRILLATION: MAGNESIUM ION INHIBITS ITS ELECTRICAL INDUCTION AND ISCHEMIA ABORTS IT. J. Morgan*, C. Powell* and L. Cohen University of Chicago, Chicago IL 60637

We have developed a model of electrically-induced ventricular fibrillation (EIVF), using the spontaneously beating (250-325 bpm) Langendorff isolated rat heart. Each heart was surrounded by a 38°C heating jacket and perfused (100 cm H_2O) at 38°C with Krebs-Henseleit solution (5.9 mM K^+; 1.2 mM Mg^{2+}), aerated with 95% O_2, 5% CO_2 (pH 7.4). The coronary flow (CF) was 5-10 ml/min. After a 30 min stabilization period, the left ventricle was stimulated with two stainless steel wire electrodes. Four such sequences were carried out at 30 min intervals with 2 sec pacing bursts (2.5 msec pulses; 200 Hz). The voltage was increased in small increments, at 10 sec intervals, from 0.1-10 volts (V) up to the voltage at which the heart had been in EIVF for 100 sec. The threshold capture voltage (TCV) was 2.0 ± 0.5 V. EIVF was first observed (EIVF-1) at 4.2 ± 0.8 V, and reached a cumulative time of 100 sec (EIVF-100) at 5.8 ± 1.7 V. EIVF-100 was aborted within 49 ± 32 sec of stopping CF at 1.2 mM Mg^{2+}. Similar studies were performed using 3 hearts at each of 10 different $[Mg^{2+}]$, from .01 to 5 mM. The cumulative time in VF was inversely related to the $[Mg^{2+}]$. EIVF-100 occurred in 23/30 studies and the voltage required was hyperbolically related to $[Mg^{2+}]$ (K_m 0.82 ± 0.25 mM). TCV was $[Mg^{2+}]$-independent. Thus, VF was electrically induced in the isolated rat heart; Mg^{2+} inhibited EIVF; and, ischemia aborted EIVF.

124.4

EFFECTS OF TWO CALCIUM CHANNEL BLOCKING AGENTS ON THE VAGAL CHRONOTROPIC RESPONSE IN DOGS. **D.W. Wallick, P.J. Martin, and S.L. Stuesse. Neurobiology Department, NEOUCOM, Rootstown, OH 44272 and Div. of Invest. Med., Mt. Sinai Med. Center, Cleveland, OH 44106**

A brief burst of electrical stimuli (3 pulses per burst, 1ms duration, 10ms interpulse interval) delivered to the vagus nerve during the cardiac cycle elicits a triphasic cardiac chronotropic response. The cardiac cycle length initially increases, then briefly decreases, and subsequently increases again. We compared the effects of two calcium channel blocking agents, verapamil and nifedipine, on these responses to vagal stimulation in morphine-chloralose anesthetized, open-chest dogs (N=4 per group) in sinoatrial nodal rhythm. Verapamil (two doses of 100ug/kg each) did not significantly affect the triphasic chronotropic response. Nifedipine (three separate doses of 10ug/kg, 40ug/kg, and 50ug/kg respectively) attenuated the initial increase in cardiac cycle length caused by vagal stimulation but did not affect the other components of the chronotropic response. Thus the initial slowing caused by vagal stimulation is blocked by nifedipine while other components of the vagal chronotropic response are not.

124.5

THE EFFECTS OF PROPAFENONE, PROPRANOLOL, AND VERAPAMIL ON CLOFILIUM-INDUCED PROARRHYTHMIC RESPONSES IN CONSCIOUS DOGS. K.J. Sansone* and M.E. Sullivan* (SPON: E.H. Cantor). Berlex Laboratories, Inc., Cedar Knolls, NJ 07927.

We investigated the effects of propafenone (P), propranolol (Prop), and verapamil (V) on proarrhythmic responses induced by clofilium (C, a selective class III antiarrhythmic agent). The study was conducted in conscious dogs 4-7 days following myocardial infarction produced by a two-stage occlusion of the LAD. Prior to administration of C (1-2 mg/kg,iv) the mean percent ectopic activity was 1+1% (n=49). Proarrhythmic responses were observed in 29 of the 49 animals treated and consisted of an increase in ectopic activity to 39+3% ($p<0.05$) or the appearance of non-sustained ventricular tachycardia. These 29 animals were randomized into 4 groups for subsequent treatment with saline (n=7), P (2 mg/kg,iv; n=8), Prop (1 mg/kg,iv; n=6), or V (0.1 mg/kg,iv; n=8). Neither saline nor Prop had any effect on the C-induced proarrhythmic response. However, both P and V reduced the C-induced ectopic activity from 34+7% to 14+6% ($p<0.05$) and from 46+8% to 24+9% ($p<0.05$), respectively. These data indicate that beta-adrenergic blockade has no effect on the C-induced proarrhythmic response; however, class I (P) or class IV (V) agents decrease this effect. This study suggests that class I and/or class IV agents may be useful in suppressing potential proarrhythmic effects of class III agents.

124.6

INTERACTIONS OF PROLONGED EXPOSURE TO COCAINE AND SPRINT TRAINING ON VENTRICULAR ARRHYTHMIAS IN RATS. Paul V. Fiore, Robert L. Hamlin, Robert L. Bartels* Exercise Physiology and Veterinary Physiology and Pharmacology, The Ohio State University, Columbus, OH 43210-1092.

The purpose of this study was to evaluate the interaction of cocaine (C) and sprint training (ST) on ventricular arrhythmia (VA) in rats. Sixty rats were separated into four groups: (I) sedentary (S)/no C; (II) ST/no C; (III) ST/C; (IV) S/C. ST was performed for 14 weeks, 4 days per week. Twenty-five mg/kg C was given subcutaneously 4 days per week. After 14 weeks, rats were anesthetized with 2% halothane, watched for spontaneous arrhythmias, and then given graded doses of epinephrine (epi) to determine the arrhythmic dose. Rats were euthanatized. They were weighed (BW), hearts were weighed (HW), and hearts were graded for percent myocardial fibrosis (MF). Trained rats or rats receiving cocaine had significantly greater HW:BW. There were no differences in % MF among groups. No rats manifested spontaneous arrhythmias. There was no differnce in arrhythmic doses of epi among groups. Groups receiving C developed more serious ventricular arrhythmias (*eg.*, sustained tachycardia, R on T, torsad de pointe) and fewer rats in those groups recovered from those arrhythmias than rats denied C. Conclusions are that ST neither aggravates nor protects rats, treated with C for 14 weeks, from ventricular arrhythmias provoked by epi.

124.7

SYNERGISTIC ANTIARRHYTHMIC ACTION OF PROPRANOLOL AND SEMATILIDE IN EXPERIMENTAL MYOCARDIAL INFARCTION. C.M. Doroshuk* and M.E. Sullivan* (SPON: E.H. Cantor). Berlex Laboratories, Inc., Cedar Knolls, NJ 07927.

We investigated the antiarrhythmic activity of propranolol (P), sematilide (S, a specific class III agent), and the combination of P + S against programmed electrical stimulation-induced reentrant ventricular tachyarrhythmias (PES-VT) in conscious dogs 3-22 days following an experimental myocardial infarction produced by an occlusion (120 min)/reperfusion technique. S administered alone (0.1-10 mg/kg,iv) was effective in preventing PES-VT in 7 out of 8 experiments at a mean dose of 1.6 mg/kg,iv. P administered alone (0.3-3 mg/kg,iv; n=5) was ineffective. In another group of dogs, a sub-therapeutic dose of S (0.3-1 mg/kg,iv; n=5) was administered and found not to protect from PES-VT. The addition of P (0.5 mg/kg,iv) to these dogs following the sub-therapeutic dose of S protected against PES-VT in 4 out of 5 dogs. No adverse cardiovascular or CNS effects were noted at any dose tested in animals receiving the agents alone or in combination. In animals receiving the combination therapy, ventricular refractory period (RP) increased 8% following S and 18% with the addition of P. P used alone at this dose had no effect on RP. These data suggest a synergistic action between these two agents. Modulation of beta-adrenergic tone can enhance class III activity and may provide additional therapeutic benefit in preventing PES-VT in dogs following myocardial infarction.

124.8

ANIMAL MODELS OF VENTRICULAR FIBRILLATION (VF)/SUDDEN CARDIAC DEATH (SCD) FOR ASSESSMENT OF ANTIARRHYTHMIC/ANTIFIBRILLATORY AGENTS. P.S. Chan, T.K. McLendon*, G.J. Quirk*, M.A. Ronsberg*, S. Bielen* and P. Cervoni. Cardiovasc. Res., Med. Res. Div., American Cyanamid Company, Pearl River, NY 10965.

SCD is a major killer in the U.S. today and efficacious agents for its prevention are lacking. VF is considered the immediate cause or final event of SCD. Accordingly, using VF/SCD as an end point we examined members of all 4 classes of antiarrhythmic agents in 6 animal models in order to shed light on their efficacy in the prevention of VF/SCD. In coronary artery occlusion- and reperfusion-induced VF/SCD in domestic pigs, sotalol was the most active, followed by β-adrenoceptor blockers (Class II), procainamide and quinidine (Class IA). Typical Class III and IV agents were inactive. Class II agents were very active in coronary artery occlusion- and reperfusion-induced VF/SCD in rats and thevetin (cardiac glycoside)-induced SCD in guinea pigs. In $CaCl_2$- or KCl-induced VF in mice, Class IA, IB and amiodarone and clofilium of Class III were active, while Class IC (flecainide and encainide), Class II, sotalol and bretylium of Class III as well as Class IV agents were inactive. In isoproterenol-induced VF/SCD in heavy rats, Class IV agents were inactive while most members of other classes were generally active. In conclusion, several models of VF/SCD have to be employed to assess the potential of an agent for the prevention of VF/SCD.

124.9

AN IN VITRO GUINEA PIG HEART MODEL PROVIDING CONTRACTILITY AND ELECTROPHYSIOLOGY DATA ON ANTIARRHYTHMIC COMPOUNDS. Kim E. Miller *, John F. Carpenter*, and Robert R. Brooks. Norwich Eaton Pharmaceuticals, Inc., Norwich, NY 13815, A Procter & Gamble Company.

Langendorff guinea pig hearts perfused with Krebs-bicarbonate solution were paced at 180 bpm after removal of the right atrium. Following baseline determinations of coronary flow rate (CF), right ventricular pressure (RVP), the rate of pressure increase (+dP/dt) and decrease (-dP/dt), electrocardiographic intervals PR, QS, and QT, R-wave amplitude, and effective refractory period (ERP), hearts (4-6/compound) were perfused with solutions containing reference antiarrhythmic agents (0.03-100 uM) for 20 min and measurements were repeated. The Class I agent **quinidine** decreased CF, RVP, +dP/dt, -dP/dt, and increased PR, QT, and ERP. **Procainamide** also increased PR and QT. Of Class II agents, **atenolol** had insignificant effects on measured parameters, while **propranolol** was negatively inotropic and increased PR, QS, and QT. The Class III drug **clofilium** increased both QT and ERP. **Sotalol** acted like propranolol, but also increased QT and ERP. A plot of ERP vs QT approximated a straight line with Class III drugs in the upper right quadrant. Class IV drugs, **bepridil** and **verapamil**, had chiefly negative inotropic actions. This model may be used to characterize potential new anti-arrhythmics and identify those with Class III actions.

124.10

EFFECTS OF HIGH DEXTROSE, AND ADENOSINE DURING REPERFUSION IN PURKINJE FIBER. M.L. Bhattacharyya and M. Pattanayek. Meharry Medical College, Nashville, TN 37208

We reported earlier that reperfusion of an ischemic Purkinje fiber (ischemia mimicked with a hypoxic, hyperkalemic and acidotic solution containing 20 mM lactate) leads to arrhythmia (uncontrolled electrical and mechanical activity), subsequent depolarization and cessation of contractile force. In the present study we report that, the manipulation of $(Ca)_o$, $(Mg)_o$, and/or the presence of calcium channel blockers in the perfusate or reperfusate could not prevent the subsequent electrical and mechanical dysfunction during reperfusion. However, the presence of high dextrose (55 mM) during reperfusion stopped arrhythmia in 8 out of 11 experiments. Sucrose in similar concentration was much less effective (prevention of arrhythmia in 1 out of 5 expt.). Adenosine (3 mM), if present during reperfusion, was also protective against reperfusion induced arrhythmia. High dextrose was also able to restore normal electrical and mechanical activity of a depolarized tissue (depolarized due to arrhythmia) much sooner (10-15 min.) if administered after the onset of arrhythmia during reperfusion. These studies seem to indicate that maintenance of an optimum $(ATP)_i$ is more important than other interventions, in preventing reperfusion induced dysfunction.

124.11

DIFFERENT MODES OF RECOVERY OF EXCITABILITY AS DETERMINANTS OF PERIODIC AND NONPERIODIC DYNAMICS IN CARDIAC PURKINJE FIBERS. D.R. Chialvo*, D.C. Michaels*, and J. Jalife. SUNY Health Science Center, Dept. of Pharmacology, Syracuse, NY 13210.

Supernormality (SN), which can be defined as greater than normal excitability during or immediately following action potential repolarization, has been observed in a variety of cardiac preparations. However, as yet, no description of the dynamics of tissue response to repetitive stimulation in the presence of SN has appeared. Isolated sheep cardiac Purkinje fibers (2-5 mm in length) were superfused with Tyrode solution and stimulated with depolarizing current pulses through a suction pipette. The recovery of excitability and the response pattern were measured in each fiber for a wide range of current amplitudes and stimulation frequencies. When the KCl concentration of the Tyrode solution was decreased from 8 to 2 mM, the recovery function consistently changed from monophasic ("normal", N), to biphasic ("supernormal" type 1, SN1), to triphasic ("supernormal" type 2, SN2). In all cases, SN2 corresponded to the boundary between quiescence and spontaneous pacemaker activity. During repetitive stimulation, type N preparations responded only with phase-locked responses (i.e., Wenckebach-like patterns, etc). However, type SN1 and SN2 preparations yielded complex nonperiodic response patterns. An analytical model was developed to further investigate the nature of these behavioral transitions. Model results sugest that the topological changes in the recovery function from SN2 to N (produced by the change in KCl concentration) might be due to "damping" by an unknown subcellular process that is absent in the oscillatory condition and strongly present in the N case. Our results provide a more accurate description of the "supernormality" phenomenon, predict the expected behavior under such conditions, and suggest a hypothetical subcellular process which induces the three types of recovery.

124.12

INDUCTION BY RESIBUGENIN OF DELAYED AFTERDEPOLARIZATIONS IN RABBIT HEART IN VIVO JT.Xie,LH.Xie,CL.Li, N.Liang and X.Feng.(Spon:DH.Singer) Medical Research Center, Nankai Univ. Tianjin,PR.China 300072

Epicardial monophasic action potential (MAP) electrode recordings were used to determine whether resibufogenin (RBG) induced the development of delayed afterdepolarizations(DADs) invivo in the anesthetized rabbit heart (n=20) similar to those described in vitro. Exposure to RBG 0.3mg/kg IV for 30 min was associated with a decline in spontaneous heart rate (from 280±24 to 277±22 BPM) together with decrease in MAP ampletude (from 30.2±5 to 16.7±4 mV) and in $MAPD_{50}$ (from 91.5±13 to 86.7±17 ms).DADs develop in 4/20 animals. They occurred during the terminal portion of phase 3, and exhibited a phasic depolarization process.

DAD characteristics were similar to those described in single cardiac fibers exposed to digitalis glycosides in vitro. The DAD's did not reach threshold and triggered rhythms did not appear. The findings are furhter evidence for the electrophysiologic similarities between RBG and digitalis and suggest that DAD's may contribute to dysrhythmias induced by toxic doses of the former.

124.13

HYSTERESIS IN THE EXCITABILITY PROPERTIES OF THE SINGLE VENTRICULAR MYOCYTE. Carmen Delgado*, Mario Delmar*, Paco Lorente* and Jose Jalife. SUNY/Health Science Center. Syracuse , NY 13210.

Hysteresis is a property common to many physical and biological systems, in which the transition from one stable state (A) to another (B), follows a path that varies according to the direction of the transition (i.e., $A \Rightarrow B \neq B \Leftarrow A$). We have analyzed the existence of hysteresis in the excitability properties of the single ventricular myocyte when changing it from its active (A) to its quiescent (Q) state, or vice versa.

The membrane potential of freshly dissociated guinea pig ventricular myocytes was recorded with suction pipettes in the whole cell, current clamp configuration, and the minimum amount of current necessary to generate an active response following a long (>1min) period of quiescence (I_{th} $Q \Rightarrow A$) was compared to the amount of current needed to maintain cell activation after the cell had been excited regularly at a basic cycle length of 1000 ms (I_{th} $A \Rightarrow Q$). In all instances, I_{th} $Q \Rightarrow A$ was greater than I_{th} $A \Rightarrow Q$, hence exhibiting hysteresis.

The transition $Q \Rightarrow A$ showed a temporary period of "accommodation" in which the delay between the beginning of the current input and the action potential upstroke decreased from one action potential to the next, until a steady-state was achieved. On the other hand, $A \Rightarrow Q$ transition was characterized by a "relaxation" process, in which the amplitude of the subthreshold response decreased progressively following the first failure. Finally, after the addition of $CoCl_2$ to the bath solution, both hysteresis and the transitional state were significantly diminished or abolished, thus suggesting that activation of the calcium current plays a significant role in determining these phenomena.

124.14

PROVOCATIVE TESTING OF SINUS NODE FUNCTION IN THE DOG. Faiza Besbasi* and Robert L. Hamlin. Department of Veterinary Physiology and Pharmacology, The Ohio State University, Columbus, OH 43210-1092

Dysfunction of the sinus node (SN) has been recognized since sick sinus syndrome (SSS) was reported in dogs. One of the most commonly employed electrophysiologic tests used to diagnose SN dysfunction in man is overdrive atrial pacing (AP). The response of the SN to AP in both human and dog (experimental research) is a transient suppression generally known as sinus node recovery time (SNRT). Further investigation of the SNRT in normal dogs is necessary to establish its usefulness in detecting SN dysfunction, particularly SSS, in dog. The purpose of this study was to establish the optimum duration and frequency that consistently yielded the longest SNRT in healthy dogs before and after cholinergic blockade. In 15 anesthetized dogs, the right atrium was paced at varying rates (10-90 beats above basic sinus rhythm) and for varying durations (15-120 sec). This study showed that within a certain range (frequency of 10 to 60 beats above sinus rhythm and duration of 15 up to 60 sec) there is a consistent direct relationship between corrected sinus node recovery time (CSNRT) and duration and frequency of AP. The longest average CSNRT (336 ± 45 msec) was observed when AP was performed for 60 sec at a frequency of 60 beats above basic heart rate. Atropine administration significantly shortened the average CSNRT (136 ± 16). This data could serve to standardize duration and frequency and provide the normal limits against which CSNRT obtained from dogs with suspected SSS can be compared.

124.15

SURGICAL INTERRUPTION OF VAGAL POSTGANGLIONIC PATHWAYS TO THE CANINE SINOATRIAL NODE. M.F. O'Toole, W.C. Randall, R.D. Wurster, W.H. Wehrmacher, M.F. Duff* and J.L. Ardell. Loyola University Medical Center, Maywood, Il 60153

Synapses mediating right and left vagal control of sino-atrial node (SAN) function have been localized in the fat pad near the right superior pulmonary vein (PVFP). Vagal postganglionic pathways to SAN were surgically delineated in 7 dogs. Changes in SAN and atrioventricular node (AVN) functions to electrical stimulation of the right and left cervical vagi and of the sympathetic ansae subclaviae were assessed before and after sequential surgical lesions placed caudal from the junction of the superior vena cava and the pericardium, past the PVFP, and across the suclus terminals to a point midway across the ventral surface of the right atrium. In 4/7 dogs these incisions totally interrupted vagal input to the SAN. A 10-15% residuum of right vagal input remained in 3/7 dogs, which was ablated by lesions of fat pads on the right superior pulmonary vein, suggesting a dorsal route to the SAN. Neither right nor left vagal input to the AVN nor the sympathetic supply to SAN and AVN was interrupted. Thus, the major parasympathetic postganglionic innervation to the SAN is via the free wall of the right atrium, and vulnerable to surgical interventions in this region. Research supported by NIH Grant HL 27595.

124.16

Control of junctional conductance in heart; the phosphorylation hypothesis. W.C De Mello Department of Pharmacology,Medical School,UPR,San Juan PR,00936, USA

The hypothesis that cAMP increases the junctional conductanca(gj) through the activation of a protein kinase and phosphorylation of junctional proteins(De Mello,1983) was tested in cell pairs obtained by enzymatic dispersion of adult rat ventricle.The junctional resistance was obtained by using two separated voltage-clamp circuits.After giga-ohms sealing was achieved the membrane was broken and whole cell clamp was produced. The holding potential of both cells was -40 mV.Cell 1 was then pulsed to -20 mM while the membrane potential of cell 2 was maintained unchanged.gj was estimated from junctional current and transjunctional voltage.Isoproterenol(10-6M) added to the bath increased gj by 40% within 20 sec.When an inhibitor of cAMP-dependent protein kinase was dialyzed into both cells no change in gj was found in the first 5 min.Isoproterenol(10-6M) added to the bath had no effect on gj in cell pairs exposed to protein kinase inhibitor. These results support the view that cAMP is a fast modulator of gj in heart and that the activation of protein kinase is an essential step in the process of enhancement of gj.
Supported by grant HL-34148 from NIH.

124.17

Effect of Adenosine on Potassium and Calcium Currents in Guinea Pig Atrial Myocytes. S. Visentin*, S-N Wu*, L. Belardinelli, Univ. of Florida, College of Medicine, Gainesville, FL 32610.

Adenosine (Ado) decreases the atrial action potential duration (APD) and increases the acetylcholine (Ach) regulated potassium current (iK,Ach). In atrial cells Ado like Ach causes a small decrease in calcium inward current (iCa). In this study we examined the relative contribution of iK,Ach and iCa to the Ado-induced shortening of atrial APD. Experiments were carried out in single atrial myocytes isolated by enzymatic digestion. Both membrane potentials and currents were measured by the whole-cell patch clamp technique. Myocytes were superfused (2-4 ml/min) with prewarmed (35±1°C) Krebs-Henseleit solution (pH 7.4) containing 2.0 mMCa++. Ado caused a dose-dependent a) shortening of APD, b) increase in iK,Ach and c) decrease in peak inward current (iCa). Ado at 3μM and 10 μM increased ik,Ach by 41.5±5.3 and 83±3%, whereas it decreased iCa by only 20±5 and 26±7%, respectively. This apparent decrease in iCa is overestimated because of a concurrent increase in iK,Ach. The same concentrations of Ado decreased APD at 90% repolarization by 35±8 (3μM) and 56±9.0% (10μM). In conclusion, the shortening of atrial APD is mainly due to an increase in iK,Ach and the contribution of iCa to this effect is negligible.
Supported by a grant from NIH (HL35272)

124.18

AMIODARONE-DIGOXIN INTERACTION IN RATS: A REDUCTION IN HEPATIC EXTRACTION. C. Lambert*, D. Lamontagne*, H. Hottlet*, P. du Souich. Research Center, Hôpital du Sacré-Coeur, and Department of Pharmacology, Univ. de Montréal.

The mechanism of the amiodarone (Am)-digoxin (D) interaction, resulting in a marked increase in the D serum concentrations, is still unexplained. The influence of Am on D hepatic extraction in rats was investigated. The D hepatic extraction ratio (ER) was determined using isolated-perfused livers of control and Am-treated (25, 50 and 100 mg/kg/day for 5 days) rats. When plotted as a function of time, an inverse relationship between the ER and the dose of Am was evident. Dose-related reductions were observed in mean D hepatic clearance (from 20 ± 1 to 3 ± 1 at 12 min. and from 17 ± 1 to 2 ± 0.3 ml/min at 20 min. of D infusion; $p < 0.05$, X ± SEM). An analysis of the hepatic effluent by HPLC revealed only D in both control and Am-treated rats. Using liver homogenates, no differences were found in the ability of control or Am-treated (50 mg/kg/day for 5 days) rats to metabolize D. These changes occur without significant lesion of the hepatocytes, as the SGPT and SGOT concentrations were not affected by the administration of Am. Furthermore, a microscopic study of the liver revealed no significant cellular modifications. It was concluded that Am increases the D serum concentrations by inhibiting its hepatic extraction.
(Funded by CAFIR, Université de Montréal).

124.19

COMPARISON OF THE ANTICHOLINERGIC AND α -ADRENERGIC BLOCKING ACTIVITIES OF LNC-834 AND QUINIDINE IN ISOLATED SMOOTH MUSCLE. Chau-Ting Huang and R. H. Burns*. Norwich Eaton Pharmaceuticals, Inc., Norwich, NY 13815

LNC-834 (LNC) (3S-hydroxy-10,11-dihydroquinidine hydrogen sulfate pentahydrate) is an analog of quinidine (Q), the benchmark of antiarrhythmic therapy. In anesthetized dogs, LNC might not have anticholinergic or α-adrenergic blocking activity. To confirm these findings, we compared the effects of LNC and Q in both isolated quinea pig ileum and rabbit aortic strip. At 1 to 100 μM, both LNC and Q decreased ileal responses to acetylcholine (ACh) in a concentration-related manner, yet their effects were much weaker than that of atropine. Unlike atropine, both LNC and Q shifted the curve to the right and similarly reduced the maximum ACh (300 nM) responses. In isolated aorta, like phentolamine, Q (30 and 100 μM) shifted ($p<0.001$) the norepinephrine (NE) concentration-response curve to the right in a parallel manner, suggesting a specific, competitive inhibition. LNC (up to 100 μM), on the other hand, produced much less effect on NE responses. In addition, the aortic response to histamine was not affected by Q or LNC. These results indicate that Q exerts greater antagonism of α-adrenoceptor-mediated effects than LNC, a difference that may be clinically significant as hypotension and vasodilation are undesirable side effects of Q in antiarrhythmic therapy.

125.1

MOLECULAR CHARACTERIZATION OF THE BOVINE ADRENAL ATRIAL NATRIURETIC FACTOR-R_1 RECEPTOR. S. Meloche*, B. Liu*, N. McNicoll*, H. Ong* and A. De Lean. Clin. Res. Inst. and Faculty of Pharmacy, Univ. of Montreal, Montreal, Canada H2W 1R7

Atrial natriuretic factor (ANF) potently inhibits aldosterone secretion by interacting with specific receptors in adrenal zona glomerulosa cells. The adrenal receptor serve as a model for the functional ANF-R_1 subclass or receptors. The ANF-R_1 receptor was purified 13,000-fold, to apparent homogeneity, by affinity chromatography on ANF-agarose and steric exclusion HPLC. SDS-PAGE and silver staining of the purified receptor revealed the presence of a single protein of Mr 130,000. This protein showed the same pharmacological specificity as the native receptor and was sensitive to modulation by amiloride. The receptor also displayed high guanylate cyclase activity insensitive to ANF. Trypsin treatment of the protein produced a 70 KDa fragment which has lost guanylate cyclase (GC) activity but retains intact binding properties and sensitivity to neuraminidase. These results suggest the presence of a region highly sensitive to proteolysis distal to the glycosylated binding domain and the membrane spanning domain, but proximal to the GC catalytic domain.

125.2

COVALENT PHOTOAFFINITY LABELING OF BOVINE ADRENAL ZONA GLOMERULOSA (BAZG) ANF RECEPTOR BY UNDERIVATIZED ^{125}I-ANF. L. Larose*, N. McNicoll*, E. Escher, S. Meloche*, H. Ong* and A. De Léan. IRCM, Univ. Montreal and Univ. Sherbrooke. Quebec, Canada.

The potent physiological effects of atrial natriuretic factor (ANF) on cardiovascular homeostasis suggest that it could be involved in the pathophysiology of certain forms of hypertension. These effects are mediated through specific high affinity ANF receptors, which have been characterized and purified from BAZG. In the process of further studying the molecular properties of ANF receptor binding domain, we have uncovered an unexpected reactivity of the hormone-receptor complex to near-U.V. light exposure. Incubation of BAZG membranes or solubilized ANF receptors with ^{125}I-ANF (99-126) followed by U.V. light exposure revealed by SDS gel electrophoresis and autoradiography a single band with an apparent molecular weight of 130 KDa. Prevention of the photoaffinity labelling was dependent on the concentration of native ANF (99-126) and was not observed with ANF (103-123). Upon photolysis, the label became irreversibly associated with the ANF receptor as suggested by its resistance to heat, guanidine-HCl, urea and trichloroacetic acid denaturation. The yield of covalent labeling of ANF receptors by photolysis was time dependent and reach 2.5% of the specific ^{125}I-ANF bound after 30 min. of U.V. exposure. This method could be very helpful for further studies of the specific active binding site on ANF receptor.

125.3

INTRINSIC AND EXTRINSIC RECEPTORS FOR ATRIAL NATRIURETIC PEPTIDES IN BOVINE AORTIC ENDOTHELIAL CELLS. A.Y. Jeng, M. Lehmann, J. Iljus, and L.P. Wennogle (SPON: G.B. Weiss). Research Department, CIBA-GEIGY Corp., Summit, NJ 07901

Two receptor subtypes for the twenty-eight amino acid atrial natriuretic peptide, ANP(99-126), were characterized in cultured bovine aortic endothelial cells. [^{125}I]-labeled-ANP(99-126) binding was resolved into two components by displacement with ANP(103-123): a predominate high affinity component (K_d = 0.24 nM, B_{max} = 145 fmol/mg protein) and a less-abundant low affinity component (K_d = 700 nM, B_{max} = 28 fmol/mg protein). Two bands with approximate molecular weight of 65,000 and 135,000 were detected by crosslinking followed by SDS/polyacrylamide gel electrophoresis. These bands were present in a 85:15 proportion and the 65K band was preferentially displaced by ANP(103-123). The 65K band could be removed by sonication or by trypsin digestion of intact cells, whereas the 135K band was resistant to either treatments. When trypsin digestion was performed in broken cell preparations, the 135K band was degraded to a band of 75K. These results indicate that the 65K receptor is peripherally associated with the membrane while the 135K receptor is a transmembrane protein which is sensitive to protease digestion only from the cytoplasmic surface of the membrane.

125.4

ANF RECEPTOR SUBTYPES IN RAT AND BOVINE TISSUES. Matthew A. Sills* and Michael Williams. (SPON: J. Liebman). CIBA-GEIGY Pharmaceutical Corp., Research Department, Summit, NJ 07901.

The presence of ANF receptor subtypes has been the focus of increased attention (Maack et al., Science 238: 675). In the present study, competition experiments with rat ANF(1-28) and the C-receptor selective compounds ANF (5-25)(atriopeptin I; API) and the ring-deleted analog C-ANF(4-23) were performed in demedullated rat kidney, rat adrenal and bovine adrenal. In rat kidney, API and C-ANF(4-23) inhibited only 55% of specific [^{125}I]ANF binding, with IC_{50} values of 3 and 1 nM, respectively. ANF(1-28) inhibited 60% of the binding with an IC_{50} value of 0.15 nM and the remainder of the binding with and IC_{50} value of about 30 nM. In bovine adrenal, only ANF (1-28) was able to inhibit [^{125}I]ANF binding with high affinity (0.34 nM). In contrast, API inhibited 64% of the binding in rat adrenal with an IC_{50} value of 2 nM, and the remainder of the binding with an IC_{50} value of 140 nM. ANF(1-28) inhibited all of the binding in rat adrenal with an IC_{50} value of 0.44 nM, whereas CANF(4-23) was unable to inhibit a significant degree of binding. These results indicate that rat kidney contains both B- and C-type ANF receptors and that bovine adrenal contains primarily the B-type ANF receptor. In addition, the ability of API to inhibit a component of binding in rat adrenal that is insensitive to C-ANF(4-23) may be indicative of additional components (states or subtypes) of the ANF receptor.

125.5

IDENTIFICATION OF ATRIAL NATRIURETIC PEPTIDE (ANP) RECEPTORS IN MOUSE Y-1 ADRENOCORTICAL TUMOR CELLS. Seymour Heisler and Bernard P. Schimmer*. Université Laval, Quebec, Que. G1V 4G2 and University of Toronto, Toronto, Ont. M5G 1L6

ANP inhibits glucocorticoid synthesis and secretion from bovine and human adrenal cells. Currently, we sought to establish whether ANP could bind to mouse Y-1 adrenal tumor cells alter,cyclic nucleotide metabolism,and affect steroidogenesis. Y-1 cell membranes specifically bound [^{125}I]-ANP. Association (22°C) was rapid with equilibrium reached within 45 min. Binding of [^{125}I]-ANP was inhibited in a concentration-dependent manner by unlabelled ANP and atriopeptin I ($IC_{50} \simeq 1.2\times10^{-9}$ M and 1.6×10^{-8} M, respectively), but not by C- or N-terminal deleted ANP fragments, ACTH or AVP (up to 10^{-6} M). Scatchard analysis revealed a single class of high affinity binding sites with a K_D of 1.6×10^{-10} M and a Bmax of 2.8×10^{-11} M/mg protein. ANP stimulated cGMP synthesis and secretion by Y-1 cells ($EC_{50} \simeq 3.5\times10^{-9}$ M); the latter process was inhibited by probenecid ($IC_{50} \simeq 5\times10^{-5}$ M). While the atrial peptide partially inhibited ACTH-stimulated cAMP formation ($IC_{50} \simeq 10^{-8}$ M), it had no effect on basal or ACTH-stimulated steroidogenesis. The data demonstrate the presence of specific and saturable ANP receptors in Y-1 cells which are coupled to cyclic nucleotide generating effector enzymes, but which are not coupled to the steroid response of the cells. (Supported by the Medical Research Council of Canada and the Quebec heart Foundation)

125.6

ATRIAL NATRIURETIC FACTOR (ANF) RECEPTORS ARE COUPLED TO ADENYLATE CYCLASE IN RAT PLATELET MEMBRANES. M.B. Anand-Srivastava, A.K. Srivastava, J. Gutkowska* and M. Cantin*. Clinical Research Institute of Montreal, Montreal, Quebec, Canada H2W 1R7.

We have shown previously that ANF receptors in various tissues are negatively coupled to adenylate cyclase. Human platelet membranes, which are shown to be devoid of particulate guanylate cyclase activity have also been reported to have ANF receptors. The present studies were undertaken to investigate if the ANF receptors in platelets are coupled to adenylate cyclase system. ANF inhibited adenylate cyclase activity in a concentration and GTP-dependent manner. The maximal inhibition observed was about 50% with an apparent Ki between 1-5 nM. ANF was also able to inhibit the stimulatory effects of hormones and forskolin on adenylate cyclase to various degrees. In addition, ^{125}I-labeled ANF bound specifically to rat platelet membranes with an apparent Kd of ~ 200 pM. Affinity cross-linking of ^{125}I-ANF to its binding sites in rat pletelet membranes and analysis by SDS-polyacrylamide gel electrophoresis followed by autoradiography showed a predominant labelling of a protein band with an apparent Mr of 66,000. However, no significant labelling of a protein band with a Mr of 130,000 was observed under these conditions. These data suggest that in the platelets the ANF receptor of Mr 66,000 may be coupled to adenylate cyclase system. (Supported by grants from MRC & QHF).

125.7

CHARACTERIZATION OF ATRIAL NATRIURETIC FACTOR RECEPTORS ON PC12 CELLS. A. Rathinavelu, D.B. Wainscott, J. Giridhar, G.E. Isom. Dept. of Pharmacol. & Toxicol., School of Pharmacy & Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.

Atrial Natriuretic Factor (ANF) is a peptide hormone with potent diuretic, natriuretic and vasorelaxation properties. Specific high affinity receptors for ANF have been identified in the adrenal zona glomerulosa in which ANF inhibits both basal and stimulated aldosterone synthesis. We have identified and characterized high affinity receptors for ANF on PC12 cells, a rat pheochromocytoma cell line widely used as a model for catecholamines synthesis and secretion. The apparent dissociation constant for ANF was 6.29×10^{-10}M and Bmax was 1.94×10^{-10}M. The receptor density in these cells calculated as 194,000 ± 20,000/cell. Photoaffinity labelling with [^{125}I]-ANF as the ligand on the membrane revealed two classes of receptors with apparent M_r 70,000 and 130,000. Labelling of both classes of receptors was inhibited in the presence of 2 μM cold ANF in the incubation medium. Internalization of the ligand-receptor complex was observed when the cells were incubated at 37°C with [^{125}I]-ANF and the rate of internalization increased with increasing concentration of ANF. These results suggest that PC12 cells have functional ANF receptors which can be used as model for studying ANF receptor regulation. (Supported by grants from the Indiana Heart Association and PHS SO75505586).

125.8

NEUTRAL ENDOPEPTIDASE (24.11) PLAYS A ROLE IN THE CLEARANCE OF ATRIAL NATRIURETIC PEPTIDE FROM THE CIRCULATION. M. Asaad, N. Delaney, C. Dorso, V. Kratunis, H. Cheung, R. Tung, R. Neubeck, and J. Norman. The Squibb Institute for Medical Research, Princeton, N.J. 08543-4000

The effects of exogenously administered rat ANF-(99-126) on plasma cGMP and ANF concentrations were examined in Sprague-Dawley rats in the presence of the selective neutral endopeptidase inhibitor SQ 28,603. Plasma ANF concentration was determined by a radioreceptor binding assay, which detects only biologically active forms of the peptide. Plasma cGMP concentration was determined by an RIA procedure. SQ 28,603 (100 μmol/kg @ 1 ml/kg i.v.) alone produced modest but statistically significant increases (about 2-fold) in plasma cGMP concentration that lasted for at least one hour, but it did not elicit statistically significant changes in the endogenous levels of plasma ANF. ANF (3 nmol/kg @ 1 ml/kg i.v.) produced marked, but transient increases in plasma cGMP and ANF concentrations. SQ 28,603 (100 μmol/kg @ 1 ml/kg i.v.) administered 30 minutes before ANF (3 nmol/kg @ 1 ml/kg i.v.) markedly augmented both the magnitude and the duration of the ANF-induced increases in the concentration of plasma cGMP and ANF. These results support the hypothesis that neutral endopeptidase contributes to the inactivation and clearance of ANF from circulation.

125.9

RENAL EFFECTS OF A LOW DOSE OF ATRIOPEPTIN III ARE INDEPENDENT OF DA1 DOPAMINE RECEPTOR ACTIVATION. A.S. Bass* and M.B. Murphy* (SPON: L.I. Goldberg). Clinical Pharmacology, University of Chicago, Chicago, IL 60637

The renal effects of a pharmacologic dose of atriopeptin III (ANF) are independent of DA1 dopamine (DA1) receptor activation (Clin Res 34:712A, 1986). To determine if physiologic concentrations of ANF might mediate its renal effects through DA1 receptors, we examined the renal responses to an infusion of ANF at 2.5 ng/kg/min iv, in anesthetized, euvolemic dogs with and without the DA1 receptor antagonist, SCH 23390 (SCH, 0.5 μg/kg/min iv). At this dose SCH completely blocks the increments in urinary sodium excretion (UNaV) and renal blood flow (RBF) produced by an intrarenal-arterial infusion of the DA1 receptor agonist, fenoldopam. Results:

	UNaV (μEq/min)		GFR (ml/min)	
	VEH	SCH	VEH	SCH
Control	24±16.0	37±20.1	31±5.4	27±3.9
ANF	64±29.5*	83±38.0*	34±5.9*	30±4.1*
Recovery	29±10.2	58±22.1	33±5.2	30±3.9

mean ± SEM; *p<0.05, control vs ANF; GFR-glomerular filtration rate; VEH-vehicle; VEH: n=7, SCH: n=9

Infusion of ANF produced comparable increments in UNaV and GFR both in the absence and in the presence of SCH. RBF and mean arterial pressure were not affected by ANF or ANF in the prescence of SCH. These results demonstrate that the renal effects of physiologic concentrations of ANF are independent of DA1 receptor activation. NIH grant GM-22220.

125.10

HEMODYNAMIC AND HEMATOLOGIC RESPONSES TO PHYSIOLOGIC DOSE ATRIAL NATRIURETIC PEPTIDE (ANP) IN CONSCIOUS SHEEP. B. A. Breuhaus, North Carolina State Univ., Raleigh, NC 27606.

Six conscious unstressed sheep with chronic catheters in their right atria and aortic roots and electromagnetic flowmeters on their main pulmonary arteries were used to test the effect of 28 amino acid ANP (25 ng/kg/min for 60 minutes) on arterial pressure (AP), heart rate (HR), right atrial pressure (RAP), cardiac output (CO), total peripheral resistance (TPR), stroke volume (SV), packed cell volume (PCV), total solids (TS), plasma osmolality (OSMO), plasma volume (PV), and mean circulatory filling pressure (MCFP). ANP decreased RAP, CO, and SV ($p<.05$) without changing AP, HR, PV, or MCFP. TPR, PCV, and TS were increased ($p<.05$). These data support the hypothesis that ANP decreases RAP and CO by increasing the resistance to venous return to the heart.

	AP mmHg	HR bpm	RAP mmHg	CO L/min	TPR mmHg·min/L	SV ml
Control	82	70	2.8	5.47	14.89	78.1
ANP (60 min)	80	74	-0.5*	4.59*	18.00*	61.6*
Post Control	80	71	0.4*	4.56*	17.67*	64.4*

	PCV %	TS gm/dl	OSMO mOsm/L	MCFP mmHg	PV ml
Control	24.1	6.1	307	12.2	2851
ANP (60 min)	25.5*	6.2*	301	11.2	2610
Post Control	25.5*	6.1	304	10.3	

* p<.05 re Control

Supported by HL37085

125.11

EFFECTS OF LONG-TERM ELEVATION OF CIRCULATING LEVELS OF ATRIAL NATRIURETIC FACTOR ON ARTERIAL PRESSURE REGULATION IN ANGIOTENSIN II HYPERTENSIVE DOGS. **J.P. Granger, D.L. Stacy, M.J. Solhaug# and M. La Rook#. Dept. of Physiology, Eastern Virginia Medical School, Norfolk, Virginia 23501**

In vitro studies have suggested that ANF is a potent antagonist of the vasoconstrictor actions of angiotensin II. The purpose of this study was to determine the effects of long-term elevation of circulating levels of ANF on arterial pressure regulation in conscious dogs (N=9) with angiotensin II-induced hypertension. Infusion of angiotensin II at a rate of 10 ng/kg/min for 7 days increased mean arterial pressure from 85±3 to 133±4 mmHg. This increase in MAP was associated with an increase in total peripheral resistance and decrease in cardiac output. After 7 days of angiotensin II infusion, ANF was then infused simultaneously at a rate of 20 ng/kg/min for 7 days. Plasma levels of ANF increased from 59±15 to 285±28 pg/ml. Increasing plasma ANF levels for 7 days had no significant long-term effect on arterial pressure (133±4 vs 125±6 mmHg) or cardiac output and total peripheral resistance. There were also no significant changes in sodium balance, glomerular filtration rate or plasma aldosterone concentration during the 7 days of ANF infusion. These data indicate that long-term increases in circulating levels of ANF have minimal chronic hypotensive effects in dogs with angiotensin II hypertension.

125.12

EFFECT OF ATRIAL NATRIURETIC PEPTIDE ON BLOOD PRESSURE AND VASCULAR PERMEABILITY: INFLUENCE OF SODIUM INTAKE. Jean Pierre Valentin*, Jean Ribstein* and Albert Mimran* (SPON: D. Casellas). CHU, 34059 Montpellier, France.

Atrial natriuretic peptide (ANP) antagonizes the effects of some vasoconstrictors, and induces a fluid transfer from the intra - to the extravascular compartment. The influence of sodium intake (LS: low and HS: high sodium for 4 weeks) on the effects of 2 infusion rates of ANP (LD: low = 0.1 and HD: high dose = 1 μg/kg/min for 30 min) was assessed in binephrectomized anesthetized rats by measuring blood pressure (BP), hematocrit (Hct) and plasma protein concentration (PPC). During HD infusion, BP decreased more markedly in LS (-23.7%) than HS rats (-5.5%), whilst the response to LD was similar in LS and HS. The increase in Hct was significantly blunted in LS when compared to HS rats for both ANP infusion doses. The increase in PPC associated with ANP infusion was similar in all groups, and was less marked than that of Hct. These results indicate that chronic sodium intake exerts opposite influence on ANP-induced vasodilation and permeability; the vascular renin system may be a major determinant of the responses to ANP.

125.13

MYOCARDIAL DEPRESSION BY ATRIAL NATRIURETIC FACTOR AT PATHOPHYSIOLOGICAL LEVELS IN THE ANESTHETIZED RABBIT. Andrew J. Rankin and Fred V. Swift*. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Nfld., CANADA A1B 3V6.

Several studies have shown that pharmacological levels of atrial natriuretic factor (ANF) decrease cardiac contractility; others have suggested that this effect is secondary to hemodynamic changes. This study was designed to investigate the cardiac actions of ANF at levels within the pathophysiological range while monitoring plasma levels of the peptide by radioimmunoassay. Inotropic state (dP/dt max) was measured during ANF infusion in two groups of anesthetized rabbits: one in which hemodynamic variables that influence inotropic state, heart rate and afterload, were prevented from changing in response to ANF; and a second group in which hemodynamic variables were allowed to freely change. Myocardial depression was noted in both groups suggesting that ANF has some direct action on cardiac contractility.
Supported by MRC and Canadian Heart Foundation.

125.14

EFFECT OF MASSIVE WEIGHT REDUCTION UPON ATRIAL NATRIURETIC FACTOR IN SPONTANEOUSLY OBESE RATS. David L. Crandall, Robert J. Lozito*, Gregory D. Ferraro* and Peter Cervoni. Dept. Cardiovascular Biological Research, American Cyanamid Company, Medical Research Division, Lederle Laboratories, Pearl River, NY, 10965 U.S.A.

Spontaneously obese rats (799±21 g) were subjected to a program of gradual weight reduction ultimately resulting in an average 40.8% decrease in body weight, or a quantitative reduction of 326 g/rat. Values obtained from these weight-reduced rats were compared to obese, control rats which exhibited a final body weight of 721±23 g. Transition from the obese to lean condition was associated with a reduction in heart weight, atrial weight and left ventricular thickness, while body length remained constant. Adipose tissue depot weight decreased significantly during body weight reduction, and adipocyte volume decreased from 611±79 pl to 104±17 pl. An acute oral dose of furosemide (10 mg/kg) 72 hr before sacrifice yielded 12.3±1.6 ml of urine in obese rats and 8.7±1.5 ml of urine in reduced rats over a 4 hr test period. Circulating α-rANP was 191±13 pg/ml plasma in reduced and 123±18 pg/ml in obese rats when blood was collected directly from the tail artery in the conscious state, yet mean arterial blood pressure remained normotensive for both groups. These results provide insight toward the role of ANP during experimental obesity, as well as during the transition to the lean condition when significant changes in fluid volumes are documented to occur.

125.15

EFFECT OF SHORT TERM TREATMENT WITH EITHER LEAD OR CADMIUM ON THE ATRIAL NATRIURETIC FACTOR (ANF) SYSTEM. Jaisri Giridhar and Gary E. Isom. Dept. of Pharmacol. & Toxicol., Sch. of Pharmacy & Pharmacal Sci., Purdue Univ., W. Lafayette, IN 47907.

Changes in the ANF content has been associated with the progression of hypertension. In this study, the interactions of lead or cadmium, metals associated with altered cardiovascular function, were observed on the ANF system. Male rats (150-175 gms in wt) were treated with 0.01-1.0 mg/kg of Pb or Cd twice a day for 7 days and then maintained for a total of 30 days. The treatment did not alter water consumption and all Pb doses and only the 1 mg/kg Cd dose produces significant decreases in urine output ($p<0.01$). On day 30, the rats were sacrificed and the atria, hypothalamus and plasma were extracted and the content of ANF estimated by RIA. Pb slightly decreased ANF content in the atria and hypothalamus and appeared to have no effect on the release of ANF, whereas Cd appeared to enhance both synthesis and release of ANF. Incubation of PC12 cells with Cd (50 μM) for 90 min decreased the ANF receptor binding by almost 4 fold (Kd=0.18 nM, Bmax=10.7 pM) as compared with binding in control cells (Kd=0.91 nM, Bmax=48.1 pM). These observations indicate Cd produces a significant interaction with the ANF system and these effects may contribute to its cardiovascular toxicity.
(Supported by grants from the Indiana Heart Association and PHS SO75505586).

125.16

NATRIURESIS AND ANF RELEASE DURING VOLUME EXPANSION IN ADRENALECTOMIZED RATS. Victoria Cachofeiro, Gaétan Thibault, Marc Cantin* and Raul Garcia. Clinical Research Institute of Montreal, Montreal, Quebec, Canada, H2W 1R7

The effect of adrenalectomy and replacement therapy on the natriuretic response and release of atrial natriuretic factor (ANF) during acute blood volume expansion (2 increments of 10% of calculated total blood volume) was investigated. Basally, adrenalectomized (ADR) animals showed lower urinary volume (UV). Blood volume expansion increased UV in all groups except in DOC-treated ADR rats. Urinary sodium excretion was significantly increased in sham-operated animals ($p < 0.001$), and ADR rats ($p < 0.05$). ADR+DEXA and ADR DOC rats had lower plasma ANF levels than controls, volume expansion did not modify these values in any group. However, plasma levels of ANF (1-98) increased after volume expansion only in sham-operated animals ($p < 0.05$). ADR and ADR-DEXA rats had higher hematocrit than the other 2 groups ($p < 0.05$). This pattern was not modified by the volume expansion, although a gradual and marked decline in hematocrit was seen in all 4 groups ($p < 0.001$). The lower BP values observed in ADR rats as compared to controls ($p < 0.001$) raised after volume expansion ($p < 0.05$). Similar changes in left atrial pressure were observed in all groups. It can be concluded that adrenalectomy attenuates the natriuretic response during acute blood volume expansion which was not restored to normal with replacement therapy. This lower response was not associated with changes in plasma ANF.

125.17

TACHYCARDIA, ATRIAL DIMENSIONS AND ATRIAL NATRIURETIC PEPTIDE (ANP) IN ANESTHETIZED RABBITS. J.R. Ledsome and K.A. King*. Department of Physiology, University of British Columbia, Vancouver, B.C. V6T 1W5

Tachycardia has previously been shown to increase mean right atrial pressure (RAP) and plasma immunoreactive ANP (iANP) although it was not clear whether ANP release was mediated by the increase in heart rate (HR) or atrial stretch or both. During tachycardia the increase in RAP may be due to increased amplitude of the "a" wave rather than an increase in atrial filling. Therefore, the effects of tachycardia on right atrial dimensions (RAD), a more direct measure of atrial size was investigated in urethane/chloralose anesthetized rabbits. Pacing the heart at a rate of 6 Hz (control=4.2 Hz) did not affect mean arterial pressure (MAP) or RAD, although it did significantly increase RAP and iANP (from 12.9 +/- 2 to 29.6 +/-8.6 pg/ml; n=7). Administration of atenolol (0.4 mg/kg) increased RAP, decreased MAP and HR and increased both RAD and iANP. Tachycardia after atenolol caused a decrease in MAP, an increase in both RAP and RAD and a much larger increase in iANP (from 24.7 +/- 3.6 to 298.6 +/- 71.1 pg/ml; n=7). The results suggest that both an increase in HR and an increase in atrial dimensions can increase iANP independently. When both stimuli occur simultaneously, iANP release is potentiated.
Supported by the B.C. Heart Foundation and MRC of Canada.

125.18

NATRIURETIC/KALURETIC PROFILES OF SIX ATRIAL NATRIURETIC PEPTIDES/ANALOGS AND THREE REFERENCE DIURETICS. P.R. Kastner, L. Emmert*, R. Bohnke*, G. Hage*, F. Hassman*and J.M. Berman* Merrell Dow Research Institute, Indianapolis, IN 46250 and Cincinnati, OH 45215

Studies with atrial natriuretic peptides (ANP) suggest some may be potassium sparing. Experiments were carried out to assess and compare the natriuretic/kaluretic activities of ANP_{1-28}=I(n=9), ANP_{3-28}=II(n=4), ANP_{5-28}=III(n=7), 8-amino octanoic acid substituted ANP_{5-28} ie $[Aoa^{8-10}]ANP_{5-28}NH_2$= Aoa^{8-10}(n=2), linear ANP fragments: $ANP_{8-15}NH_2$=8-15(n=3) and cinnamic acid substituted $[cin^8]ANP_{8-15}NH_2$=cin^8(n=2) plus hydrochlorothiazide (HTZ)(n=4),furosemide (F)(n=3) and amiloride (A)(n=4). Each compound (except HTZ=.3 and 3 mg/kg IP) was dissolved in saline and was infused through a needle-tipped catheter into a renal artery of an anesthetized dog. Both ureters were cannulated for urine collection. Successive doses of A (.0048-244.8 nmol/kg/min),F(.03-1000 μg/kg/min),I,II,III and Aoa^{8-10} (1.2-153.6 pmol/kg/min) and 8-15 and cin^8 (10-10,000 pmol/kg/min) were infused to obtain peak excretory responses. Vol output, Na and K excretion ($U_{Na}V$,U_KV) renal blood flow, BP and HR were measured. Based on peak $U_{Na}V$ and U_KV, the order of natriuretic potency was F> III> II> HTZ> cin^8> I> 8-15> A> Aoa and the order of kaluretic potency was F>III>HTZ>II>I>cin^8>8-15>Aoa>A. Only A decreased U_KV. Peak Na/K excretory ratio greatly favored A(51.4) with 8-15 second(12.0)and I last (5.8). Based on these results, ANPs are not potassium sparing in anesthetized dogs.

125.19

PREGNANCY-INDUCED REFRACTORINESS TO THE UTERINE RELAXANT ACTIVITY OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN RATS. W. Potvin* and D. R. Varma. Dept. Pharmacol. Ther. McGill Univ. Montreal, Quebec, Canada H3G 1Y6

Despite its general smooth muscle relaxant properties, synthetic rat ANP was ineffective in relaxing pregnant rat uterine strips. To determine if this was due to high levels of progesterone, virgin rats were either treated with progesterone (2 mg/kg for 3 d) or 1 uterine horn was ligated before mating. Progesterone abolished the tocolytic activity of ANP. ANP relaxed the nongravid but not the gravid horn of the uterus from pregnant rats. Ovarectomy decreased the tocolytic activity of ANP, but it could be restored by estrogen pretreatment. Refractoriness to the tocolytic activity of ANP was not associated with significant changes in its effects on aortic strips nor in the effects of isoprenaline; also, it was unrelated to prostaglandins and leukotrienes. Data suggest that the tocolytic activity of ANP is modulated by estrogen and progesterone. Pregnancy-induced refractoriness of the uterus to ANP is probably due to high levels of circulating and placentally produced progesterone. Progesterone may alter ANP activity by affecting ANP receptors and/or second messenger. These possibilities are currently being studied. The lack of effect of ANP on pregnant uterus might be physiologically and pharmacologically relevant especially because ANP lowered the blood pressure and increased placental blood flow in rats.

NEUROTRANSMITTER RECEPTORS

126.1

RECEPTOR-COUPLING OF THE M_1 MUSCARINIC ACETYLCHOLINE RECEPTOR (mAChR) EXPRESSED IN A MURINE FIBROBLAST CELL LINE. H.I. Yamamura, L. Mei*, J. Lai*, W.R. Roeske, and J.C. Venter*, Univ. of Ariz, Tucson, AZ 85724 and NINCDS, NIH, Bethesda, MD 20892.

A gene for the mAChR was cloned from a rat genomic library and transfected into a murine fibroblast (B82) cell line (Lai et al., 1988). The mAChRs expressed in these transfected cells were characterized with the non-selective muscarinic antagonist [^{3}H](-)QNB. Kinetic studies showed that association and dissociation of the ligand occurred at 37°C with a K_d value of 14 pM. Saturation studies showed that the binding was of high affinity with a K_d of 12 pM and a Bmax of 17 fmol/10^6 cells. The M_1 selective antagonist pirenzepine was more potent than the M_2 selective antagonist AF-DX 116 in competition studies. The molecular weight of the mAChR was 80 kD. Carbachol stimulated the hydrolysis of inositol lipids in the transfected cells. Pirenzepine was more effective in blocking the stimulation than AF-DX 116. Pertussis toxin and the phobol ester (PMA) both reduced the stimulation. Carbachol did not alter the basal or agonist-induced stimulation of cAMP formation. We conclude that the mAChRs in the transfected B82 cells are of the M_1 type and are associated with the hydrolysis of inositol lipids through a G-protein.

126.2

CHARACTERIZATION OF MUSCARINIC RECEPTORS MEDIATING PHOSPHOINOSITIDE HYDROLYSIS IN 132-1N1 HUMAN ASTROCYTOMA CELLS BY AGONISTS AS M2 TYPE. B. V. Rama Sastry and Clifford C. Stephan*, Dept. of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Stimulation of muscarinic receptors increases phosphoinositide (PI) hydrolysis in 132-1N1 human astrocytoma (AC) cells. To evaluate whether M1 or M2 subtype of receptor mediates PI hydrolysis in AC cells, different muscarinic agonists were studied. The cell pools of inositol-phospholipids were prelabeled by incubating AC cells in a low inositol containing media (CMRL-1066) supplemented with [^{3}H]inositol (2 µCi/ml) for 20-24 hours at 37°C. The cells were washed with a physiological salt solution (PSS, composition in mM, NaCl, 118; KCl, 4.7; $CaCl_2$, 3.0; $MgSO_4$, 1.2; KH_2PO_4, 1.2; EDTA, 0.5; glucose, 10; HEPES, 10). The cells were resuspended in PSS, and PI hydrolysis was measured by accumulation of [^{3}H]inositol-1-phosphate in the presence of 10 mM LiCl. These studies gave the following results: (a) 5-methylfurfuryltrimethylammonium, carbachol, and dl-muscarine were full agonists (EC50 in µM: 14, 37, and 50 respectively); (b) M1 receptor agonist (McN-A-343), partial M2 receptor agonist (5-hydroxymethylfurfuryltrimethylammonium), acetylcholine-releasing agent (5-methoxyfurfuryltrimethylammonium) and nicotine did not exhibit significant effects. These results indicate that the M2 receptor is linked to PI hydrolysis in AC cells. (Supported by The Smokeless Tobacco Research Council, Inc. and The Council for Tobacco Research, USA, Inc.)

126.3

M2ß SUBTYPE OF MUSCARINIC RECEPTORS INVOLVED IN THE STIMULATION OF PHOSPHOINOSITIDE HYDROLYSIS IN 132-1N1 HUMAN ASTROCYTOMA CELLS. Clifford C. Stephan* and B. V. Rama Sastry, Dept. of Pharmacology, Vanderbilt Univ. Sch. Med., Nashville, TN 37232.

Muscarinic (M) agonist-induced phosphoinositide (PI) hydrolysis in 132-1N1 human astrocytoma (AC) cells suggests that M2 muscarinic receptors are linked to PI hydrolysis. To evaluate these M2 receptors, pA_2 values of different muscarinic antagonists for PI hydrolysis in AC cells were determined. The cell pools of inositol phospholipids were prelabeled by incubating the cells in a low inositol containing medium (CMRL-1066) supplemented with [^{3}H]inositol (2 µCi/ml) for 20-24 hours at 37°C. The cells were washed with a physiological salt solution (PSS, composition in mM, NaCl, 118; KCl, 4.7; $CaCl_2$, 3.0; $MgSO_4$, 1.2; KH_2PO_4, 1.2; EDTA, 0.5; glucose, 10; HEPES, 10). The cells were resuspended in PSS, and PI hydrolysis was measured by accumulation of [^{3}H]-inositol-1-phosphate (IP) in the presence of 10 mM LiCl. The following results were obtained: (a) All M antagonists caused a concentration dependent decrease in the level of [^{3}H]-IP formed in response to carbachol (100 µM); (b) The rank order of potencies of antagonists (pA_2 values) has the following order: atropine (8.8) > HHSiD (hexahydra-sila-difenidol, 7.6) > pirenzapine (6.8) > methoctramine (6.0) > AF-DX 116 (an analog of pirenzapine, 5.8). These results support the concept that M2ß receptors are linked to PI hydrolysis. (Supported by The Smokeless Tobacco Research Council, Inc. and The Council for Tobacco Research, USA, Inc.)

126.4

ANOMALOUS PIRENZEPINE-INDUCED ANTAGONISM OF MUSCARINIC RESPONSES. W. Surichamorn*, C.L. Amrhein*, M. McKinney*, E. Richelson, S. Stenstrom*, C.L. Cioffi and E.E. El-Fakahany. Univ. of Maryland Sch. Pharm., Baltimore, MD 21201, Abbott Labs., Chicago, IL 60064 and Mayo Fnd., Rochester, MN 55905.

Pirenzepine (PZ) selectively antagonized muscarinic receptor (MR)-mediated cGMP formation in a noncompetitive fashion in mouse neuroblastoma N1E-115 cells. These effects of PZ were time and concentration dependent and they were also reversible. Interestingly, while atropine elicited competitive antagonism of the cGMP response at low concentrations, it also behaved like a noncompetitive antagonist at higher concentrations and its effects were partially reversible. We investigated whether this deviation from competition could be due to the short time of exposure to MR agonists (30 s) employed in cGMP measurements. Our data indicated that the mode of PZ-induced antagonism of ligand binding to MR was different when assessed using nonequilibrium (30 s) or equilibrium (1 hr) incubations. Thus, PZ appeared to be noncompetitive and competitive under these two conditions, respectively. Furthermore, although PZ blocked receptor-mediated phosphoinositide hydrolysis competitively when the response was measured at 20 min, it was clearly noncompetitive using 5 min incubations. Therefore, the noncompetitive antagonism by PZ detected in cGMP assays might be only apparent and might be attributed, at least in part, to a lack of an equilibrium state under the specific conditions of these assays.

