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The Physiologist

INSIDE

2009 Professional Service Award

The Secret of Service

Rudy Ortiz

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I'd like to thank the Trainee Advisory Committee (TAC) and the APS Council for selecting me as the 2009 Professional Service award winner. I am truly grateful for this great honor. I would also like to thank Marty Frank, Marsha Matyas, Melinda Lowy, Linda Allen and Brooke Bruthers for their years of friendship that have allowed me to participate and serve so broadly in the APS. Without a doubt, these relationships have led to such a distinction. I look forward to assisting them in achieving the Society's many goals, especially training the next generation of distinguished physiologists and promoting physiology in general.

As part of accepting this honor, I agreed to write an article on my professional service experience; hence my feeble attempt. Nonetheless, I looked forward to this challenge and approached it as an opportunity to inspire other young physiologists with great potential to take advantage of the numerous opportunities to serve APS and help promote its mission.

I have participated in APS conferences since I was an undergraduate, and I owe special gratitude to my professors (most of whom are APS members) for supporting my attendance. By attending conferences and networking I was able to meet leading physiologists who enlightened and inspired me to pursue my research interests in physiology. One of my graduate mentors, Leo Ortiz, first introduced me to Marty and the rest is history.

Marty proceeded to introduce me to his supportive staff as well as other physiologists; that was instrumental in facilitating my networking within the Society. The cultivation of these networking opportunities was a key to my professional development, and continues to be so. This professional growth guided me to serve the Society, which has been a privilege.

So what's the secret to service?

I can't say I know any secret in particular. However, I am confident that meeting with Marty was the first step and networking was the subsequent step that facilitated my professional development.

The key is finding an individual who is well-established within an organization, who has many associations and can guide you. Start by speaking with him/her to gain insight on how best to pursue service opportunities. To my knowledge, professional and academic service is one of the three core

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requirements for tenure and promotion at most academic institutions (along with teaching and research). At some point all new assistant professors must get involved in service at an institution or professional society. In my initial meetings and conversations with Marty he conveyed the importance of pursuing an advanced degree (especially in physiology!) and ultimately the importance of getting involved in a professional society. Getting "one's foot in the door" is an important first step to becoming involved and, during my relatively short time of service with APS, I have learned that there is no shortage of need.

Whether or not professional service should be viewed as an obligation may be debatable. While I have no intention of weighing the pros and cons of this debate here, I do want to address this issue. Given how much the Society has guided my professional growth and development, there is no doubt in my mind that I have an obligation to the Society to "repay" the years of financial and professional support they have given to me. At this time, my repayment comes in the form of service. I don't view my service as an obligation out of guilt or requirement (the type your mother would lay on you for missing church or disobeying her!), but rather an obligation of courtesy.

The only words of caution I have for others is to take measures to balance your academic career, professional service and personal life so you can achieve your greatest potential. While I have greatly enjoyed my numerous service commitments to APS as Physiologist-In-Residence for the summer teacher's workshop, as the representative from the Comparative Section on the inaugural TAC and as panel member for the Education Committee events at Experimental Biology to name a few, serving on committees can become contagious and potentially hazardous at an early stage in the development of a young scientist's career.

While I have not (and hopefully will not) learned this lesson the hard way, I know I have been extremely fortunate to have had great mentorship from many APS members. My mentors taught me early on to not spread myself too thin and to learn to balance my academic responsibilities. Sometimes this means having to say "no," but this can be done in a politically correct manner so as to leave the window of opportunity open for a more suitable time. Saying "yes" too often can potentially impede the development of a young scientist's career. This may be the biggest "secret" to service which I think most senior physiologists within the Society recognize and appreciate. It is critical to allow trainees to get involved at an early stage in their careers, but there has to be balance. In short, learning to say "no" can be as important as realizing when it is acceptable to say "yes."

With that said, if you manage your commitments accordingly, the benefits of service definitely outweigh any downside. I have learned a great deal from my service within the Society, at the institutions where I have worked and in my community. Networking can open doors to opportunities to serve, and in turn, service can lead to networking opportunities, which is beneficial both academically and professionally. As Dr. Diane Munzenmaier (2008 Professional Service award winner) mentioned in her article last year, service can be mutually beneficial. Service is an opportunity to give and receive, which can succor one's professional and academic progress. Furthermore, it is difficult to put value on the experiences and new acquaintances made by participating in the various service opportunities. There is nothing like getting hustled in a "friendly" game of poker by a group of high school teachers or, even worse, getting hustled in billiards by renowned APS leaders!

In closing, I hope my attempt to enlighten others about my professional service experience will prove to be useful in recruiting the next generation of young scientists within the Society. I would like to restate my gratitude to TAC, Marty Frank and the entire APS staff who I have had the pleasure and honor with which to work, my APS colleagues who I have had the honor and pleasure of serving with on various committees, my APS mentors who have played a critical role in my professional development and growth, and my wife and daughters for their patience and understanding, which have enabled me to participate in many service opportunities. ❖

APS Bylaw Change

The following bylaw changed allows the Committee on Committee to increase or decrease the number of members as deemed necessary, such as adding a young faculty member to the committee.

SECTION 2. Finance Committee. A Finance Committee, composed ~~five of~~ of at least five regular members of the Society appointed by Council, shall receive the total coordinated budget proposals annually from the Executive Director and shall determine the annual budgets, reserve funds and investments of the Society, subject to approval by the Council. The term of each member of the Finance Committee shall be three years; a member may not serve more than two consecutive terms. The Council shall designate the Chairperson of the Committee who shall be an ex officio member of the Council, without vote. On advice of the Finance Committee and consent of Council, the Executive Director shall be empowered to appoint and compensate a Director of Finance who shall assist in carrying out the functions of the Finance Committee under the supervision of the Executive Director. The Past President shall serve as a voting member of the Finance Committee. The President-Elect, President, Executive Director, the Chairperson of the Publications Committee, and the Director of Finance shall be ex officio members of the Finance Committee, without vote. ❖

FASEB Grant Writing Workshop

APS Members Participate in FASEB Grant Writing Workshop

The FASEB Office of MARC and Professional Development Programs sponsored a Leadership Development and Grant Writing Seminar Program for Graduate Students and Postdocs on August 28–29, 2009 in Virginia Beach, VA.

The enthusiastic attendees were not distracted by the beautiful weather and eagerly participated in the intensive two-day seminar.

David C. Morrison of Grant Writers' Seminars & Workshops, LLC, opened the program with "Getting Started as a Successful Grant Writer and Academician." Aimed at new investigators, the seminar focused on writing a successful grant application, a subject missing from most of the educational backgrounds of junior faculty members. The pace of the presentation was well suited to the participants and was enhanced by extensive question-and-answer sessions. Morrison received high marks and rave reviews for his presentation.

Howard G. Adams of H.G. Adams & Associates offered sessions on strategic planning, goal setting and prioritizing, which aimed to help participants maximize the leadership potential that is crucial in bringing about innovation and change. This symposium, "Leadership Development: Training Tomorrow's Leaders Today," included a team-building activity in which participants were divided into small groups and assigned individual case studies. Following the group breakout sessions, the participants reunited to assess and discuss results. Adams also received excellent reviews.

There were a total of 45 participants, nine of which were members of The American Physiological Society and who were also recipients of FASEB MARC Program travel awards:

Jose Garcia, Univ. of Puerto Rico, Medical Sciences Campus; Mary Garcia-Czarin, Univ. of Kentucky; Shea Gilliam-Davis, Wake Forest Univ.; Tanecia Mitchell, Univ. of Arkansas for Medical Science; Marisa Pulido-Covington, Medical Univ. of South Carolina; Odrick Rosas, Univ. of Puerto Medical Science Campus; Iris Salgado, Univ. of Puerto Rico Medical Science Campus; Jose Torres-Hernandez, Texas Southern Univ.; and Tanganyika Wilder, Univ. of Illinois at Chicago. ❖

Participants of the FASEB-sponsored Leadership Development and Grant Writing Seminar Program for Graduate Students and Postdocs.

Tennessee Physiological Society Holds First Annual Meeting

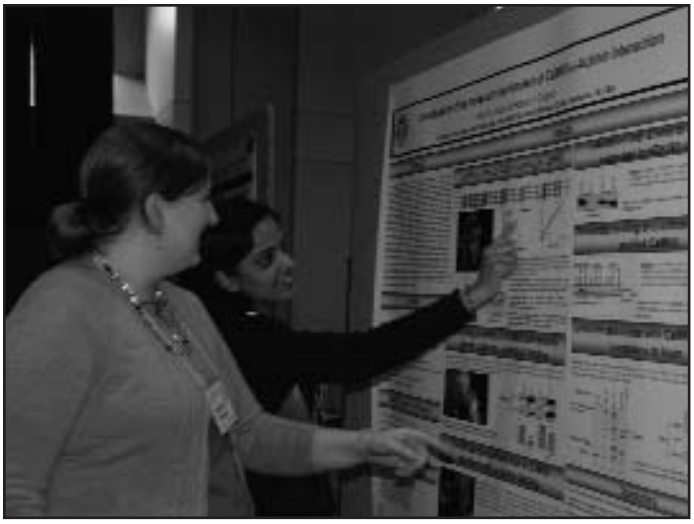
The Tennessee Physiological Society (TPS) held its first annual meeting on Friday, October 23, 2009 at the Vanderbilt University Student Life Center. The meeting was hosted by the Vanderbilt Department of Molecular Physiology & Biophysics and also supported by the Department of Anesthesiology. Peter K. Lauf, Chair of the APS Chapter Advisory Committee, officiated and witnessed the birth of TPS. By a majority vote, attendees approved the bylaws and thereby officially created the Society. The bylaws had been previously approved by APS council, officially recognizing TPS as the Tennessee Chapter of the American Physiological Society. By a majority vote, the first slate of TPS officers was approved. Eric Delpire from the Department of Anesthesiology at Vanderbilt University was recognized as TPS President, Donald Thomason from the Department of Physiology at University of Tennessee Health Science Center recognized as TPS President-elect, and Tom W. Ecay from the Department of Physiology at East Tennessee State University recognized as the TPS Treasurer.

Alan Verkman presents the Keynote Address.

The one day meeting was organized with morning talks, lunch, afternoon poster session, keynote address, and dinner. Four Tennessee institutions were represented in the morning talks: Ronald Emeson from the Department of Pharmacology at Vanderbilt spoke on Prader-Willi syndrome and serotonin receptor editing. Zhongmao Guo from the Meharry Medical College spoke on the protective role of the aryl hydrocarbon receptor pathway against atherosclerosis. Carole Williams from the Department of Physiology at East Tennessee State University spoke on neuropeptide, neuromodulation and cardiac ischemia. Charles Leffler from the Department of Physiology at University of Tennessee Health Science Center, spoke on carbon monoxide and newborn cerebral blood flow. Finally, representing the Vanderbilt Postdoctoral fellows, Kevin Erreger presented his work on the regulation of amphetamine-induced dopamine release by glucagon-like peptide-1.

Three events were held in the afternoon: the first event was a poster session with 29 posters featuring a variety of physiology topics. The second event, which occurred concomitantly to the poster session, was a gathering of high school students with two Vanderbilt physiologists: Maureen Gannon and Alyssa Hasty. Maureen and Alyssa, who work on diabetes and obesity, respectively, cleverly used these two disorders to introduce Physiology and talk about integration of the different organs and systems involved. They both told the students how they got interested in science, how their individual paths led them to end up working in the same institution, and what they like and dislike in their job. The third event was the keynote address by Alan Verkman from the University of California San Francisco. The title of the address was "Chloride channels: discovery and applications of small-molecule modulators." Verkman spoke on systemic diarrhea in third world countries, traveler's diarrhea, cystic fibrosis, chloride channels, the multiple processes involved in their regulation, and the additional transport pathways involved in chloride movement, all as possible targets for the development of new therapeutics. He presented an impressive synopsis of all his successes and efforts in developing new chloride channels inhibitors.

With this initial success, and with a determination to reach out to more institutions and physiologists, TPS will hold its next meeting at UTHSC in Memphis in the fall of 2010. ❖



Nidhi N. Jalan (back) and Heather Gosnell (front), both Vanderbilt graduate students, discuss a presented poster.

Don Thomason, Alan Verkman, Eric Delpire, Peter K. Lauf, Norma Adragna, and Tom W. Ecay.

Ethics and Integrity IUPS 2009

The Ethics Committee of the International Union of Physiological Sciences (IUPS) sponsored a symposium at the 36th Congress in Kyoto Japan on "Best Practices in Physiological Research: Ethics and Integrity." The symposium was held on August 1, the last day of the meeting and, in spite of a very full program that morning, we had a full room for this session.

Much like water's pervasive presence in any physiological system, ethics and integrity are pervasive throughout the study of physiology. The advancement of physiology depends upon the assumptions that the results we present are conducted using the most ethical and humane procedures and based on an honest interpretation of the data. The symposium at the 36th Congress addressed these two topics with four keynote speakers: Colin Blakemore (UK), Naoko Kagiyama (JP), Kim Barrett (US) and Penny Moody-Corbett (CA). The session was divided into two parts; focusing first on the issues of the use of animals in physiological experiments and then on issues of integrity and scientific misconduct.

Colin Blakemore introduced the session with his lecture on "Animals in Medical Research: Magic or Tragic." Blakemore reviewed the history of animal research in the UK, the rise of a very violent anti-vivisectionist movement and the response of researchers to defend both their right and society's need to continue the regulated use of animals in physiological experimentation. Blakemore reminded us that early research was done without anesthetic and the current knowledge of methods to reduce pain, but that animal research was re-introduced to Britain late in the nineteenth century. He also reminded us that opposition to this area of research has been long standing. However, there was an unprecedented rise of violence against the research community in Britain through the 1980s and 1990s, including attacks on scientists, animal suppliers, and industry that resulted in huge property damage and danger to both individuals directly involved and their families. Initially unprepared for these attacks, the research community was either silent or reacting defensively against a wave of attacks from activists and the media, which generated public outrage. However, as Blakemore presented, the research community did not succumb to this violence: researchers, businesses and families impacted by medical research defended the rights of the research community to use animals in humane and responsible manner for the pursuit of medical research. They were supported in this defense by support from medical charities and research funders, and the attitude of the media and then the public shifted dramatically. Changes in laws and regulations specifically addressing acts of violence and intimidation were put in place and, as a result, there has been a significant reduction in violent acts against the research community. Studies show that a clear majority of the British public now supports the use of animals for medical research. Blakemore cautioned that we should not become complacent about our use of animals in research and reminded us of the absolute requirement and responsibility that we have as researchers, using public funds; that public engagement must become part of the job of being a scientist. It is with these tools, the continued refinement of the humane treatment of animals in experimentation and continued oversight of regulations that we will continue to have the support of the majority of the public in our work.

Following the introductory lecture, Kagiyama presented, "Guidelines for proper treatment of animals in research" (Kagiyama, Ito and Demers). Her presentation provided information on the guidelines in place internationally through the International Council on Laboratory Animal Science (ICLAS), as well as the specific programs in place in Japan. ICLAS has its roots in the International Committee on Laboratory Animals (ICLA) which was established in 1956 under the initiative of UNESCO, the Council for International Organizations of Medical Sciences (CIOMS) and the International Union of Biological Sciences (IUBS). Kagiyama explained that ICLAS is "dedicated to advancing human and animal health by promoting the ethical care and use of laboratory animals in research worldwide." There are 35 national, 43 scientific and 42 associate organizations and nine honorary members. The goal of ICLAS is to support the harmonization of policies and guidelines in animal care worldwide and is based on five principles which include establishment of policies on humane endpoints, animal euthanasia, animal user training programs, experimental program reviews and the care and use of genetically engineered animals. The organization does not regulate by international standards; rather it is based on the applications of the five principles within the context of the cultures, traditions, religions, laws and regulations of each of the member countries. Kagiyama then described how the Science Council of Japan has applied these principles. In this country, the "Law for the humane treatment and management of animals in research" was established in 1973 and recently (2005) revised. The amendment to the Law in 2005 was based on consultation with various organizations including 13 scientific associations and adherence to the 3Rs: replacement, reduction, and refinement. Kagiyama provided a description of the guidelines in place for institutions and went through an example of an interaction between an individual researcher and their institutional review board. Kagiyama was able to cover the wide spectrum of the importance of international harmonization on care and management of animals used in research to the responsibilities of individual researchers in their laboratories and institutions.

The second half of the symposium focused on the issues of scientific misconduct. Moody-Corbett gave a presentation titled, "PIE: Physiology, Integrity and Ethics - Working Together." Her presentation began with a description of the problems of falsification, fabrication and plagiarism and the intentional misrepresentation of scientific data. Recent estimates from the US indicate that the number of cases of misconduct that are reported is probably much lower than the actual number that occurs. However, the ones reported are the high profile cases that often hit the front page and garner considerable media attention. They result in a loss of confidence in science and scientists, and the media are quick to present a very negative picture of scientific behavior. However, Moody-Corbett pointed out that a more serious problem is that of "questionable research practices" which may include a variety of behaviors such as self-plagiarism, cleansing data, questionable authorship, misrepresenting publication status, misrepresenting other researchers' work, maligning colleagues, etc. These practices are much more dif-

ficult to document but are likely much more prevalent, forming the basis for poor training environments, disruption in scientific advancement and loss of credibility. Misconduct has both personal and financial costs at the institutional level; impacting reputation and morale and leaving a problem of how the scientific record is to be corrected. Scientific misconduct has a huge global impact and discovering and rectifying the problem and protecting both whistleblowers and wrongfully accused is a challenge. Moody-Corbett gave a brief description of the recent world conference on scientific integrity (Lisbon, 2007). Recommendations from the Congress focused on recognition, setting standards, education and training and suggested a "code of conduct" for scientists; stressing that basic standards for responsible research behavior must be global. Moody-Corbett finished her presentation by reminding the audience of the importance of fostering a culture of integrity, being academic role models, ensuring institutional policies and harmonizing within and across borders.

The final presentation of the session was by Kim Barrett: "Publishing Physiological Research: Integrity and Misconduct." Barrett, who is the Chair of the APS Publications Committee, reinforced the importance of intellectual honesty, accurate assignment of credit, fairness in peer review, collegiality in scientific interactions, transparency in conflicts of interest and protection of human and animal subjects. She reported that while most scientists are completely ethical a significant proportion have admitted to "questionable practices." APS has 14 publications and Barrett presented data indicating a dramatic increase in the number of ethical cases from under 40 cases a year in 2005 to a projection of over 100 cases for 2009. The types of ethical issues included: redundant publications, animal welfare concerns, authorship disputes, duplicate publications, human welfare concerns, data fabrication/falsification (inappropriate manipulation of figures), plagiarism, conflicts of interest, etc. Barrett reviewed several of these topics and gave examples of detection of inappropriate manipulation of figures as detected by the APS Art Department, demonstrating the ease with which questionable practices become apparent. The problem of unethical practices is not isolated to the researcher submitting work for publication but also involves issues of reviewer bias, or conflict of interest or reviewers misappropriating privileged information. Barrett described the procedure for handling cases of suspected misconduct and the importance for APS to be consistent in its approach and constantly vigilant. Following a charge of misconduct several outcomes are possible from cases being dismissed (rare) or allowing authors to supply revised material to outright rejection or sanctions for one year or up to a lifetime. Barrett pointed out that there are a number of challenges in dealing with misconduct including the Society's limited ability to conduct a detailed investigation and cultural and language differences which create barriers. In summarizing, Barrett reminded us that science is based on trust and that ethical lapses betray that trust. The APS is challenged to detect and investigate ethical transgressions but is committed to maintaining the integrity of the literature which they publish. In the end it is the scientists who are responsible for understanding the rules and educating the next generation of investigators.

The importance and interest of these topics was evident by the discussion following the session and the committee welcomes further comments on these topics.

Acknowledgements: The Ethics Committee would like to acknowledge financial support from the IUPS, ICLAS, APS, CPS and the respective institutions of the speakers.

Co-chairs: Tadashi Isa, National Institute for Physiological Sciences, Myodaiji, Okazaki, Japan and Penny Moody-Corbett, Memorial University of Newfoundland, St. John's NL Canada

Speakers:

Colin Blakemore, Professor of Neuroscience, Universities of Oxford and Warwick;

Naoko Kagiyama, Professor of Veterinary Medicine Hokkaido University;

Kim Barrett, Dean of Graduate Studies, University of California, San Diego, and Chair of the Publications Committee for the American Physiological Society; and

Penny Moody-Corbett, Associate Dean for Research and Graduate Studies, Memorial University of Newfoundland and Chair of the Ethics Committee of IUPS. ❖

Penny Moody Corbett

Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

Broomfield, CO, July 15-18, 2009

The 2009 APS Conference: Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology, was held in Broomfield, CO. The conference took place over four days at the Omni Interlocken Resort. The resort offered an excellent panorama of the majestic Rocky Mountains, shopping opportunities and world-class golf. The Organizing Committee included Chair, Jane Reckelhoff, Univ. of Mississippi Medical Center; Vice-Chair, Christine Maric, Univ. of Mississippi Medical Center; Kathryn Sandberg, Georgetown Univ.; Carmen Hinojosa-Laborde, Univ. of Texas Health Science Center at San Antonio; J. Michael Wyss, Univ. of Alabama at Birmingham; John Stallone, Texas A&M Univ.; Virginia Miller, Mayo Clinic; and, Meredith Hay, Univ. of Arizona. The committee-organized program that included symposia, oral presentations for students and postdoctoral fellows, interactive poster sessions, and social networking opportunities, made the conference a valuable experience for those who attended.

The conference was attended by 85 total registrants: of whom 21% of registrants were represented by young scientists, including five postdoctoral and 13 students. Thirty-six (42%) attendees identified themselves as APS members, and ten (12%) registered as nonmembers, invited chairs and speakers made up the remaining 21 (25%) attendees. Table 1 (below) shows the breakdown of the different registration types. This conference also attracted a large group of registrants from outside the United States. Out of the 85 registrants, 20 (15%) represented countries from Argentina, Australia, Belgium, Brazil, Canada, Germany, Nigeria, South Korea, Switzerland, Thailand, and the United Kingdom.

The conference program consisted of one plenary lecture and seven symposia on a wide variety of topics related to sex steroids and gender physiology. The audience was encouraged to share their ideas and thoughts with the speakers at the end of their talks. There were six oral presentation sessions that were dedicated to the postdoctoral fellows and students attending the conference, several of the trainees presented their very first oral talk at this meeting. During the conference, Magdalena Alonso-Galicia hosted a workshop sponsored by the APS Career Opportunities in Physiology Committee that gave young scientists the opportunity to gain information and valuable career skills on authorship of abstracts. The conference also had several social activities including a Welcome and Opening Reception, which was designed to give attendees a chance to meet with long time colleagues, create new friendships, and enjoy some hot and cold hor d' oeuves and beverages before the evening presentations. There were two afternoon poster sessions where scientists presented their work and discussed their findings with other attendees. In addition, the conference program allowed for some free time in the afternoons for participants to network and explore the posters that were submitted for the conference.

Table 1. Registration Statistics

Registrant Type	Number of Attendees (%)
APS Member	36 (42%)
Nonmember	10 (12%)
Postdoctoral	5 (6%)
Student	13 (15%)
Invited Chairs/Speaker	21 (25%)
Total	**85**

A total of 58 abstracts were submitted for the conference. Forty-three of these abstracts were programmed as poster presentations (photo 2). The remaining 15 abstracts were submitted by invited speakers. Of the abstracts submitted for the conference, 33 (57%) were submitted by a female first author; nine (16%) were submitted from institutions outside of the United States, including two abstract from both Argentina and Europe respectively, one abstract from Australia, Brazil, Canada, Nigeria, and South Korea.

Conference Organizers, Jane Reckelhoff (left) and Christine Maric (far right) present certificates to the abstract travel award winners. Limor Raz, Medical College of Georgia (first place), Avantika Kekatpure, University of the Pacific (second place), and Kristen Osterlund, University of Arizona (third place).

On Friday evening, Reckelhoff hosted the Banquet and Awards Presentation Dinner. Attendees gathered at the hotel's dining room for dinner, wine and conversation. During the event, 13 postdoctoral fellows and students were recognized as the recipients of the Research Recognition Award for Outstanding Abstract by a Graduate Student or Postdoctoral Fellow. The following individuals were presented with a certificate and cash prize: Vicky Rands, Tulane Univ.; Limor Raz, Medical College of Georgia; Megan Wenner, Yale Univ.; Jewell Jessup, Wake Forest Univ.; Louis Mattar, Univ. of Western Ontario, Canada; Ahmed Oloyo, Univ. of Lagos, Nigeria; Kristi Pogue, Univ. of Texas Health Sciences Center, San Antonio; Michaela Manigrasso, Univ. of Mississippi Medical Center; Avantika Kekatpure, Univ. of the Pacific; Kristen Osterlund, Univ. of Arizona; Rebecca

Conference attendees viewing posters.

Schneider, Univ. of Missouri, Columbia; Marcia Venegas-Pont, Univ. of Mississippi Medical Center; and, Michael Mestek, Univ. of Colorado, Boulder (photo 1).

In addition Cheryl Bell, University of Connecticut; Rayna Gonzales, University of Arizona, Phoenix; Camila Manrique, University of Missouri, Columbia; and, Minolfa Prieto, Tulane University, were the recipients of the Porter Physiology Development Committee's Minority Travel Fellowship Award, which is provided to encourage participation of under-represented minority students in the physiological sciences. With support from the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK), the fellowship provides reimbursement of all expenses associated with travel and participation in the conference. The recipients of the award were matched with APS members: Pascale Lane, University of Nebraska College of Medicine and Lourdes Fortepiani, University of Texas Health Sciences who attended the conference, offered guidance and made introductions to other scientists.

The American Physiological Society and the Organizing Committee gratefully acknowledge the financial support provided though generous educational grants from NIH, National Institutes of Diabetes and Digestive and Kidney Diseases. ❖

ET-11: APS International Conference on Endothelin

Montreal, Canada, September 9-12, 2009

The ET-11: APS International Conference on Endothelin was held in the metropolitan city of Montreal, Canada. The conference took place over four days at the Montreal Marriott Chateau Champlain Hotel. Located in the downtown area, the hotel was steps from the historic district, cultural activities and a host of shopping and dining opportunities. The Local Organizing Committee (LOC) was chaired by Pedro D'Orleans-Juste, Univ. of Sherbrooke, Canada, and included Co-Chair, David Pollock, Medical College of Georgia; Bruno Battistini, Acasti Pharma, Inc.; Jocelyn Dupuis, Univ. of Montreal, Canada; Alan Fournier, Univ. of Quebec, Canada; Ernesto L. Schiffrin, McGill Univ., Canada; Duncan Stewart, Univ. of Ottawa, Canada; and Eric Thorin, Univ. of Montreal, Canada. The committee organized a program that would include symposia, oral presentations for students and postdoctoral fellows, interactive poster sessions, and social networking opportunities to make this conference a valuable experience for those who attended. In addition to the LOC, the International Scientific Advisory Committee and the Endothelin International Advisory Committee assisted the organizers in reviewing and programming all of the abstracts that were submitted to the conference.

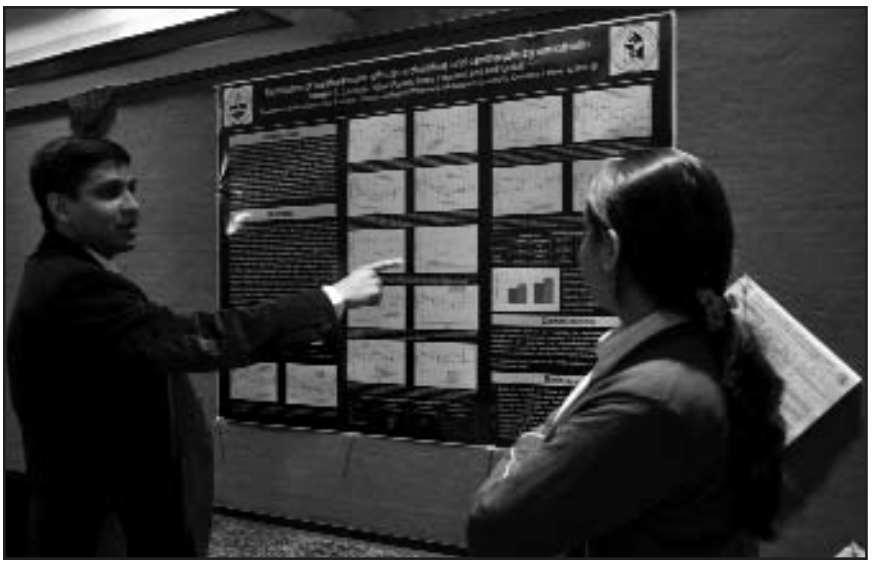
The conference was attended by 201 total registrants: 38% of registrants were represented by trainees, including 14 postdoctoral and 62 students. Twenty (10%) attendees identified themselves as APS members, and 65 (32%) registered as nonmembers, invited chairs and speakers made up the remaining 40 (20%) attendees. Table 1 (below) shows the breakdown of the different registration types. This conference also attracted a large group of registrants from outside the United States. of the 201 registrants: 136 (67%) represented countries from Brazil, Canada, Czech Republic, Denmark, France, Germany, Israel, Italy, Japan, The Netherlands, Spain, Sweden, Switzerland, and the United Kingdom.

The conference program consisted of one key note lecture and 12 symposia on a wide variety of topics related to endothelin. The audience was encouraged to share their ideas and thoughts with the speakers at the end of their talks. Ten of the symposia provided the opportunity for selected oral presentations from the volunteered abstracts that were submitted for the conference. The remaining two symposia were ded-

Pedro D'Orleans-Juste (left) and David Pollock (far right) present Michelle Gumz, University of Florida and Aditya Goel, Johns Hopkins Medical Institute with the postdoctoral abstract travel award.

Table 1. Registration Statistics

Registrant Type	Number of Attendees (%)
APS Member	20 (10%)
Nonmember	65 (32%)
Postdoctoral	14 (7%)
Student	62 (31%)
Invited Chairs/Speaker	40 (20%)
Total	**201**



ET-11 attendees during the poster sessions.

icated to clinical trials and careers. Both sessions had lively debate and were well attended. The conference also had several social activities, including a Welcome and Opening Reception, which was designed to give attendees a chance to meet with long time colleagues, create new friendships, and enjoy some hot and cold hor d'oeuves and beverages while listening to live classical music. There were also three afternoon poster sessions where scientists presented their work, and discussed their findings with other attendees. On the last evening of the conference, a special ticketed event took place at the Montreal Museum of Fine Arts. The attendees enjoyed cocktails, a three-course dinner, musical entertainment and the opportunity to enjoy some of the artwork in the museum.

A total of 152 abstracts were submitted for the conference. 90 of these abstracts were programmed as poster presentations. Fifty-one volunteered abstracts were programmed as oral presentations. The remaining 11 abstracts were submitted by invited speakers. Of the abstracts submitted for the conference, 45 (30%) were submitted by a female first author; 98 (64%) were submitted from institutions outside of the United States, including 29 from Canada, nineteen from the United Kingdom, 12 from Japan, and eight from Brazil. The remaining abstracts came from Czech Republic, Denmark, France, Germany, Greece, Israel, Italy, Poland, The Netherlands, Spain, Sweden, and Switzerland.

During the Career Symposia, D'Orleans-Juste and Pollock presented travel awards to nine postdoctoral fellows and students who were recognized as the recipients of the Research Recognition Award for Outstanding Abstract by a Graduate Student or Postdoctoral Fellow. The following individuals were presented with a certificate and cash prize: Brian Weil, Univ. of Colorado; Boulder, Merlijn J. P. M. T. Meens, Maastricht Univ.; The Netherlands; Bambang Widyantoro, Kobe Univ., Japan; Iain MacIntyre, Univ. of Edinburgh, United Kingdom; Nicholas Kirkby, Univ. of Edinburgh, United Kingdom; Rafaela Claudino, Univ. Federal de Santa Caterina, Brazil; Aditya Goel, Johns Hopkins Medical Institute; Michelle Gumz, Univ. of Florida; and, Melissa Li, McGill Univ., Canada.

In addition Fernanda Giachini, Univ. of Sao Paulo/Medical College of Georgia; Aisha Kelly-Cobbs, Medical College of Georgia; Victor Lima, Medical College of Georgia; and Cornelius Nwora, Texas Southern Univ., were the recipients of the Porter Physiology Development Committee's Minority Travel Fellowship Award, which is provided to encourage participation of under-represented minority students in the physiological sciences. With support from the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK), the fellowship provides reimbursement of all expenses associated with travel and participation in the conference. The recipients of the award were matched with APS members: Craig F. Plato, Gilead Sciences, Inc.; Ulla C. Kopp, Univ. of Iowa, Carver College of Medicine; Gregory D. Fink, Michigan State Univ.; and, R. James White, Univ. of Rochester, who offered guidance and made introductions to the other scientists.

Pedro D'Orleans-Juste (Organizing Committee Co-Chair; University of Sherbrooke, Canada) present the student abstract travel awards to: Rafaela Claudino, University Federal de Santa Caterina, Brazil, Nicholas Kirkby, University of Edinburgh, UK, Merlijn J. P. M. T. Meens, Maastricht University, The Netherlands, Brian Weil, University of Colorado, Boulder, Iain MacIntyre, University of Edinburgh, UK, Bambang Widyantoro, Kobe University Graduate School of Medicine, Japan, and David Pollock (Organizing Committee Co-Chair; Medical College of Georgia).

The American Physiological Society and the Organizing Committee gratefully acknowledge the financial support provided though generous educational grants from NIH, National Institutes of Diabetes and Digestive and Kidney Diseases, Abbot Laboratories, Actelion Pharmaceuticals, Ltd., Gilead Sciences, Inc., Pfizer, Ltd., and AstraZeneca, UK. ❖

New Regular Members

***Transferred from Student Membership**

Roslida ABD Hamid
Univ. of Putra, Malaysia
Reem R. Abraham*
Melaka Manipal Med. College, India
Nilufa Akhter
Dhaka Shishu Hosp., Bangladesh
Taskina Ali
Banabandhu Sheikh Mujib-Med. Univ., Bangladesh
Namasivayam Ambalavanan
Univ. of Alabama, Birmingham
Alexandra Andreeva
Univ. of Illinois, Chicago
Ugochukwu B. Anyachie
Univ. of Nigeria, Enugu
John W. Apolzan*
Medical College of Georgia, Augusta
Adesina P. Arikawe*
Univ. of Lagos, Luth, Nigeria
Michelle M. Arnhold*
Univ. of Wisconsin, Superior
Sudip Bajpeyi*
Pennington Biomed. Res. Ctr., LA
Salah Abu Baker
Univ. of Nevada, Reno
Jonathan M. Beckel*
Univ. of Groningen, Netherlands
Tara M. Blank*
Univ. of North Texas
Sarah Wright Bottjer
Univ. of Southern CA, Los Angeles
Eric Alan Bridenbaugh*
Texas A&M Univ.
Keith R. Brunt*
Toronto Gen. Hosp., ON, Canada
Thomas Buford*
Univ. of Florida
Uriel A. Buitrago-Suarez
Mount Marty College, Yankton, SD
Mehmet Bulbul*
Medical College of Wisconsin
Douglas G. Burrin
USDA Child. Nutr. Res. Ctr., Houston, TX
Philippe P. Caimmi
Univ. Maggiore Della Carita, Italy
Flavia Regina Carreno
Univ. of North Texas, Fort Worth, TX
A. Brent Carter
Univ. of Iowa
Jie Chen
Univ. of Illinois, Urbana-Champaign
Yan-Hua Chen
East Carolina Univ., NC
Bopaiah P. Cheppudira
Mayo Clinic, Rochester, MN
Narendranath Reddy Chintagari*
Ctr. for Vet Health Sci., Stillwater, OK
Mark T. Clunes
St. George's Univ., Grenada
Christopher T. Coburn
Western Carolina Univ., NC
Summer Baldwin Cook*
Univ. of New Hampshire
Emily Ann Cordas*
NIH, NIDDK, Bethesda, MD
Zelieann Rivera Craig*
Univ. of Illinois, Urbana
Clayton Eugene Curtis
New York Univ.
Charles A. Darveau*
Univ. of Ottawa, Canada
Daisy La Dawn Daubert*
Ferris State Univ., MI
Daniele Mike De Luca
Women's & Children Hosp., Italy
Geert J. De Vries
Univ. of Massachusetts
Ndeye Khady Diop-Bove
Albert Einstein College of Med., NY
William W. Dowd
Stanford Univ., Pacific Grove, CA
Jianhai Du
Med. Coll. of Wisconsin, Milwaukee
Serge Olaf Dumoulin
Utrecht Univ., Netherlands
Oetras Dzeja
Mayo Clinic, Rochester, MN
Stefan G. Maia Etelvino
Flinders Univ., Adelaide, Australia
Andrea Nicole Flynn
Univ. of Arizona, Tucson
Guo-Hua Fong
Univ. of Connecticut Hlth. Ctr.
Matthew S. Ganio*
Texas Hlth Presbyterian Hosp., Dallas
Heath Gregory Gasier*
Naval Sub. Base, New London, CT
Hélène Girouard
Univ. De Montreal, Canada
Brian Glancy*
NIH, Bethesda, MD
Alan L. Goldin
Univ. of California, Irvine
Elena Grossini
Univ. Del Piemonte Orientale A Avogadro, Italy
Mangala Gunatilake
Univ. of Colombo Fac. Med., Sri Lanka
Shereen M. Hamza*
Univ. of Mississippi Med. Ctr.
Johanna Lucy Hannan
Medical College of Georgia
Parisa Hasanein
Bu-Ali Sina Univ., Hamadan, Iran
Kim Henige*
California State Univ., Northridge
Andrew Hill
Rutgers Univ., Newark, NJ
Yoshimi Homma
Fukushima Med. Univ., Japan
Oiaobing Huang
Southern Med. Univ., China
Kristen L. Hutchins*
Howard Payne Univ., Brownwood, TX
Nese Imeryüz
Marmara Univ. Sch. of Med., Turkey
Caron Yumi Inouye
California State Univ., East Bay
Sarvesh Jajoo
Southern Illinois Univ., Springfield
Mahnrdad Jazayeri
Univ. of Washington, Seattle
Jamie Johnston
Univ. of Victoria, Canada
Mohammad Khaksari Haddad
Kerman Med. Fac., Kerman, Iran
Kanchana M. Karuppiah
Univ. of Florida
Karen Rachele Kelly*
Cleveland Clinic, OH
Shakil Ahmad Khan
Univ. of Chicago, IL
James Derek Kingsley*
Indiana State Univ., Terre Haute
Erik Mason Kolb*
Chaffey College, Rancho Cucamonga, CA
Marion Korach-Andre
Karolinska Inst., Huddinge, Sweden
Jean-Claude Lacaille
Univ. of Montreal, PQ, Canada
Samuel Todd Lamitina
Univ. of Pennsylvania
Vladimir Ljubicic*
Univ. of Ottawa, ON, Canada
Xingsheng Li
Univ. of Alabama, Birmingham
Battur Lkhagvaa
Health Sci. Univ. Mongolia
Michael M. Lockard*
Willamette Univ., Salem, OR
Fei Ma
Univ. of Kentucky, Lexington
Hongtao Ma*
Weill Cornell Med. Coll., NY
Irene Mackraj
Univ. of KwaZulu Natal, South Africa
Guruprasad Madhavan*
Natl. Acad. of Sci., Washington, DC
Daniel Margoliash
Univ. of Chicago, IL
Gustavo Matute-Bello
Univ. of Washington, Seattle
Stuart J. McDougall
Oregon HSU, Portland
Edward K. Merritt*
Univ. of Alabama, Birmingham
Michael Louis Mestek*
Univ. of Colorado, Boulder
Denshun Miao
Nanjing Med. Univ., China
Michelle Mielke*
Univ. of the Pacific, Stockton, CA

Stephanie A. Moeckel-Cole
Univ. of Massachusetts
Frank B. Mojiminiyi
Usman DunFodio Univ., Nigeria
Cephas T. Musabayane
Univ. of KwaZulu-Natal, South Africa
Kazuhiro Nakamura
Kyoto Univ., Japan
Michael P. Nusbaum
Univ. of Pennsylvania Sch. of Med.
Hidehiko Okamoto
Westfalian Wilhelms-Univ., Germany
Lawrence A. Olatunji
Coll. of HSU, Ilorin, Nigeria
Gabriela Ravanelli de Oliveira-Pelegrin*
Univ De São Paulo, Brazil
Simen Gylterud Owe
Univ. of Oslo, Norway
Pablo Pacheco
Univ. Nacional Autonoma De Mexico
Nicole M. Palenske*
Central College, Pella, IA
Christopher A. Pearl
Western Michigan Univ.
Christopher G.R. Perry*
East Carolina Univ., Greenville, NC
Thomas A. Quinn
Univ. of Oxford, UK
Erik P. Rader
Univ. of Iowa
Marcella A. Raney*
Occidental Coll., Los Angeles, CA
Malte J. Rasch
Chinese Acad. of Science, China
Sultana F. Ratna
Banabandhu Sheikh Mujib Med. Univ., Bangladesh
R. Scott Rector*
Univ. of Missouri
Palanivel Rengasamy
McMaster Univ., Hamilton, Canada
Brian F. Renna*
Community College of Philadelphia, PA
Nour-Eddine Rhaleb
Henry Ford Hospital, Detroit, MI
Bryan Charles Rourke
California State Univ., Long Beach
Shu-ichi Saitoh
Fukushima Medical Univ., Japan
Keith K. Schillo
SUNY, Oneonta, NY
Michael B. Schwarzer
Univ. of Leipzig Heart Ctr., Germany
Maria Kraemer Schwartz*
Mayo Clinic, Rochester, MN
Marilia C. Seelaender
Univ. of Sao Paulo, Brazil
Chris Sekirnjak*
Salk Inst. for Bio. Scis., La Jolla, CA
Elena Y. Senchenkova
Louisiana State Univ., Shreveport
Siamak Shahidi
Hamadan Univ. of Med. Sci., Iran
Purnima Sharma
Agency for Sci. Tech. & Res., Singapore
Peng Shi*
Univ. of Florida
Najeeb A. Shirwany*
Univ. of Oklahoma HSC
Grant H. Simmons*
Univ. of Missouri, Columbia
Jalal Solati
Islamic Azad Univ. Karaj Branch, Iran
Noriko Tagawa
Kobe Pharmaceutical Univ., Japan
Luc Tappy
Univ. of Lausanne, Switzerland
Ali Tavakkolizadeh
Brigham and Women's Hospital, MA
Kirk Gerald Thompson
NIH, NEI, Bethesda, MD
Andrei V. Tkatchenko
Wayne State Univ., MI
Malgorzata B. Tokarska-Schlattner
Joseph Fourier Univ., France
Kumika Toma
Ohio Univ. Coll. of Osteo. Med.
Matthew C. Trudeau
Univ. of Maryland
Toru Tsujimoto
Nat'l. Inst. Phys. Scis., Okazaki, Japan
Christine I. Turenius*
Loma Linda Univ., Medical Center, CA
Jason Daniel Vescovi*
York Univ., Toronto, ON, Canada
Anton Vonk-Noordegraaf
Vrije Univ. Med. Ctr., Netherlands
Joanne Wang
Univ. of Washington, Seattle
Xian Wang
Peking Univ. HSC, Beijing, China
Yanhua Wang
Emory Univ. Sch. Med., Atlanta, GA
Yuhong Wang
Univ. of Oklahoma
Kenneth J. Warring-Davies
Leeds Gen. Infirm., West Yorkshire, UK
Robert G. Weiss
John Hopkins Univ., Baltimore, MD
Katherine A. Wilkinson*
Emory Univ., Atlanta, GA
Trevor Williams
Univ. of Colorado, Denver
Geoffrey F. Woodman
Vanderbilt Univ., Nashville, TN
Susan Wray
Univ. of Liverpool, UK
Yuming Wu
Hebei Med. Univ., China
Hao Xu
Medical Coll. of Wisconsin, Milwaukee
Yan Chun Xu
West Virginia Univ.
Don Yuan
West Virginia Univ.
Gengoian Zhang
West Virginia Univ.
Sarah Xin Zhang
Univ. of Oklahoma HSC
Meihong Zhu
Univ. of Nevada Sch. of Med., Reno

New Student Members

Padzlina Amir Shapuddin
Univ. of Putra, Malaysia
Farhad Asskaryar
Sir Ramachandra Univ., India
Emma Louise Bahe
Univ. of Minnesota
Nathan David Barrows
California State Univ.
Sumit Barua
Ajov Univ., South Korea
Pradeep Bhandari
BP Kairala Inst. of Health, Nepal
Josip Andjelo Borovac
State Univ. of New York, Plattsburgh
Nadejda I. Bozadjieva
Univ. of Minnesota
Rebecca Sue Bruning
Pennsylvania State Univ.
Yen-Jui Chang
National Yang-Ming Univ., Taiwan
Patrick Leon Crosswhite
Univ. of Oklahoma
Tracy Yvette Dodd
Univ. of South Alabama
Ladan Eshkevari
Georgetown Univ., Washington, DC
Seth Tyler Fairfax
Univ. of Missouri, Columbia
Tanuj Gulati
Drexel Univ., PA
Kaylan Michelle Haizlip
Ohio State Univ.
Bradley R. King
Univ. of Maryland
Melissa Anne Linden
Univ. of Illinois, Urbana-Champaign

Marcose G. Lopez
Mayo Medical School, MN
Rudo F. Mapanga
Univ. of Kwazulu Natal, S. Africia
Namyr A. Martinez
Univ. of Puerto Rico
Prakhar Maru
R.N.T. Med. College, India
Steven Charles Miles
Univ. of Texas, Austin
Nicholas Ara Mischel
Wayne State Univ., MI
Trevor Nyakudya
Univ. of Witwatersrand, S. Africa
Gregory O'Grady
Univ. of Auckland, New Zealand
Florence E. Orim
Nagasaki Univ., Japan
Amanda C. Pike
Univ. of Wisconsin
Erin Gail Rosenbaugh
Univ. of Nebraska
Caroline Mary Schik
Univ. of Georgia
Wan M. Shaik Mossadwq
Univ. of Putra, Malaysia
Lindsay Marie Parker
Macquarie Univ., Australia
Martin G. Rosario
Univ. of Puerto Rico
Scott Andrew Saunders
Univ. of Alberta, Canada
Ruth Renee Steadman
Univ. of Nebraska
Xin Tang
Vanderbilt Brain Inst., TN
Erkan Tuncay
Ankara Univ., Turkey
Onyeka N. Uzomah
Purdue Univ., IN
Patricia C. Villalta
Univ. of South Alabama
Kathryn C. Welliver
Des Moines Univ., IA
Courtney M. Wheatley
Univ. of Arizona
John H. Wolpers
Univ. of Missouri
Sulie Xu
West Virginia Univ.
Suchen Kumar Yadav
B P Koirala Inst. of Hlth. Scis., Nepal
Gina L.C. Yosten
St. Louis Univ., MO

Affiliate Member

Seterino Farias
Methodist Hospital, IN

Recently Deceased Members

Thomas A. Lesh
Hagerstown, IN
Jeffrey T. Potts
Fort Worth, TX
Kenneth L. Zierler
Baltimore, MD

Communications

Communications Update

The Communications Department issued nine press releases from September 1-October 22 promoting physiology-related topics, most of which came from APS journals:

From *Advances in Physiology Education*: A Woman in Space; The Story of The Development of Noninvasive Heart Care.

From *AJP: Heart and Circulatory Physiology*: A Simple Way for Middle Aged and Older Adults to Assess How Stiff their Arteries Are: Reach for their Toes.

From *AJP: Regulatory, Integrative and Comparative Physiology*: Exercise Minimizes Weight Regain By Reducing Appetite, Burning Fat, And Lowering 'Defended' Body Weight; How Alcohol Blunts The Ability Of Hamsters To 'Rise And Shine'.

We also distributed releases related to the *APS International Conference on Endothelin*: Leading Expert Examines Status, Promise Of Key Human Protein –Endothelin—At Conference Hosted By American Physiological Society (APS); Endothelin-Related Drugs Benefit Patients With Pulmonary Hypertension; Males May Experience Greater Physical Pain Due To Lower Levels Of A Key Protein, Endothelin.

In addition, we issued one podcast-related release, based on a study that had appeared in *Physiology*:

APS Podcast Updates Research on Elephant Seismic Communication

USA Today ran a front-page story on the Women in Space study. That story was also picked up by the *New York Times* blog, Freakonomics and online by Wired magazine.

Other major online outlets that ran coverage of these releases included:

ABCNews, *Science*, *Los Angeles Times*, MSN Health & Fitness, Voice of America, *Poughkeepsie Journal*, *U.S. News & World Report*, *Sun-Sentinel* (Florida), *Orlando Sentinel*, *Denver Post*, *Arizona Daily Star*, *Atlanta Journal Constitution*, *Austin American-Statesman*, *New Scientist*, *San Jose Mercury-News*, *Calgary Sun*, *Ethiopian Review*, *Science Daily*, *Zimbabwe Star*, *Telegraph* (U.K.), and *Times of India*.

If you are reading this online, you can click on the press release title to read more. If you are reading hard copy, go to http://www.the-aps.org/press/journal/index.htm for a complete list and links to all the releases. You can also find some of the media coverage highlights by clicking on the "Physiology in the News" link under the "For the Public" heading on the Press Room page (http://www.the-aps.org/press).

We have issued two new podcasts since our last report: **Elephant E-mail?**—We coined a new word in Episode 25 of ***Life Lines***, "elecomm," short for elephant communication. Caitlin O'Connell-Rodwell of Stanford University and the author of *The Elephant's Secret Sense*, discovered that elephant vocalizations travel through the ground, sometimes for great distances. Other elephants pick up these seismic communications and understand them; and **A chapter in the history of non-invasive heart care—**In Episode 26, Dusty Sarazan explains how his mentor, Dean Franklin, developed the first instruments to carry out exercise experiments in animals as they roamed freely. Franklin's inventions led to some surprising findings and resulted in the development of the non-invasive cardiovascular monitoring instruments we have today.

You can find those episodes and others at the Life Lines homepage at http://life-lines.tv. ❖

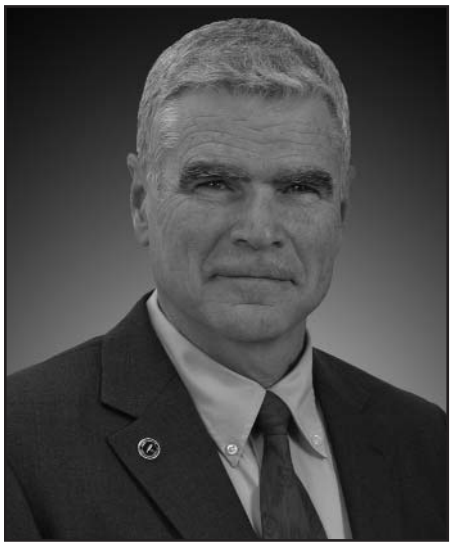
The Physiologist
Vol. 52, No. 6, 2009

Laughlin Receives 7th Schmidt-Nielsen Distinguished Mentor and Scientist Award

The APS Women in Physiology Committee is pleased to announce that M. Harold Laughlin, Curators' Professor and Chair, Department of Biomedical Sciences, College of Veterinary Medicine, University of Missouri-Columbia, has been selected as the seventh recipient of the Bodil M. Schmidt-Nielsen Distinguished Mentor and Scientist Award. The Committee was extremely impressed with both his mentoring excellence and his outstanding contributions to physiological research.

Laughlin received his PhD at the University of Iowa. He did his postdoctoral training also at the University of Iowa and then at the USAF School of Aerospace Medicine. In 1980, he was hired as an Assistant Professor of Physiology at Oral Roberts University Medical School in Tulsa. In 1985, Laughlin moved to the Department of Biomedical Sciences, College of Veterinary Medicine, and the Dalton Cardiovascular Research Center at the University of Missouri-Columbia. In 1987 he received a joint appointment to the Department of Physiology in the School of Medicine at the University. Laughlin was named interim chair of the Department of Biomedical Sciences in 1992 and then Chair in 1994. In 2007, he received the title of Curator's Professor.

Laughlin is currently regarded as one of the world leaders in research on the adaptations of the coronary and skeletal muscle beds to physical activity and inactivity, both in normal animals and in a variety of pathophysiological models of disease, in particular atherosclerosis. In addition, he has made his department one of the leading departments in integrative physiology in the country. Laughlin has more than 230 publications in peer-reviewed journals, including some of the most highly cited reviews in the field (including a chapter in the Handbook of Physiology). The seminal nature of his work has been recognized by an unusual number of named and plenary lectureships. His laboratory has been continuously funded by the NIH since 1981 and he has the longest funded NIH Program Project Grant ever awarded to the University of Missouri.



M. Harold Laughlin

Laughlin's success as a mentor has been through three primary roles: as an instructor, as a mentor, and as chair of the Department of Biomedical Sciences at the University of Missouri. As an instructor, Dr. Laughlin serves as a role model to both students and faculty alike. Despite being Chair, Dr. Laughlin continues to teach Veterinary Anatomy and Physiology courses on a voluntary basis because he considers teaching an integral component to the scientific process, for both student and instructor. As a mentor, Dr. Laughlin has worked for over 20 years to provide resources and foster independence in aspiring graduate students, veterinary residents, and postdoctoral fellows. A central component of his mentoring strategy is unwavering support of his trainee's individual goals. Consequently Laughlin trainees have been successful in careers outside of research, including teaching, medical practice, and administration. As chair of the Department of Biomedical Sciences, Dr. Laughlin has had the unique opportunity to directly impact faculty development. He implemented an individualized faculty mentor panel that advises each new faculty member as they navigate the challenges for new faculty within and outside of the University.

Laughlin holds membership in numerous scientific societies, and is very active in the Exercise and Environmental Physiology Section of APS. He has served on editorial boards for major journals in the field, including two of APS' journals, *Journal of Applied Physiology* and *American Journal of Physiology: Heart and Circulatory Physiology*. He has also served as Association Editor twice for *Journal of Applied Physiology* and for *Medicine & Science in Sports & Exercise*. Dr. Laughlin has committed years of service on NIH and Canadian study sections.

There will be a reception in Dr. Laughlin's honor at which he will give a talk on mentoring during the 2010 Experimental Biology meeting in Anaheim, CA. It will be held on Monday, April 26 at 12:00 pm at the Marriott Anaheim Hotel. All trainees and mentors are invited to attend.

APS congratulates Dr. Laughlin on this well-deserved honor.

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Vol. 52, No. 6, 2009

APS Minority Travel Fellows Attend the 2009 APS Conferences: Sex Steroids and Gender in Cardiovascular-Renal Physiology and ET-11: APS International Conference on Endothelin

The APS regularly awards Travel Fellowships for underrepresented minority scientists and students to attend APS scientific meetings with funds provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). These Fellowships provide funds for registration, transportation, meals, and lodging. Four Fellows attended the APS Sex Steroids and Gender conference in Broomfield, CO from July 15-18, 2009 and four Fellows attended the ET-11 Conference in Montreal, Canada from September 9-12, 2009.

The travel awards are open to graduate students, postdoctoral students, and advanced undergraduate students from minority groups underrepresented in science (i.e., African Americans, Hispanics, Native Americans, and Pacific Islanders). The specific intent of this award is to increase participation of pre- and postdoctoral minority students in the physiological sciences.

Fellows in the NIDDK Minority Travel program not only received financial support to attend these meetings, but were also

Martin Frank (APS Executive Director), Minolfa Prieto, Jane F. Reckelhoff (Organizing Committee Chair; Univ. of Mississippi Medical Center), Rayna Gonzales, Cheryl Bell, and Camila Manrique at the Sex Steroids and Gender Conference.

Pedro D'Orleans-Juste (Organizing Committee Co-Chair; University of Sherbrooke, Canada), Fernanda Giachini, Victor Lima, Aisha Kelly-Cobbs, Cornelius Nwora, and David Pollock (Organizing Committee Co-Chair; Medical College of Georgia) at the ET-11 Conference.

Fellows at the 2009 Sex Steroids and Gender Conference, Abstract Titles, and Meeting Mentors

Fellow	Meeting Mentor
Cheryl L. Bell, Univ. of Connecticut *Current Research Focus:* Estrogen treatment on male foreskin may increase keratinization of the foreskin thereby reducing the transmission of HIV into the body.	Pascale H. Lane, Univ. of Nebraska College of Medicine
Rayna Gonzales, Univ. of Arizona COM-Phoenix *Abstract Title:* Dihydrotestosterone Alters Endotoxin and Cytokine Induced Increases in Cyclooxygenase-2 in Human Coronary Artery Smooth Muscle Cells but has no Effecton Human Brain Microvascular Endothelial Cells.	Mentor to Camila Manrique
Camila Manrique, Univ. of Missouri *Abstract Title:* Female Gender Protects Against Oxidative Stress and Impaired Insulin-Stimulated Glucose Uptake in Skeletal Muscle in TGR (mRen-2)27 Rats.	Rayna J. Gonzales, Univ. of Arizona COM-Phoenix
Minolfa C. Prieto, Tulane Univ., School of Medicine *Abstract Title:* Sex Dependent Differences of Renin Gene Expression in Distal Nephron Segments during High Salt Intake in Chronic Angiotensin II (AngII)-infused hypertensive rats with endogenous renin angiotensin system (RAS) blockade with Losartan.	Lourdes A. Fortepiani, Univ. of Texas Health Sciences Center

provided professional guidance through pairings with APS members who served as mentors to the Fellows for the duration of the meeting. Thanks to the time and expertise offered by mentor volunteers, Fellows were able to maximize their time and more fully experience the many aspects of this meeting.

For more information, contact Brooke Bruthers, Minority Programs Coordinator, in the APS Education Office at 301-634-7132 or bbruthers@the-aps.org, or visit http://www.the-aps.org/education/minority_prog/index.htm on the APS website. ❖

Fellows at the 2009 ET-11 International Conference, Abstract Titles, and Meeting Mentors:

Fellow	Meeting Mentor
Fernanda Giachini, Univ. of Sao Paulo/Medial College of Georgia *Abstract Title:* Differential regulation of ERK1/2, via downregulation of mitogen-activated protein kinase phosphatase-1 (MKP-1), mediates sex-differences in vascular reactivity in DOCA-salt hypertension.	Craig F. Plato, Gilead Sciences, Inc.
Aisha Kelly-Cobbs, Medical College of Georgia *Abstract Title:* Chronic and acute dual blockade of endothelin receptors display different effects on cerebrovascular function in type 2 diabetes.	Ulla C. Kopp, Univ. of Iowa Carver College of Medicine
Victor Lima, Medical College of Georgia *Abstract Title:* O-GlcNAcylation contributes to augmented vascular reactivity induced by ET-1.	Gregory D. Fink, Michigan State Univ.
Cornelius Nwora, Texas Southern Univ. *Current Research Focus:* Signaling mechanisms relevant to understanding the pathogenesis of hypertension and other cardiovascular diseases.	R. James White, III., Univ. of Rochester

APS Launches New Online Professional Skills Training Program

APS is pleased to announce that it has just finished holding its first beta-test of the online Professional Skills Workshop on "Writing and Reviewing for Scientific Journals" from September 1 to October 15, 2009. The online course is supported by a grant to APS from the National Institute of General Medical Sciences at the NIH (Grant #GM073062-01).

This online workshop allowed 23 graduate students, postdoctoral fellows and early career faculty to: improve their skills at writing and submitting manuscripts; learn how to better respond to reviewer criticisms; learn how to be a good reviewer themselves; find out how their skills in these areas will impact their career advancement; discover how diversity issues or experience may be influencing how they write and review manuscripts; and learn about resources that can further develop their writing and reviewing skills.

The trainees who participated as beta-testers were: **Melissa Bates,** Univ. of Wisconsin; **Marika Bergenstock,** 3D Biotek, NJ; **Maile Ceridon,** Mayo Clinic, MN; **James Ching,** St. Louis Univ., MO; **Winyoo Chowanadisai,** Univ. of California, Davis; **Jacqueline Crissey,** Univ. of Missouri; **Melanie Fraites,** US Environ. Protection Agency, NC; **Martin Frasch,** Univ. of Western Ontario; **Melyn Galbreath,** Inst. for Exercise & Environ. Med., TX; **Shea Gilliam-Davis,** Wake Forest Univ. School of Med., NC; **German Gonzalez,** Henry Ford Hospital, MI; **Melanie Goodman,** Univ. of Oklahoma Health Sci. Ctr.; **Brittany Gorres,** Univ. of Kansas Medical Center; **Torrance Green,** Tulane Univ., LA; **Sara Jarvis,** Inst. for Exercise & Environ. Med., TX; **Annet Kirabo,** Univ. of Florida College of Medicine; **Jesus Lopez-Guisa,** Seattle Children's Res. Inst./Univ. of Washington; **Dhiman Maitra,** Wayne State Univ. School of Med., MI; **Kristen Osterlund,** Univ. of Arizona College of Med., Phoenix; **Fatima Sert-Kuniyoshi,** Mayo Clinic, MN; **Prachi Singh,** Mayo Clinic, MN; **Daniela Terson de Paleville,** Univ. of Louisville, KY; and **Jose Vazquez-Medina,** Univ. of California, Merced.

APS members participating as instructors were: **Dale Benos,** Univ. of Alabama, Birmingham; **Sue Barman,** Michigan State Univ.; **Robert Hester**, Univ. of Mississippi; **Thomas Schmidt**, Univ. of Iowa; and **Irving Zucker**, Univ. of Nebraska Medical Center.

The workshop was especially designed to attract underrepresented minority students and brought together physiology trainees with experienced mentors and scientists in an online blackboard-based environment.

Participants worked in small groups of four or five trainees matched with a biomedical researcher in their field to better enable them to receive individualized training and to allow for networking opportunities within their field of study.

During the course, participants received hands-on training at writing and reviewing their own writing and that of their colleagues. They were required to complete readings, turn in exercises, view audio/PowerPoint presentations, send in a draft manuscript, post comments and critiques of other students' abstracts, as well as take quizzes on what they learned in each lesson.

After this intensive six-week course, trainee participants were able to have: a clear concise abstract for a manuscript; a detailed plan for improving their draft manuscript; hands-on experience at critiquing manuscripts; a network of peers and mentors to share critiques and advice; and tools and resources for developing future manuscripts and abstracts.

Future Writing and Reviewing online workshops will be conducted. For more information about future courses, email education@the-aps.org. Departments or people interested in running such a course should also email the APS Education Office.

A second online course on "Making Scientific Presentations: Critical First Skills" will be beta-tested beginning in January 2010. Interested trainees should send in their email addresses to be notified of the application process for that course.

Also note that the last live versions of both these courses will be given again in January 2010. For more information on the live courses, see http://www.the-aps.org/education/profskills/upcoming.htm. ❖

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Essentials for Effectively Supervising Employees

Virginia M. Miller and Priscilla M. Flynn, Mayo Clinic, Rochester, MN

After years of study and working for others as a student and postdoctoral fellow, successfully navigating the job search and interview process, you have finally landed your first "real" job as an independent investigator with a laboratory of your own. Now what? Most graduate and postgraduate programs do not prepare you for this new position as a "manager/boss." Certainly, managing your time and resources enabled you to attain success. However, with your new independence and job title come responsibility for not only setting the long-term goals for your laboratory, securing enough funds to sustain employment for technicians and students, and assuring that institutional and governmental regulations are adhered to, but, most importantly, managing a productive team of technicians, students, and postdoctoral fellows. Graduate school does not prepare you for addressing, managing and perhaps negotiating disagreements among employees and dealing with personnel and integrity problems. These problems can disrupt the work flow and, in extreme cases, may result in termination of employment for those working with you. This article highlights key points and provides suggestions to help navigate the new path toward management.

Priscilla M. Flynn and Virginia M. Miller

Establishing your management style

Being the head of the laboratory naturally carries authority of position, and most employees and students respect that authority. The execution of that authority will depend upon your personality and personal expectations. Some self-reflection, which follows the age old adage "know thyself" and "to thine own self be true," will help you understand your leadership/managerial style. Insight derived from your new understanding will influence your decisions about the type of people who you will (or should) hire and the type of students who will be attracted to your laboratory. Answering the following questions will help you to define your style:

Are you an innovator, bridger or implementer? That is, are you an idea generator but can't stay to task long enough to complete a paper (i.e., an innovator)? Or given a single idea, you enjoy the detail (i.e., an implementer)? Or are you energized by ideas generated by interdisciplinary teams and then delegate tasks to others to complete (i.e., a bridger)? Understanding where you fit along this spectrum may influence the type of technical support needed to complement your strengths and maximize the work flow with limited resources. Whatever you do, don't hire clones of yourself. Although it's difficult to admit you have weaknesses, recognizing and hiring staff that strengthen your team will enhance the success of your laboratory.

What is your trust level? Can you delegate tasks? The answer to this question may change with time as you learn the skills and personality of personnel in your laboratory. Scientists tend to be type A (or AAAA) personalities but learning to delegate non-critical tasks can save you time for thinking about the big ideas. There is risk associated with delegating tasks. Miscommunication and different work styles may result in work not being accomplished the same way you would do it or not on the same time schedule and mistakes can occur along the way. Consider the cost of potential errors and how willing you are to "not sweat the small stuff" as long as the work is accomplished accurately and ethically. Remember "All roads lead to Rome."

What is important to you? Do you want to manage a large laboratory, work 24-7, become President of APS, sustain relationships with family and friends, remain active in hobbies, etc.? Writing a personal mission statement can help sort out these priorities and influence your expectations for laboratory personnel and how you deal with planning, deadlines and disputes.

Common Problems and Common Sense Solutions

Diversity in research staff creates a dynamic and exciting environment for discovery. However, diversity in language of origin, culture, ethnicity, training background and generation (age ranges) means that not all persons in the laboratory share your communication skills or style, have the same work ethic, or moral compass. The likelihood for problems and conflicts to arise in a fixed laboratory space is directly proportional to the number of creative, independent and career-minded people occupying that space. Most performance problems are related to lack of understanding of a set of expectations between you and the employee/student (Sample 1). A brief orientation discussion with the new technician, student or fellow regarding expectations for work hours, use of cell phones in the laboratory, personal use of internet and email during work, music and dress code will go a long way to reduce minor problems about performance (Sample 2). To reduce errors related to persons for whom English is a second language, a short description with these expecta-

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Priscilla Flynn is Coordinator of the Mayo Clinic Office of Women's Health and Instructor in the Mayo Clinic College of Medicine. She received her B.S. from the University of Minnesota - Minneapolis, her MPH from the University of Wisconson- LaCrosse and her PH from the University of North Carolina - Chapel Hill. She has extensive experience in supervising a diverse range of employees in government, non-profit and business environments.

tions bulleted or itemized is useful.

Most problems that are encountered in the laboratory along the management-road to success can be classified as "bumps in the road." These are the minor disagreements among individuals regarding use of shared equipment/supplies, scheduling, etc. These disagreements can usually be resolved without intervention on your part.

Major detours along the path include personal crises, such as an accident resulting in physical injury, prolonged illness, death of a family member, marriage, pregnancy, and divorce. These life events can create stress in the work, which can lead to staff conflicts. In instances where there are accidents leading to permanent disability or diagnosis of terminal illness, compassion and sensitivity must be employed so as to retain the dignity of the person involved. Working with your institution's department of human resources will assure that legal requirements surrounding family leave, American Disabilities Act and OSHA, etc. are met.

Landmines, which, if not detected early and dealt with properly, blow up to destroy the road to success. Events in this category include scientific misconduct, substance abuse and harassment. Again, for these issues, help from your institution's human resource department or research administration is essential. It is naïve to think that these issues will never happen in your laboratory, because over the course of 20 years of managing a laboratory, I have encountered all of them.

Mistakes Managers Make

In assessing the various types of conflicts and interpersonal issues that arise, it is helpful to avoid these common mistakes:

Conflict avoidance: The natural tendency is to avoid conflict, hope that it will go away or resolve itself. However, this natural tendency may lead to the second common mistake, waiting too long to intervene.

Waiting too long to intervene: This mistake can lead to irreparable working relationships among individuals in the laboratory. Experiments may fail and data can be lost, both costing time and money. Keep a pulse on the laboratory and observe personal interactions and team performance. Be aware that laboratory personnel expect you to maintain a productive work environment. Therefore, sooner is better than later for appropriate intervention. Repetition of minor conflicts between the same two individuals or one individual with multiple people should trigger action on your part. Early intervention is essential if you suspect substance abuse, harassment or scientific misconduct.

Fear of tough decisions: Fear of making a tough decision is understandable because of both immediate and long-term consequences that could ultimately affect your or another's career or health. Inevitably, with most tough decisions, not all will agree or be happy with your decision. However, the outcome of your laboratory and overall welfare of your staff need to be your focus.

Criticism without enough praise: A little bit of praise goes a long way in promoting productivity and loyalty, especially if the performance issue in question has been improved. What would *you* like to hear? *Thank you* goes a long way.

Lack of creativity in approaching conflict resolution: There are many ways to reach a solution and one size does not fit all. Specific circumstances, the personalities involved and the desired outcome should be considered in developing a strategy to deal with the conflict.

Documentation: Although your goal should be to help your staff be successful, some situations do not improve. Make sure that you document interactions that are serious enough to lead to dismissal and inform the employee that you are doing so.

Five-step Plan Toward Conflict Resolution

If the situation requires your intervention, these five elements should be considered in planning a creative strategy to resolve a conflict:

1. Define the goal prior to the meeting.

2. Determine the setting: your office, a neutral place, does a third party need to be present, across a table or open seating. These elements will help to formalize or defuse tension depending upon the circumstances.

3. Control your emotions. Easier said than done, right? But step 4 can help with this.

4. Practice what you will say. Talk out loud to yourself. Create a dialogue that you anticipate and say the words out loud. This simple exercise can help you "hear" how the words sound, measure the intonation in your voice and help you to anticipate responses. Repetition helps to diffuse an emotional delivery as you become used to delivering the message.

5. Avoid Email and Voicemail: Although these means of communication are acceptable and quick for conveying factual information, they are not appropriate for conflict resolution. Face to face is best, but in some circumstances, a phone call with back-and-forth dialog may be sufficient.

Once a meeting begins, the problem should be described. The impact and potential consequences of the continued conflict/problem should be addressed. Although you have practiced a scenario prior to the meeting, listen to what is being said by others. If you need to calm yourself, a deep breath and repeating what you have heard helps to slow the pace. This also allows the others involved to see that you understand their point of view. Options for resolution need to be presented. In some cases, the option is clear-cut and can be dictated by you. For example, deadlines that need to be met or performance that needs to be changed. In other cases, a compromise resolution can be discussed and agreed upon. In the latter case, follow-up, which should include encouragement and praise, where appropriate, is essential.