126.5

INHIBITION BY FORSKOLIN OF SMOOTH MUSCLE CONTRACTION IS POTENTIATED BY CHOLERA AND PERTUSSIS TOXINS R.M. Smejkal*, J.W. Covington*, H.K. Yeung*, B.F. Hill*, K.B. Seamon+ and P.K. Chiang Walter Reed Army Institute of Research, Washington, DC 20307 and +NIH, Bethesda, MD 20892

The control of adenylate cyclase through the G-proteins has been implicated in the regulation of many receptor-mediated processes. Stimulation of the muscarinic receptor of guinea pig ileum by acetylcholine (Ach) induces a concentration-dependent contraction. We have found that forskolin inhibits Ach-induced contraction of guinea pig ileum, yielding a pA_2 of 6.1. 7-Deacetyl-forskolin, which has 10% of the cyclase-stimulating activity of forskolin, yielded a pA_2 of 5.1. The stimulation of cAMP production in ileum homogenate by forskolin and its 7-deacetyl analog showed the same 10-fold difference in potency. 1,9-Dideoxy-forskolin was inactive in both assays. The effect of forskolin on ileum contraction was maximal when added 45 seconds prior to the addition of Ach. Cholera toxin (10^{-7} M) and pertussis toxin (10^{-8} M), which affect the G_s and G_i proteins, respectively, have no effect on Ach-induced ileum contraction by themselves at these concentrations, but potentiated the effects of forskolin. 3-Isobutyl-1-methylxanthine (IBMX) (10^{-5} M) and Ro20-1724 (10^{-7} M) also potentiated the inhibitory effects of forskolin These results show that the regulation of smooth muscle contraction may be mediated by adenylate cyclase and the G-protei

126.6

MUSCARINIC RECEPTORS IN THE MUCOSA OF THE CANINE LARYNX AND UPPER TRACHEA. Shigeru Kano*, Jung H Kim* and Jamshid Latifpour* (SPON: R.M.Weiss). Department of Surgery, Yale University, School of Medicine, New Haven, CT 06510

Mucous secretion has a fundamental role in the physiological function of the larynx (lubrication of the vocal folds). Because laryngeal secretion can be influenced by anticholinergic drugs, we studied the muscarinic receptor characteristics in the mucous membranes of various parts of the larynx and upper trachea using radioligand binding techniques. The mucosa was stripped off under a surgical microscope from the following regions : supraglottis (laryngeal surface of the epiglottis), glottis (ventricle), subglottis and trachea (2nd to 7th ring). Muscarinic receptors labeled with ^{3}H-quinuclidinyl benzylate (QNB) showed the following characteristics:

	female		male	
Tissue	Bmax (fmol/mg)	KD (pM)	Bmax (fmol/mg)	KD (pM)
Supraglottis	26± 4	91±16	21±5	155±49
Glottis	11± 2	228±11	13±2	156± 8
Subglottis	91±12	85± 6	48±9*	72± 4
Trachea	22± 2	94± 3	17±6	54± 3

Bmax = maximum number of receptors, KD = equilibrium dissociation constant
*significantly different from comparable value in female ($p<.025$)

The data indicate the presence of sex and regional differences in muscarinic receptor densities in various parts of larynx and upper trahcea. (Supported in part by NIH grant DK 38311)

126.7

N-METHYL-D-ASPARTATE RECEPTORS IN THE GUINEA PIG ILEUM MYENTERIC PLEXUS. Barry D. Sawyer* and Harlan E. Shannon. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

L-GLU, NMDA and carbamyl choline produced concentration-dependent contractions of guinea pig myenteric plexus-longitudinal muscle preparations in Mg^{2+}-free Krebs'. Responses to L-GLU or NMDA, but not carbamyl choline, were blocked noncompetitively by 0.6 mM Mg^{2+}. In the absence of Mg^{2+}, concentration-dependent responses also were elicited by L-ASP, L-homocysteate and D-GLU, but not by quisqualate, kainate or quinolinate. L-GLU was antagonized competitively by D-AP5, CPP (3×10^{-6} to 3×10^{-5}M) or GDGG (3×10^{-4}M), and noncompetitively by GDEE (3×10^{-4}M). GAMS (3×10^{-4}M) did not block L-GLU-induced contractions. Etoxadrol, dextromethorphan and MK-801 noncompetitively antagonized L-GLU. The present results confirm that excitatory amino acid receptors of the NMDA subtype are present in the guinea pig myenteric plexus, and are consistent with the interpretation that this receptor is associated with an ion channel which can be blocked by Mg^{2+} as well as MK-801 and other phencyclidine-like drugs.

126.8

RECONSTITUTION OF BOVINE AORTIC α_1-ADRENOCEPTORS INTO PHOSPHATIDYLCHOLINE DOES NOT RESTORE HIGH AFFINITY PRAZOSIN BINDING. S. Martin Shreeve* and Julia E. Valliere* (SPON: Miles P. Hacker). Univ. of Vermont, Burlington, VT 05405

Solubilization of the membrane-bound bovine aortic α_1-adrenoceptor with digitonin reduces the affinity of prazosin while having no effect on the affinities of several other adrenergic ligands (Shreeve, J. Pharmacol. Exp. Ther., in press). We hypothesized that the native lipid environment regulates the membrane-bound α_1-adrenoceptor and can affect prazosin affinity. This was tested by reconstituting the soluble receptor into phospholipids. Digitonin solubilized receptors were mixed with egg phosphatidylcholine (solubilized in sodium cholate) and reconstituted by gel-filtration on Sephadex G-50. In other experiments the receptor was applied to DEAE-Sephacel and digitonin was then exchanged for sodium cholate. The partially purified receptor was eluted into solubilized phosphatidylcholine and reconstituted by treatment with Biobeads followed by gel-filtration chromatography. In both cases cloudy fractions were pooled and assayed using [^{3}H]prazosin and [^{125}I]HEAT. Approximately 55% of the unpurified receptors and 15% of purified receptors were detected in reconstituted preparations. Phosphatidylcholine did not restore high affinity prazosin binding.
Supported by USPHS Grant HL38853 to S.M.S.

126.9

MODULATION OF BOVINE AORTIC α-2 ADRENERGIC RECEPTORS BY GUANYL NUCLEOTIDES (GN), Na^+ AND AMILORIDE (AML). G. Jagadeesh and R.C. Deth. College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA 02115

The interaction of bovine aortic plasma membrane α-2 receptors with the agonist epinephrine (EPI) and its modulation by Na^+, GN and AML analogs have been examined in radioligand binding studies with the antagonist ligand ^{3}H-Rauwolscine (RAU). The mean K_D and B_{max} for ^{3}H-RAU were 3.6 nM and 120 fmol/mg protein. Addition of Na^+ and GN increased the number of ^{3}H-RAU binding sites by 15-20% and decreased the K_D by 2-fold. However, AML analogs caused a concentration dependent-decrease in K_D and B_{max}. EPI bound with differing affinities (K_{DH} 50 nM, % R_H 40-60; K_{DL} 3 μM, % R_L 40-60) to two different binding sites of α-2 receptors. Na^+ and GN significantly decreased (2-4-fold) the affinity of EPI for ^{3}H-RAU. The simultaneous addition of Na^+ and GN decreased the affinity of EPI 4-5-fold at both the high and low affinity classes of sites, thus indicating a synergistic effect of these two agents. Such synergism reflects different, albeit, closely linked, sites of action for Na^+ and GN acting on α-2 receptors. AML (K_i=54 μM) decreased the affinity of EPI for α-2 adrenoceptors in both high and low affinity binding states. However, it did not alter the % of sites in each state. The data presented here indicate that α-2 receptors possess allosteric binding sites that are sensitive to Na^+ and AML. Further, it is suggested that these allosteric sites are on the α-2 receptor protein itself.

126.10

[^{3}H]L-657,743 (MK-912): A NEW, HIGH AFFINITY, SELECTIVE RADIOLIGAND FOR BRAIN α_2-ADRENOCEPTORS. D.J. Pettibone, S.D. Flagg,* J.A. Totaro,* B.V. Clineschmidt, J.R. Huff*, and R. Chen*, Merck, Sharp and Dohme Research Laboratories, West Point, PA 19486 USA

L-657,743, a highly potent and selective α_2-antagonist (Naunyn-Schmied. Arch. Pharm. 336: 169, 1987) was tritiated to a high specific activity and its binding characteristics to brain tissue were determined. The specific binding of [^{3}H]L-657,743 (50 mM Tris·HCl, pH 7.4, 80 mins at 22° C) to rat cerebrocortex was saturable, reversible, and dependent on tissue concentration. [^{3}H]L-657,743 binding was resolved into two high affinity components exhibiting Kd values of 81 and 880 pM with densities of 81 and 660 fmole/mg protein, respectively. [^{3}H]Rauwolscine saturation binding also exhibited 2 components with somewhat lower affinities (Kd's; 0.25 and 14 nM) but similar densities (Bmax; 38 and 790 fmoles/mg protein). Based on the potencies of various compounds with differing receptor selectivities, the sites labeled by [^{3}H]L-657,743 were characteristic of α_2-adrenoceptors. In contrast to α_2-antagonists, α_2-agonists displayed shallow competition curves. In the presence of 100 μM GTP, Gpp(NH)$_p$ or 150 mM NaCl, the competition curve for epinephrine was shifted to the right, whereas that for yohimbine was unaffected. In human cerebrocortical tissue, [^{3}H]L-657,743 also bound with high affinity to sites characteristic of α_2-adrenoceptors.

126.11

EFFECTS OF 2-BENZYLIMIDAZOLINE AND 2-BENZYLIMIDAZOLE ANALOGS ON ALPHA$_2$-ADRENOCEPTORS IN HUMAN PLATELETS. Urusa Intrasuksri*, Gamal Shams*, Karl J. Romstedt*, Adeboye Adejare*, Michael Clark*, Akihiko Hamada*, Duane D. Miller* and Dennis R. Feller. Div. of Med. Chem. and Pharmacol., Coll. of Pharm., The Ohio State Univ., Columbus, OH 43210.

Benzylimidazolines represent a major chemical class of alpha-adrenoceptor agonists. We have examined the effect of 2-(3',4'-dihydroxybenzyl)-imidazoline [DHBI] and imidazole analogs on human platelet function. DHBI and the 2-fluoro (2-F), 5-F- or 6-F- phenyl ring analogs inhibited epinephrine (E)-induced primary phase aggregation (AGG) with similar IC_{50} values (26,40,62 and 31 μM, respectively). 2-(3',4'Dihydroxybenzyl)imidazole [Cmpd I] and its alpha-hydroxy imidazole analog [Cmpd II] were inhibitors of E-induced AGG (IC_{50} = 131 and 631 μM, respectively. Only Cmpd II was an agonist (EC_{50} = 39 μM) and blocked AGG by arachidonic acid, ADP (secondary wave) and U46619 [(15S)-hydroxy-11α,9α-(epoxymethano) prosta-5Z,13E-dienoic acid, a thromboxane A_2 agonist]. The compounds reversed PGE_1-mediated inhibition of ADP-induced AGG with a rank order of inhibitory potency of 5-F-DHBI > Cmpd I > DHBI > Cmpd II > 2-F-DHBI > 6-F-DHBI. Thus, these drugs act as partial agonists of alpha$_2$-adrenoceptors in human platelets; the alpha-hydroxyl group is important for aggregatory activity, and these results differ from their reported activities on vascular alpha$_1$-adrenoceptors. [Supported by GM 29358].

126.12

LACK OF CROSS-DESENSITIZATION BETWEEN IMIDAZOLINES (Imid) AND PHENETHYLAMINES IN THE RAT VAS DEFERENS (VD): A RECEPTOR BLOCKADE PHENOMENON. Peter J. Rice*, Anwar Hamdi*, Thomas Abraham* & Judy Hardin* (SPON: E.A. Daigneault). Dept Pharmacology, East Tennessee State Univ., Johnson City, TN 37614.

VD repeatedly exposed to 10μM oxymetazoline (OXY) become desensitized to Imid agonists but continue to respond to phenethylamine agonists (Ruffolo et al, 1977). This study examines the role of receptor blockade and agonist efficacy in desensitization and response of the VD.

VD were mounted under 300 mg isotonic tension in PSS at 37°C aerated with 95% O_2/5% CO_2. β-adrenoreceptors, neuronal uptake, extraneuronal uptake and COMT were blocked. Following a cumulative (-)-epinephrine (EPI) concentration-effect (C-E) curve, paired VD were exposed to either 3nM phenoxybenzamine (PBZ) or five x 2 min 10μM OXY at 20 min intervals; another EPI C-E curve was constructed. EPI K_d and fraction of receptors remaining were estimated.

Both PBZ and OXY treatment blocked ≈50% of α-adrenoceptors in the VD; paired pK_ds for EPI (7.2±0.3 vs 6.8±0.4 (n=4)) did not differ significantly, suggesting OXY occupies α-adrenoreceptors as an antagonist with a slow washout. Imids tetrahydrozoline or naphazoline also appeared to block EPI response with individual washout characteristics.

These results suggest that the cross-desensitization differences between some imidazoline and phenethylamine agonists can be explained by kinetic and receptor differences. (Research supported by TN Affiliate Amer. Heart Assn & ETSU).

126.13

POTENTIATION OF ISOPROTERENOL INDUCED CAMP ACCUMULATION IN HUMAN LYMPHOCYTES BY PROTIEN KINASE A OR C INHIBITORS. Ka Kit Hui and Jun Liang Yu*. UCLA Sch of Med, Los Angeles,CA,90024.

To investigate the interaction of various protein kinase A (PKA) or protein kinase C (PKC) inhibitors on beta adrenergic agonist-induced cAMP accumulation, intact human lymphocytes from healthy subjects were incubated with 10^{-8}–10^{-4} M isoproterenol (ISO) and phosphodiesterase inhibitor (IBMX, 1.0 mM) at 37°C for 12 min after 20 min preincubation in the presence or absence of various concentrations of PKA inhibitors [type III, purified from porcine heart (PKI III) and synthetic peptide by rabbit sequence (PKIS)] or structurally different PKC inhibitors [tamoxifen (TAM), clomiphene (CLO), neomycin (NEO) polymyxin B (POLB), N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) or calmidazolium (CALM)].

All PKA inhibitors increased basal cAMP levels by 20-50% while all PKC inhibitors showed inhibitory effects (0-80%) on basal cAMP levels in a dose dependent manner. In contrast, they all, at certain concentrations, [PKI III (1.5 ug/ml), PKIS (0.3 ug/ml), TAM (0.1 uM), CLO (0.1 uM), NEO (10 uM), POLB (0.1 mM), W-7 (10 uM) and CALM (0.1 uM)], potentiated ISO-incuced cAMP accumulation (by 56%, 36%, 55%, 18%, 36%,24% 68% and 42% respectively). These data suggest that the potentiation may be related to the inhibition of PKA and/ or PKC, which have been shown to be involved in beta$_2$ adrenoceptor phosphorylation and desensitization.

126.14

COMPARISON OF DISSOCIATION CONSTANTS ESTIMATED BY β-ADRENOCEPTOR INACTIVATION AND DESENSITISATION R.M. Eglen*, W.W. Montgomery*, and R.L. Whiting* (Spon: A.M. Strosberg) Inst. of Pharmacology, Syntex Research, Palo Alto, CA 94304

Receptor inactivation by exposure to BAAM (bromoacetylalprenolol) or desensitisation by isoprenaline reduces the inotropic responses of rat isolated left atria to β-agonists. Using methods developed by Furchgott (1966), estimates of the dissociation constant (-log Ka) of prenalterol were calculated after receptor inactivation and desensitisation. The q values refer to the remaining receptor number.

The pKa values calculated after exposure to BAAM at .032, .1, .32, 1.0 and 3.2 μM (60 min) were 6.7, 7.0, 7.1, 6.5, and 6.2, respectively, with corresponding q values of .37, .15, .05, .03 and .004. The pKa values calculated after exposure to isoprenaline at 3.2, 32, 320, and 560 nM (120 min) were 7.1, 6.9, 6.9 and 6.8, respectively, with corresponding q values of .52, .34, .15 and .08. Utilizing methods developed by Stephenson (1956) pKa values calculated after exposure to BAAM at .1 and 3.2 μM were 7.1 and 6.0, respectively. The pKa value calculated after exposure to isoprenaline at 560 nM was 7.1.

In contrast to receptor inactivation by BAAM, desensitisation with isoprenaline provides estimates of the Ka value independent of the level of inactivation. Desenitisation provides the more reliable estimate of the dissociation constant of partial β-agonists.

126.15

ADRENERGIC INHIBITION OF HUMAN NEUTROPHIL OXIDANT PRODUCTION. James D. McCafferty*, Robert A. Clark*, and Ross D. Feldman. Department of Internal Medicine, University of Iowa, Iowa City, IA 52242.

Adrenergic receptor agonists may be important modulators of neutrophil activation. To determine the role of α-adrenergic receptor (AR) activation on neutrophil function, we studied the effect of AR agonists on superoxide anion (SOA) generation as assessed by superoxide dismutase-inhibitable ferricytochrome C reduction in intact human neutrophils. The αAR agonist, phenylephrine (PE) did not activate SOA generation nor did it augment threshold fMLP (30nM)-induced SOA generation. PE reduced the rate of submaximal fMLP (100-500nM)-stimulated SOA generation by 72±9% with an ID_{50} of 95nM (-24nM, +33nM). However, the βAR agonist isoproterenol (ISO), also inhibited submaximal fMLP-stimulated oxidant production (91±3%) with an ID_{50} of 8.85nM (-1.72nM, +2.15nM). Moreover, inhibition of oxidant production by both PE and ISO was attenuated by the βAR antagonist, nadolol with an EC_{50} of 132nM (-47nM, +73nM) and 139nM (-15nM, +17nM), respectively. Further, PE inhibition of SOA generation was not blocked by the α_1AR antagonist, prazosin nor by the α_2AR antagonist, yohimbine. The data confirm that βAR activation inhibits human neutrophil oxidant production and that αAR activation does not. Consistent with data from radioligand binding studies, our findings suggest that there is not a functional alpha-adrenergic receptor population on circulating human neutrophils.

126.16

INVESTIGATION OF THE GENETIC REGULATION OF β_2-ADRENERGIC RECEPTORS IN 3T3-L1 FIBROBLASTS. M.T. Nakada*, K.M. Haskell*, D.J. Ecker*, J.M. Stadel*, and S.T. Crooke. Univ. of Pennsylvania, Phila, PA 19104 and Smith Kline and French Labs, Phila, PA 19101

β-adrenergic receptors (β-ar) from mouse 3T3-L1 fibroblasts are up regulated through genetic mechanisms by glucocorticoids (gluc) and butyrate. We cloned and sequenced a 5 kb region of genomic DNA from 3T3-L1 cells containing the β_2-ar gene and a significant amount of 5' and 3' flanking sequence. Analyses of the sequence revealed an exact match to the reverse of an 8 bp consensus sequence which can confer phorbol ester (PE) responsiveness to genes. Further investigation into the role of this site revealed that PE attenuated the regulation of β_2-ar expression by gluc but did not affect the regulation by butyrate. This effect was likely due to the observed PE-induced decrease in gluc receptor number. We examined the methylation of a CG-rich region occurring immediately 5' to the coding region the 3T3-L1 β_2-ar gene with methylation-sensitive restriction enzymes and found no changes in methylation of this region upon gluc or butyrate treatment. Within the 5 kb sequence, 16 putative gluc regulatory elements were found which may mediate the gluc-induced increase in β_2-ar. Five of these sites are conserved in the human β_2-ar sequence. Interestingly, long stretches of 5' and 3' flanking regions from mouse and human β_2-ar genes seem to have been subject to selective pressure to preserve their sequences, suggesting that these regions may be necessary for genetic regulation of β_2-ar. The β_2- and β_1-ar genes differ not only in their coding sequences, but also in their 3' and 5' flanking sequences. This difference is reflected in the abilities of gene activating agents such as gluc and butyrate to independently regulate the two β-ar subtypes. The genetic analyses of regulatory sequences of the two subtypes confirm the observed physiologic controls of receptor subtype expression and offer an explanation as to why the subtypes differ in genetic regulation.

126.17

"ATYPICAL" β-ADRENERGIC RECEPTOR ANTAGONISTS STIMULATE ADENYLATE CYCLASE. J.R. Jasper*, M.C. Michel*, B. Conner* and P.A. Insel, Department of Pharmacology, University of California, San Diego, La Jolla, CA 92093

Several compounds, such as celiprolol (CEL), dichloroisoproterenol (DCI), and pindolol (PIN), which act as antagonists at the β-adrenergic receptor (βAR), also possess a small agonistic component and stimulate βAR-mediated responses without measurable increases in adenylate cyclase (AC) activity. Because the diterpene forskolin can potentiate βAR agonist (e.g. isoproterenol [ISO])-stimulated cAMP accumulation several fold, we reasoned that incubation of target cells with forskolin might reveal stimulation of AC by the partial agonists. As recently reported (FASEB J., 2:A782, 1988), incubation of wild-type (WT) S49 lymphoma cells with 1 μM forskolin plus CEL, DCI, or PIN, but not propranolol, stimulated cAMP accumulation 2- to 5-fold. We now report that this potentiation by forskolin occurs in another cell type (BC_3H_1 muscle cells) as well as in S49 cells. Forskolin fails to induce a response to these partial agonists in cyc^- or unc S49 variants with absent or altered G_s, respectively. We also have found that the partial agonists can elicit cAMP accumulation in WT S49 cells even in the absence of forskolin, if cAMP is assayed using a very sensitive RIA. Thus, these partial agonists induce a very modest stimulation of AC by βAR and forskolin appears to amplify this stimulation by enhancing interaction with the G_s protein.

126.18

STRATEGIES OF MAKING MONOCLONAL ANTIBODIES AGAINST DOPAMINE RECEPTORS. L.K. Srivastava*, S.B. Bajwa* and R.K. Mishra. Depts. of Psychiatry & Neurosciences, HSC-4N52, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

The biochemical characterization of central nervous system dopamine receptors (both D_1 and D_2 subtypes) has so far been rather slow mainly due to the lack of suitable biochemical tools. A monoclonal antibody (McAb) against the receptor would greatly help in purification, localization and structure-function studies of the receptor. Recently, we made several attempts to produce McAbs against both dopamine D_1 and D_2 receptors, and observed that the solubilized receptors are better antigens in eliciting antibody production than membrane-bound or partially purified receptor. As assayed by their ability to inhibit radioactive ligand binding to the receptor, a panel of hybridomas were selected that secreted antibody against D_1 and D_2 receptor. However, these hybridomas ceased to produce anti-receptor antibody following subcloning. We have now devised an extremely sensitive and rapid sandwich immunoassay method to screen large number of hyridomas. Using this method, we now show that mice injected with solubilized receptors show a high serum titre of antibodies against the receptors, which should be an advantage in successful development of hybridomas secreting antibodies against the receptors.
(Supported by OMHF, Canada.)

126.19

DOPAMINE D_1 RECEPTORS IN RABBIT CEREBRAL CORTEX AND NEOSTRIATUM T.A. Reader, L. Grondin*, B. Montreuil* and K.M. Dewar*. Département de physiologie, Faculté de médecine, Université de Montréal, Montréal, Québec, H3C 3J7.

The novel benzazepine compound (R)-(+)-8 chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol hemimaleate or SCH23390, has been employed to specifically label dopamine receptors of the D_1 subtype in the rat striatum [1]. The use of [^{3}H]SCH23390 has enabled the characterization of dopamine D_1 receptors in the rat cerebral cortex [2]. In the present survey, membrane preparations were obtained from the cerebral cortex (CTX) and the neostriatum (CPU; caudate-putamen) of adult male New-Zealand rabbits (2.5-3.0 kg). The saturation binding isotherms revealed that [^{3}H]SCH23390 bound with high affinity in both tissues, with densities of 150 fmol/mg protein for CTX (K_d 25°C = 0.15 nM) and of about 1,000 fmol/mg protein for CPU (K_d = 0.10 nM). The specificity of binding to the cortical D_1 receptor was verified in competition experiments with the enantiomers of the D_1 agonist 1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol or (+)SKF38393 (IC_{50} = 100 nM) and (-)SKF38393 (IC_{50} = 11 μM) as well as with Ketanserin (IC_{50} = 7 μM), thus confirming the stereospecificity of [^{3}H]SCH23390 and excluding the labelling of a serotonin S2 site.
[1] Billard W. et al. (1984) Life Sci. 35: 1885.
[2] Reader et al. (1988) J. Neurochem. 50: 451.
Supported by the MRC (Canada) and the FRSQ (Québec).

126.20

[^{3}H]GBR-12935 BINDING IN SOLUBILIZED CANINE STRIATUM. F.R. Sallee*, R.L. Stiller, H.B. Niznik*, N.H. Bzowej*, P. Seeman, and J.M. Perel. Clinical Pharmacol. Prog. WPIC, Univ. of Pittsburgh, Pittsburgh, Pa. 15213 and Depts. of Medicine and Pharmacol. Univ. of Toronto, Toronto, Ont. M5S1A8

[^{3}H]GBR-12935 (1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)-piperazine) binding sites were solubilized from canine striatal membranes with the detergent digitonin. The binding of [^{3}H]GBR-12935 to solubilized preparations was specific, saturable and reversible with a dissociation constant of ~3 nM and a binding site density of 3.4 pmol/mg protein. [^{3}H]GBR-12935 also bound to solubilized sites in a sodium-independent manner with a K_D of ~6 nM and a 60% decrease in site density. Dopamine uptake inhibitors and substates inhibited [^{3}H]GBR-12935 binding in a stereoselective and concentration dependent manner with a rank order of potency matching the literature for the membrane bound site. The log K_D values for these compounds in the solubilized preparation correlated with [^{3}H]GBR-12935 binding in the native state (r=0.84, p<.005). The log K_D for these compounds in the solubilized preparation also correlated with their IC_{50} values on [^{3}H]dopamine uptake (r=0.95, p<.002). The [^{3}H]GBR-12935 binding site appears to be a transmembrane glycoprotein by virtue of its absorption and specific elution from WGA-lectin column. Solubilization of the [^{3}H]GBR-12935 binding site with full retention of binding activity now allows for purification.

126.21

DOPAMINE RECEPTOR BLOCKADE AUGMENTS CERVICAL SYMPATHETIC NERVE ACTIVITY INDEPENDENT OF CHEMOREFLEX EFFECTS. S. Lahiri, W. Huang*, and A. Mokashi*, Dept. of Physiol., Univ. of Penna., Sch. of Med., Phila., PA 19104-6085.

Dopamine and dopamine receptors are associated with oxygen sensing in the peripheral chemoreceptors. Since the cervical sympathetic nerves (SN) respond to hypoxia with and without peripheral chemoreflex and the sympathetic ganglia contain catecholamines we hypothesized that dopaminergic mechanisms may play a role in regulating the SN activity. We studied the effects of dopamine (D_2) receptor blocker on the pre- and post ganglionic SN along with the phrenic nerve (PN) responses to hypoxia in the anesthetized cats which were vagotomised, paralyzed and artificially ventilated. In one series, domperidone (0.5 mg/kg) was administered before carotid sinus nerve (CSN) section and in another, after CSN section. In either case domperidone significantly stimulated the SN activity regardless of PN activity. We conclude that dopamine (D_2) receptors are inhibitory to the sympathetic neurons just as it is for the peripheral chemoreceptors. (Supported in part by grant NS-21068).

126.22

CONDITIONS FOR [^{3}H]RACLOPRIDE BINDING TO DOPAMINE D_2 RECEPTORS. K.M. Dewar*, B. Montreuil*, L. Grondin* and T.A. Reader. Département de physiologie, Faculté de médecine, Université de Montréal, Montréal, Québec, H3C 3J7.

The substituted benzamide Raclopride has been proposed to label specifically central nervous system dopamine D_2 receptors [1]. To establish the conditions of optimum binding, competition experiments were conducted with the D_2 antagonist (±)Sulpiride using membrane preparations from rat and rabbit neostriatum in Tris-Cl buffer 50 mM (pH 7.4). The addition of NaCl (150 mM) was found to be essential for [^{3}H]Raclopride binding, and the IC_{50} for (±)Sulpiride was determined to be of 250 nM. On the other hand, the addition of calcium and magnesium reduced binding by 30% [2], although the IC_{50} for (±)Sulpiride was unchanged. Thereafter, nonspecific binding was defined as the counts in the presence of 300 M of (±)Sulpiride. To determine the selectivity of labelling, inhibition curves were performed with dopaminergic agents in Tris-Cl buffer containing 120 mM NaCl and 5 mM KCl. In this incubation medium, the rank of potency of dopaminergic drugs to displace [^{3}H]Raclopride binding was (+)Butaclamol > Spiperone > (-)Apomorphine > (±)Sulpiride > (+)Apomorphine > Ketanserin > (-)Butaclamol, demonstrating the stereospecificity of this ligand for the dopamine D_2 receptor.
[1] Köhler, C. et al. (1985) Biochem. Pharmacol. 13: 2251.
[2] Stefanini, E. et al. (1987) Pharmacol. Res. Comm. 19: 777.
Supported by the MRC (Canada) and the FRSQ (Québec).

126.23

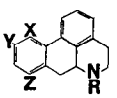
DOPAMINE AND SEROTONIN BINDING POTENCY OF APORPHINES. P.A. Leonard*, J.P. Long, H. Jackson*, R.K. Bhatnagar, T.K. Chatterjee, P. Mohan, and J.G. Cannon. Depts. of Pharmacol. and Med. Chem., Univ. of Iowa, Iowa City, IA 52242

We formerly showed 10-methyl, 11-hydroxy aporphine is a potent 5-HT_{1a} receptor agonist essentially devoid of dopaminergic action in contrast to the potent dopamine receptor agonist apomorphine. 5-HT_{1a} and D_2 binding affinities of some related aporphines using [^{3}H]8-OH-DPAT in rat cortex and [^{3}H]spiperone in rat striatum are shown below.

X	Y	Z	R	5-HT_{1a} K_i(nM)	D_2 K_i(nM)
spiperone				27	0.045
8-OH-DPAT				2.1	3500
OH	CH_3	H	CH_3	3.6	2500
OH	H	H	CH_3	30	96
†OH	OH	H	CH_3	330	28

X	Y	R	5-HT_{1a} K_i(nM)	D_2 K_i(nM)
OCH_3	H	CH_3	19	740
OCH_3	H	C_3H_7	100	820
OCH_3	OCH_3	CH_3	8.6	260
OCH_3	OCH_3	C_3H_7	240	950

(†Apomorphine)



These results suggest that 1) substitution at Y is sterically tolerated at both binding sites; the greater selectivity and potency that results relates to lipophilicity or to electronic or conformational effects; 2) replacement of hydroxy with methoxy at X does not abolish binding at these sites; X may be a hydrogen bond acceptor; 3) these sites do not always prefer the N-propyl moiety. Supported in part by NIH Grant HL-38136.

126.24

[^{3}H]-ETHYLKETAZOCINE AND [^{3}H]-BREMAZOCINE LABEL MORE THAN μ-, δ- and κ-OPIOID BINDING SITES IN THE GUINEA-PIG SPINAL CORD. M. Tiberi* and J. Magnan* (SPON: G. Caillé). Univ. de Montréal, Montréal (Québec), H3C 3J7.

The present study investigates whether the non-selective ligands [^{3}H]-Ethylketazocine (EKC) and [^{3}H]-Bremazocine (BREM) label only μ-, δ- and κ-opioid sites in guinea-pig spinal cord. Using saturation studies, the equilibrium dissociation constant (K_D) and the binding capacity (R) of selective and non-selective opioid ligands were evaluated by computerized curve fitting program. The results show that μ-, δ- and κ-selective opioid ligands each label one single class of binding sites with respective R values of 4.59, 0.95 and 1.96 pmoles/g for a total of 7.51 pmoles/g. EKC discriminates two binding classes with R values of 4.40 pmoles/g for the high-affinity sites (K_D = 0.41 nM) and 5.82 pmoles/g for the low-affinity sites (K_D = 4.73 nM) that corresponds to a total of 10.23 pmoles/g opioid sites. Furthermore, BREM binds also to high- and low-affinity sites with R values of 7.54 pmoles/g (K_D = 0.30 nM) and 10.32 pmoles/g (K_D = 2.63 nM). Thus, the total R obtained with BREM was 17.9 pmoles/g, a value statistically different from that obtained with EKC. In conclusion, the results show that EKC and BREM recognize more that μ-, δ- and κ-binding sites and might suggest that the classification of opioid receptors in guinea-pig spinal cord is more complex than the one previously proposed.

126.25

COMPARISON OF THE BINDING PROFILES OF [^{3}H]-BREMAZOCINE AND [^{3}H]-ETHYLKETAZOCINE IN THE GUINEA-PIG CEREBELLUM. J. Magnan* and M. Tiberi* (SPON: G. Caillé) Univ. de Montréal, Montréal, Canada H3C 3J7

We have reported that [^{3}H]-bremazocine (Brem) labels more than μ-, δ- and κ-opioid binding sites in the guinea-pig brain. The present study was undertaken to assess the presence of this additional, non-conventional opioid site in the guinea-pig cerebellum. We have measured the relative equilibrium dissociation constants (K_D) and binding capacities of non-selective opioids such as Brem and [^{3}H]-ethylketazocine (EKC) and selective μ-, δ- and κ-ligands. Results show that there are respectively 0.96, 0.65 and 5.11 pmoles/g tissue μ-, δ- and κ-sites in this preparation for a total opioid capacity of 6.71 pmoles/g tissue. EKC labels the same total population of opioid sites (6.55 pmoles/g tissue) with a K_D of 0.19 nM. The saturation curve for Brem is best-fitted to a two-sites model with a high-affinity K_D of 0.12 nM and a capacity of 10.97 pmoles/g tissue and a low-affinity component with a K_D of 3.54 nM and a capacity of 7.67 pmoles/g tissue. Thus Brem has a total binding capacity of 18.6 pmoles/g tissue, a value which is significantly higher than that of EKC or of the sum of μ + δ + κ sites. We conclude that Brem labels additional opioid sites different from the conventional μ-, δ- and κ-sites and that EKC does not recognize those new sites with high-affinity.

126.26

TEMPERATURE EFFECTS ON [^{3}H]-Ro5-4864 BINDING TO THE PERIPHERAL BENZODIAZEPINE RECEPTOR IN RAT LIVER. A.L. Parola*[1], L.R. Contardi*[1], A.R. Buckley*[2], C.W. Putnam*[3,4,5], D.H. Russell[2], and H.E. Laird II[1]. Depts. of Pharm/Tox[1], Pharm.[3], and Surgery[4], Colls. of Pharm/Med., U of Arizona, V.A.M.C.[5], Tucson, AZ; Dept. of Pharm/Therap[2], U of S. Florida, Tampa, FL.

In the present study, we studied the effects of temperature variation on the PBZR. Temperature changes from 4°C to 25°C resulted in a 30% decrease in the specific binding of 2nM [^{3}H]-Ro5-4864 (4'-chlorodiazepam). The loss of specific binding at 25°C occurred by 15 minutes. To study the effects of temperature variation on ligand binding, a liver membrane preparation at 4°C was then equilibrated at 25°C. [^{3}H]- Ro5-4864 was added and the temperature was reduced to 4°C to determine recoverable specific binding. 90% of the specific binding was recovered by 3 hours with 10% of the specific binding irreversibly lost, even after 24 hours at 4°C. Saturation binding experiments were performed at constant temperatures and by varying the temperature as stated above to determine the loss of activity due to changes in receptor affinity or binding sites. Scatchard analysis of the constant temperature saturation data revealed the K_d for [^{3}H]-Ro5-4864 at 4°C and 25°C was 2.4 ± 0.5 nM and 10.3 ± 1.9 nM respectively, with the same Bmax for both temperatures. When the temperature was varied, there was no change in receptor affinity but the number of binding sites decreased by 15% versus controls maintained at 4°C. Therefore, a change in temperature inactivates 10-15% of the ligand binding sites and has no apparent effect on the affinity of the remaining PBZR. (Supp. by the Amer. Fnd. Pharm. Ed., AZ Dis. Ctrl. Res. Com., and The Flinn Fnd.)

AGING - GENERAL

127.1

ISOLATION OF ACATALASEMIC MUTANTS AND A GENETIC CHARACTERIZATION DURING THE DEVELOPMENT AND SENESCENCE OF DROSOPHILA MELANOGASTER. William J. Mackay* and Glenn C. Bewley* (SPON: M. Frank). North Carolina State Univ., Raleigh, NC 27695-7614

Oxygen free radicals are highly reactive in biological systems and are capable of disrupting the structural and functional integrity of the cell. The biological consequences of free radical damage are thought to be contributing factors to aging, tumor promotion, and a number of aging related disorders. Catalase (H_2O_2:H_2O_2 oxidoreductase, E.C. 1.11.1.6) is one of several enzymes involved in scavenging activated oxygen species. At present, no true acatalasemic alleles have been isolated from any multi-cellular eukaryotic organism. Initially we recovered a gamma-ray induced deficiency that uncovers the catalase structural gene ($Df(3L)Cat^{DH104}$). We have subsequently isolated 41 mutations uncovered by this deficiency. Thirty-five mutations are lethal as homozygotes and none affect the catalase structural gene. We also recovered six viable acatalasemic mutants from this screen. Subsequent analysis has revealed that these mutants have extremely shortened life-spans in addition to severe effects on their relative viability and reproductive capacity. These acatalasemic mutants are also hypersensitive to the dietary administration of hydrogen peroxide. (Supported by NIH Grants No. AG01739 and No. AG05380).

127.2

PULMONARY FUNCTION RESPONSES OF HEALTHY OLDER ADULTS TO REPEATED OZONE (O3) EXPOSURES. D.M. Drechsler-Parks, S.M. Horvath and J.F. Bedi*. Institute of Environmental Stress, University of California, Santa Barbara, California 93106

Young adults exposed to O3 on 3-5 consecutive days usually have larger decrements in pulmonary function on the second day, followed by desensitization over days 3-5. Although older adults appear to be less responsive to O3 than young adults, it is unknown whether they have the same pattern of responses to consecutive days of exposure. The pulmonary function of 16 non-smokers, 60-89 yrs, was measured pre and post 2-hr exposures to filtered air (FA), and 0.45 ppm O3 on three consecutive days and three days later. Subjects alternated 20-min periods of rest and exercise (ventilation = 25 L/min). Forced vital capacity (FVC) and its associated factors were measured pre- and post-exposure. Data were analyzed by repeated measures ANOVA (time period, day). Data on forced expiratory flow in one sec (FEV-1) suggests: (1) FA had no effect, (2) the first and second O3 exposures induced decrements of 5.8 and 6.5%, respectively, (3) FEV-1 returned to the FA level fol-lowing the third O3 exposure, and remained at the FA level following the O3 exposure three days later. However, the responses of the individual subjects were quite variable. Three subjects had responses similar to those of young adults. (EPA R813049-01-1)

127.3

EFFECT OF AGE ON THE RESPONSE OF SKIN BLOOD FLOW IN THE HUMAN FOREARM TO DIRECT HEAT. Daniel Richardson. Dept. Physiology, Univ.of Kentucky,Lexington, KY 40536. For this study three groups each of 20 male subjects: young (Y - 20 to 39 yr); middle aged (M - 40 to 59 yr) and elderly (E - 60 to 79 yr), consented to have total forearm blood flow (FBF) measured by strain gauge plethysmography and skin blood flow (SBF) in the forearm measured by a Laser Doppler Flowmeter. The laser Doppler, a TSI Model 403, combines frequency and amplitude modulation of the reflected light into a measurement of volume flow. With the forearm in a 30°C water bath there were no significant between group differences in either FBF or SBF. Elevating bath temperature to 40°C increased FBF and SBF in all groups ($P < 0.001$). However, the increments were less in the M and E groups compared to the Y group ($P < 0.05$). Respective mean values of FBF at 40°C for the Y, M and E groups were 9.7, 7.9, and 6.9 ml/min/100 ml. Corresponding respective means for SBF were 6.3, 5.0 and 4.2 ml/min/100 ml. The frequency modulation of the laser light, an index of flow velocity, was also lower in the M and E groups at 40°C ($P < 0.03$). It is concluded that aging decreases the response of human skin blood flow to the direct effects of heat. The laser Doppler data suggest that this is mediated by a reduced flow response of individual microvessels. Supported by the KY Heart Assoc. and NIH grant AG05082.

127.4

THE EFFECT OF AGING ON ENDOTHELIAL MODULATION OF RAT VASCULAR CONTRACTILE RESPONSES TO ADRENERGIC NERVE STIMULATION. R.R. Gonzalez, Jr., and S.P. Duckles. Departments of Physiology and Pharmacology, School of Medicine, Loma Linda University, Loma Linda CA 92350 and College of Medicine, University of California, Irvine CA

Basal release of endothelially derived relaxing factor (EDRF) attenuates vascular contraction due to adrenergic nerve stimulation; however, the effect of aging on this modulation is unknown. Proximal tail artery segments (4 cm) from male Fisher-344 rats (6, 12, 20, 27 mo) were perfused (2 ml/min) *in vitro* with Krebs-Henseleit solution. The vessels were contracted with methoxamine (3 μM) and endothelial integrity was confirmed by relaxation to methacholine (20 nmol). Responses to transmural nerve stimulation (40 V, 0.3 ms, 0.1 - 3 Hz) were not significantly different across the age groups, but were greater than responses by 250 g Sprague-Dawley rats. Treatment of the segments with methylene blue (10 μM, 30 min) tripled the response to stimulation at 0.1 to 0.6 Hz and doubled it from 1 to 3 Hz in all age groups except 6 mo, where the effect of methylene blue was twice that of the other ages. These data imply that while modulation of vascular tone by the basal release of EDRF appears greatest in 6 mo rats, this effect remains constant thereafter.

Supported by NIH grant #RO1 AG06912.

127.5

AGING - INDUCED MYOCARDIAL DAMAGE IN THE LEFT VENTRICULAR MYOCARDIUM OF MALE FISCHER 344 RATS. Laura M. Quattrocci, Joseph M. Capasso, Giorgio Olivetti, Piero Anversa. New York Medical College, Valhalla, NY 10595.

To determine whether aging affects the structural characteristics of the left ventricle, hearts of male Fischer 344 rats at 4, 12, and 20 months after birth were fixed by perfusion of the coronary vasculature and the myocardium examined morphometrically. The quantitative analysis involved the evaluation of the number of foci of replacement fibrosis present within the tissue of the outer, middle, and inner layers of the wall. Consistently, there was an increase in the density of lesions as a function of age. Whereas comparable numbers of areas of scarring were found in the epicardium and midmyocardium at any age, the endocardium showed values which were 2.9-, 2.9-, and 2.0-fold greater than those of the remaining tissue at 4, 12, and 20 months, respectively. From 4 months to 20 months, the numerical concentration of lesions in the outer region increased from 1.6 ± 0.6 per mm2 to 7.3 ± 0.6 per mm2 and this difference was found to be statistically significant ($p<0.0001$). Corresponding results for the midwall were 2.0 ± 0.3 per mm2 and, 7.7 ± 0.6 per mm2 ($p<0.0001$). In the subendocardium, values of 4.6 ± 0.4 per mm2 and 14.9 ± 0.8 per mm2 ($p<0.0001$) were obtained. In conclusion, aging of the heart is accompanied by a significant amount of myocyte cell loss which preferentially occurrs in the subendocardium.

(Supported by NIH HL 38132 and HL 39902)

127.6

INTRAVENOUS NICOTINE INFUSION STUDIES IN ALZHEIMER'S DISEASE AND YOUNG NORMAL VOLUNTEERS. Paul A. Newhouse*, Trey Sunderland*, Cynthia Galinski*, Dennis L. Murphy* (Spon: G. J. Kant). Univ. of Vermont Coll. of Med., Burlington, VT 05405 and NIMH, Bethesda, MD.

A significant loss of central nicotinic cholinergic receptors has been described in the brains of patients suffering from Alzheimer's disease (AD). Pilot data from a series of nicotine bitartrate infusions in 6 AD patients at 0.125, 0.25, and 0.5 μg/kg/min (Psychopharmacol; in press) showed a small decrease in intrusion errors on a free recall task with several subjects developing significant short-lived negative mood changes. We have now completed dose-response studies in 14 AD patients and 22 young normal volunteers. Results to date in the AD patients continue to show a decrease in immediate intrusion errors after the 0.25μg dose and an improvement in recall consistency at 8 hours. IV nicotine also produced significant ($p<.05$) dose-related increases in ACTH and cortisol in both groups consistent with central nicotinic stimulation. More mildly demented patients appear to show less mood changes during nicotine infusions. However, young normals also show a dose-related increase in negative affect, suggesting that nicotine may have specific effects on mood. The degree of the mood changes in the AD patients may be a factor of the severity of the disease and the extent of damage to the central nicotinic cholinergic system. Intravenously administered nicotine may be a useful probe of the integrity of central nicotinic cholinergic systems. Whether chronic nicotinic stimulation will be a useful therapeutic strategy in AD will have to be assesed in longer term studies.

127.7

Molecular Forms of Acetylcholinesterase (AChE) in Subcortical Areas of Age-Matched Normal and Alzheimer (DAT) Brain. Judith K. Marquis, Gordon C. Siek*, Eric B. Fishman*, and Lori S. Katz*.

We previously reported that the 10s membrane-bound form (G4) of AChE is selectively lost from several cortical areas of DAT brain. We have since microdissected the hippocampus, amygdala and cingulate areas of non-demented and DAT brain. Tissue homogenates were analyzed for ChAT activity; sucrose density gradient fractions were assayed radiometrically for AChE activity to define both the 10s and 4s (G1) molecular forms. Hippocampal areas, in particular, exhibited a distinct pattern of alteration of G4/G1 activity consistent with the histopathology reported in DAT. The contribution of detergent- and salt-soluble species to the overall enzyme loss in DAT was also investigated. (Supported in part by U.S. ARO DAAG29-K-85-0071 and the Eppley Foundation.)

127.8

EFFECT OF AGE ON K^+STIMULATED ALDOSTERONE SECRETION (AS) BY RAT ADRENAL CAPSULES. Karen Radke, School of Nursing and Department of Physiology, School of Medicine, University of Rochester, Rochester, NY 14642.

The effect of age on basal and K^+-stimulated AS by adrenal capsules from male Fischer 344 rats, 3-5 and 19-21 mos. of age, was examined. Capsules were perifused with either normal (KCl=4.0 mM) Ringer's-Bicarbonate solution (RBS), gassed with 95% O_2 and 5% CO_2, or modified RBS with various concentrations (3.0-9.0 mM) KCl. Basal AS by adrenal capsules from young rats was 82 ± 15 pg/min/adrenal capsule (n=24) as compared to 12 ± 2 pg/min/adrenal capsule (n=24) by capsules from aged rats ($p < 0.05$). At each concentration of KCl in the range of 5.0-9.0 mM, K^+-stimulated AS was markedly ($p<0.05$) elevated above basal levels within each age group. The threshold for K^+-stimulated AS occurred at a concentration of 5.0 mM KCl for both young and old animals. However, the magnitude of change in K^+-stimulated AS by capsules from aged rats was significantly ($p<0.05$) less than that for young rats at each concentration of KCl in the range of 5.0-9.0 mM. Thus, there is an age-associated significant decrease in basal AS and a significant reduction in capacity of the rat adrenal capsule to produce aldosterone under K^+-stimulated conditions.

(Supported by University of Rochester School of Nursing BRSG and Alumni Seed Fund, and American Heart Association - NY State Affiliate)

127.9

AGE-RELATED ADAPTATION OF PITUITARY-ADRENOCORTICAL RESPONSES TO STRESS. M. Odio* and A. Brodish, Wake Forest University Medical Center, Winston-Salem, NC 27103

An important functional characteristic of the hypothalamic-pituitary-adrenal system (HPAS) is its adaptation from an acute to a chronic stress by attenuation of response to repetitive stress exposure. Because the HPAS has been implicated as an important endocrine link in the process of biological aging, an understanding of the effects of age on adaptational capatity of the HPAS is vital. Studies were carried out on F-344 male rats, 6 months of age (young) and 22 months of age (old), on day 1 and day 3 acute exposure to a 2-way shock-escape stress and on day 28 and day 56 chronic stress exposure. On each of these days rats were sacrificed at various times during the stress session. Corticosterone (CORT) and adrenocorticotropin (ACTH) determinations showed that: a) CORT responses increased from Day 1 to Day 3 in young but not in old rats; b) attenuation of ACTH and CORT responses to chronic stress was greater in young than in old rats; c) exposure to chronic stress appeared to delay/reverse age-related changes in HPAS function. In conclusion, short- and long-term adaptive capacity of the HPAS declines with age. However, it appears that chronic activation may sustain the functional integrity of the HPAS of aged rats. (Supported by NIH grant #AG-04207).

127.10

THE EFFECT OF OMEGA CONOTOXIN ON RELEASE OF NOREPINEPHRINE IN THE HEART AS A FUNCTION OF AGE. J. Roberts, M.L. Mortimer,* P.J. Ryan*and N. Tumer.* Med. Coll. PA, Dept. Pharmacol., Phila., PA 19129.

Increased age is associated with a decrease in adrenergic control of cardiac function. It has been shown that the release of norepinephrine (NE) is diminished in older rat heart preparations as compared to young rat hearts (Daly et al, in press). We have found that increasing the extracellular calcium (Ca^{2+}) in old hearts is not sufficient to restore Ca^{2+}-dependent release of NE (Mortimer et al, 1988). To investigate this observation further we studied the effect of omega conotoxin (a neuronal Ca^{2+} channel blocker) on release of NE in hearts isolated from male Fischer-344 rats at two ages, 6 mo and 24 mo. Hearts were perfused with Krebs-Ringer solution and the right cardiac sympathetic nerve was stimulated electrically at frequencies of 2,6,and 12 Hz. 10 nM omega conotoxin inhibited the release of NE in preparations from both 6 and 24 month-old animals at each of the frequencies employed. For example, the reduction in NE released at 2 Hz in heart preparations from 24-month-old animals was approximately 76% (0.2 ng/1.4 ng), while in the 6-month old it was only about 56% (1.7 ng/3.8 ng).

The data suggest that there might be quantitative differences in the response to omega conotoxin at the different ages. (Supported by NIA grant AG03326)

127.11

EFFECT OF LONG-TERM CALORIC RESTRICTION ON BRAIN MONOAMINES IN AGING MALE AND FEMALE FISHER 344 RATS. Malak G. Kolta*, Peter Duffy*, and Ronald Hart* (SPON: W. Slikker, Jr.). Div. Repro. & Develop. Toxicol., National Center for Toxicological Research, Jefferson, AR 72079

Dietary restriction has been shown to alter numerous physiological and biochemical parameters. The present study examines the changes in central monoamines and their metabolites in aged male and female rats after chronic caloric restriction. Fisher 344 rats of both sexes (n=5-10/group) were maintained after weaning and for 18 mo on one of 2 dietary regimes: ad libitum (AL) to NIH 31 diet or restricted (RES) to 60% of AL intake supplemented with vitamins and minerals. At 18 mo of age, rats were sacrificed; their brains were quickly removed and several regions were dissected. Caudate nucleus (CN), hypothalamus (HYPO) and olfactory bulb (OB) were assayed for content of dopamine (DA), serotonin (5-HT) and their metabolites (DOPAC, HVA and 5-HIAA) and norepinephrine (NE) using HPLC/EC. Relative to AL groups, the RES rats of both sexes showed drastic decline in NE content in CN, HYPO and OB. Significant decrease in DA and 5-HT contents were also found in CN and HYPO of male rats, while 5-HT and 5-HIAA were markedly decreased only in HYPO of female RES groups. These preliminary results suggest that long-term caloric restriction alters brain monoamines, an effect which may in turn alter the normal physiological aging process.

127.12

ABILITY OF ETHANOL TO EXACERBATE DIABETES AS A FUNCTION OF AGE. D.G. Patel. Div. of Pharmacology & Medicinal Chemistry, College of Pharmacy, University of Cincinnati, OH 45267-0004.

Although ethanol and aging individually show diabetogenic effects, the interaction between them is not reported. Fisher 344 male rats of 3 and 13 months were divided into three subgroups; ethanol, control ad lib and pair fed. Ethanol diet was isocaloric with control diets except that ethanol (30% of calories) was substituted for fat. Rats were fed respective diets for two months. Oral glucose (0.75 g/kg) tolerance tests were performed in the non-fasted state. Insulinogenic indices were computed as the ratio of the areas under the curve for insulin and glucose after oral glucose loads. In young rats fed control diets there was significant difference between pair and ad lib fed rats (0.44 ± 0.01 vs 0.51 ± 0.02, $p < 0.05$), but no such difference was observed for old control rats. Ethanol feeding induced significantly lower insulinogenic indices in both young (0.33 ± 0.01 vs 0.44 ± 0.01, $p < 0.05$) and old (0.25 ± 0.01 vs 0.36 ± 0.01, $p < 0.05$) rats when compared with those in respective control rats. Mean insulinogenic indices were significantly lower in aged rats compared to their respective diet fed young rats. These results indicate that aged rats exhibit diabetogenic glucose homeostasis compared to young rats. Chronic ethanol feeding deteriorates glucose tolerance in both young and old rats. In conclusion diabetogenic effects of aging were observed to be exacerbated by ethanol. (Funded by NIAAA #1 RO1 AA6701.)

127.13

EFFECT OF FOOD RESTRICTION ON BILE FORMATION, MEMBRANE LIPID COMPOSITION AND Na^+K^+ATPase ACTIVITY.
M. Messier*, B. Tuchweber, M. Audet*, I.M. Yousef*
Department of nutrition and Department of Pediatrics
Université de Montréal, Montréal, Québec, Canada H3C 3J7.

We previously demonstrated that life long food restriction exerts a beneficial effect on age related decline of bile formation in rats (Life Sciences, 41, 2091, 1987). The underlying mechanism is not known,thus we examined Na^+K^+-ATPase activity, cholesterol and phospholipids content in liver cell plasma membrane fractions enriched in canalicular complexes (LCPM). Female Sprague Dawley rats were subjected soon after weaning to a restricted diet (RD) (60% diet consumed by rats fed ad libitum) and bile formation determined at 5 months of age. Bile flow and bile acid secretion were significantly increased (about 25%) when compared to ad libitum fed group (AL). Enzyme enrichment of LCPM from rats of RD group did not differ significantly from AL group. Values were for RD and AL groups, respectively: leucine aminopeptidase 10x and 9x; 5' nucléotidase 15x and 13x, Na^+K^+-ATPase 9.6x and 10.4x homogenate specific activity. The cholesterol/phospholipids ratio of the membranes was not modified by RD (.70±.08 in RD vs .59±.08 in AL). In conclusion, DR results in an increase in bile formation which is not associated with structural and functional changes of hepatocytes membrane. The improved bile formation may be related to enhanced bile acid pool and secretion. (Supported by NSERC).

127.14

EFFECT OF AGING AND FOOD RESTRICTION ON BILE ACID METABOLISM IN RATS. G. Ferland*, B. Tuchweber, and M. Yousef*.
Département de Nutrition et Département de Pharmacologie, Université de Montréal, Montréal, Québec, H3C 3J7.

We examined in female Sprague-Dawley rats the influence of aging and life long food restriction (FR) (60% of the ad libitum intake) on bile flow, total bile acid (BA) secretion as well as BA composition and conjugation pattern. Rats were cannulated at 3.5, 12 and 24-27 months of age and bile collected for BA analysis. With age, there was a significant reduction in bile flow and total BA secretion (expressed per g liver or per 100g body weight). FR exerted a beneficial effect on the age-related decline of bile formation. Studies of BA composition indicated that 12α hydroxylated BA (cholic acid and deoxycholic acid) secretion decreased in aged rats compared to rats at 3.5 months of age. This was associated with a corresponding increase in secretion of 6β hydroxylated BA (muricholic acids and hyodeoxycholic acids) and of chenodeoxycholic acid. A similar pattern of change was observed in aging rats subjected to FR. Aging did not affect significantly BA conjugation with taurine and glycine. The increase secretion of BA in FR group was associated with higher secretion rate of tauroconjugates. A striking change at 24-27 months of age was an increase in secretion rate of glyco BA. This may reflect a sparing of taurine or its precursors for other physiologic functions. (Supported by NSERC).

128.1

THE FRACTAL DIMENSIONS OF CULTURED VERTEBRATE CENTRAL NEURONS. T. G. SMITH, W. B. MARKS, G. D. LANGE, W. H. SHERIFF* AND E. A. NEALE. Labs. of Neurophysiologyand Neural Control, and Instrumentation and Computer Section, NINCDS and Lab. of Developmental Biology NICHD, NIH, Bethesda, MD 20892.

Tissue cultured neurons were injected with horseradish peroxidase and viewed with a light microscope. Their images were captured with a TV camera and analyzed with an image processor. The fractal dimensions (D) of the borders of 27 CNS neurons were determined by three different methods. For each neuron the three methods gave stastically insignificant differences in D, but across the group, D varied from 1.14 to 1.60. The estimated D's correlated well with other, but ad hoc, methods of measuring morphological complexity of borders. Therefore, the borders of neurons may be considered fractal objects.

The methods were based on the fractal geometry (FG) of Mandelbrot (The Fractal Geometry of Nature, W.H. Freeman & Co. N.Y.,1982). The methods were calibrated against fractal images of known D and their estimated D's differed from the known D's by <10%. Thus, these methods may provide a "tool" to provide a quantitative morphological measure of cellular complexity, D, in the context of a general theory of geometry (FG). In particular, D may be the dependent variable of any experiment where in an independent variable may be thought to influence cellular morphology. These concepts and methods may, therefore, become the basis of a quantitative cellular morphometry.

128.2

INDUCTION AND POTENTIATION OF NEURITE OUTGROWTH IN THE PC-12 CELL LINE BY 1,1,3 TRICYANO-2-AMINO-1-PROPENE. John P. DaVanzo, J. West Paul* and Bruce K. Schrier. Dept. of Pharmacol., Sch. of Med. East Carolina Univ., Greenville, NC 27858, Molec. Neurobiol., NICHD, NIH, Bethesda, MD

In 1961 Egyhazi and Hyden demonstrated that 1,1,3 tricyano-2-amino-1-propene (Triap) increased the protein, lipid and RNA content of isolated nerve cells (J Biophys Biochem Cytol 10, 1961). Later in our laboratory, Triap was shown to reduce recovery time in sciatic nerve crush studies and to induce neurite formation in chick spinal ganglia. Using the PC-12 cell line we compared the induction of neurite outgrowth caused by Triap and nerve growth factor (NGF). Results show that Triap has a maximum effect on inducing neurite outgrowth at a concentration of 20 ug/ml (151 uM) with toxicity occurring at 50 ug/ml or greater. These concentrations of Triap correlate well with those seen to increase the rate of hind limb regeneration in the newt (Houlihan & DaVanzo, Exp Neurol 10, 1964). Triap at a concentration which did not induce neurite outgrowth (1nM) potentiated the morphological effect of NGF. This potentiation occurs at concentrations of NGF that do not produce neurite outgrowth. The mechanism by which Triap exerts its effect remains to be completely elucidated. (We acknowledge Hoechst-Roussel Pharmaceuticals, Inc. for support of these studies and Dr. Gordon Guroff for supplying us with the PC-12 cell line.)

128.3

INFLUENCE OF FATTY ACIDS ON GAP JUNCTION-MEDIATED INTERCELLULAR COMMUNICATION IN WILMS' TUMOR CELLS. C.M. Hasler,* M.H. Bennink,* and J.E. Trosko*(SPON: R.A. Roth), Michigan State University, E. Lansing, MI 48824

Gap junction-mediated intercellular communication (GJIC) is critical in the regulation of cell growth and differentiation. An inhibition of GJIC may play a significant role in tumor promotion. Dietary fat has been shown to act as a tumor promoter in vivo, possibly by modulating GJIC. Unsaturated fatty acids (UFA), but not saturated fatty acids (SFA), have been shown to inhibit GJIC in the V79 Chinese hamster metabolic cooperation assay. The objective of this study was to assess the effect of fatty acids (FA) on GJIC in Wilms' tumor cells in vitro, utilizing two techniques recently developed in our laboratory (scrape-loading/dye transfer and Fluorescence Redistribution After Photobleaching). Palmitic (PA, 16:0), oleic (OA, 18:1), linoleic (LA, 18:2), linolenic (LN, 18:3), and docosahexaenoic (DHA, 22:6) acids were tested by incubating with concentrations ranging from 25 to 250 uM for 5 min to 24 hr. 12-O-Tetradecanoylphorobl-13-acetate (TPA), known to inhibit GJIC in vitro, was used as a positive control. Ethanol served as a solvent control. OA, LA, LN, PA, and solvent had no effect on GJIC, while DHA completely inhibited GJIC at concentrations > 125 uM within 5 min. GJIC returned to normal within 1 hr. This study demonstrates that DHA inhibits GJIC in this cell system, while other FA tested had no effect.

128.4

ISOLATION AND CHARACTERIZATION OF PERITONEAL MESOTHELIAL CELLS. J. Thomas Hjelle[1], Barbara Golinska[2], David R. McCarroll[3] and James Dobbie[4]. Basic Sciences[1] and Pediatrics[3], Univ. Illinois College of Medicine at Peoria, and HOIR-Amer. Red Cross[3], Peoria, IL 61606, Inst. Biochem. Univ. Agric.[2], Poznan, Poland and Renal Therapy[4], Baxter Healthcare, Round Lake, IL 60073.

Although continuous ambulatory peritoneal dialysis (CAPD) represents a life support system for approx. 50,000 patients worldwide, little is known of the physiology and pharmacology of the mesothelial cells that constitute the primary site of fluid exchange during CAPD. In this report, methods for isolation and tissue culture of peritoneal mesothelial cells are described. Cells were harvested from the inner surface of the abdominal walls of rats and rabbits using a solution of HAM's F-12 medium containing 0.5 mg/ml collagenase (Worthington, Type II). Cells digested from the surface were grown in HAM's F-12 containing 20% horse serum. Cells grew readily to confluence and were easily subcultured following dispersal using EDTA in Ca^{++}- & trypsin-free Hank's solution. The mesothelial origin of these cells was supported by 1) immunochemical staining for cytokeratin and vimentin, but not vonWillebrand's factor and 2) electron microscopy (EM) that showed microvilli and cytoplasmic vesicles typical of these cells (Stylianou et al., Periton. Dialys. Int. 8:Abst.#36, 1988). EM also revealed the presence of membrane limited vesicles on the cell surface and in the culture medium suggesting a secretory role for these cells. (Supported by a grant from Baxter Healthcare.)

128.5

SPECIFIC HIGH-AFFINITY UPTAKE OF ASCORBATE BY RAT OSTEOBLASTS IN VITRO. John X. Wilson and S. Jeffrey Dixon. University of Western Ontario, London, Ontario, Canada N6A 5C1

Ascorbic acid is essential for the formation of bone by osteoblasts in vitro, but the mechanism by which osteoblasts transport ascorbate has not previously been investigated. We examined the uptake of L-[^{14}C]ascorbate by the osteoblast-like cell line ROS 17/2.8 and by primary cultures of rat calvarial cells. Cells took up [^{14}C]ascorbate from the external medium during incubations of 1-30 min at 37°C, pH 7.3. Uptake proceeded more quickly than did efflux, so the cellular accumulation of radioactivity increased with incubation time. Consistent with a carrier-mediated mechanism, uptake was both saturable and stereoselective. Competition experiments indicated that unlabeled L-ascorbate was a much more potent inhibitor (IC_{50} = 3 x 10^{-5}M) of L-[^{14}C]ascorbate transport than was isoascorbate (IC_{50} = 3 x 10^{-4}M). Uptake was dependent on both the incubation temperature and the extracellular concentration of sodium (Na^+_o), since it was largely inhibited by cooling to 4°C, as well as by the substitution of K^+, Li^+ or N-methyl-D-glucamine$^+$ for Na^+_o. We conclude that osteoblasts possess a specific, high-affinity, Na^+_o-dependent uptake system for ascorbate. This system may play an important role in bone formation. [Supported by the Medical Research Council of Canada].

128.6

A COMPARISON OF PICHINDE VIRUS INFECTION AND RAS ONCOGENE INSERTION ON THE 1H NMR SPECTRA OF CLONED CELLS. J.W.Frazer[1]*, A.W.Boddie,jr.[1]*, C.Hazlewood[2], P.Wydel[2]*, and L.Dennis[3]* (Spon:C.T.Liu) 1. M.D.Anderson Hosp.&Tumor Inst.Houston,Tx 77030,2. Depts. Physiol.and Microbiol&Immunol. Baylor Col.Med. Houston,Tx 77030. 3. Exxon Res.&Eng Co.,Baytown,Tx.

Ras oncogene insertions in rat fibroblasts or 3T3 cells were accomplished by transfection from spleen cells. Pichinde virus, an arenavirus selectively affecting strain 13 guinea pigs, was applied to VERO cells and to a guinea pig lung cell line, JH4, in culture. NMR spectra were obtained at 200 and 300 mHz using deuterium exchange or suppression by constant irradiation to avoid the large water peak. Major differences in the region of the spectrum dominated by glycosidic groups (3.2-5.0 ppm) appeared when the RAS oncogene was present.These differences were less marked when 3T3 cells were transfected than when rat pulmonary fibroblasts were transfected. Although the precise distribution of protons and their coupling to wa-ter was different from the spectral alterations found with RAS oncogene, the results suggest that Pichinde virus infec-tion produces alterations in glycoprotein expression at cul-tured cell surfaces which are detectable and quantifiable with a variety of NMR analytical procedures.

Partially supported by contract DAMD-17-87-M-9102, U.S. Army Institute of Infectious Diseases.

128.7

STRUCTURAL REQUIREMENTS OF PHENOXYCARBOXYLIC ACID COMPOUNDS FOR PEROXISOME PROLIFERATION IN PRIMARY CULTURED RAT HEPATOCYTES. T. Esbenshade*, F. Loiodice*, V. Tortorella*, H. A. I. Newman, D. Witiak*, G. Krishna and D. Feller. Dept. Med. Chem., Univ. of Bari, Bari, Italy; NHLBI, Bethesda, MD 20892; Coll. of Medicine and Pharmacy, The Ohio State Univ., Columbus, Ohio 43210.

The hypolipidemic agent clofibrate and chemically related phenoxycarboxylic acids produce hepatic peroxisome proliferation in rodents which has been linked to chemical carcinogenesis. We evaluated the concentration-dependent (0.03 to 1.0 mM) effects of a series of phenoxycarboxylic acids on the induction of peroxisomal fatty acyl-CoA oxidase (FACO) and microsomal laurate hydroxylase (LH) activities in cultured hepatocytes exposed to the compounds for 72 hours. Optimal induction of the peroxisome proliferation-associated enzymes FACO and LH was produced by phenoxyacetic acids possessing 1) a chlorine atom at the 4-position of the phenyl ring, 2) a di-methyl or mono-ethyl substitution at the alpha-carbon atom of the carboxylic acid side chain and 3) the S(-)-orientation, for the chiral analogues which have a mono-ethyl substituted alpha-carbon atom. These results show that changes in the chemical and stereoisomeric structures of phenoxyacetic acids alter peroxisome proliferation and are consistent with a receptor mediated mechanism. (Supported by NIH HL-12740)

128.8

A TECHNIQUE TO ESTABLISH SEPARATE PRIMARY CULTURES OF MICROVASCULAR ENDOTHELIAL CELLS AND ASTROGLIAL CELLS FROM RAT FOREBRAIN. Ellen L. Gordon*, Per E. Danielsson* and H. Richard Winn. Univ. of Washington, Seattle, WA 98195.

We have developed a technique to prepare separate cultures of cells from rat forebrain that are enriched for astrocytes (AC) and microvascular endothelial cells (EC). After an initial homogenization step, filtration through a 149 μ filter removes most of the large blood vessels. Additional enzymatic treatments and density gradient centrifugations result in a mixture of microvessel fragments and single cells of several different types. Subsequent key aspects of the protocol are: (1) filtration through a 10 μ nylon mesh to separate individual cells from microvessel fragments; (2) preincubation on poly-D-lysine-coated plates for 24 hours to select for AC; and (3) the use of feed mixes and matrices to optimize growth of either cell type. Vessel isolation has been monitored by SEM and cells lines characterized by SEM and TEM. EC cultures possess Factor VIII antigen and take up acetylated LDL labeled with fluorescent probe, 1,1'-dioctadecyl-3,3,3', 3'-tetramethyl-indocarbocyanine perchlorate (DiI-Ac-LDL). EC cultures analyzed by flow cytometry with DiI-Ac-LDL are >98% pure in primary and subculture. AC are reactive with antiglial fibrillar acidic protein (GFAP) antibody. An additional cell type, possibly pericyte, is also obtained. This method provides a means to study cells separately and in co-culture which have been prepared from the same tissue source. (Supported by AHA and NIH 21076.)

128.9

EVALUATION OF A NOVEL ANTIESTROGEN ANALOG II USING MCF-7 HUMAN BREAST CANCER CELLS IN CULTURE. P.T. JAIN.* J.T. PENTO, R.A. MAGARIAN.* M. GRIFFIN* and D.C. GRAVES.* University of Oklahoma, Health Sciences Center, Oklahoma City, OK 73190

Analog II (1,1 dichloro-cis-2,3-diphenylcyclopropane, A-II) is antiestrogenic in the mouse (J. Pharm. Sci.70:399,1981) and inhibited the growth of DMBA-induced tumors in the rat (Nutr. Cancer 7:239, 1985). A series of derivatives of A-II was synthesized in an attempt to enhance antitumor activity while retaining pure antiestrogenic activity. In the present study the antitumor activity of AII was examined using MCF-7 cells in culture. Cells were plated in multiwell plates at a density of $5x10^4$ cells/well in 3 ml of RPMI 1640 media (without phenol red) supplemented with 5% calf serum, 5 mM L-glutamine and antibiotics. Cells were allowed to attach and were in logarithmic growth during drug treatment. Cell growth was measured on alternate days using the hemocytometric Trypan blue exclusion method. Cell growth was enhanced with estradiol (10^{-9} to 10^{-7} M) and inhibited with tamoxifen (10^{-7} to 10^{-5} M) in dose-dependent manner. Further 10^{-6} M tamoxifen inhibited estradiol stimulated cell growth which confirmed the estrogen-dependent nature of the MCF-7 cells used in this study. Our results indicate that A-II inhibited the growth of MCF-7 cells at the concentration of 10^{-9} to 10^{-6} M. In addition to the lead compound (A-II), a series of cyclopropyl derivatives will be evaluated for antineoplastic activity using MCF-7 cells in culture. (This study was supported in part by NIH grant (RAM & JTP) CA 40458).

128.10

TRIAP IS TROPHIC FOR CULTURED PERIPHERAL NEURONS B.K. Schrier, A-M. Duchemin*, T.T. Quach*, W. Paul* and J.P. DaVanzo. Mol. Neurobiol. Unit, NICHD, NIH, Bethesda MD 20892; Neuropsychiatry Br., NIMH, St. Eliz. Hosp., Washington D.C. 20032 and Dept. Pharm. E. Carolina Univ. Sch. Med., Greenville NC 27858

Triap (1,1,3-tricyano-2-amino-1-propene) enhances sciatic nerve regeneration in dogs and promotes neurite outgrowth from chick DRG explants. We tested Triap for trophic activity in a miniaturized assay (Terasaki wells) with neurons from 12 d.o. chick embryo sympathetic ganglia which do not survive the first 20 h of culture without trophic support (NGF, wounded cerebral cortex factor, frog oocyte translation products of wounded cortex mRNAs, etc.). All cells bearing neurites were counted and results for each [Triap] were compared to control (no trophic support) and NGF (at its optimal 20 ng/ml) in each assay. The combined data from 5 assays (8-26 wells per datum) show a broad biphasic [Triap] curve with a peak activity (3-fold > control, 95% of NGF effect) at 10^{-11} M and 30% increments over control at 10^{-14} and 10^{-6} M. Morphologically, Triap-treated cells were indistinguishable from NGF-treated cells. We conclude that Triap can function as a survival trophic factor for chick sympathetic neurons in culture.

128.11

HYALURONATE RECEPTORS ARE DIFFERENTIALLY EXPRESSED ON DIVIDING EPITHELIAL CELLS Anna Maria Alho* and Charles B. Underhill* Department of Anatomy and Cell Biology, Georgetown University Medical Center, 3900 Reservoir Road, Washington D.C. 20007 (SPON:W.Wojcik)

The extracellular matrix component hyalunorate (HA) has been implicated in a variety of phenomena involving cell behavior, such as cell to cell adhesion, migration, phagosytosis and locomotion. These phenomena may be mediated through the cell surface receptor for HA. We have studied the distribution of HA and its 80 kD receptor in adult hamster epithelial tissue using a monoclonal antibody (K3 MAb) for immunostaining of the HA receptor and a proteoglygan probe for histochemical detection of the HA. HA showed a widespread distribution, being present in basement membranes and between the cells in stratified epithelium. Positive staining with K3 MAb was detected in basal layers of the epidermis as well as on the surface of a variety of other epithelia. The K3 immunoreactive material from skin was identified as the HA receptor by Western blot analysis. When present, the HA receptor was preferentially located in regions of active cell growth, such as in the basal layers of stratified epithelium and in the cypts of Lieberkuhn of intestinal epithelium. In most tissues there was a co-localization of HA and HA receptor. The differential distribution of the HA receptor on dividing epithelial cells suggests that the receptor regulates cell adhesiveness of the epithelial cells at different stages of maturation.

128.12

DOXORUBICIN AFFECTS TAU PROTEIN IN HUMAN NEUROBLASTOMA CELLS. A. Argasinski*, H. Huynh*, B. Fingado*, H. Sternberg*, P.S. Timiras. University of California, Berkeley, California, 94720.

Tau protein, a microtubule-associated protein found primarily in neurons, was detected in a human neuroblastoma cell line, LAN-5. LAN-5 cells treated with 2.0×10^{-5}M retinoic acid differentiate and form processes morphologically similar to neurons. Both differentiated and undifferentiated LAN-5 cells were treated with varying concentrations of doxorubicin, an anthracycline antibiotic with antineoplastic activity. Doxorubicin killed many of the dividing undifferentiated cells but few differentiated cells. After 2 or 4 days of treatment with doxorubicin, the cells were harvested, protein concentration was determined and a SDS-PAGE was performed. Proteins were blotted onto nitrocellulose paper and immunostained with either rabbit antisera or mouse monoclonal antibody to tau. Undifferentiated LAN-5 cells treated with 4.0×10^{-8}M doxorubicin for 4 days and cells treated with 8.0×10^{-8}M doxorubicin for 2 days showed a distinct lower tau band (just below 50kd) that was either absent or very faint in the controls. Currently, we are trying to determine whether this low molecular weight tau band is due to a difference in the phosphorylation of tau. (Supported by State of California, Department of Health Services, Alzheimer's Disease Program)

129.1

TURNOVER OF PLATELETS ADHERED TO FIBRINOGEN-COATED SURFACES.
C.J. Jen* and H.M. Wang*(SPON: H.I. Chen). Department of Physiology, College of Medicine, National Cheng-Kung University, Tainan, Taiwan, R.O.C.

The dynamic interactions between platelets and fibrinogen-coated surfaces were investigated by using a tube-flow device and a rotating rod device. Platelet accumulation to the fibrinogen-coated surfaces of both devices required Ca^{2+} and reached the maximal values of 30-40 platelets/1000 um^2. The density of platelets adhered to the tube surface increased with flow time and decreased with distance from the tube inlet. These adhered platelets were difficult to be washed off the tube surface and showed little turnover. The platelet accumulation kinetics in a rotating rod device increased with rotation speed from 300 rpm to 1200 rpm. At 1200 rpm, about half of these platelets adhered to the rod were exchangeable with platelets in the suspension whereas the rest of them were permanently attached. The detachment of platelets from the rod surface depended on the presence of erythrocytes and platelets in the suspension and it was facilitated by Ca^{2+} removal from the suspension. These observations suggest that the turnover of platelets adhered to fibrinogen-coated surfaces depends on experimental conditions.

129.2

INACTIVATION OF CALPAIN BY LEUPEPTIN ALTERS THE KINETICS OF PROTEIN PHOSPHORYLATION IN IONOMYCIN STIMULATED PLATELETS.
Lynn M. Brumley* and Robert W. Wallace. Univ. of Alabama at Birmingham, Birmingham, Al. 35294

Protein kinase C (PKC), myosin light chain kinase (MLCK) and a Ca^{2+}/calmodulin-dependent phosphatase (calcineurin) are degraded by calpain upon ionomycin-induced platelet activation. Hydrolysis by calpain activates the phosphatase and PKC, but inactivates MLCK. ^{32}P-Labelled platelets were stimulated by ionomycin in the presence and absence of 1 mM leupeptin to determine the effects of calpain on the phosphorylation of myosin light chain (LC), a substrate for MLCK, PKC and calcineurin. Calpain activation was monitored by following the autolysis of the 80 kDa catalytic subunit on western blots. In the absence of leupeptin, significant autolysis occurred within 5 sec and was complete by 20 sec; leupeptin inhibited autolysis. The initial rate of incorporation of ^{32}P into the LC was increased, but the final level of ^{32}P was significantly decreased, when platelets were treated with leupeptin. These data indicate that inhibition of calpain alters the kinetics of phosphorylation of the LC in ionomycin stimulated platelets possibly by blocking proteolysis of PKC, MLCK and calcineurin. Supported by NIH grant HL29766.

129.3

STUDIES ON THE BIOSYNTHESIS OF 5-LIPOXYGENASE IN HL60 CELLS.
S. Kargman¥*, R.A.F. Dixon†*, R.E. Jones†*, R.E. Diehl†* and C.A. Rouzer¥* (SPON: A.W. Ford-Hutchinson), ¥Merck Frosst Canada Inc. P.O. Box 1005, Pointe Claire-Dorval, Québec H9R 4P8 and † Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

Exposure of human HL60 cells to DMSO results in their differentiation to mature granulocyte-like cells which concomittantly acquire the capacity to synthesize leukotrienes. Immunoblot analysis of protein from differentiated HL60 (dHL60) cells revealed an 80 kD species comigrating with 5-lipoxygenase (5-LO) purified from human leukocytes. The dHL60 cells contained approximately 5-fold more 5-LO protein and 5-LO enzyme activity than did undifferentiated cells. Analysis of HL60 cell total RNA, using a human 5-LO cDNA probe, detected a single 2700 nucleotide RNA species in the dHL60 cells only; therefore 5-LO appears to be transcriptionally regulated. Metabolic labelling studies demonstrated that in dHL60 cells, 5-LO is a quantitatively minor protein with a t 1/2 of ~ 30 hours. Activation of dHL60 cells with Ca^{2+} ionophore A23187 resulted in the loss of 5-LO protein and activity from the cytosol and the accumulation of inactive 5-LO protein in a membrane fraction. Despite the loss of total 5-LO activity after ionophore treatment, no augmentation of 5-LO synthesis occurred. Finally, we were unable to detect any glycosylation, fatty acid acylation or phosphorylation of human 5-LO in HL60 cells.

129.4

ALKALINE PHOSPHATASE AS A MARKER OF NEUTROPHIL (PMN) MATURITY IN PERIPHERAL BLOOD AND BONE MARROW OF SHEEP.
D.L. Rosolia*, F. Cerasoli, Jr.*, P.J. McKenna*, S.P. Peters*, M.H. Gee, and K.H. Albertine. Jefferson Medical College, Philadelphia, PA, 19107.

Infusion of zymosan-activated plasma (ZAP) in awake sheep is associated with down regulation of PMN superoxide anion (O_2-) release 24h after the infusion. This effect appears to be on leukocytic stem cells in bone marrow. Therefore, the maturational status of releasable PMNs may be an important determinant of PMN inflammatory function. The present study was done to compare PMN maturation cytochemically in blood and bone marrow from control sheep and sheep 24h after ZAP infusion. Smears of arterial blood and bone marrow aspirates from the superior iliac crest were stained for PMN alkaline phosphatase (PAP) activity (Sigma Kit 86-R). PAP activity in 100 PMNs was ranked from 0 to 4. The table summarizes the average results as percentages.

Source (n)	Period	PAP 0	PAP 1	PAP 2	PAP 3	PAP 4
Blood (5)	Baseline	0	1	16	34	49
Blood (2)	24h after ZAP	0	2	14	51	33
Marrow (4)	Baseline	16	21	17	24	22

24h after an *in vivo* ZAP infusion, there is a shift in the percentage of circulating PMNs which exhibit PAP 3 and PAP 4 activity, suggesting that younger cells have entered the circulation, most likely from bone marrow. This apparent shift toward less mature cells may help explain the observed decrease in PMN inflammatory function after ZAP-induced acute lung injury. [Supported by NIH grants HL36237, HL34014, HL38075, AI24509, and the Center for Critical Care Research]

129.5

EFFECT OF IN VIVO ENDOTOXIN ON NEUTROPHIL (PMN) SUPEROXIDE (O_2-) RELEASE IN AWAKE SHEEP.
F. Cerasoli, Jr.*, Y. Ishihara*, S.P. Peters*, K.H. Albertine and M.H.Gee. Jefferson Medical College, Philadelphia, PA 19107

We have previously shown that phorbol myristate acetate (PMA) stimulated O_2- release from PMNs isolated 24 h after intravascular complement activation is decreased. To determine if endotoxemia has a similar effect on PMN function we infused 5.0 µg/kg (0.17 µg/min) into chronically instrumented sheep. PMNs were isolated before, 24, 48, and 72 h after infusion and PMA stimulated (10^{-11} M to 10^{-5} M) O_2- release was measured *in vitro*. Circulating PMN counts decreased to 20% of baseline for 6 h after endotoxin. Recruitment occurred within 24 h resulting in PMN counts 3 to 4 times baseline on day 3. Twenty four h after endotoxin the sensitivity of these PMNs to subthreshold doses of PMA was increased (3.6 nmoles/45 min at 10^{-11} M) compared to baseline (0.7 nmoles/45 min at 10^{-11} M). There was no change in O_2- release due to maximal stimulation (5.51 nmoles/45 min with 10^{-7} M vs 6.22 nmoles/45 min with 10^{-7} M at baseline). Sensitivity to subthreshold PMA decreased 48 and 72 h after infusion with no change in maximal release. These data show that *in vivo* endotoxin enhances O_2- release from PMNs stimulated with subthreshold doses of PMA. *In vivo* endotoxin may augment PMN mediated tissue injury by enhancing the effect of PMN stimuli which would normally produce a minor injury. [Supported by NIH grants HL 36237, HL 34014, HL38075, AI 24509 and the Center for Critical Care Research]

129.6

SUPEROXIDE (O_2-) RELEASE FROM MATURING NEUTROPHILS (PMNs) ISOLATED FROM SHEEP BONE MARROW.
P.J. McKenna*, F Cerasoli Jr.*, D. Rosolia*, M.H. Gee, K.H. Albertine. Jefferson Medical College, Philadelphia PA 19107

We have shown previously that PMN "down regulation" occurs 24 hrs after *in vivo* zymosan activated plasma (ZAP) infusion into awake sheep. Since the half life of circulating PMNs cannot explain this result, we hypothesized that ZAP affects maturing PMNs within the bone marrow. The purpose of these initial studies was to characterize O_2- release from maturing neutrophils isolated from normal sheep peripheral blood and bone marrow aspirated from the superior iliac crest . Neutrophils were isolated on a Percoll-plasma discontinuous gradient. Mature PMNs obtained from peripheral blood and marrow separated at the 80/70% layer while PMN precurser cells from marrow separated at the 70/60% and 60/50% layer. 250,000 cells from each layer were plated in microtiter wells and stimulated (10^{-11} to 10^{-5}M) with phorbol myristate acetate (PMA). PMNs collected from the 80/70% and 70/60% layers released 5 to 7 nmoles O_2- upon maximal stimulation (10^{-7}M PMA) while PMNs collected from 60/50% released 50% less O_2-. There was no difference in the sensitivity of the density layers to PMA. These data suggest 1) immature and mature PMNs can be separated based on density, 2) mature PMNs exist in marrow and are capable of O_2- release, and 3) immature PMNs in marrow release less superoxide than mature PMNs. Comparison of PMN populations and function in blood and marrow may lead to an explanation of PMN "down regulation". [Supported by NIH grants HL 36237, HL 34014, HL 38075, AI 24509 and the Center for Critical Care Research]

129.7

INTRACELLULAR pH REGULATION IN SQUID LEUCOCYTES. T.A. Heming*, S.E. Brown*, C. Vanoye* & A. Bidani. Univ. of Texas Medical Branch, Galveston, TX 77550

Intracellular pH_i of squid (Sepioteuthis lessoniana) leucocytes was followed using the fluorescent dye BCECF (2,7-biscarboxyethyl-5,6-carboxyfluorescein). Leucocytes were obtained from branchial heart hemolymph (~$5 \cdot 10^6$ cells/mL) and loaded with BCECF (40 min, 25 °C). Leucocyte pH_i was 7.31±0.01 in nominally CO_2-free saline (mM, 425 NaCl, 10 KCl, 63 $MgCl_2$, 0.6 HEPES; 16 mg% dextrose, pH_o 7.4 at 25 °C). pH_i varied little over the extracellular pH_o range 6.8-7.8; pH_i=6.52+0.11 pH_o. Acidification with weak acids, K-acetate or Na-propionate (40 mM), rapidly reduced pH_i by 0.20± 0.02 units ($t_{1/2}$~20 sec). pH_i then recovered with $t_{1/2}$=3.5±0.5 min. pH_i recovery was independent of external Na, insensitive to 0.1-1 mM amiloride, but was blocked by 1 mM NEM (N-ethylmaleimide). The data indicate that in squid leucocytes, pH_i recovery from an intracellular acid load ($pH_i \geq 7.1$) does not involve a Na/H antiporter. The results support the presence of a previously unrecognized mechanism, specifically a NEM-sensitive H-ATPase, in these invertebrate leucocytes, similar to the H-ATPase recently described in mammalian alveolar macrophages (Brown et al., FASEB J. 2:A719, 1988).

MECHANISMS OF MICROSOMAL OXIDATION

130.1

INTERFERON EVOKED CHANGES IN THE mRNA LEVELS FOR CYTOCHROME P-452. L.C. Knickle* and K.W. Renton. Dept of Pharmacology, Dalhousie Univ., Halifax, N.S., Canada B3H 4H7

Interferon and immuno-modulators are well known to depress cytochrome P-450 and related drug biotransformation. In this study the effect of interferon on the synthesis of the cytochrome P-452 apo-protein was investigated using a synthetic oligodeoxyribonucleotide hybridization probe to assess cytochrome P-452 mRNA levels in rat liver. All animals were induced with clofibrate. RNA was isolated from the livers of induced animals treated with poly rI.rC (an interferon inducer) or saline. The levels of cytochrome P-452 mRNA were determined by Northern blot experiments using a 20 base oligomer probe specific to cytochrome P-452 selected from the cytochrome P-452 cDNA sequence (1). Cytochrome P-452 mRNA levels were found to be depressed in poly rI.rC treated animals compared to controls. These changes in mRNA levels will be correlated with microsomal cytochrome P-452 content as determined by lauric acid hydroxylation.

(1) Hardwick, J.P. et al. (1987). J. Biol. Chem. 262, 801-810.

Supported by Medical Research Council of Canada.

130.2

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A DOG LIVER CYTOCHROME P-450. Paul J. Ciaccio and James R. Halpert.* University of Arizona, Tucson, AZ 85721.

A cytochrome P-450 called PBD-1 isolated from liver microsomes of an adult male Beagle dog treated with phenobarbital (PB) possesses structural and functional similarities to steroid-inducible P-450 forms from rat liver microsomes. The sequence of the first 28 amino acids is identical in 16 and 15 positions to that of rat PCNa and PCNb, respectively. In addition, upon immunoblot analysis, polyclonal antibodies raised against PBD-1 recognize PCNb and polyclonal antibodies raised against PCNb cross-react with PBD-1. Like rat steroid-inducible forms, PBD-1 loses catalytic activity upon purification. Triacetyloleandomycin (TAO) complex formation and erythromycin demethylase, marker activities for steroid-inducible forms from the rat, increase 4- and 5-fold in dog liver microsomes upon PB-treatment. Antibodies raised against PBD-1 inhibit greater than 70% of TAO complex formation and 50% of erythromycin demethylase activity in microsomes from PB-treated dogs. TAO complex formation is not inhibited by chloramphenicol, a selective inhibitor of the major PB-inducible dog liver cytochrome P-450 PBD-2. These data suggest that PBD-1 is responsible for a major portion of macrolide antibiotic metabolism by microsomes from PB-treated dogs. However, in contrast to the rat, microsomes from untreated and PB-treated dogs exhibit markedly lower steroid 6-β hydroxylase activity, another marker activity of rat steroid-inducible P-450 forms. Anti-PBD-1 IgG has little or no effect on this activity. (Supported by NIH grants ES 00151 and ES 03619).

130.3

TIME-DEPENDENT, IRREVERSIBLE INACTIVATION OF CYTOCHROME P-450 BY 8-METHOXYPSORALEN IN LIVER MICROSOMES OF MICE. D.C. Mays,* J.B. Hilliard,* D.D. Wong,* M.A. Chambers* and N. Gerber. Departments of Family Medicine and Pharmacology, Ohio State University, Columbus, Ohio 43210.

8-Methoxypsoralen (8-MOP) is metabolically activated by cytochrome P-450 to reactive intermediates and is a potent inhibitor of drug metabolism in experimental animals and humans (Mays et al., J. Pharmacol. Exp. Ther. 243: 227, 1987; Clin. Pharmacol. Ther. 42: 621, 1987). Investigations in liver microsomes isolated from mice pretreated for three days with 70 mg/kg/day of β-naphthoflavone were undertaken to help elucidate the mechanism of inhibition of drug metabolism by 8-MOP. Preincubation of liver microsomes with 40 uM 8-MOP for 1, 4, or 10 min in the presence of an NADPH-generating system decreased the activity of 7-ethoxycoumarin deethylase (ECD) in a time-dependent manner. Vmax for formation of 7-hydroxycoumarin decreased from 3.4 to 1.4 nmol/mg protein/min after preincubation with 40 uM 8-MOP for 10 min. Addition of 1 mM cysteine reduced covalent binding of reactive metabolites of 8-MOP to microsomal protein from 5.6±0.2 to 1.6±0.2 nmol/mg protein, but did not block the loss of ECD activity or the 45% loss of cytochrome P-450, measured spectrally as CO-binding pigment. There was no evidence of MI-complexes. This study shows that metabolism of 8-MOP in mouse microsomes leads to irreversible inhibition of cytochrome P-450 and the trapping of reactive intermediates of 8-MOP by cysteine does not protect against inhibition of drug metabolism.

130.4

ANTIBODY INHIBITION OF THE SPARTEINE / DEBRISOQUINE MONOOXYGENASE, VARIATION OF THE CATALYTIC SITE. R.F. TYNDALE*, T. INABA and W. KALOW. Univ. of Toronto, Toronto, Ont, M5S 1A8.

A genetic polymorphism in the oxidative metabolism of Debrisoquine (DB) and Sparteine (SP) is well known. A large number of restriction fragment polymorphisms exist for both phenotypes (FJ Gonzalez et al Nature FEB 1988, UA Meyer personnal comm.) suggesting variation within as well as between the phenotypes. This variation has been reflected in the variation of K_m of SP metabolism which we observed with a human liver bank. We used antibody against rat P450db1 to recognize the human enzyme involved in the DB polymorphism. Using SP as a prototype substrate for the human polymorphic enzyme we examined the micro-heterogeneity of the catalytic site. Anti-(rat)P450db1 serum was found to inhibit the metabolism of SP in an in vitro human enzyme assay. The percent to which the antibody was able to inhibit the SP metabolism varied in different liver samples reflecting variation at the catalytic site. Western Blots of the liver microsomes confirmed the variation in the of binding of this antibody to the primary db1 band. The main finding of this study was that the variability that is known from sequencing and kinetic data is reflected in the range of inhibition of the SP metabolism by QD and by this specific antibody. (Thanks to Dr. FJ Gonzalez of NIH for his help. Supported by MRC MT-4763)

130.5

AZOREDUCTION OF DIMETHYLAMINOAZOBENZENE (DAB) IN PRIMARY CULTURES OF RAT HEPATOCYTES. INDUCTION BY HYPOLIPIDEMIC AGENTS. A.M. Stoddart* and W.G. Levine. Dept. of Mol. Pharmacol., Albert Einstein Coll. Med., Bronx, NY 10461.

This laboratory has investigated DAB azoreduction by hepatic microsomal cytochrome P-450 and its specific induction by clofibrate. To extend these studies, a primary hepatocyte culture system was developed as a model. Hepatocytes isolated from male S-D rats were incubated in a medium containing fetal calf serum and hydrocortisone for up to 96 hours with varying concentrations of clofibrate or nafenopin, a related hypolipidemic. DAB azoreductase and laurate hydroxylase activities decreased rapidly in untreated cultures. However, there was gradual marked induction of both activities in media supplemented with clofibrate and/or nafenopin. Responses of both activities were concentration dependent. Maximum induction of both enzymes was seen at 72 hr. An additive effect of both drugs was observed at this period. Despite induction of both enzyme activities, total cytochrome P-450 could only be detected from its reduced-CO spectrum during the first few hours of culture. Nevertheless, inhibitors of cytochrome P-450 activity blocked DAB azoreductase activity. The results demonstrate that a primary culture of rat hepatocytes is a useful model for studying the regulation of DAB azoreductase activity.

130.6

INTERACTIONS OF DIMETHYL SULFOXIDE (DMSO) WITH DIMETHYL NITROSAMINE-N-DEMETHYLASE (DMND) IN MOUSE LIVER. E.H. Jeffery, K.C. Arndt* and W.M. Haschek*. Univ. of IL, Urbana, IL 61801.

We have previously shown that DMSO protects against acetaminophen hepatotoxicity, but not respiratory toxicity, in the mouse (Jeffery and Haschek, Toxicol. Appl. Pharmacol. 93:452-461; 1988). We therefore considered the possibility that DMSO or one of its two metabolites dimethyl sulfide or dimethylsulfone could inhibit an hepatic cytochrome P-450 involved in acetaminophen toxicity, that is not found in lung. The ethanol-inducible cytochrome P-450 falls into this category, and is further implicated by the fact that chronic ethanol enhances acetaminophen toxicity, while acute ethanol protects. The in vitro effect of DMSO on mouse liver DMND was carried out using 1 or 50 mM dimethyl nitrosamine (DMN). 1 mM DMN was used to specifically measure the high affinity (Km 0.07 mM) activity due to the ethanol-inducible cytochrome, while 50 mM DMN measured non-specific activity (Km 77 mM) representative of general cytochrome P-450 activity. Kinetic analysis indicated that only the low Km form of DMND is inhibited by in vitro DMSO. The inhibition was competitive, with a K_I of 0.93 ± 0.14 mM. However, DMSO treatment of mice (4 g/kg BW 24 h prior to death) resulted in no inhibition of either the low or high Km forms of hepatic microsomal DMND. In conclusion in vitro DMSO inhibition of cytochrome P-450 activity is specific to the low Km DMND, but that in vivo DMSO does not inhibit microsomal DMND.

130.7

INDUCTION BY N-BENZYLIMIDAZOLE ALTERS SEDIMENTATION PROPERTIES OF HEPATIC SUBCELLULAR ORGANELLES. Wendy L Hopson and Michael R Franklin Univ. of Utah, Salt Lake City, UT 84112.

N-benzylimidazole (NBI)(75 mg/kg/day x3 days) increased liver cyt.P-450 concn 3-4 X in rats, similar to induction by clotrimazole and higher than that after phenobarbital and B-naphthoflavone. The yield of microsomal P-450 per g tissue, in contrast to the other inducers, was not increased above that from untreated rats, however, due to a large drop in the yield of microsomal protein per g liver. To examine this anomaly, liver homogenates(20% w/v in 0.25M sucrose) were prepared and centrifuged at various g forces for 20 min. The supernatants were assayed for protein, MAO activity (mitochondrial marker),P-450, and p-nitroanisole demethylase activity (pNA). At any g force, all parameters were removed to a greater extent from the homogenates from NBI-treated rats. At 5,000g, the above parameters were decreased respectively, 15, 75, 15, and 25% in untreated and 25, 85, 35, and 45% in NBI-treated. At 18,000g, 50% of the original P-450 and pNA in the untreated had been removed, while the corresponding values for NBI were 70 and 85%, and 95% for 7-ethoxyresorufin deethylase activity. Microsomal parameters removed from the homogenate, when expressed per mg of removed protein, approached 150,000g pellet (microsome) values with increasing g forces. The results show that NBI treatment alters sedimentation characteristics of at least two subcellular organelles. Whether N-BI is unique among P-450 inducers in this regard requires further study.
(Supported by USPHS grant # GM 39335)

130.8

INDUCTION OF PEROXISOMAL ß-OXIDATION (pßox) BY SODIUM 10-UNDECYNYL SULFATE (SUS) IN PRIMARY CULTURES OF RAT HEPATOCYTES. R.L. Hawke*, G.L. Hodgson*, J.S. Zulkoski*, H.B. Marr*, M.J. Clarke* and R.M. Welch. The Wellcome Research Laboratories, Research Triangle Park, NC 27709.

It has been suggested that a cascade of metabolic events, possibly including ω-hydroxylation of fatty acids, may trigger the peroxisomal proliferative response of the liver following exposure of rats to agents such as ciprofibrate (CIP). 10-Undecynoic acid and SUS, the sulfate ester analogue, have been shown to be highly specific inactivators of lauric acid (LA) hydroxylases *in vitro* [CaJacob and Montellano, Fed. Proc., 44, 1611 (1985)]. Therefore it was of interest to determine the effects of these fatty acid analogues on pßox and on CIP induction of pßox in cell culture. Treatment of rat hepatocytes with 100 µM-CIP for 72 hrs resulted in 14- and 17-fold induction of LA 12-hydroxylase (12-OH) and pßox, respectively. The addition of 1-10 µM SUS to the culture media during the 72 hrs treatment with CIP resulted in a dose-dependent decrease in the induced levels of LA 12-OH (85% inhibition at 7.5 and 10 µM). No effect was observed on the induced levels of pßox at this dose or over the entire dose-response curve. Treatment of rat hepatocytes with higher doses of SUS only, resulted in dose-dependent increases in pßox (10 fold at 250 µM). This induction was not observed with sodium dodecyl sulfate or with 10-undecynoic acid. Perfluorooctanoic and perfluorodecanoic acids were found to be potent inducers of LA 12-OH but weak inducers of pßox. These results suggest that while both CIP and the metabolically inert fatty acids can elicit a proliferative response in cell culture, ω-hydroxylation is not a prerequisite for this response.

PULMONARY CIRCULATION II — THURSDAY AM

134.1

DISTRIBUTION OF LYMPHATIC STOMATA OVER THE PERITONEAL AND PLEURAL DIAPHRAGMATIC SURFACE IN RABBITS.
D. Negrini, S. Mukenge, M. Del Fabbro, G. Miserocchi. Istituto di Fisiologia Umana, Universita' di Milano, Italy.

The abdominal cavity of 8 anesthetized, supine, apneic rabbits was opened by a median incision: the diaphragm was rinsed with heparinized saline and fixed by instilling 0.2% gluteraldehyde in the peritoneal cavity. After 10 min the diaphragm was removed and its pleural side was rinsed with heparinized saline. The whole piece was then left in gluteraldehyde for 48h. Samples of either the pleural or peritoneal side of the tendineous portion of the diaphragm were cut out and processed for scanning electronmicroscopy. The average diameter of pleural and peritoneal stomatas (n=441) was 3.3 ± 2.7 (SD) microns. We counted, on the average, 37000 and 6600 stomatas per cm on peritoneal and on pleural side of the tendineous portion, respectively. The stomatas covered .56 and .05% of the total peritoneal and pleural inspected area respectively. For a diaphragmatic peritoneal liquid absorption of 2.7 microL/minxcm^2 (previously estimated under physiological conditions), the velocity of fluid progression in the initial lymphatics was calculated at 6mm/min.

134.2

MONOCLONAL MURINE ANTI-LIPID A ANTIBODY (E5) FAILS TO PREVENT THE ENDOTOXIN REACTION IN SHEEP. A.P. Wheeler*, G.R. Bernard*, K.L. Brigham Division of Pulmonary Medicine, Vanderbilt Univ Medical Ctr, Nashville, TN 37232.

In sheep, endotoxin (LPS) causes pulmonary hypertension, hypoxemia, leukopenia, protein rich lung lymph, reduction in dynamic compliance (C_{dyn}) and increases resistance to airflow (R_L), changes similar to those seen in human sepsis and ARDS. In awake lymphing sheep, we used a whole body plethysmograph to measure C_{dyn}, R_L, and functional residual capacity (FRC). Pulmonary artery (PA), left atrial (LA), and systemic arterial (SA) pressures were recorded continuously. Arterial blood gases (for calculating A-aDO_2), leukocyte counts and lymph samples were collected every 30 min. Animals received a 30 min (2 mg/kg) infusion of E5 (XOMA Corp., Berkeley, CA) 4 hours before LPS (0.75 mcg/kg) challenge (n=4), or were given a mixture of LPS and E5 in identical doses over 30 minutes (n=6). A control group given only E5 (n=4) showed no change in any measured parameter while a control group receiving LPS alone (n=6) exhibited a typical endotoxin response. Mixing E5 with the LPS prior to infusion blunted the early PA pressure rise (33.7 ± 4 cm H_2O vs 43.2 ± 8) when compared to LPS controls, however, no other measured parameter was improved in the pretreatment group, or the in vitro group. These studies suggest that E5 does not effectively neutralize LPS, or that very small amounts of unbound LPS are able to produce the endotoxin response in sheep, an animal extremely sensitive to the effects of LPS.

134.3

NEUTROPHIL (PMN) EMIGRATION INTO ALVEOLI FOLLOWING TRACHEAL INSTILLATION OF LPS DEPENDENTS ON PMN CD11/CD18 ADHERENCE COMPLEX AND ON PROTEIN SYNTHESIS. R. Winn, C.L. Rice, and J.M. Harlan*. University of Washington, Seattle, Washington 98195.

PMN adherence and emigration into dermis following endotoxin (LPS) injections depends on both PMN CD11/CD18 and newly synthesized expression of endothelial leukocyte adherence molecules (ELAMs). We examined whether PMN adherence and emigration into the lung involved similar mechanisms. PMN adherence due to CD11/CD18 was blocked with the anti-CD18 monoclonal antibody (MAb) 60.3, and expression of ELAMs was prevented with cycloheximide (Cx). Catheters were placed in the vena cava and trachea (above the carina) of anesthetized rabbits. Each animal had 2 ml of saline containing 20 ug of LPS instilled into the trachea. Three groups of 4 animals were studied: 1) Control (LPS only), 2) MAb 60.3 (pretreated with 2 mg/kg MAb), 3) MAb 60.3 plus Cx (pretreated with 2 mg/kg 60.3 and 500 ug/kg Cx, 100 ug of Cx intratracheally, and 500 ug/kg Cx at 1, 2 and 3 hrs. after LPS). At 4 hrs., the left lung was lavaged with 10 ml of saline then total and differential cell counts done on lavage fluid. PMN counts and reduction from control values are shown.

	Control	MAb 60.3	MAb 60.3 + Cx
PMNs/ml	$4.1 \pm 3.4 \times 10^6$	$1.1 \pm 0.04 \times 10^6$	$0.2 \pm 0.2 \times 10^6$
% reduction	--	74%	95%

These results indicate the endotoxin induced PMN emigration in the lung is mediated primarily by PMN CD11/CD18. (Supported by NIH Grants RR 05432 and HL 30542).

134.4

EFFECT OF LUNG INFLATION ON VENULAR AND PERIVENULAR INTERSTITIAL PRESSURES IN NEWBORN RABBIT LUNGS. CD Fike, SJ Lai-Fook, & RD Bland. Cardiovasc. Res. Inst., Univ. of CA, San Francisco & Dept. of Peds., Baylor College of Medicine, Houston, TX. To determine the effect of lung inflation on the hydrostatic pressure gradient for fluid flux across venules, we isolated and perfused with blood the lungs from 17 newborn rabbits, 7-14 days old. We maintained constant blood flow and monitored left atrial (Pla) and pulmonary arterial (Ppa) pressures. We used the direct micropuncture technique to measure 20-50 um diameter venular (Pmv) and perivenular interstitial (Pi) pressures relative to pleural pressure. In 6 lungs we measured Pi at airway (Paw) pressures of 5 and 15 cm H_2O. In 11 other lungs we measured Pmv at both Paw pressures. During micropuncture we kept Pla > Paw (zone 3).

Table summarizes pressures (cm H_2O), mean $\pm$ SD.

Paw	Ppa	Pmv	Pi	Pla
5	20.5 ± 2.0	11.9 ± 1.3	3.3 ± 0.8	8.6 ± 0.7
15	28.5 ± 2.1	21.0 ± 1.9	6.7 ± 1.4	16.7 ± 1.1

With lung inflation Pmv increased by 9.1 cm H_2O and Pi increased by 3.4 cm H_2O. Thus, the hydrostatic pressure gradient for fluid flux across venules increased by 5.7 cm H_2O. This suggests that the tendency for edema formation might increase at high inflation pressures during positive pressure ventilation of newborn lungs if zone 3 conditions are maintained. (Supported by HL-25816 and BRSG to CF.)

134.5

EFFECT OF METHYLPREDNISOLONE ON NITROGEN DIOXIDE (NO_2)-INDUCED PULMONARY EDEMA IN GUINEA PIGS. M. Vassilyadi and R.P. Michel. McGill Univ., Montréal, QC., Canada, H3A 2B4

The treatment of NO_2-induced lung edema is controversial, and the mechanisms and patterns of interstitial fluid accumulation in this form of permeability edema are unclear. To ascertain the role of methylprednisolone (MP) in the therapy of NO_2-induced edema, we exposed 108 prone guinea pigs, in groups of 12, to 277-448 ppm·hr NO_2: in 60, we administered the MP just before, and in 48 immediately after exposure. In each group, half the animals received 30 mg/kg MP IP., the other half saline. Mortality rates and lung wet weight/dry weight (W/D) ratios were calculated. Alveolar edema, periarterial interstitial edema, and acute bronchiolitis were graded semiquantitatively by light microscopy on sections of the middle (ML) and lower lobes (LL) fixed by freeze-substitution. We found NO_2 produced an exposure-dependent increase in lung water ($R = 0.70$, $p<0.01$). Pre-treatment with MP produced a four-fold reduction in mortality, a significant fall in W/D ratios and in alveolar and interstitial edema, but no difference in bronchiolitis. Treatment with MP after NO_2 was ineffective. Both LL and ML had equally abundant alveolar edema, but LL had significantly more interstitial edema, supporting our previous findings in supine dogs that in NO_2-induced edema, interstitial accumulation may follow alveolar flooding, with interlobar discrepancies probably due to differences in lung volume or in ventilation.

Supported by the MRC of Canada.

134.6

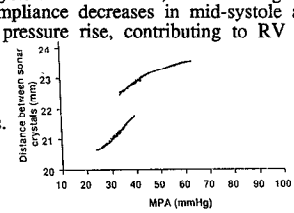
NON-INVASIVE, ON-LINE MEASUREMENT OF REGIONAL PULMONARY HYPOXIC VASOCONSTRICTION IN THE CONSCIOUS ANIMAL. Daniel W. Sheehan, Robert A. Klocke and Leon E. Farhi, State Univ. of New York at Buffalo, Buffalo, NY 14226

Most previous studies of the pulmonary vascular response to regional hypoxia have been complicated by one or more of the following: anesthesia, surgery, a rise in pulmonary arterial pressure and changes in PaO_2 and $PaCO_2$. Our non-invasive technique, which allows one to alter the O_2 level in the right apical lobe (RAL) of the conscious sheep while measuring continuously the fraction of pulmonary blood flow to that lobe (Q_{RAL}), relies on the fact that some of the methane produced in the animal's gut enters the blood. Since CH_4 is cleared by the lungs nearly *in toto*, its fractional output from the RAL reflects the lobe's share of the perfusion, normally about 12%. A double-lumen tracheal divider separates the gas exchange of the RAL from that of the rest of the lungs (RL). Expired gas is entrained into a constant gas flow (5 and 25 l/min for the RAL and RL respectively), in which CH_4 is measured with a flame ionization detector. Q_{RAL} is calculated from flows and CH_4 fractions, averaged every half minute. In ten experiments on three sheep, when the RAL inspirate was switched from air to N_2, PaO_2 remained above 80 torr; cardiac output, pulmonary vascular pressures and RL gas pressures were unaffected. Q_{RAL} decreased to 31% of its control value in 16 min but rebounded to 51% at 67 min before stabilizing at 36% in 92 min. This shows that one can study the local response in the absence of systemic changes and that some of the existing data may have been obtained prior to steady state. (Supp. by NHLBI)

134.7

COMPLIANCE IN THE CALF MAIN PULMONARY ARTERY DECREASES IN MID-SYSTOLE IN ACUTE PULMONARY HYPERTENSION. E.C. Orton*, P.R. Bakerman*, K.R. Stenmark*, J.T. Reeves. U. of Colorado Health Sci. Ctr., CVP Res. Lab and Webb-Waring Lung Inst. and Children's Hosp., Denver, CO 80262

In pulmonary hypertension, a late systolic rise in the right ventricular (RV) pressure suggests an additional load contributing to impaired function. We hypothesized that compliance in the large conduit arteries decreased in mid-systole causing the late RV load. In calves, sonar crystals were implanted in the main pulmonary artery (MPA) wall (diameter) and Millar catheters placed in the RV and MPA for simultaneous pressure measurements over a wide range of pulmonary artery pressures. In normotension, MPA pressure/diameter relationship was relatively linear and steep. In acute pulmonary hypertension, diameter did not rise as rapidly with pressure increments and this situation worsened abruptly in mid-systole. We conclude, that assuming diameter is a function of volume, compliance decreases in mid-systole and could produce the late systolic pressure rise, contributing to RV failure in pulmonary hypertension.



Characteristic normal and pulmonary hypertensive curve during systole, each with 10 superimposed beats.

134.8

INCREASED *a*-SKELETAL ACTIN MESSENGER RNA OCCURS IN RIGHT VENTRICULAR HYPERTROPHY SECONDARY TO SEVERE PULMONARY HYPERTENSION IN NEONATAL CALVES. P.R. Bakerman*, K.R. Stenmark*, J.H. Fisher*, SPON: J.T. Reeves. U. of Colorado Health Sci. Ctr., CVP Res. Lab and Webb-Waring Lung Inst., and Children's Hospital, Denver, CO 80262

Calves raised at 4300m simulated altitude, rapidly develop suprasystemic pulmonary artery pressures. Right ventricular hypertrophy and failure occurs as a consequence of the increased pressure load. Alterations in contractile proteins may allow for improved myocyte performance or reflect myocyte differentiation. We have chosen to study the contractile protein actin. Since actin isotypes in the heart are difficult to quantitate by protein analysis, we compared mRNA levels in tissue minces of the right (RV) and left ventricles (LV) in control and hypertensive calves. Northern and slot blots were probed with cDNA probes specific for *a*-skeletal and *a*-cardiac actin (pHM*a*A-3UT-Fnu and pHMcA-3UT-DB) respectively. Control animals primarily express cardiac actin with minimal skeletal actin expression in the RV and LV. Skeletal actin mRNA was dramatically increased in the RV of hypertensive animals whereas cardiac actin mRNA is decreased significantly. This isotype switch does not occur in the LV of these animals. Although the physiologic significance of this change is unknown, a change in the RV myocyte phenotype has occured. Skeletal actin may have a different contractile and/or function or may represent an adaptation of the RV myocyte to pulmonary hypertension.

134.9

UNILATERAL HYPOXIC PULMONARY VASOCONSTRICTION IN THE DOG, PONY, AND MINIATURE SWINE. A.R. Elliott, E.P. Steffey, K. Jarvis and B.E. Marshall. Dept. of Surgery, School of Veterinary Med. UCD, Davis, CA 95616. Dept. of Anesth., School of Med., U. of PA., Philadelphia, PA. 19104.

The hypoxic pulmonary vasoconstrictor (HPV) response to unilateral hypoxia was analyzed in pentobarbital anesthetized dogs (n=5), swine (n=5), and ponies (n=5). The left and right lung lobes (LL, RL) were differentially ventilated with the LL being exposed to inspired oxygen concentrations (C_TO_2) of 100%, 12%, 8% or 4% while the RL always received an C_TO_2=100%. Cardiac output, mean pulmonary pressures, and arterial and mixed venous blood gases were measured, as well as percent pulmonary blood flow distribution using 15μ radioactive microspheres. LL P_AO_2, hypoxic stimulus ($P_sO_2 = P_AO_2^{0.62} \times P_{\bar{v}}O_2^{0.38}$)[1], and percent flow diversion (%FD) were calculated at each C_TO_2. At C_TO_2 = 4% there were significant differences ($p>0.05$) between the %FD (mean ± S.E.) responses of each species: the % FD_{swine} (95.14 ± 1.32) > $\%FD_{pony}$ (76.02 ± 4.62) > $\%FD_{dog}$ (50.09 ± 9.45). For all species the magnitude of %FD is inversely related to the level of regional hypoxia. There are marked species differences in the magnitude and sensitivity for stimulation of HPV between the swine, the strongest responder; the pony, an intermediate responder; and the dog, the weakest responder. [1]Marshall, C. and B.E. Marshall. J. Appl. Physiol. 53:711-716, 1983.

134.10

LUNG REPERFUSION INJURY IN UNANESTHETIZED SHEEP. J.E. Lovd*, N.E. Wickersham *, K.L. Brigham.
Center for Lung Research, Vanderbilt Univ, Nashville, TN 37232.

To investigate the mechanisms of reperfusion lung injury, we sought to develop a model in unanesthetized sheep. To occlude the left main pulmonary artery (PA) we surgically implanted an inflatable cuff and allowed full postoperative recovery. We found only small changes relative to baseline (Bl) in lung lymph flow and hemodynamics during or after ischemic periods of up to 24 hours. Bronchial arterial ligation in a few animals had no apparent influence on the physiologic response to unilateral pulmonary artery reperfusion (Reperf). After the hypothesis that the lung was protected from ischemia by oxygen supplied via airways, we obstructed ventilation of the left lung by a balloon catheter in the left mainstem bronchus during a 12 hour occlusion of the left PA. Ischemia was associated with minimal physiologic abnormality, but during reperfusion all 5 animals developed marked physiologic changes characterized by hypoxemia (Bl=86 ± 4 torr, Reperf=60 ± 6), a 3-fold increase in lung lymph flow (Bl=1.6 ± 0.2 ml/15 min, Reperf=5.4 ± 0.7) with a concomitant increase in lung lymph L/P ratio (Bl=0.69 ± 0.04, Reperf=0.80 ± 0.02). Pulmonary artery pressure rose slightly (Bl=25.5 cmH_2O, Reperf= 29.6). An increase in lung lymph flux of conjugated dienes (Bl=0.46, Reperf= 2.43) suggests free radical lipid peroxidation is present. We conclude that unilateral occlusion of lung perfusion in awake sheep does not result in significant reperfusion injury, but severe reperfusion injury does occur after release of unilateral occlusion of both perfusion and ventilation.

DRUG ABUSE II

135.1

PUPIL SIZE AND THE LIGHT REFLEX OVER A COURSE OF BUPRENORPHINE (B) ADMINISTRATION. W.B.Pickworth* and H.Lee* (SPON:T.-P. Su). NIDA,Addict.Res.Cntr. Baltimore, Md. 21224.

Opiate-induced changes in pupillary size correlate with euphoria in nonaddicted subjects; pupillary response to opiates in addicts indicate degree of narcotic dependence. We studied the pupillary effects of buprenorphine (B), a partial opiate-agonist, in 17 opiate-dependent volunteers. On each day they received a sublingual ethanol solution of B or placebo. B dosage was elevated rapidly to a daily dose of 8 mg which was maintained for 12 days. One group (n=8) received B (8 mg) or placebo on alternate days; the other group (n=9) continued to receive B (8 mg) daily. This pattern continued for 17 days then each group was given placebo for 20 days. Pupillary size and the light reflex were measured on selected days throughout the study. During B maintenance, pupils size averaged 2.2mm. On B withdrawal pupil size increased to 4.8mm. Maximal pupillary size occurred 5 days after B withdrawal. The latency of the light reflex was constant (150 msec) throughout the study. As pupil size increased the light flash caused a larger constriction; furthermore, the rate of change of pupillary size which averaged 2.6 mm/sec during B maintenance increased to 5.3mm/sec on B withdrawal. These data indicate that pupillary measures of B withdrawal persist for several days and agree with animal studies where acute opiate administration diminished the light reflex.

135.2

ALPRAZOLAM: SUBJECTIVE EFFECTS, BEHAVIORAL EFFECTS AND ABUSE LIABILITY IN DRUG ABUSERS John J. Guarino* and Roland R. Griffiths* (SPON: N.A. Ator) The Johns Hopkins Univ., School of Medicine, Baltimore, MD 21224.

There is increasing recognition that there are differences among the benzodiazepines with respect to abuse liability. The present study was undertaken to provide information about the abuse potential of the recently introduced and widely prescribed benzodiazepine, alprazolam (Xanax®,Upjohn: ALP) in subjects with histories of sedative abuse. On a residential ward the acute effects of placebo, ALP (1, 2, 4 and 6 mg/70 kg) and four lorazepam doses were assessed using a within subject double blind Latin square design. Subjects were 9 healthy male volunteers with histories of sedative and alcohol abuse. Drug effects were assessed with objective performance tasks, staff ratings of drug effect and subject ratings of drug effect, sleep, mood, drug liking and monetary street value. The peak effect for most measures was at 3 hours and effects were mostly gone by 12 hours. When asked to identify the specific drug effect, subjects identified ALP as benzodiazepine-like at low doses, but reported substantially more barbiturate-like identifications at higher ALP doses. ALP produced dose related decrements in performance on the following psychomotor tasks: digit symbol substitution test, number recall, circular lights, balance, sequential acquisition of behavioral chains and picture memory. There were dose related increases in subject ratings of drug effect, drug liking and monetary street value. The dose effect curves for most performance tasks and staff ratings increased monotonically with dose. In contrast, there was no corresponding differentiation among the three highest doses of ALP in terms of subject rated drug effect. This suggests that subjects were unable to accurately gauge the magnitude of impairment produced by the higher doses of ALP, a conclusion consistent with previous reports with other benzodiazepines (e.g., JPET, 234:120, 1986). Overall, the results of this study suggest that ALP has abuse potential. This conclusion is based on the similarity of subjective and performance effects of ALP to those produced by other abused benzodiazepines and barbiturates. Supported by NIDA Grant DA-03889

135.3

ABUSE POTENTIAL EVALUATION OF TRANSNASALLY GIVEN BUTORPHANOL IN HUMANS. Donald R. Jasinski, Kenzie L. Preston and Margaret Testa*, F. S. Key Medical Center and The Johns Hopkins University School of Medicine, Baltimore, MD 21224.

To compare the effects of butorphanol tartrate administered by a nasal spray (transnasal, TN) and by intramuscular (IM) injection a double-blind, double-dummy, balanced latin square cross-over comparison was conducted. Six healthy male subjects with histories of substance abuse including opiates participated. The effects of placebo, TN butorphanol 1 and 2 mg, and IM butorphanol 1, 2, and 4 mg were assessed on measures of subjective, behavioral and physiological response including signs and symptoms, Addiction Research Center Inventory scales, and onset of drug effects. The onset and time course of butorphanol administered by the IM and TN routes were similar. In general, IM butorphanol produced effects which were similar to those described previously including miosis, some opiate-like behavioral and subjective effects, no significant respiratory or cardiovascular effects and increasing dysphoric sedation and perceptual effects with increasing dose. The dysphoric sedation and perceptual effects were prominent only for the IM butorphanol 4 mg dose. These changes were accompanied by decreased subject-rated liking and identifications as an opiate and increased observer- and subject-rated disliking. TN butorphanol 1 and 2 mg produced effects which were qualitatively and quantitatively similar to those produced by IM butorphanol 1 and 2 mg. There is no pharmaco-dynamic evidence to suggest that the abuse potential of TN butorphanol is different from that of IM butorphanol since there is not a more rapid onset of action, the profile of effects is not changed, and there is no greater efficacy. (Supported by Bristol-Myers Co).

135.4

SIMILARITIES BETWEEN THE EEG AND BEHAVIORAL EFFECTS OF ACUTELY ADMINISTERED MORPHINE, AMPHETAMINE, PENTOBARBITAL, NICOTINE, ETHANOL AND MARIHUANA IN HUMAN SUBJECTS. Scott E. Lukas. Alcohol and Drug Abuse Research Center, Harvard Medical School/McLean Hospital, Belmont, Massachusetts 02178.

One theory of drug abuse suggests that it is a form of *stimulus* administration. Drugs are then used to change the organism's behavior and the *direction* of this change is unimportant. Adult male volunteers provided informed consent to be prepared with scalp EEG electrodes and i.v. catheters for blood withdrawal. Subjects then received either i.v. morphine (5, 10 or 20 mg), amphetamine (5, 10 or 20 mg), pentobarbital (50, 100 or 200 mg), nicotine (0.75, 1.5 or 3.0 mg), oral ethanol (0.35 or 0.7 g/kg) or smoked marihuana cigarettes (1.26 or 2.53% Δ^9-tetrahydrocannabinol). Subjects reported drug-induced behavioral effects by operating an instrumental joystick device. At least 1 dose of each drug resulted in multiple paroxysmal bursts of euphoria that occurred within a few minutes of drug administration and typically lasted 1-10 min each. However, subjects continued to detect the drugs' unique pharmacological effects (e.g., stimulation, sedation, etc.) for 0.5 to 3 hr. A microanalysis of the EEG after low and intermediate doses revealed that EEG alpha activity increased during these episodes of drug-induced euphoria. The highest doses caused immediate and profound stimulative or depressive effects which may have obscured the EEG alpha response observed after the lower doses. These data suggest that the reinforcing properties of these drugs may have a common neurophysiological mediator. This finding may help explain the basis for polydrug abuse as well as why drugs belonging to a variety of pharmacologic classes are self-administered by both human and animal subjects. (Supported in part by NIDA Grant DA 03994 and Research Scientist Development Award DA 00115.)

135.5

STRUCTURAL REQUIREMENTS FOR NICOTINIC ANTAGONISTS IN THE CNS. T.J. Martin*, J. Suchocki*, E.L. May, and B.R. Martin. Dept. of Pharmacology/Toxicology, Medical Coll. VA/VA Commonwealth Univ., Richmond, VA 23298.

The structure-activity relationship for anti-nicotinic activity in the CNS has yet to be thoroughly documented. The antagonism of nicotine-induced antinoiception was studied for a variety of mecamylamine (N,2,3,3-tetramethyl-bicylco-[2.2.1]-heptan-2-amine) analogs using the mouse tail-flick procedure of Dewey, et al. (1975). All compounds were injectd s.c. 10 min prior to nicotine administration s.c. (2.56 mg/kg, ED84) and tail-flick latency was determined 5 min after nicotin administration. The AD50's of pempidine, (±)-exo-mecamylamine, (-)-exo-mecamylamine, (+)-exo-mecamylamine and (±)-endo-2-desmethyl-mecamylamine were 0.13 (0.05-0.29), 0.08 (0.02-0.29), 0.09 (0.04-0.23), 0.24 (0.10-0.57), and 0.13 (0.07-0.27), respectively. The endo and exo isomers of bicyclo-[2.2.1]-heptan-2-amine as well as their N-methyl analogs produced a non-dose- responsive antagonism of no greater than 50% at doses up to 1 mg/kg. Several N-alkylpyridine-substituted analogs of exo-bicyclo-[2.2.1]-heptan-2-amine displayed similar effects. Therefore, mecamylamine antagonizes nicotine- induced antinociception with a modest stereoselectivity ((+)exo/(-)exo=2.7) and dialkyl substitutions at the 3 position appear to be necessary for full antagonism. Supported by CTR Grant #2130 and NIDA grant #DA07027.