The bottom line

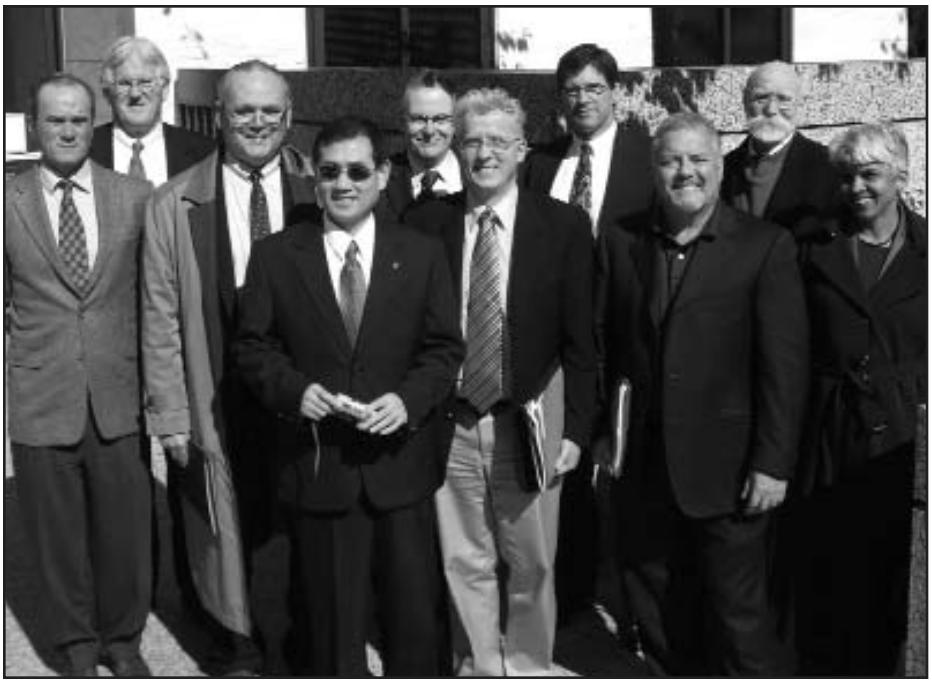
Surrounding yourself by eager young people from diverse backgrounds creates an exciting, dynamic laboratory for discovery. Recent studies show that although diverse teams are more creative, they take more time to form effective collaborations due to cultural or age differences. If you understand your managerial style and have clear expectations for yourself and others in the workplace, on most days work flows easily, everyone is productive and the trip along the road to success is smooth. However, life stresses, miscommunications and work ethics may create circumstances that disrupt the trip. The responsibility will fall to you, as the head of the laboratory, to intervene appropriately and in some cases make the "hard call." Your actions may not be accepted by all but "to thine own self be true." You will make mistakes along the way. Learn from them, admit when you were wrong, forgive yourself and others, then and move on down the road. ❖

APS Members Meet with Congress to Discuss Research Funding

On Monday, October 18, 2009, members of the APS Public Affairs Committee met with their Senators to discuss federal funding for biomedical research at the NIH, NSF, VA and NASA. Over the last several months, the scientific community has expended considerable effort to propose, review and undertake research projects with the unprecedented influx of funds from the American Recovery and Reinvestment Act of 2009 (ARRA). At the same time, there are serious concerns about what will happen when those funds expire at the end of 2010. For example, if the ARRA funds are not built into the NIH budget, the agency could experience a precipitous drop in success rates as ARRA-funded scientists apply for grants to continue their research.

APS members thanked their Senators for supporting the ARRA funds for research, and reminded them that science needs predictable, sustainable funding increases in order to maintain momentum and best take advantage of scientific opportunities. They also emphasized that cycles of "boom and bust" funding should be avoided, as this is particularly disruptive to the training of the next generation of scientists.

Twelve Congressional offices were visited in total. Senators from both sides of the aisle expressed support for research funding, but also uncertainty about how the current economic crisis will affect future funding for many priority programs, including biomedical research.



APS Public Affairs Committee Members (from left to right) James Galligan, Robert Hester, David Harder, Zhongjie Sun, Bill Yates, John Chatham, Michael Brands, Michael Portman, Richard Paul and Mrinalini Rao.

Two Animal Research Sessions Planned for EB 2010

The American Physiological Society's Animal Care and Experimentation Committee (ACE) will sponsor two events at the Experimental Biology 2010 meeting in Anaheim. The first is a symposium on a variety of tactics that are being used to harass and intimidate those whose research involves animals. This session is intended to help researchers educate themselves to minimize risk and increase public support for their work. The second event is an informal discussion where researchers who serve on animal care and use committees can discuss regulatory challenges they are facing. Both sessions are open to scientists from all societies who are registered for the EB meeting.

"Trends in Animal Rights Activism and Extremism" will be held from 3:15-5:45 on Saturday, April 24, 2010 in Room 303B of the Anaheim Convention Center. Animal rights activists now frequently make requests for information under the federal Freedom of Information Act or state open records laws. These are laws intended to promote openness in government, which means that in most cases the information must be released unless it falls under specific exemptions. Due to concerns about how this information might be used, UCLA Senior Counsel Amy Blum has been asked to discuss how researchers and their institutions can minimize this risk. In recent years, the radical fringe of the animal rights movement has increasingly adopted violent tactics pioneered by animal rights extremists in the UK. The second speaker will be University of Iowa Director of Animal Resources Paul Cooper, who will discuss how institutions should prepare themselves to respond effectively to an animal rights break-in. Due to a growing recognition among researchers that keeping a low profile does not provide protection against activists, the symposium will feature two talks on public outreach. UCLA neuroscientist David Jentsch, who organized the first large scale US march in support of humane animal research, will discuss ways for scientists to become pro-active research advocates. In addition, UCLA neurobiology doctoral candidate Megan Wyeth will discuss public outreach for the early career scientist. Jentsch founded an organization at UCLA called Pro-Test for Science, and Wyeth has received a Michael D. Hayre Fellowship in Public Outreach from Americans for Medical Progress to assist other institutions in establishing Pro-Test chapters. Therefore, the last half hour of the session will be an informal discussion with Jentsch and Wyeth for those interested in establishing a Pro-Test for Science chapters.

"The Challenges of Running an IACUC: An Open Forum for IACUC Chairs and Members" will be held in Salon J-K of the Anaheim Marriott from 7:30-9 a.m. on Tuesday, April 27. This will be an informal event that is intended to promote discussion among IACUC chairs and members about regulatory challenges they face. There are no scheduled speakers because the purpose of this session is to foster an open exchange of ideas and experiences. Attendees are encouraged to bring questions and a willingness to brainstorm solutions with others. Current and former IACUC members as well as those interested in regulatory matters are welcome to attend.

Review: *An Odyssey with Animals*

Whether you are a researcher or a lay person, pro-research or pro-animal rights—or anywhere in between—Adrian Morrison's *An Odyssey with Animals: A Veterinarian's Reflections on the Animal Rights & Welfare Debate* (2009) will give you much to pause and consider. Despite having been personally harassed by extremists for years, his own lab destroyed by Animal Liberation Front vandals, Morrison has written no polemic. Instead, he uses the attacks against him as a jumping off point from which to delve into the issues that surround human use of animals. In doing so, he uncovers layers of nuance in a debate notorious for its polarization.

Morrison brings a multi-faceted perspective to the debate: he grew up on a farm with working animals, studied first to become a veterinarian and then a research scientist, and has even placed himself in the guinea pig's shoes by volunteering for human clinical research trials. Over the last 15 years, he has made a methodical study of the animal rights issue—its history, its messages, and the many schools of thought within and around it.

In addition to his thorough study of the topic, Morrison's experiences also provide unique insight. His description of the infamous Silver Spring Monkey case early in the book, for instance, is made all the more fascinating by virtue of his personal involvement. His bucolic childhood gives him a very different perspective on animal use than many members of our increasingly post-agrarian, or what Morrison calls post-domestic, society. The insights from this become clear in chapter 8, when Morrison expands the discussion to include animal uses beyond research.

Morrison offers a strong and convincing case for animal research. He describes his own research into REM sleep to illustrate the complex nature of scientific discovery. He explains the specific role animal research has played in many monumental discoveries, and then he refutes both scientific distortions and philosophical presumptions of the animal rights movement.

He does not, however, let researchers off the hook. He encourages his fellow scientists to avoid dismissing all advocates for animals out of hand, lest they disregard legitimate welfare recommendations as efforts to stymie discovery—a mistake he admits to having made in the past himself. Now, he draws distinctions not only between *animal rights* and *animal welfare*, but also between the general term *animal rights* and the *animal rights movement*.

Odyssey carries through it a theme of responsibility: responsibility of scientists to keep looking deeper and the responsibility we all have to the animals in our care. Morrison supports the humane use of animals, but only so long as we remember our responsibility.

Inspired by personal experience, *Odyssey* is written with an easy, personable tone. The author discusses his own struggle with using cats in his research in light of his affection for his pet cat, Buster. He makes his own views clear, along with his reasoning for them, while leaving room for readers to come to their own, likely divergent, decisions. No matter where you eventually fall in the debate yourself, reading *An Odyssey with Animals* will enrich your understanding of a difficult and complex issue and add depth to whatever conclusions you draw. ❖

Claire Edwards
American Physiological Society

Moving?

If you have moved or changed your phone, fax or Email address, please notify the APS Membership Office at 301-634-7171 or Fax to 301-634-7241. Your membership information can also be changed by visiting the Members Only portion of the APS Website at http://www.the-aps.org. ❖

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- Why can't they use computers instead?
- What types of animals are needed?
- Who makes sure that research animals are treated well?
- Why do some people say that animal research is wrong?
- Why do companies test cosmetics and other products on animals?
- Why do some new drugs that are tested in animals still have serious side effects?
- Is research painful?

Answers from the American Physiological Society

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Physiology in Perspective: The Walter B. Cannon Award Lecture (Supported by the Grass Foundation)

Jeffrey J. Fredberg
Harvard Univ. School of Public Health

"A Hard Day in the Life of a Soft Cell: Physical Laws Governing Cytoskeletal Deformation, Contraction, and Remodeling"

Saturday, April 24, 5:45 pm

Henry Pickering Bowditch Award Lecture

Paul Janssen
Ohio State Univ.

"Myocardial Contraction-Relaxation Coupling"

Sunday, April 25, 5:45 pm

Carl Ludwig Distinguished Lectureship of the Neural Control and Autonomic Regulation Section

Shaun F. Morrison
Oregon Health Sciences Univ.

"Central Pathways for Thermoregulation"

Sunday, April 25, 8:00 am

Claude Bernard Distinguished Lectureship of the Teaching of Physiology Section

Robert A. Bjork
Univ. of California, Los Angeles

"Making Things Hard on Yourself, But in a Good Way: Creating Desirable Difficulties to Enhance Learning"

Sunday, April 25, 10:30 am

August Krogh Distinguished Lectureship of the Comparative & Evolutionary Physiology Section (Supported by Novo Nordisk Foundation)

William H. Karasov
Univ. of Wisconsin

"Digestive Physiology: A View From Molecules to Ecosystem"

Sunday, April 25, 2:00 pm

Edward F. Adolph Distinguished Lectureship of the Environmental and Exercise Physiology Section

John M. Johnson
Univ. of Texas Health Sci. Ctr., San Antonio

"Mechanisms of Control in the Human Cutaneous Circulation"

Sunday, April 25, 3:15 pm

Robert M. Berne Distinguished Lectureship of the Cardiovascular Section

Frank Faraci
Univ. of Iowa

"Mechanisms of Protection for Vascular Disease in Brain"

Monday, April 26, 8:00 am

Joseph Erlanger Distinguished Lectureship of the Central Nervous System Section

Allan Basbaum
Univ. of California, San Francisco

"The Generation and Control of Pain: From Molecules to Circuits to Behavior"

Monday, April 26, 10:30 am

Ernest H. Starling Distinguished Lectureship of the Water and Electrolyte Homeostasis Section

Kirk P. Conrad
Univ. of Florida

"Maternal Vasodilation in Pregnancy: The Emerging Role of Relaxin"

Monday, April 26, 2:00 pm

Julius H. Comroe, Jr. Distinguished Lectureship of the Respiration Section

Eugene Nattie
Dartmouth Medical School

"Central Chemoreception: Then and Now"

Monday, April 26, 3:15 pm

Carl W. Gottschalk Distinguished Lectureship of the Renal Section

P. Darwin Bell
Medical Univ. of South Carolina

"Tubular Flow Sensing; The Good, the Bad and the Ugly. Lessons Learned From Macula Densa Cells and Polycystic Kidney Disease"

Tuesday, April 27, 8:00 am

Hugh Davson Distinguished Lectureship of the Cell and Molecular Physiology Section

Sergio Grinstein
Hospital for Sick Children, Toronto

"Imaging Phagocytosis: Receptors, Phospholipids and the Cytoskeleton"

Tuesday, April 27, 3:15 pm

Horace W. Davenport Distinguished Lectureship of the Gastrointestinal & Liver Section

Michael Gershon
Columbia Univ. Medical School

"The Birth of a Neuron: Serotonin and Neurogenesis in the Adult ENS"

Tuesday, April 27, 10:30 am

Solomon A. Berson Distinguished Lectureship of the Endocrinology and Metabolism Section

Iain Robinson
National Institue for Medical Research, United Kingdom

"The Growth Hormone Axis: In Sickness and Health"

Tuesday, April 27, 2:00 pm

Walter C. Randall Lecturer in Biomedical Ethics

Debra Anne Schwinn
Univ. of Washington

"Scientific Integrity: Positive and Negative Academic/Industry Collaboration"

Tuesday, April 27, 2:00 pm

Saturday, April 24, 2010 8:00 AM-12:00 PM

Symposium
Refresher Course in Cardiovascular Physiology
Education Track
Robert L. Hester and Tom Pressley

Saturday, April 24, 2010 9:00 AM-4:20 PM

Symposium
Microcirculatory Society President's Symposium
William F. Jackson and Jonathan H. Jaggar

Saturday, April 24, 2010 10:30 AM-12:30 PM

Symposium
The Role of Magnesium and Novel Cation Channels in Cardiovascular/Renal/Metabolic Disease: From Molecule to Patient
Translational Physiology Track
Metabolism and Metabolic Diseases Track
Andrea Rosanoff and Robert K. Rude

Saturday, April 24, 2010 1:00 PM-3:00 PM

Workshop
Computational Modeling and Simulation as a Tool for Studying Physiological Processes
Education Track
Irene C. Solomon and Christopher Wilson

Symposium
Science Beyond the Laboratory: From Grad School Through Retirement
Public Policy Track
Career Development Track
Gina Schatteman

Saturday, April 24, 2010 2:00 PM-4:30 PM

Workshop
Hot Topics in Renal Microvascular Control
Edward Inscho and William F. Jackson

Workshop
Productive Translational Research: Tools for Connecting Research Cultures and Managing Conflict
Career Development Track
Education Track
Deborah Zucker

Saturday, April 24, 2010 3:15 PM-5:15 PM

Workshop
Nanotechnology and Nano/Microfluidics
Education Track
Emily Gibson and Moshe Levi

Symposium
Trends in Animal Rights Activism and Extremism
Public Policy Track
Bill Yates

Saturday, April 24, 2010 5:45 PM-6:45 PM

Lecture
Physiology in Perspective — The Walter B. Cannon Memorial Award Lecture
Jeffrey J. Fredberg

Sunday, April 25, 2010 8:00 AM-10:00 AM

Lecture
Carl Ludwig Distinguished Lectureship of the APS Neural Control and Autonomic Regulation Section
Shaun Morrison

Symposium
Development of Endocrine Tissues
Jeff Schwartz and Paul Thomas

Symposium
Government Careers in Physiology Revealed
Career Development Track
Kathy L. Ryan and Kamal Rahmouni

Symposium
Novel Opportunities for the Treatment of Heart Failure
Systems Biology (-omics) Track
Magdalena Alonso-Galicia and Craig F. Plato

Featured Topic
Autoregulation and Renal Injury
Blood Pressure Regulation Track
Heather Drummond

Featured Topic
Helping Students Put the Pieces Together: Fostering Integrative Learning of Physiology
Education
Career Development Track
William H. Cliff

Featured Topic
Matrix Metalloproteinases in Mitochondrial, Cytoskeletal and Nuclear Remodeling
Suresh C. Tyagi

Featured Topic
Reactive Oxygen Species in Vascular Tone and Remodeling
Hypoxia and Oxidative Stress Track
Steven J. Miller

Featured Topic
Regulation of Epithelial Ion and Water Channels
Roger T. Worrell

Sunday, April 25, 2010 10:30 AM-11:30 AM

Lecture
Claude Bernard Distinguished Lectureship of the APS Teaching of Physiology Section
Education Track
Robert A. Bjork and Peter Snyder

Sunday, April 25, 2010 10:30 AM-12:30 PM

Physiology InFocus—Physiology and Biomedical Engineering: Partners in Translational Research
Airway Smooth Muscle: Where Does it Come From, How Does it Work, What Does it Do
Jeffrey J. Fredberg and Merryn Tawhai

Symposium
Autonomic Adjustments to Stress
Blood Pressure Regulation Track
Chester A. Ray and Jason R. Carter

Symposium
Endoplasmic Reticulum Stress at the Crossroad Between Fatty Liver, Leptin Resistance, Obesity and Diabetes
Ivan Torre-Villalvazo

Symposium
Ion Transport in Cancer
Ion Channels and Transporters Track
Cathy Fuller and Ken Gagnon

Symposium
So You Want to Phenotype Your Mouse? Challenges to Evaluating the Cardiovascular and Metabolic Systems
Systems Biology (-omics)
Metabolism and Metabolic Diseases Track
Owen P. McGuinness and Kate Ellacott

Symposium
Update on Prorenin and Its Receptor
Janos Peti-Peterdi and Genevieve Nguyen

Featured Topic
Age, Sex, and Control of Breathing
Aging, Gender and Sex Differences Track
Evelyn Schlenker

Featured Topic
Angiogenesis, Neurogenesis and Brain Recovery from Injury
Frank C. Barone and Michael Chopp

Featured Topic
Cerebral Challenges and Consequences of Exercise
Thermal Regulation and Environmental Stress Track
Samuel N. Cheuvront

Featured Topic
Extracellular Matrix and Pathology of Cardiovascular Disease
Jason D. Gardner and Pamela A. Lucchesi

Sunday, April 25, 2010 2:00 PM-3:00 PM

Lecture
August Krogh Distinguished Lectureship of the APS Comparative and Evolutionary Physiology Section (supported by Novo Nordisk Foundation)
William H. Karasov

Sunday, April 25, 2010 3:15 PM-4:15 PM

Lecture
Edward F. Adolph Distinguished Lectureship of the APS Environmental and Exercise Physiology Section
John M. Johnson

Sunday, April 25, 2010 3:15 PM-5:15 PM

Lecture
Microcirculatory Society Landis Award Lecture

Symposium
History of Comparative Physiology
David L. Goldstein

Symposium
Interaction Between Respiratory and Limb Muscle Blood Flow During Exercise
Blood Pressure Regulation Track
Peter D. Wagner and Jerome Dempsey

Symposium
Molecular Physiology of Iron Homeostasis and Its Disorder
Translational Physiology Track
Tomas Ganz and Elizabeta Nemeth

Symposium
Orexin and the Control of Breathing
Eugene Nattie and Tomoyuki Kuwaki

Symposium
Personalized Medicine for the 21st Century: The Implications of a Systems Approach
Systems Biology (-omics)
Translational Physiology Track
Susan E. Mulroney and Howard Federoff

Symposium
Shear Stress and Vascular Biology
Anthony Passerini and John Frango

Featured Topic
Molecular Mechanisms and Genetics of Hypertension
Blood Pressure Regulation Track
Carol Moreno Quinn and Anna Dominiczak

Sunday, April 25, 2010 5:45 PM-6:45 PM

Lecture
The Henry Pickering Bowditch Award Lecture
Paul Janssen

Monday, April 26, 2010 8:00 AM-9:00 AM

Lecture
Robert M. Berne Distinguished Lectureship of the APS Cardiovascular Section
Frank Faraci

Monday, April 26, 2010 8:00 AM-10:00 AM

Symposium
Cannon's Voodo Death 2010: Autonomic Triggers and Adverse Cardiac Events
William T. Talman and Alan Kim Johnson

Symposium
Focus on the Big Picture: Integration of Undergraduate and Medical Curricula
Education Track
Lynelle Golden

Symposium
Non-erythropoietic Properties of Erythropoietin: Implications for Tissue Protection and Cancer
Translational Physiology Track
Abdulla K. Salahudeen and Stephanie S. Watowich

Featured Topic
CNS Regulation of Body Temperature
Shaun Morrison

Featured Topic
Gastrointestinal Development and Disease
Systems Biology (-omics) Track
Jessica A. Dominguez and Jennifer K. Uno

Featured Topic
Hyperoxia- and Reactive Oxygen Species-induced Stress in the Lung
Hypoxia and Oxidative Stress Track
Kaushik Parthasarathi and Christopher M. Waters

Featured Topic
Neural Control of Blood Pressure and Fluid Volume Homeostasis: Sodium Metabolism
Blood Pressure Regulation Track
Thomas Cunningham

Featured Topic
Renal Section Young Investigator Awardee Featured Topic
Ion Channels and Transporters Track
Pablo Ortiz

Featured Topic
Translational Research in Metabolic Syndrome and Cardiovascular Disease: Swine Versus Mouse Models
Metabolism and Metabolic Diseases
Translational Physiology Track
Michael Sturek

Monday, April 26, 2010 10:30 AM -11:30 AM

Lecture
Joseph Erlanger Distinguished Lectureship of the APS Central Nervous System Section
Allan Basbaum

Monday, April 26, 2010 10:30 AM-12:30 PM

Tutorial
Publishing 101: The Dos and Don'ts of Publishing in APS Journals
Career Development Track
Kim E. Barrett and Rita Scheman

Symposium
Current Understanding of the Mechanisms and Regulation of Intestinal Vitamin, Trace Elements and Metal Transport
Ion Channels and Transporters Track
Hamid M. Said and Robert J. Cousins

Symposium
Microcirculatory Society Young Investigator Symposium
TBA

Physiology InFocus --Physiology and Biomedical Engineering: Partners in Translational Research
Preparing Students for Physiological Complexity: Emphasizing Quantitative Skills
Dee U. Silverthorn

Symposium
RNAi Interference in Cardiovascular Disease
Blood Pressure Regulation
Systems Biology (-omics) Track
Zhongjie Sun

Symposium
Stem Cells: Nature's Own Nanotechnology
William M. Chilian and Thomas H. Hintze

Featured Topic
Caveolar Microdomains, Signaling and Disease
Systems Biology (-omics) Track
Paul A. Insel

Featured Topic
Hyperkalemic and Hypokalemic Periodic Paralysis in Skeletal Muscle: New Insight from New Mouse Models
Jean-Marc Renaud

Featured Topic
Neural Mechanisms of Sympathetic Activation in Cardiovascular Diseases
Blood Pressure Regulation Track
Hui-Lin Pan

Featured Topic
Pulmonary Hypertension: Mechanisms and Mediators
Blood Pressure Regulation Track
Larissa Shimoda and Thomas C. Resta

Monday, April 26, 2010 12:00 PM-2:00 PM

Symposium
Drug Development 101
Translational Physiology Track
Career Development Track
Nansie McHugh

Monday, April 26, 2010 12:45 PM-2:00 PM

Symposium
The "Scientific Foundations for Future Physicians" *Report and Its Implications for Medical and Pre-medical Education*
Education Track
Public Policy Track
Dee U. Silverthorn

Monday, April 26, 2010 2:00 PM-3:00 PM

Lecture
Ernest H. Starling Distinguished Lectureship of the APS Water and Electrolyte Homeostasis Section
Kirk P. Conrad

Monday, April 26, 2010 3:15 PM -5:15 PM

Lecture
Julius H. Comroe, Jr. Distinguished Lectureship of the APS Respiration Section
Eugene Nattie

Symposium
Humoral Factors in Renal Injury and Hypertension
Joey P. Granger

Symposium
Interactions Between Myosin Light Chain Kinase and Phosphatase in Arteiolar Myogenic Tone
Blood Pressure Regulation Track
Michael A. Hill and William C. Cole

Symposium
Neuromodulatory Cytokines in Cardiovascular Functions
Immune Responses
Blood Pressure Regulation Track
Mohan K. Raizada and Julian Paton

Symposium
Publish, Not Perish: How to Survive the Peer Review Process
Career Development Track
Jessica A. Dominguez and Sender Lkhagvadorj

Symposium
Redox Control of Skeletal Muscle Adaptation to Exercise and Disuse
Scott Powers and Michael Reid

Symposium
Regulation of Neuronal Cell Volume: From Activation to Inhibition to Degeneration
Glenn Toney and Sean D. Stocker

Symposium
Systems Biology Approach to Monitor Intercellular Networks
Systems Biology Track
Sylvie Breton and Bruce Molitoris

Featured Topic
Endocrine Adaptations to Exercise: How Important is Energy Balance?
Metabolism and Metabolic Diseases Track
Peter A. Farrell

Featured Topic
Novel Gastrointestinal Nutrient Sensors
Metabolism and Metabolic Diseases Track
Stephen Crozier and Helen E. Raybould

Monday, April 26, 2010 5:45 PM-7:45 PM

Special Session
GIL Trainee Poster Symposium

Tuesday, April 27, 2010 8:00 AM-9:00 AM

Lecture
Carl W. Gottschalk Distinguished Lectureship of the APS Renal Section
P. Darwin Bell

Tuesday, April 27, 2010 8:00 AM-10:00 AM

Symposium
A Primer for the New PI: How to Herd Cats AND Keep Your Boss Happy
Career Development Track
Francisco H. Andrade and Angela J. Grippo

Symposium
Airway Protective Behaviors: Cough and Swallow
Donald R. Bolser and Paul W. Davenport

Symposium
Novel Molecular Targets for Modulating Cardiac Cell Death and Survival
Hypoxia and Oxidative Stress Track
David M. Roth

Featured Topic
Cardiovascular Adjustments at High Altitude
Hypoxia and Oxidative Stress Track
Mikael Sander

Featured Topic
Coordinate Regulation of Vascular Smooth Muscle Gene Expression, Cell Phenotype, and Vessel Function
William J. Pearce

Featured Topic
Epithelial Barrier Function in Inflammatory Bowel Diseases
Immune Responses Track
Michael Fromm

Featured Topic
Physiological Biomechanics
Matthew McHenry

Featured Topic
Regulation of Epithelial Transporters
Nuria M. Pastor-Soler and Judith Blaine

Featured Topic
Sex Steroids in Cardiovascular-Renal Physiology and Pathophysiology
Aging, Gender and Sex Differences Track
Barbara T. Alexander and Donna H. Korzick

Special Session
Trainee Highlights in Physiological Genomics
Lin Liu

Tuesday, April 27, 2010 10:30 AM-11:30 AM

Lecture
Horace W. Davenport Distinguished Lectureship of the APS Gastrointestinal and Liver Physiology Section
Michael D. Gershon

Tuesday, April 27, 2010 10:30 AM-12:30 PM

Featured Topic
Cardiovascular Consequences of the Metabolic Syndrome
David Stepp and Jefferson Frisbee

Symposium
Inflammatory Responses and Hypoxia-Inducible Pathways
Hypoxia and Oxidative Stress Track
Sean Colgan and Holger K. Eltzschig

Symposium
Intrinsic and Extrinsic Regulation of Activity in the Hypothalamus: Mechanisms and Consequences
Colin Brown and Mike Ludwig

Featured Topic
Membrane Estrogen Receptors
Willis K. Samson

Featured Topic
Mitochondrial Oxidants and Antioxidants in Autonomic Regulation and Cardiovascular Function
Hypoxia and Oxidative Stress Track
Matthew C. Zimmerman

Physiology InFocus --Physiology and Biomedical Engineering: Partners in Translational Research*One Hundred Years of Starling: His Contributions to Physiology*
Jane F. Reckelhoff and Rafael Rubio

Featured Topic
PGC-1alpha in Health, Exercise and Disease
David Hood

Symposium
Point-Counterpoint: An Update in Endothelial Barrier Function
Diego F. Alvarez and Konstantin G. Birukov

Symposium
STIM Proteins: Calcium-sensors with Multiple Functions
Anant B. Parekh

Tuesday, April 27, 2010 2:00 PM-3:00 PM

Lecture
Solomon A. Berson Distinguished Lectureship of the APS Endocrinology and Metbolism Section
Iain C. A. F. Robinson

Lecture
The Walter C. Randall Lecture on Biomedical Ethics
Public Policy Track
Debra Anne Schwinn

Tuesday, April 27, 2010 3:15 PM-4:15 PM

Lecture
Hugh Davson Distinguished Lectureship of the APS Cell and Molecular Physiology Section
Sergio Grinstein

Tuesday, April 27, 2010 3:15 PM-5:15 PM

Symposium
Mouse Models of Diabetic Renal Disease
Metabolism and Metabolic Diseases Track
Lisa M. Harrison-Bernard and Christine Maric

Symposium
Regenerative Medicine in the 21st Century
Translational Physiology Track
Emmanuel C. Opara and Anthony Atala

Symposium
The Control and Consequences of Renal Electrolyte Transport
David Mount and John Geibel

Symposium
Vascular Tissue Engineering
Christopher Brauer

Featured Topic
Comparative Metabolic Physiology
Hypoxia and Oxidative Stress Track
Thermoregulation and Environmental Stress Track
Karen Sweazea

Featured Topic
Donald Reis Memorial Featured Topic

Symposium
Habitual Exercise and Arterial Aging
Aging, Gender and Sex Differences Track
Douglas R. Seals and Hirofumi Tanaka

Featured Topic
Wiggers Award Featured Topic: Hypertension, Inflammation and Adaptive Immunity
Julian H. Lombard and David Harrison

Tuesday, April 27, 2010 5:45 PM-7:45 PM

Business Meeting
APS Business Meeting

Wednesday, April 28, 2010 8:00 AM-10:00 AM

Symposium
A. Clifford Barger Memorial Symposium: New Insights into the Relationship Between Sodium Metabolism and Blood Pressure Regulation
Blood Pressure Regulation Track
Michael H. Humphreys and Peter Bie

Symposium
Cytoskeleton-Associated Motors and Tethers in Epithelial Polarity and Trafficking
Bruce Stanton and Dennis Brown

Symposium
Integrins: New Insights and Therapeutic Targets
Systems Biology (-omics) Track
Shaila Basavappa

Symposium
Mechanobiology and Oxidative Stress
Brett Blackman and Hanjoong Jo

Symposium
New Insights into the Pulmonary Circulation
Blood Pressure Regulation
Hypoxia and Oxidative Stress Track
John B. West and Frank L. Powell

Featured Topic
Mechanisms of Peripheral Chemoreception
Hypoxia and Oxidative Stress Track
Daniel K. Mulkey

Featured Topic
Regulation of Vascular Caliber and Contractility
Ed van Bavel and Michael A. Hill

Featured Topic
Sarcopenia: Signal Transduction and Metabolism in Senescent Skeletal Muscle
Aging, Gender and Sex Differences Track
Thomas H. Reynolds

Featured Topic
The Brain, Behavior and Autonomic Function in Health and Disease
Angela J. Grippo and Julia A. Moffitt

Symposium
Vitamin D Deficiency and Its Impact on Health
Translational Physiology Track
Diane L. Kamen and Vin Tangpricha

Wednesday, April 28, 2010 10:30 AM-12:30 PM

Symposium
Novel Redox Signaling in Ion Channel Regulations and Pathophysiology
My N. Helms

Physiology InFocus --Physiology and Biomedical Engineering: Partners in Translational Research
Physiology at the Crossroads of Biomedical Engineering and Medicine
Y.S. Prakash

Symposium
Protein O-GlcNAcylation: A New Signaling Paradigm for the Cardiovascular System
Hypoxia and Oxidative Stress Track
Lance Wells and Natasha Zachara

Symposium
The Role of the Ubiquitin Proteasome System in Cardiac Disease, Diabetes, and Aging
Aging, Gender and Sex Differences
Metabolism and Metabolic Diseases Track
Monte S. Willis and Xeujun Wang

Symposium
To Exercise or Not to Exercise: Can We Replace Physical Activity With a Pill?
Eva R. Chin and Espen E. Spangenburg

Featured Topic
Hypoxia Effects on Cardio-respiratory Function and Integration
Hypoxia and Oxidative Stress Track
Kendall F. Morris

Featured Topic
Physiological Role of Vascular Endothelial Growth Factors as Homeostatic Regulators
David Bates

Postdoctoral Positions

Postdoctoral Position in Pulmonary Vascular Physiology: An NIH-supported postdoctoral position is available in the laboratory of Dr. Nikki Jernigan to study smooth muscle signaling mechanisms that regulate vasoreactivity in the hypertensive pulmonary microcirculation. A variety of experimental approaches are utilized including in vivo hemodynamic studies using chronically-instrumented animals, dimensional analysis of isolated and pressurized small pulmonary arteries, ratiometric calcium imaging, laser scanning confocal microscopy, electrophysiology, isolated perfused lungs, cell culture, and molecular biology. A background in cardiovascular, molecular or electrophysiology is desirable. Salary is commensurate with experience and based on current NIH guidelines. Applicants should have a PhD or MD degree and send a cover letter, CV and names and contact information of two to three references to: Nikki L. Jernigan, PhD, Assistant Professor, Vascular Physiology Group, Department of Cell Biology and Physiology, University of New Mexico Health Sciences Center, MSC08 4750, Albuquerque, NM 87131-0001, Email: njernigan@salud.unm.edu. Home Page: http://hsc.unm.edu/som/cbp/faculty/jernigan.shtml.

Postdoctoral Position: University of Pittsburgh, Department of Medicine, Renal-Electrolyte Division: Position is available for a highly motivated postdoctoral fellow to pursue studies on trafficking and regulation of the epithelial Na+ channel ENaC. A variety of experimental approaches will be employed in these studies. Experience in techniques of cell biology, particularly immunofluorescence, cell fractionation and organelle isolation and surface biotinylation is valued along with basic biochemical and molecular biology skills (transfection, knockdowns, PCR, phosphorylation and blotting). Electrophysiological techniques (two-electrode voltage clamp, and Ussing chamber measurements) are also used but may be acquired. State-of-the-art equipment and facilities are available. Interested applicants with experience in cell biology, molecular biology or electrophysiology are encouraged to apply. Applicants must by a US citizen or permanent resident and possess a PhD degree (or equivalent), should have excellent oral and written communication skills, and display initiative as well as independence. The candidate will be expected to present research findings at scientific conferences, compose manuscripts, assist in experimental design, and apply for extramural postdoctoral funding during the first year of work. Salary is based upon experience and NIH salary levels. Please send a statement of research interests, curriculum vitae, and the names and contact information of three references to J.P. Johnson, Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh, 935 Scaife Hall, 3550 Terrace Street, Pittsburgh, PA 15261; Email: johnsonj@pitt.edu. Webpage: http://www.dept-med.pitt.edu/renal/faculty_info.aspx?fp=5104.

Postdoctoral position: Available immediately for individuals with PhD in neuroscience or related fields. Experiences with molecular technique such as DNA plasmid making and viral packaging desirable. Prospective candidates will participate in the molecular and cellular basis of electrophysiological and imaging studies in the hippocampal neuron anatomy and plasticity. Please send a short letter of application, curriculum vitae and details of three references to: Dr. Masako Isokawa (email: isokawalab@gmail.com), Department of Biological Sciences, University of Texas at Brownsville, 80 Fort Brown, Brownsville, TX 78520. UTB is an equal opportunity, affirmative action employer.

Postdoctoral Positions, Physiology: The Department of Integrative Physiology at the University of North Texas Health Science Center (UNTHSC) is seeking highly motivated individuals for postdoctoral fellowships. Applicants must hold a PhD or equivalent doctoral degree and be ready to participate in a well-funded research program addressing fundamental aspects of neuroendocrine regulation of cardio-respiratory function. We study mechanisms underlying the plasticity of central neurons following chronic changes in physiological state such as hypertension, COPD, heart failure and sleep apnea. Individuals who have completed or will complete their PhD training in physiology, pharmacology, neuroscience or a related field are encouraged to apply. Applicants with expertise using in vivo and in vitro electrophysiological approaches will be given high priority. Interested applicants should apply online at http://www.unthscjobs.com. Applicants should attach curriculum vitae, a brief statement of research interests and career goals, and the names of three references to: Questions concerning this position can be sent to Steve Mifflin (smifflin@hsc.unt.edu). UNTHSC is an equal opportunity employer.

Postdoctoral Fellow: A Postdoctoral Fellow position is available at the University of Alabama at Birmingham in the department of Physiology and Biophysics for a highly motivated researcher to pursue several projects involving the characterization and regulation (e.g., by phospholipids) of both endogenous acid-base transporter (e.g., bicarbonate –dependent ones) expressed in neurons and glia, as well as heterologously expressed transporter in *Xenopus* oocytes and mammalian cells. An additional project involves characterizing ion channels and transporter activity in cell models of polycystic kidney disease (PKD). Applicants are expected to have electro-physiology experience to pursue projects involving patch clamping and two-elegrode voltage clamping. Experience with molecular techniques and mutagenesis approaches for structure-function studies is desired. Opportunities will be available to learn additional techniques (e.g., ion-sensitive fluorescence imaging) and to submit independent grant proposals. Salary is very competitive. Send a current CV, a letter describing research interests, and three references to: Dr. Mark Bevensee, University of Alabama at Birmingham, 1918 University Blvd., MCLM 812, Birmingham, AL 35294-005. [AA/EOE]

Postdoctoral position: Postdoctoral position is available at the Institute for Clinical and Experimental Medicine in Prague, Czech Republic to conduct research designed to provide new insights on hypertensinogenic mechanisms mediated by intrarenal interaction between renin-angiotensin system and eicosanoid metabolites. A variety of angiotensin II-dependent models of hypertension including two-kidney, one-clip Goldblatt hypertensive rats and transgenic rats with inducible expression of the mouse Ren-2 renin gene

(Cyp1a1-Ren-2 rats) are routinely employed in our laboratory. In vivo renal clearance and blood pressure measurements procedures are utilized to evaluate renal hemodynamics and cardiovascular function. Applicants should hold a doctoral degree and preferably have background in cardiovascular research and physiology. Background experience in other fields such as pharmacology or biochemistry is also welcome. The ideal candidate will be motivated and enthusiastic about working in a fully equipped, modern laboratory. This position is ideal for a scientist looking to relocate to Prague. To apply, submit a letter including research interest and CV to Dr. Ludek Cervenka, luce@medicon.cz, Videnska 1958/9, Praha 4, 140 21, Czech Republic.

Postdoctoral position: An NIH-funded postdoctoral position is available immediately at the Penn State University College of Medicine Department of Pediatrics in Hershey, PA. The project is focused on the regulation of the human pulmonary surfactant proteins. Pulmonary surfactant, a lipoprotein complex, is essential for normal lung function. Length variant polymorphisms residing on the first half of intron 4 of the pulmonary surfactant protein B (SP-B) have been associated with several lung diseases. This region is characterized by the presence of repeated motifs and several deletion and insertion variants have been identified. Recent evidence indicates (Lin 2005, *Bioch J.* 389:403-412) that deletion variants associate with abnormally spliced SP-B RNA. The goal of this project is to study in a systematic way the role of SP-B intron 4 variants on splicing to determine whether sequence composition and/or size is (are) critical for correctly spliced RNA, as well as study potential cis and trans-elements within this region. Applicants should hold a PhD and be residents or citizens of the USA. They must have a proven record of theirs expertise in molecular biology. Please send to Joanna Floros, PhD, jfloros@psu.edu, a CV, a brief statement of research interests and career goals, and three letters of recommendation from individuals that the applicant has interacted with the most in the course of his/her academic training. [EOE]

Postdoctoral Fellow position: Available immediately in the Department of Obstetrics/Gynecology, Wake Forest University Health Sciences, Winston-Salem, NC. (WFUHS position number is 9941) We are seeking a PhD (or equivalent degree) in Physiology (or related field) to join an active research group investigating maternal vascular vasoreactivity / remodeling during pregnancy using rodent and human models. A candidate with experience in smooth muscle physiology and vascular reactivity studies using small vessel myography is preferred. Additional experience working with translational genomic approaches is beneficial as the research project will address mechanism(s) underlying pathological complications involved with pregnancy at both the functional and genomic levels. A successful applicant is expected to work independently; therefore strong organizational as well as verbal and written communication skills are essential. Starting salary will be commensurate with experience. *Applicants should send curriculum vita and letter of interest to Dr. Lorna G. Moore, Graduate School of Arts & Sciences, Wake Forest University, Winston-Salem, NC 27157-1001.*

PhD Training Program in Space Life Sciences: The National Space Biomedical Research Institute (NSBRI)-sponsored PhD Training Program in Space Life Sciences at Texas A&M University (TAMU) is currently accepting applications for Fall 2010. Students participating in this program work toward a PhD in Nutrition, Kinesiology or Nuclear Engineering (Health Physics), or a MD/PhD or PhD in Medical Sciences from the Texas A&M University Health Sciences Center Graduate School of Biomedical Sciences. Students will focus their research on space environment-induced bone loss, muscle wasting, cosmic radiation damage and/or changes in metabolism. The Space Life Sciences graduate program at TAMU is designed with immersive components including: fundamental courses in space life sciences, individual research and an experiential component with work at Johnson Space Center, Brookhaven National Laboratory and/or University of Texas Medical Branch. All trainees participate in outreach by teaching elements of space life sciences in a K-12 setting. To learn more about the program, please visit http://SLSGraduateProgram.tamu.edu. The deadline for submitting an application package is February 15, 2010. For more information please contact: Chelsea L. Bishop, Texas A&M University, Program Coordinator, Ph.D. Training Program in Space Life Sciences, 213 Kleberg Center, 2253 TAMU, College Station, TX 77843. Phone: 979-845-0850. Fax: 979-862-1862. Email: CLBishop@tamu.edu.

Academic Fellowships: New opportunities for postdoctoral training under the University of Maryland Baltimore School of Medicine's Training Program in Cardiac & Vascular Cell Biology (http://medschool.umaryland.edu/cardiovascular/). These fellowships offer funding for stipend, travel and training related expenses. Trainees will obtain an integrated understanding of 21st century biomedical science, from molecule to man, with a focus on cardiovascular physiology and pathology, including the molecular basis and the genetics of cardiovascular disease, renal disease and stroke. Training within the labs of our 32 faculty mentors (http://medschool.umaryland.edu/cardiovascular/mentors.asp) takes place within a highly collaborative training environment, and includes: molecular determinants of muscle cell and heart function, Ca2+ signaling, biophysics and trafficking of ion channels and membrane transport proteins, the function of the cytoskeleton, genome wide association studies (GWAS), molecular mechanisms contributing to heart failure, cardiac arrhythmias, hypertension, sleep disorders, circadian activity, renal disease and stroke. Applicants must have a PhD, MD, or comparable degree. Funded positions are restricted to US citizens and permanent residents. Please send curriculum vitae, a statement of research interests and the names of three references to: Katherine Frankel at kfrankel@som.umaryland.edu.

Faculty Positions

Physiology Faculty Position: Ross University School of Medicine, located on the beautiful Caribbean island of Dominica in the West Indies, invites applications for a faculty post at any rank in Physiology. We invite applicants from any area of expertise, but are currently very interested in an individual

who can teach Endocrine and Reproductive Physiology. Our mission is to prepare highly dedicated students to become effective, successful physicians in the United States. Basic science coursework is taught in Dominica and students then complete their clinical studies in the United States. After passing all prerequisite examinations, Ross graduates are licensed to practice medicine in all 50 states of the US. Ross University School of Medicine is a division of DeVry, Inc (NYSE:DV). Education is the primary focus of the faculty. The academic year is divided into three semesters with a new class of students admitted each semester. Lectures and other educational responsibilities continue throughout the year. Effective teachers are sought, particularly individuals who are interested in improving medical education and who work well on a team. Research opportunities exist, primarily in the area of medical education. Essential Duties and Responsibilities: *1*) the preparation of course material (handouts etc.); *2*) the delivery of effective lectures; *3*) the preparation, administration, marking and reporting of examinations; *4*) undergo training to qualify as a facilitator in the problem-based learning program; *5*) supervise educational activities of students under actual or simulated situations; *6*) prepare instructional plans and career analyses to reflect current changes in the field; *7*) advise individuals or groups of students in academic matters and exercise professional judgment in referring students to appropriate personnel; *8*) develop new instructional materials and teaching techniques with participation in on-going reviews and revision of curriculum planning; *9*) actively participate in relevant professional activities in order to improve teaching and subject matter competence; *10*)serve on faculty committees as appointed or elected, and confer with advisory groups in order to modify course content; *11*) prepare, administer and evaluate examinations to assess the development of student accomplishments; *12*)participate in other activities as assigned by the department chair or executive dean. Qualifications: *1*) content expertise in endocrine, reproductive, respiratory, renal or GI physiology; *2*) ability to relate physiology to clinical scenarios; *3*) experience in computer-assisted delivery of course content; *4*) excellent communication skills in English; *5*) strong teaching skills and experience or evidence of potential; *6*) interest in medical education; 7) desire for self improvement; *8*) flexibility and ability to work well on a team. Education, Experience, Knowledge and Skills: PhD, MD or MD/PhD degree in physiology. Enthusiastic teacher with previous teaching experience at a North American or United Kingdom medical school. Ross University offers a competitive potentially tax-free annual salary, relocation assistance to and from the island, a deferred pension program, tuition assistance benefit, scholarship program for dependents, 100% medical benefits paid for the employee, travel benefits, a living allowance, 25 days of paid annual leave is provided along with opportunities for professional development, which includes a conference and book allowance. To apply, please visit our website www.rossu.edu; select Careers and complete our online application process. [EOE]

Endowed Professorships and Faculty positions in Health: Michigan Technological University invites applicants for new tenure-track positions at any rank in the broad areas of health sciences and engineering. This campus-wide Strategic Faculty Hiring Initiative (SFHI) is projected to bring up to 10 new faculty members, including possible endowed positions, to campus over the next two years to strengthen the key focus areas of biochemistry, bioengineering, bioethics, biomaterials, biomechanics, human factors, medical informatics, cell biology, physiology, and statistical genetics. Faculty hired through this initiative are expected to establish a vigorous, nationally competitive research program and to be committed to excellence in both undergraduate and graduate education. The application review process will begin on November 15, 2009. Details on the SFHI and application instructions are available at http://www.mtu.edu/sfhi. Michigan Tech is an internationally renowned doctoral research university located in Michigan's scenic Upper Peninsula, on the south shore of Lake Superior. Houghton provides a unique setting where natural beauty and exceptional year-round outdoor activities, culture, education, and a diversity of residents from around the world come together to share a superb living and learning experience. As part of its strategic focus, Michigan Tech is experiencing remarkable growth in research. In the last five years, research expenditures have doubled, up to $60 million in 2008. The university has also recently initiated efforts to advance health-related research capabilities with the establishment of animal facilities and the formation of the Departments of Biomedical Engineering and Exercise Science, Health and Physical Education. Michigan Tech is an ADVANCE institution, one of a limited number of universities in receipt of NSF funds in support of our commitment to increase diversity and the participation and advancement of women in STEM. Applications from women and minorities are encouraged. [AA/EOE]

Faculty Position: The College of Pharmacy at Southwestern Oklahoma State University (SWOSU) invites applications for a tenure track academic year faculty position in the Department of Pharmaceutical Sciences at the rank of assistant or associate professor. Historically, employment has been available every summer. Candidates should possess a PhD in the pharmaceutical or biomedical sciences with an interest in pharmacogenomics. Preference will be given to candidates who have an entry-level pharmacy degree and/or postdoctoral research experience. Responsibilities will include teaching in the professional PharmD program in the physiology, pathophysiology, and pharmacology course sequences. Course development relevant to the applicant's background and expertise will be encouraged. The successful candidate will be expected to develop curricular content in pharmacogenomics, independent and collaborative research and with undergraduate or entry-level Doctor of Pharmacy student involvement. Salary will be commensurate with existing salary structure.

Review of applications will begin immediately and continue until the position is filled. The anticipated starting date is January 1, 2010. SWOSU has an enrollment of about 5,000 students and is located in Weatherford, OK, which is one hour west of Oklahoma City on historic Route 66. The College of Pharmacy has approximately 85 students in each of the professional program years. Applicants should send a cover letter referencing Position 10-F004, vitae, unofficial transcripts, and the names and contact information of three references to: Human Resources, Southwestern Oklahoma State University, 100 Campus Drive, Weatherford, OK 73096, or Email application materials to:jobs@swosu.edu. For more information visit http://www.swosu.edu. Southwestern Oklahoma State University is an AA/EEO employer and encourages applications from minorities and women.

Assistant Professor: Penn State Abington seeks a Physiologist for appointment to a tenure-track position at the Assistant Professor level to begin August 2010. The successful candidate will teach undergraduate lecture and laboratory courses in introductory human physiology, advanced mammalian physiology, the physiology and development of animals and plants, and advanced courses in his/her area of expertise. The candidate is expected to establish an active research program with a strong record of scholarly activity, and include undergraduates in his/her research activities. The candidate is expected to advise students and provide career guidance, as well as participate actively in campus, university and community service. Applicants must have completed a PhD in Physiology or closely-related area. Preference will be given to candidates with strong teaching experience at the college level. The review process will begin November 15, 2009 and continue until the position is filled. Applicants should submit an electronic dossier including: a cover letter, curriculum vitae, statement of their research interests and a statement of their teaching philosophy to: Chair, Physiologist Search Committee, Division of Science and Engineering, cld5@psu.edu. Penn State is committed to diversity in its workforce. [AA/EOE]

Assistant/Associate Professor in Pharmacology: The Department of Pharmaceutical Sciences at North Dakota State University invites applications for a tenure-track faculty position at the rank of Assistant/Associate Professor, with appointment beginning on or after August 15, 2010. Candidates must hold a doctoral degree in pharmacology, physiology, or closely related field, have at least two years of postdoctoral experience with a strong record of scholarship, and possess good interpersonal skills and effective written and oral communication skills. Preference will be given to applicants with training and research expertise in areas that complement existing departmental strengths in cancer, cardiovascular, and vaccine research. The successful candidate will be expected to establish an externally funded research program, teach and mentor graduate students, and participate in team-taught pharmacology courses offered to pharmacy students. A highly competitive salary and a start-up package commensurate with qualifications and experience are available. Currently, the fast growing department has 12 faculty members, 30 doctoral students and eight post-doctoral fellows/research associates, has a Center of Excellence, Center of Biopharmaceutical Research and Production (CBRP), and participates in a NIH-funded ($10.5 million) Center of Biomedical Research Excellence. Additional information about the Department and University can be obtained at http://www.ndsu.edu/pharmsci/. Application deadline is November 30, 2009, or thereafter until the position is filled. The application portfolio containing the curriculum vitae, statement of teaching philosophy, description of research interests and future plans, and three letters of reference must be submitted electronically: http://www.jobs.ndsu.edu/applicants/Central?quickFind=51125. For more information, please contact Dr. Stephen O'Rourke (Email: stephen.orourke@ndsu.edu), North Dakota State University College of Pharmacy, Nursing and Allied Sciences, Fargo, ND 58105. NDSU is an equal opportunity institution. Women and traditionally underrepresented groups are encouraged to apply.

Assistant Research Professor: The Indiana University-Purdue University Indianapolis School of Medicine, Department of Medicine, Division of Nephrology invites applications for Assistant Research Professor or above, appointment type and rank to be determined by qualifications and interest. The Division of Nephrology is seeking an outstanding researcher in live cell and live animal imaging utilizing confocal and multiphoton microscopy. The individual will be responsible for research in the area of biological imaging with additional responsibilities in the daily operation of a core microscopy facility. Applicants for this position must have either a MD or PhD with postdoctoral experience. Training and experience in anatomy, cellular biology and molecular biology in addition to microscopy is desirable. Experience is also desired in 3D and 4D image processing. Experience in microinjection and Zebrafish studies is preferred. Candidates must be sensitive to the needs of and possess an interest in working in an academic community that is diverse with regard to gender, race, ethnicity, nationality, sexual orientation, and religion. Applicants should submit curriculum vitae and arrange to have three letters of recommendation sent to: Submit CV and letter of interest to: Madelynn Cowley, Human Resource Specialist, Indiana University Division of Nephrology, 950 W Walnut, Suite 202, Indianapolis, IN 46202. IUPUI is an Equal Opportunity/Affirmative Action Institution M/F/D.

Animal Physiologist: Tenure track position, Assistant Professor beginning August 2010. PhD required. Teaching responsibilities include upper-division animal physiology, participation in non-majors courses in human anatomy and physiology, and other majors and non-majors courses. Active research in animal physiology is required. The successful candidate will be expected to develop

and maintain a research program involving Southeast students. Advising biology majors and biomedical scholars is required. Send CV, undergraduate and graduate transcripts, three reference letters, and statements of teaching philosophy and research interests by November 30, 2009 to Dr. W. R. Eddleman, Dept. of Biology, MS 6200, Southeast Missouri State University, Cape Girardeau MO 63701. *An Equal Opportunity/M-F/Affirmative Action Employer.*

Assistant/Associate/Full Professor Exercise Science Program: The George Mason University School of Recreation, Health, and Tourism invites applications for a nine-month tenure-track faculty appointment (open-rank) in the exercise science program beginning August 2010. Primary responsibilities include teaching undergraduate and graduate courses (e.g., biomechanics, motor control, exercise physiology, sport nutrition, motor learning, and/or research methods); maintaining an active line of research and publication record in area of expertise; and securing external funds. Additional responsibilities include serving on school, college, and university committees; developing professional partnerships with public and private agencies; and contributing to the continued development and delivery of the exercise science program. Applicants must have completed a doctorate in exercise science or a closely related field. Candidates must demonstrate potential for scholarly activity, contribute to collaborative interdisciplinary research, and show a strong commitment to teaching and advising undergraduate and graduate students. George Mason University, recognized internationally for its innovation, diversity and entrepreneurial spirit, is a state-supported university located in the vibrant Northern Virginia-Washington, DC. Metropolitan Area. George Mason University currently enrolls approximately 30,000 students in undergraduate, master's, and doctoral programs. The university has emerged in the last decade as one of the premier institutions of higher learning in the state and the nation. Contact ghaller@gmu.edu with any questions. Candidates should apply online at http://jobs.gmu.edu for position F7005z and attach a letter of interest, current vitae and references. Review of applications will begin Monday, November 1, 2009, and continue to be accepted until the position is filled. [AA/EOE].

Assistant Professor for Bloomsburg University of Pennsylvania, Human Biologist: Bloomsburg University of Pennsylvania, Department of Biological and Allied Health Sciences invites applications for a tenure-track position at the Assistant Professor level (AA#50-9-83) to begin in the Fall 2010. Candidates must possess a doctorate with a background in human physiology, anatomy, or a related health science field. Candidates will be expected to teach anatomy and physiology, introductory biology courses, as well as upper level courses that support the allied health programs. Additionally, candidates are expected to develop an active research program that involves both undergraduates and graduate students. Candidates must also be committed to the recruitment, retention, and advisement of students. Experience teaching anatomy and physiology and/or introductory biology courses is preferred. Salary will be commensurate with experience. Send letter of application, curriculum vitae, copies of transcripts (undergraduate and graduate), a one page statement of teaching philosophy, statement of research interests, and contact information for three professional references to: Dr. Angela R. Hess, Chairperson of BAHS search committee, Department of Biological and Allied Health Sciences, AA# 50-9-83, Bloomsburg University of PA, 400 East 2nd Street, Bloomsburg PA 17815. For more information about the department, visit: http://departments.bloomu.edu/biology. Review of complete applications will begin on 12/14/2009 and will continue until the position is filled. Demonstrated ability to work with diverse populations preferred. Recommendation for hiring is needed by the majority of the regular, full-time department faculty. Finalists for the position must communicate well and successfully complete an interview process, teaching demonstration, and research seminar, as judged by the department faculty. Bloomsburg University of Pennsylvania encourages applications from historically under-represented individuals, women, veterans, and persons with disabilities and is an AA/EEO employer.

Chair of the Department of Exercise Science: Syracuse University invites applications and nominations for the tenure-track position of Chair of the Department of Exercise Science in the School of Education to begin summer of 2010. To be considered, candidates must have earned a doctorate in exercise science, exercise physiology or related discipline and demonstrate a record of teaching effectiveness and scholarship commensurate with appointment at the associate professor/full professor level. They must possess a strong record of publication and have obtained a level of extramural funding demonstrating excellence in research. They must show readiness to contribute to both undergraduate and graduate teaching, advise Master's and PhD student research, and perform University and professional service. The department supports a number of academic programs and pursues a range of project and research interests. It is one of seven departments in the School of Education and offers undergraduate majors in physical education and health and exercise science, and a master's degree program in exercise science. A program in physical therapy is offered through an articulation agreement with a neighboring medical university. Currently, students may pursue doctoral studies in exercise physiology through the PhD program in Science Education. The department consists of seven faculty members, 150 undergraduate students and 60 graduate students. Candidates must have demonstrated or have the potential to lead and administer such an academic department. This includes interacting effectively with students, faculty members, and other administrators; collaborating with others in a multidisciplinary teaching and research environment; engaging in the local community and promoting the engagement of others; showing a com-

mitment to diversity in the actions and plans of the department. Unique opportunities exist for participation in translational and interdisciplinary research, collaborating with basic science departments on campus and the neighboring medical university. In such an environment, candidates must have and be able to express a vision for the department that will move it forward. Although outstanding candidates in all areas of exercise science will be considered, special consideration will be given to investigators with a record of research in human cardiovascular, ventilatory, or metabolic physiology, as well as those that complement the new institutional commitments in cell signaling and disability studies. Special consideration will also be given to those applicants with experience in both applied and basic physiology. Initial screening of applications is ongoing and applications will be accepted until the position is filled. **Important:** as part of the application process, you must complete a Dean/Senior Executive/Faculty Application at http://www.sujobopps.com and apply to job# 025184; to be considered, a CV must be attached to the application. You must also send letter of application outlining your accomplishments and future directions, and three letters of recommendation to Dr. James Bellini, Search Committee Chair, Exercise Science, Room 201 Women's Bldg, Syracuse University, Syracuse, NY 13244-5040; jlbellin@syr.edu; Tel.:315-443-2114; Fax: 315-443-9375; for additional information on the department go to http://www.soe.syr.edu. [AA/EOE]

Assistant Professor: Eastern Washington University (EWU) Department of Biology invites applications for a full-time, tenure-track Assistant Professor position beginning September 2010. The Department of Biology seeks a candidate who complements the strengths of our faculty in scholarship and teaching and who, by working with women and minority students, reinforces our commitment to enhancing diversity. Responsibilities: 1) teaching duties include human anatomy & physiology for majors or non-majors and animal physiology for seniors; other teaching assignments may include electives in area of specialization, contributions to the introductory biology curriculum, participation in graduate-level classes, and involvement in the medical and dental programs to which our department contributes; 2) developing and sustaining a productive research program supported by external funding; 3) supervising and mentoring of master's theses and undergraduate research; 4) advising undergraduate students; and 5) becoming professionally engaged with the local community. Qualifications: applicants must have a PhD in biological sciences or equivalent. The successful candidate must be committed to teaching a diverse student population. The Biology Department includes a diverse faculty and over 450 majors and Masters-level graduate students focusing on ecological, molecular, physiological, microbial, and health sciences. Our main campus is located 16 miles SW of Spokane in Cheney, WA. Send: letter of application, curriculum vitae, statement of teaching philosophy and experience, statement of research plan, up to three recent reprints, copies of transcripts, and three letters of reference (under separate cover) to the Physiologist Search Committee, Department of Biology, Eastern Washington University, 258 Science Building, Cheney, WA 99004-2440. Review of applications will begin after December 15, 2009, and will continue until position is filled. Information about the department may be found at www.ewu.edu/biology. AA/EEO Employer: EWU is committed to affirmative action and equal opportunity. We encourage applications from members of historically underrepresented groups and from all qualified individuals. The successful candidate must have the ability to promote and support cultural competency, pass a background check, and show proof of eligibility to work in the US pursuant to US immigration laws. ❖

Senior Physiologists' News

Letter to Frank Knox

Jim Parker writes: "Thank you for your interest. I have mixed feelings about qualifying as a senior physiologist. I am currently not retired and started a new research grant this July. This grant relates to the interaction of coagulation factors in amplifying the mechanical effects of the TRPV4 channel in mechanical lung injury. Ventilator induced lung injury has been a long-standing standing interest of our lab and we actually published the first paper identifying a vascular permeability lesion during lung overdistention approximately 25 years ago. Patients are now routinely ventilated with small tidal volumes but

we are hoping that defining the mechanism whereby such a small decrease in volume can account for such a large increase in survival will further improve treatment in acute respiratory distressed patients.

"I also am continuing to write papers and some book chapters including one for the upcoming Handbook and I am continuing with my teaching assignments in the medical physiology and graduate student courses.

"My advice to younger colleagues is to become broadly trained in integrated physiology and whole animal methods as well as molecular and genetic techniques so that their research can be related to physiologic and pathophysiological conditions in intact animals and patients.

"My hobbies include Masters swimming and saltwater and freshwater fishing." ❖

APS Members Elected to the Institute of Medicine

Five APS members were among the group of new members announced by the Institute of Medicine (IOM) in conjunction with its 39th annual meeting. A total list announced by the IOM included the names of 65 new members and five foreign associates. Election to the IOM is considered one of the highest honors in the fields of health and medicine and recognizes individuals who have demonstrated outstanding professional achievement and commitment to service.

The APS members elected to the IOM include:

Alfred L. Goldberg, Professor of Cell Biology, Department of Cell Biology, Harvard Medical School, Boston, MA;

Joanne J. Lupton, Distinguished Professor, Regents Professor, and William W. Allen Endowed Chair in Nutrition, Department of Nutrition and Food Science, Texas A&M Univ., College Station;

Roger A. Nicoll, Professor, Departments of Cellular and Molecular Pharmacology and Physiology, Univ. of California, San Francisco;

Daniel K. Podolsky, Philip O'Bryan Montgomery Jr., Distinguished Presidential Chair in Academic Administration, and Doris and Bryan Wildenthal Distinguished Chair in Medical Science, Department of Internal Medicine, Univ. of Texas Southwestern Medical Center, Dallas; and

Clifford B. Saper, James Jackson Putnam Professor of Neurology and Neuroscience, Harvard Medical School; and Professor and Head, Department of Neurology, Beth Israel Deaconess Medical Center, Boston, MA.

Moss Appointed Senior Associate Dean for Basic Research, Biotechnology and Graduate Studies

Richard L. Moss, the Robert Turell Professor of Physiology and chair of the physiology department at the University of Wisconsin School of Medicine and Public Health, has been appointed senior associate dean for basic research, biotechnology and graduate studies. Moss will succeed Paul DeLuca, who recently was named Univ. of Wisconsin-Madison provost and vice chancellor for academic affairs. The associate dean position has been expanded to encompass biotechnology.

Robin Looft-Wilson Recognized for Teaching Excellence

The William and Mary Alumni Association announced its Alumni Fellowship Awards on September 24, 2009, recognizing five younger faculty members who are particularly outstanding as teachers. Among this year's winners was APS Member Robin Looft-Wilson, who was recognized for her outstanding teaching work.

The early years of her career were spent focused largely on space physiology, astronaut health. After conducting experiments for NASA on how the circulatory system adapts to zero-gravity, Looft-Wilson got a PhD and started focusing on the basics of blood vessels. Specifically, she studies how blood vessel mechanics influence cardiovascular disease, one of the most dangerous and lethal conditions in American health. In her work, Looft-Wilson looks at an amino acid called homocysteine, high levels of which are a major risk factor for atherosclerosis along with cholesterol.

The real-world application of her research is not lost on her students, many of whom have relatives with cardiovascular problems. "It seems like a lot of the students just want to know as much as they possibly can. In my physiology classes I present much more about pathologies and treatments," she says. "It's more of the medical aspects than I would have presented otherwise, but it all comes from student demand." Looft-Wilson has her students read primary literature along with her lectures to ensure that she stays fresh and her students know about the cutting edge of research in her field. By learning to criticize and evaluate published findings, she prepares her students for even more rigorous graduate work. Her obvious enthusiasm is contagious.

Sebastien Banzet has moved to Environnements Operationnels at the Institut de Recherches Biomedical Des Armees, Bretigny sur Orge, France. Prior to this move, Banzet was in the Department of Human Factors at the Center de Recherches Du Service De Sante, La Tronche, France.

Andreas M. Beyer is currently a Postdoctoral Fellow in the Department of Physiology at the Medical College of Wisconsin, Milwaukee. Previously, Beyer was a Postdoctoral Fellow in the Department of Internal Medicine at the University of Iowa.

Ann C. Bonham is currently the Chief Scientific Officer at the Association of American Medical Colleges, Washington, DC. Bonham had been the Executive Associate Dean in the Department of Medical Pharmacology and Toxicology School of Medicine, Sacramento, CA.

Siu Lung Chan is currently a Postdoctoral Associate in the Department of Neurology at the University of Vermont, Burlington, VT, having left the position of Postdoctoral Fellow in the Department of Pathology at the University of Iowa, Iowa City.

Joseph Anthony Covi is an Assistant Professor in the Department of Biology at the University of Wisconsin, Stevens Point, WI. Prior to this position, Covi was a Postdoctoral Research Associate in the Department of Biology at Colorado State University, Fort Collins, CO.

Zellieann Rivera Craig is a Postdoctoral Research Associate in the Department of Veterinary Biosciences at the University of Illinois, Urbana, IL. Previously, Craig was in the Department of Physiology at the University of Arizona, Tucson, AZ.

Rebecca Lynn Cunningham is now in the Department of Pharmacology and

Neurosciences at the University of North Texas Health Sciences Center, Fort Worth, TX. Cunningham had been in the Department of Pharmacology at the University of Texas Health Science Center, in San Antonio, TX.

John James Durocher has moved to Saint Francis University in the Department of Physical Therapy, Loretto, PA, having left the position of Assistant Professor in the Department of Health, Exercise Science and Sport Management at the University of Wisconsin, Kenosha, WI.

Amy Leanne Firth is now in the Laboratory of Genetics at the Salk Institute, La Jolla, CA. Previously, Firth was in the Department of Medicine at the University of California at San Diego, La Jolla.

Richard J.B. Francis has taken a position of Assistant Professor at the University of Pittsburgh, Department of Developmental Biology, Pittsburgh, PA. Prior to this position, Francis was a Staff Scientist at National Institutes of Health, Bethesda, MD.

Ryan A. Harris is currently an Assistant Professor in the Georgia Prevention Institute at the Medical College of Georgia, Augusta, GA. Previously, Harris was a Postdoctoral Research Fellow in the Department of Medicine, University of California, San Diego, La Jolla, CA.

Jeffrey W. Holmes is currently an Associate Professor in the Department of Biomedical Engineering at the University of Virginia, Charlottesville. Prior to this position Holmes was an Assistant Professor in the Biomedical Engineering Department at Columbia University, New York.

Robert Tyler Morris has moved to the Department of Molecular Physiology at Vanderbilt University, Nashville, TN. Morris had been in the Department of Biology at Missouri Southern State University, Joplin.

Carmel Nottle is currently a Lecturer in Human Movement at University of South Australia, Salisbury Downs, Australia. Prior to this position, Nottle was a Sessional Tutor at Wintec, Hamilton, New Zealand.

Naro Ohashi has moved to the Department of Nephrology at Numazu City Hospital, Numazu, Japan, having left the First Department of Medicine at Hamamatsu University School of Medicine, Hamamatsu, Japan.

Ivan Mark Olfert has moved to the Center for Cardiovascular and Respiratory Sciences at West Virginia University, Morgantown. Prior to this move, Olfert was in the Department of Medicine at the University of California, San Diego, La Jolla, CA.

Qining Qin is now a Postdoctoral Fellow in the Department of Biological Chemistry at Johns Hopkins University School of Medicine, Baltimore, MD, having left the University of California, Los Angeles, CA.

Carmelle V. Remillard is currently Manager, Scientific Web Content in the Scripps Genomic Medicine at Scripps Translational Science Institute, La Jolla, CA. Prior to this position, Remillard was a Postgraduate Researcher in the Department of Medical Pulmonary at the University of California, San Francisco.

Stefan B. Sigurdsson is currently a Professor Rector in the Department of Solborg at the University of Akureyri, Iceland. Previously, Sigurdsson was a Professor in the Department of Physiology at University of Iceland, Reykjavik, Iceland.

Jamilur Rashid Talukder is currently an Adjunct Professor in the Department of Biology, Lemoyne-Owen, Memphis, TN. Prior to this move, Talukder was a Postdoctoral Research Fellow in the Department of Medicine at West Virginia University, Morgantown.

Gail D. Thomas is now at Cedars-Sinai Medical Center, Los Angeles, CA, having left the Division of Hypertension, University of Texas Southwestern Medical Center, Dallas.

Miao Tian is now in the Department of Molecular and Cell Biology, University of California, Berkeley, CA. Prior to this position, Tian was in the Department of Ophthalmology at Northwestern University, Chicago, IL.

Masato Tustsui is currently Professor and Chairman in the Department of Pharmacology at Faculty of Medicine, University of Ryukyus, Okinawa, Japan. Tsutsui had been an Associate Professor in the Department of Pharmacology at the University of Occupational and Environmental Health School of Medicine, Kitakyushu, Japan.

Serge P. Von Duvillard is now a Professor in the Department of Biology & Physical Education at College of Idaho, Caldwell. Von Duvillard left the position of Professor in the Department of Sport Science and Kinesiology at University of Salzbaurg in Hallein, Austria.

Andrew Voss is currently an Assistant Professor in the Department of Biological Sciences at California State University, Pomona. Prior to this position, Voss was a Postdoctoral Fellow in the Department of Physiology at University of California, Los Angeles.

Yang Wang is currently an Associate Professor in the Department of Pediatrics at the University of Chicago, Chicago, IL. Previously, Wang was an Assistant Professor in the Department of Pediatrics at University of Louisville, KY. ❖

The Physiologist
Vol. 52, No. 6, 2009

The Wine Wizard

Peter Wagner

The summer may be officially over, but where I reside the heat is still on: 90 F here today. Here are some refreshing whites, and then a few reds. After all, can't survive on whites alone, and I guess there may even be parts of the US that are not as hot as San Diego right now.

Whites

Four New Zealand Sauvignon Blancs that carry on the great tradition of interesting, flavorful, mostly clean and varietal wines. By the way, you don't know (and I understand if you do not care) how much this hurts, what with my coming from Australia. Bias aside, they are presented in order of my preferences, Kim Crawford first:

2008 Kim Crawford, Marlborough $13. As prices inch upward, this wine remains a cut above its competitors in richness of flavors. Great gooseberry/grassy/herbal fruit on the nose and palate and just the right amount of acid – not too bracing, but by no means dull. Very clean, long finish.

2008 Whitehaven, Marlborough $14. Not quite as powerful as the Kim Crawford. Yet solid varietal herbal gooseberry/passionfruit fruit and medium-soft acid.

2007 Drylands, Marlborough $12-50. I am not sure why the 2007 is still selling – most current NZ's are 2008. I have mentioned before that Sauvignon Blanc is a wine to drink young and not a wine to cellar, so personally, I would go after this one only if I wanted to drink it within three to six months. This year's effort is a bit less lush that earlier vintages, but still herbal, grassy, and tasty. The acidity is medium.