135.6

REVERSAL OF THE DELTA-9-TETRAHYDROCANNABINOL INHIBITORY EFFECT ON LUTEINIZING HORMONE SECRETION BY INTRACEREBROVENTRICULAR (ICV) NOREPINEPHRINE INFUSION. L.L. Murphy*, A. Bartke* and V. Chandrashekar* (SPON: R. Browning) Southern Illinois University, Carbondale, IL 62901-6512.

The inhibition of luteinizing hormone (LH) secretion by delta-9-tetrahydrocannabinol (THC), the primary psychoactive constituent of marihuana, may be due to alterations in hypothalamic noradrenergic activity and subsequent suppression of LH-releasing hormone release. The current study was designed to determine if ICV infusion of norepinephrine (NE) could reverse the inhibitory effect of THC on LH release. Adult castrated, testosterone (T)-treated rats were implanted with a stainless steel cannula in the third ventricle and allowed 1 week to recover. Blood samples were taken via intra-atrial cannulae every 10 min for 60 min pre- and 90 min post-THC (1.0 mg/kg BW, iv) or vehicle treatment. Twenty min post-THC, NE (100 µg) or acid saline was infused ICV at a rate of 2 µl/2 min. THC administration reduced LH levels to 50% of control values within 10 min ($p<0.01$) and up to 90 min after the injection and abolished the pulsatile release of LH characteristic of the castrated, T-treated animals. During the period of LH suppression, ICV NE induced a significant increase ($p<0.01$) in plasma LH levels at 10 min when compared to acid saline controls. These results indicate that ICV infusion of NE abolishes the inhibitory effect of THC on LH secretion and further suggest that hypothalamic noradrenergic alterations may mediate the THC inhibitory effect on LH secretion. Supported by DA 03875).

135.7

THE EFFECTS OF SOME CANNABINOIDS IN A MODEL OF ANXIETY IN RATS AND MICE. Emmanuel Onaivi* and Billy Martin. Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA, USA 23298

Different subjective effects are known to occur after smoking marihuana or following the injection of the extract and, in some instances opposing effects have been reported. The mechanisms via which cannabinoids produce anxiolytic and/or anxiogenic effects still remains largely elusive. In the present study we assessed the action of some cannabinoids to modify indices of anxiety in rats (Sprague Dawley) and mice (ICR) following 30 min intraperitoneal pretreatment using the elevated plus-maze. Δ^9-THC (0.3-20mg/kg) produced an aversion to the open arms characterized by significant reduction in the time spent and number of entries into the open arms of the maze the rat being more sensitive than the mouse. The aversion was intense even at 2 hr after treatment. Similar responses were obtained with lower doses of levonantradol (0.05-1mg/kg) and (-)-11-OH-Δ^8-THC-DMH (0.05-0.5mg/kg) and the effect was stereospecific as (+)-11-OH-Δ^8-THC-DMH (1-10mg/kg) was inactive even at a dose that was 200 times greater than the (-) - isomer. In contrast mice treated with cannabidiol (10-100mg/kg) were less aversive to the open arms with significant increase and decrease in time and number of entries into the open and closed arms, respectively. In addition, 11-COOH-Δ^8-THC (3-20 mg/kg) had no effect. This simple test system can dissociate between the anxiogenic and anxiolytic properties of cannabinoids. Supported by NIDA grant DA-03672 and the VA Commonwealth Center of Drug Abuse.

135.8

DIFFERENTIAL EEG AND BEHAVIORAL EFFECTS OF SELECTIVE SIGMA AND PCP RECEPTOR AGONISTS IN THE RAT. N. Khazan and G.A. Young. Dept. Pharmacol. Toxicol., U. Maryland Sch. Pharm., 20 N. Pine St., Baltimore, MD 21201.

In vitro studies have shown that the benzomorphan opioid N-allyl-normetazocine (SKF 10,047) labels two binding sites in rat and guinea pig brain. A high affinity site, which is stereoselective for the (+)-isomer, represents the putative sigma receptor, where (+)3-(3-hydroxyphenyl)-N-(1-propyl)piperidine ((+)-3-PPP) is a selective agonist. A low affinity site represents the putative phenyclidine (PCP) receptor with [1-(2-thienyl)cyclohexyl]piperidine (TCP) being a selective agonist. In the present study we characterized in vivo EEG and behavioral effects of (+)-3-PPP and TCP. TCP, like PCP, was found to induce EEG synchrony and increase low frequency EEG spectral power, while (+)-3-PPP produced a relative increase in high frequency EEG. Furthermore, TCP increased motor activity, and produced ataxia and stereotypic circling, while (+)-3-PPP induced stereotypic sniffing. These in vivo results further demonstrate differential profiles of effect following sigma and PCP receptor stimulation. (Supported by NIDA Grant DA01050.)

135.9

STEREOSELECTIVE [3H]-COCAINE BINDING TO MEMBRANES OF MONKEY CAUDATE-PUTAMEN. B.K. Madras*, J. Bergman, M. Fahey*, D. Canfield*, R.D. Spealman. Harvard Medical School, NERPRC, Southborough, MA 01772.

Ongoing studies have examined the reinforcing and other behavioral effects of cocaine, cocaine analogs, and indirect agonists in monkeys. In the present study the affinities of these drugs at [3H]-cocaine binding sites in monkey caudate-putamen (Macaca fascicularis) were determined. Membranes were incubated at 0°C in Tris-HCl buffer (NaCl 120 mM) for 60 min in the presence of [3H]-cocaine (2.8 nM). Cocaine analogs displaced 85-100% of specifically bound [3H]-cocaine with the following rank order: WIN 35,428 > WIN 35,065-2 > WIN 35,981 = (-)-cocaine > norcocaine > WIN 35,065-3 > (+)-cocaine > (+)-pseudococaine > (-)-pseudococaine, MDL 72222, ICS 205-930, benzoylnorecgonine, benzoylecgonine. Indirect agonists displaced 60-80% of [3H]-cocaine binding sites with the following rank order: methylphenidate = mazindol > nomifensine > (-)-cocaine => GBR 12909 > nisoxetine > bupropion > amphetamine > citalopram. The dopamine agonists SKF 38393, (+)-PHNO and quinpirole had low affinity for the site(s). The studies indicate that the affinities of cocaine analogs for [3H]-cocaine binding sites paralleled the rank order of potency of these drugs for increasing schedule-controlled behavior in monkeys. Certain indirect agonists, however, were less potent in vivo than would be predicted by their binding affinities in vitro.
Supported by USPHS Grants DA00499 and RR00168.

135.10

SIMILARITIES IN BINDING SPECIFICITY BETWEEN RABBIT ANTI-TCHAP ANTIBODIES AND THE PHENCYCLIDINE RECEPTOR. M. Zorbas* and S.M. Owens. Dept. of Pharmacology, U. Arkansas for Med. Sci., Little Rock, AR 72205.

Rabbit antibodies were produced against an amino acid analog of the highly potent PCP receptor ligand TCP. Three rabbits were immunized with 5-(N-[1'-(2-thienyl)cyclohexyl] amino)pentanoic acid (TCHAP) coupled to bovine serum albumin. All three rabbits produced high affinity antibodies with Kd's for PCP of 5.2, 4.3 and 4.5 nM, respectively). The cross reactivity patterns of these antibodies were studied in a RIA using [^{3}H]PCP and various arylcyclohexylamines. From the IC_{50} values of these dose-response curves relative potencies to PCP were determined. Linear regression analysis was used to correlate these data with relative potency data from a receptor binding study. Correlation of antibody relative potency data with receptor binding relative potency data was significant for all three rabbit antiserum (r^2=0.68, r^2=0.62, r^2=0.45, respectively; $p<0.05$ in all cases). These data suggested that the epitope recognized by the anti-TCHAP antibodies could represent critical molecular features necessary for arylcyclohexylamine binding to the PCP receptor. These data support and refine previous findings that the amino acid analog of PCP, PCHAP, can provide the antigenic stimulus to produce antibodies which can mimic arylcyclohexylamine binding to the PCP receptor. (Supported by NIDA grant DA 04316, UAMS Student Research Fund, and NIDA RSDA (SMO) K02 DA 00110).

135.11

ANTI-PCHAP MONOCLONAL ANTIBODIES AS MODELS OF PHENCYCLIDINE (PCP) RECEPTOR BINDING TO ARYLCYCLOHEXYLAMINES. S.M. Owens, L.W. Arnold*, D. Sasser* and M. Gunnel*. Dept. of Pharmacology, U. of Arkansas for Med. Sci., Little Rock, AR 72205 and Dept. of Microbiology and Immunology, U. of North Carolina, Chapel Hill, NC 27514.

PCHAP (5-[N-(1'-phenylcyclohexyl)amino]pentanoic acid) has been shown to stimulate production of rabbit antibodies which could recognize the pharmacologically active features needed for arylcyclohexylamine binding to the PCP receptor. In the current studies, three hybridoma lines secreting monoclonal antibodies (MAb) against PCHAP were produced after C57Bl/10.A mice were immunized with a PCHAP-bovine serum albumin conjugate. The binding affinities of these MAb for arylcyclohexylamines and other PCP receptor ligands were studied in a [^{3}H]PCP radioimmunoassay. The Kd values for PCP for the three MAb were 132 nM, 54 nM and 21 nM. Similar high affinities were found for other potent PCP receptor agonist of the arylcyclohexylamine class. The antibodies had low affinity for non-arylcyclohexylamine PCP receptor agonists, such as dexoxadrol and SKF 10,047, and virtually no recognition for other drugs such as morphine. These MAb should prove useful as PCP receptor models for studying the chemical interaction between arylcyclohexylamines and binding proteins and for making anti-idiotypic antibodies that recognize the PCP receptor. (Supported by NIDA grant DA 04136 and NIDA Res. Sci. Dev. Award (SMO) K02 DA 00110).

135.12

SPECIFICITY AND ACCURACY OF COMMERCIAL URINE SCREENING ASSAYS FOR THE DETECTION OF COCAINE USE. E.J. Cone, S. Menchen* and J. Mitchell*. NIDA Addiction Res. Ctr., Baltimore, MD 21224 and Navy Drug Screening Lab., NAS, Norfolk, VA 23511

Eight commercial urine screening assays for detection of cocaine use were evaluated for sensitivity, specificity, accuracy and GC/MS confirmation rate. Assays evaluated included: TDx^R Cocaine Metabolite assay; Toxi-Lab^R Drug Detection Systems; Coat-A-Count^R Cocaine Metabolite RIA; Cocaine Metabolite Double Antibody RIA; KDI Quik Test^{TM} Drug Screen; Abuscreen^R RIA; Emit^{R}dau^{TM} Cocaine Metabolite Assay and Emit^{R}st^{TM} Urine Cocaine Metabolite Assay. Results were compared to assay results by GC/MS for benzoylecgonine (BE). The immunoassays and the TLC procedure reliably detected BE at concentrations of 500 and 1000 ng/ml, respectively. KDI Quik Test^{TM} produced numerous inaccurate results and was not a valid test for detection of cocaine use. The crossreactivity of cocaine in the immunoassays varied from 1% to >5000% (BE = 100%) and appeared to be the basis for production of occasional "apparent" false positives in the analyses of clinical specimens by the Coat-A-Count^R and Double Antibody RIA.

BLOOD PRESSURE

136.1

RELATIVE CONTRIBUTION OF PULSE AMPLITUDE AND FREQUENCY TO POST-PULSATILE PRESSURE SENSITIZATION OF BARORECEPTORS. M.W. Chapleau, S.L. Johnson,* G. Hajduczok, and F.M. Abboud. CV Ctr. and Univ. of Iowa Coll. of Med., Iowa City, IA 52242

We have shown recently that baroreceptor (BR) sensitivity is increased following exposure to pulsatile pressure. The purpose of this study was to determine the contribution of pulse amplitude and frequency to the post-pulsatile pressure (PPP) sensitization of BR. Multi-unit BR activity was recorded from the isolated carotid sinus of anesthetized dogs (chloralose). Activity during static pressure (99±4 mmHg) immediately before a 5 minute exposure to pulsatile pressure was compared to the activity at the same static pressure after pulsing. Various pulse amplitudes at constant frequency (2.1 Hz) and frequencies at constant amplitude (27±2 mmHg) were tested. PPP-sensitization was related to amplitude (n=8, r=.90±.03) and frequency (n=6, r=.72±.06) with amplitude having the greater effect (*different than before pulsing, P<.05).

Increase in Activity (spikes/sec) After vs. Before Pulsing

Pulse Amplitude (mmHg)			Pulse Frequency (Hz)		
10	30	50	0.2	1.6	2.4
1±4	43±5*	71±20*	2±2	20±7*	34±12*

Carotid sinus diameter (sonomicrometers) was not changed after pulsing suggesting increased strain sensitivity of BR. We conclude that increases in pulse amplitude and frequency sensitize BR and suggest that this may contribute to sympathetic inhibition following increases in pulse pressure and heart rate such as occurs during exercise (AHA87G9, HL14388).

136.2

CHRONIC RESETTING OF BARORECEPTORS DIFFERS IN HYPERCHOLESTEROLEMIC VS. RENAL HYPERTENSIVE RABBITS. P. Xie,* M.W. Chapleau, T.S. McDowell,* G. Hajduczok, and F.M. Abboud. CV Ctr. and Univ. of Iowa Coll. of Med., Iowa City, IA 52242

Structural vascular changes have been proposed to account for reduced baroreceptor (BR) sensitivity in both hypercholesterolemia (atherosclerosis) and hypertension. The purpose of this study was to test whether the pressure threshold (Pth) of BR and BR gain (Δ Activity/Δ Pressure) are altered in hypercholesterolemic (HC) rabbits (1% cholesterol diet-12 wks) and to contrast the findings with those found previously in renal hypertensive (RHT) rabbits. Blood cholesterol in normal (N) and HC rabbits averaged 58±14 and 3064±157 mg/100 cc, respectively. BR activity was recorded from the isolated carotid sinus (CS) after chloralose anesthesia. Pth and the pressure-activity relation were determined during ramp increases in CS pressure. The results from N, HC, and RHT rabbits are shown below (*different from N, P<0.05).

	MAP (mmHg)	Pth (mmHg)	BR Gain (spikes/sec/mmHg)
N (n=6)	87±2	51±3	0.94±0.16
HC (n=8)	84±1	50±1	0.60±0.07*
RHT (n=7)	141±5*	75±5*	0.84±0.11

BR gain was markedly suppressed while Pth was normal in HC rabbits. In contrast, Pth was markedly elevated and gain only mildly reduced in RHT rabbits. The results suggest that the prevailing level of MAP is the primary determinant of Pth and that the mechanism of chronic BR resetting is different in RHT and HC rabbits (Supported by NIH HL14388).

136.3

BEAT-BY-BEAT CHANGES IN CARDIAC CONTRACTILITY DURING RAMPED NECK PRESSURE-SUCTION. Peter B. Raven, Beatriz Parra*, Glen H.J. Stevens* and James A. Pawelczyk*. Department of Physiology, TCOM, Fort Worth, TX 76107.

M-mode echocardiographic measures of left ventricular end-systolic dimensions (ESD) were made on five volunteer healthy male subjects in the supine position during ramped neck pressure and suction of the carotid sinus. Radial artery catheterization was utilized to obtain end-systolic pressure (ESP). The calculated end-systolic pressure dimension ratio (ESP/ESD) was used as an index of contractility for each beat. Mean arterial pressure increased ($\bar{x}$ = 5.25 mmHg) during neck pressure (carotid hypotension) and decreased ($\bar{x}$ = 7.2 mmHg) during neck suction (carotid hypertension). The calculated ESP/ESD ratio progressively increased to an average maximum change of 2.5 mmHg/cm during carotid hypotension and progressively decreased to an average maximum change of 2.85 mmHg/cm during carotid hypertension. These data indicate that rapid decreases and increases in carotid sinus transmural pressure reflexly increase and decrease cardiac contractility, respectively.
(Supported in part by N.I.H. grant #HL34397)

136.4

MODULATORY EFFECTS OF ADENOSINE ON BAROREFLEX ACTIVATION IN THE NUCLEUS OF THE SOLITARY TRACT. Rogelio Mosqueda-Garcia*, Ching-Jiunn Tseng* and David Robertson. Vanderbilt University, Nashville, TN 37232

Adenosine (ADO) elicits hypotensive and bradycardic responses in the Nucleus of the Solitary Tract (NTS). The NTS contains the first synapse of the baroreflex and L-glutamate (GLU) has been proposed as the neurotransmitter of that synapse. We investigated how adenosine affects baroreflex activity and its interaction with GLU. BP and HR were recorded intraarterially in urethane anesthetized Sprague-Dawley rats. They were placed in a stereotaxic and the renal nerve was dissected for recording of sympathetic renal nerve activity (RNA). ADO 2.3 nmol/60 nl was injected unilaterally into the right NTS. Baroreflex responses were elicited by i.v. phenylephrine (Phe) before and after intra-NTS administration of the ADO antagonist 1,3-dipropyl-8-p-sulphenylxanthine (DPSPX, 0.92 nmol). Basal BP, HR and RNA were decreased by ADO but not by DPSPX. Phe elicited an increase in BP of 62±8 mmHg with an increase in pulse period (pp) of 154±38 msec and inhibition of RNA. After DPSPX, a similar increase in BP (63±6 mmHg) elicited a slight increase in pp (55±12 msec). Kynurenic acid (33 nmol into the NTS), a GLU antagonist, inhibited the cardiovascular responses elicited by similar administration of ADO. DPSPX did not inhibit the hemodynamic effects of GLU in the NTS. In binding experiments, GLU did not interact with ADO receptors or ADO did not displace GLU binding from brainstem membranes. These results suggest that ADO has a modulatory role in the baroreflex activation and this effect can be mediated by an interaction with GLU in the NTS.

136.5

$MgCl_2$ ALTERS BAROCEPTOR REFLEXES IN NEONATAL PIGS. P.M. Gootman, M. Brust, B.W. Hundley*, H.L. Cohen*, G. Condemi*, B.T. Altura, B.M. Altura, Dept. Physiology, SUNY-Hlth Sci. Ctr. Bklyn., Bklyn, NY 11203

The effects of changes in plasma concentration of magnesium on arterial blood pressure (AoP) and heart rate (HR) responses to baroceptor stimulation with phenylephrine (PE: 20 ug/kg) or inhibition with nitroprusside (NP: 30 ug/kg) were examined in 19 piglets <1 day to 50 days old, lightly anesthetized with Saffan, paralyzed, tracheotomized and artificially ventilated on 100% O_2; pO_2,pCO_2 and pH were monitored. Infusion of $MgCL_2$ for 15 min increased arterial plasma levels of Mg from 1.82 mg/dl to 4.61 mg/dl.

	Before		During $MgCl_2$	
% Change	AoP	HR	AoP	HR
<1-5 Days Old (n=9)				
PE	+47.8	-13.8	+24.4	-6.7
NP	-38.4	+ 6.8	-23.0	-2.4
12-21 Days Old (n=6)				
PE	+33.8	-30.0	+20.9	-15.4
NP	-31.0	+ 5.5	-38.7	-10.8
>30 Days Old (n=4)				
PE	+32.1	-29.5	+36.8	+24.8
NP	-32.6	+10.2	-34.2	+13.4

The results showed that elevations in plasma Mg altered responses to PE and NP. (Supported by NIH grants HL-20864 and HL-29600).

136.6

TIME COURSE OF RECOVERY OF ARTERIAL PRESSURE CONTROL IN CONSCIOUS DOGS AFTER CAROTID SINUS DENERVATION. D.S. O'Leary* and A.M. Scher. Dept. Physiology and Biophysics, Univ. Washington, Seattle, WA 98195.

We previously reported that 9 or more days after carotid denervation the strength of arterial pressure control was not different from that prior to denervation (Faseb J. 2:A717, 1988). The present study examined the time course of recovery of pressure control in four conscious dogs with AV block. Changes in total peripheral resistance and atrial rate in response to step changes in cardiac output were used as indices of baroreflex strength. One day after carotid baroreceptor denervation arterial pressure was increased by 16.5% ($p < .02$) and both indices of baroreflex strength decreased to < 40% of control ($p < .001$). These indices increased daily to reach control levels by 8-9 days. The increases in both indices were highly correlated to the recovery from the initial hypertension (peripheral resistance $r = -.98$; atrial rate $r = -.94$). Carotid sinus baroreceptor denervation causes an initial hypertension and decrease in the strength of control of arterial pressure however, both recover after 9 days. There is apparently plasticity within the autonomic pathways such that the strength of control by the aortic baroreceptors increases to compensate for the loss of the carotid baroreceptors. (Supported by HL16910 and HL07090)

136.7

CAPTOPRIL AND HYPOXIA STIMULATE MYOCARDIAL PROSTAGLANDIN SYNTHESIS TO CAUSE A BEZOLD-JARISCH-LIKE REFLEX IN CONSCIOUS DOGS. David M. Nganele* and Thomas H. Hintze. New York Medical College, Valhalla, N.Y. 10595

Prostaglandins (PG) are known to activate inhibitory cardiac receptors to elicit a Bezold-Jarisch-like reflex. The goal of this study was to determine if stimuli that are known to cause the release of PG could activate inhibitory cardiac receptors in conscious dogs. Dogs were instrumented for the measurement of arterial pressure (AP), heart rate (HR) and left ventricular (LV) internal diameter. A catheter was inserted into the LCX coronary artery for the local administration of Captopril into the coronary circulation. Intracoronary Captopril (0.1 mg/Kg) reduced AP by 21±3.7% from 98±2.3 mmHg and LV end diastolic diameter by 2.4±0.3% from 35±2.3 mm with no change in HR ($p<0.05$). This dose of Captopril had no effect when administered i.v.. Indomethacin completely reversed the effects of Captopril. Dogs were trained to wear a mask through which low oxygen gas mixture was delivered. Short term exposure to hypoxia (8% 02 for 5 min) reduced pO2 from 90.2±3.0 mmHg to 53.0±3.4 mmHg with no change in pCO2. There was no change in AP but HR was reduced by 12±3.7% from 86±5.0 b/min ($p<0.05$). Prior administration of indomethacin prevented this reduction in HR. The myocardium, therefore, can be stimulated to release PG which activate inhibitory cardiac receptors. Supported by NIH HL 36264.

136.8

EFFECTS OF AGE AND PREMEAL BLOOD PRESSURE ON THE CARDIOVASCULAR RESPONSE TO MEALS. Timothy C. Fagan, Kenneth A. Conrad*, Paula V. Mayshar* and Mary J. Mackie*, College of Medicine, University of Arizona, Tucson, Arizona, 85724

The effects of age and premeal blood pressure (BP) and heart rate (HR) on the cardiovascular responses to a meal were examined in 82 subjects aged 19-79, 41 male and 41 female, with untreated premeal supine diastolic BP of 62-120 mmHg. The subjects fasted for at least 5 hours, and BP and HR were measured 4 times at hourly intervals. A meal chosen by each patient was ingested after the second measurement. The immediate premeal and 2 hour postmeal measurements were compared. After the meal, mean BP was reduced from 147/93 to 139/83 mmHg in the supine position and from 148/101 to 142/94 mmHg in the standing position, all $p<0.001$. HR increased by 8 bpm in both positions, $p<0.05$. The changes in supine and standing systolic BP and supine diastolic BP were all related to the age of the patient, $r=-0.35$ to -0.45, all $p<0.001$; older subjects had greater reductions in BP. Older subjects also had higher premeal systolic and diastolic BP, and subjects with higher premeal BP had greater reductions after the meal, $r=-0.40$ to -0.58, all $p<0.001$. After correction for premeal BP, the reduction in BP after the meal was no longer related to age, but after correction for age, the reduction in blood pressure was still greater in subjects with higher premeal blood pressure. Meals reduce supine and standing systolic and diastolic blood pressure. Older subjects have a greater reduction in blood pressure than younger subjects, and this difference is primarily mediated through higher initial blood pressure.

136.9

AGE-RELATED BAROREFLEX IMPAIRMENT IN FEMALE SPRAGUE-DAWLEY RATS. Ruben D. Buñag and Shinichi Tanabe*, Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103.

Reflex chronotropic and sympathetic responses to drug-induced changes in blood pressure were compared in 2 or 8 month-old female Sprague-Dawley rats anesthetized with urethane-chloralose. Reflex bradycardia elicited as blood pressure was elevated with phenylephrine was consistently smaller at 8 than at 2 months of age, but attendant reductions in splanchnic nerve activity were significant only when pressor responses exceeded 30 mm Hg. By contrast, reflex responses elicited by lowering blood pressure with sodium nitroprusside did not differ between rat groups. Although graded electrical stimulation of the aortic depressor nerve lowered blood pressure equally, reductions in heart rate and splanchnic nerve activity were always smaller in 8 month-old than in 2-month old rats, thereby suggesting defects in central regulation. However, peripheral baroreceptors may also be involved because afferent aortic nerve activity recorded during infusions of phenylephrine or sodium nitroprusside were likewise reduced in 8 month-old rats. These results indicate that baroreflex sensitivity becomes reduced with age because baroreceptor and central components of the reflex arc no longer function normally. (Supported by NIH Research Grant HL 37980).

136.10

SPECIFIC MONOAMINE OXIDASE INHIBITORS ENHANCE PRESSOR RESPONSIVENESS IN AWAKE RATS. Laszlo Kerecsen*, Edward Walaszek and Ruben D. Buñag. Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103.

(-)Deprenyl, a monoamine oxidase-B inhibitor used clinically in Europe for the treatment of parkinsonism, does not affect pressor responses to tyramine but since it could alter cardiovascular reactivity, we compared it with clorgyline, a monoamine oxidase-A inhibitor. Cardiovascular responses to phenylephrine, angiotensin II and tyramine were recorded in awake rats pretreated with daily subcutaneous injections (0.25 mg/kg) of either clorgyline or (-)deprenyl. Indwelling vascular catheters were implanted chronically into a femoral artery and vein, and a Doppler flow probe was used to monitor blood flow in the iliac artery. After 1 or 7 days of clorgyline pretreatment, pressor responses to tyramine and angiotensin (but not to phenylephrine) were enhanced, and the attendant reflex bradycardia was also considerably increased. By contrast, rats similarly pretreated with (-)deprenyl had only slightly enhanced pressor responses to tyramine and bradycardic responses to phenylephrine, while all other cardiovascular responses were unaltered. Neither monoamine oxidase inhibitor affected iliac arterial flow. These results suggest that cardiovascular side-effects would be prominent with clorgyline, but not with (-)deprenyl. (Supported by NIH Research Grant HL 37980).

136.11

CHRONIC ICV INFUSION OF ENALAPRIL ALTERS BAROREFLEX SENSITIVITY IRREGULARLY IN RATS. Lea Eriksson*, Shinichi Tanabe* and Ruben D. Buñag. Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103.

Enalapril, a converting enzyme inhibitor, was infused (100 mcg/day) into a lateral cerebral ventricle (ICV) using osmotic minipumps in female Wistar rats for two weeks, after which baroreflex sensitivity was tested by infusing phenylephrine or sodium nitroprusside intravenously (IV). Reflex bradycardia elicited by elevating blood pressure with phenylephrine was only slightly enhanced in awake rats, but when the same rats were later anesthetized with urethane-chloralose then the increase in reflex bradycardia in rats infused with enalapril ICV was significantly greater than that in controls infused with either the vehicle alone ICV or enalapril IV. By contrast, reflex tachycardia produced by lowering blood pressure with sodium nitroprusside was smaller in awake rats infused with enalapril ICV than in the controls; reflex tachycardia was greatly suppressed by subsequent anesthesia so that the differences between groups diappeared. Our results suggest that central converting enzyme inhibition affects baroreflexes in two different ways: reflex bradycardia is enhanced, while reflex tachycardia is suppressed. (Supported by NIH Research Grant HL 37980).

RENAL TRANSPORT AND BODY FLUID REGULATION II

137.1

RENAL ADRENERGIC CONTROL IN PROXIMAL TUBULE AND LOOP OF HENLE FLUID REABSORPTION. B.J.Tucker, S.C.Thomson*, and R.C.Blantz Univ. of Calif., San Diego and VAMC, San Diego, CA 92161

Increased renal nerve traffic enhances reabsorption of fluid from the renal tubule. An important role for α-adrenergic subtypes has been previously demonstrated. However, the influence of β-subtypes is less well defined. Micropuncture measurements were performed in rats treated with propanolol, a β-blocker, (P) (n=6), 25 mg/kgBW/day p.o., for 4-6 days and compared to untreated rats (C)(n=6). Measurements of nephron filtration rate (SNGFR), proximal tubule (APR) and loop of Henle (LR) fluid reabsorption, proximal (FRp) and distal (FRd) tubule fractional reabsorption were performed before and after renal denervation (DNX) to assess both the β- and α-adrenergic influences. Blood pressure (MAP) and pulse rate (HR) were also measured. P treatment decreased HR from 384±16 to 304±9 (P<0.05). (*P<0.05 to first period, ¤P<0.05 to C period)

	MAP	SNGFR	APR	LR	FRp	FRd
	[mmHg]	[nl/min]			[%]	
C	113±4	45±3	18±2	17±1	40±3	79+2
C+DNX	109±4	44±3	12±2*	18±2	27±3*	70±3*
P	103±3¤	38±2¤	16±1	13±1¤	44±3	79±2
P+DNX	101±2¤	35±3¤	11±1*	14±1	32±3*	73±2*

P did not alter FRp or FRd indicating little, if any, β-adrenergic influence on tubular fluid reabsorption in euvolemic conditions. DNX decreased FRp and APR in both P and C, but neither LR or the distal portion of FRd changed indicating an α-adrenergic effect primarily in the proximal tubule.

137.2

EFFECT OF SOME STEROIDS ON SODIUM RETENTION IN PERFUSED RAT KIDNEY. Joe Kuraya-Ziadeh*, Muna El-Kasti*, Raed Hawwa* and Anwar B. Bikhazi. American University of Beirut, Faculty of Medicine, Beirut, Lebanon.

A modified Sprague Dawley rat kidney perfusion technique was employed in situ to study the effect of steroids on Na retention. After anesthesia and abdominal resection, the kidneys were perfused via the abdominal aorta with heparinized Krebs improved Ringer containing tracer ^{22}Na. After perfusion, kidneys were removed, decapsulated, homogenized, and Na retention measured by tracer quantitation as μg Na per mg protein. Compared to controls, kidneys of prednisone, dexamethasone and corticosterone treated rats (i.p. injection 5 mg/100 g body weight for each steroid) showed a reduction (15%), insignificant change, and increase (22%) in Na retention respectively. In K-free and Mg-free perfusates, similar treatments with each steroid respectively indicated a decrease (17%, 49% and 92% for K-free; 39%, 104% and 139% for Mg-free) in Na retention compared to controls. Ouabain (15 mM) resulted in decrease in Na retention after prednisone (161%) and dexamethasone (79%) treatment compared to the ameliorating effect of corticosterone (27%). Prednisone and dexamethasone, contrary to corticosterone, competed with ouabain for the peritubular Na-K-ATPase. This is further documented by the K-free and Mg-free data. (Supported by the Lebanese National Research Council and the Nadim Andraos Foundation).

137.3

INHIBITION OF PROXIMAL TUBULAR TRANSPORT (PT) IN ISCHEMIC RENAL FAILURE (IRF) IN THE RAT. J.E. Bird, O.W. Peterson*, and R.C. Blantz. University of California, San Diego and VA Medical Center, San Diego, CA. 92161

Previous studies from this laboratory (JCI, 1988) have shown that ischemia and 24 hours reflow (I-R) caused a decrease in single nephron filtration rate (SNGFR) and absolute proximal reabsorption (APR), and that treatment with the antioxidant probucol (P) improved SNGFR but not APR, with an increase in proximal tubular necrosis. Current studies examined the effects of PT inhibition with benzolamide (B) during I-R in uninephrectomized control (CB), ischemic (IB) and P treated ischemic (IPB) rats. C=control, I=ischemic and IP=P treated ischemic without B. GFR=glomerular filtration rate.*= p<.05 vs. CB, ¤=p<.05 vs. corresponding non-B group.

	CB	IB	IPB	C	I	IP
GFR	1.4±.1	0.3±.1*	0.4±.1*¤	1.4±.1	0.2±.1*	0.1±.1*
SNGFR	58±3	40±4*¤	46±6¤	45±4*	15±3*	28±3*
APR	19±2	11±2*¤	15±3¤	15±2	6±1*	6±1*

B improved SNGFR and APR in IB and IPB rats. B and P had an additive beneficial effect in IRF. GFR in IB and IPB rats was significantly lower than in CB rats, suggesting persistent tubular backleak. Conclusions: 1) Proximal tubular function, measured by APR was improved by B treatment in IRF. 2) B treatment in IB rats improved SNGFR independent of antioxidant therapy. 3) Treatment with an inhibitor of PT transport and an antioxidant produced major improvement in glomerular and tubular function in IRF.

137.4

THE EFFECT OF EXTRACELLULAR $[H^+]$ ON THE CONVERSION OF CORTICOSTERONE TO ALDOSTERONE. Todd V. Robinson* and Edward G. Schneider. Univ. of Tennessee, Memphis, Memphis, TN 38163

Changes in H^+ concentration ($[H^+]$) have been found to affect aldosterone secretion. Whether $[H^+]$ has an effect on the early or the late part of the aldosterone synthetic pathway is not known. Cyanoketone was used to block the early part of the biosynthetic pathway of aldosterone in isolated, perfused canine adrenal glands. Deoxycorticosterone was then added to the perfusate, and the effects of changing $[H^+]$ on the formation of corticosterone, 18-hydroxycorticosterone, and aldosterone were examined. Changes in extracellular $[H^+]$ were induced by altering the bicarbonate ion concentration of the perfusate. During alkalosis (18.1 ± 0.5 nM $[H^+]$), the conversion of corticosterone to both 18-hydroxycorticosterone (+28 ± 12%) and aldosterone (+23 ± 2%) was significantly greater than the conversion of corticosterone into 18-hydroxycorticosterone (-18 ± 18%) or aldosterone (-13 ± 7%) during acidosis (70 ± 0.8 nM $[H^+]$). The conversion of deoxycorticosterone to corticosterone was not significantly affected by changes in $[H^+]$. These results demonstrate that changes in $[H^+]$ alter aldosterone, in part, by a direct action on the enzyme, 18-methyloxidase, that converts corticosterone to aldosterone, the late site. (Supported in part by grants-in-aid from the American Heart Association, Tennessee Affiliate.)

137.5

Lithium Clearance Increases During Head-out Water Immersion in Conscious Dogs.

J.L. Sondeen, S.K. Hong, J.A Krasney. Dept. of Physiology, SUNY, Buffalo, NY 14214.

Head-out water immersion induces a natriuresis in conscious dogs but it is unclear whether the decreased sodium reabsorption response is located in the proximal tubule or the distal tubule. Therefore, we measured lithium clearance, which has been proposed as a marker for proximal tubular sodium reabsorption, in conscious dogs during head-out water immersion in thermoneutral water. The dogs were studied under mildly hydrated conditions and all blood and urine sample losses were replaced with 0.9% NaCl. Shown are urine flow (V), creatinine (CCr), free water (CH2O), osmotic (COSM), sodium (CNa), and lithium (CLi) clearances, and fractional Na clearance from the distal nephron (CNa/CLi).

	Pre-immersion	100 min immersion
V (ml/min)	0.89 ± 0.08	4.23 ± 0.81*
CCr (ml/min)	112 ± 7	116 ± 17
CH2O (ml/min)	-0.87 ± 0.08	0.20 ± 0.15*
COSM (ml/min)	1.76 ± 0.05	4.03 ± 0.79*
CNa (ml/min)	0.75 ± 0.09	3.39 ± 0.76*
CLi (ml/min)	19 ± 2	30 ± 4*
CNa/CLi (%)	4.30 ± 0.67	12.55 ± 3.77*

These results suggest that the natriuretic response to head-out water immersion is due to inhibition of Na reabsorption in both the proximal and distal tubule.

Supported by NIH Grant PO-1-HL-28542.

137.6

LEUKOTRIENE B_4 (LTB_4) INHIBITS VASOPRESSIN (AVP)- INDUCED OSMOTIC WATER FLOW IN RABBIT CORTICAL COLLECTING DUCTS.

R.L.HEBERT, M.D.BREYER,* H.R.JACOBSON. DIV. OF NEPHROLOGY, VANDERBILT UNIV. NASHVILLE, TN, 37232, U.S.A.

Leukotriene B_4 (LTB_4), a metabolite of arachidonic acid, is produced via the 5-lipoxygenase pathway . LTB_4 was originally shown to be released by inflammatory cells such as neutrophils, macrophages and eosinophils, and to possess potent chemotactic properties . In addition to its obvious origin from inflammatory cells, LTB_4 may be produced by native renal cells. To determine if intrarenal LTB_4 exerts any physiologic actions , we examined the effect of LTB_4 on arginine vasopressin (AVP) induced osmotic water permeability in the rabbit cortical collecting duct (CCD). Tubules were perfused in vitro at 37°C and exposed to submaximal bath AVP (10μU/ml). Hydraulic conductivity (Lp X 10^{-7} cm/atm/sec. mean ± SE) as a parameter of osmotic water flow was measured. Control CCD had a basal Lp of 14±3, and a peak AVP induced Lp of 220±12 (n=20). Upon pretreatment of CCD with 10^{-7}M LTB_4, basal Lp was 4±2 (N.S. vs. control). CCD pretreated with LTB_4 upon exposure to AVP had a peak Lp of 114±10 (n=10) a reduction in peak AVP-induced Lp of 48% (p<0.0005). Chlorophenylthio (Cc-AMP) was used in order to determine if LTB_4 inhibition results from decreasing the generation of c-AMP.10^{-4}M (Cc-AMP) alone increases the peak Lp from a basal value of 10±3 to 220±24 (n=10). Pretreatment of the CCD with LTB_4 did not change the peak Lp-induced response. Following the infusion of Cc-AMP, the basal Lp was 13±3 and the peak Lp response to Cc-AMP and LTB_4 was 230±21(n=10), We conclude: 1) Leukotriene B_4 inhibits the hydroosmotic response of cortical collecting duct to vasopressin but not to chlorophenylthio-c-AMP, therefore this inhibition occurs at a pre-c-AMP step. 2) These results support a role for leukotrienes in the modulation of water transport in pathophysiologic state

137.7

SEXUAL DIMORPHISM IN CENTRAL α_1-ADRENERGIC STIMULATION OF VASOPRESSIN RELEASE IN RATS.

J.D. Stone*, J.T. Crofton, and L. Share. University of Tennessee, Memphis, TN 38163.

To determine whether sexual differences exist in the adrenergic control of vasopressin secretion, conscious, unrestrained male rats and females in specific estrous cycle phases were studied. Plasma levels of vasopressin (P_{AVP}) and mean arterial blood pressure (MABP) were measured before and 5 and 15 min after central administration of norepinephrine (NE, 10 μg) or the α_1-agonist phenylephrine (PE, 50 μg) in rats previously fitted with intracerebroventricular (icv) cannulae and femoral artery and vein catheters. Vehicle alone did not affect P_{AVP} and MABP. Injection of NE or PE stimulated release of AVP in all groups ($p < 0.05$). NE-induced rises in P_{AVP} were 2-4 times greater in females than in males, but the differences were significant only for proestrus ($p < 0.01$) and metestrus ($p < 0.05$). Icv NE increased MABP similarly in all groups ($p < 0.01$). After PE, diestrous and proestrous females increased P_{AVP} 3 times and 5 times that of males, respectively ($p < 0.05$, $p < 0.01$), and had 2-4 times greater elevations of MABP than males ($p < 0.05$). These data suggest that gonadal steroid hormones influence α_1-adrenoceptor mediated control of AVP release and blood pressure.

Supported by USPHS grants HL-07339, HL-12990, and HL-19209 from the National Heart, Lung and Blood Institute.

137.8

VASOPRESSIN (AVP) AND CARDIOVASCULAR RESPONSES TO HEMORRHAGE IN MALE AND FEMALE RATS.

J. T. CROFTON AND L. SHARE. UNIVERSITY OF TENNESSEE, MEMPHIS, TN 38163.

We have examined the question of sexual dimorphism in the volume control of vasopressin release. Blood volume (BV) was measured in female (F) and age-matched male (M) rats by the indicator dilution technique (^{125}I-RISA) and was similar between the sexes (8.2±0.2 vs. 8.2±0.1 ml/100 g body wt, respectively). Subsequently, conscious, unrestrained M and F rats were subjected to two consecutive hemorrhages (H1 and H2), each 10% of BV. The plasma vasopressin concentration (P_{AVP}) was measured prior to and 10 min after each hemorrhage. Mean arterial blood pressure (MAP) was monitored throughout the experiment. No changes in MAP and P_{AVP} were noted in M rats in response to H1. In F rats, MAP decreased ($p<0.01$) 9±2 mmHg and P_{AVP} increased ($p<0.01$) by 2.5±0.7 μU/ml. MAP in F was lower ($p<0.05$) than in M after H1. In response to H2, MAP decreased ($p<0.01$) to similar levels in F and M rats (-36±4 vs. 30±7 mmHg, respectively). As expected, P_{AVP} increased ($p<0.01$) in response to the fall in MAP in both sexes, but was 2 times greater ($p<0.01$) in F than M rats (50±6 vs. 23±3, respectively). These data, plus our earlier observation that pressor responsiveness to vasopressin is attenuated in female rats, suggest that, in severe hemorrhage, a greater increase in P_{AVP} levels is required to maintain MAP in female rats.

Supported by USPHS grants HL-12990 and HL19209.

137.9

THE EFFECTS OF HYPOXIA ON BLOOD-GASES, ACID-BASE BALANCE AND ION EXCHANGE IN AMBYSTOMA TIGRINUM.

Colleen R. Talbot* and Daniel F. Stiffler. Calif. State Polytechnic Univ., Pomona, CA 91768.

Larval Ambystoma tigrinum were chronically cannulated in the truncus arteriosus and subjected to approximately 4 hrs of hypoxia. Blood samples were taken before, during and for 24 hrs following hypoxia. During hypoxia blood lactate levels increased almost 7 fold (from 1.7 to 11.5 mM). This produced a severe metabolic acidosis which decreased blood pH from 7.9 to 7.3 and [HCO_3^-] from 12.4 to 4.2 mM. There was a 15 torr decrease in P_{O2} but no change in P_{CO2}. All parameters had returned to control levels by 24 hrs. The effects of hypoxia-induced anaerobic metabolism on ion flux rates were examined using $^{22}Na^+$ and $^{36}Cl^-$. There were no significant changes in the influx of Na^+ or Cl^- during hypoxia or recovery. There was, however, a significant decrease in Na^+ efflux (from -1.8 to -1.2 μEq/10g h) and a possible decrease in Cl^- efflux (-1.66 to -0.29 μ Eq/10g h). These results suggest that reduced amounts of O_2 effect cutaneous ion exchange in recovery from a hypoxia-induced lactacidosis.

This work was supported by NSF grant DCB 86-17073 to DFS.

137.10

ACID-BASE AND CUTANEOUS ELECTROLYTE TRANSPORT RESPONSES TO HYPERCAPNIA IN TERRESTRIAL, ADULT AMBYSTOMA TIGRINUM.

Daniel F. Stiffler. Calif. State Polytechnic Univ, Pomona, CA 91768

Aquatic, larval Ambystoma tigrinum increase the rate of cutaneous Na^+ influx during hypercapnia (JEB 130:389) and following exercise-induced lactacidosis (JEZ 244:39) and decrease Cl^- influx during hypercapnia. These ion fluxes presumably involve exchanges of Na^+ for H^+ and Cl^- for HCO_3^- so that the observed changes in aquatic larvae lead to acid excretion and base conservation. Adult A. tigrinum are primarily terrestrial and their skin is only intermittently in contact with an ionic environment. Because of this it is of interest to see if the skin of these adults has retained it's acid-base regulatory function. When adults were subjected to hypercapnia (3 % CO_2) they showed a typical amphibian compensation response in that they elevated extracellular [HCO_3] from 14 to 22 mM to partially restore the arterial pH. The skin does not, however, appear to be involved in this response as neither Na^+ nor Cl^- influx changed significantly.

(Supported by NSF DCB 86-17073)

137.11

PROGRESSIVE HYPERCAPNIA IN THE TOAD (BUFO MARINUS) AND THE BULLFROG (RANA CATESBEIANA). Daniel P. Toews* and Daniel F. Stiffler, Calif. State Polytechnic U., Pomona, CA 91768

Several authors have shown that when amphibians are exposed to higher than ambient levels of CO_2 the compensatory plasma HCO_3^- increase is relatively small (compared to most fish). It has been suggested that a bicarbonate reabsorptive threshold might exist at one or more of the exchange surfaces. When we exposed toads and bullfrogs to a progressive CO_2 increase (increased 2%/day for 4 days), plasma HCO_3^- levels were raised to values well above the proposed threshold of 30 mM. Nonetheless, even though the HCO_3^- reabsorptive potential exists at lower CO_2 levels for complete pH compensation, extracellular compensation is limited. There were differences between the aquatic bullfrog and the terrestrial toad with respect to the maximal observed HCO_3^- concentration. The bullfrog achieved a concentration about 10 mM higher than the toad which appeared to level out at about 40 mM. This may be related to the fact that the toad's cutaneous Na^+/H^+ exchange transport system did not respond to the acidosis with the increased transport rate typical of aquatic amphibians.

(Supported by NSF DCB 86-17073).

137.12

BODY COMPOSITION USING ISOTOPE DILUTION AND INDIRECT CALORIMETRY IN MALNOURISHED PATIENTS. SA Goldstein*, DH Elwyn*, J Askanazi, V Kvetan*, J Wang* and RN Pierson, Jr. Columbia Univer, NY, NY 10032, Albert Einstein Med Coll, Bronx, NY 10467.

This study examines the impact of weight (wt) loss in patients with chronic obstructive pulmonary disease (COPD) on the aqueous phase of total body composition. Six malnourished patients (69±2 years) with COPD were measured with ^3H_2O for total body water (TBW), $^{35}SO_4$ for extracellular water (ECW), and ^{24}Na for total exchangeable sodium, and anthropometrics for body fat. Intracellular water (ICW) = TBW-ECW. Indirect calorimetry and N balance were used to estimate changes in ICW and ECW during hypercaloric enteral or parenteral feeding.

AT ADMISSION (X ± SEM)

Wt (kg)	CHI (% pred)	TBW (l)	ECW (l)	ICW (l)	ECW:ICW (% pred)	FAT (%)
45±5	54±5	29.0±3	13.5±2	15.5±2	156±12	11±3

CHI = Creatinine height index, pred = predicted

With 2 weeks of feeding, there was an increase in body wt (0.5±0.2 Kg, $p < .025$) with a decrease in ECW (40±10 g/Kg, $p < .005$) and an increase in ICW (22±5 g/Kg, $p < .005$). This shows that with repletion, there is a shift in the water distribution in malnourished patients, due to changes in both components (ECW and ICW).

PITUITARY/NEUROENDOCRINE

138.1

REGULATION OF PROLACTIN mRNA LEVELS BY SEX STEROIDS IN RAT ANTERIOR PITUITARY GLAND. Yiai Tong*, Hui Fen Zhao*, Jacques Simard*, Claude Labrie*, Luc Petitclerc*, Fernand Labrie and Georges Pelletier*, MRC Group in Molecular Endocrinology, Laval University Medical Center, Quebec G1V 4G2, Canada.

Estrogens are known to exert specific stimulatory effects on basal and dopamine-inhibited prolactin (PRL) release and synthesis as well as on PRL mRNA gene expression. On the other hand, androgens, in the presence of 17β-estradiol (E_2), exert an inhibitory action on PRL release both in vivo and in vitro. Using castrated male rats, we have studied the effect of E_2 (0.25 µg), progesterone (P, 2 mg) or dihydrotestosterone (DHT, 100 µg) administered twice daily for 14 days alone or in combination on PRL mRNA levels measured by in situ hybridization and dot blot hybridization. The cDNA probe encoding rat PRL was kindly provided by Dr. R. Maurer. Similar results were obtained with both techniques. Treatment with E_2 increased PRL mRNA levels by 2.5 to 3.5-fold, the values being similar to those observed in intact female rats. Administration of DHT or P alone had no effect on PRL mRNA accumulation. Concomitant administration of DHT, however, decreased the stimulatory effect of E_2 on PRL mRNA levels by 50 to 80%. All the hormonal treatments described above did not affect growth hormone mRNA levels. The results obtained clearly indicate that in vivo the stimulatory effect of E_2 on PRL gene expression is partially reversed by androgens. These results also demonstrate that quantitative in situ hybridization is a powerful tool to study the regulation of gene expression.

138.2

ANTAGONISM OF ARGININE-VASOPRESSIN-INDUCED ACTH SECRETION IN VIVO. Themis C. Kamilaris, Renato Bernardini, Mark A. Demitrack*, Bill Gallucci*, Philip W. Gold*, and George P. Chrousos. (SPON: P.A. Deuster) DEB/NICHD and *BPB/NIMH, Bethesda, MD 20892.

Arginine-vasopressin (AVP) participates in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis by acting synergistically with corticotropin-releasing hormone (CRH) to stimulate pituitary ACTH secretion. Whether and to what extent AVP is involved in the circadian and/or stress-induced activation of the HPA axis, however, remains unknown. In vivo active AVP antagonists that would specifically antagonize the stimulatory effect of AVP on the corticotroph would allow elucidation of the physiologic role of AVP in HPA axis function. We examined the abilities of 3 in vitro antagonists [d(CH_2)5 Tyr(Et^2)VAVP, desGly9d(CH_2)5Tyr(Et^2)VAVP and desGly9d(CH_2)5D-Tyr(Et^2)VAVP] to inhibit AVP-induced ACTH secretion in vivo, in chronically cannulated male Sprague-Dawley rats. Each of the antagonists was given intravenously alone, or 2 minutes prior to an IV injection of 0.3 ug/100 gr body weight of AVP, at the dose of 25ug/100gr body weight. Whereas, none of the three compounds had any agonist effect of its own, all three peptides inhibited AVP-induced ACTH secretion by 64, 47, and 46%, respectively. These results suggest that the compounds tested are potent AVP antagonists in vivo and could be used in physiological experiments to elucidate the role of AVP in HPA axis function.

138.3

ANGIOTENSIN ACTS AT SUBFORNICAL ORGAN TO INCREASE PLASMA [OXYTOCIN]. A.V.Ferguson and N.W.Kasting Depts. of Physiology, Queen's University, Kingston,K7L 3N6; and UBC Vancouver V6T 1W5; Canada.

The subfornical organ (SFO) contains angiotensin II (AII) receptors and is the CNS site at which this peptide acts to influence plasma [vasopressin], blood pressure and drinking. Our own studies have also suggested that systemic AII may stimulate oxytocin [OXY] release through an action at the SFO. We have therefore examined the effects of systemic AII administration on plasma [OXY] in freely moving intact and SFO lesioned male Sprague Dawley rats fitted with atrial catheters a minimum 5 day period prior to experimentation. Electrolytic SFO lesions were placed in the lesion groups at this time. On the day of study animals were placed in isolation boxes and following a 60 m equilibration period a control blood sample was withdrawn. 30 m later a 30 m atrial AII infusion (1.0 ug/kg/m) was initiated, after which a second sample was taken. A third blood sample was withdrawn a further 30 m later. After collection all blood samples were immediately centrifuged and the plasma retained for OXY radioimmunoassay. Post mortem histology allowed each animal to be assigned to SFO intact, rostral SFO lesion or caudal SFO lesion groups. Systemic AII increased plasma [OXY] in both intact (means 6.8 to 44.8 pg/ml), and caudal SFO lesioned (6.8 to 45.1 pg/ml) groups, while it had no significant effect in the rostral SFO lesioned animals (17.4 to 22.8 pg/ml) although basal [OXY] were elevated in this group. These data support the hypothesis that systemic AII acts at the SFO to increase plasma OXY concentrations.

Supported by the MRC of Canada.

138.4

SUBFORNICAL ORGAN STIMULATION INCREASES PLASMA LUTEINIZING HORMONE CONCENTRATIONS IN THE CONSCIOUS RAT. S.D. Donevan, D.A. Van Vugt*, and A.V. Ferguson. Dept of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The subfornical organ (SFO), a circumventricular structure of the brain, plays an important role in control of pituitary hormone secretion. Anatomical studies have identified SFO projections to the medial septum, a region known to contain a large proportion of luteinizing hormone releasing hormone (LHRH)-immunoreactive neurons which project to the median eminence, while lesion studies have shown that ablation of the SFO disrupts the estrous cycle in rats. In the present study, the effect of electrical stimulation in the SFO on plasma luteinizing hormone (LH) concentrations were examined in the conscious rat. Male, Sprague Dawley rats (n=11) were anaesthetized with sodium pentobarbital (60 mg/kg). An indwelling atrial catheter was inserted into the right jugular vein and a stimulating electrode was stereotaxically implanted into the SFO and cemented in place. Following a 4-5 day recovery period, animals were placed in isolation boxes and blood sampling was initiated following a 60 min equilibration period. Seven blood samples were obtained at 10 minute intervals over one hour. A 3 min, 30 s on/off stimulation (100 uA, 10 Hz, 0.1 ms pulse width) period was begun immediately prior to the the third sample. SFO stimulation increased plasma [LH] to 246.9±82.1 pg/ml from pre-stimulation control levels of 59.8±4.2 pg/ml. These observations support a role for the SFO in the regulation of gonadotropin secretion.

Supported by MRC of Canada.

138.5

PHOSPHOLIPID TURNOVER IN A DOPAMINE (DA)-SENSITIVE, PROLACTIN (PRL)-SECRETING RAT PITUITARY ADENOMA AND IN TWO DA-RESISTANT, PRL-SECRETING RAT PITUITARY TUMORS. H. Forget* and R. Collu* (SPON: L. Dumont). Centre de Recherche, Hôpital Ste-Justine, Univ. de Montréal, Montréal, Canada

The secretion of PRL by the pituitary gland is under a tonic inhibitory control exerted by DA. Recently, it has been suggested that DA may exert its action by inhibiting inositol phosphates (IPx) production and mobilization of intracellular calcium. To study the effects of DA on IPx production and PRL release we have utilized an estrone-induced, DA-sensitive rat pituitary adenoma and two malignant, transplantable and DA-resistant rat pituitary tumors, 7315a and MtTW15. Purified cells obtained from the three tissues were incubated for 30 min in media with drugs (TRH and ANGIOTENSIN II) stimulating IPx production and PRL release, in the presence or the absence of DA. Baseline IPx production and PRL release by the adenoma were inhibited by DA. TRH and ANGIOTENSIN II stimulated IPx production by adenomatous and 7315a cells; this effect was antagonized by DA in adenomatous cells. PRL release by adenomatous cells only was stimulated by TRH and ANGIOTENSIN II and this stimulation was inhibited by DA. Our results show the presence of differences between adenoma and transplantable pituitary tumors in the mechanisms of regulation of PRL release.

138.6

INHIBITORY EFFECT OF THE LHRH AGONIST [D-Trp^6, des-Gly-NH$_2$^{10}]-LHRH ETHYLAMIDE AND AN LHRH ANTAGONIST ON PITUITARY LUTEINIZING HORMONE BETA- SUBUNIT MESSENGER RNA IN THE RAT. L. Petitclerc*, C. Labrie*, J. Simard*, M. Badr*, H.F. Zhao*, G. Pelletier, D.H. Coy and F. Labrie, MRC Group in Molecular Endocrinology, Laval Univ. Medical Center, Quebec G1V 4G2

We have studied the effect of the LHRH antagonist [D-Nal$_1$, C-Cpa^2, D-Trp^3, D-Arg^6, D-Ala^{10}]-LHRH and the LHRH agonist [D-Trp^6, des-Gly-NH$_2$^{10}]LHRH ethylamide (LHRH-A) on plasma LH concentrations as well as pituitary β-LH mRNA levels in castrated male rats. β-LH mRNA was measured by dot-blot hybridization using rat LH cRNA probes derived from the cDNA fragment kindly provided by Dr. J.L. Roberts. Plasma LH was at 4.14 ± 0.30 ng/ml after 3 weeks of castration. Administration of 200 µg of the LHRH antagonist caused a 78% fall in plasma LH two hours after the injection. After one week of daily LHRH antagonist administration, plasma LH was 96.5% lower than the value found in control castrated animals. Pituitary β-LH mRNA levels fell 43% within 10 hours of injection and reached a low of 20% relative to control after one week of treatment. Administration of 5 µg LHRH-A caused 45% and 95% reductions in plasma LH after 7 and 14 days of treatment, respectively, while β-LH mRNA fell 50% and 80%, respectively. The present results demonstrate the potent inhibitory effects of the LHRH antagonist and LHRH-A on LH release and LH β-mRNA expression, thus offering models for study of the direct action of other modulators of LH secretion, especially sex steroids, at the pituitary level.

138.7

BRAIN ANGIOTENSIN BUT NOT PERIPHERAL ANGIOTENSIN TRIGGERS DRINKING IN RESPONSE TO HEMORRHAGE. M. Ian Phillips, Frank Heininger* and Birgitta Kimura*, Department of Physiology, University of Florida, Gainesville, FL. 32610.

It is well known that hemorrhage induces drinking. Since hemorrhage increases renin release due to volume depletion, it might be assumed that the drinking is stimulated by increased peripheral Ang II. However, we investigated if brain Ang II is responsible for the drinking response. To test this, we measured brain Ang II in hemorrhaged rats and blocked synthesis with Captopril. Male Sprague-Dawley rats were hemorrhaged 33% and 44%. Thirty minutes after hemorrhage the brains were removed and dissected. Ang II was measured in hypothalamic blocks and in plasma by radioimmunoassay (Phillips & Stenstrom, Circ. Res., 1985). Significant increases in brain Ang II levels were found in the hemorrhaged rats (145 ± 18, 147 ± 11 pg/g) compared to controls (85.4 ± 5.0 pg/g). The levels were higher than could be accounted for by raises in plasma Ang II. To prevent Ang II from being formed, Captopril (25 µg/µl) was injected i.v.t. immediately prior to hemorrhage (33%) and the rats tested for drinking. Another group was tested with Captopril i.p. (50 mg/kg) injections before hemorrhage. The results showed that i.v.t. Captopril inhibited the drinking response to hemorrhage ($p < 0.01$) whereas i.p. Captopril or ACS i.v.t. had no effect. It is concluded that brain Ang II is the major stimulus for drinking to hemorrhage and not the peripheral Ang II. (Supported by NIH grant R01-27334.)

138.8

PLASMA PROLACTIN PARALLELS RESPONSE OF ADRENOCORTICOTROPIN TO MODERATE HEMORRHAGE IN UNANESTHETIZED SWINE. D. E. Carlson, H. G. Klemcke, and D. S. Gann, Brown U./R. I. Hospital, Providence, RI 02902 and USDA, Agricultural Research Service, USMARC, Clay Center, NE 68933.

Prolactin (PRL) responds to several stimuli that elicit release of adrenocorticotropin (ACTH) but does not increase in response to hemorrhage in fetal animals. To determine if PRL increases after hemorrhage in postnatal pigs, eleven immature, female swine (15-25 Kg) were prepared chronically under halothane and conditioned behaviorally to lie in a sling. They were bled 14 ml/kg/5 min. PRL, ACTH, cortisol (F), vasopressin (LVP), and renin (PRA) were measured by RIA. Epinephrine (EPI) and norepinephrine (NE) were separated by HPLC. Samples were taken at -0.1, 0, .25, .5, .75, 1, 1.5, 2, 4, and 7 h from the onset of hemorrhage. Arterial PRL increased at 0.75 and 1 h ($P<0.01$) and paralleled ACTH and F that peaked at 0.75 h ($P<0.05$ and $P<0.01$, respectively). All 3 hormones recovered significantly by 4 h. In contrast, PRA and LVP peaked transiently at 0.25 h after hemorrhage and recovered by 1.5 h ($P<0.05$, in each case). EPI and NE did not change significantly. In individual pigs, ACTH and F showed correlations (Spearman) with PRL that were positive in 10 pigs and significant in 6 pigs. The pig with the smallest ACTH change (8.4 pg/ml peak) showed no increase in PRL. Peaks in PRL were simultaneous with (5 pigs) or delayed by 0.25 h from (5 pigs) peaks in ACTH. Significant correlations of PRL with PRA and with LVP occurred in only 2 pigs and in 1 pig, respectively. We suggest that a common mechanism may elicit release of ACTH and PRL after hemorrhage. Supported in part by NIH grant GM27946.

138.9

CAPTOPRIL BLOCKS PITUITARY-ADRENAL RESPONSE TO MODERATE HEMORRHAGE, BUT AUGMENTS THOSE TO LARGE HEMORRHAGE. J.S.Cross*, M.P.Lilly, D.S.Gann, Brown University/ Rhode Island Hospital, Providence, RI 02903

We have shown captopril(CAP) inhibits the ACTH, AVP, Angiotensin II (A-II) and adrenal medullary responses to 20% hemorrhage(hem) in dogs. To see if a larger stimulus would overcome the effect of CAP, we studied pentobarbital anesthetized dogs, two days after splenectomy, and placement of adrenal vein, and femoral artery and vein catheters. Dogs were bled 30% of blood volume (Evan's Blue) over 3 min. CAP (20µg/kg/min) or vehicle was infused iv 10min before hem. Adrenal venous and arterial blood was sampled at -20,-15,-10,-5,2,4,6,8,10,15,20 & 30 min. Adrenal epinephrine was assayed by HPLC and secretion rate(E) was calculated from adrenal blood flow. AVP, ACTH, and A-II were assayed by RIA; data(mean ± SEM) were analyzed by ANOVA.