2008 Oyster Bay, Marlborough $10. This has in my mind always been a second tier label for NZSB's, but still decent value. This year's effort started off surprisingly stinky (ie, smelling sulfurous), which has been quite rare for NZSB.

Peter Wagner

Happily, this blew off within a few minutes of swirling in the glass. It was accordingly not attractive out of the bottle, but opened quite well with the usual herbal gooseberry flavors. It is a bit restrained, and has medium acidity. PS: just tried the 2009 version of the Oyster Bay, and while it was a bit thin when cold, as it warmed up it was clean and really improved with richness and clean herbal flavors.

Reds

2005 Faldo Cabernet Sauvignon, Coonawarra, South Australia $9. Coonawarra is a primary (red) wine region in Australia, and produces some of the best and most complex, but not lushest, wines, because it has a generally cooler climate that Barossa and McLaren Vale (whence come the big boys). When a $9 (= cheap) Coonawarra comes along, you have to try it. Out of the gate it smelled and tasted quite mature (smelled of earth, tea leaf, slight tobacco, rather than primarily of fresh fruit), even over the hill. But with swirling in the glass for a few minutes, much of the old character dissipated leaving quite forward, spicy dark berry fruit with some eucalyptus (the latter also typical of the region and adding a nice complex character). Tannins were medium soft, with balanced acid. If you go after it, please do not cellar it – drink it now, and only after decanting.

2007 Rancho Zabaco Zinfandel, Heritage vines, Sonoma County $11. This wine has a forward, almost sweet and lush ripe dark berry fruit nose with vanilla. The palate is medium in weight (ie, not too big or extracted), and the raspberry and dark cherry fruit is quite concentrated and tasty. Tannins are medium soft, acid is just right, and length is good. There is a sense of a touch of sweetness and some heat (sweet ethanol taste on the sides of the tongue) at the finish. Alcohol is high – 14.9%. I like it because the fruit is intense while the wine is not forced/extracted. And it is not too expensive.

2006 Mettler Zinfandel, Lodi, "old vine," CA $15. Fasten your seat belts, or on second thoughts, don't even get into your car unless you want to risk a DUI arrest. This wine has 15.6% alcohol, and it shows at the finish as heat. This is a bad boy, that's about all I can say. The nose has lots of dark berry fruit and even more American oak (evidenced by dill and coconut aromas). The palate follows in kind. It is rich, viscous, big, extracted, with lots of dark berry and raspberry fruit, soft tannins and balanced acids. The American oak (see above) is very strong on the palate as well. Sometimes I liken wines to well-known characters or stereotypes. This wine reminds me of a bouncer at a Playboy club, or at least of how I imagine such a person would look and behave, had I ever visited such an establishment, which of course, and as you well know, I have not!! So, if you crave big, powerful, oaky, alcoholic Zins, this one is made for you. Don't plan on working the next day. ❖

2010

February 10-13

Fifth International Conference SUMO, Ubiquitin, UBL Proteins: Implications for Human Diseases, Houston, TX. Information: Amy Heaton. Email: aheaton@mdanderson.org; Internet: http://www.mdanderson.org/education-and-research/departments-programs-and-labs/departments-and-divisions/cardiology/sentrin/index.html.

February 15-18

The Con-Joint Meetings of Biology and Synchrotron Radiation (BSR) and Medical Applications of Synchrotron Radiation (MASR), Melbourne, Australia. *Information:* Internet: http://www.masr2010.org.

February 20-24

Biophysical Society 54th Annual Meeting, San Francisco, CA. *Information:* Alexandra Frager. Tel: 301-634-7326; Fax: 301-634-7133; Email: afrager@biophysics.org; Internet: http://www.biophysics.org/2010meeting.

March 17-20

XVIII World International Family Therapy Association (IFTA) Congress, Buenos Aires, Argentina. *Information:* Victoria Tomsky, CLA 2010 - Industry Liaison & Sales, Paragon Conventions - Part of Liberty International Group, 18 Avenue Louis Casai; 1209 Genève, Switzerland. Tel: +41 (0)22-5330-948; Fax: +41(0) 22-5802-953; Email: vtomsky@paragon-conventions.com; Internet: http://www.paragon-conventions.net/IFTA2010/.

March 21-25

6th World Congress for Neurorehabilitation, Vienna, Austria. *Information:* Internet: http://www.wcnr2010.org.

May 6-8

The Power of Programming: International Conference on Developmental Origins of Health and Disease, Munich, Germany. *Information:* Melinda Széll, Ludwig-Maximilians- University of Munich, Dr. von Hauner Children's Hospital, Div. Metabolic Diseases and Nutrition Medicine, Lindwurmstr.4, D- 80337 Munich. Tel: +49 (0) 89 5160 2816; Fax: +49 (0) 89 5160 4939; Email: Melinda.Szell@med.uni-muenchen.de.

May 14-19

2010 American Thoracic Society International Conference, New Orleans, LA. *Information:* ATS International Conference Department. Tel.: 212-315-8652; Email: conference@thoracic.org; Internet: http://www.thoracic.org.

September 2-4

6th International Muscle Symposium, Vienna, Austria. *Information:* Internet: http://www.musclesymposium2010.at.

September 13-16

14th European Congress on Biotechnology, Barcelona, Spain. *Information:* Chiara Angelucci, IBS 2010 Organizing Secretariat, Adria Congrex Srl, Via Sassonia, 30, 47900 Rimini. Tel: +39 0541 305896; Fax: +39 0541 305842; Email: c.angelucci@adriacongrex.it; Internet: http://www.adriacongrex.it.

September 26-30

23rd Scientific Meeting of the International Society of Hypertension, Vancouver, Canada. *Information:* Meeting Secretariat: Sea to Sky Meeting Management Inc., Suite 206, 201 Bewicke Avenue, North Vancouver, BC Canada, V7M 3M7. Tel: 604-986-6455; Fax: 604-984-6434; Email: info@vancouverhypertension2010.com; Internet: http://www.vancouverhypertension2010.com/.

December 2-5

14th Asia-Oceania Congress of Endocrinology, Kuala Lumpur, Malaysia. Information: Congress Secretariat, Console Communications Sdn Bhd, Suite 11.8, Level 11, Wisma UOA 11, 21, Jalan Pinang, 50450 Kuala Lumpur. Tel: +603 2162 0566; Fax: +603 2161 6560; Email: aoce2010@console.com.my.

2012

September 1-6

AAPS 2012 Congress, Alexandria, Egypt. *Information:* African Association of Physiological Sciences, Office of the Secretariat, 82 Bulwer Road, Durban 4001, South Africa. Tel.: +27 31 2011392; Fax: +27 31 2013950; Internet: http://www.aapsnet.org/conferences.htm.

2013

July 21-26

37th Congress of the International Union of Physiological Sciences (IUPS 2013), Birmingham, United Kingdom. *Information:* Internet: http://www.iups2013.org/.

MEMBERSHIP APPLICATION FORM
The American Physiological Society

1. Check membership category you are applying for: ❑ Regular ❑ Affiliate ❑ Graduate Student ❑ Undergraduate Student

2. Name of Applicant: _____/_____/_____
Last Name or Family Name *First Name* *Middle Name*

3. Date of Birth _____/_____/_____ Optional: Male ❑ Female ❑
Month *Day* *Year*

4. Institution Name _____ Department _____
(Please do not abbreviate Institution Name)

5. Institution Street Address _____

6. City/State/Zip/Country _____

7. Home Address ***(Students Only)*** _____

8. Work Phone _____ Home Phone _____

9. Fax _____ E-mail _____

10. Educational Status: ▶ **IMPORTANT for STUDENTS: ** If you are enrolled as a graduate student for an advanced degree, or as an undergraduate student, please include the month and year you expect to receive your degree.**

Dates**	Degree	Institution	Major Field	Advisor

11. WHAT IS YOUR SECTION AFFILIATION? Please identify your primary sectional affiliation with a "1" and check (✓) up to two additional sections with which you would like to affiliate. **There can be only one "Primary" affiliation.**

___Cardiovascular	___Endocrinology & Metabolism	___Renal Physiology
___Cell & Molecular Physiology	___Environmental & Exercise Physiology	___Respiration Physiology
___Central Nervous System	___Gastrointestinal & Liver Physiology	___Teaching of Physiology
___Comparative & Evolutionary Physiology	___Neural Control & Autonomic Regulation	___Water & Electrolyte Homeostasis

12. DO YOU WORK IN INDUSTRY? ❑ YES ❑ NO

13. SPONSORS (Sponsors must be Regular APS Members. If you are unable to find sponsors, check the box below, and we will locate them for you.) *Undergraduate Students do not require sponsors but must supply proof of enrollment such as transcripts or letter from your advisor.*

CHECK THIS BOX IF APPLICABLE: ❑ Please locate sponsors on my behalf.

#1 Sponsor Name_____
Mailing Address_____
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Mailing Address_____
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_____
Phone_____
Fax_____
E-mail_____
Sponsor Signature*_____

**signature indicates that sponsor attests applicant is qualified for membership.*

▶ **Please turn over for more questions...and mailing instructions.**

Membership Application (Continued...)

Applicant Last Name (please print) _____

14. OCCUPATIONAL HISTORY [Check if student ❑]

Current Position:

Dates	Title	Institution	Department	Supervisor

Prior Positions:

Dates	Title	Institution	Department	Supervisor

15. LIST YOUR MOST SIGNIFICANT PUBLICATIONS, WITH EMPHASIS ON THE PAST 5 YEARS

(Publications should consist of manuscripts in peer-reviewed journals. List them in the same style as sample below.)

Sample: MacLeod RJ and Hamilton JR. Volume Regulation initiated by Na^+-nutrient contransport in isolated mammalian villus enterocytes. Am J Physiol Gastrointest Liver Physiol 280: G26-G33, 1991.

16. DOCTORAL DISSERTATION TITLE (if applicable):

_____

_____

17. POSTDOCTORAL RESEARCH TOPIC (if applicable):

_____

_____

18. WHICH FACTOR INFLUENCED YOU TO FILL OUT OUR MEMBERSHIP APPLICATION?

❑Mailer ❑Meeting (Which meeting? _____) ❑Colleague ❑Other _____

Mail your application to: Membership Services Department, The American Physiological Society
9650 Rockville Pike, Bethesda, Maryland 20814-3991 (U.S.A.)
(or fax to 301-634-7264) (or submit online at: www.the-aps.org/membership/application.htm)

Send no money now—you will receive a dues statement upon approval of membership.

Approval Deadlines: Membership applications are considered for approval on a monthly basis.

Questions? Call: 301-634-7171 • Fax: 301-634-7264 • E-mail: members@the-aps.org • Web: www.the-aps.org

The American Physiological Society

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- Organize an APS conference
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- Free print subscriptions to 3 of APS's 15 research journals ***(Physiology, Advances in Physiology Education,*** and ***The Physiologist)*** and over 50% discount on the other 12 APS print research journals
- Up to 75% discount on APS scholarly books
- 20% discount on subscription to *Annual Reviews*
- Over 30% discount on subscription to the AAAS Signal Transduction Knowledge Environment
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2009 American Physiological Society Conference

Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

Broomfield, Colorado
July 15 - 18, 2009

MEETING PROGRAM & ABSTRACTS

www.the-aps.org/interlocken

2009 APS Conference

Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

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Jane F. Reckelhoff (Chair)
Univ. of Mississippi Med. Ctr.

Christine Maric (Co-Chair)
Univ. of Mississippi Med. Ctr.

Meredith Hay
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Kathryn Sandberg
Georgetown Univ.

Virginia M. Miller
Mayo Clinic & Fdn.

Carmen Hinojosa-Laborde
Univ. of Texas Hlth. Sci. Ctr., San Antonio

J. Michael Wyss
Univ. of Alabama at Birmingham

John Stallone
Texas A&M Univ. Col. of Vet. Med.

Acknowledgements

The Conference Organizers and The American Physiological Society gratefully recognize the generous financial support provided through unrestricted educational grants from:

NIH, National Institute of Diabetes and Digestive and Kidney Diseases

2009 APS Conference

Sex and Gender in Cardiovascular-Renal Physiology and Pathophysiology

July 15 - 18, Omni Interlocken Resort, Broomfield, Colorado

Wednesday, July 15	Thursday, July 16	Friday, July 17	Saturday, July 18
3:00 PM **Registration** 6:00 – 7:50 PM **Opening Reception** 7:50 – 8:00 PM **Opening Comments:** **Jane F. Reckelhoff,** Univ. of Mississippi Med. Ctr. 8:00 – 8:40 PM **Plenary Lecture:** Participants: **Christine Maric,** (Chair) Univ. of Mississippi Med. Ctr. **Elizabeth Barrett-Connor,** Univ. of California, San Diego	7:00 AM **Registration** 8:00 - 8:40 AM Symposia I: **Sex and Sex Steroids in Renal Function** Participants: **Jane F. Reckelhoff,** (Chair) Univ. of Mississippi Med. Ctr. **Heddwen Brooks**, Univ. of Arizona **Chris Baylis,** Univ. of Florida 8:40 – 10:00 AM Oral Presentations 10:00 - 10:30 AM Break 10:30 – 11:10 AM Symposia II: **Sex Steroids and Hypertension** Participants: **Carmen Hinojosa-Laborde,** (Chair) Univ. of Texas, San Antonio **Michael J. Ryan,** Univ. of Mississippi Med. Ctr. **Daniel Ely,** Univ. of Akron 11:10 AM – 12:30 PM Oral Presentations 12:30 – 5:00 PM Lunch and Social Activities 5:00 - 6:30 PM Poster Session: **Sex Steroids: Kidney, Brain and Hypertension** 6:30 – 8:00 PM Dinner 8:00 – 9:00 PM Symposia III: **Sex Hormones and the Brain** Participants: **J. Michael Wyss,** (Chair) Univ. of Alabama at Birmingham **Louise McCullough,** (Chair) Univ. of Connecticut Hlth. Ctr. **Darrell W. Brann,** Med. Col. of Georgia **Patricia Hurn,** Oregon Hlth. Sci. Univ. **Farida Sohrabji,** Texas A&M Univ. Col. of Med.	7:00 AM **Registration** 8:00 – 8:40 AM Symposia IV: **Sex Differences in Vascular Reactivity** Participants: **John Stallone,** (Chair) Texas A&M Univ. Col. of Med. **Richard White,** Med. Col. of Georgia **Virginia Huxley,** Univ. of Missouri 8:40 - 10:00 AM Oral Presentations 10:00 - 10:30 AM Break 10:30 – 11:10 AM Symposia V: **Sex Steroids and Cardiovascular Disease** Participants: **Michael Ryan,** (Chair) Univ. of Mississippi Med. Ctr. **Art Arnold,** UCLA. **Kathryn Sandberg,** Georgetown Univ. 11:10 AM – 12:30 PM Oral Presentations 12:30 – 4:00 PM Lunch and Social Activities 4:00 – 5:00 PM **APS Career Workshop** 5:00 – 6:30 PM Poster Session: **Sex Steroids, Cardiovascular Disease and Metabolic Syndrome** 6:30 – 8:00 PM **Conference Banquet and Awards Presentations** 8:00 - 8:30 PM Symposia VI: **Sex Steroids and the Heart** Participants: **Barbara Alexander,** (Chair), Univ. of Mississippi Med. Ctr. **Jane F. Reckelhoff,** Univ. of Mississippi Med. Ctr. 8:30 PM – 10:00 PM Oral Presentations	7:30 AM **Registration** 8:00 – 8:40 AM Symposium VII: **Sex Steroids, Metabolic Syndrome and Diabetes** Participants: **Matthais Barton,** (Chair) Univ. of Zurich **Christine Maric,** Univ. of Mississippi Med. Ctr. **Maciej Tomaszewski,** Univ. of Leicester, UK 8:40 – 10:00 AM Oral Presentations 10:00 – 10:10 AM **Closing Comments:** **Jane F. Reckelhoff,** Univ. of Mississippi Med. Ctr.

Location:

The 2009 APS Conference, Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology will be held July 15 - 18, 2009 at the Omni Interlocken Resort, 500 Interlocken Blvd., Broomfield, CO 80021, telephone (303) 438-6600, FAX: (303) 438-7224.

Onsite Registration Hours:

Wednesday, July 15 …….....3:00—8:00 PM
Thursday, July 16 ……..7:00 AM—1:00 PM
Thursday, July 16 ……..... 4:30 PM— 8:00 PM
Friday, July 17……..……..7:30 AM—1:00 PM
Friday, July 17…….…….. 3:30 PM— 8:00 PM
Saturday, July 18 …….....7:30—10:00 AM

On-Site Registration Fees:

APS Member..... $450
Retired Member $375
Nonmember..... $500
Postdoctoral..... $400
Student $375

The registration fee includes entry into all scientific sessions, opening reception and dinners.

Payment Information:

Registrants may pay by institutional or personal check, traveler's check, MasterCard, VISA or American Express. Checks must be payable to *"The American Physiological Society"* and drawn on a United States bank payable in US dollars.

Student Registration:

Any student member or regularly matriculated student working toward a degree in one of the biomedical sciences is eligible to register at the student fee. Nonmember postdoctoral fellows, hospital residents and interns, and laboratory technicians do not qualify as students. Nonmember students who register onsite must provide a valid university student ID card. APS student members should present their current APS membership card indicating their student category status.

Postdoctoral Registration:

Any person who has received a Ph.D. degree in physiology or related field, within four years of this meeting, as attested to by the department head is eligible to register at the postdoctoral fee. **A statement signed by the department head must accompany the registration form and remittance when registering.**

Press:

Press badges will be issued at the APS registration desk, only to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public affairs, etc.) must register as nonmembers.

Ancillary Session:

APS Career Workshop: This special session entitled: "Writing Your First Paper: The *Ins* and *Outs* of Authorship" will be presented by Magdalena Alonso-Galicia, member of the APS Career Opportunities in Physiology Committee. Discuss the criteria for authorship and various roles authors can play during the research process and preparation and publication of a manuscript. Through case studies, explore real-life scenarios and how best to deal with the various issues that can arise with authorship.

Program Objective:

Upon completing the program, participants should gain more knowledge in the field of sex steroids and how they affect genders in the physiology of the cardiovascular-renal systems. The goal of the conference is to accumulate together a critical mass of scientists who have interests in sex steroids in physiology of the cardiovascular and renal systems to promote the exchange of ideas and potential collaborations in the future.

Target Audience:

The intended audience for this meeting includes all levels of researchers working in the field of sex steroids, particularly as it pertains to the cardiovascular and renal systems.

This meeting has been made possible through the generous support from:

NIH, National Institute of Diabetes and Digestive and Kidney Diseases

DAILY SCHEDULE

WEDNESDAY, JULY 15, 2009

Introduction

1.0 **OPENING COMMENTS**
Wednes., 7:50 - 8:00 PM, Centennial Ballroom F.

7:50 PM **1.1** Opening Comments. **Jane F. Reckelhoff.** *Univ. of Mississippi Med. Ctr.*

Plenary Lecture

2.0 **PLENARY LECTURE**
Wednes., 8:00 - 8:40 PM, Centennial Ballroom F.

Chair: **Christine Maric,** *Univ. of Mississippi Med. Ctr.*

8:00 PM **2.1** Novel Risk Factors in Cardiovascular Disease. **Elizabeth Barrett-Connor.** *Univ. of California, San Diego.*

THURSDAY, JULY 16, 2009

Symposia I

3.0 **SEX AND SEX STEROIDS IN RENAL FUNCTION**
Thurs., 8:00 - 10:00 AM, Centennial Ballroom F.

Chair: **Jane F. Reckelhoff,** *Univ. of Mississippi Med. Ctr.*

8:00 AM **3.1** Introduction. **Jane F. Reckelhoff.** *Univ. of Mississippi Med. Ctr.*

8:05 AM **3.2** Vasopressin, Steroid Hormones and Renal Function. **Heddwen L. Brooks.** *Univ. of Arizona.*

8:25 AM **3.3** Sex Differences in Susceptibility to Renal Disease: Importance of the NO System. **Chris Baylis.** *Univ. of Florida.*

8:45 AM **3.4** Cardiovascular Response to Chronic Estrogen and Angiotensin II Infusion in Aromatase Knockout Mice. **Kate Denton.** *Monash Univ., Australia.* **(5.1).**

9:00 AM **3.5** Extra-testicular Origins of Estradiol in Diabetes. **Michaele Manigrasso.** *Univ. of Mississippi Med. Ctr.* **(5.2).**

9:15 AM **3.6** Greater Ang (1-7) in the Renal Cortex of Female Compared to Male Spontaneously Hypertensive Rats. **Jennifer Sullivan.** *Med. Col. of Georgia.* **(5.3).**

9:30 AM **3.7** Renal Protein Excretion: Sex Differences in Young Mice Consuming High Protein Diets. **Al Rouch.** *Oklahoma State Univ.* **(5.4).**

9:45 AM **3.8** Beneficial Effects of Estogen Therapy on Cardiovascular and Renal Parameters in Old Normotensive Female Rats. **Lourdes Fortepiani.** *Univ. of Texas Hlth. Sci. Ctr., San Antonio.* **(5.5).**

Symposia II

4.0 **SEX STEROIDS AND HYPERTENSION**
Thurs., 10:30 AM - 12:30 PM, Centennial Ballroom F.

Chair: **Carmen Hinojosa-Laborde,** *Univ. of Texas, San Antonio.*

10:30 AM **4.1** Introduction. **Carmen Hinojosa-Laborde.** *Univ. of Texas Hlth. Sci. Ctr., San Antonio.*

10:35 AM **4.2** Does Estrogen have a Role in Systemic Lupus Erythematosus Hypertension? **Michael J. Ryan.** *Univ. of Mississippi Med. Ctr.*

10:55 AM **4.3** Testosterone Influences Renal Electrolyte Excretion in Experimental Models of Hypertension. **Daniel L. Ely.** *Univ. of Akron.*

11:15 AM **4.4** Sex Dimorphism of Systolic Blood Pressure, Proteinuria and Oxidative Stress During High Salt Intake in Angiotensin II-dependent Hypertension. **Vicky Rands.** *Tulane Univ.* **(5.7).**

11:30 AM **4.5** Sexual Dimorphism of Angiotensin II Hypertension After Blockade of the Endogenous Renin Angiotensin System. **Marcia Venegas-Pont.** *Univ. of Mississippi Med. Ctr.* **(5.8).**

11:45 AM **4.6** Effect of Female Sex Hormones on the Expression of Renal Sodium Transporters in Ovariectomized Rats. **Ki Young Na.** *Seoul Natl. Univ. Col. of Med., Korea.* **(5.9).**

12:00 Noon **4.7** Enhanced Responsiveness to Angiotensin II is Androgen Dependent in a Rodent Model of Intrauterine Growth Restriction Induced by Placental Insufficiency. **Barbara Alexander.** *Univ. of Mississippi Med. Ctr.* **(5.10).**

12:15 PM **4.8** Contrasting Roles of the Renin-angiotensin System in Maternal Uterus and Fetal Placenta in Normal and Pre-eclamptic Surgery. **K. Bridget Brosnihan.** *Wake Forest Univ.* **(5.11).**

Don't forget…..Pick up your Banquet Tickets by 10:00 AM on Thursday

The banquets are free but you MUST have a ticket for entry

Poster Session

5.0 SEX STEROIDS: KIDNEY, HYPERTENSION AND THE BRAIN

Thurs., 5:00 - 6:30 PM, Centennial Ballroom E.

Board #

1 **5.1** Cardiovascular Response to Chronic Estrogen and Angiotensin II Infusion in Aromatase Knockout Mice. **K. Denton, T-P. Nguyen-Huu, and G. Head.** *Monash Univ., and Baker IDI Heart & Diabetes Inst., Australia.*

2 **5.2** Extra-testicular Origins of Estradiol in Diabetes. **M. Manigrasso, E. D. Lephart, and C. Maric.** *Univ. of Mississippi Med. Ctr., and Brigham Young Univ.*

3 **5.3** Greater Ang (1-7) in the Renal Cortex of Female Compared to Male Spontaneously Hypertensive Rats. **J. Sullivan, and A. El-Marakby.** *Med. Col. of Georgia.*

4 **5.4** Renal Protein Excretion: Sex Differences in Young Mice Consuming High Protein Diets. **A. Rouch, T. Hanner, J. Carroll, W. Hanner, and D. Johnson.** *Oklahoma State Univ.*

5 **5.5** Beneficial Effects of Estrogen Therapy on Cardiovascular and Renal Parameters in Old *Normotensive* Female Rats. **L. Fortepiani.** *Univ. of Texas Hlth. Sci. Center, San Antonio.*

6 **5.6** Sex Dependent Differences of Renin Gene Expression in Distal Nephron Segments During High Salt Intake in Chronic Angiotensin II-infused Hypertensive Rats with Endogenous Renin Angiotensin System (RAS) Blockade with Losartan. **M. Prieto, V. Rands, K. L. Kavanagh, B. M. Lowenburg, and V. Martin.** *Tulane Univ.*

7 **5.7** Sex Dimorphism of Systolic Blood Pressure, Proteinuria and Oxidative Stress During High Salt Intake in Angiotensin II-dependent Hypertension. **V. Rands, S. Shenouda, B. M. Lowenburg, J. A. Planchard, V. L. Martin, and M. C. Prieto.** *Tulane Univ. and Louisiana State Univ. Hlth. Sci. Ctr.*

8 **5.8** Sexual Dimorphism of Angiotensin II Hypertension After Blockade of the Endogenous Renin Angiotensin System. **M. Venegas-Pont, J. Sartori-Valinotti, P. Glover, J. Reckelhoff, and M. Ryan.** *Univ. of Mississippi Med. Ctr.*

Board #

9 **5.9** Effect of Female Sex Hormones on the Expression of Renal Sodium Transporters in Ovariectomized Rats. **K. Y. Na, K. W. Joo, N. J. Heo, and J. S. Han.** *Seoul Natl. Univ. Col. of Med., Korea.*

10 **5.10** Enhanced Responsiveness to Angiotensin II is Androgen Dependent in a Rodent Model of Intrauterine Growth Restriction Induced by Placental Insufficiency. **B. Alexander, T. P. Royals, and N. B. Ojeda.** *Univ. of Mississippi Med. Ctr.*

11 **5.11** Contrasting Roles of the Renin-angiotensin System in Maternal Uterus and Fetal Placenta in Normal and Pre-eclamptic Surgery. **K. B. Brosnihan, L. Anton, and D. C. Merrill.** *Wake Forest Univ. Hlth. Sci.*

12 **5.12** Differential Effect of Conjugated Equine Estrogens and Benzoate of Estradiol on Blood Pressure and Natriuretic Peptide System in Female Rats. **A. dos Reis, L. Firmes, and N. Belo.** *Univ. Fed. de Minas Gerais, Belo Horizonte, Brazil.*

13 **5.13** Estrogen and the Development of Hypertension in the Female Spontaneously Hypertensive Rats. **L. Mattar, E. Noble, and J. K. Shoemaker.** *Univ. of Western Ontario.*

14 **5.14** Sex Steroids, Hypertension and Menopause: Positive Effects of Angiotensin Converting Enzyme Inhibition on Both the Aorta and Renal Nitric Oxide System. **J. Zilberman, M. Romero, R. Elesgaray, A. Costa, and C. Arranz.** *Univ. of Buenos Aires, Argentina.*

15 **5.15** Androgens Increase Blood Pressure by Upregulating the Intrarenin-angiotensin and Endothelin Systems in Female SD Rats. **L. Yanes, J. Sartori-Valinotti, D. Romero, H. Zhang, D. Davis, and J. Reckelhoff.** *Univ. of Mississippi Med. Ctr.*

16 **5.16** 17β-Estradiol Attenuates Pro-apoptotic p53/PUMA Signaling Pathway in Hippocampus CA1 Following Global Ischemia. **L. Raz, Q. G. Zhang, and D. Brann.** *Med. Col. of Georgia.*

17 **5.17** Cutaneous Adrenergic Responses to Estradiol in Women with High and Low Orthostatic Tolerance. **M. Wenner, H. S. Taylor, and N. Stachenfeld.** *Yale Univ. Sch. of Med.*

18 **5.18** The Protective Effect of Central Nitric Oxide Against Aldosterone/NaCl-

Board #

induced Hypertension in Female Rats. **B. Xue, T. G. Beltz, F. Guo, M. Hay, and A. K. Johnson.** *Univ. of Iowa and Univ. of Arizona.*

19 **5.19** Circadian Suprachiasmatic Nuclei and Renal Gene Expressions in Peri-menopausal Female C57Bl/J6 Mice. **C. Chaperon, N. Rasmussen, and P. Lane.** *Univ. of Nebraska Med. Ctr. and NINR, NIH.*

19A **5.20** Dietary Equol (4', 7 Isoflavandiol) Reduces Oxidative Stress and Protects Rats Against Focal Cerebral Ischemia. **Y. Ma, J. Sullivan, and D. A. Schreihofer.** *Med. Col. of Georgia.*

Don't forget to attend the Friday Evening Banquet and Awards Presentation

Get your ticket at the Registration Desk

Symposia III

6.0 SEX HORMONES AND THE BRAIN

Thurs., 8:00 - 9:10 PM Centennial Ballroom F.

Co-Chairs: **J. Michael Wyss,** *Univ. of Alabama at Birmingham.*

Louise McCullough, *Univ. of Connecticut Hlth. Ctr.*

8:00 PM **6.1** Introduction. **J. Michael Wyss.** *Univ. of Alabama at Birmingham.*

8:05 PM **6.2** Long-term Estrogen Deprivation Leads to Loss of Sensitivity in the Brain. **Darrell W. Brann.** *Med. Col. of Georgia.*

8:25 PM **6.3** Sex Differences in Experimental Stroke: The Underpinning for Steroid Hormonal Modulation. **Patricia D. Hurn.** *Oregon Hlth. Sci. Univ.*

8:45 PM **6.4** The Aging Blood Brain Barrier: Implications for Stroke and Repair in Acyclic Females. **Farida Sohrabji.** *Texas A&M Univ. Col. of Med.*

FRIDAY, JULY 17, 2009

Symposia IV

7.0 SEX DIFFERENCES IN VASCULAR REACTIVITY

Fri., 8:00 - 10:00 AM, Centennial Ballroom F.

Chair: **John N. Stallone,** *Texas A&M Univ. Col. of Med.*

8:00 AM **7.1** Introduction. **John N. Stallone.** *Texas A&M Univ. Col. Of Med.*

8:05 AM **7.2** NOS Uncoupling and Oxidative Stress: Does Aging Alter how we Respond to Estrogen? **Richard E. White.** *Med. Col. of Georgia.*

8:25 AM **7.3** Role for Angiopoietin-1 in Estrogen-dependent Control of Vascular Stability. **Virginia H. Huxley.** *Univ. of Missouri.*

8:45 AM **7.4** Release of EETs in Response to Shear Stress in Arteries of Female NO Deficient Rats. **Dong Sun.** *New York Med. Col.* **(9.1).**

9:00 AM **7.5** Sex-differences in Endothelin-1 Mediated Vasoconstrictor Tone in Middle-aged Adults. **Brian Stauffer.** *Univ. of Colorado, Denver.* **(9.2).**

9:15 AM **7.6** Estrogen Receptor Erα and Erβ Activation Exert Differential Effects on Mesenteric Vascular Responsiveness to Vasopressin in Normotensive and Hyper-tensive Female Rats. **Minga Sellers.** *Texas A&M Univ. Col. Of Med.* **(9.3).**

9:30 AM **7.7** Gender Specific Role of Tyrosine Kinase Receptor Transactivation in Wistar Rats. **John Passmore.** *Univ. of Louisville Hlth. Sci. Ctr.* **(9.4).**

9:45 AM **7.8** Effect of Orchidectomy on Vascular Relaxation Responses to cAMP and Potassium Channel Activation in Sprague-Dawley Rats Fed a High Salt Diet. **Ahmed Oloyo.** *Univ. of Lagos, Nigeria.* **(9.5).**

Symposia V

8.0 SEX STEROIDS AND CARDIOVASCULAR DISEASE

Fri., 10:30 - 12:30 PM, Centennial Ballroom F.

Chair: **Michael J. Ryan,** *Univ. of Mississippi Med. Ctr.*

10:30 AM **8.1** Introduction. **Michael J. Ryan.** *Univ. of Mississippi Med. Ctr.*

10:35 AM **8.2** Mouse Models for Studying Sex Differences in Physiology and Disease. **Art Arnold.** *UCLA.*

10:55 AM **8.3** Sex Chromosome Dosage in Blood Pressure Control. **Kathryn Sandberg.** *Georgetown Univ.*

11:15 AM **8.4** Estrogen Enhances SERCA Expression via Protein Kinase G in Coronary Arteries. **Brent Hill.** *Univ. of Central Arkansas.* **(9.8).**

11:30 AM **8.5** Ovariectomy but not Orchiectomy Reduces Large-conductance Ca^{2+}-activated K^+ Channel Activity in Porcine Coronary Smooth Muscle. **Darla Tharp.** *Univ. of Missouri, Columbia.* **(9.9).**

11:45 AM **8.6** Early Gestation Exposure to Excess Testosterone Reduces Cardiomyocyte Proliferation and Maturation in Near-term Female Fetal Sheep. **Sonnet Jonker.** *Oregon Hlth. & Sci. Univ., Heart Res. Ctr.* **(9.11).**

12:00 Noon **8.7** Dihydrotestosterone Alters Endotoxin and Cytokine Induced Increases in Cyclooxygenase-2 in Human Coronary Artery Smooth Muscle Cells but has no Ef-fect on Human Brain Microvascular Endo-thelial Cells. **Kristen Osterlund.** *Univ. of Arizona Col. of Med.* **(9.12).**

12:15 PM **8.8** Effect of Age and Estrogen Loss on Estrogen Receptor Alpha and Beta in Hearts of Dahl Salt Sensitive Rats. **Kristi Pogue.** *Univ. of Texas Hlth. Sci. Ctr., San Antonio.* **(9.13).**

Career Workshop

WRITING YOUR FIRST PAPERS: THE *INS* AND *OUTS* OF AUTHORSHIP

Fri., 4:00 - 5:00 PM, Centennial Ballroom F.

A special workshop presented by the APS Career Opportunities in Physiology Committee.

Poster Session

9.0 SEX STEROIDS: CARDIOVASCULAR SYSTEM AND METABOLIC SYNDROME

Fri., 5:00 - 6:30 PM, Centennial Ballroom E.

Board #

20 **9.1** Release of EETs in Response to Shear Stress in Arteries of Female NO Deficient Rats. **D. Sun, G. Kaley, and A. Huang.** *New York Med. Col.*

21 **9.2** Sex-differences in Endothelin-1 Mediated Vasoconstrictor Tone in Middle-aged Adults. **B. Stauffer, C. Westby, G. Van Guilder, J. Greiner, and C. DeSouza.** *Univ. of Colorado, Denver.*

22 **9.3** Estrogen Receptor Erα and Erβ Activation Exert Differential Effects on Mesenteric Vascular Responsiveness to Vasopressin in Normotensive and Hypertensive Female Rats. **M. Sellers, F. Xu, and J. Stallone.** *Texas A&M Univ. Col. Of Med.*

23 **9.4** Gender Specific Role of Tyrosine Kinase Receptor Transactivation in Wistar Rats. **J. Passmore, J. Fleming, and J. Falcone.** *Univ. of Louisville Hlth. Sci. Ctr.*

Board #

24 **9.5** Effect of Orchidectomy on Vascular Relaxation Responses to cAMP and Potassium Channel Activation in Sprague-Dawley Rats Fed a High Salt Diet. **A. Oloyo, O. Sofola, R. Nair, V. S. Harikrishnam, and A. Fernandez.** *Univ. of Lagos, Nigeria and Sree Chitra Tirunal Inst. for Med. Sci. & Tech., Trivandrum, India.*

25 **9.6** Osteopenia is Associated with Increased Endothelin-1 Vasoconstrictor Tone in Postmenopausal Women. **M. Mestek, B. Weil, C. Westby, G. Van Guilder, J. Greiner, B. Stauffer, and C. DeSouza.** *Univ. of Colorado, Boulder.*

26 **9.7** CYP2C29 and RXRg in the Regulation of EET-mediated Flow-induced Dilation of Female Mice. **A. Huang, D. Sun, and G. Kaley.** *New York Med. Col.*

27 **9.8** Estrogen Enhances SERCA Expression via Protein Kinase G in Coronary Arteries. **B. Hill, E. Muldrew, and A. Reed.** *Univ. of Central Arkansas.*

28 **9.9** Ovariectomy but not Orchiectomy Reduces Large-conductance Ca^{2+}-activated K^+ Channel Activity in Porcine Coronary Smooth Muscle. **D. Tharp, J. Ivey, R. Shaw, V. Ganjam, and D. Bowles.** *Univ. of Missouri, Columbia.*

29 **9.10** Ovariectomy Enhances L-type Ca^{2+} Channel Activity in Porcine Coronary Smooth Muscle. **D. Tharp, J. Ivey, R. Shaw, V. Gamjam, and D. Bowles.** *Univ. of Missouri, Columbia.*

30 **9.11** Early Gestation Exposure to Excess Testosterone Reduces Cardiomyocyte Proliferation and Maturation in Near-term Female Fetal Sheep. **S. Jonker, and C. Roselli.** *Oregon Hlth. & Sci. Univ., Heart Res. Ctr.*

31 **9.12** Dihydrotestosterone Alters Endotoxin and Cytokine Induced Increases in Cyclooxygenase-2 in Human Coronary Artery Smooth Muscle Cells but has no Effect on Human Brain Microvascular Endothelial Cells. **K. Osterlund, A. Gutierrez, R. Handa, and R. Gonzales.** *Univ. of Arizona Col. of Med.*

32 **9.13** Effect of Age and Estrogen Loss on Estrogen Receptor Alpha and Beta in Hearts of Dahl Salt Sensitive Rats. **K. Pogue, L. Fortepiani, and C. Hinojosa-Laborde.** *Univ. of Texas Hlth. Sci. Ctr., San Antonio.*

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33 **9.14** GRP30 Receptor Activation Improves Cardiac Function in Intact Female mRen2 Lewis Rats. **J. Jessup, S. Lindsey, M. Chappell, and L. Groban.** *Wake Forest Univ.*

34 **9.15** Improved TIMP-1/MMP-9 and TIMP-2/MMP-2 Balance in Volume Overloaded Hearts of Ovariectomized Rats After Estrogen Replacement. **T. Voloshenyuk, and J. Gardner.** *Louisiana State Univ. Hlth. Sci. Ctr.*

35 **9.16** Testosterone Replacement Alters Coronary Vascular Function in Male Yucatan Miniature Swine. **C. Emter, J. Ivey, D. Tharp, and D. Bowles.** *Univ. of Missouri, Columbia.*

36 **9.17** Sex-specific Effect of Estogen Receptor β on the Transition from Cardiac Hypertrophy to Heart Failure. **D. Fliegner, C. Schubert, A. Penkalla, C. Westphal, U. Kintscher, J. A. Gustafsson, and V. Regitz-Zagrosek.** *Charite Univ., Germany and Karolinska Inst., Sweden.*

37 **9.18** Gender-specific Changes in Gene Expression Profiles During Acute Severe Mg Deficiency in the Rat Heart. **M. I. Tejero-Taldo, and W. Weglicki.** *George Washington Univ.*

38 **9.19** Female Gender Protects Against Oxidative Stress and Impaired Insulin-stimulated Glucose Uptake in Skeletal Muscle in TGR (Mren-2) 27 Rats. **C. Manrique, G. Lastra, L. Appesh, and J. Sowers.** *Univ. of Missouri, Columbia.*

39 **9.20** Impact of Aging and Abdominal Obesity in the control of Hypertension in Women. **J. Zilberman, N. Vainstein, G. Cerezo, A. Vicario, M. Carasa, and M. Del Sueldo.** *Argentine Fed. of Cardiology and Clinica Especialidades, Buenos Aires, Argentina.*

40 **9.21** Role of Gender in Insulin Resistance and Myocardial Oxidative Stress in the TG (mRen2)27 Rat. **R. Schneider, M. S. Johnson, J. Habibi, A. T. Whaley-Connell, and J. R. Sowers.** *Univ. of Missouri, Columbia.*

41 **9.22** Androgen Promotes Angiotensin II Induced Abdominal Aortic Aneurysms in Female Hyperlipidemic Mice Through Vascular Smooth Muscle Atla Receptors. **X. Zhang, A. Daugherty, and L. Cassis.** *Univ. of Kentucky.*

Board #

42 **9.23** Gender Differences in Aortic Endothelial Function in a Rat Model of Type 1 Diabetes: Possible Role of Super-oxide and Cyclooxygenase. **A. Kekatpure, L. Anderson, and R. Rahimian.** *Univ. of Pacific.*

Symposia VI

10.0 **SEX STEROIDS AND THE HEART**

Fri., 8:00 - 10:00 PM, Centennial Ballroom F.

Chair: **Barbara Alexander,** *Univ. of Mississippi Med. Ctr.*

8:00 PM **10.1** Hormone Replacement Therapy: What's New Since WHI? **Jane F. Reckelhoff.** *Univ. of Mississippi Med. Ctr.*

8:30 PM **10.2** GRP30 Receptor Activation Improves Cardiac Function in Intact Female mRen2 Lewis Rats. **Jewell Jessup.** *Wake Forest Univ.* **(9.14).**

8:45 PM **10.3** Improved TIMP-1/MMP-9 and TIMP-2/MMP-2 Balance in Volume Overloaded Hearts of Ovariectomized Rats after Estrogen Replacement. **Tetyana Voloshenyuk.** *Louisiana State Univ. Hlth. Sci. Ctr.* **(9.15).**

9:00 PM **10.4** CYP2C29 and RXRg in the Regulation of EET-mediated Flow-induced Dilation of Female Mice. **An Huang.** *New York Med. Coll.* **(9.7).**

9:15 PM **10.5** Sex-specific Effect of Estogen Receptor β on the Transition from Cardiac Hypertrophy to Heart Failure. **Vera Regitz-Zagrosek.** *Charite Univ., Germany.* **(9.17).**

9:30 PM **10.6** Gender-specific Changes in Gene Expression Profiles During Acute Severe Mg Deficiency in the Rat Heart. **M. Isabel Tejero-Taldo.** *George Washington Univ.* **(9.18).**

SATURDAY, JULY 18, 2009

Symposia VII

11.0 **SEX STEROIDS, METABOLIC SYNDROME AND DIABETES**

Sat., 8:00 - 10:00 AM, Centennial Ballroom F.

Chair: **Matthais Barton**, *Univ. Hosp. of Zurich, Switzerland.*

8:00 AM **11.1** Introduction. **Matthais Barton.** *Univ. Hosp. of Zurich, Switzerland.*

8:05 AM **11.2** Sex Steroids, Diabetes and Diabetic End-organ Complications. **Christine Maric.** *Univ. of Mississippi Med. Ctr.*

8:25 AM **11.3** Estrogens, Androgens and Metabolic Risk in Men. **Maciej Tomaszewski.** *Univ. of Leicester, UK.*

8:45 AM **11.4** Female Gender Protects Against Oxidative Stress and Impaired Insulin-stimulated Glucose Uptake in Skeletal Muscle in TGR (Mren-2) 27 Rats. **Camila Manrique.** *Univ. of Missouri, Columbia.* **(9.19).**

9:00 AM **11.5** Impact of Aging and Abdominal Obesity in the Control of Hypertension in Women. **Judith Zilberman.** *Argentine Fed. of Cardiology, Buenos Aires, Argentina.* **(9.20).**

9:15 AM **11.6** Role of Gender in Insulin Resistance and Myocardial Oxidative Stress in the TG (mRen2)27 Rat. **Rebecca Schneider.** *Univ. of Missouri, Columbia.* **(9.21).**

9:30 AM **11.7** Androgen Promotes Angiotensin II Induced Abdominal Aortic Aneurysms in Female Hyperlipidemic Mice Through Vascular Smooth Muscle Atla Receptors. **Xuan Zhang.** *Univ. of Kentucky.* **(9.22).**

9:45 AM **11.8** Gender Differences in Aortic Endothelial Function in a Rat Model of Type 1 Diabetes: Possible Role of Super-oxide and Cyclooxygenase. **Avantika Kekatpure.** *Univ. of the Pacific.* **(9.23).**

Closing

12.0 **CLOSING COMMENTS**

Sat., 10:00 - 10:10 AM, Centennial Ballroom F.

Chair: **Jane F. Reckelhoff,** *Univ. of Mississippi Med. Ctr.*

This meeting has been made possible through the generous support from:

NIH, National Institute of Diabetes and Digestive and Kidney Diseases

NOTES

2009 APS Conference
Sex Steroids and Gender in Cardiovascular-Renal Physiology and Pathophysiology

Abstracts of Invited and Contributed Presentations

2.0: PLENARY LECTURE

2.1

NOVEL RISK FACTORS FOR CARDIOVASCULAR DISEASE

Elizabeth Barrett-Connor[1]
[1]Family & Preventive Medicine, UCSD, 9500 Gilman Dr., MC 0607, La Jolla, CA, 92093-0607.

Novel risk factors should improve the diagnosis of CVD or the prediction of future risk, or differential diagnosis, or allow earlier intervention, giving added value to classical CVD risk factors. Ideally they are cheap, fast, and reliable. Although C-Reactive Protein (CRP) is a nonspecific inflammatory marker, it has the distinction of having been shown to identify patients at otherwise low CVD risk and to improve prognosis when reduced by statin therapy. Other old (WBC and fibrinogen) and new (IL-6) inflammatory markers may be equally good but are less well studied. Lipoprotein-associated phospholipase (Lp-PLA2) is thought to reduce inflam-mation and oxidized phospholipids; blood levels correlate with classic lipoprotein risk factors and improve the prediction of CVD. Very low levels of troponin are associated with subclincal myocardial ischemia and predict future CVD events. The risk of CVD is greatly increased if both troponin and NTproBNP, a marker for heart failure, are increased. In a recent analysis of 50 putative biomarkers, the combination of troponin, elevated BNP, and CRP was the strongest predictor of CVD. The interleukin ST2 is a marker for myocardial stretch and for heart failure. It is used to differentiate heart failure from other causes of shortness of breath. The hepatic protein fetuin A is related to calcium, phosphorus, insulin resistance, and diabetes. Levels are inversely associated with vascular calcification and renal disease. Support: NIH/NIA AG07181 & AG028507 & NIDDKD DK31801. Daniels, L.B. 2009. Multiple biomarker assessment in primary prevention of cardiovascular disease. *Curr Cardiovasc Risk Rept* 3:131-136.

3.0: SEX AND SEX STEROIDS IN RENAL FUNCTION

3.2

VASOPRESSIN, STEROID HORMONES AND RENAL FUNCTION

Heddwen Brooks[1], Maggie Diamond-Stanic[1], Jill Romero-Aleshire[1], Patricia Hoyer[1]
[1]Physiology, University of Arizona, 1656 E Mabel St/Rm 417, Tucson, AZ, 85724.

Perimenopause is increasingly recognized as a critical period in the development and treatment of many diseases. The 4-vinylcyclohexene diepoxide model of menopause progresses gradually through perimenopause to post-menopause and preserves the postmenopausal ovarian production of androgens. Using this model with a type I model of diabetes (STZ) we demonstrated that changes in renal structure and function associated with diabetic kidney disease occurred more rapidly in the post-menopause kidney than in cycling controls. Gene array studies were used to identify approximately 80 genes whose expression was altered by menopause in diabetic kidneys but not in non-diabetic kidneys. Previous gene array studies had identified 3βHSD4 as a novel target of vasopressin regulation in normal and diabetic kidneys. Abundance of 3βHSD4 was significantly lower in the renal cortex of post-ovarian failure diabetic (Meno/STZ) mice than in cycling diabetic mice (STZ). Protein expression of Midkine (Mdk), a heparin-binding growth factor, was significantly increased in renal cortex of Meno/STZ mice compared to STZ mice after 8 weeks of diabetes (Meno/STZ:176±13 vs STZ:100±9%,P<0.05). After 12 weeks protein expression of IEX-1, an immediate early response gene involved in proliferation was significantly increased in the renal cortex of Meno/STZ mice compared to STZ mice (Meno/STZ:146±11 vs STZ:100±11%,P<0.05). 24 hour urine albumin excretion (UAE) was significantly elevated in Meno/STZ mice compared to STZ mice after 5 weeks of diabetes (Meno/STZ: 222±44 vs STZ:122±11 µg/day,P<0.05). Glomerular area was significantly increased in Meno/STZ compared to STZ mice after 8 weeks of diabetes (Meno/STZ:3921±75 vs STZ:3721±76,P<0.05). Changes in ovarian hormone production that occur at menopause exacerbate the development of diabetic kidney disease and highlight the importance and utility of the VCD model of menopause, as it provides a physiologically relevant system for determining the impact of diabetes on the kidney during the menopausal transition. **References:** Hormonal status affects the progression of STZ-induced diabetes and diabetic renal damage in the VCD mouse model of menopause. Keck M, Romero-Aleshire MJ, Cai Q, Hoyer PB, Brooks HL. Am J Physiol Renal Physiol. 2007 Jul;293(1):F193-9. Effects of water restriction on gene expression in mouse renal medulla: identification of 3betaHSD4 as a collecting duct protein. Cai Q, Keck M, McReynolds MR, Klein JD, Greer K, Sharma K, Hoying JB, Sands JM, Brooks HL. Am J Physiol Renal Physiol. 2006 Jul;291(1):F218-24. The follicle-deplete mouse ovary produces androgen. Mayer LP, Devine PJ, Dyer CA, Hoyer PB. Biol Reprod. 2004 Jul;71(1):130-8.

3.3

SEX DIFFERENCES IN SUSCEPTIBILITY TO RENAL DISEASE: IMPORTANCE OF THE NO SYSTEM

Chris Baylis[1]
[1]Dept Physiol and Med, Univ Florida, Gainesville, FL.

Animal and clinical studies suggest that that renal disease progresses at a slower rate in the female vs. male. Aging results in a slowly evolving renal disease with falls in GFR due to renal vasoconstriction and structural damage and there is a sexual dimorphism in kidney aging with females being protected. Pre-menopausal women produce more NO than men and total NO production falls with age. NO is a major factor in regulation of vascular tone, growth and structural integrity and becomes deficient in the cardiovascular system with advancing age, as endothelial dysfunction develops. Although the substrate, L-arginine is maintained, the concentration of the circulating endogenous nitric oxide synthase (nNOS) inhibitor ADMA increases with age; this is delayed in women. There are also likely to be falls in the activity of the endothelial nitric oxide synthase (eNOS) due to the cumulative oxidative stress of aging. Within the kidney, declines in abundance and activity of the neuronal (nNOS) correlate with development of kidney disease in the male rat, whereas in the protected female, renal nNOS abundance is maintained. Some of the sex difference reflects the cardio- and renoprotective effects of estrogens, but estrogen has multiple actions, not all of which are beneficial. Androgens also have multiple actions and while much animal data suggests that a net damaging action, the clinical data is more positive. Deficiency of eNOS is associated with increased cardiovascular risk and intrarenal NO deficiency is linked with progression of chronic kidney disease in animal models. Baylis C. Nature Reviews Nephrol. In press.

4.0: SEX STEROIDS AND HYPERTENSION

4.3

TESTOSTERONE INFLUENCES RENAL ELECTROLYTE EXCRETION IN EXPERIMENTAL MODELS OF HYPERTENSION

Dan Ely[1], Jonathan Toot[1]
[1]Biology, University of Akron, 185 E. Mill St., Akron, OH, 44325.

Our laboratory has focused on the influence of testosterone (T) and the Y chromosome(Yc) on blood pressure (BP) and sodium excretion. We developed a Yc consomic strain that is genetically identical to a normotensive WKY male except the Yc is derived from a SHR (SHR/y male) hypertensive father and they have higher BP. Castration of SHR/y and WKY resulted in an increase in Na excretion (118%, 68% respectively). T replacement normalized Na excretion in both strains. AR blockade with flutamide increased Na excretion by 24% in WKY and 62% in SHR/y. The SHR/y male has a 2 week earlier rise in plasma T during development compared to that of WKY. The Yc gene responsible for the elevated BP in SHR/y males is Sry, a transcription factor expressed in testis, brain, heart adrenal glands and kidney. This latter finding prompted the question of whether exogenous Sry1 injected into the kidney would elevate BP and change Na excretion. Exogenous Sry1 increased BP but did not elevate Na excretion, however urinary dopamine increased 36% and plasma NE 57%, and renal tyrosine hydroxylase 47%. In conclusion, T conserves renal Na and Sry may function to regulate T and renal catecholamines (NHLBI RO1-HL71579-01A3).REFERENCES: Turner M, et al. Which SRY Locus is the Hypertensive Y Chromosome Locus? Hypertension, 2009,53{part 2}:430-435.;Toot J, et al. Testosterone influences renal electrolyte excretion in SHR/y and WKY males. BMC Physiology 2008, 8:5. Different Sry copies are expressed in many tissues and testosterone influences Na excretion.

5.0: SEX STEROIDS: KIDNEY, HYPERTENSION AND THE BRAIN

5.1

CARDIOVASCULAR RESPONSE TO CHRONIC ESTROGEN AND ANGIOTENSIN II INFUSION IN AROMATASE KNOCKOUT MICE

Kate Denton[1], Thu-Phuc Nguyen-Huu[2], Geoff Head[2]
[1]Physiology, Monash Univ., Bldg. 13F, Australia, [2]Neuropharmacology, Baker IDI Heart & Diabetes Inst., 75 Commercial Rd., Melbourne, 3004, Australia.

Low-dose angiotensin II (AngII) decreases arterial pressure in female rats, suggesting an estrogen/renin-angiotensin interaction. To confirm the role of estrogen, we investigated the cardiovascular response to AngII in the estrogen deficient aromatase knockout (ArKO) mouse. Four-month old wildtype (WT; n=10) and ArKO (n=29) female mice were implanted with telemetry devices. After recovery, all WT mice received placebo, whereas ArKO mice were allocated into four groups; placebo (ArKO; n=12), low-dose 17beta-estradiol (E2) (ArKO+LE2; n=6), mid-dose E2 (ArKO+ME2; n=5), or high-dose E2 (ArKO+HE2; n=6). After 2 weeks of E2, AngII (200 ng/kg/min s.c.) was infused for 1 week. Mean arterial pressure (MAP) was greater in the ArKO placebo as compared to the WT placebo treated mice (5mmHg; P<0.04). No dose of E2 reduced MAP in ArKO mice. AngII infusion did not influence BP in WT, but increased MAP in placebo-treated ArKO mice (+4±2 mmHg; P < 0.01), ArKO+ME2 mice (+11±4 mmHg; P<0.001) and ArKO+HE2 mice (+5±3 mmHg; P<0.02). However, MAP decreased in ArKO+LE2 mice (-3±2 mmHg; P<0.02). These studies suggest that a lack of estrogen and surprisingly, higher doses of estrogen renders mice susceptible to the pressor effects of AngII. In contrast, only the low-dose E2 was found to attenuate these pressor effects, mimicking what we have observed in female rats. In conclusion, our findings support the view that the depressor effects of low-dose AngII are likely to be due to estrogen (Funding NHMRC#490919).

5.2

EXTRA-TESTICULAR ORIGINS OF ESTRADIOL IN DIABETES

Michaele Manigrasso[1], Edwin D. Lephart[2], Christine Maric[1]
[1]Physiology and Biophysics, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS, 39216, [2]Physiology & Developmental Biology, Brigham Young University, 785 WIDB, Provo, UT, 84602.

Our previous study has shown that type 1 diabetes in males is associated with reduced circulating testosterone but increased estradiol levels, an effect that persisted even after castration, suggesting extra-testicular origins of estradiol in diabetes. The present study examined the tissue source of estradiol and the activity of aromatase, enzyme necessary for its production in male Sprague-Dawley non-diabetic and streptozotocin (STZ)-induced diabetic rats after 90 days of diabetes. Diabetes was associated a decrease in aromatase activity (ND, 26.3±4.2; D, 18.4±1.5 fmol/hr incubation/mg protein) compared with non-diabetics, suggesting that increased estradiol levels in diabetes are not due to increased testicular production. No differences in aromatase activity in the adrenal gland were observed. Surprisingly, the diabetic kidney (ND, 1.8±0.92; D, 7.9±1.4 ROD) and eye (ND, 23.0±3.7; D, 87.0±28.0 ROD) both showed increased aromatase activity compared with non-diabetics. These findings suggest that increased circulating estradiol levels in diabetes may originate from the kidneys and eyes. Indeed, the diabetic kidney is associated with increased tissue levels of estradiol (ND, 313±14.0; D, 512±111 pg/mg protein). Our preliminary data also show that increased estradiol levels in diabetes, at least in males, may have adverse effects on the kidney. Thus, inhibiting aromatase activity may protect from end-organ damage associated with diabetes.

5.3

GREATER ANG (1-7) IN THE RENAL CORTEX OF FEMALE COMPARED TO MALE SPONTANEOUSLY HYPERTENSIVE RATS

Jennifer Sullivan[1], Ahmed El-Marakby[2]
[1]Vascular Biology Center, Medical College of Georgia, 1459 Laney Walker Blvd, Augusta, GA, 30912, [2]Oral Biology, Medical College of Georgia, 1120 15th St. CL2126, Augusta, GA, 30912.

We previously published that renal cortical Ang II levels are similar in male and female SHR. This was surprising since Ang II contributes to hypertension in SHR and females have a lower blood pressure compared to males. We hypothesized that the discrepancy in MAP and Ang II levels was due to (1) lower AT1 receptor activity in female SHR, or (2) greater Ang (1-7) in female SHR. 12 week old male and female SHR were treated with the AT1 receptor antagonist telmisartan (10 mg/kg/day) for 10 days and mean arterial pressure (MAP) was measured by telemetry. Prior to starting telmisartan treatment, male SHR had a greater MAP compared to females (149±3 and 134±3 mmHg, respectively, $p<0.05$). After telmisartan treatment, MAP was significantly decreased in both male (96±2 mmHg) and female SHR (85±1 mmHg) and the decrease in MAP was comparable between the sexes, indicating that AT1 receptor activity was comparable. We next measured ACE2 and neprilysin levels by real-time RT-PCR and Ang (1-7) levels by EIA in the renal cortex of male and female SHR. Although ACE2 and neprilysin mRNA levels were similar in the renal cortex of male and female SHR, Ang (1-7) levels were significantly greater in the renal cortex of female SHR compared to males (333±66 and 192±62 ng/g cortex, respectively, $p<0.05$). In conclusion, greater Ang (1-7) production in the renal cortex and not differential AT1 receptor activity likely contributes to lower levels of MAP in female SHR despite comparable Ang II levels.

5.4

RENAL PROTEIN EXCRETION: SEX DIFFERENCES IN YOUNG MICE CONSUMING HIGH PROTEIN DIETS

Al Rouch[1], Tess Hanner[2], Joe Carroll[2], Will Hanner[2], Derek Johnson[2]
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We measured protein excretion (PE) in young male and female mice on high protein diets. The first study compared PE in mice consuming either 40% soy protein or 40% casein for 25 days. All mice consumed the same amount of protein. PE (mg/day) on the 25th day from the casein group exceeded PE of the soy group in both sexes (males: 22.9±2.5 vs 12.7±.9, $p<.009$; females: 10.2±1.7 vs 5.4±0.8, $p<.03$). PE was lower for females. Thus the protein source and gender matter. The second study compared PE in normal and castrated males (NM, CM) and normal and ovariectomized females (NF, OF) (n=6 each group). Mice consumed 40% casein for 25 days. PE for males: (Day 2: NM = 5.5±0.8 vs CM = 4.2±0.7 NS); (Day 25: NM = 31.0±0.8 vs CM = 5.9±0.9 $p<0.0001$). PE for females: (Day 2: NF = 3.8±0.7 vs OF = 2.5±1.0 NS); (Day 25: NF = 8.3±0.9 vs OF = 5.6±0.9; $p<0.02$). Real-time quantitative PCR data on kidney cortex showed higher expression of estrogen receptor alpha (ERα), androgen receptor (AR), and transforming growth factor beta (TGFβ) in NF compared to OF whereas no differences existed between NM and CM. Kidney weight/body weight ratios determined on day 25 were: NM>NF>OF=CM. PE data suggest that the presence of androgen, not the absence of estrogen, promotes high PE. Androgen appears to be required for dietary-protein-induced kidney hypertrophy. Data suggest sex differences in ERα, AR, and TGFβ expression, but correlation with PE requires further studies.

5.5

BENEFICIAL EFFECTS OF ESTROGEN THERAPY ON CARDIO-VASCULAR AND RENAL PARAMETERS IN OLD "NORMOTENSIVE" FEMALE RATS

Lourdes Fortepiani[1]
[1]Physiology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX, 78229.

Decreases in estrogens have been associated with increased cardiovascular (CV) and renal risk during aging. However, clinical trials and animal studies have documented increased CV disease with hormone replacement therapy after menopause. This study tested the hypothesis that long-term estrogen supplementation (ES) does not have detrimental effects on CV and renal parameters in aged rats when they are normotensive. Twelve month old female Wistar rats were implanted with silastic pellets, empty or containing 5mg of 17 beta estradiol and aged for 11 wks. After 11 wks, arterial catheters were implanted and following 4 days of stabilization, blood pressure (BP) and heart rate (HR) were monitored during 4 hr in conscious unrestrained rats. Changes in renal function were assessed by proteinuria and glomerular filtration rate (GFR). ES decreased BP (102 +/- 3.5 vs 112+/-3.2 mmHg) and HR (355.6 +/- 6.6 vs 370 +/- 7.6 bpm). In addition, ES decreased food intake and adiposity index, mainly visceral fat (5.38 +/- 0.7 vs 9.79 +/- 0.9 per 100g body weight), parameters that may contribute to the decrease on BP and HR. However ES did not modify renal function (GFR) or hemodynamics measured as renal plasma flow and renal vascular resistance. Contrarily to the effects observed in hypertensive rats, these results suggest that ES exerts a beneficial effect on CV parameters in the absence of changes in renal function in "normotensive" aged female Wistar rats. This work was supported by NIH AG029250-01.

5.6

SEX DEPENDENT DIFFERENCES OF RENIN GENE EXPRESSION IN DISTAL NEPHRON SEGMENTS DURING HIGH SALT INTAKE IN CHRONIC ANGIOTENSIN II-INFUSED HYPERTENSIVE RATS WITH ENDOGENOUS RENIN ANGIOTENSIN SYSTEM BLOCKADE WITH LOSARTAN

Minolfa Prieto[1], Vicky F Rands[1], Kimberly L Kavanagh[1], Benjamin M Lowenburg[1], Victoria L Martin[1]
[1]Physiology (SL-39) & Renal Hypertension Ctr., Tulane University School of Medicine, 1430 Tulane Ave, New Orleans, LA, 70112.

Collecting duct (CD) renin mRNA, protein and activity are stimulated during AngII-dependent hypertension; however if CD renin gene expression differs between male and female rats has not been determined. CD renin gene expression was examined in male (M, n=23) and female (F, n=22) SD rats subjected to chronic infusion of AngII (80ng/min, 14 days), high salt diet (HS, 8%NaCl), and AT1 receptor blockade with Losartan (LOS, 30mg/L) distributed in five groups (n=3-5 rats/group) 1) Controls normal salt diet (C), 2) High salt diet (HS, 8% NaCl), 3) AngII 4) HS+AngII; and 5) AngII+HS+LOS]. Renin mRNA measured by qRT-PCR was higher in M-C rats than in F-C rats (C-M: 1.0±.4 vs C-F: 0.2±0.06 au). However, HS increased renin mRNA levels in female but not in male rats (HS-M: 1.1±0.1 vs HS-F: 2.5±0.4 au). Renin Western blot performed on medullary tissues revealed similar levels of mature renin in C-M and C-F rats but higher for prorenin in C-F rats. HS increased prorenin only in female rats (HS-M: 0.4±0.3 vs HS-F: 3.1±.4 au). Male and female rats similarly increased renin mRNA and protein levels during AngII infusions and responses back to baseline with LOS. There is sex dimorphism of renin in distal nephron segments during basal conditions. HS intake appears to stimulate CD renin only in female rats, thus sex differences in CD renin during high salt diet may differentially impact blood pressure. Grants from [2]NIH (P20-RR-017659) and [3]Tulane-BIRCWH Program (K12HD043451).

5.7

SEX DIMORPHISM OF SYSTOLIC BLOOD PRESSURE, PROTREINURIA AND OXIDATIVE STRESS DURING HIGH SALT INTAKE IN ANGIO-TENSIN II-DEPENDENT HYPERTENSION

Vicky Rands[1], Sylvia Shenouda[2], Benjamin M Lowenburg[1], Jeffrey A Planchard[1], Victoria L Martin[1], Minolfa C. Prieto[1]
[1]Physiology, Tulane University, 1430 Tulane Ave, SL-39, New Orleans, LA, 70112, [2]Pharmacology, Louisiana State University Health Sciences Center, 1901 Perdido, New Orleans, LA, 70112.

Salt intake inhibits the renin angiotensin system (RAS), but when AngII levels are not suppressed, increased HS intake exacerbates hypertension and renal injury. We investigated whether the pressor response to AngII and related proteinuria exhibit sex differences during HS intake and endogenous RAS blockade. In this study 35 Sprague Dawley rats distributed in 4 groups 1) Sham + HS [male n=5, female n=4]; 2) AngII [male n=3, female n=3]; 3) AngII + HS [male n=5, female n=5]; 4) AngII+ HS + LOS [male n=5, female n=5], were infused with AngII (80ng/min; 14 days), fed with HS diet (8% NaCl), and treated with losartan (LOS, 30mg/L). Baseline systolic blood pressure (SBP) was not different between genders (116±3 vs. 119±2 mmHg) or after 2-wks of AngII infusions (228±9 vs. 217±20 mmHg). HS diet did not modify the SBP (228±13 vs. 211±16 mmHg). LOS similarly ameliorated the SBP (156±15 vs 157±10 mmHg). HS intake increased proteinuria in AngII-infused rats (45±5 vs 19±6 mg/day) and augmented

progressively with a greater increase in males than females 79±6 vs 47±6 mg/day). Proteinuria was ameliorated by LOS in females. Oxidative stress, measured by Western blot of N-tyrosine proteins increased more in HS males than HS females. SBP under HS intake does not exhibit sexual dimorphism; however augmented proteinuria and N-tyrosine proteins in male rats may reflect a greater degree of renal injury than in female. Grants from [2]NIH (P20-RR-017659) and [3]Tulane-BIRCWH Program (K12HD043451).

5.8

SEXUAL DIMORPHISM OF ANGIOTENSIN II HYPERTENSION AFTER BLOCKADE OF THE ENDOGENOUS RENIN ANGIOTENSIN SYSTEM

Marcia Venegas-Pont[1], Julio Sartori-Valinotti[1], Porter Glover[1], Jane Reckelhoff[1], Michael Ryan[1]
[1]Physiology, UMMC, 2500 North State Street, Jackson, MS, 39215.

Recent data suggests that male rats have a greater blood pressure (BP) response to Angiotensin II (AngII) infusion than females. However, with endogenous renin angiotensin system (RAS) blockade, female rats have a greater BP response to AngII. We tested whether, like rats, female mice have a greater BP response to AngII when the endogenous RAS is blocked. 20 week old male and female C57BL/6J mice (n≥6/group) received either AngII (800 ng/kg/min) or saline for 16 days via osmotic minipumps. All mice were treated with the angiotensin converting enzyme inhibitor, enalapril (40 mg/kg/day in drinking water) for the 4 days prior to, and throughout the AngII or vehicle infusion. BP was measured in conscious mice on day 16 via carotid catheters. BP was higher in males than females given Ang II with enalapril (Male: 144±3 vs. Female: 121±6 mmHg, $p<0.05$) but not different in mice receiving enalapril alone (Male: 99±3 vs. female: 100±3 mmHg). Oxidative stress is one possible mechanism for AngII mediated hypertension in male rodents. However, its importance remains unclear for AngII hypertension in females. Therefore, urinary F2-isoprostanes were used as a marker of oxidative stress. AngII did not significantly increase F2-isoprostanes in either sex; however, female mice with AngII had higher levels (female: 10.7±0.7 vs. male: 6.1±0.6 mg/ng creatinine, $p<0.05$). Urinary albumin was not different between male and female mice in any group. These data suggest that there are species specific differences in the MAP response to AngII. In addition, oxidative stress may have a limited role in the development of AngII hypertension in female mice. Therefore, different mechanisms may contribute to AngII hypertension in male and female rats and mice.

5.9

EFFECT OF FEMALE SEX HORMONES ON THE EXPRESSION OF RENAL SODIUM TRANSPORTERS IN OVARIECTOMIZED RATS

Ki Young Na[1], Kwon Wook Joo[1], Nam Ju Heo[1], Jin Suk Han[1]
[1]Internal Medicine, Seoul National University College of Medicine, 28 Yeonggeon-dong, Jongno-gu, Seoul, 110-799, Republic of Korea.

The incidence of hypertension and cardiovascular disease is lower in young women than in age-matched men. Because this gender-linked advantage is lost after menopause, female sex steroids have been believed to play an important role in this phenomenon. In this study, we observe the effects of estradiol and progesterone on the expression of renal sodium transporters in ovariectomized rats. Female, Sprague–Dawley rats underwent ovariectomy and were randomized into four groups receiving daily injections of hormones for 10 days: (1) vehicle treated after ovariectomy; (2) 17β-estradiol benzoate; (3) progesterone; and (4) 17β-estradiol benzoate + progesterone. Expression of major protein in renal sodium transporters was determined by semiquantitative immunoblotting of rat kidney. Estradiol significantly decreased the protein abundance of Na-Cl cotransporter (NCC), γ-subunits of the epithelial sodium channel (ENaC), and Na-K-ATPase, which was associated with reduced plasma aldosterone levels, blood pressure (BP), and body weight. Estradiol combined with progesterone significantly decreased the abundance of Na-K-2Cl cotransporter (NKCC2). Although estradiol with progesterone also decreased BP and plasma aldosterone levels, the difference did not reach statistical significance. Estradiol decreased plasma aldosterone levels, BP, and the expression of renal sodium transporters. In combination with progesterone, the effect of estradiol on BP and aldosterone levels was attenuated.

5.10

ENHANCED RESPONSIVENESS TO ANGIOTENSIN II IS ANDROGEN DEPENDENT IN A RODENT MODEL OF INTRAUTERINE GROWTH RESTRICTION INDUCED BY PLACENTAL INSUFFICIENCY

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Intrauterine growth restriction (IUGR) induced by placental insufficiency in the rat leads to hypertension in male offspring that is androgen dependent. Blockade of the renin angiotensin system (RAS) abolishes hypertension in male IUGR; yet, no difference in peripheral or intrarenal RAS is observed. However, male IUGR exhibit an enhanced responsiveness to angiotensin II (ANG II). Thus, we hypothesized that enhanced responsiveness to ANG II in male IUGR is androgen dependent. Renal hemodynamics were determined before and after an acute infusion of ANG II (100 ng/kg/min for 30 minutes) in intact and castrated (CTX) adult male rats pretreated with an ACE inhibitor (enalapril, 250 mg/L for 1 week). The increase in mean arterial pressure (MAP) (28±6 vs. 48±4* mmHg) and renal vascular resistance (RVR) (4±1vs. 13±5* mmHg/ml/min) and the decrease in glomerular filtration rate (GFR)(1±1 vs. 3±0.2* ml/min) and renal blood flow (RBF)(15±2 vs. 30±6*) were greater in response to ANG II in intact IUGR (Control vs. IUGR); castration abolished the enhanced response to ANG II in IUGR (MAP: 19± 6 vs. 20±4†; RVR: 2±2 vs. 6±1†; GFR: 2±1 vs. 1±1†; RBF: 2± 7 vs. 13± 11†; Control vs. IUGR) and reduced plasma testosterone (Control: 182±12 vs. 5.7±0.2; IUGR: 322±26 vs. 5.5±0.2 ng/dL; Intact vs. CTX). Thus, enhanced responsiveness to ANG II in adult male IUGR is androgen dependent and may serve as an underlying mechanism in IUGR hypertension. *$P < 0.05$ vs. Control , † $P < 0.05$ vs. Intact Counterpart.

5.11

CONTRASTING ROLES OF THE RENIN-ANGIOTENSIN SYSTEM IN MATERNAL UTERUS AND FETAL PLACENTA IN NORMAL AND PREECLAMPTIC PREGNANCY

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The maternal uterine placenta bed is adjacent to the chorionic villi of the fetal placenta. It has been suggested that each has a local renin-angiotensin system (RAS), yet the regulation and the role of the RAS in these regions are unknown. The objective of this study was to determine if the RAS in these two tissues are regulated similarly in normal (NP) and preeclamptic (PRE) pregnancy. This study measured angiotensin (Ang) peptides, RAS component mRNAs, and receptor binding in the uterine placental bed and chorionic villi from NP (n=20, 25) and PRE (n=14, 21) subjects. In PRE uterine placental bed, Ang II peptide levels and renin and ACE mRNA expression were significantly higher than NP subjects. Angiotensin receptor binding was undetectable in NP and PRE uterine placental bed suggesting a loss of local uterine RAS function during pregnancy. In the chorionic villi, Ang II, angiotensinogen, and AT_1 receptor gene expression were significantly higher in PRE subjects. No differences were observed in renin or ACE gene expression. The AT_1 receptor was the predominant receptor subtype in NP and PRE chorionic villi. These findings suggest that the chorionic villi have an activated local RAS in PRE where the AT_1 receptor predominates. In contrast, due to the lack of RAS receptors, the maternal uterine placental bed may play an endocrine role by producing Ang II which then acts in the adjacent fetal placenta to vasoconstrict chorionic villi vessels. These results illustrate the marked contrasting profiles and potential roles of the maternal and fetal local RAS.

5.12

DIFFERENTIAL EFFECT OF CONJUGATED EQUINE ESTROGENS AND BENZOATE OF ESTRADIOL ON BLOOD PRESSURE AND NATRIURETIC PEPTIDE SYSTEM IN FEMALE RATS

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The effects of estrogen hormone therapy on blood pressure are controversial and seems to be depedent on the type of estrogen used. The aim of the present study was to compare the effects of conjugated equine estrogens (CEE), largely used in hormone replacement therapy, with estradiol benzoate (E2) on blood pressure and the involvement of Natriuretic Peptide System. Female spontaneously hypertensive rats (SHR) and Wistar rats were selected after determination of blood pressure by tail plethismography. The animals were ovariectomized and 3 weeks after were daily injected for 4 days with E2 (5µg/100g/day), CEE (Premarin: 50µg/100g/day) or vehicle (Veh: 0.1ml of corn oil/100g/day). Blood pressure was measured and one day later the rats were decapitated and the blood collected in tubes containing heparin sulfate for ANP and estradiol determinations by RIA. Right and left atria were excised for determination of ANP and ANP mRNA expression. Kidneys were quickly excised, frozen in liquid nitrogen for analysis of natriuretic peptide receptor A (NPr-A) and clearance receptor (NPr-C) mRNA. Parametrial, retroperitoneal and mesenteric adipose tissues were collected, weighed and stored at -80°C for determination of clearance receptor (NPr-C) gene expression. Significant reduction of blood pressure was observed in SHR after E2 treatment, but CEE treatment did not change blood pressure. E2-treatment increased plasma ANP levels as well ANP content and mRNA in right atrium of SHR. CEE-treatment did not change plasma ANP levels or ANP content or mRNA in right atrium of SHR. In conclusion, our data show that short-term E2 but not CEE treatment decreased blood pressure of ovariectomized SHR and this reduction was accompanied by increase of ANP synthesis and release. Financial Support: CAPES, CNPq, FAPEMIG.

5.13

ESTROGEN AND THE DEVELOPMENT OF HYPERTENSION IN THE FEMALE SPONTANEOUSLY HYPERTENSIVE RATS

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Following menopause rates of female cardiovascular disease surpass rates in males, indicating a role for estrogen in cardioprotection (1). This study tested the hypothesis that estrogen plays a protective role in the development of hypertension

through an impact on vascular mechanics. 3-week old female spontaneously hypertensive rats were left intact (SHR), ovariectomized (before 3 weeks of age; SHR+OVX) or were ovariectomized but supplemented with 17-β Estradiol (0.25mg, 21d release; SHR+OVX+E). SHR+OVX animals were heavier upon arrival *vs.* SHR (86 ± 2 *vs.* 62 ± 2, $p < 0.001$), while estrogen blunted weight gain. Non-invasive blood pressure was taken weekly, and *in vivo* measurements of pulse wave velocity (PWV) were made to assess vascular mechanics after 3 weeks. MAP increased similarly from 110 ± 2 and 118 ± 2 ($p<0.001$) at week 1 to 138 ± 2 and 153 ± 1 ($p<0.001$) in week 3 in the SHR and SHR+OVX groups, respectively. The rise in MAP was blunted in SHR+OVX+E (from 133 ± 2 to 143 ± 2, $p=0.001$). PWV (an index of vascular stiffness) was not different between groups. These data suggest a protective role for estrogen in the early weeks of hypertension development. Further, the between-group differences observed in MAP were independent of mechanical changes in the vessel walls. Supported by CIHR. (1) Barton, M. *Arterioscler Thromb Vasc Biol*. 2007; 27: 1669–1672.

5.14

SEX STEROIDS, HYPERTENSION AND MENOPAUSE: POSITIVE EFFECTS OF ANGIOTENSIN CONVERTING ENZYME INHIBITION ON BOTH THE AORTA AND RENAL NITRIC OXIDE SYSTEM

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Background: Sex steroids change and cardiovascular risk increases after menopause. Estradiol may provide cardiovascular protection by controlling the renin-angiotensin system. Objective: To evaluate the effects of the ACE inhibitor enalapril on systolic blood pressure (SBP (mmHg)), sex steroids and NO synthase(NOS) activity in the aorta and kidney in spontaneously hypertensive postmenopausal rats (SHR). Methods: 16-month old SHR received enalapril (SHR-E, 250 mg/L drinking water) or tap water (SHR-C) for 30 days. At the end of treatment, NOS activity (pmol.^{14}C-citrulline/g tissue.min) was measured in aorta and renal cortex and medulla. Plasma sex steroids: testosterone, estradiol, dehydroepiandrosterone and dehydroepiandrosterone sulfate were measured. Results: Enalapril decreased SBP and increased NOS activity in the kidneys and aorta of SHR. No changes in sex steroid levels were observed.

	SBP	NOS Activity Aorta	NOS Activity Cortex	NOS Activity Medulla
SHR-C n=6	174±8	362.4±9.2	395.3±7.1	496.0±7.3
SHR-E n=6	152±9#	412.3±14.9*	471.4±3.8*	584.8±7.4*

* $p<0.05$ or #$p<0.01$ vs control.

Conclusions: Although sex steroid plasma levels were not modified by treatment, our results evidence that ACE inhibition improves vascular and renal NO system in kidneys of postmenopausal hypertensive rats. This fact could be beneficial for the renal function in menopausal stage. These data clearly supports the therapeutic benefits of inhibition of renin angiotensin system in hypertensive menopausal women.

5.15

ANDROGENS INCREASE BLOOD PRESSURE BY UPREGULATING THE INTRARENIN-ANGIOTENSIN AND ENDOTHELIN SYSTEMS IN FEMALE SD RATS

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Polycystic ovary syndrome (PCOS) affects 15 % of reproductive age women, and is characterized by hyperandrogenism, polycystic ovaries and amenorrhea. Cardiovascular disease risk is higher in PCOS patients than age-matched controls. To test the hypothesis that hyperandrogenism in PCOS cause hypertension through upregulation of intrarenal angiotensin and endothelin system, 3 week-old female SD rats were administered dihydrotestosterone (DHT) (7.5 mg/90 days, pellets) or placebo. After 12 weeks of treatment, plasma DHT was increased 3-fold and plasma estradiol was unchanged, similar to changes with PCOS in women. DHT increased mean arterial pressure measured by radiotelemetry (96±2 vs. 108±2 mm Hg; $p<0.05$) in female SD rats. Intrarenal levels of angiotensinogen and angiotensin converting enzyme mRNA were upregulated while angiotensin type 1 receptor protein and mRNA expression was downregulated in DHT treated rats. Plasma renin activity and aldosterone were decreased in DHT treated rats. Intrarenal levels of preproendothelin-1 mRNA were also upregulated in DHT treated rats. In summary, androgen supplementation in young female rats increases blood pressure and promote renal injury. Androgen mediated activation of the intrarenal angiotensin and endothelin systems may be important in the hypertension in women with PCOS. This work was supported by NIH HL51971.

5.16

17 ß-ESTRADIOL ATTENUATES PROAPOPTOTIC P53/PUMA SIGNALING PATHWAY IN HIPPOCAMPUS CA1 FOLLOWING GLOBAL ISCHEMIA

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17ß-Estradiol (E_2) has been implicated to be neuroprotective against a variety of neurodegenerative diseases, including stroke. The current study examined whether E_2 neuroprotection following global cerebral ischemia may involve an epigenetic mechanism to modulate acetylation (and thus activation) of the proapoptotic protein p53, and whether this could alter expression of the p53 downstream target, PUMA, a BH3 proapoptotic protein. Our results revealed that p53 acetylation at Lys 373 and Lys 382 is markedly *increased* in hippocampal CA1 neurons in placebo-treated animals 24h after reperfusion, as compared to sham controls. Intriguingly, E_2 strongly attenuated the increase in p53 acetylation in CA1 neurons 24h after reperfusion, without affecting total p53 protein levels. The E_2-induced attenuation of p53 acetylation suggests that p53 transcriptional activation may be diminished by E_2 treatment. In support of this suggestion, Western blot and immunohistochemical studies revealed that protein expression of the p53 target gene, PUMA was significantly enhanced in placebo-treated animals at 24-48h after reperfusion (as compared to sham controls), and that E_2 strongly attenuated PUMA upregulation. As a whole, our studies suggest a *novel* epigenetic regulatory effect of E_2 in stroke to modulate p53 activation and attenuate expression of the downstream proapoptotic target gene, PUMA, thereby providing an antiapoptotic mechanism for E_2 neuroprotection. *NINDS Grant # NS050730*.

5.17

CUTANEOUS ADRENERGIC RESPONSES TO ESTRADIOL IN WOMEN WITH HIGH AND LOW ORTHOSTATIC TOLERANCE

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We tested the hypothesis that estradiol decreases skin vessel adrenergic responsiveness in women with high [HT (n=6, 23±3 y, Cumulative Stress Index, CSI=-808±79] but not low [LT (n=3, 23±3y,CSI=-410±42] orthostatic tolerance. Laser Doppler flowmetry measured skin blood flow (SkBF) during graded norepinephrine infusions via microdialysis. A GnRH antagonist suppressed estrogen and progesterone for 16 days; we added estradiol (E, 0.2 mg/day patch) for days 4-16 and progesterone (EP, 200 mg/day) for days 13-16. In HT, a rightward shift of the NE-SkBF curve shows E induces relative vasodilation (EC50: -3.5±0.7, -2.8±0.2, GnRH, E); L-NMMA (EC50: -5.0±1.9, -4.5±2.4, GnRH, E) and COX inhibition (CI, EC50: -5.1±0.4, -4.8±0.3) reduced this shift; L-NMMA+CI removed it (EC50: -5.3±0.4, -5.1±0.5). EP shifted the NE-SkBF curve left (EC50: -5.2±0.6) compared to E (EC50: -3.5±0.7) indicating relative vasoconstriction; within EP, neither L-NMMA nor CI affected the NE-SkBF curve. In LT, neither E nor EP altered the NE-SkBF curve (EC50: -5.1±0.9, -4.7±1.5, -4.7±0.7, GnRH, E, EP). Neither L-NMMA nor CI altered responsiveness during GnRH and E; CI led to a leftward shift of the NE-SkBF curve during EP (EC50: -4.7±0.7, -5.3±0.5 NE, CI) indicating enhanced vasoconstriction**.** E lowers adrenergic responsiveness via NO and COX mechanisms in HT; EP counters this effect. LT are insensitive to E or EP except during CI, which enhanced vasoconstriction, suggesting a role for prostaglandins.HL071159.

5.18

THE PROTECTIVE EFFECT OF CENTRAL NITRIC OXIDE AGAINST ALDOSTERONE/NACL-INDUCED HYPERTENSION IN FEMALE RATS

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It has been generally accepted that centrally produced nitric oxide (NO) plays an important role in the regulation of blood pressure (BP), and that sex hormones modulate expression and activity of NO synthase (NOS) in both central and peripheral tissues. Previous studies from our laboratory have shown that central estrogen infusion inhibits aldosterone (Aldo)/NaCl-induced increases in BP, whereas central blockade of estrogen receptors (ER) augments Aldo/NaCl-induced hypertension. The present study tested the hypotheses that NO is involved in attenuating the response to Aldo/NaCl in female rats. Aortic BP was measured in conscious rats with the use of telemetry implants. N^G-nitro-l-arginine methyl ester (L-NAME, an inhibitor of NOS; 50 µg/kg/day) was administrated centrally into the lateral ventricle for 28 days during Aldo (1.8 µg/kg/h) pump implantation. Central L-NAME infusion enhanced the pressor effect in Aldo/NaCl-treated female rats (n=3, Δ21.7±1.1 mmHg) when compared to Aldo/NaCl-treated females with peripheral L-NAME (n=2, Δ10.6±1.2 mmHg). Central infusions of L-NAME alone had no effect on BP. Immunohistochemical studies revealed co-localization of neuronal NOS and ER in the subfornical organ and paraventricular nucleus of female rats. These results suggest that central NO may mediate, at least in part, the protective effects of estrogen against Aldo/NaCl-induced hypertension. (Support: NIH HL-59676, HL-62261, HL-14388, & DK-66086).

5.19

CIRCADIAN SUPRACHIASMATIC NUCLEI AND RENAL GENE EXPRESSIONS IN PERIMENOPAUSAL FEMALE C57BL/J6 MICE.

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Women's cardiovascular health declines rapidly after menopause. This preliminary study describing temporal SCN regulation of renal function during natural perimenopause in nine-month-old C57Bl/6J female mice was approved by IACUC. Mice were acclimated in individually housing for 2 months eating and drinking *ad libitum* under standard 12:12 light/dark cycle and then randomly assigned to six different temporal groupings (n = 2-4 mice/time point) (0400, 0800, 1200, 1600, 2000, and 2400). SCN and renal medullary tissues were analyzed for mRNA expression at each time point using RT-PCR. SCN mRNA gene expression was greatest at 0400 and lowest at 1600 hours for Clock, and Trk C and Esrα receptors. The SCN Per1 and Per2 gene expressions and renal NT3 gene and renal Esr α receptor gene expressions were the greatest at 1600 hours and lowest at 0400 hours. Time series cross correlations of SCN Clock and renal NT3 gene expressions indicated that SCN clock gene expression was inversely correlated (-.876) with renal NT3 gene expression with a 12 hour time lag. Good and George (2001) had indicated that renal NT3 inhibited HCO^-_3 absorption in the medullary thick ascending limb. The current preliminary results implicated SCN regulatory synchronization of temporal renal NT3 gene expression. Research on perimenopause vulnerability of SCN regulation of temporal renal function is needed. Funded by Gerontological Nursing Interventions Research Center at University of Iowa and Dean's Grant UNMC CON.

5.20

DIETARY EQUOL (4', 7 ISOFLAVANDIOL) REDUCES OXIDATIVE STRESS AND PROTECTS RATS AGAINST FOCAL CEREBRAL ISCHEMIA

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High soy diets are neuroprotective in experimental stroke in female and male rats. We hypothesized that equol, a metabolite of dietary daidzein, is responsible for this protective effect. Male and ovariectomized female Sprague-Dawley rats were placed on a soy-free diet containing no additional isoflavone, 500 mg/kg genistien, or 250 mg/kg equol for 4 weeks. Rats underwent 90-minute transient middle cerebral artery occlusion (tMCAO) followed by reperfusion. Cerebral blood flow determined by laser-Doppler scanning did not differ among groups. Three days after tMCAO, rats were sacrificed and cerebral infarct was determined by TTC staining. Genistein (n=6-9) reduced infarct size in both sexes, but this effect was not significant. In contrast, compared to soy-free rats, equol significantly ($P<0.05$) reduced infarct size in both male (2.4±0.4%, n=8 vs. 13.2±5.4%, n=7) and female rats (3.6±0.2%; n=11 vs. 17.5±5.0%; n=10). Because equol is considered a better antioxidant than genistein, we examined whether the isoflavones reduced oxidative stress. Equol, but not genistein, significantly reduced plasma TBARS and NOS2 mRNA expression in the ischemic hemisphere 24 hours after tMCAO in female rats ($P<0.05$; n=6). Preliminary results failed to show changes in gp91phox, nitrotyrosine, MnSOD, or CuSOD among the treatment groups. These data show that dietary equol is neuroprotective in stroke and is likely responsible for the protective effects of dietary soy. Whether the moderate antioxidant effects of equol are responsible for this protection remains to be determined.

6.0: SEX HORMONES AND THE BRAIN

6.2

LONG-TERM ESTROGEN DEPRIVATION LEADS TO LOSS OF SENSITIVITY IN THE BRAIN

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The study objective was to elucidate how 17β-Estradiol (E2) exerts antioxidant and neuroprotective effects in the brain following stroke, and establish the importance of timing of E2 replacement. E2 was found to profoundly attenuate activation of neuronal NADPH oxidase and superoxide (O_2^-) production in the rat hippocampus CA1 region following global cerebral ischemia, an effect correlated with reduced oxidative damage and enhanced neuronal survival. The NOX2 NADPH oxidase was shown to be the major producer of O_2^- in neurons after stroke, and E2 attenuation of neuronal NADPH oxidase activation was found to involve ER-α-mediated nongenomic signaling leading to Akt activation, with subsequent Akt-induced phosphorylation and attenuation of Rac1 activation. Intriguingly, the ability of E2 to induce nongenomic signaling, suppress NADPH oxidase activation and exert neuroprotection in the hippocampus CA1 after stroke was *lost* after a period of long-term E2 deprivation; and this loss was *brain-specific* as the uterus remained responsive to E2. Correspondingly, a remarkable loss of ERα, but not ERβ, was observed in the hippocampus CA1 following long-term E2 deprivation, with no change observed in the uterus. As a whole, the study reveals a novel antioxidant mechanism in neurons by E2 that may underlie its broad-based neuroprotective ability in neurodegenerative disorders, and it provides support for a "critical period" of E2 replacement for induction of protective effects in the brain.

6.3

SEX DIFFERENCES IN EXPERIMENTAL STROKE: THE UNDERPINNING FOR STEROID HORMONAL MANIPULATION

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Stroke or Brain Attack is a sexually dimorphic disease in that sex is an important genetic determinant of how cerebral ischemia shapes damage to the brain. We have evaluated models of stroke in vivo and in vitro and shown that stroke sensitivity, or the amount of tissue damage that results from the insult, is not the same in males vs. females despite complex co-morbidities. Cell death after cerebral ischemia is not necessarily "hardwired" identically in both sexes either qualitatively or quantitatively. Sex steroids such as progesterone, estrogen and testosterone amplify these fundamental sex differences. Accumulating evidence suggests that selected stroke therapies work in a sex specific manner, suggesting that treatment design must consider biological sex. (NIH NR03521, NS 33668, NS 49210, Bugher Foundation of the American Heart Assn). REFERENCES: Vagnerova K, Koerner I, Hurn PD. 2008 Gender and the injured brain. Anesth Analg 107:201-14.Herson PS, Koerner I, Hurn PD. 2009 Sex, Sex Steroids and Brain Injury. Sem Reproductive Medicine, in press. Liu M, Hurn PD, Roselli CE, Alkayed NJ. 2007. Role of P450 aromatase in sex-specific astrocytic cell death. J Cereb Blood Flow Metab. 27: 135-141. McCullough LD, Zheng Z, Blizzard KK, , Hurn PD. 2005 Ischemic NO and PARP activation in cerebral ischemia: male toxicity, female protection. J Cereb Blood Flow Metab. 25:502-12.

6.4

THE AGING BLOOD BRAIN BARRIER: IMPLICATIONS FOR STROKE AND REPAIR IN ACYCLIC FEMALES

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The major cellular components of the blood brain barrier (endothelial cells and astrocytes) express estrogen receptors and our studies indicate that permeability across the BBB is modulated by both estrogen and reproductive age (Bake and Sohrabji, 2004). Overall, BBB permeability was increased in reproductive senescent females, a group that mimics salient aspects of the menopause. Moreover, estrogen treatment to this older acyclic female further increased blood brain barrier permeability. Ischemic stroke and reperfusion causes significant dysregulation of the blood brain barrier and this may contribute to the severity of infarction. In view of its actions at the blood brain barrier, we hypothesized that estrogen treatment would increase the severity of ischemic injury in older acyclic females. In an endothelin (ET)-1MCA occlusion model, acyclic older (10-12m) females sustained large infarct volumes as compared to adult (5-7m) females, and estrogen treatment while neuroprotective in younger females, paradoxically, exacerbated infarct size in the acyclic group (Selvamani and Sohrabji, 2008). Although the cellular locus of age related changes in the BBB remains to be elucidated, our recent studies suggest that astrocytes may be a key candidate. Although astrocytes from senescent and mature adult females do not differ in their ability to mount an inflammatory response, astrocytes from senescent females' impeded neuronal differentiation when co-cultured with neural progenitor cells (Lewis et al., 2008a, 2008b). In the context of stroke- induced neurogenesis and differentiation, this latter finding suggests that neurogenic repair processes may be impaired in the acyclic older brain. Support by AG019515, AG027684, AG028303).

7.0: SEX DIFFERENCES IN VASCULAR REACTIVITY

7.2

DUAL AND OPPOSITE EFFECTS OF ESTROGEN ON CORONARY ARTERIES UNDER PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL CONDITIONS

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Estrogen is a vasodilatory hormone which relaxes coronary arteries via both endothelium-dependent and –independent mechanisms involving NO production. In contrast, we reported estrogen can constrict coronary arteries by stimulating production of reactive oxygen species (ROS) in coronary artery smooth muscle cells (CASMC). The molecular target mediating both responses to estrogen appears to be Type 1 (n)NOS, which we found expressed in porcine and human CASMC. Estrogen normally stimulates nNOS activity to produce NO and relax vessels; however, when nNOS is uncoupled from NO production, estrogen may stimulate production of superoxide to contract coronary arteries. We now report that diabetes may uncouple NOS activity, and thereby enhance ROS production in

coronary arteries. Whereas 17beta-estradiol relaxes coronary arteries from normal pigs, we found estrogen is a potent constrictor of coronary arteries from STZ-diabetic pigs. This vasoconstrictor response was inhibited by ICI182780 (estrogen receptor antagonist), tempol (superoxide dismutase mimetic), or L-NAME (inhibitor of NOS activity). In addition, estrogen enhanced ROS-induced fluorescence in CASMC from diabetic animals. Expression of nNOS was increased in CASMC from diabetic pigs, whereas expression of GTP-cyclohydrolase was decreased by this disease state. We propose that diabetes uncouples NOS activity in porcine coronary arteries leading to an abnormal contractile response to estrogen. Estrogen-stimulated ROS production in diabetes may contribute to the higher incidence of diabetes-related cardiovascular disease in women compared to men, and may possibly suggest novel therapeutic measures to treat this unfortunate sexual dimorphism. (supported by HL073890).

7.3

SEX HORMONES ARE NOT THE SOLE DETERMINANT OF SEX-DIFFERENCES IN VASCULAR FUNCTION

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The presence vs. absence of the sex hormone, estrogen, has been the focus of sex-differences in susceptibility, onset, course, and outcomes of cardiovascular disease, reflecting conclusions drawn from facts such as a lower incidence of hypertension or coronary heart disease in reproductive age women relative to the rise in rates of heart disease and stroke after menopause. Ovary removal does result in changes in brain microvessel architecture that is ameliorated poorly by standard hormone replacement therapy (HRT) and minimized when HRT mimics normal cycling. With respect to microvascular exchange it may be the presence of testosterone, rather than estrogen, that differentiates men from women and is manifest in venous microvessels. Studies of sex differences in intact isolated and in situ microvasculature have been complimented by recent work on endothelium cultured from these microvessels. In this case sex differences with respect to barrier responses and expression of 2nd messenger systems were retained in culture. This work demonstrated that in the absence of externally applied or changing sex hormone levels cells retain genomic identity as XX or XY. These studies imply that sexual dimorphism involves the genome leading to the differences manifest by sexually immature children, adults exposed to cycling levels of the sex hormones, and adults following removal of the sex hormones with age. (NIH RO1 HL078816 & NASA NNJ05HF37G) Glinskii, OV, TW Abraha, JR Turk, LJ Rubin, VH Huxley, VV Glinsky. 2007. Microvascular network remodeling in dura mater of ovariectomized pigs: Role for angiopietin-1 in estrogen-dependent control of vascular stability Am J Physiol 293:H1131-37; Wang JJ, VH Huxley. 2008 Sexual and maturational differences in phosphodiesterase mRNA expression in rat skeletal muscle microvascular endothelial cells. FASEB J 22: 1145.4.

8.0: SEX STEROIDS AND CARDIOVASCULAR DISEASE

8.2

MOUSE MODELS FOR STUDYING SEX DIFFERENCES IN PHYSIOLOGY AND DISEASE

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All sex differences in phenotype arise at the genetic level from sexual inequality in the number and type of sex chromosome genes. Although most sex differences in tissue function are induced directly by gonadal hormones, XX and XY cells are also unequal because they have constitutively different expression of X and Y genes. Mouse models are available for distinguishing direct effects of gonadal hormones and sex chromosome genes. The “four core genotypes” (FCG) mouse model tests for different phenotypic effects of XX vs. XY genomes, independent of the effects of gonadal secretions. In FCG mice, the testis-determining gene Sry is deleted from the Y chromosome and inserted as a transgene onto an autosome, so that gonadal sexual differentiation is controlled by an autosome and is independent of sex chromosome complement. Comparing XX and XY mice of either gonadal sex allows one to discover sex differences in somatic tissue functions that result from the different effects of an XX vs. XY genome. We have found that the number of X chromosomes causes sex differences in expression of the prodynorphin gene in the adult mouse striatum, and causes sex differences in susceptibility to neural tube closure defects in a mouse model. Moreover, the XX vs. XY difference in sex chromosome complement causes differences in response to nociceptive stimuli, in formation of habits, in aggressive behavior, in immune response to autoantigens, and in regulation of body weight and fat mass. These sex chromosome effects are likely exerted directly on somatic tissues and are not the result of group differences in the levels of gonadal hormones. Both X and Y genes are implicated as direct causes of sex differences. Arnold AP. (2004) Nature Rev Neurosci 5:701-708; Arnold AP & Chen X, Front Neuroendocrinol 2009 30:1-9.

8.3

ANGIOTENSIN CONVERTING ENZYME 2 FEEDBACK REGULATION CONTRIBUTES TO SEX DIFFERENCES IN ANGIOTENSIN II-DEPENDENT HYPERTENSION

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ACE2 is a newly discovered monocarboxypeptidase that counteracts the vasoconstrictor effects of angiotensin II (Ang II) by converting Ang II to Ang-[1-7] in the kidney and other tissues. To further our understanding of ACE2 regulation *in vivo*, we investigated Ang II regulation of ACE2 in male (M) and female (F) mice after optimizing an assay for measuring ACE2 activity in the mouse renal cortex. Ang II increased ACE2 activity in both sexes though the magnitude of this increase was greater in females (1.45-fold) compared to males (1.22-fold). Substrate concentration curves revealed that the positive substrate feedback regulation by Ang II was due to increased ACE2 enzyme velocity (Vmax) rather than increased substrate affinity (Km). Under these conditions, peak mean arterial pressure (MAP) was higher in male compared to female mice [peak MAP (mm Hg): M-Ang II, 146±5.5 vs F-Ang II, 132±3.8, n=12, $p<0.05$]. This study suggests that up-regulation of renal ACE2 activity serves as a positive feedback mechanism for controlling the adverse effects of Ang II in pathological conditions such as Ang II-dependent hypertension. The finding that the magnitude of ACE2 up-regulation is greater in female compared to male mice suggests sex differences in ACE2 feedback regulation contribute to sex differences in blood pressure in experimental models of Ang II-dependent hypertension (R01 AG19291).

9.0: SEX STEROIDS: CARDIOVASCULAR SYSTEM AND METABOLIC SYNDROME

9.1

RELEASE OF EETS IN RESPONSE TO SHEAR STRESS IN ARTERIES OF FEMALE NO DEFICIENT RATS

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We demonstrated previously that epoxyeicosatrienoic acids (EETs)-mediated flow/shear stress-induced dilation of arterioles is estrogen- and NO deficiency-dependent. Thus, cannulated mesenteric arteries isolated from female rats that had been treated with L-NAME (in the drinking water) for 3-4 weeks, were perfused with 2 and 10 dyne/cm^2 shear stress (SS) for 5 minutes, followed by collection of the perfusate to measure EET concentrations by GC-MS. We found that shear stress dose-dependently stimulated the release of EETs in the perfusate, a response that was prevented by PPOH, an inhibitor of EET synthase. Based on the vessel length/diameter, flow rate applied and viscosity of the physiologic salt solution, we calculated EET concentrations in the perfusate at a range of 1.113 ± 0.4 pg/min/mm^2 intraluminal surface area of the vessel (SS=2 dyne/cm^2) to 9.928 ± 2.3 pg/min/mm^2 (SS=10 dyne/cm^2). In order to further characterize specific regioisomers of EETs detected in the perfusate, we perfused vessels, in separate experiments, with 10dyne/cm^2 shear stress for 10 minutes, followed by collection of perfusate samples that were then pooled after purification of EETs. By using LC-MS analysis, we found that 11,12-EET and 14,15-EET are the major mediators of EET spectrum in the perfusate, and 5,6-EET was essentially undetected. Thus, the present studies provided solid evidence of shear stress-stimulated release of EETs in female vessels in NO deficient status. (Supported by Intramural Grant 49420, NIH HL070653 and HL43023).

9.2

SEX-DIFFERENCES IN ENDOTHELIN-1 MEDIATED VASOCONSTRICTOR TONE IN MIDDLE-AGED ADULTS

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The prevalence of cardiovascular disease (atherosclerosis, heart failure (HF) and hypertension (HTN)) is lower in middle-aged women than men. Increased endothelin-1 (ET-1) mediated vasoconstriction has been linked to the etiology of a number of cardiovascular diseases including atherosclerosis, HF and HTN. Therefore, we hypothesized that ET-1-mediated vasoconstrictor activity is lower in healthy, sedentary middle-aged women than men. Forearm blood flow (FBF) in response to intra-arterial infusions of ET-1 and BQ-123 (a selective ET_A receptor antagonist) was assessed by venous occlusion plethysmography in 21 women (age: 58±1 yrs; BMI: 26.0±1.0 kg/m^2) and 25 men (age: 57±2 yrs; BMI: 26.8±0.7 kg/m^2). The vasoconstrictor response to ET-1 was higher (~40%) in the women compared to the men. In response to BQ-123, the increase in FBF from baseline was significantly higher in the men than the women (24±5% vs 9±5%; $P<0.05$). These results indicate that middle-aged men are under greater ET-1-mediated vasoconstrictor tone than middle-aged women. This sex-difference in ET-1-mediated vasoconstrictor tone may be a mechanism underlying the sex-difference in the prevalence of major cardiovascular diseases in middle-aged adults.

9.3

ESTROGEN RECEPTOR α AND β ACTIVATION EXERT DIFFER-ENTIAL EFFECTS ON MESENTERIC VASCULAR RESPONSIVENESS TO VASO-PRESSIN IN NORMOTENSIVE AND HYPERTENSIVE FEMALE RATS

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Previously we reported ER-selective agonists (PPT, ERα; DPN, ERβ) in female rats exerted differential effects on aortic contractile responses to vasopressin (VP) and on release of the prostanoids thromboxane (TXA_2) and prostacyclin (PGI_2). However, peripheral vascular responses are unknown. Mesenteric arterioles were obtained from female Sprague-Dawley rats (14-16 wks age) with aortic coarctation-induced hypertension (ACIH) or sham (normotensive; NT) and that were either left intact (INT), ovariectomized (OVX), or OVX + PPT- or DPN-treated. Reactivity to VP was measured using isometric myography. The release of PGI_2 and TXA_2 was measured using radioimmunoassay. Rats were sacrificed 12-14 days post-ACIH. In NT rats, maximal contractile response to VP was greater in PPT than in INT, OVX or DPN. These differences were abolished in ACIH. In NT, VP-induced release of TXA_2 was similar in INT, PPT and OVX while DPN was lower. ACIH dramatically upregulated TXA_2 release in INT while PPT was higher and both OVX and DPN were lower. In NT, VP produced similar PGI_2 release in INT and PPT, while OVX and DPN were lower. ACIH markedly increased VP-stimulated PGI_2 release, which was similar in INT and PPT, while DPN and OVX were lower. In conclusion, in NT, ERα enhances reactivity to VP and TXA_2 and PGI_2 release more than ERβ. In ACIH, dramatic increases in TXA_2 and PGI_2 release occur in both INT and ERα groups compared to NT, but not in OVX or ERβ groups. NIH: HL-080402.

9.4

GENDER SPECIFIC ROLE OF TYROSINE KINASE RECEPTOR TRANS-ACTIVATION IN WISTAR RATS

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EGFR receptor inhibition reduces contraction of renal afferent blood vessel to AII or NE in male rats. We have reported a gender specific, age-related decline in alpha adrenergic contraction of renal interlobar blood vessels in rats and gender specific responses that are receptor specific to alpha agonists (A61603 (Tocris) alpha 1A/C and phenylephrine all alpha 1 A/C, B and D). Contraction to A61603 and phenylephrine are much reduced by 10-months of age in male rats but female vessels demonstrate little decline until 17-months of age. To analyze gender as well as receptor differences in transactivation, Lavendustin A, a src tyrosine kinase receptor inhibitor (Calbiochem) was used. Adult male but not female rat vessels contracted less to phenylephrine when pretreated with Lavendustin A (26 %). Female but not male rat vessels pretreated with Lavendusin A and constricted with A61603 had a small significant reduction (8%). Pretreated blood vessels from rats aged to approximately 1-year-old, had no significant inhibitor dependent decline. These results indicate that male rats undergo a reduction in tyrosine kinase transactivation for alpha 1 B and/or D receptors with aging and vessels from female rats don,t utilize as much transactivation as males. Gene chip array data indicate that female wistar rats have a greater expression of alpha 1A receptors compared to males, while Alpha 1 B and D are not gender different. Supp. by Ohio Valley Heart and U of L funding.

9.5

EFFECT OF ORCHIDECTOMY ON VASCULAR RELAXATION RE-SPONSES TO CAMP AND POTASSIUM CHANNEL ACTIVATION IN SPRAGUE DAWLEY RATS FED A HIGH SALT DIET

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Forty eight weanling male rats were randomly divided into 8 groups (n = 6 each) that were either orchidectomised or sham-operated, with or without testosterone replacement (10mg/kg sustanon 250® i.m) once in 3 weeks, and/or placed on normal (0.3%) or high (8%) NaCl diet. 3mm Aortic rings were suspended in organ baths containing Hepes buffer bubbled with 100% oxygen. Relaxation responses to forskolin and diazoxide were studied in noradrenaline (0.1μM) pre-contracted rings. MABP was determined before and weekly throughout the experimental period using non-invasive tail cuff method. The results indicate a significant increase ($P < 0.05$) in the mean arterial blood pressure of rats placed on high salt diet, compared to controls. Orchidectomy elicited a reduction in MABP ($p < 0.05$) while testosterone replacement normalized MABP to values seen in intact rats fed a high salt diet. There was a significant decrease ($p < 0.05$) in the relaxation response of rats placed on high salt diet to forskolin when compared with controls. High salt diet reduced the relaxation response to diazoxide but not in orchidectomised rats while testosterone supplementation reestablished the blunted diazoxide relaxation. Thus inhibition of potassium channel or adenylyl cyclase activation appear to be part of the mechanisms by which high salt diet increases vascular tone, and these effects were reduced by orchidectomy in male Sprague Dawley rat. Unilag CRC 2007/14 and INSA JRD-TATA.

9.6

OSTEOPENIA IS ASSOCIATED WITH INCREASED ENDOTHELIN-1 VASOCONSTRICTOR TONE IN POSTMENOPAUSAL WOMEN

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Background: Osteopenia refers to bone mineral densities that are below the normal range, yet above the diagnostic threshold for osteoporosis. Low bone mineral density is a predictor of adverse cardiovascular (CV) events in postmenopausal women. A potential mechanism contributing to the increased CV risk in postmenopausal women with osteopenia is endothelial vasomotor dysfunction. Endothelin (ET)-1 is a potent vasoconstrictor peptide that is associated with endothelial vasomotor dysfunction and increased CV risk. Currently, there is little information regarding the influence of osteopenia on ET-1 vasoconstrictor activity in postmenopausal women. We tested the hypothesis that ET-1 mediated vasoconstrictor activity is greater in postmenopausal women with osteopenia compared with those without. **Methods:** Forearm blood flow (FBF) responses to intra-arterial infusion of BQ-123 (100 nmol/min for 60 min), a selective ET_A receptor antagonist, were determined in postmenopausal women: 9 with osteopenia (age: 59.4±2.7 yr) and 15 without osteopenia (age: 58.1±1.7 yr). **Results:** In women with osteopenia, FBF increased ~25% ($P<0.05$) in response to BQ-123. However, in the women without osteopenia, resting FBF was not significantly changed. **Conclusions:** These results suggest that osteopenia is associated with greater ET-1-mediated vasoconstrictor tone. Increased ET-1 vasoconstrictor activity may contribute to the elevated CV risk in postmenopausal women with osteopenia.

9.7

CYP2C29 AND RXRG IN THE REGULATION OF EET-MEDIATED FLOW-INDUCED DILATION OF FEMALE MICE

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We aimed to identify which cytochrome P450 (CYP) family member(s) is the transcriptional target for estrogen and shear stress, resulting in the synthesis of epoxyeicosatrienoic acids (EETs) to initiate vasodilation. Microarray indicated an upregulation of CYP2C29 and retinoid X receptor γ (RXRγ) in microvessels of female eNOS-KO mice, revealing a gender-specific regulation of these genes, as a function of NO deficiency. Shear stress-induced, EET-mediated dilation of arterioles in female eNOS-KO mice was assessed before and after transfection of vessels with CYP2C29 siRNA. Knockdown of CYP2C29 significantly attenuated the dilator responses, indicating that CYP2C29 is the gene responsible for the synthesis of EETs. RT-PCR and western blot confirmed the specific knockdown of CYP2C29 gene and protein expression in transfected vessels. The role of the transcription factor RXRγ in the regulation of CYP2C29 and shear stress-dependent dilation was also assessed by transfection of the vessel with RXRγ siRNA. Knockdown of RXRγ in vessels that initiated EET-mediated vasodilation to shear stress in the control condition, significantly inhibited the responses. RXRγ siRNA not only silenced vascular RXRγ expression, but also synchronously downregulated CYP2C29 expression. In conclusion, the studies revealed a specific signaling cascade, by which estrogen and shear stress activate the CYP2C29 gene to synthesize EETs that dilate microvessels, via a RXRγ-dependent mechanism. (Supported by Intramural Grant 49420, NIH HL070653 and HL43023).

9.8

ESTROGEN ENHANCES SERCA EXPRESSION VIA PROTEIN KINASE G IN CORONARY ARTERIES

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Our lab has previously reported that estrogen (17β-estradiol, E2) induces an up-regulation of the vascular sarcoplasmic reticulum Ca^{2+}-ATPase pump (SERCA2b). The purpose of this study was to determine if the known regulatory kinases responsible for increasing SERCA activity could also be responsible for the E2 mediated increase in SERCA expression. The distal ends of coronary arteries obtained from the hearts of female pigs were sectioned into longitudinal strips and incubated for 24 hrs in 1 nM E2 or its vehicle solvent. The arterial strips were homogenized for Western blot analysis. Immunoreactivity was determined using antibodies reactive to SERCA2, protein kinase A (PKA), protein kinase G (PKG), and Ca^{2+}-calmodulin kinase II (CaMKII). Our results indicate the E2 selectively increases ($p<0.05$) protein kinase expression with PKA > CaMKII > PKG. However, incubating arterial strips with E2 and selective inhibitors for PKA and PKG (KT4720 and KT5823, respectively) for 24 hrs indicate that only PKG is responsible for the selective increase in SERCA2b. Overall, our data suggest that one of the protective benefits of E2 against vascular disease in pre-menopausal women may be its ability to up-regulate selective intracellular Ca^{2+} regulatory mechanisms. Support: NCRR of the NIH, Grant #20 RR-16460.

9.9

OVARIECTOMY BUT NOT ORCHIECTOMY REDUCES LARGE-CONDUCTANCE CA^{2+}-ACTIVATED K^+CHANNEL (BK) ACTIVITY IN PORCINE CORONARY SMOOTH MUSCLE

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Gender and hormonal status play a role in the regulation of coronary ion channel activity. Previous studies show female swine have greater coronary smooth muscle (CSM) K^+ channel current (I_K) than male swine, and the TEA-sensitive component (i.e., BK component) of I_K is greater in female compared to male swine. The purpose of the current study was to determine if these sex differences are due to endogenous male and/or female hormones. Sexually mature Yucatan miniature swine were obtained from the breeder and either left intact (Intact male, IM, n=4; Intact female,IF, n= 5) or gonadectomized (Castrated male, CM, n= 4; ovariectomized female, OVX, n= 6). Basal I_K was greater in females (133 ± 7 pA/pF; n=30) compared to males (100 ± 9 pA/pF; n=25). OVX swine had decreased basal I_K (108 ± 10 pA/pF; n=29) compared to IF (133 ± 7 pA/pF; n=30). Furthermore, OVX swine had decreased Iberiotoxin-sensitive , i.e., BK channel current (49 ± 6 pA/pF; n=29) compared to IF (70 ± 6 pA/pF; n=30), however, this was not associated with a difference in BK or BK Beta 1 subunit mRNA expression. In contrast, I_K current density in CM swine was not different from IM, even in the presence of the BK channel activator NS 1619 (IM, 261 ± 25 pA/pF; n=21 and CM, 230 ± 32 pA/pF; n=18). In conclusion, endogenous sex hormones, i.e. estrogen/progesterone are responsible for increased I_K in female compared to male swine, due primarily to increased BK channel activity. NIH HL071574.

9.10

OVARIECTOMY ENHANCES L-TYPE CA^{2+}CHANNEL ACTIVITY IN PORCINE CORONARY SMOOTH MUSCLE

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Gender and hormonal status play a role in the regulation of coronary ion channel activity. We previously demonstrated increased L-type Ca^{2+} channel current (I_{Ca}) in coronary smooth muscle (CSM) of male compared to female swine. Furthermore, in male swine we demonstrated that endogenous testosterone increases I_{Ca} in CSM by enhanced expression of Ca_v 1.2. Conversely, the role of estrogen in female swine has not previously been investigated. Therefore, the purpose of the current study was to determine the effect of endogenous estrogen on L-type Ca^{2+} channel activity and expression in female Yucatan miniature swine. Sexually mature female swine were obtained from the breeder and were either left intact (IF; n=5) or ovariectomized (OVX; n=6). L-type Ca^{2+} channel current (I_{Ca}) was enhanced in OVX (-9.5 ± 0.6 pA/pF; n=25) compared to IF (-4.5 ± 0.3 pA/pF; n=26), although L-type Ca^{2+} channel alpha subunit (Ca_v 1.2) mRNA expression was unchanged. Of the L-type Ca^{2+} channel β subunits, β1 (188 ± 31; n=3) and β2a (561 ± 79; n=3) had higher mRNA expression levels (target/18S) than β3 (9 ± 1; n=3) and β4 (2 ± 0.1). While β2a, β3, and β4 mRNA and protein expressions were not different between groups, protein expression of the L-type Ca^{2+} channel β1 subunit (Ca_v β1) was decreased in OVX (7 ± 0.5 d.u.; n=2) compared to IF (16 ± 2 d.u.; n=4). In conclusion, endogenous estrogen inhibits L-type Ca^{2+} channel activity in CSM possibly due to stimulation of Ca_v β1 subunit expression. NIH HL071574.

9.11

EARLY-GESTATION EXPOSURE TO EXCESS TESTOSTERONE REDUCES CARDIOMYOCYTE PROLIFERATION AND MATURATION IN NEAR-TERM FEMALE FETAL SHEEP

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In female fetuses, early-gestation exposure to excess testosterone (T) decreases near-term liver insulin-like growth factor (IGF) 1 expression and circulating IGF binding-protein (IGFBP) 3 levels, resulting in low body weight. IGF-1 stimulates cardiomyocyte (CM) proliferation in the near-term heart and reduces the proportion of CM that are terminally differentiated (TD, indexed by nucleation), but does not alter CM size. We hypothesized that in genetically female fetuses, early exposure to excess T would result in fewer, more mature CM, but no change to CM size. Pregnant ewes were injected with 100mg T propionate in oil, or only oil, 2x weekly from 30-60 days of gestation (dG). Fetal hearts were arrested in diastole at 135dG (of 145), dissociated into free cells, and fixed. T-exposed fetuses were lighter than controls (2.8kg v 4.0kg, P=0.04, n=4 females each group) and tended to have lighter hearts (17.6g v 23.8g, P=0.07). CM dimensions (length, width) were unchanged by treatment. T-exposure lowered the right ventricular index of TD (37% v 55%, P=0.03) and tended to lower the left ventricular TD index (34.8% v 45.8%, P=0.12). Reduced cardiac weight and unchanged CM dimensions suggests that T-exposure slows CM proliferation but, contrary to our hypothesis, also slows CM maturation. Further analysis of fetal cardiac IGFBP and receptors is necessary to understand the growth response to excess early-gestation T exposure. Research funded by NIH grants K12HD043488 and R01RR014270.

9.12

DIHYDROTESTOSTERONE ALTERS ENDOTOXIN AND CYTOKINE INDUCED INCREASES IN CYCLOOXYGENASE-2 IN HUMAN CORONARY ARTERY SMOOTH MUSCLE CELLS BUT HAS NO EFFECT ON HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

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Clinically, both protective and non-protective effects of androgens on the cardiovascular system have been reported. In addition, past studies have demonstrated that androgens modulate proinflammatory mediators in vascular tissues both under normal conditions and following induction of inflammation. Previous studies show that chronic testosterone treatment exacerbates endotoxin-induced cyclooxygenase-2 (COX-2) levels in cerebral arteries isolated from rodents. Here we investigated whether dihydrotestosterone (DHT; 100nM), the more potent androgen receptor agonist, modulates COX-2 levels in primary human coronary artery smooth muscle cells (HCASMC) and primary human brain microvascular endothelial cells (HBEC) following stimulation with lipopolysaccharide (LPS; 100µg/ml) or interleukin 1 beta (IL1β; 5ng/ml). Western blot demonstrated that both IL1β and LPS treatment (6hr) increased COX-2 levels compared to vehicle in HCASMC, and to a slightly lesser extent in HBEC. DHT treatment (6hr) alone did not significantly alter COX-2 levels in either cell type. However, LPS or IL1β-induced increases in COX-2 were blunted when co-administered with DHT in HCASMC, but had no effect in HBEC. In conclusion, the reduction of cytokine- or endotoxin-induced inflammation by DHT appears to be more pronounced in the medial layer compared to the intimal layer of the blood vessel wall. *Support: AHA SDG RG.*

9.13

EFFECT OF AGE AND ESTROGEN LOSS ON ESTROGEN RECEPTOR ALPHA AND BETA IN HEARTS OF DAHL SALT SENSITIVE RATS

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Estrogen (E) therapy in older women has been shown to increase the risk of adverse cardiovascular events. We observe that Dahl salt-sensitive (DS) female rats develop age-related hypertension that is accelerated by ovariectomy (OVX), and delayed by E replacement (OVX+E); however, chronic E therapy does not provide cardiovascular protection in older DS rats. We hypothesize that aging or E loss affect cardiac estrogen receptor (ER) expression to diminish the protective effect of E. We measured ER alpha and ER beta expression by Western blot in hearts of DS rats at ages 6, 9, 12, and 15 months (M). Data are optical density ratios (mean ± S.D). ER alpha was significantly less ($p<0.05$) at 12M compared to 6M (6M:0.78±0.12; 9M:0.53±0.18; 12M:0.38±0.12; and 15M:0.69±0.26), and ER beta was not different (6M:0.74±0.05; 9M:0.58±0.08; 12M:0.57±0.07; 15M:0.60±0.20). To determine the effect of E loss, we measured ER expression in young DS rats with intact ovaries (Intact), after OVX, or OVX+E. ER alpha was significantly lower ($p<0.05$) in OVX (0.38±0.13) compared to Intact (0.71±0.09) and OVX+E (0.63±0.16), while ER beta was similar between groups (Intact:0.55±0.06; OVX:0.67±0.01; and OVX+E:0.61±0.09). We conclude that aging and E loss decrease cardiac ER alpha expression, while ER beta is unaffected. We speculate that downregulation of ER alpha in the heart may be a factor contributing to the adverse cardiovascular effects of E therapy in older women and the loss of the protective effect of E in older DS rats.

9.14

GPR30 RECEPTOR ACTIVATION IMPROVES CARDIAC FUNCTION IN INTACT FEMALE MREN2.LEWIS RATS

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GPR30 is a novel estrogen receptor expressed in various tissues including the heart. We showed that treatment with the GPR30 agonist G-1 reduced blood pressure in ovariectomized but not intact female mRen2.Lewis rats. Since this hypertensive strain exhibits salt-dependent hypertension and diastolic dysfunction, we assessed the effects of the GPR30 agonist (G-1, 40 nmol/kg/hr for 14 days,) or vehicle (V, DMSO/15% EtOH) on cardiac function and structure in intact female mRen2.Lewis rats fed a normal (NS) or a high salt (HS) diet (4% Na, 10 wks). The G-1 agonist did not reduce blood pressure in either the NS (V: 149±5 vs. G-1: 143±6 mmHg, n=5) or HS-fed rats (V: 238±9 vs. G-1: 225±4 mmHg, n=5). Independent of pressure alterations, GPR30 activation reduced cardiac hypertrophy in HS fed rats (V: 5.4±0.1 vs. 4.8±0.1 mg/g, $P < 0.05$, n=5). GPR30 activation increased myocardial relaxation as defined by tissue Doppler, e×, in the NS and HS-fed rats by 18% and 14%, respectively ($P< 0.05$). The enhanced lusitropy was further accompanied by a 23% increase in fractional shortening in the NS group ($P<0.05$). While G-1 reduced myocyte hypertrophy in HS-fed mRen2.Lewis compared to NS-fed littermates (V: 1261±46 vs. G-1: 1095±59 µm2, $P < 0.05$), it

did not limit salt-stimulated cardiac collagen deposition. These data provide the first evidence for a cardioprotective role of GPR30 in the female mRen2.Lewis rat. We conclude that activation of this novel receptor may improve diastolic dysfunction and cardiac hypertrophy in hypertensive females independent of blood pressure reductions. Funding: NIH HL-56973.

9.15

IMPROVED TIMP-1/MMP-9 AND TIMP-2/MMP-2 BALANCE IN VOLUME OVERLOADED HEARTS OF OVARIECTOMIZED RATS AFTER ESTROGEN REPLACEMENT

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Volume overload (CVO) induces cardiac structural and functional remodeling that is more severe in ovariectomized than intact female rats. Estrogen replacement attenuates adverse remodeling and functional deterioration induced by CVO, yet little is known about the influence of estrogen on the balance of MMPs and their inhibitors, TIMPs, during remodeling. We hypothesized that MMP and TIMP expression and activity are altered during CVO-induced remodeling, and that the balance of these key regulators of extracellular matrix turnover is estrogen regulated. Five groups of female rats were studied: intact sham, ovariectomized sham, intact with CVO, ovariectomized with CVO, and ovariectomized with CVO treated with estrogen. After 8 weeks of fistula-induced CVO, left ventricular tissues were collected and assessed for MMP-2, TIMP-1 and -2 protein expression, MMP-2 and -9 activity, and collagen type I and III expression. Increased MMP-9 immunostaining and elevated MMP-9 activity, concurrently with decreased TIMP-1 expression, were found in hearts of ovariectomized CVO rats. Estrogen prevented TIMP-1 down regulation and decreased MMP-9 activity. The ovariectomized CVO group exhibited the greatest LV hypertrophy and had the highest level of TIMP-2 expression and TIMP-2/MMP-2 ratio. Estrogen blocked the reduction in MMP-2 expression in the ovariectomized CVO group. Moreover, the 2-fold increased collagen type I to III ratio in ovariectomized CVO hearts was prevented by estrogen.

9.16

TESTOSTERONE REPLACEMENT ALTERS CORONARY VASCULAR FUNCTION IN MALE YUCATAN MINIATURE SWINE

Craig Emter[1], Jan Ivey[1], Darla Tharp[1], Douglas Bowles[1]
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The impact of androgens on cardiovascular health and disease is incompletely understood. Recent evidence indicates opposing stimulating and inhibitory effects of chronic and acute testosterone (T) treatment, respectively, on L-type Ca^{2+} channel ($I_{Ca,L}$) expression and activity, likely due to disparate genomic and non-genomic mechanisms. The purpose of this study was to examine coronary vascular function (CVF) in intact (IM, n=4), castrated (CM, n=4) and castrated hormone replaced (HR, n=4; T, 10 mg/day for 4 wks) male Yucatan miniature swine. *In vivo* coronary blood flow responses to intracoronary infusion of endothelin-1 (ET-1; 1-20 ng/kg/min), nifedipine (NIFED; 1-10 ug/kg/min) and adenosine (ADO; 0.5-5 ug/kg) were measured in the left anterior descending coronary artery under anesthesia. Coronary flow reserve and maximal coronary vascular conductance (CVC_{max}) were significantly attenuated ($p \leq 0.05$) in IM and HR animals following intracoronary ADO. The contribution of $I_{Ca,L}$ to basal coronary flow ($CVC_{max/NIFED}/CVC_{max/ADO}$) was greater in HR animals (89%±9) when compared to IM (64%±7) and CM (74%±8). Further, ET-1 induced maximal coronary vascular resistance (CVR_{max}) and the difference between baseline and CVR_{max} was greater in the HR group ($p=0.05$). Our results suggest daily T replacement increases genomic expression of $I_{Ca,L}$ but does not suppress its activity. Our demonstrated effects of hormone replacement on CVF should be considered when applied clinically. NIH HL071574.

9.17

SEX-SPECIFIC EFFECT OF ESTROGEN RECEPTOR BETA ON THE TRANSITION FROM CARDIAC HYPERTROPHY TO HEART FAILURE

Daniela Fliegner[1], Carola Schubert[1], Adam Penkalla[1], Christina Westphal[1], Ulrich Kintscher[2], Jan Ake Gustafsson[3], Vera Regitz-Zagrosek[1]
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Objective: The influence of ERβ on the pathophysiological and molecular mechanisms leading to sex-differences in PO induced cardiac hypertrophy and heart failure was investigated. Methods: Transverse aortic constriction (TAC) or sham surgery in male and female WT and $ER\beta^{-/-}$ mice were performed. Echocardiographic and hemodynamic measurements were done. Animals were sacrificed after 2 or 9 weeks. Hearts were analyzed by microarrays (MA), histology, RT-PCR and immunoblot. Results: Nine weeks after TAC sex differences in the development of MH appeared and more pronounced in WT males than in females. In the $ER\beta^{-/-}$animals males developed a more excentric form of MH than females after TAC. It was accompanied by the impairment of systolic and diastolic function. Male WT TAC animals showed a strong increase in cardiac fibrosis whereas female WT hearts showed no changes of collagen content after TAC. In contrast $ER\beta^{-/-}$ female animals developed more severe fibrosis after TAC. Only $ER\beta^{-/-}$ male TAC animals went into a strong apoptotic gene program. However, MA indicated a higher mitochondrial gene expression in the respiratory chain only in WT female TAC hearts, not in male TAC hearts and not in $ER\beta^{-/-}$ TACanimals. Conclusions: ERβ contribute to the maintenance of energy homeostasis in female mice and limits the development of excentric cardiac hypertrophy, fibrosis and apoptosis in female and male mice and slows consequently the progression to heart failure.

9.18

GENDER-SPECIFIC CHANGES IN GENE EXPRESSION PROFILES DURING ACUTE SEVERE MG DEFICIENCY IN THE RAT HEART

M. Isabel Tejero-Taldo[1], William Weglicki[2]
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Mg deficiency has been found associated with cardiovascular disease, metabolic disorders, and in cancer patients treated with cardiotoxic drugs. In most cases, the relationship is not well characterized and any gender-specific influence in the process unknown. Objective: to examine gender-specific alterations in gene expression patterns during acute MgD in the healthy heart. Sprague-Dawley male and female rats were fed control or low Mg diet (10% of daily recommended Mg) during 4 or 7 days. Hearts were perfused in Langendorff mode for 10 min to ensure all blood was removed, and then stored in RNA later. mRNA expression profiling was done with RaGene 1.0 arrays (Affimetrix®) on total RNA from heart ventricles. Normalized expression data was further analyzed for significant differences in gene expression. Results: Gene expression profiles are similar under basal conditions in males and females with just 21 genes differently expressed ($p<0.05$; fold changes > 1.5). The biggest difference in gene profiles was observed at 4 days of MgD with 555 genes differently expressed ($p<0.05$; fold changes > 1.5) between males and females, then decreasing in number by 7 days (308 genes, $p<0.05$; fold changes > 1.5). Preliminary pathway analysis seems to indicate changes in cell signaling, metabolism and DNA integrity networks the most important. Conclusion: Gender is a significant source of variation in gene expression profiles during acute MgD, perhaps impacting associated pathologies.

9.19

FEMALE GENDER PROTECTS AGAINST OXIDATIVE STRESS AND IMPAIRED INSULIN-STIMULATED GLUCOSE UPTAKE IN SKELETAL MUSCLE IN TGR (MREN-2)27 RATS

Camila Manrique[1], Guido Lastra[1], Lama Appesh[1], James Sowers[1]
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Insulin resistance is linked to activation of the renin-angiotensin-aldosterone system (RAAS) and increased oxidative stress. We investigated the effect of gender on the production of reactive oxygen species (ROS), NAPDH oxidase activity, skeletal muscle glucose transport in transgenic (mRen2)27 Ren2 rats which display increased tissue angiotensin II, serum aldosterone, ROS production and whole-body insulin resistance. We used 8-11 week old, male and female, Ren2 and age-matched Sprague-Dawley (SD) rats. Soleus NADPH oxidase activity was assessed by spectrophotometry, ROS production by chemiluminescence, and 2-deoxyglucose transport in the presence or absence of insulin. Systolic blood pressure was lower in Ren2 females compared to males (189.6±3.0 vs 170.8±10.5 mmHg, $p<0.05$). NADPH oxidase activity was higher in Ren2 males compared to females (6.378±0.417 vs 4.1±0.246 mOD/min/mg, $p<0.05$). ROS production was similar between females but was lower in Ren2 females compared to males (102.69±4.48vs170.69±11.33 RLU/sec/mg, $p<0.05$). Insulin-stimulated glucose uptake was similar among SD animals, but higher in Ren2 females relative to males (0.398±0.039vs0.636±0.189 nmol/mg/min, $p<0.05$). Our findings suggest the existence of gender differences in oxidative stress and insulin-stimulated glucose transport in skeletal muscle in the Ren2 model, which could reflect a modulation of RAAS by sexual steroids. Supported by NIH (R01 HL73101-01A1) and Veterans Affairs Merit System (0018) grants.

9.20

IMPACT OF AGING AND ABDOMINAL OBESITY IN THE CONTROL OF HYPERTENSION IN WOMEN

Judith Zilberman[1], Nora Vainstein[1], Gustavo Cerezo[1], Augusto Vicario[1], Mariano Carasa[2], Mildren Del Sueldo[1]
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Introduction: Hypertension (HYP) and abdominal obesity (AO) are risks factors for cardiovascular disease (CVD) in postmenopausal women (W).Little is known about blood pressure (BP) control in hypertensive W, or the relationship between HYP and AO.Aim: To study the prevalence and control of HYP according to age in W, and the relationship between HYP and AO. Methods: In a cross sectional study, 1199 W (18-85yrs.) were evaluated between 2006 and 2007 (Cardiovascular Disease Prevention Program for W in V. María, Córdoba, Argentina).AO was defined as a waist circumference (WC) ≥88 cm and HYP as a SBP and/or DBP ≥

140/90 mm Hg and controlled HYP (HYPc) as SBP/DBP <140/90 mm Hg. Results: Two age groups (G) were included: G1:<50 yrs (n=507), WC:88,73±14,4 and G2:>49 yrs (n=692), WC: 96,42±8.3.According AO >87cm G1/G2: n: 260/530 p=<0,001, Age:38,9±7,9/63,5±8,6 yrs, WC:99,97±9,7/102±9,7 cm(p=0,046), SBP/DBP/WC, G1: 121,11±17,9/ 71,83±11,4, G2:139,3±21,9/ 79,2±12,6 (p<0,001); HYP:26% (n=67)/ 73,2%(388) p<0,001; SBP/DBP/HYP G1:140,83±16,7/ 83,43±10,9; G2 145,9±21,4/ 82,2±13(p= 0,002); HYPc:25,4% (n=17)/ 30%(n=116)p=NS; SBP/DBP/HYPc G1:123,12±11/73,47±10,1,G2: 123,3±9,9/71,2±8,3 p=NS. Conclusion: in our population the BP increase was related to age and AO. Both G showed poor HYPc and no relationship with AO. Poor HYPc and incidence of AO should be improved. It is necessary to design strategies to improve HYPc, reduce AO and other risks factors in women to prevent CVD.

9.21

ROLE OF GENDER IN INSULIN RESISTANCE AND MYOCARDIAL OXIDANT STRESS IN THE TG(MREN2)27 RAT

Rebecca Schneider[1], Megan S Johnson[1], Javad Habibi[1], Adam T Whaley-Connell[1], James R Sowers[1]

[1]Internal Medicine, University of Missouri, 1 Hospital Drive, D110 Diabetes Center, Columbia, MO, 65212.

Cardiovascular disease (CVD) is among the leading causes of death in older women. Insulin resistance and hypertension are risk factors for developing CVD complications, and activation of the renin-angiotensin-aldosterone system (RAAS) has been implicated in the pathogenesis of CVD associated with insulin resistance. However, the role of gender in RAAS-mediated CVD is unknown. We investigated gender-associated differences in the development of insulin resistance and hypertension in the TG(mREN2)27 (Ren2) rat, which expresses the mouse renin transgene with subsequent RAAS activation. At 9 weeks of age, both Ren2 males and females developed elevations in systolic blood pressure (SBP) and increases in left ventricular (LV) mass compared to Sprague Dawley controls. However, SBP, LV mass, and systemic insulin resistance were more severe in Ren2 males compared to females. Both Ren2 males and females develop excess LV reactive oxygen species (ROS); interestingly, despite differences in LV mass, ROS production did not differ between genders. Additionally, no gender differences were apparent in myocardial glucose uptake as determined via micro-positron emission tomography. These data demonstrate that at 9 weeks of age, Ren2 females develop insulin resistance and hypertension to a lesser extent than males despite similarly elevated ROS levels, suggesting the presence of a protective, as yet unidentified, mechanism against an overactive RAAS in females. Support provided by NIH and VA.

9.22

ANDROGEN PROMOTES ANGIOTENSIN II INDUCED ABDOMINAL AORTIC ANEURYSMS IN FEMALE HYPERLIPIDEMIC MICE THROUGH VASCULAR SMOOTH MUSCLE AT1A RECEPTORS

Xuan Zhang[1], Alan Daugherty[2], Lisa Cassis[1]

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Objective: Previous studies demonstrated that deficiency of angiotensin type 1a receptors (AT1aR) ablates angiotensin II (AngII) induced abdominal aortic aneurysm (AAA) formation in male hyperlipidemic mice. We hypothesized that androgen stimulates vascular smooth muscle cells (VSMC) AT1aR expression in abdominal aortas to increase AAA formation in female mice. ***Methods and Results:*** Female ApoE-/- mice were ovariectomized, and 1 week later implanted with pellets containing placebo (P) or dihydrotestosterone (DHT; 10 mg). After 1 week of P/DHT administration, female mice were infused with saline or AngII (1000 ng/kg/min) for 28 days. Administration of DHT increased AT1aR mRNA abundance in abdominal, but not thoracic aortas (abdominal AT1aR/18S ratio: P, 0.11± 0.03; DHT, 0.25±0.03 P<0.05) and also increased AAA from 22% to 66%. In separate studies, female (LDLR-/-) mice that are either wild type (AT1aR f/f Cre0/0) or VSMC-specific AT1aR deficient (AT1aR f/f Cre0/+) were administered DHT and infused with AngII for 28 days. In VSMC AT1aR deficient females AAA incidence decreased from 80% to 50% and maximal abdominal aortic diameter was reduced compared to wild type mice(At1aR f/f Cre0/0,1.90±0.22; AT1aR f/f Cre0/+, 1.35±0.14 mm, P<0.05). ***Conclusions:*** These results demonstrate that upregulation of AT1aR mRNA in VSMC contributes to the effect of androgen to promote AngII induced AAAs in female hyperlipidemic mice. Fundings: AHA (predoctoral fellowship 0815513D) and NIH P01 HL080100.

9.23

GENDER DIFFERENCES IN AORTIC ENDOTHELIAL FUNCTION IN A RAT MODEL OF TYPE 1 DIABETES: POSSIBLE ROLE OF SUPEROXIDE AND CYCLOOXYGENASE

Avantika Kekatpure[1], Leigh Anderson[2], Roshanak Rahimian[1]

[1]Department of Physiology and Pharmacology, Thomas J. Long School of Pharmacy and Health Sciences, University of Pacific, 3601 Pacific Avenue, Stockton, CA, 95211, [2]Department of Anatomical Sciences, Arthur A. Dugoni School of Dentistry, University of Pacific, 2155 Webster Street, San Francisco, CA, 94115.

To date little is known of the interaction between diabetes and sex hormones in the vasculature. The objectives of this study were to investigate whether there is a gender difference in the aortic endothelial function in streptozotocin (STZ, 65 mg/kg, iv)-induced diabetic rats, and the potential role of superoxide and cyclooxygenase (COX) metabolites in diabetes-induced vascular dysfunction. Endothelium dependent vasodilation (EDV) to acetylcholine (ACh; 10^{-8} to 10^{-5}M) in aortic rings precontracted with phenylephrine (PE; 2μM) were obtained before and after pretreatment with MnTMPyP (10mM), a superoxide scavenger, or indomethacin (10μM), a COX inhibitor. Constrictor dose-response curves to PE (10^{-8} to 10^{-5}M) were also generated before and after pretreatment with indomethacin. STZ-induced diabetes impaired EDV in both genders, but the effects of diabetes was more pronounced in females. Female diabetic rats also demonstrated a greater potentiation of the PE responses after indomethacin compared with males. Although, indomethacin reduced ACh-induced dilation in both male and female diabetic animals, prior incubation of rat aorta with MnTMPyP fully restored diabetes-induced impairment of EDV in both genders. These results suggest the predisposition of female rat aorta to vascular injury in diabetes, possibly via superoxide production. Furthermore, they suggest that COX metabolites play a role in the vascular reactivity of the aorta in diabetic rats (Supported by NIH/NIDCR).

10.0: SEX STEROIDS AND THE HEART

10.1

HORMONE REPLACEMENT THERAPY: WHAT'S NEW SINCE WHI?

Jane F. Reckelhoff and Licy L. Yanes.

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In the past 7 years since the first information from the Women's Health Iniative Study reports were published, the use of hormone replacement therapy (HRT) for postmenopausal women has decreased significantly. The study did not support previous studies showing that HRT protects against cardiovascular disease. In addition, the study showed that HRT was associated with increased ischemic stroke, pulmonary emboli and breast cancer. These data were surprising since experimental studies using animal and in vitro models strongly suggest that estradiol is protective as measured by many parameters. In this talk, we will examine the new studies performed in women since the WHI and new studies underway to address the shortcomings of WHI.

11.0: SEX STEROIDS AND METABOLIC SYNDROME

11.2

SEX DIFFERENCES IN DIABETIC END-ORGAN COMPLICATIONS

C. Maric

University of Mississippi Medical Center, Jackson, MS 39216.

While the incidence of cardiovascular and renal disease is lower in pre-menopausal women compared with age-matched men, this female protection is lost in diabetes. In fact, women with diabetes have 5 times, as opposed to 2 times in men, the incidence of congestive heart failure compared to non-diabetics. Women with diabetes also have more adverse outcomes after a vascular event than men. The data with respect to sex-differences in renal disease caused by diabetes is not as well understood; however, what is certain is that diabetes erases the advantage of the female sex as a protective factor against both cardiovascular and renal disease. The question is why and how?. Several clinical as well as experimental studies have suggested that diabetes is associated with dysregulation of sex hormone synthesis or secretion, resulting in imbalances in circulating sex hormone levels. In men, diabetes is associated with low levels of testosterone while low levels of estradiol are commonly seen in diabetic women. These observations strongly imply that restoring sex hormone levels to those of non-diabetics may be protective in attenuating end-organ complications associated with diabetes. Experimental studies have shown that while supplementation of estradiol in diabetic animals is generally renoprotective, supplementation of testosterone has thus far produced variable results, from having no effect, adverse or protective. Studies from our laboratory have shown that the complete absence of testosterone exacerbates diabetic renal disease and diabetic retinopathy. Supplementation of testosterone at a lower dose is renoprotective while higher doses are detrimental. Alongside decreases in testosterone, studies in our laboratory have shown that the male STZ-induced diabetic rat exhibits increased circulating estradiol levels, suggesting that estradiol may in fact have adverse effects in males, yet protective effects in the females (as previously shown). Attenuating estradiol production in diabetic males affords renoprotection. These studies strongly support a role for sex steroids in the pathophysiology of diabetic end-organ complications. Future studies should be directed towards examining the mechanisms by which sex steroids exert their actions in non-reproductive tissues, such as the heart and the kidney.