Horm	CAP	-20min	-10min	10min	20min	30min
ACTH	-	23.8± 3.8	22.2± 3.6	76.8±16.6*	162.4±45.7*	137.4±38.4*
(pg/ml)	+ †	24.8± 2.4	25.9± 2.8	177.0±29.3*†	237.1±50*	185.4±36.9*
AVP	-	3.3± 0.6	3.3± 0.6	82.4±29.3*	71.0±25.8*	72.2±27.1*
(pg/ml)	+ †	2.2± 0.0	2.1± 0.2	131.2±42.2*	89.6±33.8*	74.5±23.9*
E	-	0.6± 0.2	0.9± 0.3	11.9± 5.7*	22.0± 9.4*	23.6±12.5*
(ng/min)	+ †	1.2± 0.6	4.0± 2.1	108.1±42.9*†	75.5±38.0*	117.6±67.1†*
A-II	-	50.5±15.9	49.9±12.4	214.6±35.4*	262.2±46.1*	269.3±43.3*
(pg/ml)	+ †	59.6± 9.0	17.3± 1.5*†	41.3±15.2*†	69.6±28.4*†	48.6±14.3*†
MAP	-	106.9± 7.0	111.6± 4.2	93.1±12.0	101.4±10.0	104.3±10.0
(mmHg)	+ †	108.5± 9.9	102.7± 9.5*	60.3±10.3	64.2± 8.7†	65.5± 8.7†

*=p<0.05 response from control, †=p<0.05 treatment effect.

ACTH, E, AVP, and A-II increased after hem in controls. Although CAP blunted the AII response to 30% hem, we saw augmented ACTH, AVP, and E responses. Arterial pressure(MAP) decreased similarly in both groups, but did not recover with CAP. These data suggest: (1) A-II is necessary for MAP defense after 30% hem, (2) A-II may be necessary for normal hormonal responses to moderate hem, but large responses can be activated by other mechanisms after larger stimuli. (Supported by NIH #DK26831)

138.10

EFFECTS OF PHENCYCLIDINE ON NEUROENDOCRINE SYSTEMS. W.O. Boggan & J.G. Ondo, Departments of Psychiatry and Physiology, Medical University of South Carolina, Charleston, SC 29425

We examined the effects of central administration of phencyclidine (PCP) on luteinizing hormone (LH) and prolactin (PRL) known to be important in reproduction. PCP inhibits a variety of actions of the excitatory amino acids (EAA) glutamate and/or N-methyl-D-aspartate (NMDA) and these compounds increase the binding of PCP or its analogues to their recognition site. Since our previous studies have indicated that EAA can promote LH secretion and may play a role in the neural control of LH, we hypothesized that PCP would alter the ability of glutamate to increase plasma concentrations of LH. Experiments were conducted using castrate and intact male Sprague Dawley rats. Two different procedures were used. One utilized animals cannulated both in the jugular vein and in the lateral ventricle. Blood samples were taken at various times before and after the infusion (5 min) of either PCP or its pH adjusted (7.4) vehicle, artificial CSF (vol = 250 nl). In the other procedure, animals were implanted with only the ventricular cannula and were sacrificed 30 min after receiving a 5-min infusion of either CSF, PCP, glutamate, AP5 (a specific NMDA receptor antagonist) or combinations of these. Plasma concentrations of LH and PRL were measured by radioimmunoassay. The data indicate that PCP administered into the lateral ventricle can decrease plasma concentrations of LH in the castrate, thus supporting the hypothesis of a direct CNS action of PCP on LH. In addition, PCP inhibited glutamate-induced LH secretion and AP5. Since there appear to be endogenous PCP-like compounds and CNS receptor sites for PCP, these findings suggest a possible role for these compounds and their receptors in EAA mediated LH secretion. In contrast to several previous studies, we found no effect of central administration of PCP on PRL.

138.11

Hormonal Modulation of the Brain Cholinergic System in the Female Rat. J. V. Lee* and K.F.A. Soliman. College of Pharmacy, Florida A&M University, Tallahassee, FL 32307.

Female Sprague-Dawley rats maintained under controlled environmental conditions were used in this experiment. Female rats were ovariectomized and two weeks later drugs were administered. The first group was treated with 5 ug of estrogen. Another group was treated with 5 ug of estrogen supplemented 48h later with 5mg progesterone. Animals were sacrificed and their brains were dissected into cerebral cortex, cerebellum, hypothalamus, hippocampus, midbrain, medulla oblongata, pons and bulbus olfactorius. Assay of choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) were performed in each brain regions. Estrogen treatment was associated with significant increase of ChAT activity of the hypothalamus, cerebellum, bulbus olfactorius, midbrain, medulla and pons. The same treatment was also found to cause significant increase in AChE activity in the pons, bulbus olfactorius and the medulla. The administration of both estrogen and progesterone was associated with significant increase of ChAT activity in all brain regions examined and significant increase in AChE activity of the cerebellum, medulla and midbrain. The results of this experiment indicate that the cholinergic system might be involved in the different physiological and behavioral changes associated with estrogen and progesterone in the female (Supported by grants from NASA NAG 2-411; and NIH RR08111).

138.12

Involvement of the Cholinergic System in Pain Threshold Alterations of Genetically Obese Rats. C. Goodman*, H. Akunne* and K.F.A. Soliman. College of Pharmacy, Florida A&M University, Tallahassee, FL 32307.

Male Zucker obese and their lean littermates kept under controlled environmental conditions were used in this experiment. Five weeks-old animals were monitored for their pain sensitivity, blood glucose levels and weight gain for 9 weeks. The results indicate that starting at the 4th week of the experiment, obese animals had significantly higher pain threshold than their lean littermates using the tail flick procedure. Moreover, obese animals exhibited an increased blood glucose level at the 6th week of the experiment. At the end of the experiment, animals were sacrificed and choline acetlytransferase (ChAT) and acetylcholinesterase (AChE) enzymes were assayed in the brain. Results indicate that obese animals had a significantly lower ChAT activity levels in the midbrain, pons, and cerebellum comparing to the lean animals. Meanwhile, the hypothalami of the obese animals had a significantly lower AChE activity than their lean littermates. It was concluded from this study that obesity is associated with elevated pain threshold and blood glucose levels. The elevation of pain threshold might be related to changes in the enzymes of the brain cholinergic system. (Supported by a grant from NASA NAG 2-411, NIH RR01118 and NIH grant RR03020).

PROSTAGLANDINS AND LEUKOTRIENES

139.1

NON-ANTIOXIDANT LIPOXYGENASE INHIBITORS DO NOT INHIBIT NEUTROPHIL FUNCTION. SB Shappell,* AA Taylor,* CW Smith,* JR Mitchell. Baylor College of Medicine, Houston, TX 77030.

The mechanism by which lipoxygenase (LOX) inhibitors decrease neutrophil (PMN) accumulation and protect against myocardial reperfusion injury is unclear. Although the protective mechanism is presumed to result from LOX inhibition, most compounds also are potent antioxidants (e.g., BW755C, NDGA, Nafazatrom) and several inhibit PMN function (Biochem Pharmacol 35: 3481),thereby questioning the intermediacy of LOX products in reperfusion injury. Other LOX inhibitors, however, are poor antioxidants but still protect against injury (e.g., REV-5901). We now report the effects of REV-5901 and SKF 86002 as well as NDGA (positive control) on PMN activation. NDGA (10 uM) completely inhibits PMA (10^{-8} M) induced PMN secretion (chemiluminescence assay). In contrast, REV-5901 and SKF 86002 in concentrations up to 50 uM are without effect. Furthermore, NDGA (10 uM) markedly inhibits fMLP (10^{-9} M) induced chemotaxis (bipolar shape change) whereas REV-5901 and SKF 86002 (up to 50 uM) do not. Conclusions: 1) The direct inhibitory effects of LOX inhibitors on PMN function correlate with antioxidant properties. 2) The protective effect of REV-5901 on reperfusion injury without inhibition of PMN function in vitro supports a role for LOX products in the injury. 3) The possibility that other antioxidant agents may protect by inhibiting PMN function should be investigated.

139.2

EFFECTS OF DIETHYL MALEATE (DEM), A GLUTATHIONE-ALKYLATING AGENT, ON PROSTAGLANDIN RELEASE IN THE ISOLATED PERFUSED SPLEEN OF RABBITS. T.Hidaka*, H.Furuno* and R.Ogura* (SPON: K.U.Malik), Department of Medical Biochemistry, Kurume University School of Medicine, Kurume 830-91, Japan.

To investigate the role of glutathione (GSH) on prostaglandin (PG) synthesis, isolated rabbit spleens were perfused with Tyrode's solution with or without the addition of DEM in concentrations up to 1mM. In the absence of DEM, PG synthesis was stimulated by the Ca^{2+} ionophore A23187 (20 nmoles) or arachidonate (0.4 μmoles). This resulted in the release of cyclooxygenase products, as assessed by radio-immunoassay. Prostaglandin (PG) E_2 was a major product, accounting for 60-70% of the total. Small amounts of $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, PGD_2 and thromboxane (Tx) B_2 were also produced. When DEM was added to the perfusion medium, GSH decreased dose-dependently with increasing DEM concentration. Lactate dehydrogenase activity was not detected in the venous effluent, indicating that DEM depleted intrasplenic GSH without causing any lysis of cellular membranes. A23187-induced production of PGs and of Tx decreased with increasing concentrations of DEM up to 0.5mM, whereas at 1.0mM of DEM, these products showed a tendency to increase as compared with levels at 0.5mM of DEM. However, this increase was only significant for TxB_2, which returned almost to its untreated control level. 1mM DEM does not cause cell lysis, however it appears to perturb the cell membrane to a degree similar to that which occurs with stimulation of phospholipase A_2. The small but significant increase of TxB_2 may be a result of PGH_2 accumulation caused by inhibition of PGE_2 isomerase. Perfusion with arachidonate gave virtually identical results : 1.0mM DEM attenuated the production of all prostanoids except for TxB_2 as compared with untreated controls. These results strongly suggest that GSH may play an important role in maintaining a normal level of PGH_2 generation.

139.3

ELUTRIATION OF GUINEA PIG LUNG CELLS: CHARACTERIZATION, ULTRASTRUCTURE AND SECRETION OF THROMBOXANE B_2. C. Robidoux*, J.-P. Pelé* and P. Sirois* (SPON: J. Barabé). *Dept. Pharmacology, Fac. Med., University of Sherbrooke, Sherbrooke, PQ, Canada.

Lungs release arachidonic acid metabolites during inflammatory and hypersensitivity reactions but the cell types responsible for the synthesis of these mediators are still unknown. In this study, guinea pig lung cells (713.4 ± 41.3 x 10^6 cells/lung) were obtained by enzymatic digestion (Protease VII) and partially purified by elutriation. Eight fractions were obtained and the cell content of each was analysed by standard stainings and by electron microscopy. Fourteen cell types were characterized. Each elutriation fraction was stimulated with selected agonists (PAF, ethoxy-PAF, phorbol myristate, ionophore A23187, f-Met-Leu-Phe) and the secretion of thromboxane B_2 was monitored. LTD_4 stimulated by 100% the basal secretion of TXB_2 by total cells. The stimulation with LTD_4 was higher in the fractions 1,2 and 8. PMA was the most potent agonist and increased by 9 folds the secretion of TXB_2 by the cells present in fraction 4. Other fractions had more discrete responses. The chemotactic peptide, f-Met-Leu-Phe, stimulated by 2 folds the secretion of total cells and the effect was more prominent in fractions 5, 6 and 7 only. A23187, PAF and ethoxy-PAF stimulated by two folds the secretion of TXB_2 from total cells and from elutriated cells. (Supported by the M.R.C.)

139.4

THROMBOXANE A_2 (TXA_2) EFFECTS ON THORACIC AORTA OF YOUNG AND OLD RATS: USE OF SELECTIVE TXA_2 RECEPTOR ANTAGONISTS. Lane J. Wallace, Gamal Shams* and Dennis R. Feller. Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210.

TXA_2 is a bioactive metabolite of arachidonic acid which produces vascular smooth muscle contraction and blood platelet aggregation. The goal of this study is to establish whether there are age dependent differences of vascular contractility to TXA_2. Thoracic aorta of F-344 rats of age 5-6 months (young) and 20-22 months (old) were used as a model to examine responses to U46619 [(15S)-hydroxy-11α,9α-(epoxymethano) prosta-5Z,13E-dienoic acid; a TXA_2 agonist] alone or in the presence of prostanoid (SQ 29,548) or nonprostanoid (trimetoquinol; TMQ) endoperoxide/TXA_2 receptor antagonists. Maximal contractile responses and EC_{50} values (23 and 55 nM, respectively) to U46619 were the same in aortic strips of young and old animals. Experimentally determined pA_2 and pK_B values for SQ 29,548 and TMQ as antagonists of U46619-mediated contraction were unchanged in aorta of young (9.09 and 6.18, respectively) and old (9.41 and 5.94, respectively) rats. We conclude that the reactivity and population of TXA_2 receptors in vascular smooth muscle are unaffected by the aging process. (Supported by HL-22533).

139.5

INCREASED LEUKOTRIENE B_4 MEASURED BY GC-MS IN MYOCARDIAL ISCHEMIA-REFLOW INJURY. A.A. Taylor,* S.B. Shappell,* H. Hughes,* and J.R. Mitchell. Center for Experimental Therapeutics, Baylor College of Medicine, Houston, TX 77030.

A variety of pharmacologic agents which reduce myocardial injury during ischemia-reflow have in common the ability to inhibit neutrophil accumulation in tissue regions at risk. Identification of the chemoattractants that cause neutrophil accumulation, adherence to vascular endothelium, and migration into myocardial tissue is essential for the rational development of safe and effective therapy for minimizing ischemia-reflow injury. Previous studies demonstrating reduction of infarct size with dual cyclooxygenase/lipoxygenase inhibitors or specific 5-lipoxygenase inhibitors suggest a role for the potent chemotactic factor leukotriene B_4 (LTB_4). Interpretations of such studies are complicated by the fact that these agents are also anti-oxidants. We have developed a highly sensitive and specific assay for quantitating LTB_4 in vivo utilizing gas chromatography-negative ion electron capture mass spectrometric analysis of the pentafluorobenzyl ester, bis-tertbutyldimethylsilyl ether derivative of LTB_4 using deuterated LTB_4 as an internal standard. In a rabbit model of circumflex artery occlusion for 90 minutes followed by 90 minutes of reflow, we have observed a marked (> 3-fold) increase in LTB_4 in myocardial tissue at risk compared to myocardium not subjected to ischemia-reflow injury.

139.6

METABOLISM AND EXCRETION OF EXOGENOUS [^{3}H]-LTC_4 IN PRIMATES. P. Tagari*, A. Foster*, M. Cirino*, D. Delorme*, Y. Girard*, J. Rokach* (SPON: A.W. Ford-Hutchinson), Merck Frosst Canada Inc. P.O. Box 1005, Pointe Claire-Dorval, Québec H9R 4P8

IV administration of [^{3}H]-leukotriene (LT)C_4 (10 μCi/kg; saphenous vein) to 4 cynamologous monkeys resulted in rapid conversion (to LTD_4 and E_4) in, and elimination from the systemic circulation. Mean urinary excretion of tritium comprised 11.4±1.6% (n=4) of the original dose after 4 hours, rising to 14.8±2.1% after 24 hours. [^{3}H]-LTC_4 was a major component in urine samples obtained during the first hour only. Minor quantities of leukotrienes D_4 and E_4, and the ω-oxidised derivatives 20-OH-LTE_4 and 20-COOH-LTE_4 were observed in samples throughout the experimental period. After 1 hour, the more polar products of β-oxidation, 18-COOH-LTE_4 and particularly 16-COOH-LTE_4 were prominant. These were identified by co-injection with synthetic standards and, for the major metabolite 16-COOH-LTE_4, N-acetylation, ozonolysis and UV spectroscopy. Biliary excretion was faster, with 43.4% of the administered [^{3}H] eliminated within 1 hour, with a similar metabolic profile. This data suggests that ω- and subsequent β-oxidation followed by urinary excretion is a major route for the transformation and elimination of peptide leukotrienes in primates. The measurement of urinary 16-COOH-LTE_4 concentrations may, therefore, prove an index of in vivo leukotriene production of clinical importance.

139.7

DIFFERENTIAL RESPONSE TO ILOPROST DURING ISCHEMIA-REPERFUSION OF NORMAL VERSUS DIABETIC MYOCARDIUM. Galen M. Pieper. Medical College of Wisconsin, Milwaukee, WI 53226.

Eicosanoid metabolism is altered in diabetes. Post-ischemic responses of diabetic hearts (DH) to eicosanoids are unknown. A synthetic analog of prostacyclin, iloprost (ILO), was given to isovolumically-beating hearts which were subjected to 20 min of total global ischemia and 30 min of reperfusion at preischemic flow rates. Acute DH (48 hrs) but not chronic DH (2 months) had pronounced postischemic dysfunction and a high incidence of ventricular fibrillation which was dramatically reversed by ILO ($3x10^{-8}$M). ILO stimulated endogenous prostacyclin release in postischemic control hearts (CH) but not DH. However, ILO significantly decreased postischemic recovery of CH which was partially blocked by the calcium entry blocker, diltiazem, or by the free radical scavengers, superoxide dismutase plus catalase (100 U/ml). Similarly, postischemic dysfunction was elicited by arachidonic acid (10^{-6}M) in CH but not chronic DH. These data suggest that ILO may promote recovery in DH at concentrations which produce dysfunction in CH. Thus, ILO may induce dysfunction in CH by a calcium ionophoretic action of ILO which appears depressed in diabetes and by concomitant free radical production presumably via prostaglandin hydroperoxidase.

139.8

RG 12525 AND RELATED COMPOUNDS, A NEW, ORALLY ACTIVE SERIES OF LEUKOTRIENE ANTAGONISTS. R. G. Van Inwegen, G. Nuss*, G. Schuessler*, S. O'Rourke*, J. Travis*, D. Sweeney*, J. Gricoski*, D. Mertz*, R. Galemmo*, F. C. Huang* and G. Carnathan*. Rorer Central Research, Horsham, PA 19044.

RG 12525 (5-(2- 4-(quinolin-2-yl)methoxy phenoxymethyl) benzyl tetrazole) is a novel, orally active LTD_4 antagonist that was developed from a chemical series which evolved from our initial observations that RG 5901 and FPL55172 are weak competitive antagonists of LTD_4. Against ^{3}H-LTD_4 binding to membranes from guinea pig lung, RG 12525 was a competitive inhibitor with a K_i of 2.5 nM. Competitive antagonism of leukotrienes was demonstrated with a guinea pig lung strip assay in vitro with K_B values vs LTC_4, LTD_4 & LTE_4 of 3 nM. At 10 μm, RG 12525 had no effect on histamine-, methacholine-, or $PGF_{2\alpha}$-induced contractions, demonstrating >4000-fold selectivity for the leukotriene receptors. RG 12525 was orally effective as an inhibitor of LTD_4-induced wheals in guinea pigs (ED_{50} ~5 mg/kg vs 100 ng LTD_4/site) with a half-life of about 9 hours. In an antigen-induced systemic anaphylaxis model, RG 12525 was orally effective in preventing death with and ED_{50} of 2 mg/kg. This represents at least a 30-fold increase in oral potency over LY171883, SC39070, L649923 and ICI198615. The structure activity relationships observed with other compounds in this series will be discussed, including the steric relationships of the tetrazole, phenyl, and the quinoline rings.

139.9

ALBUMIN (BSA) INHIBITS A23187-INDUCED STIMULATION OF ARACHIDONIC ACID (AA) METABOLISM. J. T. Herlihy, D. Samples, E. A. Sprague and M.J.K. Harper. University of Texas Health Science Center, San Antonio, Texas 78284.

The calcium ionophore, A23187, increases the production of AA and associated metabolites. Both AA and its lipoxygenase products are lipophilic and often BSA is used to trap the released AA and metabolites. A23187 is also lipophilic and this study examines the effect of BSA on the A23187-induced release of eicosanoids. Cultured baboon aortic smooth muscle cells were grown for 24 hours in medium containing 1 uCi/ml (166 Ci/mmol) 3H-AA. After washing, the A23187-induced release of radiolabelled metabolites was determined in the presence and absence of 1% BSA. In the absence of BSA, A23187 increased total radioactivity 24 fold; however only a 2 fold increase was observed when BSA was present. The ionophore was found to co-elute with BSA on gel filtration chromatography. These results show that the presence of protein alters the stimulatory effects of A23187 on AA metabolism, presumably by decreasing the concentration of free ionophore.

(Supported in part by NIH grants HL35391, HD14048 and HL26890)

139.10

CHARACTERIZATION OF PEPTIDE LEUKOTRIENES PRODUCED UPON PULMONARY ANAPHYLAXIS IN THE ANESTHETIZED RAT. A. Foster, G. Letts, S. Charleson, B. Fitzsimmons and J. Rokach (Spon. A.W. Ford-Hutchinson), Merck Frosst Canada Inc. P.O. Box 1005, Pointe Claire-Dorval, Québec H9R 4P8

Previous work in this laboratory has shown that the intravenous administration of [^{3}H] leukotriene (LT)C_4 (1μCi/kg, n=6) results in a 69±1.6% recovery of radioactivity in the bile following rapid systemic clearance. In this study, we investigated the in vivo production of leukotrienes following antigen challenge (100μL 3% ovalbumen (OA) by tracheal instillation) in bile duct cannulated, sensitized inbred rats (A). Significant elevations of LTC_4 (3.65±0.78), LTD_4 (2.80±1.11) and N-Ac LTE_4 (3.87±1.15) ng/100g BW, n=13 were measured and characterized in the bile by reverse phase HPLC RIA techniques. LTC_4 was further characterized by UV spectroscopy (λmax 280nm with shoulders at 270 and 290 nm) and by conversion to LTD_4 by γ-glutamyl transpeptidase. Pretreatment with the specific 5-lipoxygenase inhibitor, L-656,224 (7-chloro-2-[(4-methoxyphenyl)methyl]-3-methyl-5-propyl-4-benzofuranol, 15 mg/kg p.o. 2h pre) resulted in a >90% inhibition of all three metabolites. We conclude that peptide leukotrienes are produced upon pulmonary anaphylaxis in the rat, and that this model is suitable for demonstrating the biochemical efficacy of 5-lipoxygenase inhibitors in vivo.

A. Holme, G. and Piechuta, H. Immunology 42, 19-24 (1981)

139.11

MUCOSAL ARACHIDONATE METABOLISM ASSOCIATED WITH SMALL INTESTINAL ISCHEMIA-REPERFUSION INJURY. Martin J. Mangino Charles B. Anderson*, and John Turk*. Washington Univ. School of Medicine, St. Louis, MO 63110

The effects of small intestinal ischemia and reperfusion on mucosal eicosanoid synthesis were studied in anesthetized dogs (n=10). Segments of the distal ileum were subjected to ischemia for 3 hours and allowed to reperfuse for 1 hour. samples of intestine were obtained before ischemia, during ischemia (2 hours), and 1 hour after reperfusion. The results are:

	Before Ischemia	During Ischemia	After Reflow
TXB2	280 ± 73	311 ± 66	1,302 ± 280*
PGI2	1,079 ± 281	1,606 ± 211	2,122 ± 523*
PGE2	949 ± 463	1,493 ± 1,070	2,451 ± 560*
LTC4	280 ± 77	477 ± 181*	1,113 ± 247*
LTB4	605 ± 131	1,095 ± 342	4,762 ± 706*

Results expressed as pg/100 mg tissue, * $P < 0.05$ relative to values obtained before ischemia. In other experiments, the production of hydroxyeicosatetraenoic acid (HETE) isomers was determined by negative ion GC/MS. The production of 15-HETE, 12-HETE, and 5-HETE by mucosal tissue after reflow increased by 700%, 900%, and 1,000%, respectively, relative to mucosal tissue obtained before ischemia. These data demonstrate dramatic alterations in arachidonate metabolism by ischemic intestinal mucosa that may have functional significance. Supported by NIH AM-33308.

139.12

INHIBITION OF LTB_4 INDUCED LEUKOPENIA BY LY255283 AND LY223982. W.T. Jackson*, L.L. Froelich*, T. Goodson*, D.K. Herron*, B.E. Mallett*, and D.M. Gapinski*. (Spon: J.H. Fleisch) Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285.

Compounds LY255283 (1-(5-ethyl-2-hydroxy-4-(6-methyl-6-(1H-tetrazol-5-yl)-heptyloxy)phenyl)ethanone) and LY223982 ((E)-5-(3-carboxybenzoyl)-2-((6-(4-methoxyphenyl)-5-hexenyl)oxy)benzenepropanoic acid) were tested for their effects on leukopenia induced in rabbits by leukotriene B_4 (LTB_4) and N-formyl-methionyl-leucyl-phenylalanine (FMLP). In the absence of test compound, i.v. administration of 1μg LTB_4 induced a transient decrease in circulating leukocytes (70% decrease between 0.5-2.0 min after injection of LTB_4). FMLP (0.39μg) induced an equivalent amount of leukopenia. Test compounds were given intravenously 2 minutes prior to administration of LTB_4 or FMLP. Indomethacin (10mg/kg) had no effect on LTB_4-induced leukopenia. LY255283 (0.02-2.0mg/kg) inhibited LTB_4-induced leukopenia in a dose dependent manner. Complete inhibition of the LTB_4-induced response occurred at 2.0mg/kg. However, a 5.0mg/kg dose of LY255283 had no effect on FMLP-induced leukopenia. Similar results were obtained with LY223982 (2-10mg/kg). A 10mg/kg dose of LY223982 completely inhibited LTB_4-induced leukopenia but a 40mg/kg dose had only a marginal effect on the FMLP-induced response. Neither compound (LY255283, 2mg/kg; LY223982,10mg/kg), by itself, affected the circulating leukocyte count. LY255283 and LY223982 have previously been reported to be potent and specific antagonists of LTB_4-induced neutrophil aggregation in vitro (FASEB Journal 2 (5):A1110, 1988). Present studies show that the compounds are also specific LTB_4 antagonists in an in vivo setting.

139.13

TOPICAL INFLUENCE OF ALOE VERA ON ADJUVANT ARTHRITIS, INFLAMMATION AND WOUND HEALING. Robert H. Davis PA College of Podiatric Medicine, Phila., PA 19107 and Florida Food Products, Eustis Florida 32727.

Adjuvant induced arthritis closely resembles rheumatoid arthritis in its pathologic manifestation. Adjuvant arthritis induced by administering M. butyricum in oil into the paw was inhibited 25% by applying 5% Aloe + RNA + ascorbic acid in cream topically for 13 days over a 21 day period. Once established, the arthritis was regressed 45% by the same topical treatment. Topical Aloe vera showed a dose-response relationship between 0.25 to 1.0% in inhibiting croton oil induced ear swelling. This provides a good index as to where the concentration of commercial preparations should be. Low doses of Aloe vera in cream applied topically over the wound for 7 days significantly improved wound healing over controls. Thus, Aloe inhibits the early inflammation but, also, improves would healing. This study provides us with some indications of "How Aloe Works".

139.14

TRIPHASIC RESPONSE TO ARACHIDONIC ACID (AA) ON BLOOD PRESSURE IN CONSCIOUS RATS. Mahesh Mistry* and Kafait Malik, Dept. of Pharmacology, University of Tennessee, Memphis, TN 38163

Thromboxane A_2 (Tx) or structurally related prostanoids have been proposed to participate in prohypertensive mechanisms. The present study was designed to determine if Tx or prostaglandin (PG) endoperoxides generated from AA influence arterial blood pressure (b.p.) in normotensive rats. Male Sprague-Dawley rats were instrumented with PE50 catheters in the femoral artery and femoral vein under ketamine/xylazine anesthesia. Following recovery on the next day, the rats were placed in perspex holders and the arterial catheter was connected to a pressure transducer for recording mean b.p. and the venous catheter was used for drug administration. Responses to bolus intravenous (i.v.) injections (200μl) of AA (1.5-2.5mg) and Tx analogue, U46619 (0.5-5μg), were recorded before and after inhibition of cyclooxygenase with indomethacin and Tx synthetase with UK38485 and treatment with Tx receptor antagonist, SQ29548. Administration of AA resulted in a dose-dependent triphasic response, viz., transient depressor (Phase I) followed by pressor (Phase II) and then a prolonged depressor response (Phase III). Treatment with indomethacin (10mg/kg) attenuated Phase I and abolished Phase III of AA-induced response. Tx synthetase inhibitor, UK38485 (30mg/kg i.v. bolus and 15 mg/kg/hr infusion) did not alter any component of the AA response whereas Tx receptor antagonist, SQ29548 (5mg/kg i.v. bolus and 5mg/kg/hr infusion), which also blocks the actions of PG endoperoxides, significantly attenuated Phase II of the AA response. Pressor responses to U46619 were not affected by indomethacin or UK38485 but were almost abolished by SQ29548. These data suggest that Phase III and at least a part of Phase I of the b.p. response to AA in the rat is due to its conversion to vasodilatory PGs whereas Phase II of the AA response is dependent on the generation of PG endoperoxides or a pressor prostanoid(s). (Supported by NIH 19134)

139.15

BW 755C BUT NOT PEPTIDOLEUKOTRIENE ANTAGONISM REDUCES MYOCARDIAL DAMAGE AND NEUTROPHIL INFILTRATION FOLLOWING CORONARY ARTERY OCCLUSION WITH REPERFUSION. E.F. Smith III, J.W. Egan*, D.E. Griswold*, L.M. Hillegass* and M.J. Slivjak*, Depts. of Pharmacology and Immunology, Smith Kline & French Labs., King of Prussia, PA.

This study was designed to compare the cardioprotective effects of a 5-lipoxygenase inhibitor, BW 755C, with a peptidoleukotriene receptor antagonist, SK&F 104353, following coronary artery occlusion for 30 min with reperfusion (MI/R) for 24 hr in rats. BW 755C (10 mg/kg, i.v.) or SK&F 104353 (25 mg/kg, i.v.) were given 1 min prior to and 4 hr after coronary artery occlusion. SK&F 104353 antagonized LTD_4 vasopressor responses by 90% after 1 hr and 60% after 4 hr, indicating blockade of leukotriene responses. Creatine phosphokinase and myeloperoxidase (MPO) activity were measured to determine myocardial injury and neutrophil accumulation, respectively. Infarct size was reduced from 22% in MI/R-vehicle treated animals to 14% in MI/R-BW 755C treated animals ($p < 0.05$). In comparison, SK&F 104353 had no effect on myocardial injury. Myocardial MPO activity increased from 1.5±0.5 U/g in sham-MI/R animals to 4.3±0.6 U/g in MI/R-vehicle animals ($p < 0.01$). In the MI/R-SK&F 104353 group, MPO levels were 4.4±0.8 U/g. These data indicate that peptidoleukotrienes do not contribute to myocardial ischemic/reperfusion injury or PMN accumulation, but suggest that other 5-lipoxygenase products are involved.

139.16

SULOTROBAN INHIBITS THE CEREBROVASCULAR EFFECTS AND NEUROLOGIC CONSEQUENCES OF THROMBOXANE. A. Sulpizio*, C. Sauermelch*, J.P. Hieble and R.N. Willette. Smith Kline & French Laboratories, King of Prussia, PA 19406.

Elevated intracisternal levels of thromboxane B_2 (TXB_2) accompany subarachnoid hemorrhage and have been implicated in the pathogenesis of cerebral vasospasm. This study describes the cerebrovascular effects and neurologic consequences of U46619, a TX-mimetic, in the absence and presence of sulotroban (BM 13.177; SK&F 95587), a TX antagonist. In isoflurane anesthetized rats, paralyzed and ventilated (O_2), the Lt. common carotid and basilar arteries were ligated and BP, HR, ECoG and local cortical perfusion (LCP; laser flowmetry) were monitored. Internal carotid administration of U46619 elicited immediate dose-related (0.3-3.0 ug) decreases in LCP and ECoG activity unrelated to systemic changes. Pre- or post-treatment with sulotroban (10-20 mg/kg, i.v.) reduced or abolished the effects of U46619. Similar, agonist and antagonist LCP effects were observed following intraparenchymal microinjections (80-100 nl) beneath the LCP probe. Sulotroban alone had no significant effects on LCP, ECoG or the LCP-pCO_2 relationship. In vitro, sulotroban competitively antagonized ($K_B=6.4\times10^{-7}$; DR=5.7) U46619-induced contraction of the internal carotid artery (pH 7.4 & 6.8). In this model, sulotroban inhibits the LCP and ECoG effects of U46619, apparently by antagonizing TX-receptor mediated cerebrovasoconstriction in arteries and microvessels.

139.17

EFFECT OF PROSTANOID PRECURSORS AND SUPEROXIDE RADICAL ON HUMAN BLOOD PLATELET FUNCTION IN VITRO. B.E. Akinshola*, P.S. Verma* and R.E. Taylor* (SPON: W.L. West). Howard University, College of Medicine, Washington, D.C. 20059.

The level of cyclic adenosine monophosphate (cAMP) in human platelets is known to be an important regulator of platelet function. The polyunsaturated fatty acids: Dihomo-gamma-linolenic acid (DHLA), and Eicosapentaenoic acid (EPA), precursors of the prostaglandin (PG) 1 and 3 series respectively, were studied for their ability to stimulate platelet cAMP and/or PGE_1 levels and to inhibit platelet aggregation (PAg). Incubation of washed platelets (10^8/ml) with 125 uM DHLA increased intraplatelet levels of PGE_1 from 197±7.03 to 1622±9.70 picograms, cAMP from 3±0.78 to 31±1.95 picomoles, and inhibited collagen-induced PAg. To a lesser degree, 125 uM EPA also increased platelet cAMP levels and inhibited collagen-induced PAg. Addition of 1 umole of xanthine per unit of xanthine oxidase (a superoxide radical generating system) to the incubating medium potentiated the effects of both fatty acids, whereas 240 uM hydrogen peroxide (H_2O_2) inhibited these effects. These data suggest that (1) superoxide radical may activate the platelet cyclooxygenase system to increase lipid peroxidation of these fatty acid precursors which may result in the inhibition of PAg, whereas H_2O_2 may have an opposite effect and (2) DHLA may be more effective in inhibiting PAg than EPA, which has been reported to reduce the incidence of coronary disease in certain human populations.

139.18

Inhibition of Antigen-Induced Contractions of Guinea-Pig Tracheal Strips (GPTS) by 5-Lipoxygenase (5-LO) Inhibitors. D. W. Snyder, H. L. Cho*, E. D. Mihelich* and P. P. K. Ho. Lilly Research Labs, Eli Lilly and Co., Indpls, IN 46285.

The ability of several reported 5-LO inhibitors to block antigen-induced contractions of GPTS from actively sensitized animals against ovalbumin (OA) were evaluated. Paired GPTS, pretreated with indomethacin (5 μM) and pyrilamine (10 μM), were challenged with cumulative concentrations of OA (10^{-7} - 10^{-3} mg/ml) in the presence or absence of drug. Potency of the 5-LO inhibitors was assessed as % inhibition of the OA-induced response at $3x10^{-5}$ mg/ml. All compounds tested suppressed the OA-induced contractions in a concentration-related manner. REV-6866 (N-methyl-4-benzyloxyphenyl-acetohydroxamic acid) was the most potent (IC_{50} = 0.56 μM) 5-LO inhibitor tested. Three structurally related inhibitors reported by Summers et al., 1987 (J. Med. Chem. 30:574) suppressed the OA responses with IC_{50} values from 1.3 - 150 μM. AA-861, a structurally unrelated 5-LO inhibitor, suppressed the OA-induced contractions with an IC_{50} value of 7.4 μM. These IC_{50} values correlated (r = 0.97) with the inhibition (IC_{50} values) of isolated 5-LO activity from guinea-pig neutrophils. LTD_4-induced contractions were not altered by these 5-LO inhibitors. The results demonstrate that OA-induced contractions are mediated via 5-LO products and suggest this model can be used to evaluate potency and selectivity of 5-LO inhibitors in vitro.

139.19

SUPPRESSION BY HIGH-DOSE GLUCOCORTICOSTEROID ADMINISTRATION OF EICOSANOID BIOSYNTHESIS BY HUMAN ALVEOLAR MACROPHAGES. Rolf J. Sebaldt*, James R. Sheller*, John A. Oates*, Garret A. FitzGerald* (SPON: M. Abou Donia). Division of Clinical Pharmacology, Vanderbilt University, Nashville, TN 37232

Although studies in vitro suggest that steroids modulate eicosanoid formation via induction of phospholipase inhibitory proteins, we failed to depress whole blood leukotriene (LT) B_4 formation or excretion of the urinary metabolites of prostaglandin (PG) E_2, PGI_2, or thromboxane (Tx) B_2 by high dose glucocorticoid (GC) administration in man. To further assess the cell specific action of steroids, we administered prednisone 60 mg to healthy volunteers for 1 week. Alveolar macrophages were obtained by bronchoalveolar lavage, preincubated at 100-300 x 10^3 cells/well with hydrocortisone (HC, 0-10^{-5} M) and stimulated with opsonized zymosan (OZ) (100 ug/ml), ionophore A23187 (50 uM) or no stimulus. Released eicosanoids were measured by gc/ms, a highly specific and sensitive technique. In vitro HC caused dose- and time-dependent suppression of TxB_2 and PGF_2 and PGE_2 (up to 54% after 11.5 h at 10^{-5} M), but suppressed LTB_4 less markedly. One week of prednisone suppressed the 3 cyclooxygenase products ex vivo under all preincubation conditions, and with or without OZ, by 81-88% and LTB_4 by 50% (no stimulus), 75% (OZ) and 65% (A23187). Unlike the major arachidonate products of neutrophils, platelets and endothelial cells, eicosanoids formed by human alveolar macrophages are subject to inhibition by GC in human volunteers.

139.20

LACK OF EFFECT OF ADENOSINE ON RAT MESANGIAL CELL PROSTANOID PRODUCTION. Gary R. Marchand, George J. Trachte and Lois J. Heller. Univ. of Minnesota, Duluth, MN 55812

Since glomerular mesangial cells (MC) have adenosine (ADO) receptors, we tested the hypothesis that adenosine reduces glomerular filtration rate by either increasing MC TxB2 or decreasing MC PGE2 production. Experiments were done with primary cultures of rat MC incubated (30 min, 37° C, pH 7.4, room air) with vehicle, 10 uM ADO, 1 ug/ml calcium ionophore (A23187) or both ADO and A23187 (9-12 plates/treatment). Although the MC metabolized ADO, more than 79% assayed by HPLC was recovered after incubation. Prostanoid concentrations were measured by RIA. PGE2 production (mean+SEM) is in pg/mg protein/30 min. TxB2 production is in percent of plates with detectable levels.

	Vehicle	ADO	A23187	ADO + A23187
PGE2	365+66	239+45†	13039+2687*	15584+3847*†
TxB2	8.3%	0%†	40%	80%*†

*p<.05 vs. vehicle †NS vs. preceeding control

ADO alone had no effect on either PGE2 or TxB2 production. As expected, A23187 stimulated prostanoid production. However, ADO did not significantly affect ionophore-stimulated prostanoid production. We conclude that the effect of ADO on glomerular function is not mediated by MC prostanoid production. (USPHS SO7 RR05896 and 5R01 HL35869)

139.21

DEVELOPMENT OF A MEMBRANE RECEPTOR ASSAY FOR LEUKOTRIENE B4. Diana M. Patelunas[1], Alan P. Agins[2] Robert Zipkin[3] and J. Bryan Smith[1*]. [1]Temple Univ., Phila., Pa. 19140; [2]Brown Univ., Providence, R. I. 02912; [3]Biomol Research Laboratories, Plymouth Meeting, Pa. 19462.

5(S),12(R)-dihydroxy-6-cis-8,10 trans-14-cis-eicosatetraenoic acid (LTB_4) is a potent inflammatory mediator generated by human cells. A new membrane receptor assay has been developed to quantitate LTB_4 production. Membranes from HL-60 cells are incubated with [^{3}H] LTB_4 in the presence of polyethylene glycol 8000 and gamma globulin. Unlabeled LTB_4 standards or biological samples with unknown concentrations of LTB_4 compete with [^{3}H] LTB_4 for binding. The mixture is filtered through a Millipore HAWP filter, the filter is washed with Hanks buffer, and the radioactivity retained by the filter is determined. The range of sensitivity for this assay is from 10 pg to 1 ng of LTB_4. The ability of other lipoxygenase products to compete with [^{3}H] LTB_4 for the binding sites on the membranes from HL-60 cells was determined. The cross reactivity of 20-hydroxy LTB_4 is 10 %. 12(R) HETE, LTD_4, 14,15-LTD_4, and 20 amino-propyl LTB_4 have a cross reactivity of 1 % or less. 20-carboxy LTB_4, arachidonic acid, 5(S) HETE, 12(S) HETE, 5(S), 12(S)-Di-HETE, 5(S),12(R)-Di-HETE, 8(S),15(S)-Di-HETE, 5(S), 6(R)-Di-HETE, LTC_4, 14,15-LTC_4 and LTE_4 have a cross reactivity of less than 0.1 %. Thus, HL-60 cells possess stereospecific receptors which have a binding specificity analogous to that of human polymorphonuclear leucocytes (PMNs) receptors. The use of membranes from HL-60 cells for the measurement of LTB_4 provides a novel alternative to radioimmunoassay for the determination of the levels of this important eicosanoid in biological fluids.

139.22

ASSESSMENT OF RECEPTOR RESERVE IN BASAL AND DIFFERENTIATED U-937 CELLS AFTER LEUKOTRIENE D_4-INDUCED DESENSITIZATION. James D. Winkler, Henry M. Sarau*, James J. Foley* and Stanley T. Crooke. Molecular Pharmacology, Smith Kline & French Laboratories, King of Prussia, Pa. 19406

In U-937 cells, LTD_4 and LTE_4 stimulate calcium mobilization (EC_{50}s: 5 and 500 nM, respectively) and LTE_4 was shown to be a partial agonist with a linear relationship between effect and receptor occupation. LTD_4-pretreatment produced a concentration-related desensitization of LTE_4-induced Ca^{2+} mobilization in both basal and DMSO-differentiated U-937 cells. However, the maximal desensitization was greater in basal cells (66% ± 4%) than in differentiated cells (33% ± 7%). LTD_4-pretreatment resulted in a greater percentage reduction of Ca^{2+} mobilization produced by LTE_4 than that produced by LTD_4, suggesting that a receptor reserve exists for LTD_4. The extent of receptor reserve was assessed in basal and differentiated U-937 cells by comparing LTD_4 concentration-response curves from control and desensitized cells. Both basal and differentiated U-937 cells were shown to have substantial receptor reserve for LTD_4 as assessed from the K_a/EC_{50} ratios, which were 9.5 ± 3.8 and 7.2 ± 1.2, respectively, and the magnitude of receptor reserve was similar for basal and differentiated cells. The difference in sensitivity to desensitization observed in basal and differentiated U-937 cells may result from an alteration in the signal transduction mechanism.

139.23

THE EPITHELIUM-DERIVED RELAXING FACTOR (EpDRF) MODULATES THE RESPONSES OF GUINEA PIG ISOLATED BRONCHUS AND TRACHEA TO LEUKOTRIENES. S. Prié*, A. Cadieux* and P. Sirois* (SPON: J. Barabé). Dept Pharmacol., Fac. Med., University of Sherbrooke, Sherbrooke, P.Q., Canada.

The influence of respiratory epithelium on the responses of guinea pig airways to leukotrienes and histamine was investigated. The epithelial cell layer was removed from rings of trachea and upper bronchi by gently rubbing their mucosal surfaces. The responses of the tissues were studied in a cascade superfusion system. The contractions of epithelium-denuded rings to leukotrienes and histamine were markedly enhanced as compared to those with intact epithelium preparations. The responses to LTA_4, LTC_4, LTD_4, LTE_4 and histamine were increased by 2.7, 3.2, 1.9, 4.5 and 1.3 folds respectively on the trachea and by 2.3, 1.9, 2.3, 1.9 and 1.9 folds respectively on the bronchi. Histological sections of the airways were made to confirm the absence or the presence of the epithelium. The effect of indomethacin on the contractile responses induced by these agonists in absence of epithelium was evaluated. Our results showed that the myotropic activities to LTD_4 and histamine were enhanced. Furthermore, the use of tranylcypromine (an inhibitor of PGI_2 synthethase) in presence of the epithelial cell layer enhanced the contractions induced by LTD_4 without affecting those to histamine. These results suggest that arachidonic acid metabolites are, at least in part, responsible for EpDRF activity. (Supported by the M.R.C.)

ENDOTHELIUM-DEPENDENT RESPONSES II

140.1

PORCINE ENDOTHELIAL CELLS IN CULTURE RELEASE DIFFERENT RELAXING FACTORS. C. Boulanger*, Ph.D. and P.M. Vanhoutte, M.D., Dept. Physiology, Mayo Clinic, Rochester, MN 55905

Experiments were performed to determine the influence of ouabain on the release and the effect of relaxing factor(s) from cultured endothelial cells. A column of porcine aortic endothelial cells grown on microcarrier beads (in suspension culture) was perfused with modified Krebs-Ringer bicarbonate solution. The release of relaxing factor(s) by the endothelial cells was bioassayed using a ring of canine coronary artery without endothelium (contracted with prostaglandin $F_{2\alpha}$) under basal conditions and during stimulation with bradykinin (10^{-9} to 3×10^{-8}M), the calcium ionophore A23187 (10^{-8} to 10^{-7}M) or adenosine diphosphate (ADP; 3×10^{-6} to 3×10^{-4} M). Incubation of the endothelial cells with ouabain (5×10^{-6}M) did not affect the relaxation of the bioassay ring under basal conditions or upon stimulation with ADP, but reduced those evoked by bradykinin and A23187 and the relaxations induced by nitric oxide. In contrast, incubation of the bioassay ring with ouabain reduced the relaxation under basal conditions, and upon stimulation with ADP but did not affect the responses to bradykinin and A23187. These experiments suggest that porcine aortic endothelial cells in culture release two endothelium relaxing factors. One factor is released under basal conditions and upon stimulation with ADP, and the other during stimulation with bradykinin or the calcium ionophore A23187.

140.2

IDENTIFICATION AND CHARACTERIZATION OF ENDOTHELIAL THROMBOXANE RECEPTORS. Cheng-Po Sung*, Anthony J. Arleth* and Barry A. Berkowitz. Department of Pharmacology, Smith Kline and French Labs., King of Prussia, PA 19406-0939.

Thromboxane A_2 (TxA_2) is a potent inducer of platelet aggregation and constrictor of vascular smooth muscles. Since it is known that the contractile and relaxant activities of vascular smooth muscle are modulated by endothelial cells, we have investigated the possible existence of TxA_2 receptors in endothelial cells. In this study we have defined a new and useful binding assay with the radiolabelled antagonist of thromboxane, IPTA-OH. The binding of [^{125}I]PTA-OH to membrane preparations of BPAE cells was specific and saturable. The dissociation constant (K_d) at equilibrium and the maximal number of binding (B_{max}) sites were K_d = 2.0 ± 0.3 nM, and B_{max} = 28.9 + 5.2 fmol/mg, respectively. The specific binding was 60-70% of the total binding. Thromboxane B_2, prostaglandin D_2 and $F_{2\alpha}$ did not displace the ligand (0.1 nM) at concentrations up to 10 μM. However, binding was displaced by IPTA-OH (IC_{50} = 20 nM) > SQ29548 (IC_{50} = 100 nM) > U46619 (IC_{50}= 1 μM). The identification of specific TxA_2 receptors on vascular endothelial cells and the demonstration of a sensitive and selective binding assay for endothelial cells will be important for future definition of the function(s) of these high affinity binding sites as well as the design of drugs to modulate them with improved efficacy and selectivity.

140.3

ANATOMICAL AND SPECIES DIFFERENCES IN ENDOTHELIAL-DEPENDENT RELAXATION RESPONSES TO ACETYLCHOLINE W.W. Montgomery*, R.M. Eglen*, A.M. Strosberg and R.L. Whiting*
Inst. of Pharmacology, Syntex Research, Palo Alto, CA 94304

Acetylcholine (ACh) produces an endothelial-dependent relaxation in isolated vascular tissue. The extent of vasorelaxant responses to ACh is less pronounced, however, in isolated venous tissue. The following data describes the effects of ACh in contracted arterial rings of differing anatomical origin isolated from a number of mammalian species.

While thoracic aortic rings isolated from rats relaxed in response to ACh (IC_{50}=1.0μM), relaxant effects were not observed in thoracic aortic rings isolated from from guinea pigs (GP), cynomologous monkeys (CM) or rhesus monkeys (RM). Relaxant effects in response to ACh were observed in abdomenal aortic rings isolated from GP (IC_{50}=1.0μM), while relaxant effects were not observed in abdomenal tissue isolated from CM or RM. Relaxant effects in response to ACh were observed in femoral rings (12-15 mm distal to the abdomenal aorta) isolated from CM or RM (IC_{50}=1.0μM). Relaxant responses to ACh were observed in aortic and femoral rings isolated from rabbits. However, the responses in the aortic rings were not sustained.

ACh-induced relaxation varies in arteries isolated from different species. These variations are less evident at the more distal systemic levels of the vasculature. These data suggest that smaller resistance vessels are more relevant to the endothelial-dependent resistance lowering effects of ACh.

140.4

HETEROGENEITY OF ENDOTHELIUM-DEPENDENT RESPONSES TO ACETYLCHOLINE IN CANINE CORONARY AND FEMORAL ARTERIES Maria Vidal* and Paul M. Vanhoutte. Department of Physiology and Biophysics, Mayo Clinic, Rochester, MN 55905

The present experiments were designed to elucidate the mechanism underlying heterogeneity of actylcholine-induced relaxations in canine arteries. Isolated segments of carotid arteries were perfused with modified Krebs-Ringer bicarbonate solution. Rings (bioassay tissues) of left coronary circumflex or of femoral artery (without endothelium) were superfused with the solution flowing through the carotid artery. The absence of endothelium in the bioassay tissues was demonstrated by the lack of relaxation to acetylcholine. All experiments were carried out in the presence of indomethacin to inhibit the synthesis of prostanoid; the bioassay rings were contracted with prostaglandin $F2_{\alpha}$. Infusion of acetylcholine into the perfused segment evoked relaxation of bioassay rings from the coronary but not of the femoral artery. In contrast, endothelial administration of the calcium ionophore A23187 induced comparable relaxations of both bioassay tissues. The endothelium-independent vasodilators sodium nitroprusside and nitric oxide caused similar relaxations of the coronary and femoral arteries. These results suggest that acetylcholine, but not A23187, causes the release of an endothelium-derived relaxing factor from the endothelium of the canine carotid artery which differs from nitric oxide.

140.5

DIFFERENTIAL EFFECT OF CYTOCHROME P450 INHIBITORS ON ARACHIDONIC ACID (AA) AND ACETYLCHOLINE (ACh) INDUCED ENDOTHELIUM-DEPENDENT RELAXATION IN ISOLATED PERFUSED RAT MESENTERIC ARTERIES (MAS). A.S.O. Adeagbo and K.U. Malik, Department of Pharmacology, University of Tennessee, Memphis TN 38163.

AA, like ACh and other vasoactive agents is known to induce vascular smooth muscle relaxation that is dependent on an intact endothelium. The present study was undertaken to determine the contribution of cytochrome P450 products to AA actions by examining the effect of three structurally distinct cytochrome P450 inhibitors on the relaxation produced by AA and ACh in MAs preconstricted by norepinephrine. AA (1 - 100 nmol) and ACh produced dose-dependent relaxation of MAs which was not blocked by indomethacin (2.8 μM), but which was inhibited in arteries deprived of their endothelium by perfusion with distilled water for 10 min. Cytochrome P450 inhibitors, α-naphthoflavone (10 or 100 μM), ketoconazole (1 or 5 μM), and metyrapone (50 or 100 μM) inhibited ACh but not AA-induced relaxation of MAs. Sodium nitroprusside (SNP, 0.01 - 1 nmol) caused dose-dependent, but endothelium independent, relaxation of the MAs. Metyrapone (100 μM), but not other inhibitors, reduced SNP-induced relaxation of MAs. These data suggest that the endothelium-derived relaxing factor (EDRF) released by AA is different from that released by ACh, and/or the EDRF(s) released by these agents in the MAs act by different mechanisms. (A.S.O.A. is the recipient of Fogarty International Fellowship # 1 FO5 TW03931 - 01. This work was supported by USPHS NIH Grant 19134).

140.6

IMPAIRMENT OF RECEPTOR-MEDIATED, ENDOTHELIUM-DEPENDENT RELAXATION IN DIABETES AND ENHANCED SUSCEPTIBILITY TO OXYGEN-DERIVED FREE RADICALS. Garrett J. Gross and Galen M. Pieper. Medical College of Wisconsin, Milwaukee, WI 53226.

Endothelium-dependent relaxations (EDR) to muscarinic receptor, purinergic receptor and receptor-independent vasodilators were studied in thoracic aorta from chronic diabetic rats using acetylcholine, ADP and the calcium ionophore, A23187, respectively. Despite full relaxation in nondiabetic vessels (CV), relaxation to acetylcholine and ADP was only 58±8% and 56±14%, respectively, of precontracted tension in diabetic vessels (DV). EDR to A23187 in DV was unimpaired as well as endothelium-independent relaxation to nitroglycerin or papaverine. Acute exposure to an in vitro free radical (FR)-generating system of xanthine plus xanthine oxidase produced a marked (28%) decrease in contraction of DV (but not CV) which was prevented by catalase but not by nordihydroguaiaretic acid or indomethacin. H_2O_2 given alone mimicked this response in DV. Prior exposure to FR's reduced the subsequent EDR to acetylcholine by 50% in CV while abolishing EDR in DV. Abolition of EDR in DV was partially prevented by catalase. EDR to A23187 was marginally reduced by FR-exposure. Thus, impairment of EDR in diabetes appears to be receptor-dependent. Furthermore, DV are at risk to FR-mediated injury.

140.7

BLOCKADE OF ENDOTHELIUM-DEPENDENT REDISTRIBUTION OF MYOCARDIAL BLOOD FLOW BY GOSSYPOL. Lorie R. Pelc*, Garrett J Gross and David C. Warltier. Medical College of Wisconsin, Milwaukee, WI 53226.

We have shown that intracoronary (ic) infusion of acetylcholine (ACh) produces an endothelium(E)-dependent increase in the subendocardial to subepicardial blood flow ratio (Endo/Epi). In contrast, infusion of nitroprusside (NP), produces an E-independent, uniform transmural increase in regional myocardial blood flow (MBF). We studied the effects of gossypol (GP) on the distribution of MBF (via radioactive microspheres) following ic infusion of ACh or NP in anesthetized dogs. ACh (10 μg/min; n=7) or NP (40 μg/min; n=7) were administered before and after GP (1 mg/min x 30min ic). Both ACh and NP produced comparable increases in MBF before and after GP. However GP blocked the increase in Endo/Epi produced by ACh without altering the distribution of MBF produced by NP.

MBF	Control	Drug	GP	Drug+GP
ACh	0.97±0.11	2.01±0.13*	1.19±0.18	2.17±0.12*
NP	0.85±0.13	1.86±0.23*	1.02±0.07	1.75±0.28*
Endo/Epi				
ACh	1.24±0.03	1.91±0.22*	1.51±0.20	1.53±0.14
NP	1.26±0.13	1.38±0.12	1.13±0.08	1.42±0.08

These results suggest that GP inhibits ACh-mediated E-dependent redistribution of MBF to left ventricular subendocardium. Supported by NIH #HL32911.

140.8

EFFECT OF ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF) ON THE RESPONSES TO NEUTROPHIL-DERIVED RELAXING FACTOR (NDRF). D.K.H. Lee*, R.J. Sturm*, D.E. Henry*, D. Grimes and T.J. Rimele. Wyeth-Ayerst Research, Princeton, NJ 08543-8000, U.S.A.

An NDRF with a pharmacologic profile similar to EDRF has been previously reported (JPET 245: 102-111, 1988). For further characterization, rings of rat aorta, precontracted with phenylephrine ($1-3x10^{-7}$M), were incubated in organ chambers with NDRF and other smooth muscle relaxants at concentrations producing >80% relaxation. Inhibitors of EDRF-induced relaxation, methylene blue ($1x10^{-5}$M), LY-83,583 ($1x10^{-5}$M), phenidone ($3x10^{-5}$M), hydroquinone ($3x10^{-5}$M), pyrogallol ($3x10^{-5}$M), and phorbol 12-myristate-13-acetate ($1x10^{-6}$M), caused significant reversal of the relaxations induced by neutrophils, acetylcholine, A-23187, superoxide dismutase, nitroprusside, molsidomine, nicorandil and isosorbide, but had little effect on the relaxations induced by forskolin, ANF, db-cAMP, 8-Br-cGMP, prazosin, diltiazem, W-7, BRL-34915, H_2O_2, and TMB-8. In general, there was a positive correlation between the ability of these inhibitors to reverse the relaxations and the ability of a particular relaxing agent to increase intracellular cGMP levels without affecting cAMP levels. The present results provide further support for our hypothesis that NDRF displays a pharmacologic profile similar to that of EDRF.

RECEPTORS FOR PEPTIDES AND OTHER HORMONES

141.1

MOLECULAR PROPERTIES OF OPIOID RECEPTORS IN HUMAN NEUROBLASTOMA CELLS. Syed M.I. Kazmi* and Ram K. Mishra. Depts. of Psychiatry and Neurosciences, HSC-4N52, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Human neuroblastoma, SH-SY5Y cells expressed μ and δ opioid receptor subtypes. The opioid receptors were coupled to adenylate cyclase system through guanine nucleotide regulatory protein, N_i. * Pertussis toxin catalyzed ADP-ribosylation of G-protein ($N_{i\alpha}/N_{o\alpha}$) resulted in significant reduction in the high affinity agonist binding to both μ and δ receptors. The agonist mediated decrease in cyclic AMP levels and increase in low Km GTPase activity were almost completely abolished with pertussis toxin treatment. The sulfhydryl alkylating agent, N-ethylmaleimide caused similar reduction in agonist binding and opioid receptor G-protein coupling processes. Both μ and δ opioid receptors were solubilized using 3-[3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS, 6 mM) and NaCl (250 mM). The solubilized receptors displayed similar pharmacological characteristics with respect to agonist/antagonist binding parameters. However, the selectivity of various opioid ligands appeared to be somewhat reduced in the solubilized preparations as compared to membrane-bound receptors. Reconstitution studies may reveal the role of different phospholipid components in conferring the ligand selectivity and subsequently, separate entities of multiple opioid receptors. (Supported by NCI, Canada.)

*Kazmi and Mishra (1987) Mol. Pharmacol. 32, 109-118.

141.2

ACTIVATION OF ADENOSINE RECEPTOR ANTAGONIZED THE ISOPROTERENOL-STIMULATED INCREASE IN CONTRACTILITY IN CULTURED ATRIAL MYOCYTE Bruce T. Liang. Brigham and Women's Hospital and Harvard Med. Sch., Boston, MA 02115

Activation of adenosine receptors causes negative inotropic and chronotropic effects in the heart. However, the presence of adenosine receptors and the coupling of adenosine receptors to an antagonism of the stimulatory effect of isoproterenol in cultured heart cells are not known. We used atrial cells cultured from I4 day chick embryo as the model system. Isoproterenol caused a I8.7±2 % increase in the basal amplitude of contraction(n=II, ±SE, P<0.0I)and had no effect on the spontaneous rate(Isoproterenol= 3uM). Superfustion of the myocyte with R-PIA (I0 uM) in the presence of isoproterenol (3 uM) resulted in a 37.4 ± 4% decrease in the amplitude of contraction (n=II, ±SE, P<0.0I) and a I7.2±I.3% decrease in the beating rate (n=II, ±SE, P<0.0I). The atrial myocyte recovered spontaneous contractile state upon removing isoproterenol and the adenosine receptor agonist N^6-R-phenyl-2-propyladenosine (R-PIA). The onset of action of isoproterenol or of R-PIA was 60 seconds, achieving a steady state in less than 2 I/2 min. Conclusion: Adenosine receptors are present and are coupled to inhibition of isoproterenol-stimulated increase in contractility in intact cultured embryonic chick atrial myocytes; these myocytes may offer a good model system in which to study the mechanism(s) underlying the antagonism between the adenosine receptor and the β-adrenergic receptor.

141.3

CHARACTERIZATION OF [^{3}H]CGS 15943A BINDING TO ADENOSINE A1 RECEPTORS IN RAT CORTEX. Michael F. Jarvis*, Michael Williams, George Stone* and Matthew Sills* Research Depart. Pharmaceuticals Division, CIBA-GEIGY Corp. Summit, NJ 07901

The triazoloquinazoline, CGS 15943A, is the first reported non-xanthine adenosine antagonist that is selective, and has high affinity for, central adensoine receptors (Williams et al. 1987; JPET 241: 415). In the present studies, [^{3}H]CGS 15943A specifically bound to recognition sites in rat cortical membranes in a saturable and reversible fashion. [3H]CGS 15943A bound with high affinity (Kd = 4 nM) and limited capacity (Bmax = 1.5 pmol/mg protein). Competition studies revealed that [^{3}H]CGS 15943A binding was consistent with the labeling of brain A1 receptors. Adenosine agonists inhibited binding with the following order CPA (IC_{50} = 4 nM) > 2-CADO > R-PIA > NECA > S-PIA (IC_{50} = 160 nM). The potency order for adenosine antagonists was CGS 15943A > 8-PT > DPX > theophylline = caffeine. All agonist inhibition curves were shallow and best described by a two-site binding model. In contrast, antagonist inhibition curves were best described by a one-site binding model. The complex binding interactions found with adenosine agonists may indicate that [^{3}H]CGS 15943A labels both a high affinity A1 receptor and an additional low affinity binding component in rat cortex. [^{3}H]CGS 15943A represents the first radiolabeled non-xanthine adenosine antagonist which potently and selectively interacts with central purinergic receptors.