11.3

ESTROGENS, ANDROGENS AND METABOLIC RISK IN MEN

Maciej Tomaszewski[1]

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Men are more prone to – and likely to die of – cardiovascular and metabolic disorders compared with women of a similar age. This "male disadvantage" may be related to the sex-specific effects of endogenous sex hormones. Dominance of androgens over estrogens in men is unlikely to explain this sexual dimorphism as increased levels of testosterone are linked to protective rather than detrimental effects on male cardiovascular system. In contrast, increased plasma levels of estrogens (mainly estradiol) correlate with higher risk of atherosclerosis in men. The association between endogenous estrogens and male cardiovascular morbidity and mortality may be mediated, at least in part, by traditional metabolic risk factors - low HDL-cholesterol and elevated LDL-cholesterol. Further studies should focus on sexual differences underlying molecular mechanisms of sex steroids actions and their interplay with environmental factors to elucidate why endogenous estrogens (that are generally perceived as cardio-protective in women) may increase metabolic and cardiovascular risk in men. References: 1. Tivesten A, et al. Low serum testosterone and high serum estradiol associate with lower extremity peripheral arterial disease in elderly men. The MrOS Study in Sweden. J Am Coll Cardiol. 2007;50:1070-1076.* This study shows for the first time that low testosterone and high estradiol levels associate with peripheral arterial disease in men. 2. Tomaszewski M, et al. Association between lipid profile and circulating concentrations of estrogens in young men. Atherosclerosis. 2009;203:257-62. * One of the first studies to show that increased circulating concentrations of estrogens may have negative impact on lipid profile in young men. Supported by NIH Fogarty International Research Collaboration Award (R03 TW007165).

NOTES

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AUTHOR INDEX

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APS ET-11

11th International Conference on Endothelin

ET-11: APS International Conference on Endothelin

Montréal, Canada

September 9-12, 2009

MEETING PROGRAM & ABSTRACTS

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ET-11: APS International Conference on Endothelin
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Registration Opens at 3:00 PM on Wednesday, September 9, 2009

Thursday, September 10	Friday, September 11	Saturday, September 12
7:00 AM **Registration** 8:15 – 8:30 AM **Opening Remarks:** **Pedro D'Orléans-Juste**, (Chair) Univ. of Sherbrooke **David M. Pollock**, (Chair) Med. Col. of Georgia 8:30 – 9:00 AM **Key Note Lecture:** **Donald E. Kohan,** Univ. of Utah Hlth. Sci. Ctr. 9:00 – 10:00 AM Symposia I: **Cellular Regulation of the Endothelin Pathway** **Subrata Chakrabarti,** (Chair) Univ. of Western Ontario **Andrey Sorokin,** (Chair)**,** Med. Col. of Wisconsin 10:00 - 10:30 AM **Break** 10:30 – 12:00 Noon Symposia II: **Novel Physiological and Pharmacological Mechanisms** **Anthony P. Davenport,** (Chair) Univ. of Cambridge **Janet J. Maguire,** (Chair) Univ. of Cambridge **Stephanie W. Watts,** (Speaker) Michigan State Univ. 12:00 Noon – 2 :00 PM **Lunch and Poster Session I** 2:00–3:30 PM Symposia III: **Endothelin in Development and Aging** **Eric Thorin,** (Chair) Univ. of Montréal **Sandra T. Davidge,** (Chair) Univ. of Alberta **Christopher A. DeSouza,** (Speaker) Univ. of Colorado 3:30–3:45 PM **Break** 3:45–5:15 PM Symposia IV: **Pulmonary Function and Disease** **Bruno J. Battistini,** (Chair) Acasti Pharma, Inc. **Jocelyn Dupuis,** (Chair) Univ. of Montréal 5:15–6:15 PM Career Symposia V: **Pedro D'Orléans-Juste**, (Chair) Univ. of Sherbrooke **David M. Pollock**, (Chair) Med. Col. of Georgia	7:30 AM **Registration** 8:15 – 10:00 AM Symposia VI: **Roles of Endothelin-1 on Cardiac Function and Diseases** **Duncan Stewart,** (Chair) Ottawa Hlth. Res. Inst. **Ghassan Bkaily,** (Chair) Univ. of Sherbrooke **Matthais Barton,** (Speaker) Univ. Hosp. of Zurich 10:00 - 10:15 AM **Break** 10:15 – 12:00 Noon Symposia VII: **Roles of Endothelin-1 on Vascular Function and Diseases** **Ernesto L. Schiffrin,** (Chair) McGill Univ. **Adel Giaid,** (Chair) McGill Univ. **Rita C. Tostes,** (Speaker) Med. Col. of Georgia 12 :00 Noon – 2 :00 PM **Lunch and Poster Session II** 2:00–3:45 PM Symposia VIII: **Endothelin-1 Renal, Fluid and Electrolyte Physiology and Disease** **Ariela Benigni,** (Chair) Mario Negri Inst. **Donald E. Kohan, (Chair)** Univ. of Utah Hlth. Sci. Ctr. 3:45 – 4:00 PM **Break** 4:00 – 6:10 PM Ancillary Session IX: **Clinical Trials with ET Antagonists: An Update** **David Pittrow,** (Chair) Tech. Univ. Dresden **David J. Webb,** (Chair) Univ. of Edinburgh 7:00 – 11:00 PM ***Special Purchase Event:*** **Montreal Museum of Fine Arts** Experience some of the finest collection of art while enjoying fine dining and musical entertainment.	7:30 AM **Registration** 8:15 – 10:00 AM Symposia X: **Roles of Endothelin-1 in Neurophysiology, Stroke and Related Diseases** **Adviye Ergul,** (Chair) Univ. of Georgia **Pierre Moreau,** (Chair) Univ. of Montréal **Edith Hamel,** (Speaker) McGill Univ. 10:00 - 10:15 AM **Break** 10:15 – 12:00 Noon Symposia XI: **Endothelin-1 in Cancer and Blood Diseases** **Subrata Chakrabarti,** (Chair) Univ. of Western Ontario **Robert Sabbagh,** (Chair) Univ. of Sherbrooke **Anna Bagnato,** (Speaker) Regina Elena Cancer Inst. 12 :00 Noon – 2 :00 PM **Lunch and Poster Session III** 2:00 - 3:30 PM Symposia XII: **Endothelin-1 in Inflammatory Diseases and Pain** **Giles A. Rae,** (Chair) Univ. Fed. de Santa Caterina **Anil Gulati,** (Chair) Midwestern Univ. 3:30 – 3:45 PM **Break** 3:45 – 4:10 PM **Highlights of the APS/ET-11 Conference** **Masashi Yanagisawa**, Univ. of Texas Southwestern Med. Ctr. 4:10 – 4:25 PM **Closing Remarks:** **David M. Pollock**, (Chair) Med. Col. of Georgia **Pedro D'Orléans-Juste**, (Chair) Univ. of Sherbrooke

Location:

The 2009 APS Conference, ET-11: International Conference on Endothelin will be held September 9–12, 2009 at the Montreal Marriott Chateau Champlain Hotel, 1050 de la Gauchetiere West., Montreal, QC H3B 4C9, Canada, telephone (514) 878-9000, FAX: (514) 878-6161.

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Any person who has received a Ph.D. degree in physiology or related field, within five years of this conference, as attested to by the department head is eligible to register at the postdoctoral fee. **A statement signed by the department head must accompany the registration form and remittance when registering.**

Press:

Press badges will be issued at the APS registration desk, only to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public affairs, etc.) must register as nonmembers.

Special Ticketed Event:

Join your colleagues for a special evening event at the Montreal Museum of Fine Arts, one of the finest cultural venues in Montreal. Enjoy fine dining and musical entertainment, while catching up with old and new acquaintances. The cost is $45 each. To purchase a ticket please visit the registration desk located in the Viger Ballroom Foyer. Tickets are limited and are on first come, first-served basis.

Program Objective:

Upon completing the program, participants should gain more knowledge in the physiology and pathophysiology of endothelin. The goal of the conference is to accumulate together a critical mass of scientists and those in industry who have interests in the important role of endothelin to promote the exchange of ideas and potential collaborations in the future.

Target Audience:

The intended audience for this conference includes all levels of researchers working in the field of endothelin.

This conference has been made possible through the generous support from:

Abbott Laboratories
Actelion Pharmaceuticals, Ltd.
Gilead Colorado, Inc.
Pfizer, Ltd.
AstraZeneca, UK
NIH, National Institute of Diabetes and Digestive and Kidney Diseases

THURSDAY, SEPTEMBER 10, 2009

Opening Remarks

1.0 OPENING REMARKS

Thurs., 8:15 – 8:30 AM, Salle de Bal Ballroom.

8:15 AM **1.1** Opening Comments. **Pedro D' Orleans-Juste,** *Univ. of Sherbrooke, Canada,* and **David M. Pollock,** *Med. Col. of Georgia.*

Key Note Lecture

2.0 KEY NOTE LECTURE

Thurs., 8:30–9:00 AM, Salle de Bal Ballroom.

8:30 AM **2.1** Clinical Relevance of ET Antagonists: An Update. **Donald E. Kohan.** *Univ. of Utah Hlth. Sci. Ctr.*

Symposia I

3.0 CELLULAR REGULATION OF THE ENDOTHELIN PATHWAY

Thurs., 9:00–10:00 AM, Salle de Bal Ballroom.

Co-Chairs: **Subrata Chakrabarti**, *Univ. of Western Ontario, Canada.*
Andrey Sorokin, *Med. Col. of Wisconsin.*

9:00 AM **3.1** Introduction. **Subrata Chakrabarti.** *Univ. of Western Ontario, Canada.*

9:05 AM **3.2** Regulation of ET-1 mRNA Expression by the Circadian Clock Protein Period 1. **Michelle Gumz.** *Univ. of Florida.*

9:15 AM **3.3** ET-1 Regulates the Epithelial Na^+ Channel via β Pix/14-3-3 Complex. **Alexander Staruschenko.** *Med. Col. of Wisconsin.*

9:25 AM **3.4** ETB Receptor is Responsible for Nuclear Calcium Regulation by ET-1. **Levon Avedanian.** *Univ. of Sherbrooke, Canada.*

9:35 AM **3.5** ETB-dependent Inhibition of Neointimal Proliferation is not Mediated by Receptors Expressed on the Endothelium. **Nicholas Kirkby.** *Univ. of Edinburgh, UK.*

Symposia II

4.0 NOVEL PHYSIOLOGICAL AND PHARMACOLOGICAL MECHANISMS

Thurs., 10:30 AM–12:00 Noon, Salle de Bal Ballroom.

Co-Chairs: **Anthony Davenport,** *Univ. of Cambridge, UK.*
Janet Maguire, *Univ. of Cambridge, UK.*

10:30 AM **4.1** Introduction. **Anthony Davenport.** *Univ. of Cambridge, UK.*

10:35 AM **4.2** The 411 on ET Receptors: What's New? **Stephanie W. Watts.** *Michigan State Univ.*

10:55 AM **4.3** Genetic Study of ET-2 Function in Mice. **Inik Chang.** *Univ. of Texas Southwestern Med. Ctr.*

11:05 AM **4.4** Reduced Pressor Responses to Big-ET-1 in mMCP-4 KO Mice. **Martin Houde.** *Univ. of Sherbrooke, Canada.*

11:15 AM **4.5** CGRP Promotes Dissociation of ET-1 from ETA-receptors and Terminates Vasoconstriction in Rat Resistance Arteries. **Merlijn J.P.M.T. Meens.** *Maastricht Univ., The Netherlands.*

11:25 AM **4.6** Discrepancies in Antagonist Affinities for Human Vascular ET_A Re-ceptors Determined in Functional and Binding Assays. **Janet Maguire.** *Univ. of Cambridge, UK.*

11:35 AM **4.7** Greater Functional ETB Receptor Antagonism with Bosentan than Sitaxsentan in Man. **Iain MacIntyre.** *Univ. of Edinburgh, UK.*

11:45 AM **4.8** Involvement of Endothelin in the Actions of β-Amyloid. **A. Gulati.** *Midwestern Univ.*

Poster Session

5.0 POSTER SESSION I

Thurs., 12:00 Noon–2:00 PM, Viger A/B/C.

Board #

1 **5.1** Endothelin (ET-1) Increases Albumin Permeability of Isolated Rat Glomeruli. **M. Saleh, J. Pollock, V. Savin, and D. Pollock.** *Med. Col. Of Georgia and Med. Col. of Wisconsin.*

2 **5.2** Regulation of Collecting Duct Endothelin-1 Production. **D. Kohan, P. Stricklett, and K. Strait.** *Univ. of Utah Hlth. Sci. Ctr.*

3 **5.3** Role of Endothelin on Lung Structural Remodeling and Pulmonary Function in Ischemic Cardiomyopathy. **B. H. Jiang, J-C. Tardif, Y. Shi, and J. Dupuis.** *Montreal Heart Inst., Canada.*

4 **5.4** Atrasentan Reduces Proteinuria in Patients with Diabetic Nephropathy. **D. Andress, T. Rabelink, R. Padley, M. Amdahl, and P. Audhya.** *Abbott Labs and Leiden Univ. Med. Ctr., The Netherlands.*

5 **5.5** Oedemagenic Effect of Endothelin Agonists in Isolated Human Lungs. **R. Bennett, M. Cowen, and A. Morice.** *Hull and East Yorkshire Hosp., NHS Trust, UK., and Univ. of Hull, UK.*

6 **5.6** Heme Arginate Therapy Enhances Heme Oxygenase and Atrial Natriuretic Peptide to Abate Endothelin-1and

Board #

Suppress Renal Histopathological Lesions. **J. Ndisang, and A. Jadnav.** *Univ. of Saskatchewan Col. of Med., Canada.*

7 **5.7** Protective Effects of Endothelin-A Receptor Antagonist, BQ123, Against LPS-induced Oxidative Stress in Lungs. **A. Piechota, A. Goraca, and A. Polañczyk.** *Med. Univ. of Lodz, and Tech. Univ. of Lodz, Poland.*

8 **5.8** Chronic and Acute Dual Blockade of Endothelin Receptors Display Different Effects on Cerebrovascular Function in Type 2 Diabetes. **W. Li, A. Kelly-Cobbs, R. Prakash, K. Sachidanandam, E. Mezzetti, and A. Ergul.** *Med. Col. Of Georgia and Univ. of Georgia.*

9 **5.9** The Effect of RAS Blockade on ET-1 Levels in Distinct Renal Compartements. **I. Vaneckova, Z. Vanourkova, Z. Huskova, and L. Cervenka.** *Inst. for Clinical and Experimental Med., Czech Republic.*

10 **5.10** The Effects of Dietary Salt and Proteins on ET-1 Concentrations in Ren-2 Rats with Ablation Nephrectomy. **Z. Vanourkova, Z. Huskova, I. Vaneckova, and L. Cervenka.** *Inst. for Clinical and Experimental Med., Czech Republic.*

11 **5.11** Identification of Peptide Inhibitors by Phage Display: The Use of Endothelin-1 as a Model. **A. Lamoussenerie, D. Chatenet, M. Létourneau, J. Frappier, A. Louimaire, and A. Fournier.** *Univ. of Quebec, Canada.*

12 **5.12** ET-1 is Regulated by Erk5 in Diabetic Retinopathy. **Y. Wu, B. Feng, S. Chen, and S. Chakrabarti.** *Univ. of Western Ontario, Canada.*

13 **5.13** NH4Cl Loading Downregulates Pendrin in ETB-receptor KO Mice. **S. Frische, P. Kalk, F. M. Carpi, G. Serena de Fronzo, and B. Hocher.** *Univ. of Aarhus, Denmark, Charite Univ., Germany and Univ. of Camerino, Italy.*

14 **5.14** Endothelin Receptor A Antagonism Prevents Platelet-Activating Factor-Indiced Fetal Growth Restriction in the Rat. **L. G. Thaete, S. Khan, and M. Neerhof.** *North Shore Univ. Hlth. Sys.*

15 **5.15** ET Receptor Antagonist in the Treatment of Diabetic Ketoacidosis. **A. Gulati, M. Lavhale, K. Koenig, and S. Havalad.** *Midwestern Univ. and Lutheran Gen. Children's Hosp.*

16 **5.16** A Series of Novel 1-3-6-Trisubstituted-2-Carboxy-Quinol-4-One

Board #

Endothelin-A Antagonists. **N. Olgun, R. Stephani, H. Patel, and S. Reznik.** *St. John's Univ.*

17 **5.17** Frog Endothelins: cDNA Cloning, Sequence and Evolutionary Analysis of Endothelins. **K. Saida, J. Quan, S. Takizawa, J. Adur, E. Nara, and T. Uchide.** *Natl. Inst. of Advanced Indust. Sci. and Tech., Japan.*

18 **5.18** Endothelin Converting Enzyme-1: A Plausible Target Gene for Hypoxia Inducible Factor. **M. Khamaisi, J. Axelrod, R. Karry, A. Shina, and S. Heyman.** *Rambam Med. Ctr., Goldyne Savad Inst. Of Gene Therapy, Univ. of Haifa and Hadassah Med. Ctr., Jerusalem, Israel.*

19 **5.19** Human Podocytes Express Endothelin Receptors and Display ETA Receptor-dependent ET-1 and Pro-inflammatory Cytokine Production. **N. Dhaun, E. Owen, N. Johnston, J. Goddard, D. Webb, and D. Kluth.** *Univ. of Edinburgh, UK.*

20 **5.20** TLR4 Signaling-Mediated Endothelin Upregulation is Central to Ischemia/Reperfusion-Induced Fetal Growth Restriction in the Mouse. **L. G. Thaete, X. Qu, M. Neerhof, and T. Jilling.** *North Shore Univ. Hlth. Sys.*

21 **5.21** Structural Aspects of Endothelin Receptor Antagonists. **S. Andurkar and A. Gulati.** *Midwestern Univ.*

22 **5.22** Chronic Activation of the Renal Endothelin Receptors Promotes Sickle Cell Glomerulopathy in Mice. **P-L. Tharaux, N. Sabaa, C. Fligny, and M. Milon.** *INSERM & Univ. of Paris-Descartes, France.*

23 **5.23** Correlation of Saliva and Plasma Endothelin Isoforms bET-1, ET-1,ET-2 and ET-3 in Healthy Humans. **R. Gurusankar, P. Kumarathasan, A. Saravanamuthu, E. Thomson, and R. Vincent.** *Environmental Hlth. Sci. and Res. Bureau, Canada.*

24 **5.24** Chronic Intravenous Infusion of Endothelin-1 does not Change Plasma Aldosterone Level in Rat. **D. Nakano, Y. Fujisawa, N. Pelisch, H. Hitomi, Y. Nishiyama, and A. Nishiyama.** *Kagawa Univ., Japan.*

25 **5.25** ETAR and B1R Antagonism as Osteoarthritis Therapies. **G. Kaufman, C. Zaouter, P. Sirois, and F. Moldovan.** *Univ. of Montreal and Univ. of Sherbrooke, Canada.*

26 **5.26** Endothelin$_A$ Receptor Upregulated but ET$_B$ Downregulated in Pulmonary Arterial Hypertension. **R. Kuc, J. Maguire,**

Board #

M. Carlebur, Y. Zheng, M. Toshner, N. Morrell, N. Davie, and A. Davenport. *Univ. of Cambridge and Pfizer, Ltd., UK.*

27 **5.27** Endothelin-1 Impairs Skeletal Muscle Glucose Uptake via a Mechanism Involving Insulin Receptor Substrate-1 Downregulation. **A. Shemyakin, F. Salehzadeh, F. Bohm, A. Krook, and J. Pernow.** *Karolinska Inst., Sweden.*

28 **5.28** Chronic Endothelin-A Receptor Antagonism Reduces Proteinuria in Chronic Kidney Disease Through Effects on Renal Haemodynamics. **N. Dhaun, I. MacIntyre, D. Kerr, V. Melville, N. Johnston, J. Goddard, and D. Webb.** *Univ. of Edinburgh, UK.*

29 **5.29** Role of ET-1 in the Induction of Placental Endoplasmic Reticulum Stress in Pregnancy Disorders. **A. Jain.** *Univ. of Cambridge, UK.*

30 **5.30** ET_B Receptor Deficiency Enhances the Acute but not Chronic Effects of Angiotensin II on the Blood Pressure of Female Rats. **E. I. Boesen, B. R. Giles, H. M. Socha, and D. M. Pollock.** *Med. Coll. of Georgia.*

Symposia III

6.0 ENDOTHELIN IN DEVELOPMENT AND AGING

Thurs., 2:00–3:30 PM, Salle de Bal Ballroom.

Co-Chairs: **Eric Thorin**, *Univ. of Montreal, Canada.*
Sandra T. Davidge, *Univ. of Alberta, Canada.*

2:00 PM **6.1** Introduction. **Eric Thorin.** *Univ. of Montreal, Canada.*

2:05 PM **6.2** Exercise and Age-Related ET-1 Mediated Vasoconstrictor Tone. **Christopher A. DeSouza.** *Univ. of Colorado, Boulder.*

2:30 PM **6.3** Endothelin Receptor A Antagonism Prevents Placental Pathology Caused by Uterine Ischemia/Reperfusion-Induced Oxidative Stress in the Rat. **Larry Thaete.** *North Shore Univ. Hlth. Sys.*

2:45 PM **6.4** The Development of Alzheimer Disease like Pathology in YAC AbPP Transgenic Mice is Associated with the Overexpression of Endothelin-1 and Nitric Oxide Synthase. **Gjumrakch Aliev.** *Univ. of Texas South-western Med. Ctr.*

3:00 PM **6.5** Exocytosis of Endothelin-1 from Endothelial Cells Induces Rapid Vasoconstriction in Aged Arteries. **Aditya Goel.** *Johns Hopkins Med. Inst.*

3:15 PM **6.6** The Metabolic Syndrome is Associated with Greater ET-1 Mediated Vasoconstrictor Tone in Older Men. **Michael Mestek.** *Univ. of Colorado, Boulder.*

Symposia IV

7.0 PULMONARY FUNCTION AND DISEASE

Thurs., 3:45–5:15 PM, Salle de Bal Ballroom.

Co-Chairs: **Bruno J. Battistini,** *Acasti Pharma., Inc.*
Jocelyn Dupuis, *Univ. of Montreal, Canada.*

3:45 PM **7.1** Introduction. **Bruno J. Battistini.** *Acasti Pharma., Inc.*

3:50 PM **7.2** TGFβ and BMP9 Stimulation of Endothelin-1 Synthesis in Human Lung Microvascular Endothelial Cells: A Comparative Study. **Gregory Star.** *Jewish Genl. Hosp., Montreal, Canada.*

4:05 PM **7.3** $ET_{A/B}$ Blockade has no Pulmonary Vasodilator Effect After PDE5-Inhibition. **Daphne Merkus.** *Erasmus MC Hosp., Rotterdam, The Netherlands.*

4:20 PM **7.4** Disruption of Endothelin Heterodimers Alters Pulmonary Vascular Reactivity to Endothelin-1. **Stephanie Sauvageau.** *Montreal Heart Inst., Canada.*

4:35 PM **7.5** Endothelin-1 Mediates the Effect of Transforming Growth Factor-β on Wound Repair and Skin Fibrosis. **David Lagares.** *Centro de Investigaciones Biológicas, Madrid, Spain.*

4:50 PM **7.6** Involvement of Endothelin-1 in Habitual Aerobic Exercise-induced Increase in Arterial Compliance. **T. Miyauchi.** *Univ. of Tsukuba, Japan.*

Limited Dinner Tickets are Available for the Evening Event at the Montreal Museum of Fine Arts

See Registration Desk for Details

Career Symposia V

8.0 THE MARRIAGE BETWEEN ACADEMIA AND THE PHARMACEUTICAL INDUSTRY: ET SHOWS US THE WAY

Thurs., 5:15–6:15 PM, Salle de Bal Ballroom.

Moderator: **David M. Pollock,** *Med. Col. of Georgia.*

Panelists: **Pedro D'Orleans-Juste,** *Univ. of Sherbrooke, Canada.*

Craig F. Plato, *Gilead Sciences, Inc.*
Stephanie Sauvageau, *Montreal Heart Inst.*
Bruno J. Battistini, *Acasti Pharma., Inc.*
Takashi Miyauchi, *Univ. of Tsukuba, Japan.*

FRIDAY, SEPTEMBER 11, 2009

Symposia VI

9.0 ROLES OF ENDOTHELIN-1 ON CARDIAC FUNCTION AND DISEASE

Fri., 8:15–10:00 AM, Salle de Bal Ballroom.

Co-Chairs: **Duncan Stewart,** *Ottawa Hlth. Res. Inst., Canada.*
Ghassan Bkaily, *Univ. of Sherbrooke, Canada.*

8:15 AM **9.1** Introduction. **Duncan Stewart.** *Ottawa Hlth. Res. Inst., Canada.*

8:20 AM **9.2** What You Should Know About Endothelin. **Matthais Barton.** *Univ. of Zurich, Switzerland.*

8:45 AM **9.3** Endothelial Cells Derived Endothelin-1 Promotes Cardiac Fibrosis in Diabetic Heart through Stimulation of Endothelial to Mesenchymal Transition. **Bambang Widyantoro.** *Kobe Univ. Grad. Sch. of Med., Japan.*

9:00 AM **9.4** Crosstalk Between Endothelin-1 and Neuropeptide Y in Human Endocardial Endothelial Cells. **Chantale Provost.** *Univ. of Sherbrooke, Canada.*

9:15 AM **9.5** Doxorubicin-Induced Cardiomyopathy is Attenuated in ECE-1 Heterozygous Knockout Mice via Preventing the Impairment of Cardiac Mitochondrial Bio-genesis. **Kazuya Miyagawa.** *Kobe Univ. Grad. Sch. of Med., Japan.*

9:30 AM **9.6** Additional Lack of eNOS Promotes Cardiac Fibrosis in ET-1 Transgenic Mice. **Nicolas Vignon-Zellweger.** *Charite Univ., Germany.*

9:45 AM **9.7** Effect of Bosentan on Reductive Stress Induced Endothelin-1/eNOS Dysfunction in Cardiomyocytes. **Rajasekaren N. Soorappan.** *Univ. of Utah Hlth. Sci. Ctr.*

Symposia VII

10.0 ROLES OF ENDOTHELIN-1 ON VASCULAR FUNCTION AND DISEASE

Fri., 10:15 AM–12:00 Noon, Salle de Bal Ballroom.

Co-Chairs: **Ernesto Schiffrin,** *McGill Univ., Canada.*
Adel Giad, *McGill Univ., Canada.*

10:15 AM **10.1** Introduction. **Ernesto Schiffrin.** *McGill Univ., Canada.*

10:20 AM **10.2** O-GlcNAcylation: Another Mechanism for the Effects of ET-1 on the Vasculature? **Rita Tostes.** *Med. Col. of Georgia.*

10:45 AM **10.3** Effect of Endothelin-1 Overexpression on Vascular Structure and Function of Apolipoprotein E Knockout Mice. **Melissa Li.** *McGill Univ., Canada.*

11:00 AM **10.4** Attenuated Collateral Formation Under Diabetes is Improved by Genetical Suppression of Endothelin Converting Enzyme-1. **Kazuhiko Nakayama.** *Kobe Univ. Grad. Sch. of Med., Japan.*

11:15 AM **10.5** Synergy of Agonistic Autoantibodies Targeting ETA- and AT1 Receptors Increases Sensitivity to Natural Ligands. **Rusan Catar.** *Charite Univ., Germany.*

11:30 AM **10.6** Dual ET Receptor Antagonism Prevents Diabetic Remodeling of Resistance Arteries: Comparison to Selective Receptor Blockade. **Kamakshi Sachidanandam.** *Univ. of Georgia.*

11:45 AM **10.7** Upregulation and Localization of Apelins in Human Atherosclerosis: Comparison with ET-1. **Anthony Davenport.** *Univ. of Cambridge, UK.*

Poster Session

11.0 POSTER SESSION II

Fri., 12:00 Noon – 2:00 PM, Viger A/B/C.

Board #

1 **11.1** Angiotensin II Hypertension Impairs ETB-dependent Natriuresis and Reduces Renal Medullary ET_B Receptor Expression. **W. Kittikulsuth, E. I. Boesen, K. A. Hyndman, J. S. Pollock and P. M. Pollock.** *Med. Col. of Georgia and Med. Col. of Wisconsin.*

2 **11.2** SPOO2P, A Botanical Drug, Inhibits Endothelin-1 Induced Aortic Contractions *in vitro* and Lowers Blood Pressure of Anesthetized Rats. **A. Adeagbo, V. Subbiah, and I. Joshua.** *Touro Univ. Coll. of Med., PhytoPharmacom, LLC, Durham, NC and Univ. of Louisville.*

3 **11.3** Angiotensin II Increases the Expression of Kinin B1 Receptor Through an Endothelin Mechanism in Vascular Smooth Muscles Cells. **M. Morand-Contant, M. Anand-Srivastava, and R. Couture.** *Univ. of Montreal, Canada.*

4 **11.4** Activation of GPER Inhibits Contraction to Endothelin-1 and Causes NO-Dependent Relaxation of Coronary Arteries. **O. Baretella, M. R. Meyer, E. R. Prossnitz,**

Board #

and M. Barton. *Univ. of Zurich, Switzerland and Univ. of New Mexico.*

5 **11.5** Impaired Flow-Induced Arterial Remodeling during DOCA-salt Hypertension. **P. Lemkens, P. Schiffers, G. E. Fazzi, J. Nelissen, B. Janssen, and J. De Mey.** *Maastricht Univ., The Netherlands.*

6 **11.6** Endothelin-1-induced Cardiac Hypertrophy is Regulated by C-terminal Domain Phosphatase of RNA Poly-merase II. **S. Sakai, N. Shimojyo, T. Ogata, K. Aonuma, and T. Miyauchi.** *Univ. of Tsukuba, Japan.*

7 **11.7** Endothelin-1 Signaling in the Female Internal Pudendal Artery: Potential Role in Female Sexual Dysfunction. **K. Allahdadi, R. Tostes, and R. C. Webb.** *Med. Col. of Georgia.*

8 **11.8** Angiotensin Type 1 Receptor and Endothelin Type A Receptor Antigen Specific T-cells and Receptor Restricted Humoral Response in Wait-listed Patients. **A. Philippe, M. Stein, B. Hegner, M. Haase, H. Heidecke, H-C. Fischer, P. Reinke, and D. Dragun.** *Charite Univ., CellTrend, Luckenwalde and KfH-Nierenzentrum, Germany.*

9 **11.9** Dendritic Cells and the Endothelin System: Implications for Atherosclerosis. **S. Shaw, R. Spirig, I. Potapova, J. Shaw, J. Tsui, and R. Rieben.** *Univ. of Bern, Switzerland and Royal Free Hosp., London, UK.*

10 **11.10** Activating AT1R and ETAR Autoantibodies Serve as Biomarkers and Link Autoimmunity and Vasculopathy in Systemic Sclerosis. **D. Dragun, M. Naether, D. N. Müller, U. Frei, H. Heidecke, F. C. Luft, M. Gollasch, and G. Riemekasten.** *Charite Univ., and CellTrend, Luckenwalde, Germany.*

11 **11.11** Differential Regulation of ERK1/2, via Downregulation of MKP-1, Mediates Sex-differences in Vascular Reactivity in DOCA-salt Hypertension. **F. Giachini, J. Sullivan, V. Lima, R. C. Webb, Z. Fortes, D. Pollock, M. H. Carvalho, and R. Tostes.** *Med. Col. of Georgia and Univ. of São Paulo, Brazil.*

12 **11.12** Effects of Exogenous Big Endothelin-1 on Post-ischemic Cardiac Dysfunction and Norepinephrine Overflow in Rat Hearts: Involvement of ET_B Receptor/NOS1 Systems. **M. Tawa, T. Fukumoto, N. Yamashita, M. Ohkita, K. Ayajiki, T. Okamura, and Y. Matsumura.**

Board #

Shiga Univ. of Med. Sci., and Osaka Univ. of Pharma Sci., Japan.

13 **11.13** Short Sleep Duration is Associated with Greater Endothelin-1 Vasoconstrictor Tone. **B. Weil, M. Mestek, C. Westby, G. Van Guilder, J. Greiner, B. Stauffer, and C. DeSouza.** *Univ. of Colorado, Boulder.*

14 **11.14** Raloxifene Reduces ET-1 Gene Expression Through the AMPK/eNOS Pathway in Cultured Calf Pulmonary Artery Endothelial Cells. **M. Ohkita, S. Ohkuma, E. Kawabata, A. Hiramatsu, M. Nishida, and Y. Matsumura.** *Osaka Univ. of Pharmaceutical Sci., Japan.*

15 **11.15** TGF-β Increases Inward Remodeling of Cannulated Small Mesenteric Arteries. **M. Schoorl, E. Bakker, and E. VanBavel.** *Univ. of Amsterdam, The Netherlands.*

16 **11.16** Effect of Pressure-Overload Hypertrophy on the Subcellular Localization of Endothelin Receptors. **C. Merlen, N. Farhat, L. R. Villeneuve, M-C. Gillis, E. Thorin, and B. G. Allen.** *Univ. of Montreal, Canada.*

17 **11.17** Darusentan is a Potent Inhibitor of Endothelin Signaling and Function in Both Large and Small Arteries. **F. Liang, C. B. Glascock, D. L. Schafer, J. Sandoval, L. Cable, and K. Pitts.** *Gilead Science, Inc.*

18 **11.18** O-GlcNAcylation Contributes to Augmented Vascular Reactivity Induced by ET-1. **V. Lima, F. Giachini, F. Carneiro, Z. Fortes, M. H. Carvalho, M. Saleh, D. Pollock, A. Ergul, R. C. Webb, and R. Tostes.** *Med. Col. of Georgia and Univ. of São Paulo, Brazil.*

19 **11.19** Endogenous Endothelin-1 Enhances the Expression of Giα Proteins in Vascular Smooth Muscle Cells from Spontaneously Hypertensive Rats: Role of Growth Factor Receptors Transactivation. **Y-H. Gomez-Sandoval, and M. B. Anand-Srivastava.** *Univ. of Montreal, Canada.*

20 **11.20** Modulation of Cardiovascular Effects of Clonidine and Centhaquin by Endothelin. **M. Lavhale, N. Parikh, M. Leonard, and A. Gulati.** *Midwestern Univ.*

21 **11.21** Expression of Endothelin Receptors in Human Carotid Atherosclerosis. **A. Rafnsson, H. Agardh, L. Folkersen, A. P. Davenport, U. Hedin, J. Pernow, and A. Gabrielsen.** *Karolinska Inst., Sweden and Univ. of Cambridge, UK.*

Board #

22 **11.22** Gene Expression in the Vasculature of Mice Overexpressing Human Endothelin-1 in the Endothelium. **S. Simeone, P. Paradis, and E. L. Schiffrin.** *McGill Univ., Canada.*

23 **11.23** Electrical Remodeling Precedes LV Dysfunction in ET-1-Induced Cardiomyopathy. **E. Mueller, A. Momen, S. Massé, Y-Q. Zhou, R. M. Henkelman, K. Nanthakumar, D. Stewart, and M. Husain.** *Univ. of Toronto and Hosp. for Sick Kids, Canada.*

24 **11.24** Hemin via Heme-oxygenase-1 Diminishes Endothelin-1-induced Phosphorylation of ERK1/2 in Vascular Smooth Muscle Cells. **D. A. Kasal, D. Garcia dos Santos, M. Fritsch, P. Paradis, P. Ponka, and E. Schiffrin.** *Lady Davis Inst. for Med. Res., Canada and State Univ. of Rio de Janiero, Brazil.*

25 **11.25** Involvement of Calmodulin-dependent Protein Kinase II alpha in ET-1-induced ERK1/2 and Protein Kinase B Signaling and Hypertrophic and Proliferative Responses in Vascular Smooth Muscle Cells. **A. Bouallegue, and A. Srivastava.** *Univ. of Montreal, Canada.*

26 **11.26** ET-1 Implication in Vascular Abnormalities in Hereditary Cardiomyopathy. **J. Al-Khoury, C. El-Khoury, M. Chahine, D. Jacques, and G. Bkaily.** *Univ. of Sherbrooke, Canada.*

27 **11.27** Elevated Endothelin-1 Expression in Dogs with Naturally Occurring Cardiovascular Disorders. **T. Uchide, K. Saida, T. Sasaki, and M. Temma.** *Rakunogakuen Univ., Natl. Inst. Of Advanced Ind. Sci. & Tech., and Kitasato Univ., Japan.*

28 **11.28** Hyperthyroidism Upregulates Vascular Endothelin B Receptor. **M. A. Carrillo Sepulveda, F. Giachini, R. Tostes, M. L. Barreto-Chaves, and R. C. Webb.** *Med. Col. of Georgia and Univ. of Sao Paulo, and Inst. of Sci. Biomed. São Paulo, Brazil.*

29 **11.29** Reduced Coronary Vasomotor Control by Endogenous Endothelin after Myocardial Infarction: Role of Prostanoids. **V. de Beer, D. Duncker, and D. Merkus.** *Erasmus MC Hosp., Rotterdam, The Netherlands.*

30 **11.30** Effect of Uremia on Endothelial Function in the Rat Thoracic Aorta *in vitro*. **M. Lebel, M. Nadeau, and R. Larivière.** *Univ. of Laval and CHUQ Res. Ctr., Canada.*

Symposia VIII

12.0 ROLES OF ENDOTHELIN-1 RENAL, FLUID AND ELECTROLYTE PHYSIOLOGY AND DISEASE

Fri., 2:00 – 3:45 PM, Salle de Bal Ballroom.

Co-Chairs: **Ariela Benigni,** *Mario Negri Inst. for Pharmacological Res., Italy.*
Donald E. Kohan, *Univ. of Utah Hlth. Sci. Ctr.*

2:00 PM **12.1** Introduction. **Ariela Benigni.** *Mario Negri Inst. for Pharmacological Res., Italy.*

2:05 PM **12.2** High Salt Diet Attenuates Afferent Arteriolar Autoregulatory Efficiency Behavior by Enhanced ETB Receptor Activation. **Edward Inscho.** *Med. Col. of Georgia.*

2:20 PM **12.3** Sitaxsentan Increases Extracellular Fluid Volume in both Normal Salt and High Salt-Fed Dahl S Rats. **Lufei Hu.** *Gilead Science, Inc.*

2:35 PM **12.4** Aldosterone Modulates Steroid Receptor Binding to Unique Elements in the Endothelin-1 Gene. **Charles Wingo.** *Univ. of Florida.*

2:50 PM **12.5** ET-Receptor Blockade Delays Progression of Tubulointerstitial Fibrosis in a Mouse Model of Alport Syndrome. **Christoph Licht.** *The Hosp. For Sick Children, Toronto, Canada.*

3:05 PM **12.6** Add-on ET_A Receptor Antagonist to ACE Inhibitor Provides Reno and Cardio Protection in Advanced Type 2 Diabetes in Rats. **Sara Cattaneo.** *Mario Negri Inst. for Pharmacol. Res., Bergamo, Italy.*

3:20 PM **12.7** Chronic Endothelin-A Receptor Antagonism Reduces Proteinuria, Blood Pressure and Arterial Stiffness in Chronic Kidney Disease. **Neeraj Dhaun.** *Univ. of Edinburgh, UK.*

Symposia IX

13.0 CLINICAL TRIALS WITH ET ANTAGONISTS: AN UPDATE

Fri., 4:00 – 6:10 PM, Salle de Bal Ballroom.

Co-Chairs: **David Pittrow**, *Tech. Univ., Germany.*
David Webb, *Univ. of Edinburgh, UK.*

4:00 PM **13.1** Introduction. **David Pittrow.** *Tech. Univ., Germany.*

4:05 PM **13.2** Macitentan, A New Tissue Targeting Dual ERA. **Martine Clozel.** *Actelion Pharma., Ltd., Switzerland.*

4:30 PM **13.3** Zibotentan (ZD4054): An ET_A-Specific Antagonist Being Developed for the Treatment of Hormone-Resistant Prostate Cancer. **Jim Growcott and Don Newling.** *AstraZeneca, UK.*

4:55 PM **13.4** Advancing the Science of Endothelin Biology: A Clinical Update on Studies with Sitaxentan Sodium. **Neil Davie.** *Pfizer, UK.*

5:20 PM **13.5** Study Designs for Ambrisentan in Idiopathic Pulmonary Fibrosis **Brent Appleton.** *Gilead Sciences, Inc.*

5:45 PM **13.6** Diabetic Nephropathy as a Target for Endothelin Antagonists. **Dennis L. Andress.** *Abbott Labs, The Netherlands.*

SATURDAY, SEPTEMBER 12, 2009

Symposia X

14.0 **ROLES OF ENDOTHELIN-1 IN NEUROPHYSIOLOGY, STROKE AND RELATED DISEASES**

Sat., 8:15 – 10:00 AM, Salle de Bal Ballroom.

Co-Chairs: **Adviye Ergul**, *Univ. of Georgia.*
Pierre Moreau, *Univ. of Montreal, Canada.*

8:15 AM **14.1** Introduction. **Adviye Ergul.** *Univ. of Georgia.*

8:20 AM **14.2** The Role of Endothelin-1 Receptors in Cerebrovascular Dysfunctions Associated with Alzheimer's Disease. **Edith Hamel.** *McGill Univ., Canada.*

8:45 AM **14.3** Activation of ETA-R Contributes to the Impaired Renorenal Reflexes in Heart Failure. **Ulla Kopp.** *Univ. of Iowa Carver Col. Med. and VA Med. Ctr.*.

8:55 AM **14.4** Cardiovascular Actions of Endothelin B Receptors in Sympathetic Ganglia. **David Kreulen.** *Michigan State Univ.*

9:05 AM **14.5** Nociceptive and Hyperalgesic Actions of Endothelins in the Trigeminal System. **Giles Rae.** *Univ. Fed. de Santa Catarina, Brazil.*

9:15 AM **14.6** Modulatory Actions of Endothelins on Neuralglial Communication in the Hypothalamic Supraoptic Nucleus. **Krishna Naskar.** *Med. Col. Of Georgia.*

9:25 AM **14.7** *in vivo* Antagonism of Endothelin Receptors in a Mouse Model of the Vascular Pathology of Alzheimer's Disease. **Panayiota Papadopoulos.** *McGill Univ., Canada.*

9:35 AM **14.8** Novel Therapy Approach in Primary Stroke Prevention: Simultaneous Inhibition of Endothelin Converting Enzyme and Neutral Endopeptidase in Spontaneously Hypertensive, Stroke–Prone Rats Improves Survival. **C. Wengenmayer.** *Charite Univ., Germany.*

Join us for the APS/ET-11 Opening Reception in the Caf' Conc'at 7:00 PM

Symposia XI

15.0 **ENDOTHELIN IN CANCER AND BLOOD DISEASES**

Sat., 10:15 AM – 12:00 Noon, Salle de Bal Ballroom.

Co-Chairs: **Subrata Chakrabarti**, *Univ. of Western Ontario, Canada.*
Robert Sabbagh, *Univ. of Sherbrooke, Canada.*

10:15 AM **15.1** Introduction. **Subrata Chakrabarti.** *Univ. of Western Ontario, Canada.*

10:20 AM **15.2** The Importance of Endothelin Axis in Initiation, Promotion and Therapy of Cancer. **Anna Bagnato.** *Regina Elena Natl. Cancer Inst., Italy.*

10:45 AM **15.3** Development of Non-invasive PET Imaging Method to Monitor Blood Flow Increase in Tumors with SPI-1620. **Guru Reddy.** *Spectrum Pharmaceuticals, Irvine.*

11:00 AM **15.4** Efficacy of the Specific ETA Receptor Antagonist Zibotentan (ZD4054). **Mohammad Heetun.** *Univ. Col. of London, UK.*

11:15 AM **15.5** The Endothelin Axis in Carcinogen-induced Rat Colon Tumors. **Rong Wang.** *Oregon State. Univ.*

11:30 AM **15.6** Molecular Mechanisms Regulating ECE-1 Expression in Prostate Cancer. **Alison Whyteside.** *Univ. of Leeds, UK.*

11:45 AM **15.7** Chemoresistant Ovarian Cancer Cells Display Altered Endothelin A Receptor Expression and Signaling. **Laura Rosanò.** *Regina Elena Natl. Cancer Inst., Italy.*

Poster Session

16.0 **POSTER SESSION III**

Sat., 12:00 Noon – 2:00 PM, Viger A/B/C.

Board #

1 **16.1** Endothelin-1 Signalling Involving Complexed NF-kB p65, MAPKp38 and PKC Isoforms in Human Tumors and Normal Tissues. **M. von Brandenstein, J.**

Board #

Johnsons, H-P. Dienes, and J. Fries. *Univ. of Koeln, Germany.*

2 **16.2** Involvement of Imidazoline and Opiate Receptors in the Enhancement of Clonidine Induced Analgesia by ETA Receptor Antagonist. **A. Gulati, M. Boxwalla, G. Matwyshyn, and B. Puppala.** *Midwestern Univ. and Advocate Lutheran Genl. Children's Hosp. Park Ridge, IL.*

3 **16.3** Acute Effects of Endothelin Receptor Antagonists on Hepatic Hemodynamics of Normal and Cirrhotic Rats. **M. Casavin, H. Semus, K. Pitts, Y. Peng, J. Sandoval, C. Plato.** *Gilead Sciences, Inc.*

4 **16.4** Cerebrovascular Remodeling and Ischemic Brain Injury in Diabetes: Role of Endothelin-1. **W. Li, A. Kelly-Cobbs, E. Mezzetti, S. Fagan, and A. Ergul.** *Med. Col. of Georgia.*

5 **16.5** Study of the Implication of MK2 in the Vascular Response to Endothelin-1. **A. Nguyen, N. Thorin-Trescases, M. Mamarbachi, M. Gaestel, B. Allen, and É. Thorin.** *Univ. of Montreal, Montreal Heart Inst., Canada and Hannover Med. Sch., Germany.*

6 **16.6** Remarkably Long-Lasting Tachyphylaxis of Pain Response to ET-1 does not Involve the Central Nervous System. **A. Khodorova, and G. Strichartz.** *Brigham & Women's Hosp., Harvard Med. Sch.*

7 **16.7** A Nuclear Function of β-arrestin-1 in Endothelin A Receptor Signaling in Ovarian Carcinoma Cells: Regulation of Histone Acetylation and Gene Transcription. **L. Rosanò, R. Cianfrocca, S. Masi, F. Spinella, V. Di Castro, P. G. Natali, and A. Bagnato.** *Regina Elena Natl. Cancer Inst., Italy.*

8 **16.8** $Endothelin_B$ Receptor Expression is Upregulated in a Rodent Model of Glaucoma. **R. Krishnamoorthy, H-Y. Ma, M. Jiang, and T. Yorio.** *Univ. of North Texas Hlth. Sci. Ctr.*

9 **16.9** Anticancer Potential of Combining the ET_A-Specific Antagonist Zibotentan (ZD4054) with Cytotoxic Chemotherapy. **J. Growcott.** *AstraZeneca, UK.*

10 **16.10** Zibotentan (ZD4054) is an ET_A-Specific Antagonist that does not Acutely Modulate Plasma ET-1 Levels in Man. **J. Curwen, and J. Growcott.** *AstraZeneca, UK.*

11 **16.11** The Endothelin-1 Promotes Hypoxia-Inducible Factor-1 Alpha Stabilization Through Inhibition of Prolyl Hydroxylase Domain 2 Expression in Melanoma Cells. **F. Spinella, V. Caprara, L. Rosanò, V. Di Castro, M. R. Nicotra, P. G. Natali, and A. Bagnato.** *Regina Elena Natl. Cancer Inst., Italy.*

12 **16.12** A Novel *in vivo* Therapeutic Approach in Amyotrophic Lateral Sclerosis by Targeting Endothelin Receptors Synthesizing Respirator Neurons with Dendritic Nanodevices. **T. Petrov, and D. Svinarich.** *Patient Care Res. Med. Ctr., Southfield, MI.*

13 **16.13** Differential Effects of Chronic vs Acute Central Endothelin 1 on Hemodynamics and Plasma Vasopressin and VP Gene Expression. **N. Rossi, F. Zheng, and H. Chen.** *Wayne State Univ.*

14 **16.14** Endothelin-1 and Inflammation in Trypanosoma Cruzi Infected Adipose Tissue. **F. Nagajyothi, M. Desruisseaux, L. Weiss, P. Scherer, and H. Tanowitz.** *Albert Einstein Col. of Med.*

15 **16.15** Inhibitory Effects of ERAs on Human and Rat Hepatic Transporters. **A. Ray, L. Tong, K. Brouwer, L. Melvin, and J. C. Hartman.** *Gilead Science, Inc. and Qualyst, Inc., Durham, NC.*

16 **16.16** Endothelin-1 Increases the Transmembrane Resistance of Isolated Sheep Leptomeninges. **A. Filippidis, S. Zarogiannis, M. Ioannou, K. Gourgoulianis, P-A. Molyvdas, and C. Hatzoglou.** *Univ. of Thessaly Med. Sch., and Univ. Hosp. of Larissa, Greece.*

16A **16.16A** Endothelin Implicated in Referred Hyperalgesia Associated with TNBS-Induced Colitis in Mice. **R. Claudino, A. Freire Bentol, R. Marcon, J. Geremias Chichorro, D. Ferraz Leite, J. Batista Calixto, and G. A. Rae.** *Fed. Univ. of Santa Caterina, Brazil.*

17 **16.17** ECE-1 Influences PC Cell Invasion via ET-1 Mediated FAK Phosphorylation and ET-1 Independent Mechanisms. **A. Whyteside, L. Lambert, E. Hinsley, and A. Turner.** *Univ. of Leeds, UK.*

18 **16.18** The Endothelin Axis in Drug Sensitive and Multidrug Resistant Bladder Cancer Cells. **D. Pimenta, S-U. Haque, M. Heetun, M. Dashwood, X. Shiwen, N. Farooqui, D. Abraham, and M. Loizidou.** *Univ. Col. of London, UK.*

19 **16.19** Regulation of Endothelin-1 Expression and Function by Nutrient Stress in Mouse Colon Epithelia. **K. Saida, T.**

Board #

Kozakai, and M. Sakate. *Natl. Inst. of Advanced Indust. Sci. and Tech., Japan.*

20 **16.20** Characterization of CoCl2-Induced Reactive Oxygen Species. **K. Saida, and E. Kotake-Nara.** *Natl. Inst. of Advanced Indust. Sci. and Tech., Japan.*

21 **16.21** Endothelin Receptor Expression in Dorsal Root Ganglion and Sensory Changes after Spinal Nerve Injury in Rats. **G. Rae, A. R. Zampronio, C. R. Cavivhiolo Franco, and M. F. P. Werner.** *Univ. Fed. de Santa Catarina and Univ. Fed. de Parana, Curitiba, Brazil.*

22 **16.22** Study of Homologous Desensitization of the Endothelin Receptor ETA in Human OA Chondrocytes. **M. Dion, Y. Berchiche, N. Heveker, and F. Moldovan.** *Univ. of Montreal, Canada.*

23 **16.23** ET-1 Plasma Levels and Ocular Blood Flow in Retinitis Pigmentosa. **M. Cellini, E. Strobbe, C. Gizzi, and E. Campos.** *Eye Clinic, Bologna, Italy.*

24 **16.24** Response of Arteriolevenule Pairs to Endothelin-1 in the Cremaster Muscle of Leptin Deficient Mice. **J. Vigilance, and M. Frame.** *Univ. of the West Indies and Stony Brook Univ.*

25 **16.25** Endothelial Cells-derived Endothelin-1 Regulates Proteinuria in Diabetic Mice. **B. Widyantoro, N. Emoto, H. Kawachi, Y. Y. Kisanuki, M. Yanagisawa, and K-I. Hirata.** *Kobe Univ. Grad. Sch. of Med., Nigata Univ., Japan and Univ. of Texas Southwestern Med. Ctr.*

26 **16.26** Endothelin-1 Induces Pulmonary Smooth Muscle Cell Migration by Stimulating the ET-A Receptor and the ERK1/2 Pathway. **J. White, and D. Meoli.** *Univ. of Rochester.*

27 **16.27** Endothelin-1 and Angiotensin II-Induced PKB Phosphorylation is Dependant on Insulin-like Growth Factor-1 Receptor and C-Src Activation in Vascular Smooth Muscle Cells. **G. Vardatsikos, A. Bouallegue and A. K. Srivastava.** *Univ. of Montreal, Canada.*

28 **16.28** Impaired Cavernosal Response to ET-1 in Goto-Kakizaki Type II Diabetic Rats. **F. S. Carneiro, Z. N. Carneiro, F. R. C. Giachini, V. V. Lima, A. Ergul, R. C. Webb, and R. C. Tostes.** *Med. Coll. of Georgia and Univ. of Sao Paulo, Brazil.*

29 **16.29** Exercise Decreases Oxidative Stress in Mice Overexpressing Human Endothelin-1 in the Endothelium. **T. Barhoumi, P. Paradis, D. Kasal, P. Laurant, and E. L. Schiffrin.** *McGill Univ., EA Sciences Separative Biologiques et Pharmaceutiques, and UFR de Medicine et de Pharmacie, France.*

Symposia XII

17.0 ENDOTHELIN-1 IN INFLAMMATORY DISEASES AND PAIN

Sat., 2:00 – 3:30 PM, Salle de Bal Ballroom.

Co-Chairs: **Giles Rae**, *Univ. Fed. de Santa Catarina, Brazil.*
Anil Gulati., *Midwestern Univ.*

2:00 PM **17.1** Introduction. **Giles Rae.** *Univ. Fed. de Santa Catarina, Brazil.*

2:05 PM **17.2** Sexually Dimorphic Nociceotive Priming by Endothelin: Involvement of the Endothelin B. Receptor. **Sarah Sweitzer.** *Univ. of South Carolina.*

2:20 PM **17.3** Mechanisms Underlying Beneficial Effects of Atrasentan in Murine Models of Colitis. **Rafaela Claudino.** *Univ. Fed. de Santa Catarina, Brazil.*

2:35 PM **17.4** Co-Expression of ETB in Keratinocytes Enables the Coupling of ETA Receptors to Intra-cellular Calcium Release. **Gary Strichartz.** *Harvard Med. Sch.*

2:50 PM **17.5** Endothelin System Plays Role in BK Polyoma Virus Infection of Renal Epithelial Cells. **Andrey Sorokin.** *Med. Col. of Wisconsin.*

3:05 PM **17.6** Distribution of Endothelin-1 and ET-A/B Receptors in Hemorrhoids. **Varut Lohsiriwat.** *Univ. of Nottingham, UK.*

Highlights

18.0 HIGHLIGHTS OF THE APS/ET-11 CONFERENCE

Sat., 3:45 – 4:10 PM, Salle de Bal Ballroom.

3:45 PM **18.1** Highlights of the APS/ET-11 Conference. **Masashi Yanagisawa.** *Univ. of Texas Southwestern Med. Ctr.*

Closing Remarks

19.0 CLOSING REMARKS

Sat., 4:10 – 4:25 PM, Salle de Bal Ballroom.

4:10 PM **19.1** Closing Remarks. **David M. Pollock.** *Med. Col. of Georgia and* **Pedro D' Orleans-Juste.** *Univ. of Sherbrooke, Canada.*

Thank You for Attending the ET-11 Conference…
See You at ET-12!

2009 APS Conference
ET-11: APS International Conference in Endothelin

Abstracts of Invited and Contributed Presentations

2.0: KEY NOTE LECTURE

2.1

CLINICAL RELEVANCE OF ET ANTAGONISTS: AN UPDATE

Donald E. Kohan[1]
[1]Div. of Nephrology, University of Utah Health Sciences Center, 1900 East 30 North, Salt Lake City, UT, 84132.

Endothelin receptor antagonists (ETRA) are being increasingly studied in clinical trials. Currently, over 25 Phase I, II and III clinical trials using ETRA are studying and/or close to starting enrollment of patients with aneurysmal subarachnoid hemorrhage, malignancies (evaluating pain control and/or tumor progression), non-ST or ST segment elevation myocardial infarction, preeclampsia, sleep apnea, diastolic heart failure, interstitial lung disease, glaucoma, systemic sclerosis, primary pulmonary artery hypertension, asthma, systemic hypertension, proteinuric chronic kidney disease, diabetic nephropathy, and other disorders. Pre-clinical studies in experimental animals suggest that ETRA may be efficacious in the treatment of sickle cell vaso-occlusive crisis, traumatic brain injury, accelerated cardiovascular injury associated with chronic kidney disease, portal hypertension, solid organ transplantation, various autoimmune disorders, and other diseases. Endothelin-1 or endothelin receptor gene polymorphisms may help direct therapy; individuals with the endothelin-1 gene polymorphism Lys198Asn, particularly those with hypertension, obesity and diabetes, may benefit by ETRA treatment. Challenges related to the use of ETRA include determination of which diseases would best be treated, and whether toxicity would be the most limited, by targeting endothelin A receptors or combined endothelin A and B receptor blockade. Part of the difficulty in optimizing ETRA choice relates to incomplete understanding of how ET receptors interact with one another, thereby potentially affecting the action of so-called "isoform-specific" antagonists. Further studies are needed on the mechanisms of ETRA-associated toxicity, particularly with regard to fluid retention, hepatic dysfunction and testicular toxicity. Despite these challenges, therapy with ETRA holds great promise.

3.0: CELLULAR REGULATION OF THE ENDOTHELIN PATHWAY

3.2

REGULATION OF ET-1 MRNA EXPRESSION BY THE CIRCADIAN CLOCK PROTEIN PERIOD 1

Michelle Gumz[1], Lisa Stow[2], I. Jeanette Lynch[1], Brian Cain[3], Charles Wingo[4]
[1]Medicine, Univ. of Florida, 1600 SW Archer Rd., Gainesville, FL, 32610, [2]Physiology & Functional Genomics, Univ. of Florida, 1600 SW Archer Rd, Gainesville, FL, 32610, [3]Biochemistry & Molecular Biology, Univ. of Florida, 1600 SW Archer Rd Box 100245, Gainesville, FL, 32610, [4]Research Service, Veteran Affairs Medical Ctr., 1601 SW Archer Rd., Gainesville, FL, 32610

Mammalian sodium excretion is known to vary with the diurnal cycle. We have shown that the circadian clock protein Period 1 (Per1) regulates transcription of the alpha subunit of the epithelial sodium channel (αENaC). The objective of the present study was to determine if ET-1, an important regulator of renal sodium transport, is also a Per1 target. RNA silencing of Per1 was performed in two in vitro models of the renal collecting duct (CD): inner medullary CD cells (mIMCD-3) and cortical CD cells (mpkCCDc14). The effect of Per1 knockdown on ET-1 mRNA expression was assayed using quantitative real time PCR (QPCR). The data demonstrated that Per1 knockdown led to increased ET-1 mRNA expression in mIMCD-3 and mpkCCDc14 cells. This result suggested that Per1 may repress ET-1 expression. ET-1 mRNA levels were also evaluated in vivo in mice lacking all three Period genes (Per1, Per2 and Per3). Interestingly, the 24 hr mRNA expression profile of ET-1 was altered in the inner medulla and cortex of mice lacking all three Period genes compared to wild type mice. In conclusion, the results of these studies indicate a role for Per1 in the regulation of ET-1 mRNA expression and suggest a possible mechanism by which the circadian clock may regulate renal sodium excretion. Support: AHA to MLG, NIH and Dept. of Veteran Affairs to CSW.

3.3

ET-1 REGULATES THE EPITHELIAL NA^+ CHANNEL VIA β PIX/14-3-3 COMPLEX

Alexander Staruschenko[1], Tengis Pavlov[1], Ahmed Chahdi[2], Daria Vachugova[3], Oleh Pochynyuk[4], Andrey Sorokin[2]
[1]Physiology, Medical College of Wisconsin, 8701 Watertown Plank Rd, Milwaukee, WI, 53226, [2]Medicine, Medical College of Wisconsin, 8701 Watertown Plank Rd, Milwaukee, WI, 53226, [3]Physiology, Institute of Cytology RAS, 4 Tikhoretsky Ave, St. Petersburg, 194064, Russian Federation, [4]Physiology, Univ. of Texas Health Science Ctr. at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX, 78229

Endothelin-1 (ET-1) has an important role in the regulation of sodium reabsorbtion in the cortical collecting duct (CCD). ET-1 decreased the activity of epithelial Na+ channel (ENaC) in principal cells of freshly isolated CCDs and differentiated mouse principal mpkCCD14 cells, and CHO cells overexpressing α-, β- and γ-ENaC subunits. ET-1 induces translocation to focal adhesions of the guanine nucleotide exchange factor (GEF) β1Pix. Here we demonstrate that βPix is highly expressed in various kidney cells including the mpkCCDc14 cells. Coexpression of β1Pix with ENaC decreased ENaC activity. βPix's effect upon ENaC was not mediated by canonical GEF activity since coexpression of Rac1 with ENaC markedly increased channel activity, whereas coexpression of Cdc42 failed to change ENaC activity. Moreover β1Pix (Δ602-611) construct which did not bind 14-3-3 but retained GEF activity had no effect on ENaC activity. In contrast, β1Pix (L238R,L239R) mutant which has no GEF activity decreased current density to a similar extent as wild type β1Pix. Furthermore, β1Pix did not affect ENaC activity when coexpressed with γ-ENaCT628A mutant lacking the ability to be regulated by Nedd4-2. Thus, we conclude that β1Pix decreases ENaC activity via 14-3-3 recruitment but not through GEF activity and possibly participates in the ET-1 mediated decrease in ENaC activity.

3.4

ETB RECEPTOR IS RESPONSIBLE FOR NUCLEAR CALCIUM REGULATION BY ET-1

Levon Avedanian[1], Faten Choufani[1], Nahla Issa[1], Moni Nader[1], Danielle Jacques[1], Pedro D'Orléans-Juste[2], Ghassan Bkaily[1]
[1]Anatomy and Cell Biology, Univ. of Sherbrooke, 3001 12th Avenue North, Sherbrooke, Quebec, J1H 5N4, Canada, [2]Pharmacology, Univ. of Sherbrooke, 3001 12th Avenue North, Sherbrooke, Quebec, J1H 5N4, Canada

Recent work in our laboratory showed that cytosolic ET-1 modulates nuclear calcium level of several cell types, including human vascular smooth muscle cells (hVSMCs) and hepatic cells. The objective of the present study was to test the hypothesis that ETB receptors' activation would contribute to modulation of nuclear free calcium homeostasis. Using immunofluorescence and 3D confocal microscopy, we report the presence of both ETA and ETB receptors in isolated nuclei of hVSMCs and hepatic cells. Using isolated nuclei loaded with the calcium probe Fluo-4, and 3D confocal microscopy technique, our results demonstrated that cytosolic ET-1 induced increase of nucleoplasmic free calcium was solely due to activation of the nuclear membranes ETB receptors. The ETB receptor mediated increase of nucleoplasmic calcium was not due to influx through the nuclear pores, but due to both calcium entry through the nuclear membranes' R-type calcium channels and nucleoplasmic calcium release. Furthermore, ETB receptor activation seems to induce generation of nuclear calcium sparklets. In conclusion, nuclear membranes' ETB and not ETA receptors mediate cytosolic ET-1 modulation of nucleoplasmic free calcium. This work was supported by grants from CIHR and NSERC to Dr. G. Bkaily.

3.5

ETB-DEPENDENT INHIBITION OF NEOINTIMAL PROLIFERATION IS NOT MEDIATED BY RECEPTORS EXPRESSED ON THE ENDOTHELIUM

Nicholas Kirkby[1], Patrick Hadoke[1], David Webb[1]
[1]Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh, EH16 4TJ, United Kingdom

Endothelin (ET)-B receptors influence vascular lesion development with ETB-deficient mice exhibiting increased neointimal proliferation following injury (Murakoshi et al 2002). It was proposed that this anti-proliferative effect is mediated by ETB in endothelial cells (EC). Methods: Femoral artery wire-injury was performed in male EC-specific ETB knockout mice and littermate controls (n=7-10). Additional studies were performed in mice treated with antagonists to ETA (ABT627; 10mg/kg/day), ETB (A192621; 30mg/kg/day), ETA+ETB or vehicle (n=6-9). Drug activity was assessed by tail cuff blood pressure (BP) measurement. Femoral arteries were harvested at 28 days for optical tomographic and histological analysis. Results: Femoral artery injury produced large, concentric fibro-proliferative neointimal lesions. EC ETB knockout did not alter lesion size ($P=0.87$). In contrast, systemic ETB blockade increased BP and lesion size (184% of vehicle; $P<0.05$) whereas ETA blockade decreased BP and lesion size ($P<0.05$). Combined ETA/ETB antagonism reduced BP but not lesion size ($P>0.05$). Conclusions: ETB-mediated moderation of neointimal proliferation is neither mediated by ETB in EC nor dependent on BP reduction. This suggests a role for ETB expressed in other cells and that ETA-selective may be preferable to non-selective antagonists for prevention of neointimal proliferation. NK received a University of Edinburgh scholarship. Murakoshi et al (2002) Circulation 106:15.

4.0: NOVEL PHYSIOLOGICAL AND PHARMACOLOGICAL MECHANISMS

4.2

THE 411 ON ET RECEPTORS: WHAT'S NEW?

Stephanie Watts[1]
[1]Pharmacology & Toxicology, Michigan State University, B445 Life Sciences Building, East Lansing, MI, 48824-1317

Receptors are at the heart of how a molecule transmits a signal to a cell. Two receptor classes for endothelin (ET) are recognized, the ETA and ETB receptors. Intriguing questions have arisen in the field of ET receptor pharmacology, physiology and function. For example, a host of pharmacological studies support the interaction of the ETA and ETB receptor in tissues (veins, arteries, bronchus, arterioles, esophagus) but yet few have been able to demonstrate direct ETA/ETB receptor interaction; have we modeled this interaction wrong? Do we have a truly selective ETA receptor agonist? What can we learn from the recent phylogenic studies of the ET receptor family? Have we adequately addressed the number of biological molecules with which ET can interact to exert a biological effect? Recent mass spectrometry studies in our laboratory suggest that ET-1 interacts

with other hereto unrecognized proteins. Biased ligands (ligands at the same receptor that elicit distinct signaling responses) have been discovered for other receptors; do these exist for ET receptors and can we take advantage of this possibility in drug design? These and other questions will be posed. REFERENCES: Hyndman, K. A., Miyamoto, M. M. & Evans, D. H. 2009. Phylogeny, taxonomy and evolution of the endothelin receptor gene family. Mol. Phylogen Evol. (8 May 2009). Kenakin, T. 2007. Functional selectivity through protean and biased agonism: who steers the ship? Mol Pharmacol 72, 1393-1401.

4.3

GENETIC STUDY OF ET-2 FUNCTION IN MICE

Inik Chang[1], Alexa N. Bramall[2], Amy G. Baynash[1], Roderick R. McInnes[2], Duncan J. Stewart[3], Masashi Yanagisawa[1]

[1]Molecular Genetics, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX, 75390-8584, [2]Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, 101 College Street, Toronto, ON, M5G 1L7, Canada, [3]The Regenerative Medicine Program, Ottawa Health Research Institute, 725 Parkdale Ave., Ottawa, ON, K1Y 4E9, Canada

To explore the physiological function of ET-2, we generated tissue-specific and systemically inducible knockout mice. Global ET-2 null mice exhibited growth retardation and juvenile lethality. Despite normal milk intake, they suffered from an internal starvation characterized by hypoglycemia, ketonemia, and increased expression of starvation-induced genes in liver. Although ET-2 is abundantly expressed in the gut, the intestine was morphologically and functionally normal in the global mutants. Moreover, intestine epithelium-specific ET-2 null mice showed no abnormalities in growth and survival. Global ET-2 null mice were hypothermic. Housing these mice in a warm environment extended the median life span. However, neuron-specific ET-2 null mice displayed normal core body temperature, suggesting that ET-2 is not playing a role in central thermoregulation. We detected low levels of ET-2 mRNA in the lung, with transient increases soon after birth. The emphysematous structural change with an increase of total lung capacity resulted in chronic hypoxemia, hypercapnia, and increased erythropoietin synthesis in the global mutants. Finally, systemically inducible deletion of ET-2 in neonate and adulthood fully reproduced the phenotype previously observed in global knockouts. Together, these findings reveal that ET-2 is critical for growth and survival of postnatal mice by playing important roles in energy homeostasis, thermoregulation, and maintenance of lung morphology and function. This work was supported by HHMI.

4.4

REDUCED PRESSOR RESPONSES TO BIG-ET-1 IN MMCP-4 KO MICE

Martin Houde[1], Isabelle Brochu[1], Gunnar Pejler[2],Adel Giaid[3], Michael F. Gurish[4], Pedro D'orleans-Juste[1]

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Mucosal mast cell protease 4 (mMCP-4) is, among eight chymase isoforms in the mouse, the enzyme involved in the non-ACE-dependent conversion of Angiotensin I (Ang I) to Ang II. Albeit we have recently reported the important contribution of chymase in the dynamic conversion of big-ET-1 to ET-1 in the mouse in vivo (Simard et al., JPET., 2009), it remains to be determined which chymase isoform is involved in the non Endothelin Converting Enzyme dependent production of ET-1. In the present study, the pressor profiles of big- ET-1, ET-1 (1-31) and ET-1 were assessed in mMCP-4 KO mice. The loss of mMCP-4 mRNA was confirmed by quantitative RT-PCR in cardiac, pulmonary and aortic homogenates derived from mMCP-4 KO mice. Furthermore, the pressor response to big-ET-1 but not ET-1 (1-31) (1 nmol/kg) nor ET-1 (0.5 nmol/kg) was reduced by 50 % in mMCP-4 KO mice when compared to wild type congeners. Finally, a selective chymase inhibitor, Suc-Val-Pro-PheP-(OPh)2 (20 mg/kg, i.p.) (Konno et al, Eur. J. Pharmacol, 2004) significantly reduced the pressor responses to big-ET-1 in wild type but not in mMCP-4 KO mice. Our data suggest that mMCP-4 is the main chymase isoform involved in the conversion of big-ET-1 to ET-1, in the mouse, in vivo. (Supported by the Canadian Institutes of Health Research and the Clinical Research Center Etienne Lebel).

4.5

CGRP PROMOTES DISSOCIATION OF ET-1 FROM ETA-RECEPTORS AND TERMINATES VASOCONSTRICTION IN RAT RESISTANCE ARTERIES

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Aims: We tested whether i) bivalent, irreversible receptor binding causes long-lasting ETA-initiated vasoconstriction and ii) CGRP promotes dissociation of ET-1/ETA-complexes. Methods: 2nd order rat mesenteric arteries were investigated by two-photon laser scanning microscopy and wire-myography. Results: ET-receptors are present on several cell types including sensory-motor nerves (SMN). ET-1 (1-16nM) and rhodamine-labeled ET-1 (Rh-ET-1; 1-16nM) cause contractions that fade slowly after agonist removal and are not modified by ETB-antagonism, NOS inhibition or desensitization of SMN with capsaicin (CAPS). BQ123 (1iM) prevents contractions but reverses them only partly and transiently. Contractions remaining after ET-1 exposure are transiently reversed by BQ123 (3iM) and several vasodilators, but persistently inhibited by CAPS (1iM) or CGRP (0.1iM). CGRP1-receptor antagonists reduce both effects. ET-1 causes contractions with its original potency after reversal of ET-1-induced contractions by CGRP. Rh-ET-1 (16nM) binds to the tunica media of intact arteries. This persists after label removal and in presence of BQ123 (1iM) but is abolished by CGRP (0.1iM). Conclusion: In arterial smooth muscle, ET-1 interacts dynamically with a BQ123-sensitive and "irreversibly" with a BQ123-insensitive site. The latter a) contributes to signaling, b) allows ongoing interaction of the agonist with the former and c) is susceptible to allosteric inhibition initiated by CGRP1-receptor activation.