141.4

POTENT AND SELECTIVE ANTAGONISM OF ADENOSINE AGONISTS IN VIVO BY 3,7-DIMETHYL-1-PROPARGYLXANTHINE. Thomas W. Seale*, Kathleen A. Abla*, Mah T. Shamim*, John M. Carney*, and John W. Daly* (SPON: D Christensen) Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190 and National Institutes of Health, Bethesda, MD 20892

3,7-Dimethyl-1-propargylxanthine (DMPX), a caffeine analog that exhibits in vitro selectivity for A_2-adenosine receptors, compared to A_1-adenosine receptors, has now been investigated with respect to in vivo potency and selectivity. DMPX potently and selectively blocked the actions of the potent A_2 adenosine agonist 5'-N-ethylcarboxamido-adenosine (NECA) in DBA/2 mice, compared to blockade of the same responses elicited by the selective A_1-adenosine agonist N6-cyclo-hexyladenosine (CHA). DMPX was 57-fold more potent versus NECA-induced hypothermia than versus CHA-induced hypothermia and 11-fold more potent versus NECA-induced behavioral depression than versus CHA-induced behavioral depression. The hypothermia is mediated by peripheral receptors, based on blockade by 8-p-sulfophenyltheophylline (PSPT), while the behavioral depression is centrally mediated, based on lack of blockade by PSPT. DMPX was 28- and 15-fold more potent than caffeine in blocking peripheral and central NECA-responses respectively. DMPX was equipotent with caffeine versus CHA-induced hypothermia and 2.5-fold more potent than caffeine versus CHA-induced behavioral depression. The motor stimulating potency of DMPX (ED_{50} 10 μmol/kg) was slightly greater than caffeine.

141.5

ADENOSINE A1 RECEPTOR AGONISTS INHIBIT DOPAMINE RELEASE IN THE RAT STRIATUM. H. S. Kim*, W. C. Boyar*, A. Hutchison* and P. L. Wood* (SPON: G. B. Weiss). Research Dept., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901

The striatum is rich in both A1 and A2 adenosine receptors. If adenosine agonists have different effects on rat striatal dopamine (DA) release, the specific receptor subtype involvement could be demonstrated. The DA extracellular metabolite, 3-methoxytyramine (3-MT), was measured as an index of DA release. Oral injections (3 and 10 mg/kg) of cyclohexyladenosine (CHA), R-phenylisopropyladenosine (R-PIA) and 5'-N-ethylcarboxamidoadenosine (NECA) caused dose-dependent decreases in the steady-state levels of 3-MT. These agents equipotently (ED_{25}, approx. 1 mg/kg p.o.) inhibited pargyline-dependent 3-MT accumulation. Since CHA and R-PIA are relatively specific A1 receptor agonists and NECA is almost equipotent at A1 and A2 sites, our data favor an A1 receptor action. Also, the A1 receptor selective antagonist, 8-cyclopentyl-1,3-dipropylxanthine (CPDX), blocked the actions of CHA. The cerebellar cGMP level (a known A1 receptor-linked action) was also inhibited by the three A1 agonists and CPDX blocked the effects of CHA. Thus, adenosine inhibition of rat striatal DA release appears to be specifically mediated by A1 receptors.

141.6

PARTIAL PURIFICATION OF VASOPRESSIN (V_2) RECEPTORS FROM HOG KIDNEY MEMBRANES. Michael Minnich*, Nambi Aiyar*, Wendy Valinski*, Ponnal Nambi* and Stanley T. Crooke. Dept. of Mol. Pharm., SK&F Labs, Philadelphia, PA 19101

Vasopressin receptors (V_2) were solubilized from hog kidney membranes with egg lysolecithin. Binding of [^{3}H] vasopressin to the solubilized fractions was rapid, specific and saturable. The agonist dissociation constants observed in the membranes and the solubilized fractions were 1.7 ± 0.3 vs 2.3 ± 0.2 nM respectively. Cross-linking of ^{125}I-AVP to the solubilized fractions followed by SDS-PAGE analysis demonstrated the presence of a specific band at approximate mol. wt. of 60-62 KDa band which was specifically inhibited by cold AVP. The solubilized vasopressin receptors were partially purified using TSK 4000 size exclusion HPLC, fractogel DEAE 650 ion exchange, Mono-Q HR 5/5 and GTP-sepharose affinity chromatography. The fractions were analysed for vasopressin binding activity. The active fractions from each step were cross-linked through ^{125}I-AVP to identify the molecular nature. These partially purified vasopressin receptors have an apparent molecular weight of 60 KDa which compared well with the [^{125}I] AVP-cross linked receptors. Soluble AVP-binding activity could be precipitated with wheat germ lectin sepharose suggesting that the receptor may be a glycoprotein. Isoeclectric focusing of the solubilized [^{125}I] AVP crosslinked receptor demonstrated a PI of 5.8 - 6.0.

141.7

VASOPRESSIN STIMULATION OF ADENYLATE CYCLASE IN SOLUBILIZED HOG KIDNEY MEMBRANE. Wendy Valinski*, Ponnal Nambi*, Nambi Aiyar*, Michael Minnich* and Stanley T. Crooke. Dept. of Mol. Pharmacology, SK&F Labs, Philadelphia, PA 19101

Vasopressin (V_2) receptors were solubilized from hog kidney membranes using egg lysolecithin. Solubilized receptors were then mixed with dimyristoylphosphatidylcholine (DMPC) to reduce detergent concentration. Vasopressin binding in these fractions was specific, rapid and saturable. These fractions have significant adenylate cyclase activity also. Forskolin as well as guanine nucleotides such as GTP, GTPγS and Gpp (NH)p significantly (2-3 fold) increased adenylate cyclase activity suggesting the presence of guanine nucleotide sensitive adenylate cyclase in this fraction. Even in the absence of guanine nucleotides vasopressin alone stimulated adenylate cyclase activity in these fractions (80% over basal). Addition of guanine nucleotides or forskolin potentiated vasopressin stimulation of adenylate cyclase activity. This study provides an initial step towards the understanding of the molecular interactions of vasopressin receptors and kidney adenylate cyclase.

141.8

NEUROKININ RECEPTORS: PHARMACOLOGICAL AND BIOCHEMICAL CHARACTERIZATION. Stéphane Dion, Noureddine Rouissi, Guy Drapeau and Domenico Regoli. Department of Pharmacology, Medical School, University of Sherbrooke, Sherbrooke J1H 5N4.

A major breakthrough in neurokinin pharmacology has recently been accomplished with the identification of isolated organs containing a single receptor type for neurokinins. These preparations, the dog carotid artery (D.C.A.) which contains receptors for substance P (NK-1), the rabbit pulmonary artery (R.P.A.) which has receptors for neurokinin A (NK-2) and the rat portal vein (R.P.V.) which has receptors for neurokinin B (NK-3) were compared with other isolated organs containing more than one receptor type (for instance, the guinea pig ileum). Receptors were caracterized by measuring the order of potency of agonists, using four group of compounds: the natural neurokinins (SP, NKA, NKB), some tachykinins (physalaemin (PHY), eledoisin (ELE) and kassinin (KAS)) as well as some neurokinin analogues showing selectivity for one or the other receptor type. The same compounds were used to compete with appropriate ligands (^{125}IBHSP, ^{3}HNKA and ^{125}IBHELE) in binding assays. The results of biological assays demonstrated that D.C.A., R.P.A. and R.P.V. are monoreceptor systems. Moreover, a good correlation was demonstrated between the apparent affinities (pD_2 values) of agonists on the pharmacological assay organs and the inhibitory constant (K_i) measured with the binding assays.

141.9

CHOLECYSTOKININ (CCK) RECEPTOR SOLUBILIZATION FROM BOVINE CEREBRAL CORTEX. Michel A. Morency*, Jason S. Kajiura* and Ram K. Mishra. Depts. of Psychiatry and Neurosciences, HSC-4N52, McMaster Univ., Hamilton, Ont., Canada, L8N 3Z5.

High-affinity and saturable binding sites for CCK have been characterized in several regions of the mammalian brain. In the present communication, we report the first solubilization of brain CCK receptors. Receptor identification was accomplished by [^{3}H]pCCK$_8$ binding assay using polyethylene glycol precipitation followed by rapid filtration. The ability of various detergents to solubilize CCK receptors from bovine cerebral cortex was examined: CHAPS, Digitonin, n-Octylglucoside, Sodium cholate, Triton X-100, and Tween-80. Of these, sodium cholate gave the best recovery (> 50%). [^{3}H]pCCK$_8$ bound with high affinity and saturability to the solubilized cortical CCK receptors (K_D = 1.88 nM; B_{max} = 55.13 fmol/mg protein). No significant loss of [^{3}H]pCCK$_8$ binding was observed upon filtration of the soluble preparation through 0.22 μM filters or ultracentrifugation at 130,000 xg for 3 hr. In addition, the solubilized cortical CCK receptor exhibited a similar profile of relative potencies of various CCK-related peptides (CCK$_8$ ≥ caerulein > desulfated CCK$_8$ > CCK$_4$). The soluble preparation was quite stable at 4°C for 48 hrs.
This work was supported by the Natural Sciences and Engineering Research Council of Canada and the Medical Research Council of Canada.

141.10

PHARMACOLOGICAL COMPARISON OF THE RABBIT PLATELET AND NEUTROPHIL RECEPTORS FOR PLATELET-ACTIVATING FACTOR. Susan K. Paulson* and Charles P. Cox* (SPON: P.H. Stern). G.D. Searle & Co., Skokie, IL 60077

The order of potencies of several platelet-activating factor (PAF) receptor antagonists to inhibit [^{3}H]-PAF specific binding to rabbit platelet and neutrophil membranes were determined. The presence of PAF specific binding sites on both membrane preparations was confirmed using radiolabeled ligand binding assays. Scatchard analysis of PAF-specific binding to the neutrophil membranes revealed a (Kd) for PAF of 0.35 ± 0.12 nM and 1.8 ± 0.48 x 10^{11} PAF binding sites/mg protein. Scatchard analysis of PAF-specific binding to the platelet membranes revealed a single binding site with a Kd for PAF of 0.75 ± 0.22 nM and 3.2 ± 2.76 x 10^{11} PAF binding sites/mg protein. The order of potency of PAF receptor antagonists to inhibit the binding of [^{3}H]-PAF to neutrophil membranes was: PAF > Brotizolam > WEB-2086 = Kadsurenone = L-652,731 = CV-3988 > Triazolam > Alprazolam. The order of potency of PAF receptor antagonist to inhibit the binding of [^{3}H]-PAF to platelet membranes was: PAF > Brotizolam = WEB-2086 > Kadsurenone = L652,731 = CV-3988 > Triazolam > Alprazolam. In conclusion, the potencies of these PAF receptor antagonists were similar in inhibiting [^{3}H]-PAF binding to platelet and neutrophil membranes.

141.11

INTERLEUKIN-2 STIMULATION OF T-LYMPHOCYTES RESULTS IN AN INCREASE OF TYROSINE PROTEIN KINASE ACTIVITY. E. M. Saltzman*, R. E. Thom* and J. E. Casnellie* (SPON: P. M. Hinkle). Univ. of Rochester, Rochester, NY 14642.

Interleukin-2 (IL-2), a lymphokine critical to the proliferation of activated T-lymphocytes, initiates its effects through IL-2 specific membrane receptors. Although the physiological response of IL-2 receptor binding is well documented, relatively little is known about the signal transduction pathways that are activated by IL-2. Identification of these pathways has been hindered by the complex nature of the growth response to this lymphokine and the expanded period of time over which the response occurs. Utilizing the technique of immunoblotting, we have recently shown that several T-lymphocyte proteins between the molecular weights of 38,000 and 120,000 become phosphorylated on tyrosine residues following IL-2 stimulation. The degree of phosphorylation is dependent on the concentration of IL-2. Parallel dose-response curves are obtained for IL-2 induced phosphorylation and for the growth response to IL-2, indicating that the increase in tyrosine phosphorylation correlates with the ability of IL-2 to stimulate the proliferation of T-lymphocytes. These results suggest that IL-2, like other polypeptide growth factors, acts by stimulating the activity of a tyrosine protein kinase. Tyrosine protein kinase activation may possibly be the initial event in signal transduction by the IL-2 receptor.

141.12

ROLE OF TYROSINE PROTEIN KINASES IN T CELL ACTIVATION, R. E. Thom*, E. M. Saltzman*, and J. E. Casnellie* (SPON: P. M. Hinkle) Univ. of Rochester, Rochester, NY 14642.

Activation of human T lymphocytes occurs through interaction of membrane bound receptors with the specific antigen, monoclonal antibodies, or plant lectins resulting in Interleukin-2 (IL-2) production and IL-2 receptor expression. Binding of activating agents stimulate a signal tranduction pathway consisting of increased inositol lipid hydrolysis and elevation of intracellular free calcium, presumably stimulating protein kinase C. Recent studies have suggested increased tyrosine phosphorylation is also important. Using antibodies specific for phosphotyrosine in conjuncture with immunoblots, we investigated levels of tyrosine phosphorylation in the human T cell line, Jurkat, under various activating conditions. We report here that antibodies specific for the antigen receptor (CD3), the sheep erythrocyte receptor (CD2), or the plant lectins PHA and WGA, at concentrations that result in IL-2 secretion, significantly increase tyrosine phosphorylation in Jurkat cells within seconds of addition to the medium. Furthermore, related agents that do not elicit IL-2 secretion fail to stimulate tyrosine kinase activity. Compounds which increase intracellular calcium levels or stimulate protein kinase C directly, either separately or together do not activate these tyrosine kinases. These data give further evidence that tyrosine kinases are involved with the T cell activation signal transduction pathway, probably upstream from calcium or protein kinase C.

141.13

PHORBOL ESTERS MODULATE SMALL NUCLEAR RNA EXPRESSION DURING MYELOID LEUKEMIC CELL DIFFERENTIATION. Damon K. Getman* and Thomas G. Kawakami*. (SPON: E.K.Killam) Department of Pharmacology and Institute for Environmental Health Research, University of California, Davis CA 95616.

Differentiation of myeloid leukemia cells into mature end-stage cells can be induced by a variety of pharmacologically active compounds, including drugs which interfere with RNA metabolism or synthesis. We have used the differentiation of HL60 promyelocytic leukemia cells to monocyte/macrophage cells by a series of phorbol ester derivatives as a model system to study the expression of small nuclear RNA molecules (snRNAs) during cellular differentiation. SnRNAs are abundant nuclear RNA molecules which splice intron sequences from messenger RNA transcripts. HL60 cells were pulsed for one hour with the protein kinase C-activating phorbol ester tetradecanoylphorbol-13-acetate (TPA), and cells harvested after one to 48 h of incubation. SnRNA levels were then measured by Northern blot analysis. In a late passage cell line, TPA decreased the levels of U1 and U6 snRNAs in a dose-dependent fashion after 48 h incubation. Temporal studies showed that this decrease occured within one hour after drug removal. However, in early passage HL60 cells, TPA caused an induction of snRNA levels 2-5 fold above those of non-induced cells. Both cell lines exhibited mature morphology upon TPA induction. Phorbol-13-acetate, which has no differentiation effect on these cell lines, did not alter snRNA levels in parallel experiments. These preliminary data indicate that (1) small nuclear RNA expression can be regulated by factors which activate the phosphatidylinositol/protein kinase C cascade system and, (2) that changes in small nuclear RNAs may not be involved in initiating the onset of differentiation, but are instead involved in events secondary to the process of cell differentiation.

141.14

PROTEIN KINASE C (PKC) AND PERTUSSIS TOXIN (PT) EFFECT ON BRADYKININ-, CARBACHOL-, NOREPINEPHRINE-, ATP- AND IONOMYCIN- MEDIATED INTRACELLULAR CALCIUM RELEASE IN MDCK CELLS. Matthew Whitman*, Hsiao-Ling Wu*, Ponnal Nambi*, Nambi Aiyar* and Stanley Crooke. Dept. of Mol. Pharmacology, SK&F Labs Philadelphia, PA 19101

Madin-Darby Canine Kidney (MDCK) cell line is derived from the distal tubule and collecting duct and express a host of receptors that are coupled to adenylate cyclase or phospholipase C (PLC). Addition of Bradykinin (BK), Carbachol (Carb), Norepinephrine (NE), ATP or Ionomycin (I) to these cells resulted in an increase in the release of intracellular calcium as measured by Fura-2. This increase in calcium release was very rapid and depended on the concentration of compound used. Preincubation of these cells with PKC activators such as phorbol dibutyrate (PDBu) or PT inhibited BK-, Carb- and NE-mediated intracellular calcium release suggesting that a PT-sensitive G protein is involved in the coupling of the above receptors to PLC and that PKC activation may inhibit the coupling of this G protein either with these receptors or PLC. On the other hand, neither PDBu nor PT pretreatment inhibited ATP- or I-induced calcium release suggesting that PT-sensitive G protein is not involved in these responses. These data suggest that there are at least two pathways of activating intracellular calcium release in these cells.

141.15

THE SUBCELLULAR AND NONUNIFORM DISTRIBUTION OF PROTEIN KINASE C IN BOVINE RETINA. C.Z. Ou*, T.E. Donnelly, and M. Ebadi. Dept. of Pharmacol., Univ. Neb. Coll. Med., 42nd St. and Dewey Ave., Omaha, NE 68105

Protein kinase C, an enzyme that is activated by the receptor mediated hydrolysis of inositol phospholipids, relays information form a variety of extracellular signals, including neurotransmitters, neurohormones, and neuropeptides, across the membrane to regulate many Ca^{2+}-dependent processes, including regulating the strength of synaptic transmission. The activity of protein kinase C is regulated by a cooperative interaction between Zn^{2+} and Ca^{2+}. Since the retina contains the highest concentration of zinc in any living tissue, and since zinc metallothionein has been recently identified in bovine retina (Takahashi, Paliwal, and Ebadi, J. Cell. Biochem. 12D(S):353, 1988), we investigated the status of protein kinase C in retinal subcellular fractions. These were disrupted by sonication and 50,000xg pellet and soluble fractions were collected. The soluble and detergent-extracted pellet fractions were then subjected to DEAE-cellulose chromatography and the eluate assayed for protein kinase C activity. This study showed that the subcellular distribution of protein kinase C in bovine retina was nonuniform, and the P_1 fraction known to be highly enriched in photoreceptor cell synaptosomes and zinc exhibited the highest kinase C activity of 801 pmoles ^{32}P incor./mg protein/6 min. Outer rod segment exhibited a value of 56 and the remaining regions were considerably less. (Supported in part by grants from Nebraska Dept. of State and USPHS ES 03949.)

141.16

MODIFICATION OF THE GLUCOCORTICOID RECEPTOR BY MOLYBDATE- PEROXIDE. Soheil Meshinchi*, Emery H. Bresnick*, and William B. Pratt. The University of Michigan, Ann Arbor, MI 48109.

Either sodium molybdate (M) alone or hydrogen peroxide (HP) alone stabilizes the glucocorticoid receptor (GR) and inhibits its transformation to the DNA-binding form. However, when M and HP are added together to cytosol containing GR, steroid binding is destroyed. SDS-PAGE analysis of the GR reveals that M/HP-treated GR migrates with a higher apparent M_r than the control GR, suggesting a covalent modification of the receptor. Digestion of receptor into functional domains with trypsin and chymotrypsin shows that at least one site of modification lies within the steroid binding domain. Migration of the DNA binding domain appears to be unaffected by the treatment. Pretreatment of GR with NEM blocks the increase in GR mass by M/HP, suggesting that SH groups are required for covalent GR modification. M/HP-induced covalent modification may provide a tool for probing the site at which molybdate binds to steroid receptors to stabilize them in their untransformed, non-DNA binding state. (Supported by NIH Grant DK31573)

141.17

PROGESTERONE-INDUCED CHANGES IN PLASMA MEMBRANE FLUIDITY OF AMPHIBIAN OOCYTES. Gene A. Morrill, Kei Doi*, and Adele B. Kostellow, Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York 10461.

Progesterone acts at the surface of the amphibian oocyte to induce meiotic maturation. Hormone binding results in a progressive decrease in the fluidity of the Rana oocyte plasma membrane, which was detected by electron spin resonance in isolated membranes using either 5- or 16-DOXYL (4',4'-dimethyloxazolidine-N-oxyl) stearic acid probes. The 5-DOXYL probe, which inserts into the membrane with the spin label nearest to the surface, showed a 10-12% increase in the order parameter (S) within 4 h, and returned to control levels by 6 h. This parallels a similar time course for a change in membrane chloride conductance. The order parameter for the 16-DOXYL probe, which reflects the fluidity deeper within the plasma membrane, increased 35-40% by 4 h and remained elevated at 6 h. RU 38486: 17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(1-propynl)-estra-4,9-dien-3-one, a synthetic steroid that blocks progesterone receptors, prevents progesterone-induced fluidity changes and does not itself affect the order parameter. This indicates that the binding of progesterone to its receptor changes the oocyte plasma membrane structure resulting in a differential decrease in mobility near the membrane surface compared to that deeper in the membrane. (Supported in part by research grant DK 15056).

141.18

PROGESTERONE RECEPTOR POSITIVITY IN RAT MALIGNANT PHEOCHROMOCYTOMAS. James E. Crook. Medical & Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN 37831

Pheochromocytomas are primarily tumors of adrenal medullary origin that produce a catecholamine excess form of hypertension. Although they account for less than 1% of the hypertension in the U. S., the clinical symptoms may be diverse and the arterial pressure so devastatingly elevated that continued research is warranted. Because of previous work demonstrating that groups of experimental animals with high concentrations of testosterone, produced through i.m. administration of supraphysiological doses of testosterone, were the first to develop an implanted malignant pheochromocytoma, studies were undertaken to determine the receptor status of the tumor. Six male and six female New England Deaconess Hospital rats, 3-4 months old, received subcutaneous implantation of a malignant pheochromocytoma, RNC 259. Three to four weeks post implantation when the animals were markedly hypertensive, i.e., systolic blood pressure >250 mm Hg, the tumors were removed. Using a dextran coated charcoal method, ^3H-estradiol and ^3H-R5020, estrogen and progesterone receptor values (fmol/mg cytosol protein) of <3 (both sexes) and 81 ± 23 (male) and 72 ± 38 (female) respectively were obtained. These results may be useful in aiding determination of tumor status as well as providing insight into therapeutic approaches. (Supported by contract number DE-AC05-76OR00033 between the U.S. DOE and ORAU.)

141.19

INHIBITION OF ADENYLATE CYCLASE BY POLYADENYLATE. M. Bushfield*, I. Shoshani*, D. Stübner*, S.M.H. Yeung* and R.A. Johnson*. (SPON: M.B. Anand-Srivastava). Department of Physiology and Biophysics, SUNY at Stony Brook, New York 11794-8661.

Since a number of potential nucleic acid metabolites including 3'AMP, 2'd3'AMP, and dinucleotides inhibit activated adenylate cyclase through the "P"-site, we have examined whether there may be a link between nucleic acid metabolism and signal transduction via the adenylate cyclase system. We have examined the effects of physiologically relevant concentrations of poly(A) on the activity of a solubilized adenylate cyclase from rat brain. Poly(A) (≈250 nucleotides) inhibited adenylate cyclase activity with an IC_{50} of 45±4μg/ml, n=22 (equivalent to 0.45μM) The inhibition of adenylate cyclase by poly(A) was not mediated by: (a) protein contamination of the poly(A) preparation, (b) metal chelation, (c) formation of an acid-soluble inhibitor of adenylate cyclase, (d) effects on [α-^{32}P]ATP specific activity, or (e) competition with ATP for binding to adenylate cyclase. Inhibition of adenylate cyclase by poly(A) showed a different metal-dependency when compared with 3'AMP. The effect of poly(A) was slow in onset and irreversible whereas inhibition by 3'AMP was rapid and readily reversible. Thus, the effect of poly(A) was not mediated via the "P"-site. This effect was relatively specific for poly(A) as the IC_{50} for inhibtion by total RNA was 460μg/ml and poly(U) was only partially inhibitory (<50% inhibition with 2.3-300μg/ml). Deoxyribonucleic acids were ineffective. Long chains of poly(A) were required for inhibition of adenylate cyclase since $oligo(A)_{12-18}$ (0.1-31μM) had no effect. Preliminary studies with the catalytic subunit (C) of adenylate cyclase, that had been purified by forskolin-affinity chromatography, indicated that poly(A) may interact directly with C. These results suggest that poly(A) may function as an intracellular messenger to provide a link between RNA metabolism and signal transduction via the adenylate cyclase system. This work was supported by NIH grants DK 38828 and DK 33494.

142.1

METHOD FOR THE PURIFICATION AND DETERMINATION OF MOLECULAR WEIGHTS OF THREE TYPE I CYCLIC 3′,5′-NUCLEOTIDE PHOSPHODIESTERASES. Stephen D. Burrows* and Paul A. Volz* (SPON: C.B. Smith) Eastern Michigan Univ., Ypsilanti, MI 48197

Multiple molecular forms of Cyclic 3′,5′-Nucelotide Phosphodiesterase (PDE) have been previously described as having different substrate specificities depending upon their source. As an example, Type I PDE obtained from rat and guinea pig cardiac tissue and from isolated human platelet, varies greatly with respect to substrate specificity (see Table 1).

Table 1 Type I PDE Isolation DEAE-cellulose
(modification of Thompson, J.Biol.Chem., 1979)

Tissue Type	cAMP	cGMP	Calmodulin Stimulation
Rat Cardiac	-	+	+
G.P. Cardiac	+	+	+
Human Platelet	-	+	-

To purify to a more homogenous state, Type I PDEs isolated from DEAE-cellulose were applied to an affinity column of Blue-Dextran Sepharose 4B (BD-S4B). The enzyme was eluted with 4M NaCl and 0.2% Zwittergent 3-14, a zwitterionic detergent in column buffer. After purification immediate decomposition of the enzyme occurred. Although molecular weight analysis of all three enzymes was between 62-64 kD, the active site of the enzyme also seemed to have been affected since Type I PDE substrate specificity was no longer maintained by either rat cardiac or human platelet enzyme. Both enzymes now showed specificity for cAMP. However, G.P. Type I PDE still showed no selectivity between cAMP and cGMP. Apparently a component important to the stability and/or substrate specificity of the enzyme has been removed during the BD-S4B purification.

142.2

REGIONAL AND SUBCELLULAR DISTRIBUTION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE (PDE) ISOZYMES IN RAT BRAIN. M.E. Whalin*, W.J. Thompson, R.L. Garrett, Jr.* and S.J. Strada. Dept. of Pharmacology, Univ. of South Alabama College of Medicine, Mobile, AL 36688.

The regional and subcellular distribution of PDE isozymes [Type I (Ca^{2+}/calmodulin-sensitive), Type II (cGMP-sensitive) and Type IV (high affinity cAMP specific)] was examined in eight rat brain regions. Substantia nigra (SN), neostriatum (NS), frontal cortex (FC), and hippocampus (HP) contained highest specific activity Type I and Type II PDE, while SN and NS contained highest specific activity Type IV PDE. Subcellular fractionation revealed Type I PDE is cytosolic in all brain regions, Type II PDE is predominately membrane-associated and Type IV PDE is distributed equally between compartments. Further fractionation of cortical membranes showed that Types II and IV PDE reside in synaptosomes. Combined studies using immunoprecipation and pharmacological selectivity indicate that the Type II PDE is the predominate form in synaptosomes. The results support the notion that different PDE isozymes exert preferential hydrolytic roles in various brain regions and subcellular compartments. These studies were supported by grants from the USPHS (GM33538) and the USAF (49620-85-K-0014).

142.3

DIFFERENTIAL SENSITIVITY TO CYCLIC NUCLEOTIDE PHOSPHODIESTERASE INHIBITORS IN RAT BRAIN CORTICAL SLICES. R.L. Garrett, Jr.*, W.J. Thompson, M.E. Whalin* and S.J. Strada, Dept. of Pharmacology, Univ. of South Alabama College of Medicine, Mobile, AL 36688.

The effects of selective cyclic nucleotide phosphodiesterase (PDE) inhibitors on the rate of cyclic AMP (cAMP) turnover were examined in male, rat cortical slices. The % conversion of [^{3}H]-labeled adenine nucleotide pools into cAMP was determined after stimulation with maximally effective concentrations of isoproterenol (ISO) or adenosine (AD). The decay constant was measured according to Barber et al. (Mol. Pharm. 32:753, 1987) using the addition of either 200 µM propranolol or 0.55 units of AD deaminase to terminate the agonist response. Rolipram was the best inhibitor of cAMP decline in both receptor systems. However, the drug showed only 60% inhibition following AD stimulation of cAMP levels, while >90% inhibition in the ISO stimulated system. Other PDE-inhibitors tested (eg. HL-725, SQ-65442) also showed greater efficacy against the decline in cAMP levels following ISO than after AD. Some drugs (eg. RS-82856,LY-195115) were poor inhibitors and also did not discriminate between the two systems. This variation in inhibitor sensitivity may indicate the involvement of separate PDE isozymes linked to different agonist receptor systems. These studies were supported by grants from the USPHS (GM 33538) and the USAF (49620-85-K-0014).

142.4

PHARMACOLOGICAL PROFILE OF SOLUBLE AND PARTICULATE CYCLIC NUCLEOTIDE PHOSPHODIESTERASES (PDE) FROM NORMAL AND CARDIOMYOPATHIC HUMAN HEARTS. P. Silver, L. Hamel*, R. Bentley*, P. Allen and E. Pagani; Sterling Winthrop Res. Inst., Rens., NY; Brigham and Women's Hosp., Bost., MA.

In this study we characterized the pharmacological profile of PDE isozymes (Peak I, III) present in ventricular cardiac muscle from normal (N) subjects (n=4) and from patients (n=4) with end-stage cardiomyopathy (M). PDE isozymes were isolated from particulate (PAR) and soluble (SOL) fractions via DEAE-cellulose chromatography; potency (IC_{50}, µM) determined at 0.2 µM substrate for the PDE inhibitors zaprinast (ZAP), CI-930, milrinone (MIL) and rolipram (ROL), and for cGMP (% at 1 µM), are shown in the following table:

Source/Isozyme	ZAP	CI-930	MIL	ROL	cGMP
N Sol I	3	760	145	715	-
N Sol III	175	0.16	0.6	255	75%
N Par III	190	0.16	0.7	225	76%
M Sol I	3	670	120	700	-
M Sol III	154	0.25	0.8	225	68%
M Par III	161	0.16	0.9	175	63%

These data show that 1) the peak III PDE in SOL and PAR fractions in both M and N are sensitive to inhibition by MIL, CI-930 and cGMP, and insensitive to ROL 2) the potency/selectivity for MIL and CI-930 as peak III PDE inhibitors in human cardiac muscle is similar to previous studies with other species 3) there are no apparent major differences in potency of SOL or PAR PDE inhibition by MIL and CI-930 in M vs. N.

142.5

CHARACTERIZATION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASES (PDE) FROM NORMAL AND CARDIOMYOPATHIC HUMAN HEARTS. E. Pagani, R. Bentley*, L. Hamel*, P. Allen and P. Silver; Sterling-Winthrop Res. Inst., Rens., N.Y. and Brigham and Women's Hosp., Boston, MA.

PDE isozymes, in soluble (S) and particulate (P) fractions from normal (N,n=3) and cardiomyopathic (M,n=4) human ventricular tissue were resolved by DEAE chromatography. The S fraction from N and M hearts contain PDE I, II and III isozymes; the P fraction contains only PDE III. Total PDE III activity of the S fraction for cAMP hydrolysis was similar for the N and M hearts; the total activity of the P fraction from M was 2-3 fold less than N. Km and Vmax values ($\bar{x}$±SEM) for the high affinity cAMP and cGMP hydrolytic sites of PDE I and III are summarized in the following table:

Isozyme	Km(µM)		Vmax (pmole/mg/min)	
	cAMP	cGMP	cAMP	cGMP
N S I	0.38±0.06	0.43±0.02	121±14	75±13
N S III	0.23±0.09	---	212±52	---
N P III	0.26±0.05	---	219±74	---
M S I	0.39±0.07	0.42±0.02	71±14	50±17
M S III	0.25±0.04	---	241±35	---
M P III	0.29±0.05	---	124±35	---

These data suggest that 1) hydrolysis of cAMP and cGMP by PDE I is non-selective for N and M 2) Km values for cyclic nucleotide hydrolysis are the same between N and M for each isozyme 3) Vmax for cAMP hydrolysis by PDE I S and PDE III P is lower in M compared with N.

142.6

CALMODULIN DEPENDENT PHOSPHODIESTERASE (CaM-PDE) AND CYCLIC GMP SPECIFIC PDE (cG-PDE) DISTRIBUTION AND RESPONSE TO INHIBITORS IN AORTA AND NON-VASCULAR TISSUES.
H.S. Ahn*, W.N. Crim*, S.J. Moroney*, E.J. Sybertz and B.J.R. Pitts, Department of Pharmacology, Schering Research Division, Bloomfield, NJ 07003.

Immunoprecipitation with a monoclonal antibody to CaM-PDE (provided by Dr. J. Beavo) was used to assess the content of CaM-PDE in different porcine tissue extracts. The contribution of CaM-PDE to cGMP hydrolysis was: aorta, 74%; brain, 60%; heart, 34%; lung, 12%. CaM-PDE contributed minimally to the total cAMP hydrolysis. The cGMP hydrolyzing activity remaining after immunoprecipitation in aortic extracts appeared to be mainly due to cG-PDE, as it was effectively inhibited by dipyridamole, a selective cG-PDE inhibitor. A similar relative content of these enzymes was found in cultured rat aortic smooth muscle cells. The cG-PDE contribution to cGMP hydrolysis as estimated by sensitivity to dipyridamole in tissue extracts was: lung (IC_{50}=3.6 uM)> heart (13)> aorta (66). Negligible cG-PDE acivity was detected in brain. Inhibition of cGMP hydrolysis in lung extracts by M&B 22948 (2-O-propoxyphenyl-8-azapurin-6-one), an inhibitor of both cG-PDE (IC_{50}=0.5 uM) and CaM-PDE (IC_{50}=18 uM) confirmed the presence of both enzymes in this tissue and the greater importance of cG-PDE. This study directly documents the importance of CaM-PDE to cGMP hydrolysis in vascular tissue and suggests differences in relative content of CaM-PDE and cG-PDE in different tissues.

143.1

EFFECT OF PERTUSSIS TOXIN ON THE INTERACTION OF PHENYLEPHRINE & CARBACHOL IN LEFT ATRIUM. A. Ray* and K. M. MacLeod, Univ. of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

In rabbit atrium, the muscarinic agonist carbachol (CCH) has a direct cAMP-independent negative inotropic effect and can also antagonise positive inotropic responses to the cAMP-independent α-adrenoceptor agonist phenylephrine (PHE). The mechanism of this antagonism is unknown but it is possible that the ability of CCH to activate an outward going potassium current is involved. In the present study, the effect of pertussis toxin (PT), known to uncouple muscarinic receptors from potassium channels, was studied on the PHE-CCH interaction in electrically driven rabbit isolated left atrial strips. No change in cAMP levels was observed in the presence of PHE and CCH, either alone or in combination, in the presence or absence of PT (1.75 ug/kg 48 hours before sacrifice). PT treatment attenuated almost completely the inhibitory effect of CCH on PHE-induced tension. However, the direct negative inotropic response to CCH as well as its reversal of the positive inotropic response to IBMX, a phosphodiesterase inhibitor, known to occur without any change in IBMX-induced increases in cAMP levels, were only partially attenuated by PT treatment. Although it is not clear why PT affected in a differential manner the CCH-PHE interaction and the direct negative inotropic response to CCH, the results suggest the involvement of a G protein in this process. (Supported by B.C. Heart Foundation).

143.2

EFFECT OF DIABETIC DURATION ON METHACHOLINE-INDUCED RESPONSES ON THE ISOLATED RAT HEART. Ralph D. Tanz and Xiaojiang Li*, Dept. of Pharmacology, School of Medicine, Oregon Health Sciences University, Portland, OR (97201).

Isolated hearts perfused by the method of Langendorff were obtained from age-matched rats. Responses from controls, streptozotocin-treated (STZ) and STZ + insulin (2 U/day) were studied 6, 12, 24 and 52 weeks later. STZ-induced diabetes was confirmed by blood glucose levels exceeding 300 mg/dl.

Hearts from 6-24, but not 52-week, STZ animals displayed a significant bradycardia after 60 mins equilibration and were supersensitive to the negative chronotropic effect of methacholine (5.1×10^{-7} - 5.1×10^{-6}) compared to control and STZ + insulin hearts. Electrically paced hearts (5 Hz) showed no difference in coronary flow when exposed to methacholine. Nor was the dF/dt different between controls, diabetics or diabetics + insulin when hearts were paced or unpaced, or in the presence or absence of methacholine. In general, hearts from STZ + insulin animals responded similarly to their age-matched controls in the presence and absence of methacholine.

Thus, with advancing age the effect of STZ diabetes on the rate of isolated perfused rat hearts disappears. Since the coronary flow of electrically paced hearts exposed to methacholine was not different when compared to controls, our results suggest that this response to methacholine is secondary to its chronotropic action in diabetic rat hearts.

Supported by the G. & L. Pfeiffer Medical Res. Fdnt.

143.3

EFFECTS OF CARBACHOL (CARB) AND SODIUM NITROPRUSSIDE (SNP) ON CONTRACTION AND CYCLIC GMP IN FROG HEARTS. J.Diamond and D.D. W.Ng*. U. of British Columbia, Vancouver,B.C. V6T 1W5.

Previous reports in the literature (e.g. Flitney and Singh, J.Mol.Cell.Cardiol. 13:363,1981) have concluded that both cAMP and cGMP are involved in regulating contraction in frog hearts, and that they function antagonistically, with cAMP enhancing contraction and cGMP depressing it. Some earlier studies in mammalian hearts had reported dissociations between changes in cGMP and contractility, and it was suggested that these disparate results might be due to species-related differences. This possibility was tested in the present study by comparing the effects of CARB and SNP on cyclic nucleotide levels and contractility in isolated strips of frog ventricle. CARB (100 μM) reduced the contractile force of the preparations by more than 90% but had no effect on tissue levels of cAMP or cGMP. Conversely, 100 μM SNP increased cGMP levels 2.2-fold within 10 min. but had no effect on contractility. When the concentration of SNP was increased to 1 mM, cGMP levels were more markedly increased (6-fold at 5 min. and 9-fold at 10 min.) and contractile force was decreased by 12% at 5 min. and 25% at 10 min. Whether or not the cGMP increase contributes to the inhibition of contractile force seen with the higher concentration of SNP is unclear. In any case, our results suggest that cGMP elevation is not responsible for the direct negative inotropic effect of CARB in frog ventricular muscle. (Supported by the B.C.H.F)

143.4

HEART ACETYLCHOLINE TURNOVER: A MATHEMATICAL MODEL THAT PREDICTS TWO POOLS. J. Holsapple* and O.M. Brown. Neurosurg. & Pharmacol. Depts., SUNY/Health Sci. Ctr., Syracuse, NY 13210.

Much evidence indicates that a single pool precursor-product relationship model does not adequately describe the kinetics of acetylcholine (ACh) synthesis and release. We have developed a mathematical model for the treatment of ACh turnover which is based on following the molecular species of labelled precursor and product. This approach avoids the assumptions and loss of detail inherent in the usual practice of following specific activity. Deuterated and non-deuterated choline (Ch) in blood, and Ch and ACh in heart were monitored by pyrolysis-GC/MS at various times (0.5-60 min) following i.v. injection of d_4-Ch (20 μmol/kg) to rats. The differential equations describing the changes in d_4-Ch (Eq. 1) and d_4-ACh (Eq. 2) in heart, and d_4-Ch in plasma (Eq. 3) are:

Eq. 1 $dCh_h^*/dt = J(Ch_p^*/\{Ch_p^* + Ch_{pss}\}) - (k_1 + k_2)Ch_h^*$

Eq. 2 $dACh^*/dt = k_1 Ch_h^* - k_d ACh^*$

Eq. 3 $dCh_p^*/dt = -k_e Ch_p^*$

Where: Ch_p^* is [d_4-Ch] in plasma, Ch_h^* and ACh* are [d_4-Ch] and [d_4-ACh] in heart, Ch_{pss} is steady-state plasma [Ch] and k_1 is Ch to ACh, k_2 is Ch to non-ACh compounds (e.g., phospholipids), k_d is the rate of disappearance of d_4-ACh from the cell, and J is the current for Ch uptake. The calculated turnover rate for rat heart ACh in vivo is 6.2 nmol/g/min. Further, this model predicts that approximately 35% of total heart ACh is dynamic, while 65% is in a storage pool. Supported by the Amercian Heart Association.

143.5

CARDIODEPRESSANT ACTIONS OF CHLOROQUINE AND QUINACRINE IN THE ISOLATED GUINEA PIG HEART. Theodore M. Brody, Lutete Tona* and Yuk-Chow Ng*, Department of Pharmacology and Toxicology, Michigan State University, E. Lansing, MI 48824

Many anti-malarials have direct negative inotropic and chronotropic effects upon the mammalian heart. The actions of two aminoquinolines, chloroquine (C) and quinacrine (Q) were compared on electrically-driven guinea pig left atria. Both drugs decreased tension in a dose and time dependent manner. ED_{50} values for the negative inotropic effect in the presence of 1.8 mM Ca were 30 μM for Q and 60 μM for C. The effects of both drugs on various calcium pools were examined with the following results: (1) The force staircase phenomenon observed between 0.5-3.0 Hz was reduced by both drugs. (2) Q differed from C by depressing potentiated post-rest contraction. (3) Q (30 μM) also inhibited the positive inotropic effects of Bay K 8644 (1μM) and strophanthidin (0.3 μM), whereas C blocked Bay K 8644 only at high drug concentrations. The negative inotropic effect of C, in contrast to Q, was almost completely reversible. The results suggest that the two anti-malarials behave differently on cardiac calcium pools. C interferes primarily with extracellular calcium whereas Q action is more complex, involving both intra- and extracellular calcium pools as well as a possible effect on Na/Ca exchange. (Supported by USPHS grants HL-10652 and AG-02398). Dr. Tona is a Fulbright Scholar.

143.6

CARDIAC INOTROPIC ACTIONS OF CALCIUM MODULATORS AND ETHANOL IN THE RAT. A. Gallardo-Carpentier, R. P. Salvatici*, R. L. Isaacson and R. G. Carpentier. Howard U. Washington, DC 20059, and SUNY. Binghamton, N.Y. 13901.

The influence of ingestion of ethanol (E) on the acute effects of E and calcium modulators (CMs) on the atrial contractile state (ACS) was studied. Rats were isocalorically pair-fed a liquid diet with (ER) or without (NR) E (35% of total caloric intake) for 12 to 24 weeks. Atrial strips were superfused with Tyrode's solution (36 °C) and driven at 1.5 Hz while recording tension (T). The peak tension developed (PTD) and the maximum velocity of development of tension (Vmax T) were larger in NR than in ER. E 240 mg % depressed PTD and Vmax T in both NR and ER. Nimodipine 1.75 mg/l (N) also depressed PTD and Vmax T in both NR and ER. The acute negative inotropic effects of E and N were additive. Bay K 8644 250 ug/l (B) enhanced PTD and Vmax T in both NR and ER. The acute inotropic effects of E and B were subtractive. In summary, E and N exerted negative additive inotropic actions, while B had a positive effect, which was decreased in the presence of E. Ingestion of E depressed the ACS, but did not modify substantially the inotropic responses to E or CMs. Supported by NIH/MBRS and ECFMG grants.

143.7

SILVER INHIBITION OF RYANODINE BINDING IN CARDIAC SARCOPLASMIC RETICULUM VESICLES: ROLE IN CA^{2+} RELEASE?
R.A. Humerickhouse*, J.A. Wisler*, and H.R. Besch, Jr., Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46223.

Nanomolar concentrations of Ag^+ inhibit the Ca^{2+} ATPase of cardiac sarcoplasmic reticulum vesicles (SRV). This inhibition of Ca^{2+} ATPase correlated positively with Ag^+-induced release of Ca^{2+} from cardiac SRV. In contrast, in skeletal muscle SRV, others report that Ag^+ interacts with the SR Ca^{2+} release channel (ryanodine receptor), and not the Ca^{2+} ATPase. We used a subfraction of cardiac SRV highly enriched in junctional SRV and ryanodine receptors to evaluate interactions between ryanodine and Ag^+. Micromolar concentrations of Ag^+ were found necessary to inhibit ryanodine binding and to displace bound ryanodine from its receptors, IC50 occurring at approximately 2.5uM Ag^+. These data show that Ag^+ does interact with the Ca^{2+} release channel in cardiac SRV. However, the concentration-effect relationships suggest that Ag^+-induced inhibition of the Ca^{2+} ATPase is quantitatively of greater importance that its effect on the ryanodine receptors. Also, Ag^+ inhibits Ca^{2+} accumulation in the absence of ryanodine. Further experiments are required to determine precisely the relative contribution of the two effects of Ag^+ in Ca^{2+} release from cardiac vesicles. [Supported in part by the Showalter Trust].

143.8

COMPARISON OF THE EFFECTS OF AMIODARONE AND IPODATE ON RAT HEART MYOSIN.
Surath K. Banerjee*, Thomas R. Brown* and Nandalal Bagchi* (SPON: S. Dutta). VA Medical Center, Allen Park, MI 48101 and Wayne State University, Detroit, MI 48201.

We have recently shown that chronic treatment of rats with amiodarone, an antiarrhythmic agent, results in reduced body weight and heart weight and preferential synthesis of the low ATPase cardiac V_3-isomyosin. These effects are similar in direction but to a lesser degree, resembling those found in hypothyroidism. A possible mechanism for such amiodarone induced changes could involve reduction of triiodothyronine (T_3) concentration in cardiac nuclei. We have tested this hypothesis in the present study by comparing the effects of amiodarone and ipodate, a well known inhibitor of 5'-deiodinase, the enzyme that converts intracellular production of T_3 from T_4. Separate groups of rats were given dietary ipodate and amiodarone, respectively, for six weeks. Both agents increased serum T_4 and T_4/T_3 ratios, a finding consistent with the inhibition of peripheral T_4 to T_3 conversion. However, ipodate failed to produce hypothyroid-like effects on body weight, heart weight and isomyosin transitions similar to those found in the amiodarone group. These data indicate that the hypothyroid-like effects of amiodarone on the rat heart are not due to the inhibition of intracellular generation of T_3 and T_4. (Supported by the VA Medical Research Fund and the MHA)

143.9

COMPARATIVE EFFECTS OF FORSKOLIN AND DOBUTAMINE ON ADENYLATE CYCLASE ACTIVITY AND CARDIOVASCULAR RESPONSES IN NORMAL AND CARDIOMYOPATHIC HAMSTER HEARTS.
J. W. Hubbard, P. G. Conway*, F. D. Pack*, L. L. Ricker*, A. P. Angelac* and S. Fielding. Depts. of Bio. Res. & Drug Safety, Hocchst-Roussel Pharmaceuticals, Inc. Somerville, NJ 08876.

The genetic cardiomyopathic hamster (CHF 146) develops a progressive cardiac necrosis associated with end-stage decreases in cardiac cAMP and reduced cardiac performance. We examined the adenylate cyclase (AC) activity and cardiac function (Langendorff) of compensated pre-failure stage CHF and age-matched normal controls (CON) in response to dobutamine and forskolin. No differences were found in basal AC activity (RIA) between CHF (23.5 pmol/mg protein/min.) and CON (18.7 pmol/mg protein/min.). Similarly, there were no differences in basal active tension (T), heart rate (HR), or coronary flow (CF) between the two groups. Both forskolin and dobutamine (0.3-300 μM) caused significant increases in AC in the CON. However, dobutamine-induced AC was significantly less in CHF than in CON, whereas forskolin stimulation remained the same. The peak changes in T paralleled AC responses to drug treatment in the two groups, with dobutamine-treated CHF showing smaller increases in T compared to CON. Forskolin-treated CHF and CON showed equivalent increases in T between 0.001-0.03 μM. Histological analysis revealed moderate calcification and fibrosis in CHF but not in CON. It is concluded that 120-150 day old CHF show normal basal AC activity and cardiac function; however, dobutamine-induced increases in AC and T are less than in CON. Consequently, it would appear that β-receptor coupled increases in AC and cardiac function are deficit in these CHF while responses to forskolin remain unaffected.

143.10

LEUKOTRIENE D_4 PRODUCES A POSITIVE INOTROPIC EFFECT ON THE ELECTRICALLY DRIVEN GUINEA-PIG LEFT ATRIA.
R.C. Falcone*, D. Aharony[1] and R.F. Orzechowski, Philadelphia College of Pharmacy and Science, Dept. of Pharmacology, Philadelphia, PA 19104 and [1]ICI Pharmaceuticals Group, Wilmington, DE 19897.

The negative inotropic effect of peptidoleukotrienes (LTs) on the heart has been attributed to both coronary constriction and a direct action on the myocardium. The purpose of this investigation was to evaluate effects of LTs using electrically driven guinea-pig left atria (GPLA). Tissues were stimulated via puncturate electrodes at 2 Hz and 10-20% above threshold. LTD_4 (1nM to 1μM) caused a concentration related increase in contractile force. LTD_4 (1μM) evoked a 23±3% (mean±sem, n=8) increase above basal contractility which was further enhanced (47±3%, n=6) by 1μM tetrodotoxin. Repeated challenges to GPLA with 1μM LTD resulted in progressively diminished responses. LTC_4 produced a weaker positive inotropic response (17±1%, n=3) which was abolished when the tissues were pretreated with 10mM acivicin, a γ-glutamyl transpeptidase inhibitor. Various LTD_4 antagonists, ICI 198,615, LY171883 and FPL55712, were able to reverse this positive inotropic effect. The LTD_4-induced myocardial stimulation was not affected by propranolol, atropine, acivicin, or capsaicin. These data demonstrate a direct positive effect of LTD_4 on guinea-pig left atria which can be reversed by specific LTD_4 antagonists.

143.11

ALTERATIONS IN THE ADENYLATE CYCLASE SYSTEM OF THE MYOCARDIUM DURING THE DEVELOPMENT OF ADAPTIVE SUPERSENSITIVITY.
D.A. Taylor, L.E. Bennett* and W.W. Fleming. WVU Health Sciences Ctr., West Virginia University, Morgantown, WV 26506

The myocardium of several species adapts to chronic reductions in adrenergic input by an elevation in sensitivity. Unlike the adaptive supersensitivity observed in a number of other tissues, the phenomenon which develops in cardiac tissue is highly selective for beta-adrenoceptor agonists. In the guinea pig myocardium, changes in sensitivity appear in the absence of any detectable alteration in the beta-adrenoceptor population, suggesting that the molecular change responsible for adaptive supersensitivity in this tissue occurs distal to the receptors. In order to assess the role of the adenylate cyclase system in the development of supersensitivity, the cyclic AMP content of isolated atria following exposure to agonists known to activate adenylate cyclase in the myocardium was determined. Cyclic AMP content was measured using a commercially available radioimmuno-assay kit. Isoproterenol (10^{-7} - 10^{-5}M) and histamine (10^{-5} - 10^{-3}M) elevated cyclic AMP content in both right and left atria. The elevation in cyclic AMP content induced by isoproterenol but not histamine was significantly greater in tissues obtained from animals pretreated with reserpine than in control tissues. These data suggest that the portion of adenylate cyclase system specifically coupled to beta adrenoceptors may serve an integral role in the development of adaptive supersensitivity in the guinea-pig myocardium. Supported by NIH grant 5-RO1-GM29840.

143.12

LITHIUM-DEPENDENT INHIBITION OF NA-CA EXCHANGE IN CARDIAC SARCOLEMMAL VESICLES.
Rebecca Keller* and Calvin C. Hale Univ. of Missouri, Columbia, MO 65211

In an attempt to clarify the monovalent cation substrate specificity of Na-Ca exchange, we have examined the effects of Li+ on transport in cardiac SL vesicles. In all experiments, the final monovalent cation-chloride concentrations were 160 mM with 20 mM MOPS/TRIS, pH 7.4. SL vesicles equilibrated with K+, Na+, or Li+ were diluted 50-fold into KCl with 12 μM Ca-45 and assayed for Na-Ca exchange activity. No difference was observed in initial rates or total Ca2+ accumulation between K- or Li- loaded vesicles which showed significantly less activity than Na-loaded vesicles. A dose response curve of Na-loaded vesicles diluted into various mixtures of K+ and/or Li+ and Ca-45 produced a linear decrease in the initial rate of Na-Ca exchange activity from 160 mM K+ (100%) to 160 mM Li+ (63%). Similarly, mixtures of K+, Li+, or choline+ with or without Na+ showed essentially no exchange activity until the external [Na+] was reduced to 40 mM. External Li+ inhibited Na-dependent Ca-45 uptake by approximately 50% throughout the concentration range of 0 - 40 mM external Na+ compared to K+ or choline+. Na-dependent Ca-45 efflux was not affected by internal K+ or Li+. K- and Li-loaded vesicles effluxed Ca-45 in the presence of external Li+ but to a lesser extent than with external Na+. These results suggest that Li+ may substitute weakly for Na+ and is inhibitory to the exchange process. Supported by NSF DCB 8602234 and American Heart Association - Missouri Affiliate.

143.13

COMPOUND 48/80 AND CARDIAC FUNCTION. Young Hee Kang* and Gary F. Merrill. Rutgers University, New Brunswick, NJ 08903

The effects of bolus injection or continuous infusion of compound 48/80 on cardiac contracitlity (dP/dt), coronary perfusate flow (CPF), and myocardial oxygen consumption (MVO_2) were studied in isolated guinea pig hearts perfused at constant pressure (65 cmH_2O). Infusion of 48/80 (25-100 µg/min/g) decreased dP/dt, CPF and MVO_2 significantly (P<0.05) by 28±6%, 26±8%, 18±4%, respectively. Bolus injection (50-200µg) produced transient positive intropism, rapidly followed by negative inotropism and a decrease in (CPF. One minute after bolus injection, when hearts were in the steady state, dP/dt, CPF and MVO_2 decreased significantly (P<0.05) by 17±3%, 20±6%, 15±2%, respectively. The responses of dP/dt and CPF were dose dependent. Bolus injection of 100µg of 48/80 caused time-dependent release of histamine. The maximum release of histamine was seen within 10-15 seconds after injection of 48/80. These results indicate that the secondary but prolonged negative inotropism and coronary vasoconstriction seen after administration of 48/80 might be due in part to the direct effects of 48/80. Other, indirect effects such as release of endogenous coronary vaso constrictors could have contributed to the response.

143.14

CHRONOTROPIC AND INOTROPIC EFFECTS OF ATENOLOL OR BUTOXAMINE IN THE PRESENCE OF DOBUTAMINE IN ALTERED THYROID STATES. D.M. Van Wynsberghe and M. Mullett*. Univ. of Wisconsin, Milwaukee, WI 53201.

Altered thyroid states influence cardiac beta (ß) receptor density, with hyperthyroidism increasing and hypothyroidism decreasing or not changing beta receptor density. The ratio of cardiac $ß_1$: $ß_2$ adrenergic receptors is @ 83:17 in the rat. Dobutamine ($ß_1$ agonist), atenolol ($ß_1$ antagonist) and butoxamine ($ß_2$ antagonist) were used to determine the effects of altered thyroid states on adrenergic control of heart rate (HR) and left ventricular +dP/dt (LV+dP/dt) in anesthetized euthyroid, hypothyroid and hyperthyroid rats. Dobutamine concentrations (0.25-40.0 mg/Kg) were given i.v. in the absence or presence of atenolol (0.25 mg/Kg) or butoxamine (2.0 and 4.0 mg/Kg). EC_{50}s were determined from the dose-response curves. In the presence of dobutamine, with no antagonists, LV+dP/dt EC_{50} was significantly less in the hyperthyroid group, reflecting the increased ß receptor density with thyroid hormones. Atenolol decreased LV+dP/dt and HR in all groups, with a 7-9 fold >EC_{50} for LV+dP/dt and 3-4 fold >EC_{50} for HR. Butoxamine resulted in no significant differences in LV+dP/dt in the 3 groups but did increase significantly the EC_{50} for HR in the hypothyroid group with 4 mg/Kg butoxamine. This indicates a decreased $ß_1$ receptor population. Butoxamine, a $ß_2$ antagonist, has $ß_1$ effects at high doses (4mg/Kg). Butoxamine could not be given to hyperthyroid rats because even minimal concentrations caused small airway closure in ventilated animals due to the upregulation of $ß_2$ receptors in bronchial smooth muscle.

CARDIAC PHYSIOLOGY

144.1

VALIDATION OF TWO INDICES OF MYOCARDIAL RELAXATION. T-CONSTANT AND ECHO-DERIVED ISOVOLUMIC RELAXATION TIME. Elaine E. Nelson*, Kirk E. Kanady*, Moysey M. Povzhitkov,* (Spon. A.P. Roszkowski).
Syntex Research, Palo Alto, CA 94304

Impairment of left ventricular relaxation (VR) may occur as the earliest clinical evidence of cardiac dysfunction. Two basic techniques are commonly used to characterize VR, viz., relaxation T-constant (T), which requires cardiac catheterization, and non-invasive M-mode echo-derived isovolumic relaxation time (IVRT). We studied the effects of i.v. nifedepine (N) on VR in closed chest pentobarbital anesthetized dogs. Routine hemodynamic parameters were recorded along with T and IVRT. Administration of N at doses of 5-50 µg/kg produced a significant (p<0.05) and dose related decrease in aortic mean blood pressure and an increase in left ventricular (LV) dp/dt max. VR time, as evidenced from changes in both T and IVRT, significantly decreased (p<0.05). Conversely, 100-200 µg/kg doses of N increased the duration of T and IVRT, and decreased LV dp/dt max. The correlation between changes in T and IVRT were found to be high and significant (r = 0.95, P < 0.05). Results of our study suggest that M-mode echo-derived IVRT is at least as sensitive as T for the evaluation of changes in myocardial relaxation.

144.2

A COMPARISON OF VARIOUS ALGORITHMS USED TO DETECT CORONARY ARTERY EDGES. William P. Santamore, Ph.D. N. Samuel Negin, B.S., Michael A. Kutcher, M.D., George Rebecca, M.D. Michael Negin, Ph.D. Wake Forest University Medical Center, Winston-Salem, NC 27103

Coronary angiography is the clinical standard for assessing coronary artery disease. To aid in the analysis of the coronary angiograms, recently several methods have been developed which automatically delineate the arterial borders. These methods have been validated by comparisons with objects of known dimensional size and by the reproducibility of their measurements. To compare the validity and accuracy of the various algorithms we simulated the density profile across a coronary artery. The artery was considered to have a linear density which was convoluted with a Gaussian "blur" function to account for x-ray dispersion. To this density profile, random noise could be superimposed. Using this density profile the various algorithms were compared. The first derivative algorithm consistently under estimated vessel size 1.0 vs 0.93, 2.0 vs 1.83mm, 3.0 vs 2.80mm). Adding noise to the density profile decreased the reproducibility (increased the standard deviation from 0.05 to 0.34mm). Second derivative algorithm consistently overestimated vessel size and further decreased the reproducibility. Thus, the simulated density profile can be a useful tool to rapidly assess the accuracy, limitations, and reproducibility of the various algorithms used to identify coronary arteries.

144.3

PARASYMPATHOMIMETRIC INFLUENCE (PI) ON LEFT VENTRICULAR DP/DT. H.S. Lowensohn, R.W. Caldwell, C.B. Nash and M.A. Montgomery.*
WRAIR, WRAMC, Wash, D.C. 20307 and MCG, Augusta, GA 30912.

Previous studies claimed vagal stimulation reduced cardiac inotropy, yet anatomical evidence precludes vagal innervation of the left myocardium. We studied Na pentobarbital anesthetized dogs monitoring ECG, heart rate (HR), stroke volume (SV), Dp/Dt and aortic end diastolic pressure (EDP) for pre-infusion baseline, 15 min infusion (I), and 2 hr post-infusion (PO) periods. We tested PI on the following groups of 6 dogs: saline, 0.5 mg/kg pyridostigmine bromide (PB), 2.0 mg/kg PB, 5.0 mg/kg PB. Maximum variable response coincided with the end of infusion. Dp/Dt did not change significantly from control; it rose slightly during I as HR fell and SV increased due to PB. EDP slightly, insignificantly decreased after PB. Only HR (r=-.83) and SV (r=.76) correlated (C) with PB during I, and HR (r=-.76) C with PB post-infusion. Dp/Dt C poorly with PB during I (r=.43) and PO (r=.19). Dp/Dt response after PB treatments was not significantly different from saline (ANOVA, α >.05) for I and PO periods. Neither PB, HR, SV, nor EDP exerted a strong influence on Dp/Dt during I. A fair C of Dp/Dt to EDP (r=.63) took place PO, yet Dp/Dt C poorly with BP, HR, SV, PO. ECG showed P-R and Q-T changes, particularly at the highest PB dose. We conclude PI doesn't cause a negative Dp/Dt, and it's unlikely the parasympathetics promote reduced ventricular inotropy in the normally functioning dog heart.

144.4

INFLUENCE OF EXOGENOUS INOSINE UPON CARDIAC INTERSTITIAL [ADENOSINE] IN THE ABSENCE AND PRESENCE OF ISOPROTERENOL STIMULATION. Lois Jane Heller, Joni C. Sherin* and David E. Mohrman. University of Minnesota, Duluth, MN 55812

We tested the hypothesis that high levels of inosine (INO) may interfere with the processes that remove adenosine (ADO) from interstitial fluid and thereby increase interstitial [ADO]. HPLC techniques were used to determine the [ADO] of venous effluent (v) and surface exudate (i) from isolated rat hearts perfused at constant flow with colloid-free salt solutions. The latter value was taken as an estimate of interstitial [ADO]. Hearts (n=8) were metabolically stimulated by 6 minute infusions of 10nM isoproterenol (ISO) in the absence and presence of 100µM exogenous INO. Oyxgen consumption was not altered by addition of INO nor was the increase achieved in the presence of ISO altered by INO. Values given below represent mean ± SEM.

	[ADO]v,nM		[ADO]i,nM	
[INO]	pre	+ISO	pre	+ISO
0	15±3	101±30*	407±52	1122±216*
100µM	57±15§	155±28*	831±112§	1387±131*

*,§ p<0.05 as compared to "pre" and "0 INO," respectively

Our data indicate that exogenous INO may enhance vascular and interstitial levels of ADO under control conditions but does not significantly influence the levels achieved during isoproterenol stimulation. (Supported by NIH grant HL35869.)