4.6

DISCREPANCIES IN ANTAGONIST AFFINITIES FOR HUMAN VASCULAR ET_A RECEPTORS DETERMINED IN FUNCTIONAL AND BINDING ASSAYS

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Endothelin receptor affinity and selectivity of antagonists has usually been determined in cell based assays rather than native human tissues. However, it is not certain to what extent these data predict in vivo efficacy in man. In human coronary artery we determined the affinity of endothelin antagonists for vascular ETA receptors in competition binding experiments to obtain values of KD that should be comparable to KB values obtained for antagonism of ETA-mediated endothelin-1 (ET-1) vasoconstriction. Competition assays were carried out in sections of coronary artery, with 0.1nM [125I]ET-1 and increasing concentrations (20pM-100μM) of antagonists: the ETA selective peptides BQ123, FR319317, PD151242; the ETA selective non-peptides PD156707, 50235; the non-selective, non-peptides Ro-462005 and bosentan. For functional studies cumulative concentration response curves were constructed to ET-1 in the absence and presence of antagonists in coronary artery in vitro and affinities determined by Schild analysis. The affinity of antagonists for ETA receptors was 10-1000 times lower in the functional assay than predicted from the binding assay. This discrepancy was greatest for bosentan that is 1000 times less effective as an antagonist of ETA vasoconstriction than predicted from its nanomolar binding affinity and least for the structurally related Ro-462005. These data may have relevance for the prediction of in vivo efficacy of endothelin antagonists in man.

4.7

GREATER FUNCTIONAL ETB RECEPTOR ANTAGONISM WITH BOSENTAN THAN SITAXSENTAN IN MAN

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Introduction: Endothelin receptor antagonists (ETRAs) are classified as selective or mixed depending on their degree of ETA:ETB blockade. Hence, ETB blockade varies and depends on drug dose. As yet, there are no comparative studies in man that measure functional ETB blockade achieved by currently licensed ETRAs at clinically used doses. We therefore investigated the effects of bosentan, a mixed ETRA, and sitaxsentan, an ETA selective ETRA, on ETB mediated, NO dependent, vasodilatation to ET-3. Methods: In a randomised, double-blind, 3-way cross-over study, 6 healthy subjects received 7 days of placebo, bosentan 125mg bd and sitaxsentan 100mg od. On day 7 volunteers attended for forearm blood flow measurements 3h after the morning dose. ET-3 was infused at 60pmol/min for 5min into the brachial artery of the non-dominant arm. Forearm measurements were taken before, during and after infusion. Results: After placebo, ET-3 produced an initial rapid vasodilatation (26±7%), consistent with endothelial ETB receptor activation. This was followed by sustained vasoconstriction from +5min. On treatment with sitaxsentan, dilatation appeared reduced (14±4%, p=ns) with subsequent vasoconstriction unaffected. Vasodilatation was abolished by bosentan (-11±3%, $p<0.05$). Conclusion: The acute vasodilatory effects of ET-3 were abolished by bosentan, but not by sitaxsentan, suggesting greater functional ETB blockade with bosentan at clinically relevant doses. This study was funded by Encysive/Pfizer.

4.8

INVOLVEMENT OF ENDOTHELIN IN THE ACTIONS OF β-AMYLOID

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The most prominent feature of Alzheimer's disease (AD) is the extracellular neuritic plaques having β-amyloid (Aβ). ET plays an important role in regulation of cerebral vascular system. It is possible that Aβ produces cerebrovascular effects mediated through ET which causes oxidative damage leading to degeneration of neurons. We investigated the effect of Aβ on systemic hemodynamics, brain circulation, ET-1 expression and oxidative stress parameters. Male rats were treated with vehicle or Aβ (20 μg, icv) on day 1, 7 and 14 and all experiments were performed on day 15. Systemic hemodynamics and brain circulation was

determined using a radioactive microsphere technique. A significant increase in vascular resistance (83%) and decrease in regional brain circulation (47%) in Aβ group was observed compared to vehicle. It was also found that hippocampus and brain stem showed an increase in ET-1 mRNA expression. Furthermore, in Aβ group there was development of oxidative stress, indicated by elevation ($p<0.01$) in malondialdehyde (423.3±14.2nmol/g) compared to vehicle (101.66±3.1nmol/g) and decrease in glutathione (126.6±4.2µg/g) compared to vehicle (206.6±11.4µg/g). The vasoconstrictor effect of ET-1 on cortical blood flow was increased in Aβ group compared to vehicle. ET-1 might play a role in mediating the vasoactive effect of Aβ through the oxidative stress mechanism. It is possible that ET receptor antagonists may prevent the development of oxidative stress as well as vasoconstriction due to Aβ in AD.

5.0: POSTER SESSION I

5.1

ENDOTHELIN (ET-1) INCREASES ALBUMIN PERMEABILITY OF ISOLATED RAT GLOMERULI

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We hypothesized that ET-1 increases the albumin permeability of the glomerulus independent of alterations in hemodynamic factors. Glomeruli were isolated by sieving in a buffer containing 5%BSA and the glomerular permeability to albumin (Palb) determined from the change in glomerular volume induced by exposing glomeruli to oncotic gradients of defined concentrations of albumin (5%BSA switched to 1%BSA). To assess the effect of ET-1 on possible volume changes without an osmotic stimulus, concentration-response experiments were performed in 5%BSA. Concentrations of 1000, 100, 10, and 1 nM (n= 44 glomeruli/3 rats) evoked 11.9±2.7%, 8.7±1.4%, 2.7±0.3% and 0.03±0.02% contraction, respectively. To assess the effect of ET-1 on Palb, time course experiments were performed. Since the lowest concentration of ET-1 did produce a significant contraction when compared to the control group, we determined the Palb changes triggered by 1 nM ET-1. Glomeruli were incubated at 37°C in 5%BSA. After 15 min, ET-1 did not have any effect on Palb, however, Palb was significantly increase (n= 50 glomeruli /5 rats) after during the course of 5 hrs when compared with the corresponding control groups. The maximum effect was attained after 240 min (0.57±0.07 vs. 0.08±0.05 without ET-1, permeability units). We conclude that ET-1 contracts glomeruli at concentrations higher than 1 nM and that permeability changes reach their maximum effect within 4 hrs. (Supported by HL64776 and HL69999).

5.2

REGULATION OF COLLECTING DUCT ENDOTHELIN-1 PRODUCTION

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Collecting duct (CD)-derived endothelin-1 (ET-1) is an important autocrine inhibitor of renal Na reabsorption; CD ET-1 knockout causes marked hypertension. High Na intake augments CD ET-1 release; the mechanisms involved are unknown. To assess this, primary cultures of rat inner medulla CD (IMCD) were studied. Cells exposed to flow had a 3-4 fold increase in ET-1 mRNA and intracellular Ca [Ca]i. Inhibition of calmodulin (CaM) or CaM kinase II, as well as chelation of [Ca]i, markedly reduced ET-1 release and mRNA levels. Transfection with rat ET-1 promoter-luciferase constructs revealed maximal reporter activity, as well as sensitivity to CaM blockade, between the region 1319-1725 bp 5' to the transcription start site. Incubation of this 406 bp region with IMCD nuclear extracts caused an electrophoretic mobility shift that was prevented by competition with a 125 bp fragment between 1455 and 1576 bp 5' to the transcription start site. This 122 bp region contains two NFAT sites. An antibody to NFAT-5 caused a supershift, while oligos containing the NFAT consensus site reduced the shift. In vitro footprinting of the -1455 and -1576 bp region revealed protected areas at the NFAT consensus binding sites. Cyclosporine A (CyA), a calcineurin inhibitor, caused a 60% reduction in IMCD ET-1 release. In contrast, in rat aortic endothelial cells, ET-1 promoter-luciferase activity was maximal in the proximal 500 bp promoter region and was not CaM regulated; further, CyA stimulated ET-1 production. We propose that Na loading increases CD luminal flow, augmenting [Ca]i, activating CaM and calcineurin, leading to NFAT nuclear translocation and increased ET-1 gene transcription. This pathway may be unique to CD.

5.3

ROLE OF ENDOTHELIN ON LUNG STRUCTURAL REMODELING AND PULMONARY FUNCTION IN ISCHEMIC CARDIO-MYOPATHY

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Objective: Pulmonary hypertension (PH) and right ventricular (RV) dysfunction associated with congestive heart failure (CHF) carry a poor prognosis. Although endothelin receptor antagonist (ET-RA) have proven their benefits in human pulmonary arterial hypertension, the efficacy in the treatment of ischemic HF remains controversial. In this study, we evaluated the effect of Bosentan, a dual ET-RA, on established lung structural remodeling, pulmonary function, PH and RVH in rats with ischemic HF. Methods: Two weeks after myocardial infarction (MI), rats received Bosentan (100 or 200mg kg-1 d-1) or no treatment for 3 weeks and were compared to a sham group. Results: Plasma troponin value at 24-36 hours after MI and LV echo wall motion abnormality at 2 weeks and at 5 weeks were similar. CHF induced PH and RVH compared with sham: RV systolic pressure 39±5 vs. 22±0.9 mmHg and RV/LV+Septum weight 46.1±7.3 vs. 23.6±0.6% (all $p<0.01$). Bosentan therapy did not significantly modify these parameters after 3 weeks therapy. CHF caused a restrictive lung syndrome with a downward shift of the lungs pressure-volume loop and increased dry lung weight that were also not improved by Bosentan. Conclusion: Dual ET-RA with Bosentan started 2 weeks after MI did not reduce lung remodeling and dysfunction associated with CHF, and did not show prevention of RVH and PH. This work was supported by the Canadian Institutes of Health Research, the "Fondation de l'Institut de Cardiologie de Montréal".

5.4

ATRASENTAN REDUCES PROTEINURIA IN PATIENTS WITH DIABETIC NEPHROPATHY

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Proteinuric renal disease is a common complication of long-term diabetes and glomerular podocyte expression of endothelin 1 (ET-1) is markedly up-regulated in diabetic nephropathy (DN). Renoprotective strategies are a large unmet medical need. The effects of atrasentan, a highly selective endothelin ETA receptor antagonist, were compared with placebo on albuminuria in 11 subjects with Type I DN. After ACE-i washout, subjects received an open-label dose of atrasentan to quantify renal blood flow (RBF) and glomerular filtration rate (GFR). This was followed by a double-blind, placebo-controlled, 2-period crossover phase where subjects were randomized to receive 5 mg atrasentan and placebo daily in each 42 day crossover period. Atrasentan (5 mg) had no effect on RBF, GFR or mean arterial pressure (MAP), whereas it resulted in significantly lower mean log transformed albumin excretion (1.51±0.26 µg/min) compared with placebo (1.77±0.26 µg/min; P=0.008). Common adverse events were headache, rhinitis, and peripheral edema. Two subjects experienced pulmonary edema that resolved with diuretic therapy. We conclude that atrasentan effectively reduces albuminuria in patients with Type I DN, independent of changes in RBF, GFR, and MAP, suggesting a possible direct effect on podocyte function. Larger trials are needed to better assess the efficacy and side effect profile of low dose ETA receptor antagonism in the treatment of DN. This study was funded by Abbott Laboratories.

5.5

OEDEMAGENIC EFFECT OF ENDOTHELIN AGONISTS IN ISO-LATED HUMAN LUNGS

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Aim: To quantify the oedemagenic effects of endothelin agonists in isolated human lungs. Methods: Lung samples were obtained from patients undergoing elective lung (n=4) or lobe (n=10) resection for cancer. The pulmonary arterial and bronchial systems were cannulated and the lungs ventilated with room air (TV 100-300ml at 10 BPM) and perfused with Krebs buffer (1 litre, 100-300ml/min). Perfusion pressure, airway pressure and weight were recorded. Dose response curves were constructed to Endothelin-1 (ET-1), Endothelin-3 (ET-3) and BQ3020. Oedema formation was quantified gravimetrically as % of initial weight/ minute (%/min) before and after agonist response. Results: ET-1 caused a significant increase in the rate of weight gain: pre ET-1 0.35 (0.29) vs. post ET-1 1.64 (1.28) (% /min) (SEM) p=0.02. The ET B agonists ET-3 and BQ3020 did not induce significant pulmonary oedema: pre ET-3 0.31(0.36) vs. post ET-3 0.65(0.71) and pre BQ3020 1.64 (0.79) vs. post BQ3020 2.23 (1.17) (% /min) (SEM). Oedema formation did not correlate with increased hydrostatic pressure. Conclusion: ET-1 is a potent oedemagenic agent in the human lung and its effects are mediated by activation of the ETA receptor. The fulminant effect of ET-1 in the isolated lung suggests that it may have a clinically significant role in the pathogenesis of pulmonary oedema. Further clinical studies are warranted to investigate the therapeutic potential of endothelin antagonists for the treatment of pulmonary oedema.

5.6

HEME ARGINATE THERAPY ENHANCES HEME OXYGENASE AND ATRIAL NATRIURETIC PEPTIDE TO ABATE ENDO-THELIN-1 AND SUPPRESS RENAL HISTOPATHOLOGICAL LESIONS

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We investigated the effects of the heme oxygenase (HO) inducer, heme arginate (HA) and atrial natriuretic peptide (ANP) in DOCA-hypertensive rats, a volume-overload model characterized by elevated endothelin (ET-1), fibrosis and severe

renal lesions that closely mimic end-stage-renal-disease (ESRD). HA abated kidney fibrosis, hypertrophy and renal lesions including glomerulosclerosis, tubular-dilation, tubular-cast formation, interstitial mononuclear cell-infiltration, glomerular hypertrophy and renal-arteriolar thickening in DOCA rats. These were accompanied by increased renal HO-1, HO activity, ANP and urinary cGMP, a marker of ANP activity, whereas plasma and renal ET-1 were depleted. Furthermore, in HA-treated animals, TGF-β, fibronectin and 8-isoprostane, an index of oxidative stress were abated while anti-oxidants like bilirubin, ferritin, SOD, catalase and the total-anti-oxidant capacity were increased. Interestingly, the renoprotective effects of HA was associated with improved function as proteinuria and albuminuria were reduced while increased creatinine clearance increased. In contrast, the HO blocker, chromium mesoporphyrin exacerbated oxidative stress and renal lesions with aggravation of renal function. Our study highlights the synergistic interaction between the HO system and ANP that could be explored against ESRD. Since 8-isoprostane stimulates ET-1 to potentiate oxidative stress and fibrosis, upregulating HO-1 enhanced the anti-oxidant status alongside cellular targets like ANP and cGMP to suppress ET-1, TGF-β and fibronectin with corresponding decline of renal lesions, proteinuria, albuminuria, and thus improved renal function. Supported by H&SF, Saskatchewan

5.7

PROTECTIVE EFFECTS OF ENDOTHELIN-A RECEPTOR ANTAGONIST, BQ123, AGAINST LPS-INDUCED OXIDATIVE STRESS IN LUNGS

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The aim of this study was to assess whether endothelin-A receptor blocker, BQ123, influences the lipid peroxidation (TBARS), the hydrogen peroxide (H2O2) concentration and the glutathione redox system in the lung homogenates obtained from LPS-induced endotoxic shock rats (Escherichia coli 026:B6, dosage, 15 mg/kg, i.v.). The study was performed on male Wistar rats (n=6 per group) divided into groups: (1) saline-saline, (2) LPS-saline, (3) BQ123 (dosage, 1 mg/kg)-LPS, (4) BQ123 (dosage, 0.5 mg/kg)-LPS. The ET-A receptor antagonist was injected intravenously 30 min before LPS administration. Five hours after saline or LPS administration animals were sacrificed under anaesthetic and lungs were isolated for further measurements. Injection of LPS alone resulted in a marked increase in TBARS ($p<0.02$) and H202 concentration ($p<0.01$) as well as a depletion of total glutathione ($p<0.01$). Administration of BQ123 (higher dose), before LPS challenge, led to a significant reduce in H2O2 concentration ($p<0.01$) and elevated the level of total glutathione ($p<0.01$) and the GSH/GSSG ratio ($p<0.001$). Interestingly, lower dose of BQ123 was more effective in decreasing H2O2 and TBARS level ($p<0.01$ and $p<0.05$, respectively) than the higher dose of the blocker. In conclusion, these results indicated that BQ 123 is highly effective in decreasing LPS-induced oxidative stress in lungs. Moreover, the lower dose of the antagonist showed to be more effective in an increase of total glutathione level and a decrease of free radical generation. The study was supported by a grant 503-1079-1 from the Medical University of Lodz.

5.8

CHRONIC AND ACUTE DUAL BLOCKADE OF ENDOTHELIN RECEPTORS DISPLAY DIFFERENT EFFECTS ON CEREBROVASCULAR FUNCTION IN TYPE 2 DIABETES

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The endothelin (ET) system is upregulated in type 2 diabetic Goto-Kakizaki (GK) rats leading to increased sensitivity to ET-1 and decreased relaxation in the basilar artery (BA). We also found that ETA receptor blockade restored relaxation while ETB blockade caused paradoxical constriction of BA. Thus, we hypothesized that dual antagonism of ET receptors would prevent BA dysfunction, and acute vs. chronic antagonism has different effects on BA reactivity in type 2 diabetes. The reactivity of BA in GK rats treated with or without dual ET receptor antagonist, Bosentan, for 4 weeks (Ch) was measured with wire myograph. In a sub-set of untreated rats, Bosentan (3 μM) was added into the vessel chamber at 30 min before measurement (A). Blood pressure was significantly increased in Ch group (MAP, mmHg: Ch 131±2 vs. A 111±2, Ctrl 103±6, $p<0.01$). Both treatments decreased the vascular sensitivity to ET-1 (EC50 nM: A 217±63, Ch 90±48 vs. Ctrl 9±2, $p<0.05$). Acute treatment almost completely abolished the response to ET-1 while chronic treatment slightly decreased it (Rmax: A 10.2±6.4 and Ch 87.7±25.9 vs. Ctrl 158±23.2, $p<0.01$). There was no difference in endothelium-dependent relaxation to Ach (EC50: Ch 100±66, A 57±48 vs. Ctrl 31±15). These results suggest that both treatments may alter receptor function or density in diabetes. While acute antagonism completely protects against ET-1 mediated dysfunction, increased MAP may negate the protective effects of chronic antagonism in diabetes.

5.9

THE EFFECT OF RAS BLOCKADE ON ET-1 LEVELS IN DISTINCT RENAL COMPARTEMENTS

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Objective. The relationship between angiotensin II (ANG II) and endothelin-1 (ET-1) is known to be complex; both peptides can initiate and potentiate the gene expression of each other and both are involved in the activation of Janus kinase 2. This pilot study investigated the distribution of ET-1 and ANG II and the effect of RAS blockade on the concentrations of these peptides in distinct renal compartements. Methods. 3-month-old male TGR were treated either with AT1 receptor blocker losartan (5 mg kg-1 day-1) or direct renin inhibitor aliskiren (10 mg kg-1day-1) for 10 weeks. At the end of the experiment, rats were decapitated and kidneys were cut into cortex, medulla and papilla. Plasma and tissue ANG II levels were measured by RIA, tissue endothelin-1 (ET-1) concentrations by ELISA. Results. In all four groups of animals, ET-1 levels gradually increased from cortex to medulla, reaching thousand-fold levels in papilla. Papillary ET-1 concentration in untreated TGR significantly exceeded that of control HanSD and was significantly depressed to even lower values with both drugs, being negatively correlated with ANG II levels. Conclusions. Our findings indicate that in distinct renal compartements, both systems are regulated independently, and that the effect of the two drugs inhibiting RAS at different steps on ET-1 and ANG II levels is comparable. This study was supported by the grant No.305/07/J004 awarded by the Czech Grant Agency.

5.10

THE EFFECTS OF DIETARY SALT AND PROTEINS ON ET-1 CONCENTRATIONS IN REN-2 RATS WITH ABLATION NEPHRECTOMY

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Objective. High-salt intake activates endothelin system. We investigated whether increased ET-1 production resulting fromhigh-sodium diet could be counterbalanced by a diet deprived of proteins, that is known to exert positive effect on renal function. Methods. Rats transgenic for mouse renin gene (TGR) and their age-matched controls (HanSD) underwent a partial nephrectomy (NX) at the age of 52 days and were given diets with different amounts of sodium [normal-salt (0.6 % NaCl; NS), or high-salt (2 % NaCl; HS)] or proteins [normal protein-30 % protein (NP), low protein-6 % protein (LP)] for 28 days. Atrasentan (ETA selective blocker) was administered through drinking water (5 mg kg-1day-1). At the end of experiment, rats were decapitated and tissue endothelin-1 (ET-1) concentrations were determined by ELISA. Results. ET-1 cortical concentrations between sham-operated HanSD and TGR were not different on any diet. Partial nephrectomy significantly increased ET-1 levels in both groups that were further augmented by high-salt diet. While low-protein diet prevented the increase in ET-1 only in TGR on NS, it fully normalized it in both groups on HS. Atrasentan effectively depressed ET-levels both in HanSD and in TGR but had no effect on cardiac hypetrophy. Conclusions. Endothelin blockade is more effective when endothelin system is activated by HS and the effect of HS on cortical ET-1 levels is effectively opposed by LP diet. This study was supported by the grant 305/07/0167 awarded by Czech Grant Agency.

5.11

IDENTIFICATION OF PEPTIDE INHIBITORS BY PHAGE DISPLAY: THE USE OF ENDOTHELIN-1 AS A MODEL

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Although the development of antagonists and enzymes inhibitors has largely progressed, several peptide related pathologies would still benefit from new therapeutic avenues. With an aim of developing a new strategy of blocking peptide biological actions, the technique of phage display was used to identify peptide inhibitors of endothelin-1 (ET-1). Phage were selected for their ability to bind to ET-1 and higher affinity clones were isolated, sequenced and used to generate endothelin binding peptides (EBP) by solid phase peptide synthesis. Moreover, homodimers of these EBPs were constructed with various spacers of different length and rigidity. Crude peptides were purified through HPLC and submitted to binding and inhibition assays. Affinity for ET-1 was evaluated with a modified version of an enzyme-linked immunosorbent assay (ELISA) whereas inhibition of ET-1 vasoconstrictor effect was evaluated with pharmacological bioassays on rat aorta rings. Results showed that EBPs found through phage display were able to bind ET-1. Moreover, dimerization increased binding affinity compared to monomer constructs. Pharmacological assays showed that EBPs were able to decrease the efficacy and/or the potency of the vasoconstriction produced by ET-1. Thus, using the technique of phage display, it is possible to design peptides able to act as inhibitors, leading the way to the development of new therapeutic strategies. This study was sponsored by the CIHR.

5.12

ET-1 IS REGULATED BY ERK5 IN DIABETIC RETINOPATHY

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Upregulation of endothelin 1(ET-1) causing blood flow alteration and increased extracellular matrix production are characteristic features of diabetic retinopathy(DR). Several glucose induced signaling mechanisms causes ET-1 upregulation in DR. Erk5 is member of MAPK family which plays a key role in cardiovascular development. Erk kinase(MEK)5 is the specific MEK for Erk5 activation. We examined the role of Erk5/KLF2 signaling in DR. We investigated retinas from one month STZ-induced diabetic rats and human endothelial cells (HUVECs) to investigate ERK5 dependent ET-1 alterations. In parallel, we investigate expression of vascular endothelial growth factor(VEGF), another vasoactive factor of importance in DR. One month diabetes caused significant increase in retinal ET-1 and VEGF mRNA and decreased mRNA expression of Erk5 and KLF2, a downstream mediator of Erk5. To understand cellular mechanisms of such alterations, we investigated HUVECs. Twenty five mM glucose caused significant upregulation of ET-1, VEGF and downregulation of ERK5 and KLF2 after 24hrs. Simultaneously both total and phospho-ERK5 proteins were reduced. Activation of Erk5 by constitutively active(CA) MEK5 in cells exposed to 5mM glucose or 25mM glucose, upregulated KLF2 and suppressed ET-1 and VEGF expression. On the other hand, Erk5 siRNA transfection increased ET-1 and VEGF mRNA expression. These data indicate that Erk5/KLF2 signaling may regulate glucose induced ET-1 and other vasoactive factor expression in diabetes. Erk5/KLF2/ET-1 pathway may provide a potential novel target for the treatment of DR.

5.13

NH4CL LOADING DOWNREGULATES PENDRIN IN ETB-RECEPTOR KO MICE

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Does the ETB-receptor (ETB) play a role in mediating reduced abundance of the anion exchanger Pendrin in type B collecting duct intercalated cells (CD-IC) after chronic NH4Cl-loading? Male mice lacking ETB in non-neural tissues (ETB-KO) were studied. One group had NH4Cl added to the food for 5 days while a control group had regular chow. Urine pH was reduced (5.67.+/-0.08 (n=11) vs 6.22+/-0.14 (n=12); p<0.001) and excretion of titratable acid (TA) was increased (3.7.+/-0.4 (n=5) vs 2.5+/-0.3 (n=12) mmol/kg bw; p=0.013) in the NH4Cl-ETB-KO group (n=5) vs ETB-KO-controls (n=5). WB showed a reduced abundance of pendrin (0.39.+/-0.02 vs 1.00+/-0.04; p<0.001) which was also seen by IHC in the NH4Cl-ETB-KO group vs ETB-KO-controls. In parallel treated control groups of WT-mice, urine pH was reduced (5.71.+/-0.1 (n=12) vs 7.04+/-0.18 (n=12); p<0.001); TA was increased (2.2.+/-0.7 (n=3) vs 0.25 +/-0.25 (n=12) mmol/kg bw; p=0.034) and by IHC pendrin was reduced in the NH4Cl-group vs. controls. TA was increased in ETB-KO vs WT at baseline (p<0.001) and during NH4Cl-loading (p=0.045). By WB pendrin was increased in ETB-KO vs WT (baseline: 1.96.+/-0.08 vs 1.00+/-0.38; p=0.044 and NH4Cl-loading: 0.77.+/-0.05 vs 0.41+/-0.06; p=0.030). Thus, ETB-KO mice show increased TA and pendrin expression, but adapt as WT-mice to NH4Cl loading

5.14

ENDOTHELIN RECEPTOR A ANTAGONISMN PREVENTS PLATELET-ACTIVATING FACTOR-INDICED FETAL GROWTH RESTRICTION IN THE RAT

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Background: Platelet-activating factor (PAF) is a common mediator of inflammation. It is often observed with fetal growth restriction (FGR) associated with placental ischemic/inflammatory conditions. PAF can stimulate the production of endothelin-1 (ET) which has been shown to have a significant role in several models of FGR. The extent of the impact of PAF on ET and the pathophysiology of FGR has not been determined. Objective: To elucidate the relationship between PAF and ET in the pathophysiology of PAF-induced FGR in the rat. Methods: Timed-pregnant Sprague-Dawley rats (n=6 per group) were treated with carbamyl-PAF (c-PAF, a stable analog of PAF, 0.5, 1.0, 2.5 µg/kg/h or vehicle) IV by osmotic pump and with an ETA antagonist (ABT-546, 20 mg/kg/day or vehicle) SC by osmotic pump, both for 7 days beginning at gestation day 14. Fetal and placental weights and fetal viability were assessed on day 21 (term=22 days). Uterine and placental tissues were frozen for further analysis. Results are presented as mean ± SE and data were compared by ANOVA with significance at p<0.05. Results: PAF infusion resulted in dose-dependent fetal and placental growth restriction compared to vehicle-treated rats. Simultaneous ETA antagonism completely prevented the FGR produced by 0.5 µg c-PAF/kg/h but did not improve fetal or placental weights when used with higher doses of c-PAF (all p<0.001). Conclusions: PAF infusion produces dose-dependent fetal and placental growth restriction. At low doses of PAF, ET is of primary importance in the pathophysiology of the observed growth restriction. At higher doses of PAF, additional mechanisms are responsible for the growth restriction. Supported by NIH grant HD46968.

5.15

ET RECEPTOR ANTAGONIST IN THE TREATMENT OF DIABETIC KETOACIDOSIS

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Insulin dependent type I diabetes mellitus (TIDM) can be well-controlled with the use of exogenous insulin, however during illness or due to poor management of TIDM glucose levels can rise drastically resulting in diabetic ketoacidosis (DKA). We studied the involvement of endothelin (ET) in DKA. DKA was produced by streptozotocin (STZ) (150mg/kg ip) in rats. On day 4 after STZ injection various parameters were determined before and after following treatments: non-diabetic untreated; diabetic untreated; diabetic saline treated; diabetic saline + insulin treated; and diabetic BMS-182874 + saline + insulin treated. STZ induced DKA was confirmed with increased blood glucose (from 94±3 to 521±20mg/dL), ketones (from 2.86±0.16 to 33.62±2.66mg/dL) and decreased blood pH (from 7.31±0.02 to <6.80). Saline-insulin treatment in DKA rats increased the plasma ET-1 level from 11.76±1.94 to 22.17±2.67pg/ml. BMS-182874 (9mg/kg) produced an improvement in arterial blood pH (from 6.82±0.02 to 6.91± 0.02), K+ (from 4.21±0.33 to 2.75±0.27mmol/dL), and lactate (from 2.74±0.64 to 1.57±0.20mg/dL). DKA rats treated with saline-insulin produced a 33.55% increase in MAP and 58.43% increase in cerebral blood flow (CBF), while BMS-182874 treatment maintained the MAP and CBF closer to baseline. Treatment with BQ123 also improved the blood pH, ketones, blood pressure and CBF in DKA rats. ETA receptor antagonist can be of therapeutic use in the management of DKA and its complications.

5.16

A SERIES OF NOVEL 1-3-6-TRISUBSTITUTED-2-CARBOXY-QUINOL-4-ONE ENDOTHELIN-A ANTAGONISTS

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The human endothelin (ET) peptide family consists of three distinct isoforms- ET-1, ET-2, and ET-3. ET-1 is a potent vasoconstrictor which is both upregulated by inflammatory cytokines and can increase myometrial smooth muscle tone. Preterm birth (PTB), for which the single most common cause is intrauterine infection, is defined as any birth occurring before the completion of 37 weeks' gestation and is a leading cause of perinatal morbidity and death. Several lines of evidence have indicated that ET-1 plays a critical role in the molecular pathway that connects ascending bacterial infection to PTB. Two types of ET receptors, Endothelin-A (ETA), and Endothelin-B (ETB), have been identified. Because ET-1 has a strong affinity for the ETA receptor, we have synthesized a novel series of 1-3-6-trisubstituted-2-carboxy-quinol-4-one's that act as selective Endothelin-A receptor antagonists (ETA-RA's) in vitro. We show that several analogues of these compounds display significant antagonist activity, as they have Inhibitory Concentration (IC50) values for the inhibition of binding of [125I]ET-1 to the ETA receptor of less than 10 nM. In our in vivo system, we show that our series of compounds effectively controls PTB and results in a significant reduction in the number of pups being prematurely delivered (p<0.001) at doses that do not cause toxic effects. The introduction of a series of novel, highly potent and non-toxic ETA-RA's may have broad clinical impact.

5.17

FROG ENDOTHELINS: cDNA CLONING, SEQUENCE AND EVOLUTIONARY ANALYSIS OF ENDOTHELINS

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Endothelin (ET)-related receptors homologous to mammalian receptors have been cloned from Xenopus laevis, indicating that ET-related ligands may be present in this species. Here we cloned cDNAs encoding preproendothelin-1,3 (PPET-1,3) from the X. laevis intestinal cDNA library. X. laevis ET-1,3 cDNAs encode 200 amino acids, including a 20-amino-acid putative signal sequence, as well as mature ET-1,3, big ET-1,3, and ET-1,3-like sequences. This sequences together with other published PPET sequences were used to analyze the phylogenetic relationship among all ET family genes.

5.18

ENDOTHELIN CONVERTING ENZYME-1: A PLAUSIBLE TARGET GENE FOR HYPOXIA INDUCIBLE FACTOR

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Background: Experimental diabetes is characterized by diminished renal parenchymal oxygenation, particularly at the medulla, with enhanced expression of HIF. This condition, as well as ambient hypoxia per se, triggers ET-1 synthesis. We have recently reported that diabetes augment the expression of ECE-1. Study objective: To explore the potential role of HIF as the link between evolving renal tissue hypoxia and the regulation of ECE-1 expression, by the inhibition of HIF- α degradation. Methods: Rats were subjected to the prolyl-hydroxylase inhibitor L-mimosine (600 mg/kg), or to vehicle, and sacrificed 6h later. The right kidney was perfusion-fixed for immunostaining, while the left kidney was dissected and samples of cortex, outer medulla and inner medulla were analyzed for prepro ET-1 and ECE-1 mRNA, and for ET-1 and ECE-1 protein. Results: Mimosine led to HIF-1α accumulation mainly in S3 segments of the outer stripe of the outer medulla. This was associated by enhanced pSTAT-3 expression, principally in distal nephron segments both in the cortex and in the outer medulla, and with a 51% and 66% increase in pSTAT-3 in the outer and inner medulla respectively, without a significant effect in the cortex. Both induction of HIF-1□α and pSTAT-3 were associated by a three folds increase in ECE-1 protein expression in the inner and outer medulla. Conclusions: Enhanced ECE-1 expression in the hypoxic kidney might be triggered by HIF through pSTAT-3.

5.19

HUMAN PODOCYTES EXPRESS ENDOTHELIN RECEPTORS AND DISPLAY ETA RECEPTOR-DEPENDENT ET-1 & PRO-INFLAMMATORY CYTOKINE PRODUCTION

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Podocyte damage leads to proteinuria. This is associated with chronic kidney disease (CKD) progression and incident cardiovascular disease. Endothelin-1 (ET-1) is upregulated in CKD and contributes to proteinuria development. Thus, we investigated the ET system in human podocytes. Human podocytes were cultured in vitro. ETA/B receptor expression was assessed by double label immunofluorescence and PCR. Podocytes were exposed to ET-1 (0-100pg/ml), in the presence/absence of ETA (BQ123) and ETB (BQ788) blockade, and podocyte ET-1, interleukin (IL)-6 and IL-8 production assessed. Podocytes expressed both ET receptors. At all exogenous [ET-1] (10-100pg/ml), initial podocyte ET-1 uptake was followed by ET-1 production (unstimulated 17.2±2.7 vs ET stimulated 43.4±5.9pg/ml, $p<0.01$). BQ123 reduced this (22.1±8.7pg/ml, $p<0.01$ vs ET stimulated). BQ788 appeared to increase podocyte-derived ET-1 (71.5±3.7pg/ml, $p<0.01$ vs ET stimulated). Podocytes showed endogenous IL-6 (4.4±3.2ng/ml) and IL-8 production (7.5±1.6ng/ml). Both were increased by ET-1 stimulation (IL-6: 6.7±6.3ng/ml, IL-8: 13.4±2.4 ng/ml, $p<0.01$ vs. unstimulated for both). The increase in IL-6 and IL-8 production was prevented by BQ123 (IL-6: 4.6±0.9, IL-8: 6.5±2.6 pg/ml, $p<0.01$ vs. ET stimulated for both), but not by BQ788. Human podocytes possess a functional ET system. In response to ET-1, they produce ET-1, IL-6 and IL-8 ETA receptor-dependently. These effects may contribute to proteinuria development and CKD progression.

5.20

TLR4 SIGNALING-MEDIATED ENDOTHELIN UPREGULATION IS CENTRAL TO ISCHEMIA/REPERFUSION-INDUCED FETAL GROWTH RESTRICTION IN THE MOUSE

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Background: Unilateral ischemia/reperfusion (I/R) in pregnant mice leads to fetal growth restriction (FGR) in both horns. We hypothesize that I/R produces reactive oxygen species (ROS)-generated ligands which stimulate TLR4 leading to ET production and ultimately to FGR. Objective: To determine the contribution of TLR4 signaling to I/R-induced FGR in mice. Methods: Pregnant TLR4-deficient and normal mice were subjected to 30 min unilateral I/R on gestation day 15. Fetal and placental weights and fetal viability were assessed on day 19. Additional I/R-exposed normal mice were treated with: 1) antioxidant α-phenyl-N-t-butylnitrone (PBN). 2) Pep-1 (binds hyaluronan and prevents it from activating TLR4). 3) ETA antagonist. Results: Unilateral uterine I/R resulted in FGR in both horns in normal mice (Wts (g): fetal=1.12±0.02 vs 1.27±0.02, $p<.001$; placental=0.09±0.002 vs 0.10±0.003, $p<.01$). I/R in TLR4-deficient mice produced two distinct outcomes. 50% of TLR4-deficient mice miscarried. In the remaining 50%, all fetuses were alive with normal fetal and placental weights; TLR4 deficiency prevented growth restriction. PBN prevented FGR, implicating ROS. Pep-1 prevented FGR, implicating hyaluronan as a TLR4 ligand in I/R-induced FGR. Finally, ETA antagonism prevented FGR, implicating ET, a product of TLR4 signaling. Conclusions: Unilateral uterine I/R produces FGR in both horns in pregnant mice. TLR4 deficiency prevents I/R-induced FGR. TLR4 activation involves ROS-generated ligands, particularly hyaluronan fragments, and the resultant FGR is mediated by the vasoconstrictor ET. Intervention in the TLR4 pathway can prevent I/R-induced FGR in mice.

5.21

STRUCTURAL ASPECTS OF ENDOTHELIN RECEPTOR ANTAGONISTS

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Objective: To study the structures of known endothelin (ET) antagonists and understand the structural features essential to produce ET antagonism. Methods: The literature was surveyed (late 1980s to present) using Pubmed and Scifinder Scholar to obtain structural information on known small molecule ET antagonists. Structures were assessed to identify common structural features. Results: Two chemical structural features appear in the ET antagonists: an acidic group that is predominantly ionized at physiologic pH and, steric bulk in the form of aromatic/heteroaromatic rings. The acidic group is either a sulfonamide or a carboxylic acid. Carboxylic acids are predominantly ionized at physiologic pH; however, the sulfonamide group is not sufficiently acidic. Therefore, an electron withdrawing group is attached to the nitrogen, to increase acidity. Arrangement of the bulky groups and the appending substituents appear to influence the selectivity (ETA vs non-selective) of the antagonist. The acidic group and the bulky groups are arranged on different scaffolds. The more commonly employed scaffolds will be presented along with novel scaffolds being explored in emerging ET antagonists. Conclusions: Presence of an acidic functional group along with steric bulk in the form of aromatic or heteroaromatic rings are essential features required to produce ET antagonism. Selectivity appears to be related to the nature of substituents on the bulky groups.

5.22

CHRONIC ACTIVATION OF THE RENAL ENDOTHELIN RECEPTORS PROMOTES SICKLE CELL GLOMERULOPATHY IN MICE

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Up to 18% of patients afflicted develop end-stage renal disease with glomerulopathy. Glomerular hypertrophy is present in kidneys of patients with secondary focal and segmental glomerulosclerosis (FSGS), and it is linked to development of proteinuria and glomerulosclerosis. No pathophysiological mechanism for glomerular injury in SCD has been found so far. We observed a marked increase in prepro-ET-1 mRNA expression in the endothelium of renal resistive vessels and in podocytes in transgenic sickle cell SAD mice that display FSGS. After 6 months of age, focal and segmental loss of podoplanin was found in SAD mice only, with massive glomerular enlargement (mean glomerular section area: 2372+207 µm2 vs. 1519+180 µm2 in SAD and controls respectively, $p<0.001$). At this time, the dual endothelin receptors (ETR) antagonist bosentan or vehicle, were administered to SAD and normal mice for 5 months. Some mice were sacrificed after 24h and a striking normalization of glomerular size was measured in SAD mice upon ETR antagonism ($p<0.01$). After 5 months of ETR antagonism, glomerular size was still normal and glomerulosclerosis was significantly prevented (86.3 +3.8 % in untreated SAD vs. 39.4 +3.9 % in bosentan-treated SAD, $p<0.01$) with conserved podoplanin expression. In conclusion, ETR chronic activation could favor progression of SCD glomerulopathy through promotion of early glomerular hypertrophy and may represent potential therapeutic target for the prevention of such severe FSGS.

5.23

CORRELATION OF SALIVA AND PLASMA ENDOTHELIN ISOFORMS BET-1, ET-1,ET-2 AND ET-3 IN HEALTHY HUMANS

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Plasma endothelins (ET) have emerged as indicators of cardiovascular effects of air pollutants. Time-series analyses of cardiovascular physiology and endothelin dynamics are limited by the invasiveness of blood sampling. Others have reported recently a correlation between saliva and plasma ET-1 during progression of chronic heart failure. Our objective was to assess the relationships among the endothelin isoforms bET-1, ET-1, ET-2 and ET-3 in saliva and plasma in a first step to validate a potential non-invasive biomonitoring approach to study cardiovascular impacts of air pollutants. Matched plasma and saliva samples from healthy adults (n=30) were obtained from a commercial supplier. Following proteins precipitation in acid acetone, peptides were recovered by 30 kDa molecular filtration, reconstituted in acetonitrile, and measured by HPLC-fluorescence. Results show a positive correlation for all isoforms between saliva and plasma. The ratio of saliva to plasma levels for bET-1 (2.1 vs 3.4 pmoles/ml, m=0.62, correlation p=0.045), ET-1 (2.1 vs 3.5 pmoles/ml, m=0.58, p=0.002), ET-2 (0.8 vs 1.6 pmoles/ml, m=0.49, p=0.013) and ET-3 (1.5 vs 2.3 pmoles/ml, m=0.60, $p<0.0001$) was consistently 0.5-0.6, despite a 5-fold range of mean concentrations between subjects, suggesting diffusion of the peptides from plasma to saliva. Our analyses confirm correlation of plasma and saliva endothelin levels.
Funded by Health Canada (Clean Air Regulatory Agenda).

5.24

CHRONIC INTRAVENOUS INFUSION OF ENDOTHELIN-1 DOES NOT CHANGE PLASMA ALDOSTERONE LEVEL IN RAT

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Endothelin (ET)-1 is reported to induce the aldosterone secretion from zona glomerulosa and is implicated in the elevation of aldosterone level during treatment with angiotensin AT1 receptor blocker. We investigated whether chronic intravenous infusion of ET-1 increases the plasma aldosterone level and the blood pressure. Sprague Dawley rats were given ET-1 (0.5 and 5 pmol/kg/min, i.v.) by osmotic minipump and fed 8% salt diet for 2-weeks. These doses of ET-1 did not affect the blood pressure, renal blood flow and urine volume by 30-minuite intravenous infusion into the anesthetized rats. Infusion of ET-1 for 2 weeks and high-salt diet significantly elevated the systolic blood pressure (change in blood pressure: vehicle; 1.6 ±3.4 mmHg, 0.5 pmol/kg/min; 18.2 ± 2.3 mmHg, 5 pmol/kg/min; 22.3 ±3.2 mmHg, n=6, respectively). Plasma aldosterone level was not changed by ET-1 infusion in high-salt groups (vehicle; 199.3 ± 22.6 pg/mL, 0.5 pmol/kg/min; 204.0 ± 12.3 pg/mL, 5 pmol/kg/min; 209 ± 27.4 pg/mL, n=6, respectively), but was suppressed by high-salt diet compared to normal-salt diet (vehicle; 427.8 ± 29.3 pg/mL, 5 pmol/kg/min; 412.5 ± 42.0 pg/mL). Results suggest that chronic exogenous ET-1 elevates the blood pressure but does not regulate the plasma aldosterone level.

5.25

ETAR AND B1R ANTAGONISM AS OSTEOARTHRITIS THERAPIES

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In osteoarthritis (OA), endothelin-1 (ET-1) plays a critical role in early pathogenesis and disease progression. We have previously shown that ET-1 is overexpressed in OA tissues, where it promotes cartilage catabolism and subsequent synovial inflammation and pain via its A-type receptor (ETAR) by activating proinflammatory signalling pathways, promoting inflammatory mediator release, and upregulating bradykinin receptor 1 (B1R) via cross-talk activation. The specific aim of this study was to characterize the treatment potential of ETAR and B1R peptide antagonists in an in vivo OA model. OA was induced in rats by surgical transection of the right anterior cruciate ligament (ACL). Animals were treated by weekly intra-articular injections of ETAR antagonist BQ123, B1R antagonist R954, both, or saline vehicle. Rats were sacrificed two months post-operatively, and knee joints were dissected, imaged by X-ray and micro-magnetic resonance (MR), and processed for histopathology. Antagonist-treated ACL-transected rats had less subchondral bone remodelling, thicker articular cartilage, and longer cartilage transverse relaxation (T2) times than sham-treated ACL-transected animals, as detected by X-ray, MR, and histopathology. Dual antagonism appeared to be most beneficial. Thus, ETAR and/or B1R antagonist treatment may retard or stabilize the development of morphological changes occurring early in OA. Future work will examine joint pain in vivo. Funding: Arthritis Society, CIHR.

5.26

$ENDOTHELIN_A$ RECEPTOR UPREGULATED BUT ET_B DOWNREGULATED IN PULMONARY ARTERIAL HYPERTENSION

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Pulmonary arterial hypertension (PAH) involves injury to the pulmonary vasculature elevating pulmonary arterial pressure. Right ventricular heart failure is the major cause of death in PAH patients. As PAH progresses chronic pressure and volume overload cause alteration of right ventricle (RV) structure including hypertrophy and dilatation. As a result, the space taken up by the right ventricle in the pericardium increases, impeding left ventricular (LV) diastolic filling, reducing LV end-diastolic volume and altering the LV contractile function. ETA and ETB are both present in human heart and treatment by ET receptor antagonist therapy in PAH is successful, however the contribution of ETB receptors to the development of the condition and the need to block this sub-type as well as the ETA is still unclear. Our aim was to compare the density of both sub-types in RV and LV from control hearts and PAH patients undergoing cardiac transplantation. Our results show there is no change in receptor sub-type ratio in LV, but there is a significant increase in ETA with a concomitant decrease in ETB receptors in the failing RV. Modulation of ET receptors in the RV of PAH patients suggests an adaptive response to both the pressure overload and changes in autocrine/paracrine mediators such as ET-1 experienced by these patients. Davenport AP, Kuc RE (2005). Receptor Binding Techniques.Humana Press,93-120. Davenport AP, Maguire JJ (2006). Endothelin. Handb Exp Pharmacol,176,295-329.

5.27

ENDOTHELIN-1 IMPAIRS SKELETAL MUSCLE GLUCOSE UPTAKE VIA A MECHANISM INVOLVING INSULIN RECEPTOR SUBSTRATE-1 DOWN-REGULATION

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Aim: To investigate if endothelin (ET)-1 contributes to the regulation of skeletal muscle glucose uptake in subjects with insulin resistance (IR). Methods: Eleven IR subjects participated in 3 protocols with brachial artery infusions of: 1) saline followed by insulin, 2) the ETA receptor antagonist BQ123 and the ETB receptor antagonist BQ788, followed by co-infusion with insulin, 3) ET-1. Forearm blood flow (FBF) and endothelium-dependent vasodilatation (EDV) was measured with venous-occlusion plethysmography. Forearm glucose uptake (FGU) was calculated. The effect of ET-1 on insulin receptor substrate (IRS)-1 Ser636 phosphorylation was determined in cultured skeletal muscle cells. Results: ET-1 decreased FGU by 39% ($P<0.05$), reduced basal FBF by 36% ($P<0.05$) and impaired EDV ($P<0.05$) after 2 h infusion. ET receptor blockade increased FBF by 31% ($P<0.05$) and FGU by 62% ($P<0.05$) after 45 min. ET blockade combined with insulin resulted in greater FGU than insulin infusion alone ($P<0.01$). ET-1 increased phosphorylation of IRS-1 at Ser636, an effect that was blocked by bosentan. Conclusion: ET-1 impairs and ET blockade improves skeletal muscle glucose uptake in subjects with IR via mechanism that seems coupled to impaired insulin receptor signaling. Funding: The Swedish Heart and Lung Foundation, the Actelion Endothelin Research Award.

5.28

CHRONIC ENDOTHELIN-A RECEPTOR ANTAGONISM REDUCES PROTEINURIA IN CHRONIC KIDNEY DISEASE THROUGH EFFECTS ON RENAL HAEMODYNAMICS

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Sitaxsentan, an ETA receptor antagonist, reduces BP and proteinuria and in chronic kidney disease (CKD). Nifedipine does not reduce proteinuria despite a matched BP reduction. To investigate potential mechanisms, we studied renal haemodynamics. Methods: In 13 subjects PAH/inulin clearances gave measures of renal blood flow (RBF) and glomerular filtration rate (GFR) pre-dosing, on day 0 and after 6 weeks of placebo, sitaxsentan, and nifedipine in a double-blind randomised study. Results: RBF did not change from day 0 to week 6 with either placebo or nifedipine, whereas RBF tended to increase with sitaxsentan (511±63 vs 543±73ml/min). Whilst GFR was similar at day 0 and week 6 with placebo (56±7 vs 54±8ml/min), and nifedipine (59±8 vs 58±9ml/min), sitaxsentan produced a substantial fall in GFR by week 6 (57±8 vs 48±8ml/min, $p=0.02$). Serum creatinine did not rise. Filtration fraction (FF = GFR/RBF) remained unchanged between day 0 and week 6 with both placebo and nifedipine. FF was lower with sitaxsentan (21±4 vs 16±2%, $p<0.001$). These changes in renal haemodynamics had returned to baseline before starting the next phase of the study (minimum 2 weeks). Conclusion: The likely mechanism for proteinuria reduction in CKD after 6 weeks of sitaxsentan is haemodynamic. The reduction in GFR, but not RBF, with an associated fall in FF, suggests a reduction in efferent arteriolar tone analogous to ACE inhibitors. Longer term these effects should afford renoprotection. Encysive funded.

5.29

ROLE OF ET-1 IN THE INDUCTION OF PLACENTAL ENDOPLASMIC RETICULUM STRESS IN PREGNANCY DISORDERS

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Recent evidence implicates endoplasmic reticulum (ER) stress in pregnancy disorders, such as pre-eclampsia and intrauterine growth restriction (IUGR). The ER is involved in the process of synthesizing and packaging proteins, and also serves as a reservoir of Ca^{2+}. Loss of Ca^{2+} homeostasis in the ER can suppress post-translational modifications of proteins, which triggers ER stress pathways. My project investigates the role of endothelin (ET)-1 in the induction of ER stress. Both IUGR and pre-eclampsia are associated with increased plasma levels of ET-1. ET-1 acts via G-protein-coupled receptors to trigger signaling events in a wide variety of cells. One consequence of ET-1 action is the induction of Ca^{2+} release from the ER, which can disrupt Ca^{2+} homeostasis and potentially induce ER stress. Immunohistochemistry confirmed the presence of both ET-1 and the ETB receptor in the placental trophoblast. Immunoreactivity of both was increased in

pathological placental samples. Human trophoblast-like cells treated with ET-1 gave increased expression of ER stress markers, GRP-78 and GRP-94, in a dose-dependent fashion. Furthermore, a time course analysis showed that ET-1 stimulated increasing ER stress over a 48-hour period. ET-1 also stimulated p-PLC levels, implicating a possible role in Ca^{2+} signaling. Immunoprecipitation data show that ET-1 induces phospho-activation of the ETB receptor, suggesting this is the mechanism of action of ET-1 in initiating ER stress signaling. Further understanding how ER stress is generated in pathogenic pregnancies may allow the development of possible strategies to circumvent this stress as a therapeutic tool. **Funding: CTR Studentship.**

5.30

ET_B RECEPTOR DEFICIENCY ENHANCES THE ACUTE BUT NOT CHRONIC EFFECTS OF ANGIOTENSIN II ON THE BLOOD PRESSURE OF FEMALE RATS

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The ET_B receptor mediates a number of anti-hypertensive effects including endothelial-dependent vasodilation and natriuresis. This experiment determined whether the ET_B receptor protects against the pro-hypertensive actions of angiotensin (Ang)II in female rats. Acute pressor responses to i.v. administration of AngII (0.02-0.24µg/kg) were compared in anesthetized (inactin, 100 mg/kg i.p.) wild type (WT; n=6) and ET_B deficient (sl/sl; n=6) rats. Responses to AngII were assessed as the peak change in mean arterial pressure (MAP) from basal levels. AngII elicited dose-dependent increases in MAP in both genotypes ($P<0.001$), with the increments in MAP being significantly greater in sl/sl rats irrespective of the dose ($P<0.01$). To assess whether ET_B receptors also attenuate the chronic effects of AngII, the change in 24h average MAP in response to continuous infusion of AngII at 200 ng/kg/min s.c. was compared in WT (n=5) and sl/sl rats (n=7) over 14 days (telemetry). MAP prior to AngII infusion was significantly higher in sl/sl versus WT rats (119±3 and 97±3mmHg respectively, $P<0.001$), but the AngII-induced change in MAP from basal levels was not significantly different between the two genotypes at day 14 (+40±4 and +40±6mmHg in WT and sl/sl rats respectively). These results suggest that the ET_B receptor somehow buffers the pressor effect of AngII in acute settings, but is unable to protect against the pro-hypertensive effect of AngII in the chronic setting

6.0: ENDOTHELIN IN DEVELOPMENT AND AGING

6.2

EXERCISE AND AGE-RELATED ET-1 MEDIATED VASOCONSTRICTOR TONE

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Impaired vascular vasomotor function plays an essential role in the pathogenesis and prognosis of cardiovascular disease. The endothelium is central to the regulation of vascular tone through the synthesis and release of opposing vasoactive substances, particularly nitric oxide and endothelin-1 (ET-1). It has been suggested that a disruption in the balance between the opposing effects of these vasoactive substances, favoring constriction, contributes to the age-related decline in vasomotor regulation. However, data on this issue in adult humans has been limited. We recently completed a series of studies focused on the influence of aging on ET-1-mediated vasoconstrictor tone and the effects of regular aerobic exercise on the age-related effects. Using an isolated forearm model, we determined blood flow responses to intra-arterial infusion of ET-1 and selective and nonselective ET-1 receptor blockade in young and older adults. The effect of ET-1 blockade on acetylcholine-mediated vasodilation was also investigated. Advancing age, independent of other cardiovascular risk factors, is associated with increased ET-1-mediated vasoconstrictor tone. The age-related increase in ET-1 system activity contributes to impaired endothelium-dependent vasodilation in older adults. Importantly, regular aerobic exercise is an effective lifestyle intervention strategy for reducing ET-1-mediated vasoconstrictor tone in older adults. (NIH HL077450, HL076434, and MO1 #RR00051) Van Guilder GP, et al. Endothelin-1 vasoconstrictor tone increases with age in healthy men but can be reduced by regular aerobic exercise. Hypertension, 50:403-409, 2007.

6.3

ENDOTHELIN RECEPTOR A ANTAGONISM PREVENTS PLACENTAL PATHOLOGY CAUSED BY UTERINE ISCHEMIA/REPERFUSION-INDUCED OXIDATIVE STRESS IN THE RAT

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Background: Ischemia/reperfusion (I/R) of one rat uterine horn produces fetal and placental growth restriction in both horns. Endothelin (ET) is central to this process. The placental damage that occurs in response to I/R has not been described. Objective: To examine the impact of ETA antagonism on protein and nucleic acid alteration in response to oxidative stress and resultant morphologic changes in the placenta. Methods: Unilateral occlusion of uterine and ovarian arteries for 30 min, or sham operation, was performed on timed-pregnant rats (6/group) on gestation day 17 (term=22 days). Additional I/R or sham rats received an ETA antagonist (ABT-546, 20 mg/kg/day, SC by osmotic pump). Tissues were collected on day 18, morphology was examined, and immunohistochemistry (IHC) localized both nitrotyrosine (NT) and 8-hydroxyguanine (8-HG). Results: IHC revealed extensive nuclear staining for 8-HG in placental tissues from I/R but not sham rats. 8-HG was not associated with any specific region of the placenta. In contrast, NT staining was not different between I/R and sham rats. Extensive cellular damage in the placental transitional zone was present in I/R but not sham rats. Cellular structure in the glycogen-containing cells was largely absent in I/R-treated rats but intact in sham-operated rats. In I/R-exposed rats treated with ETA antagonist, the cellular structure was the same as in shams. Conclusions: The large glycogen-containing cells of the placental transitional zone are especially vulnerable to damage from uterine I/R. Nucleic acid oxidative damage is a prominent effect of uterine I/R. ET antagonism protects placental integrity during I/R challenge in the pregnant rat. Supported by NIH grant HD42581.

6.4

THE DEVELOPMENT OF ALZHEIMER DISEASE LIKE PATHOLOGY IN YAC ABPP TRANSGENIC MICE IS ASSOCIATED WITH THE OVEREXPRESSION OF ENDOTHELIN-1 AND NITRIC OXIDE SYNTHASE

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Endothelin-1 (ET-1) is implicated in the pathogenesis of cardio- and cerebrovascular diseases. We hypothesize that because of an imbalance between the nitric oxide synthase (NOS) isoforms and ET-1, a human gene that encodes for blood vessel constriction, there therefore is development of brain pathology that manifests as AD. We investigated the impact of aged dependent (6, 12 and 18-24 month old) progressive brain pathology in a yeast artificial chromosome (YAC) transgenic mice overexpression of amyloid beta precursor protein (AÃŸPP) on the ET-1 and NOSs immunoreactivities on the brain tissues by using polyclonal and/or monoclonal antibodies. Immunocytochemical pattern of the eNOS/ET-1 immunoreactivity were seen in brain parenchymal cells but not within vascular endothelium in the brains of aged YAC AÃŸPP Tg+ mice. The YAC Tg+ mouse brains show the presence of a nNOS/ET-1 and iNOS/ET-1-containing immunopositive reaction not only in neurons, but also in all of the other brain cellular compartments. Our study, for the first time, demonstrates that misbalance between different isoforms of NOS and ET plays a major role for the BBB interruption. By using pharmacological intervention with NO donors and/or NO suppressors and ET-1 receptor antagonist the brain lesions and the downstream progression of brain pathology and dementia in AD should be delayed or minimized.

6.5

EXOCYTOSIS OF ENDOTHELIN-1 FROM ENDOTHELIAL CELLS INDUCES RAPID VASOCONSTRICTION IN AGED ARTERIES

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Human aging is associated with increased levels of endothelin (ET-1) and endogenous ETA-mediated constriction. The present experiments assessed the effects of aging on ET-1 activity in aortas from young (3 mos) and aged F344 rats (18-20 mos). After inhibition of NO synthase (LNAME), thrombin (1 U/ml) caused rapid contraction of aged aortas that was prevented by the ETA antagonist BQ123 (1 µM), by endothelial-denudation or by inhibiting exocytosis with TAT-NSF (1 µM), an inhibitor of N-ethylmaleimide sensitive factor, but was not affected by control peptide TAT-CON (1 µM). Thrombin did not cause endothelium-dependent contraction of young aortas. In aged but not young aortas, thrombin caused a rapid 3.2-fold increase in ET-1 release that was not affected by LNAME, but was abolished by endothelium-denudation or TAT-NSF. Confocal microscopy localized immunoreactive ET-1 to endothelial granules distinct from Weibel-Palade bodies. Under basal conditions, ET-1 immunofluorescence was similar in young and aged aortas. However, thrombin caused a rapid and marked increase in ET-1 staining in aged but not in young aortic endothelium. Indeed, contractions to thrombin were inhibited by acute treatment with the ECE inhibitor, phosphoramidon (30 µM). Therefore, activation of aged but not young arterial endothelium is associated with the rapid generation and exocytosis of ET-1, which causes vasoconstriction and may contribute to vascular dysfunction associated with aging.

6.6

THE METABOLIC SYNDROME IS ASSOCIATED WITH GREATER ET-1 MEDIATED VASOCONSTRICTOR TONE IN OLDER MEN

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Background: The metabolic syndrome (MetS) refers to a clustering of risk factors that promote and accelerate the development of cardiovascular disease (CVD). A potential mechanism contributing to the increased CVD risk in older adults with the MetS is endothelial vasomotor dysfunction. Currently, there is little information regarding the influence of the MetS on endothelin (ET)-1 vasoconstrictor activity in older men. We tested the hypothesis that endogenous ET-1 vasoconstrictor activity is greater in older men with the MetS compared with those without the MetS. Methods: Forearm blood flow (FBF: plethysmography) responses to intra-arterial infusion of ET-1 (5 pmol/min; for 20 min) and BQ-123 (100 nmol/min; for 60 min), a selective ETA receptor antagonist, were determined in 16 older men: 8 with the MetS (age: 61.9±1.8 yr; BMI: 31.3±0.9 kg/m2) and 8 without the MetS (age: 60.1±2.6 yr; BMI: 30.3±0.7 kg/m2). Results: The vasoconstrictor response to ET-1 was significantly lower (~135%) in the older men with the MetS (+4%) versus those without the MetS (11%). In response to BQ-123, FBF was significantly increased in both groups, however, the older men with the MetS demonstrated a greater vasodilator response (100%) than those without the MetS. Conclusions: These results indicate that the MetS is associated with greater ET-1-mediated vasoconstrictor tone. Increased ET-1 vasocostrictor activity may contribute to the elevated CVD risk in older men with the MetS.

7.0: PULMONARY FUNCTION AND DISEASE

7.2

TGFβ AND BMP9 STIMULATION OF ENDOTHELIN-1 SYNTHESIS IN HUMAN LUNG MICROVASCULAR ENDOTHELIAL CELLS: A COMPARATIVE STUDY

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Introduction: Heritable pulmonary arterial hypertension (PAH) is a debilitating disease characterized by endothelial cell dysfunction and obliteration of the pulmonary microvasculature. Excess levels of the endothelial-derived vasoconstrictor and mitogen endothelin-1 (ET-1), are found. Abnormalities in the receptor cascade for various members of the TGFβ peptide superfamily, including TGFβ and bone morphogenic proteins (BMP) have been implicated as triggers for heritable PAH. BMP9 is a recently characterized vascular quiescence factor that shares receptors with TGFβ, including activin like kinase 1 (ALK1). TGFβ stimulates ET-1 release from lung derived human microvascular endothelial cells (HMVEC-LBl). It is unknown if BMP9 acts to a similar degree. We therefore compared the two peptides. Methods: HMVEC-LBl were cultured in vitro and after an overnight serum starvation were exposed to BMP9 (2.5ng/ml), TGFβ (2.5ng/ml) or both. Media were collected for ELISA measurement for ET-1 and the cells were lysed and protein content was measured. ET-1 was normalized to cell protein Results: BMP9 and TGFβ stimulated ET-1 to a similar degree, with an 49% increase in ET-1 levels after 8 hours. Exposure to both peptides simultaneously further increased ET-1 levels by 34%. Conclusions: BMP9 stimulates ET-1 production by HMVEC-LBl as potently as does TGFβ, and both molecules provide additive effect. BMP9 and TGFβ may be acting through two separate TGFβ receptors. BMP9 may be a novel mediator in the pathogenesis of PAH. Funding: Fonds de la Recherche en Sante du Quebec.

7.3

ET_{AB} BLOCKADE HAS NO PULMONARY VASODILATOR EFFECT AFTER PDE5-INHIBITION

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ETA/B blockade and PDE5-inhibition are both used as vasodilators to alleviate pulmonary hypertension and to improve exercise capacity. The aim of the present study was to investigate if the vasodilator effect of ETA/B blockade is maintained after prior PDE5 inhibition at rest and during exercise. Five swine, chronically instrumented for measurement of aortic pressure, pulmonary artery pressure, left atrial pressure and cardiac output, were studied at rest and during treadmill exercise (1-4 km/h) under control conditions, after PDE5-inhibition with EMD360527 and after ETA/B blockade with tezosentan. Exercise resulted in systemic vasodilation(systemic vascular resistance (SVR) decreased from 20±3 to 10±1 mmHg/L/min) and pulmonary vasodilation (pulmonary vascular resistance (PVR) decreased from 2.4±0.4 to 2.0±0.4 mmHg/L/min). PDE5-inhibition resulted in systemic and pulmonary vasodilation at rest (SVR 13±2, PVR 1.6±0.1 mmHg/L/min) and during exercise (SVR 7±1, PVR 1.3±0.3 mmHg/L/min). After PDE5-inhibition, tezosentan had no effect on SVR at rest, but decreased SVR during exercise (13±3 and 6±1 mmHg/L/min) while it had no effect on PVR either at rest or during exercise. In conclusion, PDE5-inhibition and ETA/B blockade have additive vasodilator effects on the systemic vasculature, while ETA/B blockade has no vasodilator effect on the pulmonary vasculature after prior PDE5-inhibition. Supported by Netherlands Heart Foundation (2000T042).

7.4

DISRUPTION OF ENDOTHELIN HETERODIMERS ALTERS PULMONARY VASCULAR REACTIVITY TO ENDOTHELIN-1

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Background: Endothelin-1 (ET-1) is a potent vasoconstrictor of pulmonary arteries that induces its effects via the activation of ETA (ETA) and ETB receptors (ETB). The role played by each receptor is questioned by evidence that they can form heterodimers. Disruption of ETA/ETB heterodimers could shed light on the role played by each receptor in the ET-1 response. Experimental approach: Heterodimerization was evaluated by co-immunoprecipitation. Disruption of heterodimers was carried out by reducing protein expression of ETA or ETB. Pulmonary arteries were incubated in the presence of antisense (AS) targeting either the rat ETA (ETA-AS) or ETB (ETB-AS) mRNA. Following AS treatment, pulmonary reactivity to ET-1 was assessed using a myograph. Results: Heterodimerization of both receptors was observed. Each AS reduced the protein expression of its targeted receptor. The use of ETA-AS significantly increased the vascular sensitivity and the maximal vasoconstriction induced by ET-1. In contrast, the ETB-AS significantly reduced the sensitivity to ET-1 without affecting the Emax. Conclusions: With suppression of the ETB, the ETA still induces maximal pulmonary vasoconstriction to ET-1 but with a reduction in sensitivity. In contrast, suppression of ETA increased both vascular sensitivity and maximal constriction to ET-1, suggesting that the ETA limits ETB-dependent contraction. This study suggests functional significance of heterodimerisation of ET receptors in pulmonary arteries.

7.5

ENDOTHELIN-1 MEDIATES THE EFFECT OF TRANSFORMING GROWTH FACTOR-B ON WOUND REPAIR AND SKIN FIBROSIS

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Fibrosis, an excessive deposition of extracellular matrix (ECM) proteins, is thought to be a failure to suppress the normal tissue repair program and is the hallmark of chronic diseases including systemic sclerosis, and pulmonary or skin fibrosis. The cytokine TGF-β plays a fundamental role in fibrotic processes. ET-1 is a target gene for the action of TGF-β and it has also been proposed as downstream mediator of profibrotic responses. The aim of the present study was to characterize the TGF-β-induced pathways leading to ET-1 expression in human dermal fibroblasts, as well as the effect of TGF-β and ET-1 on the acquisition of profibrotic phenotype. We found that the ability of TGF-β to induce the expression of profibrotic genes depends on ET-1. In experiments involving full-thickness skin wounds in mice, we found that the gene transfer of TGF-β using adenovirus resulted in accelerated healing. Mice treated with the antagonist of ET receptors, bosentan restored healing rates. ET-1-overexpressing adenovirus significantly accelerated wound closure. In experiments using the bleomycin-induced mouse model of skin fibrosis, we also found that the blockade of the TGF-β signalling by GW788388, and the antagonism of ET receptors with bosentan prevented the fibrotic response. These results demonstrate a crucial role for ET-1 in regulating cutaneous wound size and suggest that targeting ET-1 may be of benefit in controlling tissue repair and fibrogenic responses in vivo.

7.6

INVOLVEMENT OF ENDOTHELIN-1 IN HABITUAL AEROBIC EXERCISE-INDUCED INCREASE IN ARTERIAL COMPLIANCE

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Habitual aerobic exercise results in a significant increase in central arterial compliance. Endothelin-1 (ET-1) could play a role in mediating the habitual aerobic exercise-induced increase in central arterial compliance. The aim of the present study was to examine whether ET-1 involves in the mechanisms underlying the increase in central arterial compliance to aerobic exercise training. Seven healthy middle-aged and older adults underwent systemic endothelin-A/B (ETA/B)-receptor blockade (500 mg of Tracleer) before and after 12 weeks of aerobic exercise training. Basal carotid arterial compliance increased significantly after exercise training. Plasma ET-1 concentration decreased significantly after the exercise training. Before the exercise intervention, carotid arterial compliance increased significantly with the administration of ETA/B-receptor blockade. After the training, however, increases in carotid arterial compliance previously observed with the ETA/B-receptor blockade before the training were abolished. Regular aerobic exercise enhances central arterial compliance in middle-aged and older humans. The increase in arterial compliance was associated with the corresponding reduction in plasma ET-1 concentration as well as the elimination of ET-1

mediated vascular tone. These results suggest that reductions in ET-1 may be an important mechanism underlying the beneficial effect of exercise training on central artery compliance.

9.0: ROLES OF ENDOTHELIN-1 ON CARDIAC FUNCTION AND DISEASE

9.2

WHAT YOU SHOULD KNOW ABOUT ENDOTHELIN

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In the 1980s a world-wide search for the identity an endothelium-derived vasoconstrictor resulted in the discovery of the 21-amino acide peptide endothelin-1 (ET-1). The field of endothelin research has since grown extensively by on average about 3 publications per day. The development of endothelin receptor angatonists by the pharmaceutical industry enabled scientists and physicians to study the role of endothelin in preclinical models of human disease. Over the years, the understanding regarding the functions of ET-1 has increased dramatically. We now know that ET-1 it not only is vasoconstrictor and survival factor during embryonic development, but that it also acts as a growth factor, cytokine, immunomodulator, mediator of pain, carcinogen, and that is plays an important role in kidney and vascular disease. After twenty years of extensive research, endothelin receptor antagonists (ERAs) have finally arrived in the clinics, and provide us with new tools to treat diseases such as pulmonary arterial hypertension. Possible future indications include proteinuric renal, vascular diseases, connective tissue diseases, certain forms of cancer, among others, and several clinical studies in these areas are ongoing. Indeed, there is now good evidence indicating that endothelin blockade will continue in becoming new paradigm to treat or even reverse disease in humans. Barton M, Yanagisawa M. Endothelin - 20 years from discovery to therapy. Can J Physiol Pharmacol 2008; 86: 485-498; Barton M. Reversal of proteinuric renal disease and the emerging role of endothelin. Nature Clin Pract Nephrol 2008; 4: 490-501.