144.5

WITHDRAWN

144.6

FREE RADICAL SCAVENGERS AND NEONATAL MYOCARDIUM. L.J. Veit* and L.J. Kohman* (SPON: T.K. Ray). S.U.N.Y. Health Science Center, Syracuse, NY 13210

Free radical scavengers (FRS) help to reduce myocardial damage due to free oxygen radicals (FR) in the adult heart but the same effect has not been shown in neonatal hearts rendered ischemic and reperfused for 60 min. The present study was designed to evaluate whether the benefit of FRS on neonatal myocardium could be shown earlier in reperfusion. Isolated working neonatal rabbit hearts were made ischemic at 30°C for 30 or 90 min, and protected with St. Thomas' cardioplegia (pO_2 >650 mmHg), with or without superoxide dismutase and catalase (FRS). Hemodynamic recovery was equally good in all groups. Creatine kinase, measured at 20 or 25 min ischemia, did not differ among the 4 groups. Malondialdehyde (MDA) an indicator of lipid peroxidation and FR damage was lower after 30 min reperfusion (30 min ischemia) in the group with FRS than without: 1.69 ± .69 nmol MDA/mg protein vs 4.83 ± 1.97, p<.005. Hearts reperfused for 60 min (ischemia 90 min) had similar MDA levels, with or without FRS: 2.01 ± .44 vs 1.7 ± .25, p NS. Though not present in the reperfusate, FRS remain in the heart during the early stages of reperfusion. These data support the belief that FR damage occurs early in reperfusion. Lower MDA levels following extended reperfusion, despite a longer ischemic interval, may show that hearts have had time to repair FR damage, or may indicate that MDA has been washed out of the cells by prolonged reperfusion. This study demonstrates a beneficial effect of FRS on neonatal myocardium in the very early interval following a hyperoxic ischemic insult.

144.7

CONCOMITANT-ADMINISTRATION OF PENTAPEPTIDE 6A ENHANCES THE THROMBOLYTIC POTENTIAL OF t-PA. Wilmer W. Nichols, Jawahar L. Mehta*, Tom G.P. Saldeen*, Menno ter Riet*, Dan L. Lawson*, Linda V. Thompson* and William H. Donnelly*. Univ. Fla. and VAMC, Gainesville, FL 32602

Fibrin(ogen)-degradation product peptide 6A (Ala-Arg-Pro-Ala-Lys) increases coronary blood flow (CBF) and stimulates endogenous PGI_2 release (Am J Physiol 1985;249:H457). To determine if peptide 6A would enhance the thrombolytic potential of tissue plasminogen activator (t-PA), dogs with electrically-induced coronary artery thrombus, were randomly given t-PA alone or t-PA + peptide 6A intravenously. t-PA (10mg over 20 min) alone restored CBF in 2 of 5 dogs and t-PA + peptide 6A restored CBF in 4 of 5 dogs.

RESULTS (mean ± SD)

	Time to reflow (min)	Max. CBF (ml/min)	Duration of reflow (min)
t-PA	27±23	61±15	16±3
t-PA + 6A	16±9	44±25	39±20

Coronary venous 6-keto-$PGF_{1\alpha}$ conc. after t-PA + 6A were increased in each dog with restoration of CBF (mean conc., 422±161 vs. 221±71 pg/ml; P<0.05). This study indicates that addition of peptide 6A and t-PA enhances the frequency and duration of CBF restoration and decreases the time to reflow from beginning of infusion (all P< 0.05), possibly mediated via stimulation of endogenous PGI_2 release.

144.8

EFFECT OF REPLACEMENT OF DIETARY LIPID WITH FISH OIL ON CORONARY VASCULAR RESPONSIVENESS. Carl E. Hock, Michael D. Brown and Diane K. Reibel. University of Medicine and Dentistry of New Jersey-SOM, Camden, NJ 08103 and Thomas Jefferson University, Phila., PA 19107

In order to study the effect of dietary fish oil on coronary vascular responsiveness to vasoconstrictors, rats were fed purified diets in which the lipid was replaced with either corn oil (CO) or menhaden oil (MO). Following four weeks of feeding, hearts were isolated and perfused at a constant flow. Angiotensin II (AII) and leukotriene D_4 (LTD_4) were infused into the aorta in graded doses and the change in coronary perfusion pressure (Δ CPP) was monitored. The ΔCPP was significantly higher in hearts of rats fed MO vs CO for both vasoconstrictors. The maximum ΔCPP was 50% higher for AII and 54% higher for LTD_4 in hearts of MO vs CO fed rats. The coronary vasoconstrictor effect of the eicosapentaenoic acid metabolite leukotriene D_5 (LTD_5) was also studied. Similar to both AII and LTD_4, the ΔCPP produced by LTD_5 was significantly higher in MO vs CO hearts (p<0.02). When compared to LTD_4, the maximum ΔCPP produced by LTD_5 was not significantly different, however, the dose-response curve for LTD_5 was shifted to the right. Our results indicate that the omega-3 eicosanoid LTD_5 is a coronary vasoconstrictor and that MO replacement increases coronary vascular responsiveness to AII, LTD_4 and LTD_5.

144.9

VASOCONSTRICTOR ACTIVITY IN STROMA-FREE HEMOGLOBIN SOLUTION DEVELOPS DURING REFRIGERATED STORAGE. G.P. Biro, K. Lawless*, M. Masika*, B. Korecky, P.J. Anderson*. University of Ottawa, Ottawa, Ont., K1H 8M5, Canada.

Stroma-free hemoglobin solutions (SFHS) have been shown to constrict the coronary vasculature of Langendorff-perfused rat and rabbit hearts. We undertook a systematic investigation of the effect of storage on SFHS prepared by phosphate-precipitation (SFHS1) and ultrafiltration (SFHS2) methods. Perfusion (PP) and left ventricular systolic (SP) pressures were monitored in rat Langendorff-preparations perfused with oxygenated buffer, at constant flow (11-13.5 ml/min). SFHS was added to the perfusate flow (1 ml/min) for 2-minute periods. Freshly prepared SFHS1 caused a rise of PP of 55±7%, associated with 33±12% fall in SP. Freshly prepared SFHS2 caused negligible change in PP and SP. After storage for eight weeks at 4°C, SFHS2 caused significant changes in PP (+22±4%) and in SP(-74±21%), whereas the same material kept frozen (-85°C) for the same period showed no such activity. At the end of the addition of the test-substance, the depression of SP was promptly reversible and, in each case, preceded the recovery of PP. The findings indicate that in SFHS2 vasoconstrictor and contractility-depressant activity appear to be separate and develop slowly during refrigerated storage. The identity of these principles is as yet undetermined. (Supported by the Defence and Civil Institute of Environmental Medicine of Canada).

144.10

EFFECTS OF α_1-BLOCKADE ON LEFT VENTRICULAR TRANSMURAL FLOW AND FUNCTION DURING LEFT STELLATE GANGLION STIMULATION. Patricia A. Gwirtz, H. Fred Downey, Carl E. Jones and Jeffrey M. Dodd-o*. Texas College of Osteopathic Medicine, Department of Physiology, Fort Worth, Texas, 76107-2690.

An α_1-adrenergic coronary constriction may oppose metabolic vasodilation. The transmural nature of the vasoconstrictor tone and its effect on myocardial contractile function is not well established. The present study examined these questions during stellate ganglion stimulation. In 11 anesthetized dogs, left stellate stimulation significantly increased heart rate, arterial pressure, circumflex inflow, left ventricular pressure, and epicardial and endocardial segmental shortening in the posterior ventricle. Intracircumflex prazosin (0.5 mg) caused additional increases in circumflex inflow (16%), dP/dt_{max} (19%), and posterior regional endocardial shortening (20%). Epicardial shortening was not affected. In 5 dogs, regional myocardial perfusion was measured using tracer microspheres. Stellate stimulation increased both epicardial and endocardial perfusion by 30%. Intracircumflex prazosin increased both epicardial and endocardial perfusion by approximately an additional 36%. Thus, during stellate stimulation, an α_1-vasoconstriction exists uniformly across the left ventricular wall, but contractile function is limited by this vasoconstriction only in the endocardium. (Supported by NIH HL-34172 and HL-29232).

144.11

INFLUENCE OF HYPOXIA-ACIDOSIS ON SYSTOLIC TIME INTERVALS AMONG THE FETAL HEART. J.S. Lafond and J.-C. Fouron*. Centre de Recherche, Hôpital Ste-Justine, Montréal, Qc, Canada

Lengthening of the pre-ejection period (PEP) in a situation of hypoxia-acidosis has already been described. We wanted to evaluate if this lengthening is associated with a change in isovolumetric contraction time (ICT) and/or electromechanic delay (EMD), which are the two components of PEP. Six fetal lambs of known gestational age (135 days) are catheterized. After one hour of hypoxia (arterial PO_2: 11.7 ±0.4 mmHg) we infused lactic acid (1.5 mM) during two hours so that a pH of 7.0 is achieved. Arterial PO_2 and pCO_2 increase from 18.1±1.6 and 42.4±0.7 at control period to 14.1±0.8 and 44.4±1.6 mmHg respectively at the end of protocol. Systolic time interval (STI) measurements obtained are the following:

	Control	Hypoxia	Hypoxia-acidosis 1 h	Hypoxia-acidosis 2 h
PEP	50±4	44±4*	44±3*	49±3**
EMD	32±2	30±2	31±1	33±1**
ICT	18±2	14±1	13±1*	16±1**
ET	139±5	138±5	146±8	141±5
PEP/ET	0.36±0.03	0.32±0.2*	0.31±0.02*	0.35±0.02**
R-R	331±12	337±18	330±20	331±19

(ET: injection time; R-R: time between 2 R waves from ECG); *p<0.05 compared to control period; **compared to hypoxia.

We conclude that in non-compensated hypoxia, lengthening of PEP is due to a change in both ICT and EMD.

144.12

INFLUENCE OF SLEEP STATE ON CORONARY BLOOD FLOW IN YOUNG LAMBS. James E. Fewell, Colleen S. Kondo*, Victor Dascalu*. University of Calgary, Calgary, Alberta, CANADA T2N 4N1

Experiments were done to determine if sleep state influences coronary blood flow in 5 lambs. For instrumentation, each lamb was anesthetized and prepared for sleep staging and for measurements of cardiac output (Qp), left circumflex blood flow (Qlcf) and systemic blood pressure (BP). Myocardial oxygen demand was estimated by the product (PRP) of systolic blood pressure and heart rate. No sooner than 3 days after surgery, measurements were made in quiet wakefulness (QW), quiet sleep (QS) and active sleep (AS) at an ambient temperature of 25C.

Variable	QW	QS	AS
BP (mmHg)	74±6	72±4	*69±6
HR (BPM)	198±28	202±31	*168±19
Qp (ml/min)	1554±87	1533±84	*1387±113
Qlcf (ml/min)	35±8	34±7	*31±8
PRP ($X10^3$)	17.3±3.3	15.7±1.5	*13.7±1.7

(* p<0.05 by MANOVA and Duncans as compared to QW)

Qlcf decreased during AS compared to QW; however, the ratio of Qlcf to Qp did not change. The decrease in coronary blood flow during AS is most likely related to a decrease in myocardial oxygen demand. (Supported HL34377, HD24018, American Heart Association and Alberta Heritage Foundation for Medical Research)

144.13

IS THERE AN EFFECT OF LEFT VENTRICULAR HYPERTROPHY (LVH) IN PIGS ON TIME TO ISHEMIC CONTRACTURE AND METABOLISM ? Carin Wittnich, Robert J. Cusimano*, Walter Vicente*, Haysam El Dalati*, Adam Newman*, and Tomas A. Salerno*. University of Toronto, Toronto, Ontario, Can., M5b 1W8.

In rats surgically induced left ventricular (LV) hypertrophy has resulted in a marked decrease in time to development of ischemic contracture (TIC) as compared to normal hearts. In addition, it is not agreed what effects this pathology has on myocardial metabolism. To examine this phenomenon in a species whose hearts more closely resemble that of man, adult pigs (n=14) with surgically created LV hypertrophy underwent sternotomy and control LV and RV myocardial biopsies, for ATP and glycogen assay, under general anesthetic. These freeze clamp biopsies (umole/g) were compared to those taken from pigs with normal hearts (n=4) who were studied in the same fashion. The hearts were then excised from the chest and placed in a substrate-free Krebs Henseleit solution with a compliant balloon placed in the LV for measurement of TIC. It was evident that LVH resulted in a significantly (P<0.05, t test) lower ATP store (3.57±0.21 vs 2.11±0.15), lower myocardial glycogen (21.50±0.77 vs 14.30±0.78). In addition TIC was significantly shorter in the LVH hearts (65.0±3.6 vs 41.5±4.9 min). Values are Mean±SEM. This confirms the rat findings that LVH causes a decreased tolerance to ischemia possibly as a result of lower energy stores in the myocardium. (MRC of Can. support)

144.14

INTRAMYOCARDIAL BLOOD VOLUME IN DOGS WITH EXERCISE HYPERTROPHY. Xuesi Wu*, Namsik Chung*, James Stray-Gundersen* and Erik L. Ritman. Mayo Medical School, Rochester, MN 55905

The fraction of myocardium that is intramyocardial blood (FMB) of the left ventricle (LV) can be estimated in vivo using fast CT (Wu et al, The FASEB J 2:A303, 1988). Seven dogs (4 adults (S), 3 adolescents (J)) were scanned before and after 3-10 months of training, and 7 dogs (5 S, 2 J) without training. The dogs were anesthetized with Innovar and 2:1 N_2O/O_2 mixture for scans during injection of Iohexol 32cc into the right atrium for estimation of LV myocardial volume (LVMV), and again into the aortic root during infusion of adenosine (0.0-1.0 mg/kg/min) for estimation of myocardial blood flow (F) (Wang et al, Circ (Part II) 76:IV-5, 1987) and FMB. Following training J and S increased LVMV over control by a mean of 41% and 26%. FMB increased linearly with F for each group with slope, FMB offset and correlation coefficient values respectively: 0.039, 0.082, 0.931 for control and 0.038, 0.047, 0.890 for trained S; 0.041, 0.039, 0.990 for control and 0.027, 0.057, 0.982 for trained J. Using these regressions, at F = 1 ml/gm/min, J training changed FMB by +5% (mean) over control and S by -30%. Consequently for J hypertrophy the total LV intramyocardial blood volume (IMBV = FMB x LVMV) increased by 48%, whereas for S hypertrophy IMBV decreased by 12%. We conclude that training in adolescent dogs results in growth of total intramyocardial blood volume matching muscle growth but in adult dogs intramyocardial blood volume did not increase with muscle growth.

144.15

TRIIODO-L-THYRONINE (T3) INDUCED PROLIFERATION OF THE MYOCARDIAL VASCULATURE AND AORTIC ENDOTHELIAL CELLS IN SWINE. K. Milhoan*, F. White, T. Lane, S. Dobbs-Soanes* and C. Bloor. UCSD School of Medicine, La Jolla, CA 92093.

We sought to determine if there is a relationship between mitogenic activity in vivo and in vitro in the swine vasculature. The in vivo model used was an ameroid coronary artery occlusion of the left circumflex coronary artery of the pig producing a collateral dependent bed at risk (n=8). The coronary collateral blood flow was measured with radiolabelled microspheres during exercise, before and after intravenous infusion of T3 which increased serum levels from 10 to 95 ng/ml for one month. The other four pigs served as controls. In the control pigs endocardial blood flow decreased 63±6% to the endocardium during exercise and did not change over one month. In pigs treated with T3 endocardial blood flow increased by 29±4% (p<0.05). In our cell proliferation assay, porcine aortic endothelial cells, in their third passage, were treated with either 2% fetal calf serum (FCS) in Dulbecco's modified eagles medium (DMEM) or 2% FCS in DMEM with 100 ng/ml T3. At 72 hrs the cells were removed by trypsinization and the nuclei were counted with a Coulter counter. The cells treated with T3 grew 43.4% faster than the untreated cells (p<0.05). Our result agrees with other investigators that have found T3 to be mitogenic in a number of different cell types. These data suggest that the mitogenic activity of T3 seen in vitro is present in vivo.

145.1

LOCAL CEREBROCORTICAL PERFUSION ASSESSED WITH LASER-DOPPLER FLOWMETRY. R.N. Willette, M. Underwood*, A. Sulpizio*, J.P. Hieble and D.J. Reis. Smith Kline & French Laboratories, King of Prussia, PA, 19406 and Cornell University Medical College, NY, NY, 10021.

Laser-Doppler flowmetry (LDF) derives a measure of the number and velocity of moving particles in a sample volume based on the magnitude and frequency distribution of Doppler-shifted backscatter from tissue irradiated with monochromatic laser light. This study relates movement, detected from the surface of the hindlimb somatosensory cortex with LDF, to local cortical perfusion (LCP) by comparing LDF and ^{14}C-iodoantipyrine (^{14}C-IAP) evaluations of cerebrovascular responsivity to changes in arterial BP and pCO_2. In α-chloralose anesthetized rats, autoregulation of the cortical blood flow was demonstrated with LDF and ^{14}C-IAP following BP manipulations with phenylephrine and hemorrhage. The autoregulated range was approx. 60-160 mmHg with both techniques. Results obtained with LDF and ^{14}C-IAP were also highly correlated with arterial pCO_2, r=0.87 and 0.83, respectively. Most importantly, the percentile change determined with LDF did not differ from those determined with ^{14}C-IAP at equivalent pCO_2 levels. LDF was particularly useful for monitoring the time course of physiologic and pharmacologic LCP events. In conclusion, LDF provides a reliable real-time measure of relative LCP over a range of cortical flows. At very low flow, LDF may overestimate LCP.

145.2

SIMULTANEOUS DETERMINATION OF BLOOD FLOW, CHOLINE AND ACETYLCHOLINE TISSUE LEVELS IN FOCAL CEREBRAL ISCHEMIA. Donald J. Jenden and Oscar U. Scremin. UCLA School of Medicine, Los Angeles, CA 90024 and Veterans Administration Medical Center, Albuquerque, NM 87108.

The effects of middle cerebral artery occlusion (MCAO) on cerebral blood flow (rCBF), tissue choline (Ch) and acetylcholine (ACh) contents were assessed in conscious Wistar rats. After i.v. infusion of Iodo-^{14}C-antipyrine (IAP), circulation was arrested by an i.v. bolus of euthanasia solution and the brain microwaved in situ (Biostat apparatus, Gerling Laboratories, frequency=2500 MHz, nominal power=5kW). Regions of cerebral cortex were dissected out and weighed. IAP was measured by scintillation counting and rCBF calculated from tissue and blood radioactivities. Ch and ACh were measured by gas chromatography-mass spectrometry in a separate tissue fraction. rCBF (ml $g^{-1}min^{-1}$), Ch (uM) and ACh (uM) were [Mean (S.E.)] .30(.06), 89.1(19) and 15.8(1.4) in the ischemic focus and 1.2(.1), 23.5(1.8) and 17.9(1.5) in the contralateral, non-ischemic side respectively. Differences between ischemic and control cortex were significant for rCBF and Ch but not for ACh. In plots of Ch and ACh vs rCBF, Ch showed a continuous, reciprocal dependence on rCBF. On the other hand, ACh was independent of rCBF over a wide range and it decreased for values lower than 0.2 ml $g^{-1}min^{-1}$. In summary, Ch levels vary continuously with rCBF in the ischemic hemisphere while ACh is constant except at the lowest levels of perfusion. (Supported by the Veterans Administration and NIMH 17691).

145.3

BILATERAL SYMPATHETIC DENERVATION AFFECTS CANINE CEREBRAL BLOOD FLOW RESPONSE TO RAPID HEMORRHAGE. M.A. Kapin, J.A. Anthony*, F.A. Bashour. The Univ. of Texas Southwestern Medical Center at Dallas, Texas 75235.

Recent studies have shown that bilateral as opposed to unilateral ganglionectomy or stimulation may be necessary to uncover the role of peripheral sympathetic nerves in the regulation of cerebral blood flow (CBF) during normotensive and hypertensive states. However, during hypotension the effect of bilateral sympathetic denervation on CBF is not clear. Since we have previously shown that rapid hemorrhage to 65mmHg (~3 min) results in an early decline in regional CBF, we examined whether bilateral sympathectomy could attenuate this early decline. In this study, we used either *Intact* (no denervation,n=6) or *Bilaterally Denervated* (BD, superior cervical and stellate ganglia,n=6) mongrel dogs (20 to 25 kg) subjected to rapid hemorrhage to 65±1mmHg. All dogs were anesthetized with chloralose and mechanically ventilated to maintain arterial PO_2 above 100 mmHg and PCO_2 between 30 to 40mmHg. Total and rCBF was determined by microspheres at P1,pre-hemorrhage; P2,3 min; and P3,15 min following hemorrhage. ANOVA was employed to show statistical difference (*) from control for total CBF. Total CBF (ml/min/100 gm tissue) results: *Intact*: P1,33±2; P2,18±.7*; P3,25±1*; *BD*: P1,31±4; P2,31±3; P3,35±4. In the *Intact* group, the decline in total CBF was associated with a regional redistribution of CBF that favored midbrain, hypothalamic and brainstem regions. No redistribution of CBF was observed in the bilaterally denervated group. These data support the premise that elevated peripheral sympathetic nerve activity associated with rapid-onset hemorrhage may depress the brains ability to maintain its blood flow. Supported by NIH R29 HL39830-01, AHA-TA # 877-077 and Cardiology Fund.

145.4

ATTENUATION OF KAINIC ACID (KA) INDUCED INCREASES IN CEREBRAL BLOOD FLOW (CBF) WITH ADENOSINE (ADO) RECEPTOR BLOCKADE. David G.L. Van Wylen and Ana L. Moffe*. Depts. of Surgery and Physiology, SUNY at Buffalo, Buffalo, NY 14215.

The purpose of this study was to determine if ADO receptor blockade attenuates active hyperemia induced by KA, an agonist of the excitatory neurotransmitter glutamate. The brain dialysis technique was used to sample cerebral interstitial fluid, deliver drugs locally to the brain, and measure local CBF (hydrogen clearance). Dialysis probes were implanted bilaterally in the caudate nuclei of ketamine-anesthetized rats and perfused (0.1 μl/min) with artificial cerebrospinal fluid (CSF; n=5). The artificial CSF contained either no drugs (control), 10^{-3}M KA, or 10^{-3}M KA and 10^{-3}M 8(p-sulfophenyl)theophylline (SPT), an ADO receptor antagonist. After collection of a control dialysate sample, one probe was perfused with KA while the other probe was perfused with KA+SPT. KA increased dialysate ADO, inosine (INO), and hypoxanthine (HYPO) concentrations from 2.0 ± 0.4, 4.0 ± 0.5, and 10.8 ± 1.3 μM, to 5.6 ± 2.2, 9.0 ± 1.5, and 28.0 ± 5.2 μM, respectively, and increased local CBF to 236.0 ± 62.8 percent of control ($p<0.05$). The presence of SPT with KA resulted in an increase in CBF to only 158.8 ± 25.1 percent of control. Dialysate ADO, INO, and HYPO on the side with KA+SPT increased from 1.9 ± 0.4, 4.0 ± 0.5, and 9.8 ± 1.6 μM, to 8.0 ± 2.1, 11.7 ± 1.4, and 27.0 ± 2.5 μM, respectively. These data suggest that ADO contributes to active hyperemia in the brain. Supported by NIH HL/NS 40878.

145.5

CEREBRAL BLOOD FLOW AND OXYGEN CONSUMPTION IN CONSCIOUS SHEEP DURING 72 HOURS OF HYPOXIA. D.C. Curran-Everett* and J.A. Krasney, Dept. of Physiology, SUNY-Buffalo, Buffalo, NY 14214

Cerebral blood flow (CBF) is generally tightly coupled to cerebral oxygen consumption (CMRO2) during normoxia as well as acute hypoxia. However, in conscious sheep, CBF increases through 48 h of hypoxia (PaO2=40 mmHg) while whole body VO2 decreases by 50% relative to normoxia (Respir. Physiol. 57: 73-88, 1984). This experiment examined the coupling between CBF and CMRO2 in 9 sheep exposed to hypoxia (PaO2=40 mmHg, SaO2=50%) for 72 h; hypoxia was produced by N2 dilution in an environmental chamber. Arterial (aorta) and cerebral venous (superior sagittal sinus) blood samples were taken during normoxia and at 24 h intervals of hypoxia. CBF/CMRO2 and cerebral fractional extraction of O2 (FEO2) were calculated from arterial (CaO2) and cerebral venous (CvO2) O2 contents [CBF/CMRO2 = 1 / (CaO2-CvO2)]. CBF/CMRO2 increased from 15 (± 2.3 SD) ml/ml O2 during normoxia to a peak value of 31 (± 8.2) ml/ml O2 at 48 h of hypoxia. FEO2 decreased from 52 (± 8.2) % during normoxia to a nadir of 46 (± 8.4) % at 48 h of hypoxia. (Blood O2 capacity does not change in the sheep.) This increase in CBF/CMRO2 parallels the measured CBF (ml/min/100gm) response that occurs during this period of hypoxia. These data suggest that, during the early readjustment to sustained hypoxia, CBF in the sheep exceeds the metabolic requirements of the brain; the mechanisms responsible for this "luxury" perfusion are unclear. (Supported by HL-36126).

145.6

EFFECTS OF INTRALUMINAL COCAINE AND BENZOYLECGONINE PERFUSION ON CAT MIDDLE CEREBRAL ARTERIES *IN VITRO*. Robert H. Powers and Jane A. Madden*. V.A. Medical Center and The Medical College of Wisconsin, Milwaukee, WI 53295.

The cerebrovasculature appears to be particularly sensitive to the toxic effects of cocaine, including cerebral hemorrhage and/or ischemia. The effects of cocaine and its major metabolite, benzoylecgonine, were studied in an *in vitro* system in which a segment of feline middle cerebral artery was threaded onto 2 glass cannulae and all side branches tied off so that solutions could flow intraluminally. The cannulated vessel was in a chamber filled with with physiological saline solution (PSS) at 37 °C and aerated with 20% O_2 and 6% CO_2 at pH 7.4. During a 2 hour equilibration, PSS, aerated with the same gas mixture as above, was perfused through the lumen at a pressure of 80 mm Hg and a rate of about 0.5 ml/min. Following equilibration, either norepinephrine (10^{-6} M) was added to the external bath, or cocaine hydrochloride (10^{-5} M) or benzoylecgonine (10^{-5} M), dissolved in PSS, was perfused intraluminally at the same pressure and flow as above. Internal and external vessel diameter and wall thickness were observed with a camera and TV monitor and measured with a video scaling system during equilibration and after suffusion or perfusion with the drugs. The outside diameter of vessels perfused with 10^{-5} M cocaine constricted about 11%. Vessels perfused with 10^{-5} M benzoylecgonine constricted about 20%. GCMS analysis of perfusion samples showed no significant metabolism of either the cocaine or benzoylecgonine during intraluminal perfusion. Extraluminally applied norepinephrine also constricted the vessel about 20%. These preliminary data indicate that both cocaine and its metabolite, benzoylecgonine, are potent cerebral vasoconstrictors and that benzoylecgonine may be as potent as cocaine or norepinephrine.
Supported by VA Medical Research Funds

145.7

SMOOTH MUSCLE CONTRACTILITY IN HUMAN CEREBRAL ARTERIES. L.A. Mezzetta* and E.A. Zorychta*(SPON: J.B. Richardson). McGill University, Montreal, Quebec, Canada H3A 2B4

Segments of human cerebral arteries were removed from the circle of Willis during 13 autopsies conducted within 6 hours of death. Innervation and pharmacological responsiveness of the smooth muscle were studied in isolated tissue baths equipped with electrodes for field stimulation. Agents which contracted the basilar artery include 5-Hydroxytryptamine (5-HT) (ED50=8.7 x 10-7M), noradrenaline (NA) (ED50=2.6 x 10-6M) and histamine (ED50=3.9 x 10-5M). Maximal contractions produced by NA and 5-HT were 28 and 58% of the maximal response to histamine. Acetylcholine had no significant effect. Ergonovine produced a slight increase in tone in only 1 of 7 basilar arteries while the remaining 6 were either unresponsive or showed relaxation. Adenosine triphosphate (ATP) (ED50=1.0 x 10-4M) could decrease maximal contractions produced by 5-HT and histamine by an average of 56% (n=7). Sensitivity to drugs did not vary with anatomical location as similar results were obtained using basilar, middle and anterior cerebral arteries. All areas were innervated and contractions to electrical stimulation occurred within a range of 0.5 to 7 msec and 1 to 100 Hz. Our results indicate that the therapeutic effect of ergot alkaloids does not involve direct vasoconstriction of intracerebral arteries. Although neural control of human cerebral vessels has previously been questioned, we found excitatory innervation in all areas.

MECHANICS OF BREATHING II

146.1

IMMEDIATE VENTILATORY RESPONSES TO INSPIRATORY FLOW RESISTIVE LOADING IN WAKEFULNESS AND NREM SLEEP. B.R. Miller*, C.M. Shield*, and G. Bowes. Respiratory Service, Alfred Hospital, Melbourne, Australia.

Mechanisms responsible for load compensation to flow resistive loads may be altered by normal sleep. Four normal subjects (2 female;2 male) age 25-39 years, were studied during quiet wakefulness (W) and non rapid eye movement sleep (NREM) using a nasal mask connected to a Hans Rudolph valve and pneumotach. Loads of 10 (L10) and 20 (L20) cmH2O/L/sec were applied for 2 breaths. The first loaded breath was compared to the average of 5 control breaths measured prior to each load. During control breathing NREM resulted in a 13.5% fall in minute ventilation (VI) (W:7.25±1.35L/min; NREM:6.27±1.23L/min). Loading decreased VI as compared to control in 25 of 26 trials during W, and in all 53 trials during NREM. Considerable intersubject variability was seen, but in 3 subjects significantly greater falls in VI were observed with L10 during W (20.6±12.2%) as compared to NREM (35.1±8.1%) ($p<0.001$, non-paired t-test). During W group mean VI decreased from 7.25±1.35L/min to 5.73±1.88L/min with L10, and to 4.07±0.87L/min with L20. In NREM loading decreased VI from 6.27±1.23L/min to 3.96±0.55L/min with L10, and to 3.54±1.08L/min with L20. In all trials the decrease in VI was due mainly to a fall in tidal volume. We conclude that there is measurably less preservation of VI in response to flow resistive loads during NREM than W. Supported by NH&MRC Aust.

146.2

VAGAL REFLEXES INVOLVED IN PHASIC LUNG DEFLATION (PLD) IN ANESTHETIZED DOGS. Eun J. Cha* and Stanley M. Yamashiro. Biomedical Engr., Dept., Univ. of So. Calif., Los Angeles, CA 90089-1451, & Chung-Buk Medical School, Cheong-Joo, Korea*.

Expiratory muscle activation, such as occurs during normal exercise, was simulated by inflating a vest only during the expiratory phase (PLD) in 5 dogs under Chloralose anesthesia. This led to a decrease in FRC of 218±40 ml SD (52% of control V_T, $p<.0001$), a transient increase in tidal volume (V_T) of 35% ($p<.01$), and a decrease in total breath period (T_B) of 70% ($p<.05$). The V_T increase gradually disappeared within 7 breaths, even when arterial CO_2 was controlled to within ±1 Torr ($p>.4$), while T_B was only partially recovered and remained shortened by 40% ($p<.01$). When both cervical vagi were cooled in 2 dogs to 4-6°C, which blocked the Hering-Breuer reflex (apnea following lung inflation), the transient V_T increase was maintained. Further cooling to 1-4°C, where the deflation reflex (immediate tachypnea following lung deflation) was also blocked in 3 dogs, resulted in a similar V_T response to that at 4-6°C. At this temperature (1-4°C), T_B changed neither transiently nor in the steady state ($p>.5$). These results suggest that expiratory assist by PLD leads to significant V_T increase only in the absence of the Hering-Breuer reflex. Tachypnea observed during PLD is due to the lung deflation reflex, and is blocked at a lower temperature than the Hering-Breuer reflex. (Supported by NIH HL-07012 and HL-16390).

146.3

IS CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) ASSOCIATED WITH A SPECIFIC INSPIRATORY FLOW (insp $\dot{V}$) PATTERN DURING EXERCISE? G. Feiss*, D. Henson*, S. Levy*, P. Nag* and S. Levine. VAMC and Medical College of Pennsylvania, Philadelphia, PA 19104

To answer this question, we studied 11 COPD subjects (COPDs) and 8 age-matched normals (AMNs) during both rest and graded treadmill exercise (GXT). COPDs were 66±SEM 2 years and had an FEV 1.0 of 1.0±.1 liters; AMNs were 60±2 years and had an FEV 1.0 of 3.7±.2 liters. All AMNs completed the full 12 stages (ST) of the GXT, whereas COPDs completed only 6±1 ST. At rest, COPDs and AMNs exhibited peak insp $\dot{V}$ at 50% of inspiratory time (Ti). As $\dot{V}E$ increased with exercise intensity, peak insp $\dot{V}$ in both AMNs and COPDs occurred at 25% Ti. During all exercise levels, AMNs exhibited constancy of insp $\dot{V}$ from 25% through 75% Ti. In contrast, during exercise, COPDs exhibited a progressive decrease in insp $\dot{V}$ during the latter half of Ti. These results indicate that COPD is associated with a specific inspiratory flow pattern during exercise. We believe that differences in inspiratory pressure generation between COPDs and AMNs during exercise adequately account for our present observations (Levine et al, JAP, Aug 1988).

146.4

A MODEL OF IMPERFECT ELASTICITY OF THE HUMAN CHEST WALL. D. Stamenovic*, G.M. Glass*, G.M. Barnas* and J.J. Fredberg. Boston University, The Biomechanics Institute, Harvard University, Boston, MA 02215, University of Maryland, Baltimore, MD, 21201.

To study the nonlinear behavior of the human chest wall during oscillatory forcing we used four viscoplastic models. Each consisted of three compartments: a nonlinear plastoelastic compartment mechanically in parallel with a linear viscoelastic compartment both in series with a lumped inertia. The plastoelastic compartment in all four models was given as a series of Prandtl bodies (spring and dry friction), as originally proposed by Hildebrandt (*J. Appl. Physiol.*, 28:365-372, 1970). The viscoelastic (spring and dashpot) compartment was either a Maxwell body, a generalized Maxwell body, a Kelvin-Voight body, or a standard linear solid. The proposed viscoplastic models were fit to impedance measurements of the human chest wall (*J. Appl. Physiol.* in press) by a least squares technique, and parameters of the plastoelastic, viscoelastic and inertial compartments were estimated. The parameter values indicated that for the frequency range from 0.1 to 2.0 Hz, the mechanical behavior of the chest wall can be characterized as predominantly plastoelastic. Supported by HL33009 and HL36958.

146.5

ON THE IMPERFECT ELASTICITY OF LUNG TISSUES. J.J. Fredberg and D. Stamenovic*. The Biomechanics Institute, Harvard University, and Boston University, Boston, MA 02215

Out-of-phase behavior of lung tissues is expressed classically through the resistance paradigm: $\Delta P = (Edyn + j\omega Rtis)\Delta V$, wherein elasticity and dissipation are treated as independent processes. We considered the hypothesis that it is better described by the paradigm: $\Delta P = Edyn\ (1 + j\upsilon)\Delta V$, wherein dissipative and elastic processes are treated as being explicitly coupled at the level of the stress-bearing element. Using it, changes in out-of-phase behavior (υEdyn) may be apportioned into that part secondary to concomitant change in dynamic elastance, Edyn, versus that part attributable to change in hysteresivity, υ, the latter being related to K of Bachofen and Hildebrandt (*J. Appl. Physiol.* 30:493-497, 1971). Data in the literature were re-examined and reveal that whereas Rtis varies widely with frequency, tidal volume, lung recoil, volume history and lung size, υ is to a startling degree invariant, generally falling in a narrow range between 0.1 and 0.3. Such behavior is consistent with the notion that associated changes in Rtis are secondary to changes in Edyn, that elastic energy storage and energy loss reside in the very same stress-bearing elements, and moreover, that the relationship between those processes within the stress-bearing element bears a fixed relationship. Supported by HL33009 and HL36958.

146.6

IMPERFECT ELASTICITY OF RESPIRATORY TISSUES. G.M.Barnas* D. Stamenovic*, S. Loring and J.J. Fredberg. University of Maryland, Baltimore, MD, Boston University, The Biomechanics Institute, and Harvard University, Boston, MA 02215

We have found that the mechanical impedances of several respiratory system components show strikingly similar responses to sinusoidal forcing, including the intact chest wall (both with respiratory muscles relaxed and activated), intact rib cage (relaxed and activated), intact diaphragm (relaxed and activated), isolated rib cage (with and without intercostal muscles), isolated abdominal contents, isolated skeletal muscle (both relaxed and tetanized), and isolated lungs. Resistance (stress out-of phase with strain per unit rate of strain) decreases hyperbolically as frequency increases from 0.1 to 4 Hz. In the same range elastance (stress in-phase with strain per unit strain) increases to some maximum and then decreases. Both resistance and elastance decrease as amplitude of forcing increases in this frequency range. These results cannot be explained by a linear model, but are consistent with viscoplastic behavior (Hildebrandt, *J. Appl. Physiol.* 28:365-372, 1970). We conclude that various constituents of the respiratory system share similar nonlinear behavior, and that measurements of elastance and resistance are dependent upon both the frequency and amplitude of forcing. Supported by HL33009 and HL36958

146.7

THE RESONANT FREQUENCY OF CENTRAL AIRWAYS GAS INVESTIGATED WITH GASES OF DIFFERENT DENSITIES. J. Sato*, T. Abe*, P. Romero* and J.H.T. Bates. Meakins-Christie Laboratories, McGill University, Montreal, Quebec, H3A 2B4 Canada.

When flow at the airway opening of a ventilated animal is suddenly interrupted, the airway opening pressure just behind the point of interruption exhibits high frequency oscillations that are rapidly damped. Damped ringing such as this requires the presence of a mass and an elastance (to produce the oscillations) and a resistance (to produce the damping). The elastance involved may be that of the respiratory system and the resistance that of the airways. It has been postulated that the mass responsible for these oscillations is that of the gas in the central airways. We investigated this phenomenon by performing flow interruptions during passive expiration in a normal dog, with the lungs filled with three different gas mixtures: 1) air, ii) 80% helium: 20% oxygen, and iii) 50% sulfer hexafluoride: 50% oxygen. The densities of these three gas mixtures are in the ratios 1.0:0.33:3.0. We found the frequency of pressure oscillations with the three mixtures to be 258 Hz, 410 Hz and 146 Hz, respectively. The relative magnitudes of these frequencies are very similar to the relative magnitudes of the square roots of the densities of the gases. This indicates that the post-interruption ringing is due to the mass of the central airways gas.
(Supported by MRC Canada.)

146.8

PARTITIONING LUNG RESISTANCE INTO AIRWAY AND TISSUE COMPONENTS IN RATS. P. Romero*, S. Sapienza* and M.S. Ludwig* (SPON: J.H.T. Bates). Meakins-Christie Laboratories, McGill University, Montreal, PQ and Hospital Universitario de Bellvitge, Barcelona, Spain.

Total lung resistance (R_L) can be partitioned into airway resistance (Raw) and tissue viscance (Vti), the pressure drop in phase with flow between the alveolus and the pleura, using the alveolar capsule technique which allows direct measurement of alveolar pressure. Recent measurements in mongrel dogs have shown that Vti accounts for up to 80% of R_L during low frequency ventilation at lung volumes near functional residual capacity (FRC). We were therefore interested to see whether Vti was as marked in other species. In 6 anaethetized, intubated, open-chested, mechanically ventilated Sprague-Dawley rats we measured tracheal and alveolar pressure with miniature piezoresistive pressure transducers, and tracheal flow with a pneumotachograph during tidal breathing (f=1Hz, tidal volume = 5.5 ml/kg, PEEP = 5.0 cmH_2O). We calculated R_L, Raw, Vti and lung elastance (E_L). Baseline R_L = 68.0±11.7 $cmH_2O/l/s$ (mean±sd), Vti = 17.9±12.4 $cmH_2O/l/s$, Raw = 68.0±11.7 $cmH_2O/l/s$, and E_L = 1.79±.62 cmH_2O/ml. The difference in E_L measured at the alveolar and tracheal levels was < 0.5%. The mean (±sd) proportion of R_L attributable to Vti in these 6 rats was 25.9±15.1% (range 7.7-51.8%). Hence in rats, at lung volumes near FRC and at low breathing frequencies, Vti contributes less of the total resistive pressure drop than that described in mongrel dogs. In addition there is marked interanimal variability in the contribution of Vti to R_L. (Supported by MRC Canada and FIS Spain.)

146.9

THE CONTRIBUTION OF STATIC TISSUE HYSTERESIS TO THE DYNAMIC ALVEOLAR PRESSURE-VOLUME LOOP. F.Robatto*, P.Romero* and M.S. Ludwig*. (SPON:J.H.T.Bates.) Meakins-Christie Labs., McGill Univ., Montreal, PQ. and Hosp. Univ. de Bellvitge, Barcelona, Spain.

The area inside the tracheal pressure-volume (Ptr-V) loop is indicative of the energy dissipated within the respiratory system during a breath. Recently, tissue viscance, the pressure drop in phase with flow between the alveolus and the pleura, has been shown to account for most of the PV loop area during tidal breathing in mongrel dogs. However, the contribution of static hysteresis to this area is not known. We recorded dynamic and quasistatic PV loops during tidal ventilation in anaesthetized, open-chested, dogs. We measured tracheal flow and pressure (Ptr), and alveolar pressure (Palv) in three different regions using alveolar capsules. We recorded measurements during tidal ventilation, and during a quasistatic inspiration and expiration over the same tidal volume (Vt) range (Vt=15ml/kg, f=0.3Hz, PEEP=5cmH_2O). We then delivered ultrasonically aerosolized methacholine (MCh), (100mg/ml) and repeated the measurements. During the quasistatic maneuver, under control and constricted conditions, the difference between Ptr and Palv was negligible. We found that the area of the dynamic Palv-V loop accounted for 77.5±1.2% of the dynamic Ptr-V loop at baseline and 63.0±21.6%, after MCh-induced constriction. The area of the static PV loop accounted for <10% of the dynamic Palv-V loop at baseline and 23.3±9.7% after constriction. We conclude that static tissue hysteresis contributes only a small amount to the dynamic Palv-V loop. This contribution increases after MCh-induced constriction. (Supported by MRC Canada and FIS Spain.)

146.10

FITTING OF EXPONENTIAL PRESSURE-VOLUME CURVES: SENSITIVITY TO NOISE AND COMPLETENESS OF DATA. D.H. Eidelman*, H. Ghezzo* and J.H.T. Bates. Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada, H3A 2B4.

The exponential relationship $V=A-Be^{-kp}$, (V = lung volume, P = transpulmonary pressure), is frequently employed to describe pulmonary static pressure-volume relationships in humans. The parameters of this equation, particularly K, have been employed as measures of the intrinsic elastic properties of the lung. Physiological interpretation of K, however, is meaningless without knowledge of the confidence intervals about the estimate of this parameter. We developed a method for testing the sensitivity of the parameters A, B, and K to noise and to completeness of the data. A, B, and K were determined by an iterative least-squares method. Confidence intervals on the fitted parameters were determined by Monte Carlo simulation. Sensitivity to the availability of data points at high lung volumes was assessed by progressively removing the high volume data points and refitting the curve. Sensitivity to the availability of data points at low lung volumes was assessed in a similar manner. Addition of realistic levels of noise resulted in 95% confidence intervals about K as large as 50% of the value of K itself. Progressive removal of data at both high and low lung volumes resulted in large increases in the confidence intervals, and changed the best fit value of K by as much as three-fold. Thus, interpretation of the physiological significance of an estimate of the exponential index K must be preceded by an assessment of its confidence intervals. (Supported by MRC Canada.)

146.11

EFFECT OF TEMPERATURE ON LUNG PRESSURE-VOLUME(P-V) HYSTERESIS AT DIFFERENT END-EXPIRATORY PRESSURES(EEPs) IN CONTROL AND CHLORPHENTERMINE(CP)-TREATED RATS. T.A. Jones*, D.G. Frazer, E.L. Petsonk* and M.J. Reasor. Depts. of Physiol. and Pharmacol., WV Univ., and Div. Resp. Dis. Studies, NIOSH, Morgantown, WV 26505

Three sets of curves were recorded for excised rat lungs at 24,47, and 24°C. In each set of curves the lungs were degassed, inflated to 30 cm H_2O ($P_L$max) and deflated to 0 cm H_2O. Inflation-deflation cycles were then performed between $P_L$max and successively decreasing EEPs. Hysteresis area (A) and tidal volume (V_T) were determined for each cycle. This was repeated in rats treated with CP (10 days; 30 mg/kg i.p.), a drug known to cause pulmonary lipidosis. Normalized hysteresis (A/V_T) was plotted vs. EEP. In control rats at 24°C, A/V_T remained relatively constant as EEP was reduced to +3 cm H_2O. Large increases occurred in A/V_T as the EEP was further reduced. At 47°C, the A/V_T relationship shifted markedly toward higher EEPs. In the CP-treated group, no difference from controls was seen at 24°C, while at 47°C the shift toward higher EEPs was significantly less. We previously showed the closing-opening(C-O) of lung units contributes to P-V hysteresis and is EEP-dependent, increasing at EEPs <+3 cm H_2O (Physiologist 29:144). This data suggests, therefore, that at increased temperature, C-O of lung units occurs at higher EEPs, and treatment with CP partially reduces this temperature effect.

146.12

TRANSVERSE AND LONGITUDINAL ELASTIC WAVE PROPAGATION IN INFLATED LUNGS. M. Jahed*, P. K. Bhagat*, Q. Gu*, S. S.Kraman and S. J. Lai-Fook. Biomedical Engineering Center, University of Kentucky, Lexington, KY 40506.

If the lung is an elastic continuum, both longitudinal and transverse stress waves should be propagated in the medium with distinct velocities. In 5 isolated sheep lungs, we investigated the propagation of stress waves. The lungs were degassed and then inflated to a constant deflation transpulmonary pressure (Ptp). We measured signals transmitted at locations ~1, 5 and 10 cm from an impulse surface distortion using small microphones on the pleural surface. Two transit time measures were computed from the first two significant peaks of the cross-correlation of microphone signal pairs. The "fast" wave velocities averaged 301 ± 92 (SD), 432 ± 93 and 577 ± 211 cm/s for Ptp values of 5, 10 and 15 cmH_2O, respectively. Corresponding "slow" wave velocities averaged 139 ± 22, 217 ± 36 and 255 ± 89 cm/s. The fast waves were consistent with longitudinal waves of velocity $[(K+4G/3)/\rho]^{1/2}$, where K is the measured bulk modulus, G is the shear modulus and ρ is the lung density. The slow waves were consistent with transverse waves of velocity $(G/\rho)^{1/2}$, with a G value of 0.9 Ptp. Thus, stress wave velocities measured on pleural surface of isolated lungs correlated well with elastic moduli of lung parenchyma. (Supported by HL 40362 and HL 36597).

146.13

EFFECTS OF BODY POSITION & LUNG VOLUME ON IN SITU LENGTH OF CANINE DIAPHRAGM. S.S.Margulies*, G.A.Farkas, D. Olson*, K. Gosse*, J.R.Rodarte Mayo Fdn, Rochester, MN 55905.

The performance of the diaphragm (DPM) is influenced by its in situ length (L) relative to its optimal force-generating length (L_0). We sewed 3-4 lead markers along bundles of DPM in the left ventral (CoV), middle (CoM) and dorsal (CoD) regions of the costal DPM and the left crural (Cr) DPM of 6 beagles. After 2-3 weeks recovery, the dogs were scanned anesthetized and paralyzed in the DSR at TLC (+30 cm H_2O P_{ao}), FRC, and RV (-30 cm H_2O P_{ao}), and L's were determined. Each marked DPM bundle was removed, mounted in a 37°C in vitro chamber, and adjusted to L_0 (maximum tetanic force). In vitro length-tension characteristics were similar for all DPM regions. In situ lengths (%L_0 ± SD) in the various regions were not uniform. The table summarizes our results.

	Prone			Supine		
	TLC	FRC	RV	TLC	FRC	RV
CoV	78(13)†	83(16)†	96(12)	78(13)†	84(11)†	93(8)
CoM	74(10)*†	88(10)*	104(8)	84(6)*†	100(6)*	104(5)
CoD	58(8)†	86(10)†	104(12)	67(11)†	90(11)	97(16)
Cr	62(3)*†	78(10)*†	102(12)	69(6)*†	91(9)*	100(11)

*p<0.05 prone different from supine †p<0.05 different from L_0

We conclude that some regions of the DPM shorten when posture is changed from supine to prone. Variability in length is least at RV and increases with increasing lung volume. There is no clear gravity-dependent vertical gradient in L at FRC in either posture. (Supported by grants HL04664, HL21584 and HL07222.)

146.14

LUNG/ CHEST WALL SHAPES IN MINIATURE HORSES BY X-RAY CT. E.A. Hoffman, E.L. Ritman, D.E. Mason*, and L.E. Olson. U. of Penn., Mayo Clinic, and Ohio S U: Phila., PA: Roch., MN; and Col., OH.

Ppl measurements in ponies suggest that supine, the lower 50% of the lungs may be collapsed. (JAP 64: 102, 1988) These data have lead to quantification of regional equine lung and chest wall geometry and a comparison with dogs and sloths (JAP 59: 481, 1985). Two miniature horses, were anesthetized and scanned supine and prone in the Dynamic Spatial Reconstructor with stepwise lung inflation. FRC volumes were measured by a resident gas technique. Inspiratory capacity as well as FRC gas volumes were reduced in supine posture. Image data from one horse demonstrate that the change in chest wall geometry associated with reduced lung volume supine includes a flattening of the diaphragm with the dorsal surface ascending headwords and a concomitant but less pronounced caudal descent of the ventral diaphragm. Lung height increases supine with little change occurring in the lateral rib cage dimensions. With positive pressure lung inflation, the nondependent diaphragmatic region descended more than did dependent regions in both postures. With the bulk of the lung in the nondependent region, in the prone body posture a lever motion of the diaphragm facilitates nondependent lung region expansion. Directionally similar but exaggerated chest wall shape changes in the horse compared with dogs coupled with markedly different lung geometry and absence of lobar fissures may contribute to supine respiratory compromise. (funded in part by an OSU equine res. grant)

146.15

DISTORTABILITY OF THE HUMAN RIB CAGE. M. Ward*, J. Ward*, F.Bellemare and P.T. Macklem. Montreal Chest Hospital, McGill University, Montreal, H2X2P4.

We model the rib cage(RC) as 2 parts: lung apposed (RCpul); diaphragm (DI) apposed (RCabd). Agencies displacing RCpul are: 1)pleural pressure (Ppl); 2) rib cage muscles,(RCM);3) mechanical linkage(ML) between RCpul and RCabd.Agencies displacing RCabd are:1)abdominal pressure (Pab); 2) DI; 3) ML.Accordingly DI drives RCpul and RCM drive RCabd only by ML.We measured cross-sectional areas of RCpul and RCabd(Arc,pul and Arc,abd),Ppl, Pab and EMG's of RCM and DI during relaxation,quiet breaths, pure DI and pure RCM Meuller maneuvres, pure DI(1 subject) and pure RCM breaths and bilateral phrenic nerve twitches (ψ) with closed airways in 5 normal subjects. Arc,pul and Ppl during ψ and Arc,abd and Pab during RCM Meullers,relative to relaxation allowed estimate of ML.Knowing ML and distortions during quiet breaths we measured RCM contributions to Arc,pul and Ppl and DI contribution to Arc,abd.We found that ML is much tighter than in dogs and can contribute importantly to $\triangle$Ppl. Since distortion during quiet breaths is small relative to DI contraction,we conclude that RCM contributes importantly to Arc,pul during quiet breaths. Supported by the MRC of Canada.

146.16

EFFECTS OF CPAP AND POSTURE ON DIAPHRAGMATIC SHORTENING IN VIVO. A.M. Leevers and J.D. Road, Department of Medicine, University of British Columbia, Vancouver, B.C., Canada

In a previous communication (Fed.Proc.2(5)A1497) we demonstrated a marked reduction in ΔPdi, V_T and tidal diaphragmatic shortening during lung inflation to two times V_T in supine vagotomized dogs. We postulated that this was a result of length-tension properties or changes in abdominal compliance. In this study, we attempted to determine which mechanism predominated. Four pentobarbital anesthetized, vagotomized dogs were studied in the supine position during CPAP and at various degrees of upright tilt. V_T and tidal shortening of the crural diaphragm ($\%L_{FRC}$) were compared to control values in the supine position. As before, CPAP produced a prompt reduction in V_T and $\%L_{FRC}$. Initial diaphragm length also decreased but there was no change in peak EMG activity of the crural diaphragm (EMG_{cru}). With tilting, similar reductions in initial length were produced as the weight of the abdominal contents decreased and EMG_{cru} was unchanged. A change in initial length of 15% produced a reduction in V_T of 56±15 during CPAP and 68±11 (% of control ± S.E.) during tilting. Tidal crural $\%L_{FRC}$ was 76±6 during CPAP and 56±6 (% of control ± S.E.) during tilting. These results suggest that changes in initial resting length are more important than changes in abdominal load in determining crural diaphragmatic shortening.

Supported by the B.C. Lung Association and MRC of Canada.

146.17

REGIONAL DIAPHRAGMATIC WORK VERSUS PRESSURE VOLUME WORK DURING LOADED BREATHING. J.D. Road and A.M. Leevers. Department of Medicine, University of B.C., Vancouver, B.C., Canada.

The work of breathing may be estimated from the change in volume and pleural pressure. However, this measure of work does not take into account work done on distortion of the chest wall or gas decompression. Loading conditions which produce phase lags between muscle shortening, pressure and volume, may produce varied estimates of work depending on the parameter measured. We measured crural diaphragmatic shortening ($\%L_{FRC}$), ΔPdi, and V_T during expiratory threshold loading (ETL) in 6 supine pentobarbital anesthetized dogs. ETL ranged from 0 to 20 cm H_2O. At the maximum load crural $\%L_{FRC}$ increased to 160±15 and ΔPdi to 220±18 (% of control ±S.E.). However, V_T decreased to 81±24% of control. Crural peak integrated EMG activity increased to 280±21% of control. Construction of pressure (ΔPdi)-length ($\%L_{FRC}$) loops for the diaphragm gave an estimate of regional diaphragmatic work. This work increased to 260±33 % of control at the maximum work load of 20 cm H_2O. However, pressure volume plots did not support such an increase, as the inspiratory work of breathing was unchanged. We conclude that during loaded breathing, measures of external work may markedly underestimate diaphragmatic work.

Supported by B.C. Health Care Research Foundation and MRC of Canada.

146.18

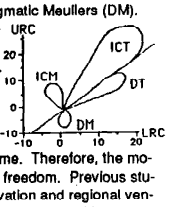
THEOPHYLLINE ACCELERATES FATIGUE AND CHANGES FORCE-FREQUENCY CURVE OF RAT DIAPHRAGM IN A TIME- AND DOSE-DEPENDENT MANNER. Karen E. Haack*, Beatriz Londoño*, Michael J. Miller*, Michael B. Reid. Harvard School of Public Health, Boston, MA 02115, and VA Medical Center, Brockton, MA 02401.

Theophylline 500 μg/ml increases fused tetanic diaphragm fatigue in vitro (Miller & Reid, Am Rev Respir Dis, 137(4): 70, 1988). Present experiments further tested this toxicity. Diaphragm strips from 28 rats were directly stimulated in vitro (optimal length; 37°C). Strips were treated with theophylline at 0 (control), 50, 150, or 500 μg/ml. Isometric forces were measured during twitch (P_1), unfused tetany (P_{45}) and fused tetany (P_{160}) every 30 min for 210 min. Fatigue was assessed by decline of P_{160}. Control P_{160} fell 14% over 210 min. Theophylline at 50, 150, and 500 μg/ml produced steeper declines of 27, 20 and 28%. Shape of the force-frequency curve, estimated by the ratios of P_1 and P_{45} to P_{160}, was stable for control strips over 210 minutes. Theophylline 500μg/ml elevated P_1:P_{160} and P_{45}:P_{160} within 30 min; these elevations were also stable. Concentrations of 50 and 150 μg/ml produced smaller, time-dependent increases. In particular, P_1:P_{160} progressively increased over 210 min. Thus, theophylline accelerates fatigue of isolated diaphragm strips. Effects on the force-frequency curve are dose-dependent and can require >4 hrs to reach equilibrium in vitro. (Supported by NIH AA-07134 and VA Medical Research.)

146.19

Rib Cage Distortion During Inspiratory Maneuvers. BA Zielinski, MG Sampson and GC Smaldone. Dept. Med. Pulm. Div., SUNY at Stony Brook, Stony Brook, N.Y.

Regional circumferential change of the rib cage was studied in six healthy male volunteers at the upper (URC) and lower (LRC) rib cage with Respitrace bands. URC was set equal to LRC during quiet breathing. Five inspiratory maneuvers were studied: quiet (QB), intercostal (ICT) and diaphragmatic tidal breathing (DT) at inspiratory flow rates < 1 l/sec., and intercostal (ICM) and diaphragmatic Meullers (DM). ΔURC was plotted against ΔLRC and data from a representative subject is shown. The line of identity was defined during QB and relaxation at various volumes against a closed glottis. Inter- costal maneuvers increased the URC/LRC ratio or caused frank LRC paradox. Diaphragmatic maneuvers decreased this ratio and/or caused URC paradox. These data show that voluntarymuscle group activation can regionally distort rib cage motion and presumably regional rib cage volume. Therefore, the motion of the rib cage cannot be described by a single degree of freedom. Previous studies investigating the relationship between muscle group activation and regional ventilation did not assess the contribution of rib cage distortion to the relationship between ΔPpl and regional ventilation. These studies did show that inspiratory efforts using select inspiratory muscle groups cause greater pleural pressure swings (ΔPpl) over their regions of activity (Irvin CG, MG Sampson, L Engel, and AE Grassino, J. Appl. Physiol.,57(1):168-175, 1984.), which in turn correlate with changes in regional ventilation (Sampson MG and GC Smaldone, J. Appl. Physiol., 56(1):196-20, 1984). However, during manuevers causing significant rib cage distortion the relationship between regional ΔPpl and ventilation has not been described. It is possible that regional ventilation may not correlate only with regional ΔPpl at low inspiratory flow rates. Regional rib cage motion may make a significant contribution. This speculation is currently being inverstigated.



146.20

RECRUITMENT OF VENTILATORY MUSCLES DURING MILD AND MODERATE EXERCISE IN HEALTHY NONATHLETIC HUMANS. L. Dal Vecchio*, R. Poggi*, M. Rossi*, L. Appendini*, and A. Rossi. Institute of Occupational Medicine, University of Padua, 35127 Padua, Italy.

We measured changes in esophageal pressure (Pes), gastric pressure (Pga), and transdiaphragmatic pressure (Pdi), in 6 normal subjects (mean±SD age: 32.8±8.7yr) at rest and during increasing moderate exercise (3 cycling at 30-60-90-180 Watt) to assess the relative contribution of the diaphragm and rib cage ventilatory muscles to the changes in ventilation (VE) (Macklem et al. JAP 1978;44:200). Average ±SD results were as follows:

WATT	0	30	60	90	180
VE (l/m)	8.9±1.8	22.2±2.3	23.8±4.1	39.7±11.1	84.8±30.9
ΔPes (cmH2O)	2.7±1.9	3.7±2.0	6.5±2.7	11.9±6.1	17.1±6.6
ΔPga (cmH2O)	4.7±3.1	5.9±3.1	6.9±2.3	7.8±6.5	6.1±4.4

The mean progressive increase in Pdi swing (ΔPes+ΔPga) with increasing work load and VE, was associated with increasing ΔPes without significant changes in ΔPga. On the average, the end-expiratory (EE) pressures changed as follows:

WATT	0	30	60	90	180
Pes (cmH2O)	-5.6±2.4	-5.7±5.1	-3.5±5.2	-1.6±6.6	2.1±6.7
Pga (cmH2O)	5.2±1.4	6.7±5.5	5.8±4.6	6.9±5.2	4.6±3.9

On the average, EEPes increased (less negative) whereas EEPga did not change. In two subjects EEPes progressively decreased (more negative) from 0 to 90 Watt, and then slightly increased (less negative) at the last step (180 watt). In three subjects EEPga progressively decreased (less positive). These findings indicate that, in healthy nonathletic subjects, the increased ventilatory demand due to exercise, at least for cycling, was matched, since the mildest load, by a significant recruitment of the rib cage muscles, more than an increase in diaphragmatic activity. (Supported by the MRC and MPI, Italy, and by a grant from ECCS).

146.21

REGIONAL COMPLIANCE AND BRONCHIAL PRESSURE-DIAMETER RELATIONSHIPS IN EXCISED PIG LUNGS. L.E. Olson, V.P. Wright* and L.M. Tobin*. The Ohio State University, Columbus, OH 43210-1092.

Parenchymal interdependence was evaluated by comparing the ratio of the specific compliance of the lung to the specific compliance of an obstructed lung region with the pressure-diameter (PD) curves of tantalum dusted airways in the obstructed lung region in excised pig lungs. Radiographs were taken when tracheal pressure (Pao) and pressure in the obstructed lung region (Ps) were equal at 0, 5, 10, 15, 20, 25 and 30 cm H_2O; the homogeneous case, and when Pao was held constant at 5, 10 and 15 cm H_2O and Ps was increased to Pao + 5, Pao + 10 and Pao + 15 cm H_2O; the nonhomogeneous case (n = 8). Pressure-volume (PV) curves of the obstructed lung region were recorded when Pao was constant at 0, 5, 10, and 15 cm H_2O and were compared with the PV curve of the lung (n = 4). Normalized diameters (D/D @ 25 cm H_2O) increased as Pao increased in the homogeneous case. D/D_{25} increased as Ps increased in the nonhomogeneous case at Pao = 5 cm H_2O only. Continuum mechanics analysis of the nonhomogeneous case at Pao = 5 cm H_2O predicted boundary pressure (Pb) at the interface between the obstructed region and the surrounding lung to be 1.0 cm H_2O at Ps = 10 cm H_2O and 1.4 cm H_2O at Ps = 15 cm H_2O and compared well with Pb's predicted from the PD curves which were 2.2 cm H_2O and 1.4 cm H_2O respectively. The small increase in Pb indicates that interdependence is minimal in pig lungs. (HL37246)

146.22

ALVEOLAR LIQUID PRESSURES IN NONEDEMATOUS AND KEROSENE WASHED RABBIT LUNG BY MICROPUNCTURE. S. Ganesan*, S. J. Lai-Fook, S. Schürch. Biomedical Engineering Center, University of Kentucky, KY 40506 and University of Calgary, Alberta T2N4N1, Canada.