9.3

ENDOTHELIAL CELLS-DERIVED ENDOTHELIN-1 PROMOTES CARDIAC FIBROSIS IN DIABETIC HEART THROUGH STIMULATION OF ENDO-THELIAL TO MESENCHYMAL TRANSITION

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Persistently high plasma endothelin-1 (ET-1) level in diabetes patients is associated with development of cardiac fibrosis. Here we hypothesize that ET-1 might contribute to accumulation of cardiac fibroblast through endothelial to mesenchymal transition (EndMT) in diabetic heart. We developed Streptozotocin-induced diabetes in vascular endothelial cell-specific ET-1 knockout (VEETKO) mice and its wild type (WT) littermates. All analysis was performed after 8, 24 and 36 weeks duration of diabetes. Diabetes increased ET-1 expression in WT heart. Electron microscopy shows disruption of mitochondria and myofibril structures, which is associated with the increasing of oxidative stress in WT but not in VEETKO heart. ET-1 further promotes endothelial cells (ECs) to undergo phenotypic conversion into fibroblast-like cells, which expressed fibroblast protein S100A4/FSP1 and ECs marker CD31; thus leads to cardiac fibrosis and heart failure in WT, but not in VEETKO mice (FS 37.55±2.73% vs. 43.63±0.91%, $p<0.01$, n=8 each). Targeted ET-1 gene silencing by siRNA in cultured human ECs ameliorated high glucose-induced phenotypic transition into fibroblast-like cell, through inhibition of TGF-ß signaling and preservation of VE Cadherin. In conclusion, these results indicate that diabetes-induced cardiac fibrosis is associated with the emergence of fibroblast from ECs origin, and this EndMT is stimulated by ET-1. Targeting ECs-derived ET-1 might be beneficial to prevent diabetic cardiomyopathy.

9.4

CROSSTALK BETWEEN ENDOTHELIN-1 AND NEUROPEPTIDE Y IN HUMAN ENDOCARDIAL ENDOTHELIAL CELLS

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The objective of the study was to determine if a dialogue exists between the NPY and ET-1 systems at the level of human right (REECs) and left (LEECs) ventricular EECs. Using immunofluorescence coupled to 3D confocal microscopy and ELISA, our results show that increasing concentrations of NPY induce the release of ET-1 from REECs and LEECs in a time and dose-dependent manner; REECs having a higher ET-1 secretory capacity than LEECs. Using selective antagonists for the Y1, Y2 and Y5 receptors and the ETA and ETB receptors, our results show that in REECs, the NPY-induced release of ET-1 is primarily due to Y2 receptors activation with the subsequent activation of the ETA and ETB receptor by the released ET-1. In contrast, in LEECs, the NPY-induced secretion of ET-1 is mainly due to Y5 receptor activation by NPY without the contribution of ET-1 receptors activation by the released ET-1. Our results suggest that NPY is a regulator of ET-1 secretion in human EECs and this secretory process is different in REECs compared to LEECs. Thus, EECs contribute to the release of factors, such as NPY and ET-1 that can affect not only the excitation-secretion coupling of EECs but also the excitation-contraction coupling of the adjacent cardiomyocytes. Supported by CIHR and NSERC grants to Dr D. Jacques.

9.5

DOXORUBICIN-INDUCED CARDIOMYOPATHY IS ATTENUATED IN ECE-1 HETEROZYGOUS KNOCKOUT MICE VIA PREVENTING THE IMPAIRMENT OF CARDIAC MITOCHONDRIAL BIOGENESIS

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Doxorubicin is an effective anticancer drug, but the clinical benefit is limited by its cardiotoxicity. The inhibition of mitochondrial biogenesis is critical for the pathogenesis of Doxorubicin-induced cardiomyopathy. Plasma ET-1 levels are elevated in patients treated with Doxorubicin, but the role of the endothelin system in Doxorubicin-induced cardiomyopathy is not fully solved. We investigated the role of the endothelin system in Doxorubicin-induced cardiomyopathy with endothelin converting enzyme-1 heterozygous knockout mice (ECE-1+/- mice). Cardiomyopathy was induced by a single i.p. injection of Doxorubicin. Five days after treatment, echocardiography revealed that fractional shortening was significantly higher in ECE-1+/- mice than that in control mice ($P<0.05$). In histological analysis, the cardiomyocyte size of ECE-1+/- mice was larger ($P<0.01$), and the damage of cardiomyocytes was less. Real time RT-PCR analysis demonstrated that PPARgamma coactivater 1alpha, a key regulator of mitochondrial biogenesis, was higher in ECE-1+/- mice ($P<0.05$). The mitochondrial DNA copy number and the ATP content of hearts were higher in ECE-1+/- mice than those in control mice ($P<0.05$). In summary, the inhibition of the endothelin system by decreased ECE-1 expression attenuated Doxorubicin-induced cardiomyopathy through preventing the impairment of cardiac mitochondrial biogenesis and the interruption of ECE-1 is a new strategy for the treatment of Doxorubicin-induced cardiomyopathy.

9.6

ADDITIONAL LACK OF ENOS PROMOTES CARDIAC FIBROSIS IN ET-1 TRANSGENIC MICE

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ET-1 is antagonized by nitric oxide (NO) mainly derived from endothelial NO-synthase (eNOS). We established cross-bred animals of ET-1 transgenic mice (ET+/+) and eNOS knock out mice (eNOS-/-) characterized by endothelial dysfunction and elevated blood pressure (BP). Using this model we aimed at elucidating the impact of lack of eNOS in ET+/+ mice on cardiac phenotype. Systolic BP was elevated in both eNOS-/- and ET+/+eNOS-/- mice compared to ET+/+ and WT mice. Cardiac interstitial fibrosis was increased in ET+/+eNOS-/- animals only. Media-to-lumen ratio of cardiac arterioles was decreased and lumen area increased in ET+/+ and ET+/+eNOS-/- group compared to WT. No difference in relative heart weight were detected. Western Blot analysis showed a significantly decreased ET-B expression in eNOS-/- and ET+/+eNOS-/- mice. Cardiac functions were reduced in eNOS-/- mice. A 2-Dimensional Electro-phoresis based proteomics study coupled to mass spectrometry (2DE/MS) revealed that proteins involved in regulation of oxidative stress, fatty acid metabolism and cytoskeletal organization were differentially abundant in the heart of our model compared to WT. ET+/+eNOS-/- mice represent therefore a novel model of myocardial fibrosis due to imbalance between the ET and NO systems in absence of hypertrophy. ET leads to dilated morphology of arterioles independently from BP. 2DE/MS study could define molecular mechanisms occuring in case of an elevated ET-1 and reduced NO production in vivo.

9.7

EFFECT OF BOSENTAN ON REDUCTIVE STRESS INDUCED ENDOTHELIN-1 / ENOS DYSFUNCTION IN CARDIOMYOCYTES

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Impairments of redox balance in cells and sub-cellular structures can lead to either oxidative or reductive stress. While oxidative stress has many known, damaging effects, specific cellular and molecular responses to a reducing environment remain elusive. Reductive stress is defined as the supra-physiological levels of intracellular reducing power (GSH/GSSG, NADH/NAD, NAPDH/NADP,

Cysteine/Cystine). In mouse hearts exhibiting protein aggregation and reductive stress (Rajasekaran et.al, Cell, 2007), the transcriptional products for endothelin (ET) and endothelin receptors (ET-A and ET-B) were significantly upregulated along with a profound decrease in the endothelial nitric oxide synthetase (eNOS). Using potential oxidizing (BSO) and reducing (DHLA or NAC) agents, we have established oxidative and reductive stress models in HL-1 cardiomyocytes in vitro. The redox state of cardiomyocytes was determined by measuring the reduced/oxidized levels of glutathione (GSH/GSSG) and reactive oxygen species (ROS) using fluorescent probes. Effects of reductive stress on cardiomyocytes hypertrophy (ANF, BNF), contractility and cell survival/death pathways were investigated. Results indicated significant impairment of Et-1/eNOS system with reductive stress, suggesting a direct role for the reductive stress on the regulation of endothelin and eNOS levels. Further, we have used bosentan, a specific antagonist/inhibitor of ET receptors, to rescue the changes associated with reductive stress in HL-1 cardiomyocytes.

10.0: ROLES OF ENDOTHELIN-1 ON VASCULAR FUNCTION AND DISEASE

10.2

O-GLCNACYLATION: ANOTHER MECHANISM FOR THE EFFECTS OF ET-1 ON THE VASCULATURE?

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O-Linked attachment of β-N-acetyl-glucosamine (O-GlcNAc) on serine and threonine residues of nuclear and cytoplasmic proteins is a highly dynamic and ubiquitous post-translational modification that plays a key role in altering the function and activity of target proteins. We recently demonstrated that O-GlcNAcylation augments vascular reactivity to contractile stimuli and O-GlcNAc-proteins are increased in the vasculature of mineralocorticoid hypertensive rats. Since ET-1 plays a major role in vascular dysfunction associated with salt-sensitive forms of hypertension, we tested whether ET-1 induces O-GlcNAc modification of vascular proteins. The effects of ET-1 on vascular function and O-GlcNAc levels were determined in vitro and in vivo. The contribution of O-GlcNAc to ET-1 effects was assessed by pharmacologic and molecular inhibition of the O-GlcNAc pathway. ET-1 increases vascular O-GlcNAc levels and reactivity to contractile stimulus. Inhibition of the post-translational modification with O-GlcNAc prevented ET-1 effects on vascular reactivity, indicating that ET-1 indeed augments O-GlcNAc levels and that this modification contributes to the vascular changes induced by this peptide. Modulation of vascular O-GlcNAcylation by ET-1 may represent a mechanism for hypertension-associated vascular dysfunction or other pathological conditions associated with increased levels of ET-1. Supported by NIH, FAPESP.

10.3

EFFECT OF ENDOTHELIN-1 OVEREXPRESSION ON VASCULAR STRUCTURE AND FUNCTION OF APOLIPOPROTEIN E KNOCKOUT MICE

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Resistance arteries from transgenic (Tg) mice with endothelial-restricted preproendothelin-1 (eET-1) overexpression exhibit vascular remodeling and endothelial dysfunction. ET-1 is implicated in atherosclerosis. It is unknown whether the effects of eET-1 will be exaggerated in atherosclerosis. We investigated this by crossing eET-1 Tg with ApoE-/-, a model of atherosclerosis. Eight-week old eET-1 Tg, ApoE-/-, eET-1/ApoE-/- crosses, and wild type littermate mice (WT) were studied. Mesenteric arteries were mounted on a pressurized myograph. At 45 mmHg, media thickness & media-to-lumen ratio (M/L) of eET-1 were greater than in WT (media: 12.5±0.4 vs 10.3±0.5 µm; M/L: 7.2±0.6 vs 5.3±0.6%). Media cross-sectional area of crossed mice was greater than in WT (8138±159 vs 6711 ±279 µm2). Acetylcholine (Ach)-induced relaxation was impaired in ApoE-/- compared to WT (Emax: 53.7±1.3 vs 84.1±6.5%). In presence of a nitric oxide synthase (NOS) inhibitor, Ach-induced maximal relaxation was reduced to 43.1±12.6% in crosses, greater than in the three other groups (15% remaining relaxation). Ach-induced NOS-dependent relaxation was decreased in crosses compared to WT (30.9±8.2 vs 67.1±3.1%). The results suggest that eET-1-induced remodeling was affected by crossing with ApoE-/-. Ach-induced NOS-dependent relaxation was replaced in part by NOS-independent relaxation, possibly through increased activity of endothelium-derived hyperpolarizing factors. The research is funded by CIHR grant 37917.

10.4

ATTENUATED COLLATERAL FORMATION UNDER DIABETES IS IMPROVED BY GENETICAL SUPPRESSION OF ENDOTHELIN CONVERTING ENZYME-1

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Many studies have indicated that the association of endothelin(ET) system and diabetic complications. However, the role of the ET system in collateral formation under diabetes is still not clarified. Endothelin converting enzyme-1(ECE-1) is a relevant enzyme not only to produce ET-1 from its precursor big ET-1 but also to degrade natriuretic peptides. We set out our study to test the hypothesis that ECE-1 inhibition possesses beneficial therapeutic effect in response to hindlimb ischemia in Type1 diabetic models using ECE-1 heterozygous knockout (ECE-1+/-) mice. Ten weeks after Streptozotocin injection, femoral artery was ligated. Then we assessed blood flow recovery and collateral flow by Laser Speckled Perfusion Imager (LSPI). DM ECE-1+/-mice significantly improved blood flow, and collateral flow as compared to DM WT mice. Lumen diameter, and wall thickness of collateral arteries were significantly augmented in DM ECE-1+/-mice. Tissue ET-1 levels were markedly upregulated in DM WT and this upregulation is attenuated in DM ECE-1+/-mice. Furthermore, tissue cGMP which is downstream signaling of NO and natriuretic peptides was significantly decreased in DM WT mice compared with non-DM mice, but DM ECE-1+/-mice prevented its downregulation. These data indicate that ECE-1 inhibition provides a novel therapeutic strategy to rescue diabetic critical limb ischemia through the improvement of collateral formation by amelioration of vasoactive substances profile.

10.5

SYNERGY OF AGONISTIC AUTOANTIBODIES TARGETING ETA- AND AT1 RECEPTORS INCREASES SENSITIVITY TO NATURAL LIGANDS

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"Renal crisis", is a major cause of death in systemic sclerosis (SSc) patients. We hypothesized that activating antibodies directed against angiotensin type 1 receptor (AT1R-Abs) and endothelin type A receptor (ETAR-Abs) may induce and amplify vascular dysfunction mediated by natural ligands Endothelin-1 (ET-1) and Angiotensin II (Ang II). Contractility was studied in microdissected rat renal interlobar artery ring segments mounted in a small vessel myograph. ERK1/2 phosphorylation was studied in primary rat vascular smooth muscle cells (VSMC) by western blot. We detected constitutive expression of AT1R, AT2R, ETAR, and ETBR mRNA in renal interlobar arteries. SSc-IgG positive for both AT1R-Abs and ETAR-Abs induced concentration-dependent contraction in renal arteries that was blocked by respective receptor blockers. Arteries treated with SSc-IgG showed significantly increased contractile responses to both Ang II (11.8 +/- 4.3%, $P < 0.05$) and ET-1 (184 +/- 14%, $P < 0.05$). The findings imply an importance of functional autoantibody-mediated crosstalk and synergy between renin-angiotensin and endothelin systems in SSc-related renal crisis pathophysiology. VSMCs incubated with SSc-IgG showed an increase in ERK1/2 phosphorylation, which was inhibited by selective receptor blockers. Our data indicate that synergies and amplification of AT1R and ETAR actions warrant close attention in SSc patients who could possibly benefit from a multimodal receptor inhibition approach.

10.6

DUAL ET RECEPTOR ANTAGONISM PREVENTS DIABETIC REMODELING OF RESISTANCE ARTERIES: COMPARISON TO SELECTIVE RECEPTOR BLOCKADE

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Vascular remodeling, characterized by extracellular matrix deposition and increased media-to-lumen (M/L) ratio, contributes to the development of microvascular complications in diabetes. We have previously shown that selective ETA receptor blockade prevents whereas selective ETB receptor blockade augments medial thickening of mesenteric arteries via regulation of matrix metalloproteases (MMP) in type 2 diabetic Goto-Kakizaki (GK) rats. The goal of this study was to determine the effect of combined ETA and ETB receptor blockade on resistance vessel remodeling. Vessel structure, MMP activity and extracellular matrix proteins were assessed in control Wistar and diabetic GK rats treated with vehicle or bosentan (100 mg/kg/day) for 4 weeks (n=7-8 per group). M/L ratio (0.1 ± 0.01 vs 0.2 ± 0.04*) and MMP-2 activity (12,593 ± 3,892 vs 21,879 ± 2,071 pixels*) were increased in diabetes (*$p<0.01$). Bosentan completely prevented these changes. Collagenase (MMP-13) activity and protein levels were significantly decreased in diabetes. Accordingly, collagen deposition was augmented in GKs (472 ± 73 vs 1003 ± 206 pixels, $p<0.01$). Dual ET receptor antagonism improved enzyme activity and normalized MMP-13 levels without any

effect on tissue inhibitor of MMPs (TIMP-2). In light of our previous data which showed that ETB receptors are vasculoprotective and blockade of this receptor increases remodeling of resistance arteries, current findings suggest that diabetes mediates the remodeling effect via activation of ETA receptors and dual blockade is an effective strategy to prevent this response.

10.7

UPREGULATION AND LOCALISATION OF APELINS IN HUMAN ATHEROSCLEROSIS: COMPARISON WITH ET-1

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We have shown that endothelin-1 (ET-1) is significantly increased in human vessels with atherosclerotic lesions, with the peptide mainly confined to infiltrating macrophages (1) and smooth muscle ECE activity is increased in human diseased coronary artery (CA) compared with non-diseased controls (2). We find that apelin peptides, acting on endothelial APJ receptors, oppose ET-1 vasoconstriction in vessels from patients with coronary artery disease (CAD) and hypothesize that apelins may function as physiological antagonists of ET-1. Using RIA and HPLC fractionation we have determined the predominant apelin isoform in heart from patients with CAD as [Pyr1]apelin-13. RIA revealed a significant increase in apelin peptide levels in human atherosclerotic CA (73.2±14.9 pg/g, n=6) compared to histologically normal CA (24.1±3.5 pg/g, n=6, P<0.01). Apelin-like immunoreactivity (-LI) was restricted to the endothelium in normal CA, whereas intense staining to the atherosclerotic plaque was observed in diseased CA. APJ-LI was present on the endothelium and smooth muscle of normal and atherosclerotic CA. Within the atherosclerotic lesion both apelin-and APJ-immunoreactivities co-localised with cell markers for smooth muscle cells and macrophages, but not T-cells. These data suggest that the apelin system is upregulated in human atherosclerosis and may oppose detrimental actions of ET-1.

1) Bacon et al. Circ Res, 1996;79:794-801. 2) Maguire & Davenport. Br J Pharmacol, 1998;125:238-240.

11.0: POSTER SESSION II

11.1

ANGIOTENSIN II HYPERTENSION IMPAIRS ETB-DEPENDENT NATRIURESIS AND REDUCES RENAL MEDULLARY ETB RECEPTOR EXPRESSION

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Since the endothelin B (ETB) receptor functions to promote sodium excretion, we hypothesized that ETB dependent natriuresis is impaired in Ang II hypertensive rats. We determined the diuretic and natriuretic response to intramedullary infusion of ETB receptor agonist sarafotoxin 6c (S6c) in rats treated with Ang II (65 ng/min s.c.) or 0.9% NaCl (vehicle control) for 14 days. Urine was collected from anesthetized rats during two consecutive 20 min baseline periods followed by four additional 20 min periods of intramedullary infusion of S6c (0.45 µg/kg/h, 0.5 ml/hr). S6c increased urine flow (5.9±0.8 vs 14.3±1.5 µl/min; p<0.05) and sodium excretion (0.86±0.20 vs 1.64±0.19 µmol/min; p<0.05) in control rats. S6c did not increase of urine flow (7.5±1.6 vs 10.8±1.0 µl/min) or sodium excretion (0.60±0.21 vs 0.95±0.07 µmol/min) in Ang II rats. S6c did not affect MAP or medullary blood flow (laser-Doppler flowmetry) in either group. To determine whether the lack of S6c-induced natriuresis could be due to reduced ETB expression, inner and outer medulla were collected from control and Ang II hypertensive rats. Ang II rats expressed less ETB receptor (Western blot) in inner medulla compared to the control group (0.66±0.10 vs 0.92±0.06 A.U., normalized to actin; p<0.05, but there was no difference in outer medullary expression. In conclusion, renal medullary ETB receptor function is impaired in Ang II hypertension that may be due to reduced ETB receptor expression.

11.2

SPOO2P, A BOTANICAL DRUG, INHIBITS ENDOTHELIN-1 INDUCED AORTIC CONTRACTIONS IN VITRO AND LOWERS BLOOD PRESSURE OF ANESTHETIZED RATS

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Elevated endothelin levels are associated with cardiovascular diseases, e.g., hypertension and chronic heart failure. An endothelin synthesis and/or receptor inhibitor is potentially beneficial for treating cardiovascular diseases. SPOO2P (PhytoPharmacon, LLC, Durham, NC) is a botanical drug that is rich in myriceric acids A and C. Myriceric acid A has been reported as a non-peptide antagonist of endothelin A (ETA) receptors. The present study determined the effects of SPOO2P on aortic muscle contractions in vitro and on blood pressure of anesthetized rats. Contraction of aortic rings bathed with physiologic salt solution (37ºC; gassed with carbogen) was measured with force displacement transducers coupled to a Tissue Force Analyzers (Micromed, Louisville, KY). Hypertension was induced with nitro-L-arginine methyl ester (L-NAME; 1 g/L) in drinking water for 3 weeks. SPOO2P (20-100 µg/ml) concentration-dependently antagonized aortic contractions elicited by endothelin-1. The effect was not reversible by short-term washing. SPOO2P (100 mg/kg; orally) also significantly reduced the mean arterial blood pressure (MAP) and heart rate (HR) of control and L-NAME hypertensive rats. The vehicle, DMSO, did not inhibit contractions or reduce MAP and HR in anesthetized rats. We conclude that SPOO2P is a potentially useful anti-hypertensive botanical drug if it scales the toxicity huddle.

[Support: University of Louisville Research Initiation Grant; Dillon Eweson granted travel support].

11.3

ANGIOTENSIN II INCREASES THE EXPRESSION OF KININ B1 RECEPTOR THROUGH AN ENDOTHELIN MECHANISM IN VASCULAR SMOOTH MUSCLES CELLS

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Kinin B1 receptor (B1R) is up-regulated by oxidative stress in diabetes and hypertension. Because angiotensin II (Ang II) and endothelin-1 (ET-1) are pro-oxidative peptides involved in cardiovascular diseases, their contribution in B1R up-regulation was determined in vascular smooth muscle cells (VSMC). Methods: B1R was measured by QRT-PCR and Western blot in VSMC derived from A10 cell line and aorta of Sprague-Dawley rats in presence of Ang II (1 nM-10 µM; 0-24 h) or ET-1 (100 nM; 0-6 h). VSMC were pretreated with selective receptor antagonists (1-10 µM) for AT1 (losartan), AT2 (PD-123319), ETA (BQ123) and ETB (BQ788) or antioxidants (N-acetyl-L-cysteine 2 mM and diphenyleneiodonium 10 µM) or inhibitors of PI3kinase (wortmannin, 100 nM) and ERK1/2 (PD098059, 10 µM). Results: Ang II increased B1R expression (mRNA and protein) in a concentration and time-dependent manner (maximum 1 µM between 3-6 h). This was blocked by losartan and wortmannin, but not by PD-123319 and PD098059. BQ123 inhibited at 6 h but not at 3 h. Both BQ123 and BQ788 prevented ET-1 induced up-regulation of B1R. B1R up-regulation by Ang II and ET-1 was prevented by antioxidants. Conclusion: Ang II increased B1R expression in VSMC via AT1 receptor in the early phase and following the release of ET-1 and ETA receptor activation in the late phase. This seems to occur through PI3kinase and oxidative stress. Results highlight new mechanisms by which B1R may contribute to diabetes and hypertension. [Supported by Grants from CIHR].

11.4

ACTIVATION OF GPER INHIBITS CONTRACTION TO ENDOTHELIN-1 AND CAUSES NO-DEPENDENT RELAXATION OF CORONARY ARTERIES

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Estrogens are important moduclators of blood pressure and vascular tone. We have recently identified the novel intracellular, transmembrane G protein-coupled receptor, GPER (formerly known as GPR30), as a modulators of blood pressure and obesity (Circ Res 2009; 104: 291). The role of GPER for epicardial coronary artery function is unknown. Porcine coronary arteries were therefore studied. Arteries were cut into 4-5mm rings and isometric tension was recorded in organ chambers. All rings were pretreated with meclofenamate to exlcude effects of cyclooxygenase. In rings precontracted with PGF2alpha, 17β-estradiol (E2), the GPER agonists G1 and ICI182780 were investigated, with EtOH as solvent control. Both G-1 and ICI caused time-dependent relaxation (p<0.05). Relaxation to G1 was blocked by removing the endothelium or by NO synthase inhibition using L-NAME (p<0.05). G1, but not E2, attenuated endothelin-1 (ET-1) induced contractions. In contrast, E2, but not G1 or ICI, attenuated serotonin-induced contractions. These data demonstrate that in epicardial coronary arteries activation of GPER attenuates ET-1-mediated contractility and induces NO-mediated, endothelium-depedndent dilation. In contrast, contractions to serotonin remained unaffected by G1. These findings point at novel vascular mechanisms involving GPER that could be important for vascular pathophysiology and disease and possibly therapy.

11.5

IMPAIRED FLOW-INDUCED ARTERIAL REMODELING DURING DOCA-SALT HYPERTENSION

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We studied the effect of elevated blood flow on arterial remodeling in endothelin-1 dependent DOCA-salt hypertensive rats. One week before the DOCA-salt treatment, selective ligation of mesenteric resistance arteries (MrA) resulted in exposure of first order MrA to high blood flow (HF, +100%). DOCA-salt

treatment was applied for either 3 or 6 weeks. First-order MrA were isolated and cannulated in a pressure arteriograph to measure lumen diameter (Ø) at 80 mmHg. Cross sectional area of the media (mCSA) was determined on cross sections. Mean arterial blood pressure was significantly increased after 3 or 6 weeks of DOCA-salt treatment as compared to control Wistar rats (117±2 vs 148±5 mmHg and 117±2 vs 172±4 mmHg resp.). After 3 weeks DOCA-salt treatment, normal flow (NF) arteries showed outward (Ø: from 385±13 to 463±14 µm) hypertrophic (mCSA: from 10380±1083 to 17540±1952 µm2) remodeling, as compared to their normotensive controls. No outward hypertrophic remodeling occurred in HF arteries of DOCA-salt treated animals (Ø: 483±24 µm; mCSA: 16980±1742 µm2). Furthermore, NF and HF vessels of DOCA-salt treated animals showed a marked reduction in calculated distensibility as compared to their normotensive controls. Similar results were obtained after a 6 week DOCA-salt treatment. For the first time we showed that outward flow-induced remodeling is impaired during the development of DOCA-salt hypertension. DOCA-salt hypertension is accompanied by arterial stiffening. This research was performed within the framework of project T2-108 of the Dutch Top Institute Pharma.

11.6

ENDOTHELIN-1-INDUCED CARDIAC HYPERTROPHY IS REGULATED BY C-TERMINAL DOMAIN PHOSPHATASE OF RNA POLYMERASE II

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Cardiac hypertrophy is associated with the increase of total amount of RNA and protein, which is in accordance with RNA polymerase II (Pol II) activation. It has been demonstrated that endothelin-1 (ET-1) phosphorylates the C-terminal domain (CTD) of Pol II; however, roles of CTD phosphatase 1 (CTDP1) on phosphorylation of CTD and cardiomyocyte size is unknown. Transient expression and/or knockdown by siRNA for CTDP1 gene using adenovirus vectors was examined in cultured cardiomyocytes. ET-1 stimulation evoked the increase of cardiomyocyte size. ET-1-induced cardiomyocyte hypertrophy was inhibited by CTDP1 overexpression and this phenomenon was augmented by CTDP1 knockdown using siRNA. Global RNA synthesis evaluated by [3H]-Uridine incorporation was parallel to the morphological findings. ET-1-induced Pol II CTD phosphorylation determined by Western blot was decreased by CTDP1 overexpression and increased by knockdown. Next, we evaluated the following mRNA expression by quantitive RT-PCR: hypertrophy associated genes, ANP, βMHC, MLC2. CTDP1 overexpression significantly downregulate all the examined genes (0.2-0.6 fold from baseline) and the knockdown significantly upregulate them (1.5-2.5 fold). However, the overexpression and/or knockdown did not alter 18S ribosomal RNA and 7SKsnRNA expression regulated by RNA Pol I and Pol III, respectively. Therefore, it suggests that endogenous CTDP1 regulates the Pol II CTD phosphorylation level and ET-1-induced cardiac hypertrophy is regulated by CTDP1 function to keep suitable cardiomyocyte size.

11.7

ENDOTHELIN-1 SIGNALING IN THE FEMALE INTERNAL PUDENDAL ARTERY: POTENTIAL ROLE IN FEMALE SEXUAL DYSFUNCTION

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Increased ET-1 constrictor sensitivity is observed in various cardiovascular diseases, as well as erectile dysfunction. The internal pudendal artery (IPA) supplies blood to the vagina and clitoris. Inadequate blood flow through the IPA leads to genital engorgement insufficiencies. Our aim is to characterize the effects of ET-1 on the IPA. The IPA from female Sprague Dawley rats (225-250 g) were mounted in microvessel chambers, maintained at 37°C. Segments were submitted to increasing concentrations of ET-1 (10-10 to 10-6 M) with and without the ETAR antagonist, atrasentan (10-8 M) or the Rho-Kinase inhibitor, Y-27632 (10-6 M). Actions mediated through the ETBR were assessed by IRL-1620 (10-10 to 10-7 M). mRNA of preproET-1, ETAR, ETBR, RhoA and Rho-kinase from IPA were measured by real time PCR. All Emax values are expressed as % KCl max. ET-1 constricted IPA concentration dependently (143.33±8.28; pD2=7.49±0.1) while pretreatment with Y-27632 (101.25±11.6; pD2=7.06±0.19) and atrasentan decreased ET-1-induced contraction (76.67±20.84, pD2=5.44±033; IRL-1620 did not induce IPA relaxation or constriction. PreproET-1, ETAR, ETBR, RhoA and Rho-kinase message were detected in IPA. We showed that the IPA, essential in vaginal and clitoral blood flow, is sensitive to ET-1, signals through the ETAR and activates Rho-kinase. These data indicate that ET-1 may play a role in impaired vaginal and clitoral blood flow in pathologies where ET-1 levels are elevated.

11.8

ANGIOTENSIN TYPE 1 RECEPTOR AND ENDOTHELIN TYPE A RECEPTOR ANTIGEN SPECIFIC T-CELLS AND RECEPTOR RESTRICTED HUMORAL RESPONSE IN WAIT-LISTED PATIENTS

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Antibodies recognizing the Angiotensin type 1 receptor (AT1R-Abs) and Endothelin type A receptor (ETAR-Abs) are involved in pathogenesis of obliterative vasculopathy in allografts and native organs. Primary sensitization events precluding antibody production may require T-helper cells. Our aim was to elucidate whether patients wait-listed for renal transplantation develop T-cell immunity against AT1R and ETAR. 19 hemodyalisis patients wait-listed for renal transplantation were studied, 11 positive for AT1R- and ETAR-Abs and 8 negative. AT1R-Ab positive patients showed an increased T cell responsiveness three-fold higher compared to AT1R-Abs negative patients (p=0.029). ETAR-Ab positive patients developed an increased T cell reactivity 1.7 times higher than for the negative patients (p=0.046). We observed significant positive correlation with Pearson coefficient between the magnitude of T-cell response and AT1R-Ab titers (0.441, p=0.029). Similar correlation was detected for T-cell response against ETAR and respective antibody titers, Pearson coefficient 0.499, p=0.015. We provide evidence for AT1R-, and ETAR-antigen specific CD4+ T-cell mediated autoimmunity in parallel to respective receptor-restricted humoral pre-sensitization.

11.9

DENDRITIC CELLS AND THE ENDOTHELIN SYSTEM: IMPLICATIONS FOR ATHEROSCLEROSIS

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Endothelin-1 and the innate immune system play important roles in atherosclerosis. Dendritic cells (DC) particularly are key antigen-presenting cells expressing toll-like receptors (TLRs) that detect signal molecules released in response to tissue injury. Currently the relationship between ET-1, TLRs and DC function is not fully understood. Aim: To evaluate effects of specific TLR ligands on ET-1 activity in human monocyte-derived dendritic cells (MoDC). Methods: Purified monocytes were incubated for 6 days in RPMI 1640 medium with 10% FCS, 1% L-Glutamine, 100 U/ml Penicillin/ Streptomycin, 10 ng/ml GM-CSF and 10 ng/ml IL-4 to generate MoDC. TLR ligands, hyaluronan and lipopolysaccharide were then added for 24 or 48 hours to induce maturation. Results: MoDC stimulated with exogenous and endogenous TLR2 and TLR4 agonists, dose and time dependently, increased ET-1 formation. DC activation in the presence of vitamin D3 (tolerogenic DC) potentiated the ET-1 response. Chetomin, an inhibitor of the transcription factor HIF-1-alpha, prevented TLR-mediated ET-1 release. Conclusion: TLR activation after vascular injury may exacerbate atherosclerosis and promote further damage via HIF-1-alpha-mediated activation of downstream effector molecules including ET-1. The role of ET-1 in DC function may differ depending upon the mode of activation and induction of DC that are either tolerogenic or initiate T-cell priming. (Funding , EU 6th Framework, SNF, Circulation Foundation UK).

11.10

ACTIVATING AT1R AND ETAR AUTOANTIBODIES SERVE AS BIOMARKERS AND LINK AUTOIMMUNITY AND VASCULOPATHY IN SYSTEMIC SCLEROSIS

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We hypothesized that autoimmune angiotensin and endothelin receptor stimulation might contribute to pathogenesis and represent the functional link between autoimmunity, vasculopathy and tissue fibrosis in systemic sclerosis. We analyzed sera of 212 patients with systemic sclerosis, 60 healthy control subjects, 120 rheumatoid arthritis patients and 124 additional control subjects with control diseases for the presence of antibodies directed against angiotensin II type 1 receptor (AT1R) and endothelin-1 type A receptor (ETAR) by a solid phase assay. Individual organ involvement, and patient survival were assessed. Vascular responsiveness to natural receptor ligands, Angiotensin II and Endothelin-1 was studied ex vivo in pulmonary resistance arteries. AT1R-AA and ETAR-AA were detected in 56.6% and 57.5% of the SSc patients, respectively, but only in 9.6% and 10.8% subjects with control diseases. AT1R-AA and ETAR-AA strongly predicted vascular complications and mortality. AT1R-AA and ETAR-AA act as functional enhancers of the vasoconstrictive responsiveness towards Endothelin-1 and Angiotensin II. Functional autoimmunity directed at AT1R and ETAR identifies SSc patients with higher risk for severe disease, vascular complications, and decreased survival. AT1R- and ETAR are functional antibodies that may serve as a novel biomarker for risk assessment in SSc.

11.11

DIFFERENTIAL REGULATION OF ERK1/2, VIA DOWNREGULATION OF MKP-1, MEDIATES SEX-DIFFERENCES IN VASCULAR REACTIVITY IN DOCA-SALT HYPERTENSION

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We hypothesized that vascular ET-1-activated ERK1/2 signaling is upregulated in male deoxycorticosterone (DOCA)-rats, contributing to sex-related differences in vascular responses. Uninephrectomized male and female Sprague-Dawley rats received DOCA pellets (200mg/Kg) and were treated with saline for 3 weeks. Control rats received vehicle and tap water. Male DOCA-rats displayed higher blood pressure (191±3 mmHg) as well as higher urinary ET-1 excretion (4.64±1.02pmol/mL), compared to female DOCA-rats (172±7mmHg; 2.55±0.37pmol/mL, respectively). Contractile responses to phenylephrine (PE) were augmented in both aorta (21.73±2.9mN) and small mesenteric arteries (13.1±1.6mN) from male, compared to female DOCA-rats (15.1±1.2mN; 10.9±0.8mN, respectively). ERK1/2 inhibition with PD-98059 (10μM) abrogated increased contraction to PE in aorta (14±2mN) and small mesenteric arteries (10±2mN) from male DOCA-rats. Phosphorylated ERK1/2 levels were increased in aorta from male DOCA-salt rats whereas mitogen-activated protein kinase phosphatase-1 (MKP-1) expression and interleukin-10 plasmatic levels were reduced. We speculate that 1) increased vascular ET-1-activated ERK1/2 signaling and 2) decreased levels of MKP-1 and IL-10, which negatively modulates ERK1/2 activation, observed in male DOCA-rats may contribute to sex-related differences in vascular function.

11.12

EFFECTS OF EXOGENOUS BIG ENDOTHELIN-1 ON POST-ISCHEMIC CARDIAC DYSFUNCTION AND NOREPINEPHRINE OVERFLOW IN RAT HEARTS: INVOLVEMENT OF ET_B RECEPTOR/NOS1 SYSTEMS

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The aim of this study was to investigate the influence of exogenously applied big endothelin (ET)-1, ET-1 precursor, in ischemia/reperfusion (I/R)-induced norepinephrine (NE) overflow and cardiac dysfunction. According to the Langendorff technique, isolated rat hearts were subjected to 40-min global ischemia followed by 30-min reperfusion. Exogenous big ET-1 (0.1, 0.3 and 1 nM) was perfused from 15 min before ischemia. As a result, higher doses (0.3 and 1 nM) of big ET-1 significantly improved I/R-induced cardiac dysfunction such as decreased left ventricular developed pressure (LVDP) and the maximum value of the first derivative of left ventricular pressure (dP/dtmax) and increased left ventricular end-diastolic pressure (LVEDP). In addition, big ET-1 significantly suppressed excessive NE overflow and increased NOx (NO2/NO3) release in the coronary effluent from the post-ischemic heart. These effects of big ET-1 were markedly attenuated by treatment with SM-19712 (selective ECE inhibitor), A-192621 (selective ETB receptor antagonist), or Nω-propyl-L-arginine [selective NO synthase-1 (NOS-1) inhibitor], respectively. From these findings, we suggest that exogenous big ET-1 has beneficial effects on I/R-induced cardiac injury. It seems likely that big ET-1 converts to ET-1, locally in the heart, and this ET-1 preferentially binds to ETB receptors and activates NOS-1 to exert its related actions.

11.13

SHORT SLEEP DURATION IS ASSOCIATED WITH GREATER ENDOTHELIN-1 VASOCONSTRICTOR TONE

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Background: Epidemiological studies indicate that short sleep duration is associated with increased cardiovascular (CV) morbidity. Endothelial vasomotor dysfunction represents a potential mechanism contributing to the increased CV risk with short sleep. Endothelin (ET)-1 is a potent vasoconstrictor peptide that is associated with endothelial vasomotor dysfunction and increased CV risk. Currently, there is no information regarding the influence of short sleep duration on ET-1 vasoconstrictor activity in adults. We tested the hypothesis that ET-1 mediated vasoconstrictor activity is greater in adults who sleep less than 7 hrs/night (short sleepers) compared with those who sleep 7 to 9 hrs/night (normal sleepers). Methods: Forearm blood flow (FBF) responses to intra-arterial infusion of BQ-123 (100 nmol/min for 60 min), a selective ETA receptor antagonist, were determined in 80 adults: 50 normal sleepers (32M/18F; age: 57±1 yr; sleep: 7.6±0.1 hrs/night) and 30 short sleepers (17M/13F; 57±1 yr; 6.1±0.1 hrs/night). Results: In response to BQ-123, short sleepers had a greater increase in resting FBF compared with normal sleepers (~20% vs. ~8%; $P<0.05$). There was an inverse relation between average sleep duration and blood flow response to BQ-123 ($r=-0.29$, $P<0.05$). Conclusions: Short sleep duration is associated with greater ET-1-mediated vasoconstrictor tone. Increased ET-1 vasoconstrictor activity may contribute to the elevated CV risk associated with short sleep duration.

11.14

RALOXIFENE REDUCES ET-1 GENE EXPRESSION THROUGH THE AMPK/ENOS PATHWAY IN CULTURED CALF PULMONARY ARTERY ENDOTHELIAL CELLS

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It has been shown that estradiol inhibits endothelin-1 (ET-1) synthesis through the increase in nitric oxide (NO) production. In the present study, we investigated the effect of raloxifene, which is a selective estrogen receptors modulator, on ET-1 production in cultured calf pulmonary artery endothelial cells (CPAECs). Raloxifene significantly decreased prepro ET-1 mRNA expression in CPAECs. This inhibitory effect was completely abolished by an estrogen receptor antagonist ICI182,780 and a nonselective nitric oxide synthase (NOS) inhibitor L-NAME. Western blot analysis showed that raloxifene increased phosphorylated endothelial NOS (eNOS) protein levels in CPAECs. In addition, raloxifene enhanced NOx output from CPAECs. On the other hand, recent studies have reported that AMP-activated protein kinase (AMPK) is closely related to eNOS activation. Therefore, we next examined whether the inhibitory effect of raloxifene on ET-1 gene expression is based on the AMPK activation. Raloxifene increased phosphorylated AMPK protein levels in CPAECs. Furthermore, treatment with an AMPK inhibitor compound C completely abolished the suppressive effect of raloxifene on prepro ET-1 mRNA expression. Taken together, it appears that raloxifene inhibits ET-1 gene expression possibly through the AMPK/eNOS pathway.

11.15

TGF-β INCREASES INWARD REMODELING OF CANNULATED SMALL MESENTERIC ARTERIES

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Small arteries remodel eutrophically in response to many physiological and pathological stimuli. We previously found no effect of phosphoramidon and bosentan on physiological remodeling of pressurized vessels in organoid culture. As a first step for testing the involvement of endothelins in pathological remodeling, we here establish a model for cytokine-induced inward remodeling. The effects of cytokines known to up-regulate endogenous ET-1 production were studied in vitro in isolated rat mesenteric arteries cultured 72 hours in a pressure myograph. Remodeling was determined by a pressure-diameter relationship under passive conditions. Incubation with a combination of TNF-α, IL-1β and IFN-γ (10 ng/ml) did not significantly affect remodeling. Vessels cultured with TGF-β (5 ng/ml) showed increased inward remodeling as compared to controls ($P\leq0.03$). Changes in mRNA expression of genes related to the endothelin system and the mechanisms underlying TGF-β-induced remodeling will be further investigated. This study was performed within the framework of the Dutch Top Institute Pharma project T2-108.

11.16

EFFECT OF PRESSURE-OVERLOAD HYPERTROPHY ON THE SUB-CELLULAR LOCALIZATION OF ENDOTHELIN RECEPTORS

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Endothelins, specifically ET-1 and ET-3, are implicated in both cardiac function and pathophysiology via their actions on ETA (selective for ET-1 >> ET-3) and ETB (non selective) receptors. ETB are present on nuclear membranes. The present study was to determine if the distribution of intracellular ETRs is altered during hypertrophic cardiomyopathy. First, we studied the endocytosis of ETs in adult rat cardiac ventricular myocytes (ACVMs) and in rat aortic endothelial cells (RAECs). In ACVMs, which express ETA and ETB, ET-3 localized to lysosomes whereas ET-1 was detected in subcellular compartments in addition to lysosomes. In RAECs, which express only ETB, ET-1 localized to lysosomes. Endocytosed ETB:ligand complexes were never observed at the nuclear membrane. We then examined the effect of pressure-overload hypertrophy induced by transverse aortic constriction (TAC). After 7 days ETB was associated with the nuclear membrane in sham hearts, whereas ETB was associated with nuclear and perinuclear structures in TAC hearts. The distribution of ETA was unaffected. Overall, these

results suggest that, in normal hearts, the localization of ETB to the nuclear membrane is not a result of post-endocytotic trafficking. Moreover, the subcellular localization of ETB is altered during pressure-overload hypertrophy, suggesting a potential role for ETB during hypertrophy.

11.17

DARUSENTAN IS A POTENT INHIBITOR OF ENDOTHELIN SIGNALING AND FUNCTION IN BOTH LARGE AND SMALL ARTERIES

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Endothelin (ET) is a potent vasoconstrictor that is upregulated in hypertension. ET vasoconstriction is mediated via the ETA receptor present on vascular smooth muscle cells, and ET receptor antagonists (ERAs) have been shown to antagonize ET-induced vasoconstriction, leading to current ERA therapies in pulmonary hypertension. We describe the pharmacology of darusentan (DAR), a propionic-acid-based ERA in phase III clinical trials for resistant hypertension. DAR was tested in a variety of assays to determine its biochemical and functional potency. Rat aortic vascular smooth muscle cells (RAVSMs) were isolated and characterized by flow cytometry. RAVSM membrane fractions exhibited moderate ET receptor density. Receptor counting showed that >95% of the ET receptors in these membrane fractions were the ETA subtype. (S)-DAR competed for radiolabeled ET binding in RAVSM membranes, exhibiting a Ki=13nM, while (R)-DAR showed no activity. In cultured RAVSMs, ET increased IP and Ca++ signaling, both of which were attenuated by (S)-DAR. In isolated denuded rat aortic rings, (S)-DAR inhibited ET-induced vascular contractility with a pA2=8.1±0.14; (R)-DAR failed to modulate contractility. The potency of (S)-DAR did not change when determined in isolated denuded rat mesenteric arterioles. (S)-DAR is an ERA with high affinity for ETA. (S)-DAR inhibits ET-induced signaling related to pro-contractile activity and is a potent inhibitor of vasoconstriction in large and small arteries.

11.18

O-GlcNACYLATION CONTRIBUTES TO AUGMENTED VASCULAR RE-ACTIVITY INDUCED BY ET-1

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The modification of proteins with O-linked beta-N-acetylglucosamine (O-GlcNAc) modulates vascular reactivity. We hypothesized that ET-1 augments vascular contractile responses via O-GlcNAc modification of proteins. Incubation of rat aortas with ET-1 (0.1μM, 1 to 24h) produced a time-dependent increase in O-GlcNAc levels (arbitrary units= 2.2±0.1 vs. 1.0±0.05 control; n=4, 24h) and decreased expression of OGT and OGA, key enzymes in the O-GlcNAcylation process. Overnight treatment of aortas with ET-1 increased phenylephrine (PE) vasoconstriction [Emax (mN) = 19±5 vs. 11±2 vehicle; n=6]. ET-1 effects were not observed when vessels were previously instilled with anti-OGT antibody or after incubation with an OGT inhibitor (ST045849). Aortas from DOCA-salt rats, which exhibit increased prepro-ET1 expression, displayed increased contractions to PE as well as augmented levels of O-GlcNAc proteins. Treatment of DOCA-salt rats with an ETA antagonist, atrasentan, abrogated augmented vascular levels of O-GlcNAc and prevented increased PE vasoconstriction. Aortas from rats chronically infused with low doses of ET-1 (2pmol/Kg/min, 14 days) exhibited increased O-GlcNAc-proteins as well as enhanced PE responses [Emax (mN) = 18±2 vs. 10±3 vehicle; n=6]. These changes are similar to those induced by PugNAc, an inhibitor of OGA. We conclude that ET-1 augments O-GlcNAc levels and that this modification contributes to the vascular changes induced by this peptide. Financial Support: NIH (HL-74167) and FAPESP.

11.19

ENDOGENOUS ENDOTHELIN-1 ENHANCES THE EXPRESSION OF GIA PROTEINS IN VASCULAR SMOOTH MUSCLE CELLS FROM SPONTANEOUSLY HYPERTENSIVE RATS: ROLE OF GROWTH FACTOR RECEPTORS TRANSACTIVATION

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Objective: Considering our finding of enhanced expression of Giα proteins in vascular smooth muscle cells (VSMC) from spontaneous hypertensive rats (SHR) and various studies showing increased endothelin-1 (ET-1) in hypertension, we investigated the role of endogenous ET-1 and underlying mechanisms in enhanced expression of Giα in VSMC from SHR. Methods: Western blot assessed expression of Giα-2 and Giα-3 proteins and phosphorylation of ERK 1/2, PDGFR, EGFR, IGF-1R and c-Src in VSMC from 12-week old SHR and age-matched Wistar-Kyoto (WKY) rats after treatment with (10-6-10-3M) ETAR (BQ123) and ETBR (BQ788) antagonists, PDGFR (AG1295), EGFR (AG1478), IGF-1R (AG1024), c-Src (PP2) and NAD(P)H oxidase (diphenyleneiodonium, DPI) inhibitors and superoxide anion (N-acetyl-L-cysteine, NAC) scavenger for 16 hours. Results: BQ123, BQ788, AG1295, AG1478, AG1024 and PP2 attenuated the enhanced expression of Giα proteins. Furthermore, BQ123, BQ788, NAC, DPI and PP2 decreased the enhanced phosphorylation of PDGFR, EGFR and IGF-1R. Additionally, AG1295, AG1478, AG1024 and PP2 attenuated the enhanced ERK 1/2 phosphorylation. Finally, DPI, NAC, AG1295, AG1478 and AG1024 diminished the increased phosphorylation of c-Src. Conclusion: Our results suggest that enhanced endogenous ET-1, through augmentation of oxidative stress and of activation of c-Src, growth factor receptor and MAP kinase, may contribute to the enhanced expression of Giα proteins in SHR. (Supported by a grant from CIHR).

11.20

MODULATION OF CARDIOVASCULAR EFFECTS OF CLONIDINE AND CENTHAQUIN BY ENDOTHELIN

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Clonidine is an antihypertensive agent acting through central α2-adrenergic receptors to lower mean arterial pressure (MAP) and peripheral α-adrenergic receptors to produce vasoconstriction. Centhaquin produces effects similar to clonidine. Endothelin (ET) has been shown to modulate the action of adrenergic receptors. In the present study, involvement of ET in cardiovascular effects of clonidine and centhaquin was investigated. Rats were anaesthetized with urethane and hemodynamic parameters were determined. Clonidine (10, 30 and 90 μg/kg, iv) and centhaquin (0.11, 0.33 and 0.99 mg/kg, iv) produced a dose dependent fall in MAP, pulse pressure and heart rate (HR). Treatment with ET-1 (100, 300 and 900 ng/kg, iv) significantly attenuated clonidine or centhaquin induced fall in MAP. In rats treated with ET-1 (900 ng/kg), clonidine (42.58%) and centhaquin (33.48%) produced a significant increase in MAP compared to untreated rats. Clonidine (37.42%) and centhaquin (21.44%) produced a significant increase in HR with ET-1 (900 ng/kg) treatment compared to untreated rats. TAK-044 (1 mg/kg) and BMS-182874 (9 mg/kg) potentiated the hypotensive effect of clonidine and centhaquin. Prazosin blocked ET-1 induced changes in cardiovascular effects of clonidine and centhaquin. It is concluded that ET modulates vascular adrenergic receptors and alters the cardiovascular effects of clonidine and centhaquin. ET antagonists potentiate the antihypertensive effects of clonidine and centhaquin.

11.21

EXPRESSION OF ENDOTHELIN RECEPTORS IN HUMAN CAROTID ATHEROSCLEROSIS

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Objective: To characterize the expression of endothelin receptors (ETA and ETB) in human carotid endarterectomies (CEA) and their correlation with clinical parameters and expression of inflammatory genes. Methods: Carotid plaques from patients undergoing CEA combined with a database including clinical and laboratory variables (Biobank of Karolinska carotid Endarterectomies; BiKE) were used. mRNA expression of the ETA and ETB receptors was analysed with microarray and realtime PCR on a subset of 107 and 138 patients, respectively. Immunohistochemistry was used to localize protein expression. Results: The ratio of ETB/ETA receptor mRNA expression was higher in lesions (1.44) than in normal arteries (0.93, $P<0.01$). There were no significant correlations between expression of ET-1 or the receptors and clinical parameters. Staining revealed that the ETB receptor was upregulated in endothelial cells and in smooth muscle cells in comparison with control arteries. Both receptors were present in inflammatory cells such as macrophages and dendritic cells in the plaque shoulder. mRNA expression of the ETB receptor correlated strongly with macrophage surface markers whereas the ETA receptor correlated with smooth muscle cell markers. Conclusion: These findings support a role for ET-1 signalling in inflammatory processes in human atherosclerosis. The upregulation of the ETB receptor and its correlation with macrophages suggest increased importance of this receptor in atherosclerosis.

11.22

GENE EXPRESSION IN THE VASCULATURE OF MICE OVEREXPRESSING HUMAN ENDOTHELIN-1 IN THE ENDOTHELIUM

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We previously showed that the mesenteric arteries of transgenic (TG) mice with endothelium-restricted human prepro-endothelin (ET)-1 overexpression exhibit vascular dysfunction, remodeling and inflammation in the absence of elevation of blood pressure. To understand the mechanisms whereby ET-1 directly induces vascular damage, we isolated RNA from mesenteric arteries of female and male mature (6-8 months) and young (6-7 weeks) TG and littermate mice (n=4) and determined the changes in gene expression by genome-wide expression profiling. Significance of ± 1.3 fold-changes was determined with $P<0.05$. Some gene

changes were validated using qPCR. In the mature female mice, the largest group of differentially expressed genes were implicated in inflammation (19/64). In mature males, the largest changes corresponded to genes involved in metabolism (64/193), mostly lipid metabolism (31/64). Young female and male mice showed similar changes with 33/119 and 27/96 genes implicated in metabolism and 14/33 and 15/27 in lipid metabolism, respectively. The increased gene expression of the lipid biosynthetic gene DGAT2, in TG males at both ages and in young females, was confirmed by qPCR for all groups ($P<0.05$) (n=3-6) except the mature males which only showed a trend (n=3-4). These findings suggest that ET-1 excess results in early changes in gene expression which increase lipid biosynthesis. This may contribute in part to ET-1-induced vascular damage. Funded by CIHR.

11.23

ELECTRICAL REMODELING PRECEDES LV DYSFUNCTION IN ET-1-INDUCED CARDIOMYOPATHY

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Binary transgenic (BT) mice with doxycycline (DOX)-suppressible cardiac expression of ET 1 exhibit progressive heart failure, QRS prolongation and death following DOX withdrawal. We hypothesized that ET-1-induced electrical remodeling precedes and contributes to heart failure, and is reversible following cessation of ET-1 expression. Adult BT vs. non-binary transgenic (NBT) littermates were withdrawn from DOX and serially studied with ultrasound biomicroscopy, octapolar catheters, multi-electrode arrays, western blot and qRT-PCR. Abnormalities in ventricular depolarization intervals (VTI) were detected as early as 4 wk after transgene activation, when cardiac structure and function remained normal. By 8 wks, systolic and diastolic dysfunction of both ventricles was observed in BT but not NBT mice. Intracardiac electrograms and epicardial mapping showed prolonged PR, QRS, atrial-His, ventricular activation, AV conduction and VTI, and reduced minimum dV/dt in BT vs. NBT mice. DOX re-introduction prevented structural and functional remodeling. Importantly, Cx43 and Scn5a expression was reduced in BT mice as early as 4 wks post transgene induction, and the associated electrophysiological abnormalities were reversible upon suppression of ET-1 expression. Summary: ET-1 mediated electrical remodeling correlated with reduced Cx43 and Scn5a expression, the sequence and reversibility of which suggest that a primary abnormality in electrophysiology participates in ventricular

11.24

HEMIN VIA HEME-OXYGENASE-1 DIMINISHES ENDOTHELIN-1-INDUCED PHOSPHORYLATION OF ERK1/2 IN VASCULAR SMOOTH MUSCLE CELLS

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Hemin is the oxidative form of heme molecules released from hemoglobin from red blood cells or heme-containing proteins from necrotic cells. Hemin may have antihypertensive effects that are attributed to upregulation of the enzyme heme-oxygenase-1 (HO-1), which degrades heme and reduces cell redox potential. We hypothesized that hemin pretreatment could reduce effects of endothelin-1 (ET-1) in cultured vascular smooth muscle cells (VSMC). Low-passage VSMCs from 11-week old male C57BL/6 mice were exposed to ET-1 (10-7M) for 30 minutes, either alone or after pretreatment for 30 minutes with hemin (2x10-5M) employing culture medium without or with 0.1% FBS . The phosphorylation of extracellular signal-regulated kinases (ERK) 1/2 and expression of HO-1 were evaluated. ET-1 caused an increase in phosphorylation of ERK1/2 (1.5-fold compared to vehicle) that was reduced by pretreatment with hemin (57 and 9% compared to vehicle, without or with FBS respectively). Hemin also caused an increase in HO-1 expression (2.5-fold compared to vehicle). Thus, hemin action mediated by HO-1, results in part from inhibition of ET-1-triggered effects. These results suggest a mechanism for the beneficial role of HO-1 upregulation in hypertension.

11.25

INVOLVEMENT OF CALMODULIN-DEPENDENT PROTEIN KINASE II ALPHA IN ET-1-INDUCED ERK1/2 AND PROTEIN KINASE B SIGNALING AND HYPERTROPHIC AND PROLIFERATIVE RESPONSES IN VASCULAR SMOOTH MUSCLE CELLS

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Endothelin-1 (ET-1), a powerful vasoactive peptide with a pathogenic role in vascular disease, requires H2O2 generation to elicit its responses. We have shown earlier that H2O2-induced activation of ERK1/2 and PKB, two key mediators of hypertrophic and proliferative responses, are mediated through Ca 2+ and Calmodulin (CaM)-dependent downstream signals in vascular smooth muscle cells (VSMC). Therefore, in the present studies we have investigated an involvement of CaM and its effector, CaM-dependent protein kinaseII (CaMKII) in ET-1-induced ERK1/2 and PKB phosphorylation and on ET-1-induced DNA and protein synthesis by using pharmacological inhibitors, an inhibitor peptide and siRNA technique. Calmidazolium and W-7, antagonists of CaM, as well as KN-93, a specific inhibitor of CaMKII, attenuated ET-1-induced responses on ERK1/2 and PKB phosphorylation in a dose-dependent fashion. KN-92, an inactive analogue of KN-93 was without effect. Transfection of VSMC with an inhibitory peptide corresponding to autoinhibitory domain of CaMKII attenuated ET-1-induced phosphorylation of ERK1/2 and PKB. Knockdown of CaMKII alpha by using siRNA also reduced phosphorylation of ERK1/2 and PKB in response to ET-1, whereas, control siRNA had no effect. Furthermore, ET-1-induced DNA and protein synthesis was significantly inhibited by both CaM and CaMKII inhibitors, W-7 and KN-93 respectively .Taken together, these data demonstrate that the activation of CaMKII alpha plays a critical role in mediating the stimulatory effect of ET-1 on ERK1/2 and PKB signaling as well as on hypertrophic and proliferative responses in VSMC. (Supported by a grant from CIHR).

11.26

ET-1 IMPLICATION IN VASCULAR ABNORMALITIES IN HEREDITARY CARDIOMYOPATHY

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The purpose of this study is to test the hypothesis that hereditary cardiomyopathy (HCM) is also a vascular disease to which ET-1 and its receptors (ETA and/or ETB) may contribute. Using hereditary cardiomyopathic hamster (HCMH) and blood pressure measurement, our results show a gradual decrease in mean arterial pressure. Using immunofluorescence and 3-D confocal microscopy, our results show that, in vascular endothelial cells (VECs) from HCMH aortas, there is a decrease in nuclear ETAR density. However, there is an increase in ETBR density both in the cytosol and in the nucleus. No change in the density of ETA or ETB is observed in vascular smooth muscle cells (VSMCs) from HCMH. The changes in ET-1 receptors densities in VECs were accompanied by an increase in the NHE-1 density as well as in cytosolic and nuclear sodium levels. However, in VSMCs, changes in NHE-1 density and in cytosolic and nuclear sodium levels take place in the absence of changes in ET-1 receptors densities. The cytosolic and nuclear sodium overloads were surprisingly upregulated by ET-1 only in VSMCs. In conclusion, our results suggest that the changes observed at the vascular level could affect the secretary functions of the vascular endothelium which contribute to the hypotension. In addition, the changes in ETA density in VECs and the increase in NHE-1 sensitivity to ET-1 in VSMCs could be compensatory mechanisms to overcome a vascular failure. This work is supported by a CIHR grant to Dr. G. Bkaily.

11.27

ELEVATED ENDOTHELIN-1 EXPRESSION IN DOGS WITH NATURALLY OCCURRING CARDIOVASCULAR DISORDERS

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We report here a pathophysiological involvement of endothelin-1 (ET-1) in naturally occurring dog cardiopulmonary disorders, including dirofilariasis (heartworm disease caused by Dirofilaria immitis), mitral regurgitation (MR), tricuspid regurgitation (TR), ventricular septal defect (VSD) and patent ductus arteriosus (PDA). Comparative quantitative expression analysis of PPET-1 mRNA was performed on organs from healthy and filarial dogs. While filarial dogs showed a significantly ($p<0.05$) higher mRNA expression level in heart (about one hundred times) and lung (about ten times) than did healthy dogs, no significant difference between the two groups was observed in the other organs examined. The plasma ET-1 level in filarial dogs (6.9±1.0 pg/ml) was significantly ($p<0.05$) increased compared with healthy dogs (1.0±0.2 pg/ml). Dogs with MR, TR, VSD or PDA, classified as New York Heart Association Class I (no clinical sign), Class II and Class III (more progressive stages) presented a significant ($p<0.05$) increase in plasma ET-1 levels relative to healthy control dogs. In surgically induced MR dogs, the ET-1 plasma concentration increased sharply, reaching a significantly ($p<0.05$) increased level at the second week post-surgery. These findings suggest that ET-1 is involved in the pathogenesis of dog cardiopulmonary disorders and that plasma ET-1 concentration can be a useful onset marker for these disorders.

11.28

HYPERTHYROIDISM UPREGULATES VASCULAR ENDOTHELIN B RECEPTOR

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Thyroid hormone (T3) has well-recognized effects on the cardiovascular system. Vasodilatation is one of the manifestations that occurs in hyperthyroidism to accommodate the increased basal metabolic rate. Considering that hyperthyroidism is associated with higher plasma endothelin-1 (ET-1) concentrations, we hypothesized that the ETB receptor, which induces vascular relaxation contributes to T3-induced vasodilation. Male Wistar rats were randomized in two groups: control and hyperthyroid (T3-20 fold the physiological dose). The hyperthyroidism was induced by daily intraperitoneal injections of 70ug/Kg 3,3',5-triiodo-L-thyronine for 14 days. Hyperthyroid state was confirmed by increased T3 and decreased T4 levels, as well as increased heart rate (HR) and cardiac mass. ET-1 contraction was decreased by 20% in aorta from hyperthyroid rats. No changes in ET-1 and ETA receptor mRNAwere observed. However ETB receptor mRNA was increased 1.3 fold in aortic segments. Our results show for the first time that hyperthyroidism up-regulates vascular ETB receptor. These findings suggest that increased ETB receptors may be involved in T3-induced vasodilation.

11.29

REDUCED CORONARY VASOMOTOR CONTROL BY ENDOGENOUS ENDOTHELIN AFTER MYOCARDIAL INFARCTION: ROLE OF PROSTANOIDS

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We have previously shown that the coronary vasoconstrictor effect of endothelin (ET) is abolished after myocardial infarction (MI). Since prostanoids have been shown to modulate the vasoconstrictor effect of ET, we aimed to investigate the interaction between prostanoids and ET in control of coronary vasomotor tone. Changes in coronary tone in response to ETA/B blockade with tezosentan alone and after cyclooxygenase inhibition with indomethacin were measured in chronically instrumented swine (9 normal swine; 8 swine with MI) at rest and during treadmill exercise. Tezosentan resulted in coronary vasodilation ($P<0.05$ by ANOVA) as evidenced by an increase in coronary venous oxygen saturation (cvSO2) in normal swine at rest, that waned during exercise. In contrast, tezosentan had no effect on coronary vasomotor tone in swine with MI. Indomethacin caused coronary vasoconstriction at rest and during exercise as evidenced by a decrease in cvSO2. The effect of indomethacin was similar in normal swine and in swine with MI. Indomethacin increased the vasodilator effect of tezosentan only during exercise in normal swine. Moreover, a coronary vasodilator effect of tezosentan was unmasked after indomethacin in swine with MI at rest and during exercise. Our results show that prostanoids exert their vasodilator influence on the coronary vasculature at least in part through suppression of the endogenous ET system, particularly after MI. Supported by Netherlands Heart Foundation (2000T042).

11.30

EFFECT OF UREMIA ON ENDOTHELIAL FUNCTION IN THE RAT THORACIC AORTA *IN VITRO*

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Hypertension in chronic renal failure is associated with endothelial dysfunction that is characterized by increased endothelin-1 (ET-1) production and inappropriate nitric oxide (NO) release. In the present study, we investigated the vasoconstriction response to ET-1 and the endothelium-dependant vasodilatation response to acetylcholine in the thoracic aorta *in vitro* of normal and uremic rats.Uremia was induced in Wistar rats by subtotal 5/6 nephrectomy. Sham-operated animals were used as control. At week 6, the thoracic aorta was harvested and segments were mounted in organ baths. Dose-response curves to ET-1 were obtained in intact and endothelium denuded vessels or in the absence and the presence of the NO synthesis inhibitor L-NAME. The vasodilatation response to acetylcholine was assessed in contracted vessels. Aortic segments were kept for immunofluorescence analyses of ET-1 and the endothelial NO synthase (eNOS) expression.As compared to the controls, rats with subtotal nephrectomy developed hypertension together with the impairment of renal function. The vasoconstriction response to ET-1 was reduced in intact aortic segments from uremic rats, which was partly restored in endothelium denuded segments as well as in the presence of L-NAME. In contrast, the vasodilatation response to acetylcholine was not affected, whereas the endothelium-independent vasodilatation response to sodium nitropruside was reduced. Immunofluorescence analyses revealed that expression of both ET-1 and eNOS was increased in the endothelium of aortic segments from uremic rats. Our results suggest that the reduction of the vasoconstriction response to ET-1 in vitro in the thoracic aorta of uremic rats is related to heightened endothelial production of ET-1 and, in part, to increased NO release due to enhanced eNOS expression. Thus, endothelial dysfunction affects the vascular reactivity, which may contribute to the pathogenesis of hypertension in uremic conditions.

12.0: ROLES OF ENDOTHELIN-1 RENAL, FLUID AND ELECTROLYTE PHYSIOLOGY AND DISEASE

12.2

HIGH SALT DIET ATTENUATES AFFERENT ARTERIOLAR AUTOREGULATORY EFFICIENCY BEHAVIOR BY ENHANCED ETB RECEPTOR ACTIVATION

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High salt diet reduces myogenic reactivity in resistance vessels and autoregulatory responses of juxtamedullary afferent arterioles. High salt diets increase endogenous endothelin levels, enhances ETB receptor expression and shifts afferent arteriolar reactivity to ET-1 to higher concentrations. Accordingly, we tested the hypothesis that high salt blunts autoregulatory behavior by activation of afferent arteriolar ETB receptors. Rats were maintained on a normal (o.4% NaCl) or high salt (8% NaCl) diet for a period of 14 days. Baseline arteriolar diameter was similar across all four groups. Autoregulatory behavior was assessed by measuring diameter changes during step-wise 15 mmHg increases in perfusion pressure from 65 to 170 mmHg. High salt diet tended to increase systolic blood pressure slightly from an average baseline of 106±3 mmHg to 114 ± 4 mmHg after 14 days ($P > 0.05$; $n = 6$). In control kidneys from normal salt rats (NS), afferent arteriolar diameter decreased by 37 ± 2% (n=6) as perfusion pressure increased from 65 to 170 mmHg. In the HS group arteriolar diameter decreased by 6 ± 4% ($P < 0.05$; $n = 8$) indicating marked attenuation of pressure induced vasoconstriction. Addition of the ETB antagonist, BQ-788 (1.0 μM) to the perfusate blood had no effect on the autoregulatory response in normal salt animals but markedly improved autoregulation in the high salt group. Increasing perfusion pressure from 65 to 170 mmHg in kidneys from normal salt rats decreased afferent diameter by 35 ± 3% (n=3), whereas in kidneys from high salt rats diameter decreased by 20 ± 7% (n=4) with BQ-788. These data suggest that ETB receptors contribute to the blunted autoregulatory efficiency that occurs with a high salt diet.

12.3

SITAXSENTAN INCREASES EXTRACELLULAR FLUID VOLUME IN BOTH NORMAL SALT AND HIGH SALT-FED DAHL S RATS

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Endothelin (ET-1) influences organ morphology, cardiovascular and renal function, and fluid homeostasis. HS feeding induces arterial hypertension and elevates circulating and renal ET-1 concentrations in DS rats suggesting a role for ET-1 in DS pathobiology. The current study evaluated the effects of HS and ETA-selective antagonism with SIT on 1) cardiac and renal morphology, 2) systemic hemodynamics, 3) bioimpedance spectroscopy determined body fluid compartment volumes (BFC), and 4) plasma volume (PV) and hematocrit (HCT) in DS. Age-matched DS (n = 32; 6-7 wk old, Harlan) were maintained on NS (0.49% NaCl) or switched to HS (8% NaCl) for 3 weeks. After measuring baseline BFC, SIT or vehicle was administered (p.o.) daily to half of NS and half HS treated groups for 18 days. At study endpoint, measurements were obtained under isoflurane anesthesia. HS feeding increased blood pressure (BP, 57.3%) and elicited cardiac (30.0%) and renal (27.7%) hypertrophy, while body weight (-13.3%), HCT (-11.6%), ECFV (-10.3%) were all reduced. SIT reduced HS-induced BP (-56.9%), expanded PV (20.4%) and exerted differential effects on heart (9.2% reduction) and renal (18.4% increase) mass despite lowering BP. SIT increased PV (26.6%), and kidney mass (14.8%) in NS-fed DS despite no apparent effects on BP. These findings indicate ETA antagonism increases PV associated with increased renal mass, suggesting that renal hemodynamic and/or sodium handling effects may contribute to PV expansion.

12.4

ALDOSTERONE MODULATES STEROID RECEPTOR BINDING TO UNIQUE ELEMENTS IN THE ENDOTHELIN-1 GENE

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Aldosterone regulates blood pressure by its action on sodium transport in the renal collecting duct (CD). Previously we identified endothelin-1 (edn1) as a novel aldosterone regulated gene and hypothesized that this interaction would be functionally important in the CD. Here, we show that aldosterone induced edn1

mRNA in isolated rat inner medullary CD cells ex vivo and in four CD cell lines in vitro (mpkCCDc14, OMCD1, mIMCD-3 and mIMCD-K2). Blockade of mineralocorticoid receptor (MR) or glucocorticoid receptor (GR) by siRNA or pharmacological antagonists demonstrated a clear role for both receptors in aldosterone induction of edn1. Chromatin immunoprecipitation assays mapped a region of the edn1 promoter containing two hormone response elements (HRE1 and HRE2) that differed in their binding site orientation. Higher resolution DNA affinity purification assays revealed dose-dependent binding of MR and GR to HRE2. However, only the highest aldosterone concentration recruited MR or GR to HRE1. Similarly, RNA polymerase II showed dose-dependent binding to HRE2 but not HRE1. HRE1 also appeared to differ in transcription cofactor binding. In conclusion, aldosterone stimulates edn1 transcription in CD cells by a mechanism that involves MR and GR binding to the high affinity HRE2. In contrast, the low affinity HRE1 may function as a transcriptional break to prevent excessive gene activation. Support by NIH, Dept of Veterans Affairs and American Heart Association fellowship to LRS.