In kerosene filled lungs, the pressure drop across the alveolar surfactant-kerosene interface corresponded to a surface tension of ~1.5 dynes/cm (Faseb. J. 2:A1694,1988). The airspaces of isolated degassed lungs were washed with kerosene and inflated with air. Alveolar liquid pressures (Pliq, cmH_2O) were measured by micropuncture at various alveolar air pressures (Palv) on deflation and inflation prior to (control) and after kerosene treatment. Table (mean±SD,n=5) summarizes results:

Volume (V)	Control (deflation)		Kerosene-washed	
%	Palv	Pliq	Palv	Pliq
100	21.6 ± 2.1	2.8 ± 1.1	25	6.2 ± 3.5
50	3.1 ± 0.2	1.0 ± 1.1	8.0 ± 1.3	-1.7 ± 0.4

Surface tension (T) was calculated using Laplace's Law for a spherical interface (radius R): Palv-Pliq = 2T/R. We assumed T was 28 dynes/cm at 100%V and that R varied as $(V)^{1/3}$. In the control and kerosene washed lungs on deflation, T decreased from 28 dynes/cm at 100%V to 2 and 12 dynes/cm at 50%V, respectively. On inflation, T was 15 dynes/cm at 50%V. These results are consistent with values obtained by the microdroplet technique. (Supported by HL 40362)

146.23

CHANGES IN AIRWAY STRUCTURE FOLLOWING REPEATED ANTIGEN CHALLENGE OF SENSITIZED RATS. S. Sapienza*, T. Du*, D.H. Eidelman*, N.S. Wang* and J.G. Martin. Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada.

Repeated inhalation challenge with antigen increases the reactivity of rats' airways to methacholine (MCh). The aim of this study was to relate morphological changes in the airways to increased MCh reactivity. We studied 17 BN rats, 6-8 weeks old, sensitized with ovalbumin (OA) and B. pertussis vaccine. OA aerosols (5% w/v) were administered 14, 19, and 24 days after sensitization. Reactivity to MCh was assessed on days 10, 25, and 29 after sensitization. 16/17 rats had early responses to OA challenge, and 6 showed increased MCh reactivity. Morphometric analysis was done in 5 randomly selected challenged animals with increased airway reactivity to MCh (R), in 5 similarly selected and challenged animals with no change in reactivity (NR), and in 2 controls (C). Lungs were fixed in formalin (10%, 24 h) in inflation at 25 cm H_2O. 5 μ sections were stained with hematoxylin-eosin and length of basement membrane (BM), wall thickness (WT), and smooth muscle area (SM) were measured in 23-75 airways per animal. Airways were divided into 2 groups: A: BM$<$900 μ; and B: BM 901-1600 u. In A and B, SM and AW were greater in R and NR than C ($p<0.05$). In B, SM and AW were greater in R than in NR ($p<0.05$).We conclude that OA exposure increases AW and SM in small and medium-sized airways and that this may account for the increased airway reactivity to MCh.

(Supported by the Medical Research Council of Canada.)

146.24

VAGAL CHOLINERGIC INNERVATION TO THE AIRWAYS OF THE NEWBORN CAT. M. A. Waldron*, B. J. Connelly* and J. T. Fisher. Departments of Physiology and Anesthesiology, Queen's University, Kingston, Ontario, K7L 3N6.

We measured the effects of vagal stimulation on lung mechanics in the newborn cat and the influence of the specific M_1 muscarinic antagonist, pirenzepine. Twenty cats (aged 2-14 days) were anesthetized with chloralose/urethane (75-100 mg/kg and 0.75-1.0 g/kg, respectively). A tracheal cannula was inserted and the animals ventilated with the chest open. The cervical vagus nerves were separated from the sympathetics and placed on bipolar stimulating electrodes. Mean inspiratory resistance (R_{Li}) and dynamic compliance (C_{Ldyn}) were measured on a breath-by-breath basis. Supramaximal stimulation, at frequencies ranging from 2-20/s caused a prompt increase in R_{Li} and a drop in C_{Ldyn}. At the highest frequencies, R_{Li} increased 257$\pm$67.6% from the control value. A 0.01 mg/kg (i.v.) dose of pirenzepine noticeably suppressed the increase in R_{Li} during stimulation (15/s) and 1.0 mg/kg inhibited the response by 94$\pm$1.0%. The M_2 receptor-mediated reduction in heart rate during vagal stimulation was unaffected by low dose ($\leq$0.2 mg/kg) pirenzepine and was only 18$\pm$6.1% inhibited by 1.0 mg/kg of the antagonist. These studies demonstrate that vagal cholinergic innervation to the airways is functional at birth and can be preferentially blocked by an M_1 antagonist. Supported by MRC and the OTS.

150.1

INTERACTIONS BETWEEN COCAINE AND d-AMPHETAMINE OR MORPHINE IN SQUIRREL MONKEYS RESPONDING UNDER A MULTIPLE SCHEDULE OF REINFORCEMENT. G.R. Wenger, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

The interactions between cocaine and d-amphetamine or morphine were determined in squirrel monkeys responding under a multiple fixed-ratio 30, fixed-interval 300 sec (mult FR30 FI300) schedule of reinforcement. When administered alone, cocaine and d-amphetamine produced dose-related decreases in FR30 responding at doses which either had no effect on or increased FI300 responding. In contrast, morphine decreased both FR30 and FI300 responding at the same doses. When the cocaine dose-response curve was redetermined in the presence of either 0.1 or 0.3 mg/kg d-amphetamine no significant interactions were observed on either FR30 or FI300 responding. When low doses of cocaine (0.03 or 0.1 mg/kg) were combined with either 0.03, 0.1, or 0.3 mg/kg morphine FR30 responding was increased compared to cocaine alone. No significant interactions were observed on FR30 responding with higher doses of cocaine. Under the FI300 schedule, 0.03 mg/kg morphine shifted the ED50 for the rate decreasing effects of cocaine to the left, 0.1 mg/kg of morphine increased the rate of responding when combined with moderate doses of cocaine without shifting the ED50 compared to cocaine alone, and 0.3 mg/kg morphine produced no significant interactions when combined with cocaine. Thus, the interactions observed between cocaine and morphine were dose and schedule specific. Supported by NIDA grant #04079.

150.2

THE EFFECTS OF COCAINE IN COMBINATION WITH d-AMPHETAMINE OR MORPHINE ON THE REPEATED ACQUISITION RESPONDING OF PIGEONS. E.B. EVANS* and G.R. WENGER, Dept. of Pharmacology, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

The effects of cocaine in combination with d-amphetamine or morphine on the repeated acquisition of a four-response chain were investigated in pigeons. Food was presented upon the completion of the sequence under a fixed-ratio schedule. Incorrect responses resulted in a five-second timeout. Low doses of cocaine (0.1 or 0.3 mg/kg) combined with 1 mg/kg d-amphetamine increased the total session percent correct (accuracy) above the effect produced when these drugs were administered alone. The interaction of 0.1 mg/kg cocaine and 1 mg/kg d-amphetamine shifted the "learning" curve (within-session accuracy) upward and to the left compared to the cocaine and d-amphetamine curves. Higher doses of cocaine combined with 1 or 1.8 mg/kg d-amphetamine produced no significant changes in total percent correct or shifts in the "learning" curves compared to the effects produced by cocaine and d-amphetamine alone. No significant interactions were observed between cocaine and 0.3 or 1 mg/kg morphine. When cocaine, d-amphetamine or morphine was given alone only dose-related decreases were observed in response rate. The combinations of cocaine with 1 or 1.8 mg/kg d-amphetamine, or 0.3 or 1.0 mg/kg morphine decreased the rate of responding in a dose dependent manner similar to the effect produced by administration of cocaine alone. (Supported by NIDA Grants #04079 and #02251)

150.3

"ANXIOGENIC" EFFECTS OF CHRONIC COCAINE TREATMENT ON BEHAVIOR IN THE CONDITIONED SUPPRESSION OF DRINKING (CSD) CONFLICT PROCEDURE. D.J. Fontana* and R.L. Commissaris* (SPON: R.T. Louis-Ferdinand). Wayne State University, College of Pharmacy & AHP, Detroit, MI 48202.

Cocaine is a highly abused drug whose subjective effects have been characterized as stimulating, euphoric and highly rewarding. However, it has been reported that repeated or chronic use of high doses of cocaine produces an anxiety state in humans. The present studies examined the effects of chronic cocaine administration on behavior in the Conditioned Suppression of Drinking (CSD) conflict paradigm, an "animal model" for the study of anxiety. In daily 10-minute sessions, water deprived rats were trained to drink from a tube which was occasionally electrified (0.25 mA), electrification being signalled by a tone. Within 3-4 weeks, control (i.e., non-drug) CSD behavior stabilized (25-50 shocks and 8-12 ml/session) and chronic cocaine (10 mg/kg, i.p., 2/day) or saline treatments were initiated. As expected, chronic saline had no effect on CSD performance over the course of 7 weeks of testing. Although having no effect on CSD behavior during the first week of treatment, chronic cocaine produced a significant decrease in punished responding over time, but no change in water intake. This "anxiogenic" action was stable over the Week 2-7 period. These results suggest that the CSD procedure may be an effective model for studying the anxiogenic effects of chronic cocaine use in man. (MH #42501-01)

150.4

SHORT-TERM MEMORY (STM) IN RATS AS ASSESSED BY SINGLE-SPATIAL ALTERNATION BEHAVIOR WITH VARIABLE RETENTION INTERVALS (RI): EFFECTS OF SCOPOLAMINE. H. E. Shannon, K. G. Bemis* and J. C. Hart*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 48285.

Male F344 rats were trained to respond under a single-spatial alternation schedule to obtain food. During trials, the houselight was illuminated and the rats were required to respond, on alternate trials, on the left and right levers. A correct response produced a food pellet, terminated the houselight and initiated a new RI. An incorrect response produced a brief feedback tone and extinguished the houselight, but the previous trial and RI were repeated. Between trials, the chamber was dark for a period (the RI) which was varied randomly between 2 and 32 sec. When the RI was 2 sec, responding was $\geq$ 90% correct. The % correct decreased as the RI was increased to 32 sec. Regression analysis provided a quantitative measure, the STM($\frac{1}{2}$), of the half-life of the short-term memory. During control sessions, the STM($\frac{1}{2}$) was approximately 22.5 sec. Scopolamine (0.01 to 0.3 mg/kg) produced dose-related decreases in the STM($\frac{1}{2}$) and in rates of responding. When the feedback tone after incorrect responses was omitted, STM($\frac{1}{2}$) decreased to approximately 10 sec. Scopolamine again decreased STM($\frac{1}{2}$) and rates of responding.

150.5

PHARMACOLOGIC SPECIFICITY OF ACUTE SENSITIZATION TO NALTREXONE (NTX) INDUCED BY OPIOID AGONIST PRETREATMENT IN RATS. Jill U. Adams* and S.G. Holtzman. Dept. of Pharmacology, Emory Univ. School of Med., Atlanta, GA 30322

Sensitization to NTX induced by a single dose of morphine may reflect the initial changes in opioid systems that underlie physical dependence. To determine pharmacologic specificity, representative μ, κ, and σ agonists, and the non-opioid, pentobarbital, were tested for their ability to sensitize rats to the response-rate-decreasing effects of NTX. Rats were trained on a multiple-trial fixed-interval 3-min schedule of food reinforcement. Agonists were administered 4 hr prior to NTX challenge; cumulative doses of NTX were given until response rates were reduced to less than 10% of control. Levorphanol (0.3 mg/kg) and fentanyl (0.06 mg/kg) induced the greatest sensitization to NTX, shifting the cumulative-dose-response curve 100-fold to the left. Smaller (10-fold), but significant shifts of the NTX curve were produced by morphine (10 mg/kg), ethylketocyclazocine (3.0 mg/kg), and (+)N-allylnormetazocine (10 mg/kg). Neither dextrorphan (3.0 mg/kg) nor pentobarbital (18 mg/kg) pretreatment altered sensitivity to NTX. Thus, acute sensitization to NTX is a stereoselective effect specific to opioid agonists; the role of the various opioid receptor subtypes in this effect remains to be determined. (Supported by Grant DA00541, RSA KO5 DA00008, and a Lilly predoctoral fellowship).

150.6

DISCRIMINATIVE STIMULUS EFFECTS OF BUTORPHANOL, NALBUPHINE, NALOXONE AND HYDROMORPHONE IN OPIOID-DEPENDENT HUMAN VOLUNTEERS. Kenzie L. Preston and George E. Bigelow*. The Johns Hopkins University School of Medicine, Baltimore, MD 21224.

To assess the stimulus properties of opioid mixed agonist-antagonist drugs in opioid-dependent humans, 5 volunteers enrolled in methadone maintenance treatment were trained in a three-choice drug discrimination procedure to discriminate between the effects of intramuscularly given saline (2 ml) (S), hydromorphone (10 mg/70 kg) (H), and naloxone (0.15 mg/70 kg) (NX). Subjects earned monetary reinforcement by correctly identifying the training drugs by letter code. Generalization curves for the training drugs and 2 test drugs, butorphanol (B) and nalbuphine (NB) were then determined. All subjects learned the H/NX/S discrimination. In generalization testing both H and NX produced dose-related increases in drug-appropriate responses and in characteristic subjective effects measures. NX produced 80% or greater NX-appropriate responding at doses of 0.075 mg and higher; H produced 80% or greater H-appropriate responding at doses of 3.5 mg and higher. B produced dose-related increases in identifications as NX and dose-related increases in those subjective effect measures increased by NX, with 1.5 mg producing 100% NX-appropriate responding. NB also produced dose-related increases in identifications as NX and dose-related increases in those subjective effect measures increased by NX, with 2.1 mg and higher doses producing 100% NX-appropriate responding. There was close concordance between the verbal subjective effect measure data and behavioral discrimination measures. (Supported by USPHS Grant DA-04089).

150.7

EFFECTS OF BUPRENORPHINE AND OTHER ORIPAVINES ALONE AND IN COMBINATION WITH VARIOUS OPIOID AGONISTS ON THE ACQUISITION AND PERFORMANCE OF DISCRIMINATIONS IN MONKEYS. J. M. Moerschbaecher. LSU Medical Center, New Orleans, LA 70112.

Responding in patas monkeys was maintained by food presentation under a multiple schedule of repeated acquisition and performance of conditional discriminations. Etorphine, the prototypical oripavine agonist, produced morphine-like effects. Etorphine decreased overall response rate while having little or no effect on accuracy of responding except at higher doses which also produced large rate-decreasing effects. Buprenorphine, an oripavine mixed agonist-antagonist, had little or no effect on rate or accuracy of responding when administered alone. The effects of the oripavine antagonist, diprenorphine, were variable among the subjects. In general, however, diprenorphine had little or no effect on responding except at extremely high doses (>1mg/kg) which decreased both accuracy and rate of responding. When buprenorphine was administered in combination with heroin and U50488H competitive antagonism obtained. The dose-effect curves for each agonist were shifted to the right at least 1-log unit by buprenorphine at a dose of 0.1 mg/kg. The data indicate that buprenorphine is an effective antagonist at both the mu and kappa receptors in old world monkeys. (DA03573 & DA04775).

150.8

ACIDIC AMINO ACIDS AND SELF-STIMULATION OF THE PREFRONTAL CORTEX IN THE RAT. M. Cobo* and F. Mora*. (SPON: C.V. Gisolfi). Dpt. Physiology. Univ. Granada. 18012 Granada. Dept. Human Physiology. Central University. 28040 Madrid. SPAIN.

To investigate whether acidic amino acids are involved in self-stimulation (SS) of the medial prefrontal cortex (MPC) in the rat, the effects of intracortical microinjections of γ-D-glutamylglycine (DGG) (an antagonist of acidic amino acid receptors) and DL-2-amino-5-phosphonovaleric acid (D-AP5) (a specific antagonist of NMDA-receptors) were studied in two different groups of male Wistar rats. In each rat, bilateral monopolar electrodes and guide cannulae were stereotaxically implanted in the MPC. SS was recorded during 5, 10 and 15 min. postinjection. Unilateral intracortical microinjections of DGG (1 µl) at doses of 2.5, 5, 10 and 20 µg/µl produced a dose-related decrease in SS of the ipsilateral side. This decrease was statistically significant. Similar microinjections of D-AP5 at doses of 10, 20 and 40 µg/µl produced a dose-related decrease that not reached statistical significance. SS of the contralateral cortex in both experiments used as control was not affected. The present series of results suggest that acidic amino acids could be part of the neurochemical substrate underlying SS of the MPC. Further research is required to elucidate wich type of receptor is involved in SS of the MPC.

150.9

A NEW METHOD OF QUANTIFYING TOXICITY FOLLOWING SYSTEMIC INJECTIONS OF EAA AGONISTS IN MICE. B.J. Connell and R.A.R. Tasker (SPON: J.F. Burka). Atlantic Veterinary College, UPEI, Charlottetown, PEI, Canada, C1A 4P3.

The recent outbreak of toxicity caused by contaminated PEI mussels has created a need for an improved method of screening commercial shellfish. In the case of PEI mussels, domoic acid (DA), an excitatory amino acid (EAA) was identified as the marine toxin involved. In mice, injection of mussel extracts containing DA initiates a series of behaviours which often lead to death. To quantify these behavioural changes, serial dilutions of extracts from toxic mussels were administered i.p. to groups of 4 female CD1 mice and behaviours were recorded with regard to both frequency and time of onset. Based on these data, 7 behavioural categories were identified and assigned a numeric value. Mice were scored according to the time spent in each category after injection. Dose-response curves (DRC) for DA in mussel extracts were constructed and compared to those obtained with another EAA, kainic acid. DA was about 10 times more potent than kainic acid based on ED50. Moreover, toxicity caused by concentrations of DA as low as 30 ug/ml could be reliably detected. We conclude that this scale represents a more sensitive and effective means of assessing toxin concentrations in contaminated shellfish and can be used as a tool for studying the role of pharmacological systems in the actions of systemically-administered EAA agonists.

150.10

PROPENTOFYLLINE PROTECTS TRANSIENT ISCHEMIC HYPERACTIVITY IN THE GERBIL. J.A. DeLeo* and J.M. Carney. Dept. of Pharmacology, University of Oklahoma, Health Sciences Center, OKC, OK 72190.

Spontaneous locomotor hyperactivity is a behavioral consequence following forebrain ischemia. To determine if a methyl xanthine derivative, propentofylline (HWA 285) protects this behavioral ischemic parameter, doses of 1, 10 or 32 mg/kg, i.p., were administered prior to or following a 10 minute period of bilateral occlusion of the common carotid arteries in the gerbil. The untreated ischemic group demonstrated the predictable hyperactivity, peaking 24 hours after the insult and a significant hypoactivity 1 hour after the occlusion. Following a 15 minute pretreatment of HWA 285 (10 mg/kg), the 1 hour hypoactivity was not observed. All groups showed significantly less stimulation from the untreated group 24 hours after the ischemic period. To determine if HWA 285 also decreased ischemic-induced hyperactivity if administered after the insult, it was given 1 and 2 hours after bilateral occlusion. At one hour post-ischemia, the treated groups were not significantly different from the untreated, but at 24 hours, the untreated group showed significantly increased stimulation from the 1 and 2 hour post-treatment groups. These data support the behavioral protection of this compound in a dose-related manner when administered prior to or following transient forebrain ischemia in the gerbil.

150.11

EFFECT OF AMANTADINE PRETREATMENT ON CHLORPROMAZINE (CPZ) AND RESERPINE (RES) PRODUCED BEHAVIORAL DEPRESSION. F.S. Messiha, Dept. of Pharmacology, Univ. of North Dakota School of Medicine, Grand Forks, ND 58201.

The RES and CPZ-produced depression of motility has been used as an animal model for Parkinson's disease and certain dyskinesias, respectively. The effect of AMN, a dopaminergic agonist, on this behavioral performance test was studied. Three groups of C57BL/6J mice were injected i.p. four times at 2 h intervals saline, CPZ or RES (0.2 mg/kg). Additional 3 groups of mice received AMN (100 mg/kg, i.p.) 15 min prior to identical dose regimens of the drugs tested. Mice motility was measured for 18 hrs. AMN enhanced motility by 3.9 ($p < 0.02$) and 4.9 ($p < 0.02$) fold above control value during the initial 1st and 2nd hrs, respectively. This was followed by behavioral depression lasting from 8 to 18 hrs which amounted to 59% ($p < 0.02$) and 52% ($p < 0.05$), respectively. The AMN pretreatment significantly antagonized CPZ-mediated reduction of motility throughout the initial 3 hrs. Reserpinized animals pretreated with AMN showed no changes in motility from corresponding control. The data indicates a biphasic effect of AMN. The initial behavioral arousal phase of AMN alleviated CPZ depression of motility while it lacked such effect on RES-treated mice. Brain monoamines data will be presented in relationship to the behavioral effect observed. (Supported in part by Grant from Ester Zahn Estate - Parkinson's Disease)

150.12

EFFECTS OF DOPAMINE AGONISTS ON SCHEDULE-CONTROLLED RESPONDING OF SQUIRREL MONKEYS. Jack Bergman, B.K. Madras*, S.E. Johnson*, and R.D. Spealman. NERPRC, Harvard Med Sch, Southborough, MA 01772

The effects of dopamine D_2-selective agonists, quinpirole (0.003-1.0 mg/kg) and (+)-PHNO (0.0001-0.03 mg/kg) and D_1-selective agonists, R-SKF 38393 (0.1-3.0 mg/kg) and SKF 75670 (0.01-0.3 mg/kg) were determined in squirrel monkeys responding under a 30-response fixed-ratio (FR) schedule of food presentation or a 3-min fixed-interval (FI) schedule of stimulus-shock termination. Both D_2-selective agonists increased rates of FI responding in a dose-related manner. Maximal effects were produced by 0.003-0.01 mg/kg (+)-PHNO and 0.1-0.3 mg/kg quinpirole; higher doses of both drugs increased responding less or decreased it. In contrast, both D_1-selective agonists only decreased rates of FI responding. FR responding under the schedule of food presentation was only decreased by D_1 and D_2-selective agonists. However, D_2-selective agonists markedly decreased FR responding at doses that had little or no effect on FI responding (0.001 mg/kg (+)-PHNO, 0.03 mg/kg quinpirole) , whereas D_1-selective agonists decreased FR responding at doses that were similar to or greater than those that decreased FI rates. The dissimilar effects and potencies of drugs in the present experiments suggest that these procedures are useful for distinguishing dopamine agonists with differing selectivities for D_1 and D_2 receptors. (Supported by USPHS Grants DA03774, DA00499, and RR00168).

PERIPHERAL CIRCULATION II

151.1

Metabolic Saturation Kinetics in an Endothelial Cell Column. R.E. Howell, F.R. Haselton and S.N. Mueller (Spon. W.J. Kinnier). Univ. of Pennsylvania and the Wistar Institute, Phila., PA 19104

The kinetics of saturable endothelial metabolic functions have been assessed in vivo by transient (indicator-dilution) measurements and in vitro (culture) by steady-state measurements. Unfortunately, comparisons between the two are difficult. Therefore, we used indicator dilution methods to assess the kinetics of angiotensin converting enzyme (ACE) activity in cultured endothelium. Bovine fetal aortic endothelial cells were grown to confluence on Biosilon microcarrier beads. Cell covered beads were poured into polypropylene columns, at a cell surface area of 250 cm^2. Columns were perfused with serum-free culture medium at 1-2 ml/min. Six injections, containing ^{14}C-albumin, ^{3}H-Benz-Phe-Ala-Pro (^{3}H-BPAP, an ACE substrate) and varying amounts of unlabelled BPAP (.05-50 nmoles), were applied separately to each column. Effluent was collected in serial samples. The Km and Vmax of BPAP metabolism were determined by a multiple non-linear regression analysis used previously to determine pulmonary endothelial ACE kinetics in vivo. The Km averaged 5 μM in three columns, which is very close to values determined in vivo (7-9 μM). The Vmax and Vmax/Km averaged 8 nmol/min and 1.6 ml/min, respectively. In conclusion, we have developed a new method that should enable better comparisons between studies of endothelial metabolic functions in vivo and in vitro.

151.2

VASCULAR TRANSPORT CAPACITY OF RAT HINDQUARTERS IS INCREASED IN STREPTOZOTOCIN (STZ) DIABETES. W.L. Sexton, R.J. Korthuis, and M.H. Laughlin. Dept Vet Biomed Sci and Dalton Res Ctr, Univ Missouri-Columbia, 65211 and Dept Physiol, LSU Med Ctr, Shreveport, LA 71130.

Male Sprague-Dawley rats were made diabetic (D, n=10) with a single injection of STZ (65 mg/kg, i.p.), while control rats (C, n=9) received an equivalent volume of sterile saline vehicle. Experiments were performed 8 wks later. Isolated hindquarters (HQ) were perfused with washed human blood cells suspended in horse serum and maximally vasodilated with papaverine. Vascular transport capacity was assessed in each HQ by determining the capillary exchange capacity (filtration coefficient, Kf) and the flow capacity at perfusion pressures of 30, 40, and 50 mmHg. Isogravimetric flows of D were 39% greater compared to C at similar perfusion pressures. Capillary pressures in D were 17% lower relative to C. Total vascular resistances (Rt) of D were reduced 34% compared to C. The lower Rt values were attributable to reductions in pre- and postcapillary resistances of 32% and 40%, respectively. However, the pre-to-postcapillary resistance ratios were not different between groups. Kf values for D were 37% greater relative to C suggesting the D HQ had a greater capillary exchange capacity. The vascular flow capacities of D HQ were elevated 76% relative to C at all perfusion pressures investigated. Thus, HQ of rats with STZ diabetes display increases in both capillary exchange capacity and flow capacity compared to C.

151.3

DIFFERENTIAL CONTROL OF BLOOD FLOW IN MUSCLES OF DIFFERENT FIBER TYPES. M.D. Delp and R.B. Armstrong. Exercise Biochem. Laboratory, University of Georgia, Athens, Georgia 30602

The purpose of this study was to investigate the potential for differential local and autonomic control of resistance vessels in rat muscles composed predominantly of different fiber types. Aortic pressure and blood flows in soleus (type I fibers) and red (type IIa fibers) and white (type IIb fibers) portions of lateral gastrocnemius muscles were sequentially measured in conscious (C), anesthetized (A, methoxyflurane), A+denervated (D, sciatic severed), A+D+alpa-blocked (αB, phentolamine), and A+D+αB+beta-blocked (βB, propanolol) conditions. Calculated resistance (R, torr/ml/min/100 g) in soleus muscle in C was 0.79 $\pm$ 0.09, increased 1232% in A, then remained unchanged in A+D, A+D+αB, and A+D+αB+βB. R in red portion of gastrocnemius muscle in C was 1.59 $\pm$ 0.26, increased 883% in A, remained unchanged in A+D, decreased 58% in A+D+αB, then increased 113% in A+D+αB+βB. R in white portion of gastrocnemius muscle in C was 7.95 $\pm$ 1.46, increased 230% in A, decreased 140% in A+D, then decreased further (-151%) in A+D+αB. R then increased 526% in A+D+αB+βB. Thus, there appears to be a differential control of vascular R between type IIa and IIb muscle, as well as between type I and type II muscle. Additionally, vascular tone was the same across fiber types in the A+D+αB+βB condition.

Supported by NIH BRSG grant #SO7RR07025-21

151.4

FELODIPINE AND MYOGENIC TONE IN BORDERLINE HYPERTENSION. J.M.O. Arnold, J.D. Spence, D.G. Bailey, J.H. Kreeft, S.G. Carruthers. University of Western Ontario, London, Ontario N6A 4G5 Canada

We have shown previously that myogenic tone is abnormal in arterioles of patients with both borderline and established hypertension (HT) as they have a decreased forearm arterial vasoconstriction to periods of venous occlusion. To determine if felodipine (FEL), a new dihydropyridine, could favorably alter this abnormality, we studied 12 patients with untreated borderline HT in a randomized, double-blind, placebo-controlled, cross-over trial of FEL (single dose, 10 mg solution). Hemodynamics were measured prior to dose and 30 min post-dose. Forearm blood flow (FBF) was measured by venous plethysmography before and immediately after 5 min of venous occlusion at 80 mmHg. There were no differences pre-dose on the two days. Maximum and minimum blood flows after venous occlusion are expressed relative to control flow as unity (mean $\pm$ s.e.; *p<0.05, **p<0.01 vs. PLAC).

	MEAN BP	MAX REL FBF	MIN REL FBF
POST PLAC	105.8±3.8	5.80±0.80	0.56±0.06
POST FEL	90.6±3.2**	2.53±0.32**	0.72±0.04*

FEL reduced BP and maximum relative flow and increased minimum flow after venous occlusion. This is consistent with a myogenic cause for the vascular responses to venous occlusion and demonstrates that it can be altered by drug therapy in borderline HT.

151.5

TOTAL SYSTEMIC AUTOREGULATION MAY INTERFERE WITH BAROREFLEX CONTROL. P. Borgdorff*, N. Westerhof*, P. Duijst* (Free Univ., Amsterdam, the Netherlands), R. Burattini* (Univ. Ancona, Italy), D.R. Gross (Texas A&M Univ., College Station, Texas 77843)

We studied total systemic autoregulation in closed chest, chloralose anesthetized, normoxic dogs (n=9) with mean arterial pressures in the range where baroreceptor function is usually maximal. Cardiac output and aortic pressure were varied by reducing venous inflow using a vena caval balloon. Compensatory action of the baroreflex was prevented by bilateral vagotomy and isolation of both carotid sinuses. Carotid sinus pressure was set at the original baseline value using a pressurized blood reservoir. With each balloon inflation cardiac output and pressure stabilized within a minute. Pressure and flow were allowed to return to baseline values between each balloon inflation to prevent the activation of slower regulatory mechanisms. The steady state values of flow versus the arteriovenous pressure difference were fit with a sigmoidal curve characteristic of autoregulation. We conclude that total systemic autoregulation may counteract baroreflex control. (Sponsored, in part, by NATO Scientific Affairs, Grant #54/87)

151.6

ARACHIDONIC ACID METABOLITES AND POSTPRANDIAL JEJUNAL BLOOD FLOW A. ALEMAYEHU* and C.C. CHOU Departments of Physiology and Medicine, Michigan State University, East Lansing, MI. 48824.

Jejunal (J) prostanoid releases (R, ng/min/100g) during nutrient absorption in the presence and absence of arachidonic acid (AA) were measured, in anesthesized dogs (n=9). J venous and aortic blood samples were collected for determination of TXB_2 (TXB), 6-keto-$PGF1_\alpha$ (PGI), (stable metabolites of TXA_2 and PGI_2 respectively), $PGF_{2\alpha}$ and PGE_2 using radioimmunoassay. During blood sampling blood flow (BF), and VO_2 (ml/min/100g) were measured when J lumen contained normal saline (NS), NS+AA, food (F), or F+AA. *, **, and + P<0.05, relative to NS, NS+AA, and F respectively. Thus, there was significant TXB and PGI releases during NS and NS+AA. F significantly enhanced BF, VO_2 and releases of all four prostanoids. AA abolished or attenuated F-induced enhancement of BF, VO_2, and PGI and PGE releases, while enhancing TXB and PGF releases. This AA action on BF and VO_2 was reversed by SQ-29548 (TXA2 receptor blocker). Conclusion: Nutrient absorption enhanced J release of all four prostanoids, which may contribute to the regulation of J BF and functions. AA action on F-induced increases in BF and VO_2 may be related to the relative production of the four prostanoids. (Support: NIH #HL-15231)

	BF	VO_2	R TXB	R PGI	R PGE	R PGF
NS	39±3	1.88±0.22	11±2	24±5	16±12	1±2
F	48±5*	2.32±0.23*	17±3*	40±13*	60±12*	8±2*
NS+AA	40±3	1.74±0.25	13±3	30±6	21±12	5±4
F+AA	37±4	1.91±0.23	20±4**	33±8	37±13**+	12±5**+

151.7

MECHANISM OF HEPATIC ARTERIAL DILATION DURING HEMORRHAGE. W.W. Lautt and J.E. McQuaker*. Hepatorenal Research Unit, Dept. Pharmacology, University of Manitoba, Winnipeg, Man., Canada, R3E OW3.

During hemorrhage the cardiac output declines and blood flow to individual organs declines in general due to peripheral vasoconstriction. The hepatic artery (HA) is unique in that it may vasodilate so that the proportion of cardiac output supplying the HA is increased. The extrahepatic splanchnic organs that supply portal blood to the liver undergo severe vasoconstriction and portal blood flow decreases. We test the hypothesis that the decrease in portal flow activates the hepatic arterial buffer response leading to dilation of the HA and protection of the liver during hemorrhage in cats. The HA buffer response is the mechanism by which changes in portal flow produce inverse changes in HA flow and is mediated by an adenosine washout mechanism. In control responses the HA conductance did not decline whereas the superior mesenteric arterial conductance decreased markedly confirming the protective effect of the HA during hemorrhage. After adenosine receptor blockade, the dilator responses to infused adenosine but not isoproterenol was blocked; the HA buffer response was blocked. Adenosine receptor blockade also resulted in elimination of the protective effect of the HA during hemorrhage so that the HA and superior mesenteric artery constricted to a similar extent. Thus, the HA buffer response is the mechanism by which the HA dilates during hemorrhage.

151.8

RESPONSES OF THE HEPATIC ARTERY DURING INTRAVENOUS INFUSIONS OF DILATOR AGENTS THAT RAISE PORTAL BLOOD FLOW. M.S. D'Almeida*, J.E. McQuaker*, L.M. D'Aleo*, and W.W. Lautt Hepatorenal Research Unit, Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada, R3E OW3.

Changes in portal blood flow (PF) produce inverse changes in hepatic arterial flow by activating the hepatic arterial buffer response (HABR). Intravenously infused dilator agents, isoproterenol, adenosine and glucagon produced significant elevations in PF but produced no such effect in the hepatic artery (HA) even though direct IA infusions of these agents into the HA elicited significant dilations of this artery. This apparent inconsistency in the hepatic arterial response was assessed in terms of the HABR. To do this, the IV infusions of the three drugs were repeated but PF was not allowed to rise, therefore eliminating the stimulus (raised PF) for the HABR. As a result, the HA conductance and blood flow rose significantly from control levels. These observations indicate that systemically administered dilator agents that elevate PF will trigger the HABR and counteract the dilating action of these compounds on the HA. The response in the HA when PF is not held at pre-infusion levels represents the combined effects of the HABR and the dilator agent. Thus, if PF is not controlled or monitored when studying the HA in response to vasoactive agents, incorrect conclusions may be drawn due to the action of the HABR. MSD holds a Research Traineeship from the Canadian Heart Foundation.

151.9

REFLEX CONTROL OF VASCULAR CAPACITANCE DURING HYPOXIA, HYPERCAPNIA OR ASPHYXIA. Carl E. Rothe, A. Dean Flanagan*, Roberto Maass*. Indiana Univ. School of Medicine, Indianapolis, IN 46223

We tested the hypothesis that the changes in venous tone induced by changes in arterial blood oxygen and carbon dioxide are via reflex control. Mongrel dogs were anesthetized with pentobarbital and paralyzed with vecuronium. Cardiac output and central blood volume were measured by indocyanine green dilution. Mean circulatory filling pressure (Pmcf), a measure of venous tone and closely related to vascular capacitance, was estimated by briefly stopping the heart by electrical fibrillation and defibrillating with DC countershock. After control experiments, cardiovascular reflexes were blocked with hexamethonium and atropine. With reflexes blocked, hypoxia (arterial PO_2=38 mmHg), hypercapnia (PCO_2=72 mmHg) or asphyxia (PO_2=41; PCO_2=69 mmHg) each caused a decrease in total peripheral resistance (TPR), but little increase in Pmcf or cardiac output, and a decrease in mean arterial pressure (Psa). With reflexes intact, hypoxia, but not hypercapnia, induced enough sympathetic outflow to increase Psa by maintaining the control level of TPR to counter the direct vasodilating effects of hypoxia or hypercapnia. With each of the experimental gas changes, the Pmcf and cardiac output were significantly increased, however. We conclude that hypoxia, hypercapnia, and asphyxia have little direct influence on vascular capacitance, but with reflexes intact, there is a significant increase in Pmcf. (Supported by NIH grant HL07723.)

151.10

INTESTINAL VASCULAR RESPONSIVENESS TO EXOGENOUS VASOPRESSIN IN CHRONIC PORTAL HYPERTENSION. J.N. Benoit, C.L. Mesh*, R.J. Korthuis and D.N. Granger, Dept. of Physiology and Biophysics, L.S.U. Medical Center, Shreveport, LA 71130.

It is generally accepted that chronic portal hypertension (CPH) lead to an intense splanchnic hyperemia that is partially related to increased plasma glucagon and a reduced vascular sensitivity to norepinephrine. In the present study, we have evaluated the vascular responsiveness of the portal hypertensive intestine to arginine-vasopressin (AVP) since: 1) exogenous AVP is used to reduce intestinal blood flow in man with CPH and 2) endogenous AVP is thought to contribute to resting intestinal vascular tone. Male Sprague-Dawley rats were made portal hypertensive by stenosis of the portal vein. Ten to twelve days after the induction of CPH, the responsiveness of the intestine to cumulative doses of AVP was studied in an isolated pump perfused small intestinal preparation. The ED_{50} for maximal vasoconstriction increased from 0.18 ± 0.01 mU in control rats to 0.42 ± 0.07 mU in rats with CPH. To determine if the impaired responsiveness to AVP was related to the hyperglucagonemia of CPH, we elevated plasma glucagon levels in control rats to levels previously measured in portal hypertensive rats (i.e., ≈450 pg/ml) and repeated the dose response studies. Glucagon significantly attenuated the responsiveness of the intestinal vasculature to AVP (ED_{50} = 0.30 ± 0.03 mU). These studies suggest that glucagon may partially mediate the altered intestinal vascular sensitivity to arginine vasopressin in animals with prehepatic portal hypertension. (Supported by American Heart Association, LA Affiliate)

151.11

ROLE OF HYDROGEN PEROXIDE, IRON, AND HYDROXYL RADICALS IN ISCHEMIA/REPERFUSION-INDUCED NEUTROPHIL INFILTRATION. Barbara J. Zimmerman* and D. Neil Granger. LSU Medical Center, Shreveport, LA 71130

Polymorphonuclear neutrophils (neutrophils) accumulate in the intestinal mucosa during ischemia with further accumulation occurring during reperfusion. Pretreatment with allopurinol, a xanthine oxidase inhibitor, or superoxide dismutase attenuates this neutrophil accumulation, suggesting a role for xanthine oxidase-derived oxidants. The objective of this study was to determine whether hydrogen peroxide, iron, and/or hydroxyl radicals also play a role in ischemia/reperfusion (I/R)-induced neutrophil infiltration in the feline small intestine. A total of 27 animals were used. Isolated intestinal segments was subjected to 3 hours of ischemia and 1 hour of reperfusion. Mucosal samples were obtained prior to and during ischemia and following reperfusion. Tissue-associated myeloperoxidase activity was used as an index of tissue neutrophil count. Treatment with either catalase or the iron chelator desferoxamine significantly attenuated the reperfusion-induced neutrophil infiltration, supporting a role for both hydrogen peroxide and iron. Two hydroxyl radical scavengers, dimethylsulfoxide (DMSO) and dimethylthiourea (DMTU), were used. DMTU significantly attenuated the I/R-induced neutrophil accumulation while DMSO had no effect. The results of this study support a role for hydrogen peroxide and iron in the I/R-induced neutrophil infiltration. The role of hydroxyl radicals remains unclear. Supported by DK33594.

151.12

INTESTINAL NEUTROPHIL (PMN) ACCUMULATION AND PLASMA LOSS IN RATS FOLLOWING LOCAL ABDOMINAL IRRADIATION. M.G. Buell* and R.K. Harding. Defence Research Establishment Ottawa and Dept. of Physiology, Univ. of Ottawa, Ottawa, Ontario, K1H 8M5.

We examined whether a single dose of 10 Gy irradiation(R) delivered to the abdomen of rats led to changes in the intestine consistent with an acute inflammatory response.

Male Sprague-Dawley rats were briefly anesthetized with Saffan and the abdomen was locally irradiated at a dose of 1(Gy using a Co-60 radiotherapy unit. I-125 albumin and Cr-51 labelled red blood cells were injected i.v. to quantitate tissue plasma volume (PV) and red blood cell volume (RBCV) respectively. Following this, animals were euthanized at 2 to 24 hrs post-R (n=6 for each time period) and the jejunum, ileum and colon were excised. Tissues were then processed for determination of PV and RBCV and prepared for the histomorphometric quantitation of mucosal PMNs.

In the 3 regions of intestine examined, tissue PV became significantly ($p<0.05$) elevated 8-12 hrs post-R. This was sustained until 24 hr post-R. A transient rise in RBCV 4-8 hrs after R (suggestive of vasodilation) was also observed. Furthermore, these changes were accompanied by a significant ($p<0.05$) transient increase in mucosal PMNs 4-12 hrs post-R.

The results support the hypothesis that an inflammatory response occurs in intestinal tissues soon after R. It is possible that this may play a role in further exacerbating the damage initiated by ionizing radiation.

RENAL PHARMACOLOGY II

152.1

HEMODYNAMIC ALTERATIONS DURING CHRONIC VERAPAMIL TREATMENT. David B. Young and Huabao Lin.* University of Mississippi Medial Center, Jackson, MS 39216

Previously we have reported that acute administration of verapamil causes a profound increase in GFR, renal blood flow (RBF) and filtration fraction (FF), and severely impairs autoregulation of RBF and GFR in anesthetized rabbits. The goal of the present investigation was to determine if these effects of verapamil persist after long-term treatment. Three groups of dogs were studied; control (N-7), acute treatment (N-6, 150 μg/kg bolus followed by 4 μg/kg/min beginning 45 minutes prior to measurements), chronic treatment (N-5, 240 mg P.O., b.i.d. starting 7 to 10 days before the study). All animals were anesthetized and renal function was measured at controlled levels of renal perfusion pressure in the surgically exposed left kidney. At 100 mmHg the RBF in control, acute, and chronic groups were: 3.7±.4, 5.6±.5, 8.0±1.4 ml/min/g KW. GFR of the three in the same order were: 0.6±.1, 1.0±.1, 1.3±.2 ml/min/g KW. In the control group the autoregulation of both variables persisted to 90 mmHg but in both the acutely and chronically treated group autoregulation failed at 100 mmHg. In dogs chronically treated with verapamil regulation of renal hemodynamics is altered, and RBF and GFR are profoundly elevated. Supported by HL 21435 and HL11678.

152.2

INHIBITION OF DOPAMINE β-HYDROXYLASE INCREASES RENAL ELECTROLYTE EXCRETION IN SPONTANEOUSLY HYPERTENSIVE RATS. G. Dytko*, M. Jugus*, E.H. Ohlstein, W.A. Mann*, and L.B. Kinter, Smith Kline & French Labs, King of Prussia, PA 1940(

Dopamine (DA) has been implicated as an endogenous vasodilator and natriuretic factor involved in the regulation of renal sodium excretion. Inhibition of dopamine β-hydroxylase (DBH) increases tissue DA levels and provides a tool by which to increase renal DA tone in vivo. In spontaneously hypertensive (SH) rats, administration of the DBH inhibitor, 1-[(3,5-difluorophenyl)methyl]-1,3-dihydro-2H-imidazole-2-thion (SK&F 102698, 100 mg/kg, p.o.), was associated with natriuresis, kaluresis and chloruresis (2-3x baseline, $p<.05$). The increases in water and electrolyte excretion were: 1) independent of changes in creatinine and urea excretion; 2) blocked by saline- but not water-loading; and 3) abolished by co-administration of the selective DA-1 receptor antagonist, SK&F 83566. SK&F 102698 has no intrinsic DA-1 agonist activity. We have shown previously that this dose of SK&F 102698 is antihypertensive in SH rats and that antihypertensive efficacy is also attenuated by co-administration of a DA-1 receptor antagonist (Ohlstein et al. FASEB J. 2:A1799, 1988). We conclude that SK&F 102698 has modest antihypertensive and saliuretic activity in SH rats and that saliuresi is the result of stimulation of renal epithelial DA-1 receptors. The results imply that renal DA levels play a physiologically significant role in the regulation of renal electrolyte reabsorption in SH rats.

152.3

SEPARATION OF THE PRESSOR AND DIURETIC EFFECTS OF B-HT 933 WITH SK&F 104078. Catherine F.T. Uyehara and Miklos Gellai, Smith Kline & French Labs, King of Prussia, PA 19406.

We assessed the effect of SK&F 104078, a novel alpha-2 adrenoceptor antagonist, on the pressor and diuretic actions of the selective alpha-2 adrenoceptor agonist, B-HT 933. In conscious, chronically instrumented Sprague-Dawley rats (n=5), we measured the effect of a sustained i.v. infusion (100 μg/kg/min) of B-HT 933 (A) on blood pressure (BP) and renal excretion of water and electrolytes. We repeated the experiments during infusion (50 μg/kg/min) of SK&F 104078 (A+B) on the same rats on different days. Results (mean ± SEM; * = $p<0.05$ vs. control; + = $p<0.05$ vs. A) were:

	RPP (mmHg)	Urine Flow (μl/min/100g)	C H_2O	Sodium Excr. (uEq/min/100g)
Control	111 ± 3	12 ± 3	-17 ± 4	0.6 ± 0.1
A	138 ± 3*	173 ± 13*	72 ± 10*	10.3 ± 1.7*
A + B	111 ± 4+	104 ± 8*+	55 ± 3*	4.1 ± 1.0*+

SK&F 104078 completely blocked the B-HT 933-induced increase in BP and partially attenuated its diuretic effect, mainly by moderating the increase in sodium excretion. The increase in free water clearance (C H_2O) was not significantly affected by SK&F 104078. We conclude that the pressor and renal tubular actions of B-HT 933 are mediated by SK&F 104078-sensitive and SK&F 104078-insensitive alpha-2 adrenoceptors, respectively. The natriuresis induced by B-HT 933 appears to be, at least in part, pressure dependent, although a direct effect of SK&F 104078 cannot be excluded.

152.4

INFLUENCE OF ALPHA AND BETA ADRENOCEPTOR BLOCKADE ON THE RENAL EFFECTS OF FENOLDOPAM. Abdallah Dlewati* and Mustafa F. Lokhandwala. Department of Pharmacology, University of Houston, Houston, TX 77204-5515

We have reported that hypotension produced by fenoldopam prevented the natriuresis and diuresis during i.v. infusion (5 μg/kg/min for 30 min). However, significant increases in sodium and water excretion were seen after terminating fenoldopam infusion when all hemodynamic parameters had returned to control levels (FASEB J. 2:1793, 1988). Because a reflex increase in sympathetic outflow may have masked the effect of fenoldopam, we have investigated the involvement of this factor in modulating the natriuretic and diuretic effects of fenoldopam. In pentobarbital-anesthetized dogs, alpha and beta blockade caused significant decreases in mean blood pressure (MBP), heart rate and renal vascular resistance (RVR), and significant increases in renal blood flow (RBF), urine volume (UV) and urinary sodium excretion (UNa^+V). In a second group of animals, i.v. fenoldopam infusion following alpha and beta blockade caused significant decreases in MBP and RVR, and a significant increase in RBF. Similar to control experiments in which fenoldopam was administered in the absence of antagonists, the natriuretic and diuretic effects of fenoldopam were seen only when blood pressure returned to control level. We conclude from these results that reflex activation of renal sympathetic nervous system in response to hypotension may not be involved in blunting of natriuresis and diuresis. However, changes in intrarenal hemodynamics or activation of the renin-angiotensin system may account for the lack of natriuresis and diuresis during fenoldopam infusion.

152.5

INVOLVEMENT OF DOPAMINE RECEPTORS IN THE DIURETIC AND NATRIURETIC RESPONSE TO FENOLDOPAM AND ACUTE VOLUME EXPANSION IN ANESTHETIZED RATS. Sharath S. Hegde* and Mustafa F. Lokhandwala. Department of Pharmacology, University of Houston, Houston, TX 77204-5515

Fenoldopam, a DA-1 receptor agonist causes diuresis and natriuresis as a result of direct tubular and/or indirect hemodynamic effects. The present study was designed to identify DA-1 receptor sites which mediate the renal response to fenoldopam. We also examined the possible contribution of endogenous DA in the diuretic and natriuretic response to acute volume expansion. In pentobarbital anesthetized rats, fenoldopam (0.5 µg/kg/min) produced diuresis and natriuresis. This effect was not accompanied by any changes in blood pressure, renal blood flow or glomerular filtration rate suggesting a direct tubular site of action. SCH-23390 antagonized the renal response to fenoldopam but produced no changes in Na-excretion and urine output by itself. In a separate group of animals, acute volume expansion, with isotonic saline (5% body weight), over a period of 60 minutes caused significant diuresis and natriuresis. Pretreatment with SCH-23390, in a dose which had previously antagonized the renal response to fenoldopam, resulted in significant attenuation in the diuresis and natriuresis during the period of volume expansion. These results suggest that fenoldopam induced diuresis and natriuresis results from activation of DA-1 receptors, perhaps at a tubular site. The findings also suggest that the natriuretic response to acute volume expansion may partly be the result of activation of DA-1 receptors, perhaps by endogenous DA produced within the kidney.

152.6

ANTIHYPERTENSIVE EFFICACY OF AQUARETIC AND SALIURETIC AGENTS IN SPONTANEOUSLY HYPERTENSIVE (SH) RATS. L.B. Kinter, M. Jugus*, W.A. Mann*, and G. Dytko*, Smith Kline & French Labs, King of Prussia, PA 19406.

Chronic administration (3 d) of hydrochlorothiazide (HCTZ, 50 mg/kg UID), captopril (C, 100 mg/kg UID), an aquaretic vasopressin V_1/V_2 receptor antagonist (d(CH_2)$_5$D-Tyr(Et)VAVP; SK&F 101498, 100 µg/kg, BID), and combinations of the above were evaluated for antihypertensive activity in conscious SH rats. HCTZ, C and SK&F 101498, administered alone, were not associated with statistically significant reductions in arterial pressure. The combinations of HCTZ + C and HCTZ + C + SK&F 101498 were significantly antihypertensive, resulting in decreases in arterial pressure of 20% or more; addition of SK&F 101498 did not significantly potentiate the antihypertensive efficacy of HCTZ + C alone. Captopril + SK&F 101498 was associated with a modest antihypertensive effect. The results show that reflex stimulation of the vasopressin axis by HCTZ or HCTZ + C is not sufficient to significantly limit the antihypertensive efficacy. On the other hand, C + SK&F 101498 is associated with modest antihypertensive activity; the effect is most likely the result of a combination of a vasodilator plus a diuaretic (V_2 antagonist activity), similar to the effect of C + HCTZ. This result suggests that reduction of circulating blood volume, whether by stimulation of salt excretion (saliuresis) or water excretion (aquaresis) is effective in potentiating the antihypertensive activity of a vasodilator agent like captopril.

152.7

FLUSHING AND HEMODYNAMIC RESPONSES TO VASOPRESSIN PEPTIDES IN THE RHESUS MONKEY. David P. Brooks, Paul F. Koster*, Frans L. Stassen*, Christine R. Albrightson-Winslow*, William F. Huffman*, Martin A. Wasserman, and Lewis B. Kinter, Smith Kline & French Labs, King of Prussia, PA 19406.

The mechanism of the flushing and hypotension associated with i.v. administration of the V_2 vasopressin partial agonist, desGly (CH_2)$_5$D-Tyr(Et)VAVP (SK&F 101926; 25 mg/kg), and the selective V_2 antidiuretic agonist, desamino-8-D-arginine vasopressin (dDAVP; 3 mg/kg), was studied in ketamine-anesthetized rhesus monkeys. The flushing associated with SK&F 101926 was reduced by pretreatment with a mast cell stabilizer (SK&F 78729-A, 5 mg/kg) and by repeated administration of peptide (within 2-4 weeks). A similar desensitization to dDAVP-associated flushing was observed on repeated administration, and treatment with dDAVP also resulted in reduced SK&F 101926-associated flushing. The hypotension associated with SK&F 101926 was not affected by pretreatment with the mast cell stabilizer. A similar degree of hypotension was observed with repeated administration of either SK&F 101926 or dDAVP. Indomethacin (5 mg/kg, i.v.) did not alter the flushing or hypotension associated with the administration of either SK&F 101926 or dDAVP. Administration of a selective V_1 vasopressin antagonist did not result in flushing or hypotension. It is concluded that the flushing response to vasopressin-like peptides in rhesus monkeys may be due to an action on mast cells, whereas the hemodynamic responses are not, but likely involve direct vasodilatory actions.

152.8

CONTRIBUTIONS OF INCREASED BLOOD PRESSURE TO THE NATRIURETIC BUT NOT WATER DIURETIC EFFECT OF THE ALPHA-2 AGONIST, B-HT 933. Miklos Gellai and Catherine F.T. Uyehara, Smith Kline & French Labs, King of Prussia, PA 19406.

The possible influence of increased blood pressure on the diuretic effect of the selective alpha-2 agonist, B-HT 933, in conscious, chronically instrumented Sprague-Dawley rats (n=6) was examined. B-HT 933 (A) was infused i.v. (100 µg/kg/min) and changes in renal perfusion pressure (RPP) and renal excretion of water and electrolytes were measured. The experiments were repeated on the same rats on different days with RPP held constant by an aortic occluder (A+B). Results (mean ± SEM) were:

	RPP (mmHg)	Urine Flow (µl/min/100g)	C H_2O	Sodium Excr. (uEq/min/100g)
Control	111 ± 3	18 ± 4	-13 ± 3	1.2 ± 0.3
A	133 ± 4*	170 ± 17*	67 ± 12*	11.0 ± 1.1*
A + B	110 ± 3+	123 ± 15*+	69 ± 11*	4.5 ± 0.8*+

B-HT 933 increased RPP, urine flow, free water clearance (C H_2O), and sodium excretion. However, when RPP was kept constant, the increase in urine flow was partially attenuated due to a decrease in the increment of sodium excretion with no change in the increase in free water clearance. Thus, the B-HT 933-induced increase in sodium excretion is partially due to an increase in perfusion pressure, whereas the increase in free water clearance is mediated by other factors, e.g., inhibition of the renal tubular action of vasopressin.

152.9

INTERORGAN GLUTAMINE FLOW DURING ACIDOSIS IN YOUNG RATS. T.C. Welbourne. LSU Medical Center, Shreveport, LA 71130.

Interorgan glutamine, gln, flow in young rats were studied during chronic NH_4Cl administration. Male Sprague-Dawley rats weighing 107±4g were placed in metabolic cages and given rat chow and 1.2% NH_4Cl in 5% Dextrose ad libitum. After 3 days arteriovenous [gln] differences were measured across the kidneys and hindquarters, major sink and source of gln respectively in acidotic adult rats, 400g. Compared to these adults, young rats consumed more NH_4Cl, 6185±431 vs 2222±292 umol/100g, but excreted similar amounts of NH_4^+, 1533±183 vs 1907±120 umol/100g; the difference was made up as increased urea production by young rats, 519±39 to 2093±85 in contrast to a decline, 3752±384 vs 2270±311 umol/100g exhibited by adults. Young rats experienced a more severe acidosis, pH 7.12±0.02 and [HCO_3^-] = 13.2±0.6mM vs 7.34±0.03 and 17.2±0.8mM; however, arterial plasma [gln] was higher in the young rats, 430±31 vs 326±21mM. Across the kidney fractional extraction, FE, of gln was similar 54±9 and 61±8 percent for young and adult rats. On the other hand, a huge release of gln occurs from the hindquarters in adults FE = -38±9 percent in contrast to a surprising uptake by young rats, FE = 23±10 percent. These results suggest the young rat utilizes a different interorgan gln flow pattern in response to chronic acidosis consistent with its regulation during well defined physiological adaptations.

152.10

INHIBITORY POTENCIES OF VARIOUS CATIONIC DRUGS (CD) ON N-METHYLNICOTINAMIDE (NMN) UPTAKE BY THE BRUSH BORDER MEMBRANE VESICLE (BBMV) OF THE DOG KIDNEY CORTEX. Reina Bendayan*, Edward M. Sellers, Anita Hui* and Mel Silverman*. Departments of Pharmacology and Medicine, University of Toronto and Addiction Research Foundation, Toronto, Canada.

The organic base transport system (OBTS) at the renal proximal tubule (RPT) is a major pathway of CD excretion. Using purified BBMV isolated from dog kidney cortex and applying a rapid millipore filtration technique (25°C), the effect on the uptake of the cationic probe NMN by various CD that undergo renal tubular secretion, paraaminohippurate (PAH) and probenecid (PB) has been determined. NMN uptake is osmotic and proton gradient dependent. Under K^+ and pH equilibrium conditions, NMN uptake is characterized by a carrier mediated component (Km = 4.6 mM) and passive diffusion. Cimetidine 10 mM has been used to correct for the passive diffusion component. Apparent inhibitory constants (Ki) calculated from kinetic analysis of NMN uptake (10 sec uptake; proton gradient) are: quinidine (0.7 uM), trimethoprim (1.5 uM), cimetidine (2.0 uM), famotidine (3.0 uM), quinine (4.0 uM), amiloride (5.7 uM), methadone (14.3 uM), procainamide (22.0 uM), ranitidine (72.0 uM), nizatidine (183.0 uM), nicotine (725 uM) and histamine (900 uM). PB and PAH do not interact with NMN uptake showing the specificity of the OBTS for organic cations. All the CD studied are competitive inhibitors of NMN uptake indicating that they all share the OBTS at the RPT. The affinity of the carrier system is different for the drugs studied explaining the competition for renal tubular secretion observed when CD are administered concurrently in vivo, e.g. trimethoprim-procainamide, cimetidine-nicotine, cimetidine-procainamide. This approach is useful to predict and explain at the molecular level drug interactions at the RPT.

TMM.1

COMPUTERIZED SIMULATION OF DOSE REDUCTION INDEX (DRI) IN SYNERGISTIC DRUG COMBINATIONS. Joseph Chou* and Ting-Chao Chou. Harvard College, Cambridge, MA 02138 and Mem. Sloan-Kettering Cancer Center, Cornell Univ. Grad. School Med. Sci. New York, NY 10021

Synergism/antagonism of multiple drug effects can be quantitatively determined by the combination index (CI) defined by Chou & Talalay (Adv. Enz. Regul. 22:27-55, 1984) using the median-effect equation (Chou, J. Theor. Biol.39:253-276, 1976). This theory can be graphically presented in two ways: (1) The dose oriented classical or conservative isobologram and (2) The effect oriented F_a-CI plot where F_a is the fractional effect. Computer software for automated generation of these graphs has been developed (Chou & Chou, Elsevier-Biosoft, Cambridge, UK, 1986). We hereby introduce a third type of graphical presentation by defining a dose reduction index (DRI) which depicts how many folds of dose reduction is allowed in a synergistic combination when compared with the dose of each drug alone for a given degree of effect (F_a) and at a given combination dose ratio (CDR). Thus, depending on experimental design, DRI can be a function of F_a or a function of CDR. DRI is readily comprehensible and can be useful for determinig whether or not it is feasible to find a dose range or CDR that is therapeutically efficacious and synergistic and yet have little or no toxicity toward the host. Computer simulation for DRI has been developed for IBM-PC.

TMM.2

DISPLAYING TEACHING AND RESEARCH INFORMATION. B.C. Blackman, G.A. Henwood*, and S. Hamill*. Bio-Pharm Clinical Services, Wynnewood, PA 19096.

A variety of methods for displaying teaching and/or research materials have been used over the years, depending on cost, time, availability and quality desired. Because of the cost and time involved in their preparation, the more polished presentations are often reserved for professional meetings. Preparation of a standard poster session display is generally costly because of graphic artist charges of $40-50 per hour to do the job. Alternative methods utilizing photographic enlargements are also expensive. Therefore, students are frequently exposed to inferior material in the classroom, often hastily scribbled on overhead projector sheets. Personal computers with graphics capability, coupled with versatile laser jet printers, now enable high quality teaching and research displays to be produced at a fraction of the cost of those commercially prepared. Although intended primarily to benefit students on a daily basis, the initial cost of the software will be offset by only a few uses for preparation of professional presentations.

TMM.3

HYPERPHARM - A CONFIGURABLE QUESTION DATA BASE THAT PROVIDES IMMEDIATE REINFORCEMENT OR CORRECTIVE MATERIAL BASED UPON USER RESPONSE. Leon Moore, Paul J. Healey*, John W. Gardner* and Michael Sanders*. Uniformed Services University, Bethesda, MD 20814.

Faced with an ever increasing amount of material for presentation and a mandated decrease of contact hours with our medical students, we have developed a 1500-question data base of national board type questions and associated graphic material to be used by students for examination preparation. Questions in the data base were those used in USU pharmacology examinations over the last four years. The questions have been classified into one of the twelve NBME categories in pharmacology. Based upon the user's response to a question, faculty generated explanations are immediately provided to reinforce and explain correct responses, or to provide a corrective explanation for an incorrect response. Graphic material associated with any question is displayed with the question and can be enlarged to fill the viewing window with a mouse click. Questions and faculty responses may be added to the data base by typing or imported from a machine readable text file. Although the example data base contains subject specific material, any questions in a format similar to the NBME format may be used. The interface between the user and the data base was developed in HyperCard scripting language for the Macintosh computer. Supported in part by an Instructional Development Award from the Uniformed Services University (E075BB).