12.5

ET-RECEPTOR BLOCKADE DELAYS PROGRESSION OF TUBULOINTERSTITIAL FIBROSIS IN A MOUSE MODEL OF ALPORT SYNDROME

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Tubulointerstitial fibrosis (TIF) is the hallmark of chronic proteinuric nephropathy. We have shown that lack of endothelin B (ETB)-receptor expression delays TIF. In humans, Alport syndrome is an example of proteinuria-induced TIF. We analyzed the effect of ETA-, ETB-, and ETA-/ETB-receptor blockade in a mouse model of Alport syndrome. COL4A3 -/- mice received an ETA- (A-192621) or ETB- (A-182086) receptor antagonist, both, or no therapy and were compared to COL4A3 -/- mice on vehicle. At 0, 4.5, 7.5 and 9.5 wks animals were sacrificed. Proteinuria and creatinine were measured. Histological changes were analyzed and quantified by Sirius red staining. Endothelin-1 (ET-1) levels were determined by quantitative RT-PCR. While ET-1 levels were equally increased in treated and untreated COL4A3 -/- mice, ET-receptor blockade resulted in a prolonged life span in COL4A3 -/- mice (ETA: 35%; ETB: 23%), which was accompanied by decreased proteinuria and creatinine. Myofibroblast dedifferentiation, macrophage infiltration, and TI matrix deposition were greatly minimized in treated COL4A3 -/- mice. Blocking ETA-receptor was more effective than ETB-receptor, and blocking both receptors did not result in additional benefit. In summary, ET-receptors blockade reduces progression of proteinuria-induced TIF which indicates an adjunct treatment strategy for chronic proteinuric nephropathy.

12.6

ADD-ON ET_A RECEPTOR ANTAGONIST TO ACE INHIBITOR PROVIDES RENO AND CARDIO PROTECTION IN ADVANCED TYPE 2 DIABETES IN RATS

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In about two-thirds of type 2 diabetic patients with overt proteinuria, ACE inhibitors provide imperfect renoprotection and cardiovascular risk remains elevated. These patients need novel therapeutic interventions which hopefully synergize with ACE inhibitors. Here we evaluated the effect of ETA receptor antagonist on top of ACE inhibitor in Zucker diabetic fatty (ZDF) rats. Animals received orally from 4mo (when they were proteinuric) to 8mo: vehicle, sitaxsentan (60mg/kg), ramipril (1mg/L) or their combination. Sitaxsentan transiently reduced systolic blood pressure, did not affect proteinuria, but had marked antiinflammatory effects with less infiltrates of monocytes/macrophages and reduced MCP-1 renal expression in respect with ZDF rats on vehicle. The combined therapy ameliorated hypertriglyceridemia and hypercholesterolemia and limited glomerulosclerosis and tubular interstitial damage more than each drug alone. Myocyte hypertrophy, an adaptive response to massive cell loss, observed in the myocardium of ZDF rats on vehicle, was reverted by the combined therapy, that also improved myocardial angiogenesis, with a significant increase of capillary number, thereby re-establishing an adequate capillary network in the myocardium of ZDF rats. In conclusion, combination of ETA receptor antagonist and ACE inhibitor can be considered a therapeutic option to lessen renal disease progression and cardiovascular dysfunction in type 2 diabetic patients who do not completely benefit by ACE inhibition.

12.7

CHRONIC ENDOTHELIN-A RECEPTOR ANTAGONISM REDUCES PROTEINURIA, BLOOD PRESSURE & ARTERIAL STIFFNESS IN CHRONIC KIDNEY DISEASE

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Blood pressure (BP) and proteinuria reduction slow chronic kidney disease (CKD) progression. Acute ETA receptor antagonism reduces BP and proteinuria in CKD. We investigated if these effects are maintained longer term. Methods: In a randomised double-blind, 3-way crossover study 27 subjects received 6 weeks of placebo, sitaxsentan 100mg and nifedipine 30mg. All subjects were optimally treated with renin-angiotensin system blockade. 24h proteinuria, protein:creatinine (PCR), 24h BP, and pulse wave velocity (PWV), as a measure of arterial stiffness (AS) were measured at baseline and week 6 of each treatment period. Results: All subjects completed the study. Compared to placebo, sitaxsentan significantly reduced proteinuria (24h proteinuria: -31±23%, $p<0.005$; PCR: -29□23%, $p=0.01$), BP (24h mean arterial BP: -4±6mmHg, $p<0.01$), and AS (PWV: -5±9%, $p<0.01$). Nifedipine matched the BP reduction with sitaxsentan ($p=0.65$) and produced a similar fall in PWV. Despite this sitaxsentan reduced proteinuria to a greater extent than nifedipine (24h proteinuria: -31±23 vs 10±46%, $p<0.01$, PCR: -29±23 vs -5±33%, $p=0.01$). Sitaxsentan did not cause clinically significant side effects, or weight gain. Conclusion: 6 weeks sitaxsentan treatment produces significant, clinically relevant reductions in proteinuria and BP in CKD subjects. These effects appear, in part, to be BP independent. AS also improves. Overall, sitaxsentan is well tolerated. Larger, longer-term studies are warranted. Encysive funded.

13.0: CLINICAL TRIALS WITH ET ANTAGONISTS: AN UPDATE

13.2

MACITENTAN, A NEW TISSUE TARGETING DUAL ERA

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Bosentan (Tracleer®), a dual ETA and ETB receptor antagonist, was the first ET receptor antagonist (ERA) to be registered for the treatment of PAH. Bosentan is now also licensed in Europe for reducing the number of new digital ulcers in patients with systemic sclerosis and ongoing digital ulcer disease, and is being studied in idiopathic pulmonary fibrosis (BUILD-3 study). Capitalizing on 20 years of research on ET, a tailored screening and discovery effort at Actelion now led to the discovery of macitentan, a tissue-targeting dual ERA. The main goals were to discover a potent molecule with reduced risk of liver enzyme elevations, dual inhibitory potency on ETA and ETB receptors, and physico-chemical properties optimized for tissue penetration. Indeed, ET-1 secretion from endothelial cells is polarised in the direction of tissues. In rats with pulmonary hypertension, macitentan reduced right ventricular hypertrophy and improved survival. In diabetic rats, macitentan decreased blood pressure and proteinuria and prevented renal structural damage. In rats with bleomycin-induced pulmonary fibrosis, macitentan decreased deposition of collagen and structural abnormalities. The clinical benefits of macitentan are now being evaluated in the phase III Seraphin study in symptomatic PAH using morbidity and all-cause mortality as the primary endpoint, and a Phase II study in idiopathic pulmonary fibrosis, called MUSIC, is being initiated. The results of these studies will tell if all of these goals have been met.

13.3

ZIBOTENTAN (ZD4054): AN ET_A-SPECIFIC ANTAGONIST BEING DEVELOPED FOR THE TREATMENT OF HORMONE-RESISTANT PROSTATE CANCER

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Zibotentan (ZD4054) is a small-molecule, specific antagonist of ETA with no detectable activity at ETB.1 In preclinical studies, zibotentan inhibited processes associated with cancer progression, including proliferation, invasion, angiogenesis and metastasis, without inhibiting apoptosis.1 In the clinical setting, single doses of zibotentan 10 or 30 mg inhibited ET-1-induced vasoconstriction in healthy volunteers, while single doses up to 240 mg had no effect on plasma levels of ET-1, confirming zibotentan's specificity for ETA.2 Zibotentan's pharmacokinetics are suitable for once-daily, oral tablet dosing. The safety, tolerability and efficacy of zibotentan 10 and 15 mg were assessed in a double-blind, randomized, placebo-controlled Phase II trial in patients with hormone-resistant prostate cancer (HRPC) and bone metastases who were pain free or mildly symptomatic (n=312).3 Although no significant improvement was observed for the nominated primary endpoint of time to progression (TTP), zibotentan was associated with prolonged overall survival (OS) compared with placebo. The most common adverse effects were those predicted from the pharmacological action of endothelin receptor antagonism: peripheral edema, headache and nasal congestion. Zibotentan is currently being evaluated in a large Phase III trial program (ENTHUSE) in patients with M0 and M1 HRPC, which will assess OS, TTP, and other endpoints including quality of life. REFERENCES: 1Growcott J. Anticancer Drugs 2009;20:83–88. 2Morris CD et al. Br J Cancer 2005;92:2148–2152. 3James ND et al. Eur Urol 2009;55:1112–1123.

13.4

ADVANCING THE SCIENCE OF ENDOTHELIN BIOLOGY: A CLINICAL UPDATE ON STUDIES WITH SITAXENTAN SODIUM

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Pathological vascular remodeling is a key contributor to the symptomatology of pulmonary arterial hypertension (PAH), and reversing this process may offer the best hope for improving this debilitating condition. The vascular remodeling process is believed to be due to endothelial cell dysfunction and to involve altered production of endothelial cell-derived vasoactive mediators. The observation that circulating plasma levels of the vasoconstrictive peptide endothelin-1 (ET 1) are raised in patients with PAH, and that ET-1 production is increased in the pulmonary tissue of affected individuals, makes it a particularly interesting target for therapeutic intervention in PAH. Clinical trials with ET receptor antagonists (ETRAs) show that they provide symptomatic benefit in patients with PAH, thereby validating the clinical relevance of the endothelin system as a therapeutic target. In addition to its key role in the pathogenesis of PAH, ET-1 has also been implicated in a number of other diseases, including chronic kidney disease and heart failure. In this paper we review the role of ET-1 together with the available data on the roles of the specific ET receptors and ETRAs in PAH and other non-pulmonary conditions. In particular we consider the possible effects of ET receptor selectivity on the pathologic process in PAH and other non-pulmonary conditions. Furthermore, we will discuss whether selective ETA or non-selective ETA/ETB blockade offers the greatest potential to improve symptoms and alter the clinical course of these diseases.

13.5

STUDY DESIGNS FOR AMBRISENTAN IN IDIOPATHIC PULMONARY FIBROSIS.

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Ambrisentan is a once-daily, oral, ET_A-selective endothelin receptor antagonist approved for the treatment of Pulmonary Arterial Hypertension (PAH). The ET_A receptor has been implicated in modulating fibrosis. Idiopathic Pulmonary Fibrosis (IPF) is a chronic fibrotic lung disease with high mortality. Improved survival has been observed in a subset of IPF patients in the BUILD-1 study. Pulmonary hypertension (PH) is a secondary complication of IPF that significantly increases mortality. PH is more prevalent in later stages and amount of honeycombing on computed tomography (CT) scan and pulmonary function severity are less in earlier disease. Therefore two global clinical studies are proposed to evaluate ambrisentan in IPF across a spectrum of the disease: early disease with minimal honeycombing and those advanced to a stage where PH has developed. ARTEMIS-IPF is event-driven, comparing ambrisentan to placebo with a primary objective of determining effect on disease progression, defined as a composite of one of 3 events: worsening in pulmonary function measures from baseline, respiratory hospitalizations, or death. Subjects must meet diagnostic criteria for IPF and have minimal honeycombing. A secondary objective in this trial is to evaluate development of PH. Right heart catheterization (RHC) is important for evaluating hemodynamics in this trial. ARTEMIS-PH is a 48 week trial comparing ambrisentan to placebo with a primary objective of determining effect on 6-minute walk distance. Eligible subjects must have IPF and also PH based on RHC. These two trials will address the question of whether ET_A antagonism may slow progression of disease in IPF in an earlier stage, delay or prevent secondary complication of PH, and determine impact on primary versus secondary disease.

14.0: ROLES OF ENDOTHELIN-1 IN NEUROPHYSIOLOGY, STROKE AND RELATED DISEASES

14.2

THE ROLE OF ENDOTHELIN-1 RECEPTORS IN CEREBROVASCULAR DYSFUNCTIONS ASSOCIATED WITH ALZHEIMER'S DISEASE

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In addition to neuronal and cognitive deficits in Alzheimer's disease (AD), there is a reduced brain perfusion associated with amyloid beta (Aβ)-induced vascular oxidative stress and transforming growth factor-β1 (TGF-β1)-mediated vascular fibrosis. Using transgenic TGF mice that overexpress TGF-β1 and recapitulate the structural vascular pathology of AD, we showed impaired dilatory function and selective decrease in the ET-A-mediated contractile response to endothelin-1 (ET-1), together with increased levels of ET-B, but not ET-A, receptors. ET-A receptor signaling through the p38 MAPK/HSP27 pathway was inhibited in cerebrovascular smooth muscle cells treated with TGF-β1, which increased mitogen-activated protein kinase phosphatase-1 (MKP-1) expression, a negative regulator of p38 MAPK. TGF mice treated with ET-A (ABT-627) or ET-B (A-192621, Abbott) receptor antagonist displayed no further decrease in ET-1-induced contraction or complete rescue of both ET-1 contractile response and basal nitric oxide (NO) production. In wild-type controls, ET-A receptor blockade reduced the ET-1 contraction to levels similar to those of TGF mice, and almost completely abrogated basal NO production. These results indicate desensitization of ET-A and upregulation of ET-B receptors by chronically increased TGF-β1, which deregulate the balance between ET-1 and NO in maintaining vascular tone. Such effects could contribute to the abnormal brain perfusion in AD. Supported by research grants from CIHR and the Alzheimer's Society of Canada.

14.3

ACTIVATION OF ETA-R CONTRIBUTES TO THE IMPAIRED RENORENAL REFLEXES IN HEART FAILURE

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Activation of renal mechanosensory nerves increases afferent renal nerve activity (ARNA) resulting in decreases in efferent renal nerve activity and natriuresis, a renorenal reflex. Activation of renal sensory nerves is enhanced by high sodium (NaCl) and reduced by low NaCl diet; the modulatory action of dietary NaCl mediated by changes in angiotensin (ANG) II. Endothelin-1 plays a differential role in the activation of renal sensory nerves, stimulatory in high NaCl diet via activation of ETB-receptors (R) and inhibitory in low NaCl diet via activation of ETA-R. Activation of ETA-R contributes to the ANG II-induced suppression of ARNA in low NaCl diet rats. The renorenal reflexes are impaired in congestive heart failure (CHF) due to increased endogenous ANG II suppressing renal sensory nerve activation. We now examine if activation of ETA-R contributes to the impaired activation of renal mechanosensory nerves in CHF. Left coronary artery was ligated between the pulmonary outflow tract and left atrium 6 weeks before the study. Left ventricular end-diastolic pressure was greater in CHF than ShamCHF, 10.3±0.8 vs 3.9±0.4 mmHg($P<0.01$). In CHF, increasing renal pelvic pressure 2.5 and 7.5 mmHg increased ARNA 3±2 and 13±3% before and 3±1 and 24±5% during renal pelvic administration of BQ123, the ARNA responses to increasing renal pelvic pressure 7.5 mmHg enhanced by BQ123 ($P<0.01$). In ShamCHF, increasing renal pelvic pressure 2.5 and 7.5 mmHg increased ARNA 7±3 and 23±5% before and 9±2 and 23±6% during BQ123 administration. Time control experiments in CHF and ShamCHF resulted in reproducible ARNA responses to increasing renal pelvic pressure 2.5 and 7.5 mmHg; CHF: 0±1 and 8±4% and 3±2 and 6±4%, respectively; ShamCHF:5±2 and 18±7% and 0±3 and 20±5%, respectively. Conclusion: activation of ETA-R in the renal pelvic area contributes to the impaired activation of renal mechanosensory nerves in CHF.

14.4

CARDIOVASCULAR ACTIONS OF ENDOTHELIN B RECEPTORS IN SYMPATHETIC GANGLIA

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Endothelin B receptors (ETBR) on sympathetic ganglion neurons are upregulated in deoxycorticosterone, high salt hypertension in rats. Chronic infusion of the ETBR selective agonist sarafotoxin 6C (S6C) causes hypertension in rats that is attenuated by celiac ganglionectomy (CGx). Comparing WKY control rats to two transgenic (TG) strains of ETBR-deficient rats, we evaluated the influence on blood pressure of activation of ganglionic ETBRs by infused S6C. TG(+/+) rats have a normal complement of wild type ETBR (WT-ETBR); TG(sl/sl) rats lack functional WT-ETBR. However, both strains of TG rat have 70 copies of an ETBR transgene driven by a dopamine-β-hydroxylase promoter, which would ensure their expression in sympathetic ganglion neurons. S6C infusion for 5 days raised blood pressure (BP) in TG(+/+) rats and CGx reduced this effect. S6C infusion did not significantly elevate blood pressure in TG(sl/sl) rats, and CGx had no effect on the response. Using primers that amplified both the WT and TG mRNA, there was significantly more ETBR mRNA in both transgenic strains compared to WKY. ETBR protein was expressed in celiac ganglia of both TG(+/+) and TG(sl/sl). Because S6C infusion did not elevate blood pressure in TG(sl/sl) rats that would be expected to express only TG ETBR, we conclude that the presence of TG ETBR receptors in sympathetic ganglia is not sufficient to mediate the neural actions of S6C to elevate blood pressure. (Support: PO1HL79687).

14.5

NOCICEPTIVE AND HYPERALGESIC ACTIONS OF ENDOTHELINS IN THE TRIGEMINAL SYSTEM

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This study aimed to characterize expression of the endothelin system in the trigeminal ganglion (TG) of male Wistar rats and its participation in nociceptive transmission, as a potential target for orofacial pain control. ET-1 and ET-3 mRNA were detected by RT-PCR in the TG and brain. Retrograde labeling of TG neurons innervating the temporomandibular joint (TMJ), upper lip or eye with fluorogold, associated with immunohistochemistry, revealed ETA and ETB receptor expression along the entire TG. TRPV1 positive neurons were widely expressed in the entire TG, and a significant proportion of these cells (~ 30%) co-expressed

ETA or ETB receptors. Our behavioral data showed that ET-1, ET-3 and IRL-1620 (3 to 30 pmol/site) or the TRPV1 receptor agonist capsaicin (0.1 or 1.0 □g/site) all induced nociceptive responses when injected into the upper lip or TMJ. Surprisingly, BQ-123, but not BQ-788 (selective ETA and ETB receptor antagonists, respectively, 10 nmol each), abolished responses induced by ET-1 into the lip. Both antagonists reduced those evoked by ET-1 in the TMJ. ET-1, ET-3 and IRL-1620 all failed to elicit eye wipes when Instilled in the eye, but ET-1 induced hyperalgesia to nociception triggered by capsaicin. Altogether, the findings suggest that endothelins, acting through ETA and/or ETB receptors, may play important roles in mediating/exacerbating pain elicited by noxious stimuli in the various trigeminal nerve branches. Financial Support: CNPq, FAPESC and PRONEX.

14.6

MODULATORY ACTIONS OF ENDOTHELINS ON NEURAL-GLIAL COMMUNICATION IN THE HYPOTHALAMIC SUPRAOPTIC NUCLEUS

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In addition to being potent vasoactive peptides, endothelins (ETs) act as neuromodulators within the CNS, influencing among others, vasopressin (VP) hormonal secretion. Still, the precise underlying mechanism for this effect remains largely unknown. Given that ET receptors are present both in neurons and astrocyte, we evaluated in this study the effects of ET receptor activation on both supraoptic nucleus (SON) neurosecretory neurons and astrocytes. Patch-clamp recordings from SON neurons in a slice preparation showed that bath application of the ETB receptor agonist sarafatoxin 6c (100 nM) diminished ongoing firing frequency (~70%) of SON neurons with a long latency of ~ 9 minutes. In addition, ET-1 (100 nM) and sarafotoxin 6c increased intracellular calcium [Ca2+]i in SON astrocytes (~80% change in F/F0), an effect blocked in the presence of thapsigargin but not Ca2+-free media. On the other hand, ET receptor activation failed to induce [Ca2+]i neuronal changes. Finally, preincubation of SON slices with the selective gliotoxin L-aminoadiptic acid (0.25 mM) prevented ET-induced effects on SON neuronal firing activity. Altogether, these results show that ET receptor activation increased [Ca2+]i in SON astrocytes, likely activating a mechanism that lead to inhibition of SON neuronal activity. Our study supports an important role for ET in the neuronal-glial communication in the magnocellular neurosecretory sytem. (Supported by AHA 0640092N to JES).

14.7

IN VIVO ANTAGONISM OF ENDOTHELIN RECEPTORS IN A MOUSE MODEL OF THE VASCULAR PATHOLOGY OF ALZHEIMER'S DISEASE

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Transforming growth factor-β1 (TGF-β1) has been associated with the vascular pathology and chronic hypoperfusion seen in Alzheimer's disease, which may contribute to cognitive decline. In transgenic TGF mice, overexpressing TGF-β1, we found reduced dilatory function, selectively impaired endothelin (ET)-1-induced contraction, and increased ETB receptor levels. Here we studied the effects of selective in vivo antagonism of ETA (ABT-627) or ETB (A-192621) receptors on cerebrovascular function and fibrosis, as well as cognitive performance. Treatment of TGF mice with either antagonist did not affect the dilatory deficit, but distinctly altered ET-1 contraction and basal nitric oxide (NO) production. While ABT-627 was devoid of any effect in TGF mice, it virtually abolished the ET-1 response and NO release in wild-type (WT) mice. In contrast, A-192621 only acted upon TGF mice with full recovery of ET-1 contraction and NO synthesis. TGF mice, treated or not, had no cognitive deficit in the Morris water maze, nor did ABT-627-treated WT controls despite drastic effects on vasomotor function. Preliminary Western blot analyses on proteins involved in vascular fibrosis showed no effect of treatments. These findings confirm that ETA receptors mediate the ET-1-induced contraction, suggest a detrimental role of ETB receptors in conditions of increased TGF-β1, and dissociate vascular dysfunction from cognitive deficit. Supported by grants from CIHR and the Alzheimer Society of Canada.

14.8

NOVEL THERAPY APPROACH IN PRIMARY STROKE PREVENTION: SIMULTANEOUS INHIBITION OF ENDOTHELIN CONVERTING ENZYME AND NEUTRAL ENDOPEPTIDASE IN SPONTANEOUSLY HYPERTENSIVE, STROKE–PRONE RATS IMPROVES SURVIVAL

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Stroke is one of the leading causes of death and neurological disabilities worldwide. In the ischemic brain, levels of endothelin-1 are raised and anti-inflammatory and neuroprotective effects of endothelin antagonists after stroke have been described previously. Based on these findings, we investigated the protective effect of the endothelin converting enzyme/neutral endopeptidase blocker, SLV 338, in salt-loaded, stroke-prone, spontaneously hypertensive rats (SHR-SP). SHR-SP were put on a high salt diet and treated with 30mg/kg, 100mg/kg SLV338 or vehicle for 27 weeks. Blood pressure, neurological outcome, body weight and mortality were assessed throughout treatment. Salt water and food intake and urine volume were measured at 2 different time points. Urinary samples were taken in weeks 1 and 9, blood samples at the same time points and in week 22. At the end of the study, all brains were analysed using MRI technique. Neurological outcome and infarct size were similar in all groups. Albuminuria was significantly reduced in treated animals. Treatment with SLV338 considerably lowered the incidence of stroke and improved survival in a blood pressure independent manner (p = 0,01).

15.0: ENDOTHELIN IN CANCER AND BLOOD DISEASES

15.2

THE IMPORTANCE OF ENDOTHELIN AXIS IN INITIATION, PROMOTION AND THERAPY OF CANCER

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The endothelin (ET) axis, which includes ET-1, ET-2, ET-3, and two G protein coupled receptors, ETA receptor (ETAR) and ETBR, promotes initiation and progression of a variety of tumors such as prostatic, ovarian, renal, pulmonary, colorectal, cervical, breast, lung, bladder, endometrial carcinoma, Kaposi's sarcoma, brain tumors, and melanoma. Acting selectively on ETAR or ETBR , ET-1 is a multifunctional peptide that regulates mitogenesis, cell survival, bone remodeling, stimulation of nociceptor receptor, tumor-infiltrating immune cells, angiogenesis, lymphangiogenesis, epithelial-to- mesenchymal transition, invasion, metastatic dissemination and drug resistance. In an age when great advances have been made in understanding the role of ET-1 axis in the molecular tumor biology, it is likely that the introduction of targeted therapies, by using ET-1 receptor antagonists, will have a major impact on the cancer management. Emerging experimental and preclinical data demonstrates that interfering with ET-1 axis pathways provides an opportunity for the development of new mechanism-based antitumor strategies by using ET-1 receptor antagonists alone and in combination with cytotoxic drugs or molecular inhibitors. ET-1 receptor antagonist treatment is currently evaluated in clinical studies to provide us with new options to treat cancer. Importantly, such strategies might allow selection of treatments based on the molecular characteristics of tumors and bring us closer to an era of personalized medicine. Supported by AIRC, AstraZeneca Anna Bagnato started working on ET-1 at ERRB, NIH, Bethesda, USA. She was the first to identify the role of the ET-1 axis in ovarian cancer progression and has published extensively on the biology of endothelin in various malignancies.

15.3

DEVELOPMENT OF NON-INVASIVE PET IMAGING METHOD TO MONITOR BLOOD FLOW INCREASE IN TUMORS WITH SPI-1620

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Aim: The ETB receptor peptide agonist, SPI-1620, has been demonstrated to selectively and transiently increase blood flow to tumors in breast, prostate and ovarian cancer animal models. However, in all the previous studies, blood flow increase in tumors was measured by the Doppler method. This study was conducted to develop and validate a non-invasive PET imaging technique to detect changes in drug-induced blood perfusion in a rat athymic xenograft head-and-neck tumor model. Materials & Methods: Tumor-bearing animals were treated with various doses of SPI-1620 (2.5, 5.0 and 7.5 ug/kg) and the blood flow was monitored by PET imaging method. Results: Consistent with previous Doppler studies, treatment of rats with SPI-1620 led to a selective and transient increase in blood flow to tumors. Blood flow to tumors was dose-dependent and lasted for about ~30 minutes (scanning was conducted only for 60 minutes post-dose). The maximum increase in blood flow to tumors was observed at 30 minutes post-dose in rats treated with 5.0 ug/kg dose. At the highest dose, blood flow to tumors decreased compared to normal tissue. Conclusion: It is concluded that SPI-1620 mediated blood flow to tumors can be detected using non-invasive PET imaging and this method is currently being used in Phase I clinical trials with SPI-1620.

15.4

EFFICACY OF THE SPECIFIC ETA RECEPTOR ANTAGONIST ZIBOTENTAN (ZD4054)

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Endothelin-1 (ET-1) contributes to growth and progression of solid cancers, mainly through ET receptor A (ETAR). Hence, ET receptor antagonism is emerging as a

potential cancer treatment. We evaluated the efficacy of the specific ETAR antagonist zibotentan (ZD4054) in blocking ET-driven cellular effects in colorectal cancer (CRC). CRC lines (HT29;SW620) & normal fibroblast strains grown from human colorectal tissues (CF36;CF56;CF65;CF75) were incubated in ET-1 with/without BQ123, zibotentan (ETAR antagonists), BQ788 (ETBR antagonist). Growth was measured by methylene blue uptake; migration by scratch wound assay; contraction in collagen gels; downstream effectors by western blotting. ET-1 driven growth (18%-45%>control) was inhibited ($p<0.01$) by ETAR (not ETBR) antagonism (BQ123=zibotentan; CRC&fibroblasts). ET-1 driven fibroblast migration & contraction were blocked by ETAR & ETBR antagonism (zibotentan=BQ123). CRC cells did not migrate/contract. ET-1-stimulated downstream effector expression was driven by ETAR or ETBR eg: (1) connective tissue growth factor blocked by ETAR antagonism (zibotentan>BQ123; CRC&fibroblasts); (2) collagen XI blocked by ETAR>ETBR antagonism (zibotentan>BQ123; fibroblasts). The specific ETAR antagonist zibotentan is at least as efficacious as BQ123 in blocking ET-1 driven growth, migration & contraction in CRC cells & colorectal fibroblasts, which form the supporting tumour stroma. Zibotentan is a strong candidate for adjuvant treatment in CRC.

15.5

THE ENDOTHELIN AXIS IN CARCINOGEN-INDUCED RAT COLON TUMORS

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Endothelin-1 (ET-1), ETAR and ETBR are over-expressed in human cancers. Here, we examined the endothelin axis in rat colon tumors induced by a mutagen from cooked meat and fish, namely 2-amino-1-methyl-6-phenyimidazo[4,5-b] pyridine (PhIP). Using ELISA assays, ET-1 peptide was 7-8 fold higher in rat colon tumors as compared with adjacent normal-looking tissue (78.1+/-29.2 vs 11.5+/-5.1 pg/mg tissue, $P<0.001$). There was no difference in preproET-1 expression, based on quantitative RT-PCR and immunoblotting. ETAR and ETBR mRNA levels were 2.1- and 1.6-fold higher in tumors compared with adjacent normal-looking tissue ($P<0.001$ and $P<0.01$, respectively). Immunoblotting confirmed that colon tumors had higher levels of ETAR and ETBR. Immunohistochemical analyses revealed that ET-1 was distributed in the apical layer of normal colonic epithelium and strongly expressed in tumor stroma, especially in vascular endothelial cells and smooth muscle cells, and also was detected in adenocarcinoma cells. Unlike ET-1, ETAR and ETBR were undetectable in tumor cells; ETAR was mainly distributed in fibroblasts and smooth muscle cells, whereas ETBR was abundant in vascular endothelial cells within tumor stroma. We conclude that the PhIP/rat model is useful for studying the endothelin axis and its role in colon tumor development. Studies approved by the Institutional Animal Care & Use Committee, and funded by NIH grants CA65525, CA090890, and CA122959.

15.6

MOLECULAR MECHANISMS REGULATING ECE-1 EXPRESSION IN PROSTATE CANCER

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It is widely known that the mitogenic peptide, endothelin-1 (ET-1), influences cancer invasion and metastasis. Plasma ET-1 levels are significantly elevated in men with metastatic prostate cancer (PC). ET-1 is also involved in the transition of hormonally regulated androgen-dependent disease to androgen-independent PC. ET-1 is produced from big ET-1 by endothelin-converting enzyme-1 (ECE-1). The 3' untranslated region (UTR) of the ECE-1 transcript contains several putative binding sites for microRNAs (miRNAs). miRNAs are small non-coding RNAs which regulate expression of target genes by binding to their transcripts. Aberrant expression of miRNAs is a common feature of a variety of malignancies, including prostate cancer. In this study we examined post-transcriptional regulation of ECE-1 by miRNAs known to be altered in prostate cancer. In order to identify regulatory miRNAs, we utilised a reporter assay in which the 3' UTR of ECE-1 was fused to the luciferase coding sequence and co-transfected into PC-3 cells alongside the appropriate miRNA precursor. The effect of specific miRNA inhibitors (anti-miRs) was also analysed in the same system. The ability of miRNAs identified in these assays to regulate ECE-1 protein expression was subsequently analysed by western blotting. The identification of miRNAs involved in post-transcriptional regulation of ECE-1 in prostate cancer may identify novel therapeutic targets. This work is supported by Yorkshire Cancer Research.

15.7

CHEMORESISTANT OVARIAN CANCER CELLS DISPLAY ALTERED ENDOTHELIN A RECEPTOR EXPRESSION AND SIGNALING

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Chemoresistance is a major challenge in ovarian cancer (OC). High-throughput analysis has shown endothelin A receptor (ETAR) overexpressed in post-chemotherapy OCs, suggesting a role in the chemoresistance onset. To identify ETAR-driven molecular determinants involved in chemoresistance to cisplatin and paclitaxel, we used the A2780 WT, cisplatin-resistant A2780 CIS, and taxol-resistant A2780 TAX human OC cells. ETAR mRNA and protein expression is higher in the resistant clones, associated with enhanced phosphorylation of MAPK, AKT and NFk-B, and PTEN down-regulation. These effects are reverted by treatment with specific ETAR antagonists. In A2780 WT cells, ETAR antagonists enhance the number of early apoptotic cells, similar to cisplatinum. Furthermore, ETAR antagonists sensitize A2780 CIS cells to cisplatinum-induced apoptosis, confirming the role of ET-1 as survival factor. In A2780 WT, as well as in A2780 CIS and TAX xenografts, the ETAR antagonist ZD4054 induces a significant tumor growth inhibition, suggesting a specific therapeutic window in which combination therapy with cytotoxic drugs is more effective in resistant OC. Analysis of 50 human OCs, with different responses to chemotherapy, shows that ETAR is overexpressed in the resistant tumors. In conclusion, ETAR blockade with specific antagonists increases the sensitivity to chemotherapeutic agents, representing a strategy to overcome chemoresistance in OC. Supported by AIRC, Ministero della Salute

16.0: POSTER SESSION III

16.1

ENDOTHELIN-1 SIGNALLING INVOLVING COMPLEXED NF-KB P65, MAPKP38 AND PKC ISOFORMS IN HUMAN TUMORS AND NORMAL TISSUES

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A cytoplasmic complex of NF-kB p65 and MAPK ß38 is formed via ETA-receptor (early) and protein kinase C (late; ET-receptors blocked) after ET-1 stimulation (BBA 1783: 1613, 2008). We analysed, whether PKC is part of this complex and the role of its isoforms in human normal renal cells, and fibroblasts, in tumor cell lines (kidney, breast, melanoma, cervix), and in renal tumor specimens by western blot, EMSA, immunprecipitation, qRT-PCR, siRNA and tissue array. PKC isoforms are part of the p65-p38 complex in all cells and tissues investigated. Blocking of the classical and novel PKC isoforms by chelerythrine reduces NF-kB intensity. Si-RNA against PKC alpha, ß2 and p38 reduces gene expression levels, such as HIF 1 alpha (PKC alpha specific) and vimentin (PKC alpha and beta 2). In renal tumors, PKC alpha is only present in oncocytoma, while PKC ß2 only in renal cell carcinoma. Thus, blocking ET-receptors does not suffice to prevent ET-1 mediated gene expression via PKC. PKC isoforms interact with p38 and p65 in a complex, while individual isoforms can target the expression of specific genes (funded by Nolting Stiftung to JWUF, and doctoral fellowship by Koeln fortune to MvB).

16.2

INVOLVEMENT OF IMIDAZOLINE AND OPIATE RECEPTORS IN THE ENHANCEMENT OF CLONIDINE INDUCED ANALGESIA BY ETA RECEPTOR ANTAGONIST

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ETA receptor antagonists have been demonstrated to potentiate opiate analgesia. Clonidine produces significant analgesia, but it is not known whether ETA receptor antagonist will affect clonidine analgesia. This study examined the influence of sulfisoxazole (ETA receptor antagonist) on clonidine analgesia. Male Swiss Webster mice were used to determine antinociceptive response of drugs by measuring tail flick latency. The effect of clonidine (0.3, 1.0 and 3.0 mg/kg, i.p.) alone or in combination with sulfisoxazole (25, 75 and 225 mg/kg, p.o.) on analgesia and body temperature was determined. Clonidine produced a dose-dependent analgesia and hypothermia. Sulfisoxazole (25, 75 and 225 mg/kg, p.o.) when administered with clonidine (0.3 mg/kg) significantly potentiated (31% increase in AUC) the analgesic effect of clonidine. Yohimbine did not affect analgesic effect of clonidine plus sulfisoxazole. Idazoxan reduced (47% decrease in AUC) the analgesic effect of clonidine plus sulfisoxazole. Treatment with naloxone reduced (46% decrease in AUC) the analgesic effect of clonidine plus sulfisoxazole. Another ETA receptor antagonist, BMS-182874 (2, 10 and 50 µg, icv) was used, and it was found that the dose of 10 µg significantly potentiated (26% increase in AUC) the analgesic effect of clonidine. These results indicate that sulfisoxazole, an ETA receptor antagonist, potentiates the analgesic effect of clonidine, which could be mediated through imidazoline and opiate receptors. (Funded by EndogenX, Inc.).

16.3

ACUTE EFFECTS OF ENDOTHELIN RECEPTOR ANTAGONISTS ON HEPATIC HEMODYNAMICS OF NORMAL AND CIRRHOTIC RATS

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The different roles of endothelin-1 (ET-1) receptors (A and B) on hepatic hemodynamics in cirrhotic livers are not completely elucidated. We studied the effects of intra-hepatic delivery of ET receptor antagonists on in vivo hepatic hemodynamics in normal and cirrhotic rats. Sprague Dawley rats underwent sham or complete bile duct ligation (BDL) surgery to induce liver cirrhosis and portal hypertension. Two weeks later, sham and BDL rats were anesthetized and administered BQ-123 (0.7, 2.1 and 7 mg/kg/hr), BQ-788 (0.03, 0.1 and 0.3 mg/kg/hr) or bosentan (3, 10 and 30 mg/kg/hr). Vehicle or incremental doses of antagonists were continuously infused for 30 minutes into the portal vein using a double lumen catheter, which allowed simultaneous recording of portal pressure (PP). Local hepatic blood flow (HBF) was measured with Laser Doppler flowmetry. Steady-state portal and systemic hemodynamics were recorded. Increasing doses of BQ-123 (ETA selective antagonist) and bosentan (non-selective) gradually decreased PP of BDL, reaching significance with the highest dose compared to vehicle (13.6±0.4 vs 15.3±0.6 mmHg for BQ-123 and 13.3±0.7 vs 14.9±0.2 mmHg for bosentan, $p < 0.05$) but had no effects in sham. In contrast, BQ-788 (ETB selective antagonist) increased PP in sham (10.4±0.6 vs 8.7±0.6 mmHg, $p < 0.05$) but had no effect in BDL. BQ-123 and BQ-788 slightly and similarly decreased HBF compared to vehicle in both sham and BDL, whereas bosentan showed no effect. In conclusion, ETA receptors are mainly responsible for mediating vasoconstriction of the hepatic circulation and contribute to portal hypertension in cirrhotic rats; whereas both receptor subtypes seem to play a similar role in hepatic blood flow regulation.

16.4

CEREBROVASCULAR REMODELING AND ISCHEMIC BRAIN INJURY IN DIABETES: ROLE OF ENDOTHELIN-1

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We have previously shown that diabetes promotes remodeling of middle cerebral arteries (MCA) characterized by increased wall:lumen ratio and matrix metalloprotease (MMP) activity in an endothelin-1 (ET-1)-dependent manner. Since diabetes increases the risk of stroke and MMP activation disrupts blood brain barrier integrity after ischemia, we hypothesized that diabetes would cause greater neurovascular damage following ischemic brain injury. Control and diabetic Goto-Kakizaki (GK) rats were subjected to permanent MCA occlusion and the infarct size, edema and cerebral perfusion were evaluated. GK rats showed lower baseline cerebral perfusion (2.8±0.3 and 3.6±0.2 pixels, n=6-9, $p<0.01$) compared to those obtained in Wistar rats. Unexpectedly, GK rats showed smaller infarcts as compared to controls (12.3±1.4 vs 49.7±4.8%, $p<0.001$). Edema was increased in GK rats (66±26 vs 41±6%, $p<0.05$). Plasma ET-1 levels were elevated approximately three-fold in diabetic animals ($p=0.001$). There was enhanced endothelial and adventitial ET-1 staining in the MCAs from diabetic animals. These findings provide evidence that ET-1-mediated cerebrovascular remodeling does not worsen the outcome of ischemic brain injury in diabetes. It is possible that this remodeling response is compensatory in nature to regulate vascular tonus and flow especially when ischemia is layered on diabetic vascular disease.

16.5

STUDY OF THE IMPLICATION OF MK2 IN THE VASCULAR RESPONSE TO ENDOTHELIN-1

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Endothelin-1 (ET-1) is a potent vasoconstrictor whose production is deregulated in many inflammatory related diseases in which the cyclooxygenase-1/2 (COX-1/2) is up-regulated. Since it is known that the p38 MAPK pathway regulates ET-1 expression at the mRNA level, we studied the implication of the downstream kinase MK2 in the post-transcriptional regulation of ET-1 and COX-2. To accomplish this, MK2-deficient mice (MK2-/-, n=20) and their wild type littermate controls (MK2+/+, n=20) were used. Arterial pressures were measured using a Millar catheter under anesthesia and isometric reactivity of the isolated femoral artery was measured subsequently using a wire myograph. Tissue and aortic endothelial cell (EC) ET-1, COX-2 and COX-1 expression was quantified by QPCR. In response to ET-1 (100 nM), EC expression of preproET-1 and COX-2 mRNA increased in a time-dependant manner ($P<0.05$): this change in mRNA was greater in MK2-/- mice. Although arterial pressure was similar in MK2+/+ and MK2-/- mice, inhibition of COX (indomethacin, 1 µM) increased ($P<0.05$) the contraction of isolated vessels to ET-1 from MK2+/+ but not MK2-/- mice. These data suggest a role of MK2 in the vascular response to ET-1 and, possibly, ET-1 post-receptor signalling in general.

16.6

REMARKABLY LONG-LASTING TACHYPHYLAXIS OF PAIN RESPONSE TO ET-1 DOES NOT INVOLVE THE CENTRAL NERVOUS SYSTEM

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A profound tachyphylaxis of the acute nocifensive flinching response to ET-1 is shown by the reduced response to a 2nd ipsilateral s.c. dose of ET-1 after the 1st one. Flinching from a second injection was 20±5%, 57±18%, 79±35% and 100±17% of that to the 1st injection of (both 200 µM) ET-1 at intervals of 24, 30, 48 and 72 h, respectively, n=6-11. Despite this tachyphylaxis to ET-1, nocifensive responses to mechanical (von Frey) and chemical (formalin) stimuli are elevated after the initial injection. Published studies on ETA receptor desensitization show that cellular responses recover in <24h, inconsistent with the prolonged behavioral tachyphylaxis.We therefore tested the hypothesis that tachyphylaxis to ET-1 occurs in the CNS. Specifically we tested: a) inhibition of initial afferent impulses by local anesthesia of sciatic nerve, for 65-80 min, reducing flinching to 6-13% of control. However, repeat ipsilateral 200 µM ET-1 at 24h still showed tachyphylaxis, 27±4% of the flinches in naive rats. b) suppression of descending inhibitory effects from endogenous opiates by naloxone (6-8 mg/kg, i.p.) given 30 min before the 2nd ET-1. This treatment also did not prevent tachyphylaxis. c) nocifensive flinching induced by a 2nd dose of ET-1 injected intraplantar 24h after the 1st s.c. dose into the tail or fore paw. Sensitization instead of tachyphylaxis occurred. In summary, prolonged pain tachyphylaxis is not due to reduced responsiveness of the CNS. NIH/NCI Grant R-01 CA80153.

16.7

A NUCLEAR FUNCTION OF β-ARRESTIN-1 IN ENDOTHELIN A RECEPTOR SIGNALING IN OVARIAN CARCINOMA CELLS: REGULATION OF HISTONE ACETYLATION AND GENE TRANSCRIPTION

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Activation of the endothelin A receptor (ETAR) by endothelin-1 (ET-1) has a critical role in ovarian cancer (OC) progression. We previously demonstrated that β-arrestin is recruited to ETAR to form molecular signaling complexes that activate β-catenin transcriptional activity and cell invasion. Here, we show that in OC cells, ETAR promotes epigenetic modification through β-arrestin-1. As shown by biochemical techniques and chromatin immunoprecipitation assays, following ETAR activation β-arrestin-1 translocates to the nucleus and is selectively enriched at specific promoters of β-catenin target genes, such as ET-1 and MMP-2, involved in invasion and metastasis. Moreover, ET-1 in a β-arrestin-1-dependent manner facilitates the recruitment of histone acetyltransferase p300 and its association with the β-catenin transcription complex on the promoters of these genes, resulting in histone H3 acetylation and enhanced gene transcription. ETAR blockade with the specific ETAR antagonist, ZD4054, abrogates the engagement of β-arrestin in the interplay between ETAR and the β-catenin pathway in developing the invasive phenotype. In ovarian cancer xenografts, ZD4054 significantly reduced peritoneal dissemination and β-catenin expression. Altogether these results reveal a novel function for β-arrestin-1 as a nuclear messenger in ETAR signalling, underpinned by an epigenetic mechanism controlling β-catenin transcriptional activity, invasion and metastasis. Supported by AIRC, AstraZeneca.

16.8

$ENDOTHELIN_B$ RECEPTOR EXPRESSION IS UPREGULATED IN A RODENT MODEL OF GLAUCOMA

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Endothelin-1 (ET-1) is a vasoactive peptide which has gained prominence in recent years for its neurodegenerative role in glaucoma. Ocular administration of ET-1 has been shown to produce optic nerve axon loss and apoptosis of retinal ganglion cells. ET-1 mediates some its neurodegenerative effects by its actions on the ETB receptor. The purpose of this study was to determine if there are changes in ETB receptor expression in vivo in the Morrison's elevated IOP model of glaucoma in rats. IOP elevation was carried out in one eye of adult male Brown Norway rats using the Morrison's method (by injection of hypertonic saline through the episcleral veins), while the contralateral eye served as control. Following intraocular pressure elevation, rats were maintained for 2 to 4 weeks and sacrificed. Retinal sections were obtained from control and IOP elevated rat eyes and analyzed for changes in ETB receptor expression by immunohistochemistry. It was found that IOP elevation for 2 to 4 weeks produced an increased in ETB receptor expression in the retinal ganglion cells, as determined by immunohistochemical analysis. These findings suggest that increased intraocular

pressure as seen in primary open angle glaucoma produces increased ETB receptor expression, which could contribute to apoptosis of retinal ganglion cells. Use of endothelin receptor antagonists could have neuroprotective effects in glaucoma.

16.9

ANTICANCER POTENTIAL OF COMBINING THE ET_A-SPECIFIC ANTAGONIST ZIBOTENTAN (ZD4054) WITH CYTOTOXIC CHEMOTHERAPY

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Zibotentan (ZD4054) is a small-molecule, specific ETA antagonist with no detectable activity at ETB.1 In prostate cancer cells in vitro, combination of zibotentan with the cytotoxic agents docetaxel or paclitaxel produced concentration-dependent reductions in proliferation and increases in apoptosis that were greater than with any of these agents alone.2 Similar results were obtained with zibotentan and paclitaxel in ovarian cancer cells.3 Furthermore, in a mouse xenograft model of ovarian cancer, zibotentan significantly inhibited tumor growth to the same degree as paclitaxel, and the combination of zibotentan and paclitaxel produced additive effects.3 These results supported evaluation of combination treatment with zibotentan and cytotoxic agents in patients with cancer. A two-part study is being undertaken in patients with hormone-resistant prostate cancer (HRPC) designed to (a) establish a maximum tolerated dose (MTD) of zibotentan when combined with docetaxel and (b) assess safety, tolerability and pharmacokinetics of the zibotentan MTD in combination with docetaxel. Moreover, the zibotentan Phase III (ENTHUSE) trial program includes an ongoing study to evaluate whether zibotentan in combination with docetaxel can improve overall survival in men with metastatic HRPC. REFERENCES: 1Growcott J. Anticancer Drugs 2009;20:83–88. 2Pflug BR et al. Mol Cancer Ther 2007;6(12)Suppl:abst A287. 3Rosanò L et al. Mol Cancer Ther 2007;6:2003–2011.

16.10

ZIBOTENTAN (ZD4054) IS AN ET_A-SPECIFIC ANTAGONIST THAT DOES NOT ACUTELY MODULATE PLASMA ET-1 LEVELS IN MAN

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The pharmacological and anti-cancer characteristics of the small-molecule ETA-specific antagonist zibotentan (ZD4054) are examined with reference to binding affinity, specificity, functional antagonism and cancer-cell phenotypic modulation. In vitro, zibotentan pIC50 for displacement of [125I]-ET-1 bound to recombinant human ETA was 21±4 nM, while concentrations up to 10 µM had no detectable effect on ET-1 binding to recombinant human ETB. In vivo, zibotentan inhibited ET-1-mediated vasoconstriction at a dose of 0.03 mg/kg iv in dogs under anesthesia, and was active for at least 7 hours at a dose of 0.1 mg/kg. In contrast, zibotentan at a dose of 1.0 mg/kg did not affect ETB-mediated vasodilation induced using the ETB-selective agonist BQ3020. Subsequent data generated in a variety of preclinical models of cancer showed that zibotentan reduced ETA-driven processes such as proliferation, cell motility, angiogenesis, and metastasis, without inhibiting apoptosis, an ETB-mediated effect. Zibotentan's specificity profile was confirmed in Phase I trials in healthy volunteers, in which single doses of 10 and 30 mg inhibited ET-1-induced vasoconstriction, while doses up to 240 mg had no effect on plasma levels of ET-1. This finding shows that plasma ET-1 increases observed in other clinical studies after dosing selective rather than specific ETA antagonists were due to residual inhibition of the ETB receptor, and disproves the idea that the ETA receptor plays a role in homeostasis of plasma ET-1. Zibotentan is currently in Phase III clinical trials in patients with hormone-resistant prostate cancer.

16.11

THE ENDOTHELIN-1 PROMOTES HYPOXIA-INDUCIBLE FACTOR-1 ALPHA STABILIZATION THROUGH INHIBITION OF PROLYL HYDROXYLASE DOMAIN 2 EXPRESSION IN MELANOMA CELLS

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The hypoxia-inducible factor (HIF)-1α conveys hypoxic stimulus in angiogenic response through the induction of specific genes such as endothelin (ET)-1 and vascular endothelial growth factor (VEGF). We demonstrate that in melanoma cells, ET-1 and ET-3 through ETBR enhance HIF-1α and HIF-2α expression in normoxia. HIF-1α silencing by siRNA reduces the expression of VEGF and ET-1. In turn,ETs regulate HIF-1α activity and stability by impairing HIF-1α oxygen-dependent degradation pathway. ETs markedly decrease the expression and promoter activity of the prolyl hydroxylase domain (PHD)2, the critical oxygen sensor controlling the degradation of HIF-1α. These effects are blocked by the selective ETBR antagonist, BQ788. In addition, we found that activation of PI3K-dependent integrin linked kinase (ILK)-AKT-mammalian target of rapamycin (mTOR) pathway is required for ETBR-mediated PHD2 inhibition, HIF-1α stability, and VEGF expression. Moreover, in vivo Matrigel plug assay indicates that ET-1 promotes neoangiogenesis and that BQ788 can effectively impair this effect. In melanoma xenografts, ETBR blockade by ETBR antagonist results in a concomitant growth inhibition, reduction of angiogenesis and HIF-1α and VEGF expression, and an increase in PHD2 protein levels. These results further indicate that targeting ETBR may represent a potential therapeutic treatment of melanoma by impairing HIF-1α stability and tumor angiogenesis through the regulation of PHD2 levels. Supported by AIRC

16.12

A NOVEL IN VIVO THERAPEUTIC APPROACH IN AMYOTROPHIC LATERAL SCLEROSIS BY TARGETING ENDOTHELIN RECEPTORS SYNTHESIZING RESPIRATORY NEURONS WITH DENDRITIC NANODEVICES

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A fatal outcome in ALS can be caused by impaired respiration due to paralysis of the diaphragm. Etr mediate respiratory neuronal activity in the spinal cord and the phrenic nerve. A systemic application of drugs for improvement of respiratory distress results in significant side effects that could be curtailed by drug delivery to specific target neurons. We aimed to establish whether nanoparticles (dendrimers, DMs ~20 nm) can be transported by phrenic axons to respective neuronal somata in the spinal cord. In vitro findings indicated that DMs overcome cellular barriers and can be conjugated to drugs resulting in greater intracellular concentrations and earlier therapeutic effect. FITC- labeled DMs (15-20 µl were injected in the diaphragms of Sprague-Dawley rats. 3-6 d later double immunofluorescence revealed that ~100% of the axons in the phrenic nerve have taken up the DMs. DMs displayed a diffuse or distinct punctuate labeling in primarily ETrB positive motoneurons suggestive of lysosomal uptake. This is the first evidence of rapid retrograde transport of DMs at long distances (~3 cm) in nerves indicating that when coupled to specific drugs (Etr antibodies, antagonists or agonists) DMs present a precise therapeutic methodology for respiratory dysfunction treatment in ALS. Acknowledgments: We thank Drs. R. Kannan and K. Nantwi, Depts. of Chem. Eng. & Mat. Sci. and Anatomy and Cell Biol., Wayne State Univ. for contributing by providing DMs and experimental animals.

16.13

DIFFERENTIAL EFFECTS OF CHRONIC VS ACUTE CENTRAL ENDOTHELIN 1 ON HEMODYNAMICS AND PLASMA VASOPRESSIN AND VP GENE EXPRESSION

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Acute central administration of ET1 results in dose dependent increases in sympathetic output and blood pressure. Plasma VP rises in response to intracerebroventricular (icv) ET1 only in sinoaortic denervated (SAD) rats due to baroreflex inhibition of VP release. These studies were designed to test whether chronic icv infusion of ET1 induces similar changes in systemic hemodynamics and VP. Male Long Evans rats with arterial and venous catheters underwent sham or sinoaortic denervation. After one week, baseline (day 0) MAP and heart rate were similar in sham 114±2 mmHg and 454±11 bpm and SAD rats 117±2 mmHg and 442±13 bpm, but VP was higher in the SAD group: 2.2±0.3 vs 1.4±0.2 pg/ml ($P<0.02$). An osmotic minipump was then placed to infuse 0.5µl/h icv with one of the following: artificial CSF, 10 pmol/h ET1, 400 pmol/h BQ123 (BQ), or ET1+BQ. On day 3, MAP rose in both ET1 treated groups: sham, +14.2±3.8 and SAD +12.7±4.3 mmHg ($P<0.05$ vs CSF) and remained elevated by day 9. BQ blocked this rise ($P< 0.05$). Heart rate decreased similarly in both groups. In contrast to the rise in VP with acute ET1 in SAD rats, VP did not change in either sham or SAD groups vs CSF on either day 3 or 9. BQ alone did not alter VP. Hypothalamic VPmRNA at day 9 was higher in CSF treated SAD vs sham rats: 547 ±126 vs 1079 ± 233 pg/HT ($P< 0.05$). BQ abolished this difference. Notably, chronic ET1 infusion decreased VPmRNA compared with CSF-treated sham and SAD rats and BQ reversed this effect. Chronic central infusion of ET1 exerts a systemic pressor effect. In contrast to observations after acute icv ET1, plasma VP does not significantly change even after SAD. The latter effect may be due to down-regulation of VP gene expression by chronically high central levels of ET1. Funded by VA Merit Award.

16.14

ENDOTHELIN-1 AND INFLAMMATION IN TRYPANOSOMA CRUZI INFECTED ADIPOSE TISSUE

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Chagas disease due to infection with the parasite T. cruzi causes cardiomyopathy. We demonstrated that T. cruzi infection of mice is characterized by an increase in plasma endothelin (ET-1) levels, expression of ET-1 in heart and an intense systemic inflammatory response. Adipose tissue is an important target of T. cruzi in a mouse model. We investigated ET-1 and the inflammatory response of adipose tissue in T. cruzi infection. CD1 mice were infected with the Brazil strain of T. cruzi and adipose tissue was analyzed at 15 and 100 days post infection. Infection resulted in an increase in prepoET-1 in adipose tissue. Infected adipose tissue also

displayed an increase in macrophages, down-regulation of adiponectin and PPARg, activation of ERK, P38 and JNK and an increased expression of Toll-like receptors- 2, 4 and 9. Infection also resulted in upregulation of cytokines and chemokines. In adipose tissue, the increase in ET-1 and cytokines likely reduced the expression of adiponectin and PPARg resulting in an inflammatory phenotype. These observations suggest that adipose tissue, through the synthesis and release of adipokines, cytokines and chemokines contributes to systemic inflammation during both acute and chronic infection. Adiponectin null mice have a cardiomyopathic phenotype. Thus, the reduction in adiponectin and PPARg and the increase in ET-1 in infected mice may contribute to T. cruzi-induced cardiomyopathy(AI-076248, AI-068538).

16.15

INHIBITORY EFFECTS OF ERAS ON HUMAN AND RAT HEPATIC TRANSPORTERS

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The potential for the endothelin receptor antagonists (ERAs) ambrisentan (amb), darusentan (dar), bosentan (bos), and sitaxsentan (sit) to inhibit hepatic transporters was studied. The effect of ERAs on the transport of probe substrates by NTCP, OATP, Pgp, MRP2, BCRP and BSEP was studied in transfected cell lines, membrane vesicles and intact human (hSCH) or rat sandwich-cultured hepatocytes (rSCH). In assays studying individual transporters, ERAs only inhibited BSEP with relative potencies of sit >bos>>dar>amb (IC50 of 25, 49, 225 and >300μM, respectively). In hSCH, amb or dar did not reduce transport. Bos significantly attenuated NTCP and BSEP transport (at 100μM to 33 and 78% of control, respectively). Sit inhibited NTCP, OATP and BSEP (at 100μM to 2, 52 and 85% of control, respectively). In rSCH, amb had no inhibitory effects on any of the transporters. Dar had no effect on either Bsep or Mrp2, but inhibited Ntcp and Oatp to approximately 50% of control when tested at 100μM. Bos and sit were the most potent ERAs, inhibiting Ntcp, Oatp, Bsep and Mrp2 activity to <35% of control when studied at 100μM. Bos and sit were consistently found to be more potent inhibitors of both human and rat hepatic transporters. BSEP inhibition has been implicated in drug induced hepatic cholestasis. Bos and sit were found to inhibit BSEP activity in the 3 different assay systems. In contrast, amb and dar were not potent inhibitors of BSEP.

16.16

ENDOTHELIN-1 INCREASES THE TRANSMEMBRANE RESISTANCE OF ISOLATED SHEEP LEPTOMENINGES

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Objective: Ionic permeability is a basic property of epithelia surrounding spaces, which contain biological fluids and are involved in fluid turnover. The aims of this study were to investigate 1) the endothelin-1 (ET-1) effect in leptomeningeal membrane by measuring the transmembrane resistance and thus the changes in ionic permeability, 2) the existence of ETa and ETb receptors in leptomeningeal tissue. Method: Leptomeningeal tissue (arachnoid and pia mater) was obtained from 24 adult sheeps. The leptomeningeal transmembrane resistance (RTM, in Ω ·cm2) was measured with Ussing chambers, under open circuit mode, in controls and after the application of 10-9M and 10-8M ET-1 in each side (6 experiments for the arachnoidal side and the pial side). Immunohistochemical samples of leptomeningeal tissue for ET-1 and ETa and ETb receptors were prepared. Results: The mean RTM of the leptomeninges in sheep was increased after the application of 10-8M ET-1 for 30 minutes but the results were significant only for the arachnoidal side and the first 10 minutes. The addition of 10-9M ET-1 increased the mean RTM for 10 minutes in arachnoidal side and 3 minutes in pial side without statistical significance. The histological preparations revealed the existence of ET-1 and ETa and ETb receptors in leptomeninges. Conclusion: The application of ET-1 increases the RTM of arachnoid and pia mater and thus decreases their ionic permeability. The existence of endothelin-1 and endothelin receptors in leptomeningeal tissue was confirmed with immunohistochemistry.

16.16A

ENDOTHELIN IMPLICATED IN REFERRED HYPERALGESIA ASSOCIATED WITH TNBS-INDUCED COLITIS IN MICE

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This study evaluated the time course of mechanical hyperalgesia in the lower abdomen and hind paw in a murine model of colitis, as well as its susceptibility to reversal by ET_A and ET_B receptor antagonist treatments. Colitis was induced in male Balb/c mice by intracolonic TNBS (0.5, 1.0 or 1.5 mg in 0.1 ml), and the frequency of withdrawal responses to 10 consecutive applications of von Frey probes to the abdomen (0.07 g) or hind paw (0.4 g) was assessed starting 24 h after treatment. Mice given 0.5 mg TNBS displayed pronounced hyperalgesia to mechanostimulation of the lower abdomen (frequency at 24 h: saline 11.0 ± 3.1, TNBS 48.0 ± 6.9%) and hind paw (frequency at 24 h: saline 12.5 ± 4.7, TNBS 47.1 ± 7.1%), which persisted up to 72 and 48 h, respectively. Higher doses of TNBS induced freezing behavior but failed to elicit hyperalgesia. Atrasentan (3, 10 and 30 mg/kg, i.v.), given 24 h after TNBS, abolished mechanical hyperalgesia of both hind paw and abdomen for up to 2 and 3 h, following 10 and 30 mg/kg, respectively. A-192621 (7 and 20 mg/kg, i.v.) attenuated abdominal mechanical hyperalgesia at 3 h after injection of the higher dose. Morphine (2.5 mg/kg, s.c.) blocked mechanical hyperalgesia at both abdomen and hind paw for up to 2 h. Maintenance of mechanical hyperalgesia of the abdominal region and hind paw during TNBS-induced colitis involves mechanisms signaled by endothelin ET_A and to a lesser extent ET_B receptors. **Financial Support**: CAPES, CNPq, PRONEX, FAPESC.

16.17

ECE-1 INFLUENCES PC CELL INVASION VIA ET-1 MEDIATED FAK PHOSPHORYLATION AND ET-1 INDEPENDENT MECHANISMS

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Plasma concentrations of endothelin-1 (ET-1) are significantly elevated in men with metastatic prostate cancer (PC). ET-1 also contributes to the transition of hormonally regulated androgen-dependent to independent PC. ET-1 is generated from big-ET-1 by endothelin-converting enzyme (ECE-1). We have previously shown that ECE-1 is present in PC cell lines and primary tissue and is elevated in primary malignant stromal cells compared to benign. Specific inhibition of endogenous ECE-1 activity in these stromal cells significantly reduced epithelial cell invasion. siRNA or shRNA-mediated knockdown of endogenous ECE-1 in either the epithelial or stromal compartment significantly reduced PC cell (PC-3) invasion and migration. The re-addition of ET-1 only partially recovered the effect, suggesting ET-1-dependent and -independent functions for ECE-1 in PC. We considered that the ET-1-dependent effect of ECE-1 on PC invasion may be due to modulation of downstream signalling events. Addition of an ECE-1 specific inhibitor to PC-3 cells reduced phosphorylation of focal adhesion kinase (FAK), a signalling molecule known to play a role in PC. ECE-1 knockdown by siRNA resulted in a significant reduction in FAK phosphorylation. Accordingly, transient ECE-1 overexpression in PC-3 cells increased FAK phosphorylation. We conclude that ECE-1 influences PC cell invasion via both ET-1-mediated FAK phosphorylation and ET-1 independent mechanisms. This work is supported by Yorkshire Cancer Research.

16.18

THE ENDOTHELIN AXIS IN DRUG SENSITIVE AND MULTIDRUG RESISTANT BLADDER CANCER CELLS

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The endothelin (ET) axis is overexpressed in bladder cancer tissues, though underlying tumourigenic mechanisms are unexplored. We investigated the ET axis in drug-sensitive and multidrug resistant (MDR) bladder cancer cells. ET-1 and receptor A & B (ETAR;ETBR) expression was detected immunohistochemically in the (drug-sensitive) bladder cancer line MGH-U1 and its MDR subclone MGH-U1/R. Cells were incubated in ET-1 with/without BQ123 (ETAR antagonist); BQ788 (ETBR antagonist). Growth was measured by alamar blue assay; migration by scratch wound assay; downstream effectors by western blotting. Immunostaining detected ETBR>ETAR>ET-1 in MGH-U1 & MGH-U1/R. ET-1 stimulated MGH-U1/R (not MGH-U1) growth (13%>control, $p<0.01$). Both control and ET-1-driven MGH-U1/R growth was inhibited by ETAR, not ETBR, antagonism (33%, $p<0.01$), suggesting endogenous ET-1 activity. Interestingly, ETBR antagonist alone slightly but consistently increased MGH-U1/R growth (p=NS). BAX expression (pro-apoptotic) was decreased by ET-1 addition and reversed by ETAR blocking. ET-1 did not affect migration. ET-1 via ETAR drives MDR bladder cancer cell growth, partly by downregulating apoptosis. This is the first report investigating the ET axis in MDR cancer cells. Since MDR develops in the vast majority of cancers and is the main obstacle to successful chemotherapy, further investigations are warranted to determine whether ET receptor antagonism is a viable option for novel adjuvant therapies in MDR cancers.

16.19

REGULATION OF ENDOTHELIN-1 EXPRESSION AND FUNCTION BY NUTRIENT STRESS IN MOUSE COLON EPITHELIA

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OBJECTIVE: The endothelin (ET) system is influenced by a variety of stress conditions in many tissues. However, the effects of nutrient stress conditions on ET expression and its function are not well understood in the intestinal tract, while ET-1 gene expression and peptide were found in the intestinal tract. The aim of this study was to investigate the effect of feeding and fasting on the expression of ET-1

and short-circuit current (Isc) induced by ET-1 in mouse colon. MATERIAL AND METHODS: Mice were fed freely, fasted for 48 h, and re-fed after fasting, respectively. ET-1 mRNA levels and peptide concentrations were analyzed using real-time polymerase chain reaction (PCR) and sandwich ELISA, respectively. Isc of epithelial tissue was measured under short-circuit conditions using a Ussing chamber. RESULTS: ET-1 mRNA expression and peptide concentrations in epithelial colonic tissue were significantly increased 48 h after fasting, and decreased within 2 h of re-feeding after a 48-h fast. Furthermore, the addition of ET-1 to the serosal but not the mucosal side increased Isc in colonic epithelia. An increase in Isc was caused by chloride ion (Cl(-)) secretion because Isc induced by ET-1 was blocked by bumetanide and Cl(- -) free conditions. In addition, an increase in Isc induced by ET-1 in colon excised from fasted mice was much lower than that obtained from free-fed mice. CONCLUSIONS: Gene expression, peptide concentration, and the function of ET-1 in mouse colonic epithelia are regulated by nutrient stress.

16.20

CHARACTERIZATION OF CoCl2-INDUCED REACTIVE OXYGEN SPECIES

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CoCl(2) and MnCl(2) are hypoxic mimetic agents. We previously found that expression of ET-2/VIC, one of hypoxia-related factors, and the induction of neurite outgrowth in PC12 cells through ROS induced by CoCl(2). MnCl(2) also are known to induce neurite outgrowth in PC12 cells. However, it is unclear whether the mechanism of the effect induced by these metals is same. In the present study, we evaluated biological effects induced by MnCl(2) and compared with those induced by CoCl(2). Furthermore, we analyzed sources of CoCl(2)-induced ROS generation. MnCl(2) up-regulated ET-2/VIC gene expression and ET-2/VIC peptide production as CoCl(2) did, but not affect ET-1 gene expression, in the neurite outgrowth of PC12 cells. NAC did not at all inhibit the effects induced by MnCl(2). Furthermore, addition of MnCl(2) to the culture medium did not generate ROS as CoCl(2) did. These results indicate that ET-2/VIC expression is a common pathway in neurite outgrowth induced by CoCl(2) and MnCl(2), but the effects induced by CoCl(2) are ROS dependent, whereas the effects induced by MnCl(2) are ROS independent. Taken together, the mechanism for the effects by CoCl(2) was different from that by MnCl(2). The ROS, were not decomposed by catalase or SOD, were rapidly generated by reaction of CoCl(2) mainly with components of HS rather than with FBS or DMEM. Some ROS generated by reaction of CoCl(2) with components of HS may participate in the observed neurite outgrowth of PC12 cells.

16.21

ENDOTHELIN RECEPTOR EXPRESSION IN DORSAL ROOT GANGLION AND SENSORY CHANGES AFTER SPINAL NERVE INJURY IN RATS

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Neuropathic pain induced by L5L6 spinal nerve ligation injury (SNL) is associated with an up-regulation of endothelin receptor-operated mechanisms in sensory nerves (Werner et al., ET-10 Conference, 2007). The current study investigated, in Wistar rats, which cell types express endothelin receptors in dorsal root ganglia (DRG), as well as the effects of treatment with ETA (Atrasentan, 10 mg/kg, i.v.) or ETB (A-192621, 20 mg/kg, i.v.) receptor antagonists on the sensory changes induced by SNL. Atrasentan or A-192621 reduced SNL-induced cold and mechanical hind paw hyperalgesia (Cold at 2 h: by 61 ± 7% and 54 ± 6%, Mechanical at 90 min: by 29 ± 6% and 42 ± 9%, respectively) and heat hyperalgesia (peak at 90 min: by 59 ± 13% and 53 ± 13%, respectively) for up to 3 h, on Days 6 or 12 after surgery, respectively. Immunohistochemistry revealed ETA receptors localized on IB4-positive C and myelinated (NF-200) A neurons of L4 and L5L6 DRG of sham-rats and of L4 DRG of SNL-rats. In L5L6 injured DRG of SNL-rats, ETA receptor expression in neurons positive for IB4 and NF-200 was markedly decreased. Immunoreactive ETB receptors were seen on GFAP-positive glial cells in all DRGs in both groups, as well as on both IB4- and NF-200-positive neurons in DRGs from SNL-rats only. Thus, ETA and ETB receptors play a role in promoting the sensory hyperalgesic changes induced by SNL and their expression in DRG are differentially regulated following nerve injury. Supported by: CAPES, CNPq and PRONEX.

16.22

STUDY OF HOMOLOGOUS DESENSITIZATION OF THE ENDOTHELIN RECEPTOR ETA IN HUMAN OA CHONDROCYTES

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We have previously demonstrated that endothelin-1 (ET-1) plays a role in cartilage catabolism in osteoarthritis (OA) by increasing NO and metalloproteinase production, and by inhibiting collagen and proteoglycan synthesis. In cartilage, ETA receptor (ETA) is the principal pharmacologically active ET-1 receptor. The purpose of this study was to determine the response of chondrocytes following acute and chronic ET-1 stimulation by examining ETA receptor desensitization. The cultured chondrocytes used were derived from consenting OA patients who underwent a total knee replacement. These chondrocytes were exposed for 2 hours with or without ET-1 (10nM) to desensitize the ETA receptor. The cells were then reexposed to ET-1 (10nM) for 5 minutes. Receptors were examined by immunofluorescence (IF) and western blot. IF showed that chronic stimulation with ET-1 reduces the ability of ETA to internalise (receptor is present at the cell surface) in contrast to a single exposure where ETA internalizes into the cytoplasm. By western blot we observed a significant increase in the signal of p-Akt when chondrocytes are exposed to chronic ET-1 when compared to an acute exposure of ET-1. This indicated that the receptor is overly active when stimulated chronically. Taken together these results suggest a defect in ETA receptor desensitization. Therefore, the ETA receptor could be a therapeutic target for OA in order to protect cartilage integrity.

16.23

ET-1 PLASMA LEVELS AND OCULAR BLOOD FLOW IN RETINITIS PIGMENTOSA

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Retinitis pigmentosa (RP) is an inherited retinal disorder clinically characterized by a pale nerve head, attenuated retinal blood vessels and bone spicule pigment in the retina. Hemodynamic studies have demonstrated that RP is associated with a reduction in the retinal and choroidal blood flow. We studied 20 patients, 12 males and 8 females, aged between 26 and 42 years (mean 34.6 years) affected by simplex RP. The institutional ethic committee of the S. Orsola-Malpighi Hospital approved the study. The patients had a visual acuity of 0.8 ± 0.2, visual field MD 10.523 ± 3.582 dB and b-wave electroretinogram amplitude of 26.000 ± 0.757 µV. An increase in plasma levels of endothelin-1 (ET-1) was found as compared with healthy controls: 1.91 ± 0.61 pg/ml vs. 1.18 ± 0.21 pg/ml ($p < 0.021$). Moreover we performed a color Doppler imaging (CDI) of the ophthalmic artery (OA) and posterior ciliary arteries (PCA's) and a finger laser Doppler velocimetry after a cold test. CDI in the OA showed a PSV of 31.17±5.19 vs. 36.70±3.15 cm/sec ($p<0.007$) and in the PCA's of 8.56±3.40 vs. 14.10±2.57 cm/sec ($p<0.001$). The laser Doppler velocimetry showed a baseline peak flow (PF) of 86.50±21.78 vs. 186.71±70.89 cm/sec (0.001). After cold test we found a decrease of PF of 67.5±21.4% vs. 74.8±22.8% and a recovery time of 50.3 vs. 22.4 sec ($p<0.004$). It is thought that the increase in ET-1 in RP lead to vasoconstriction and the decrease of retinal blood flow worsening the abiotrophic process.

16.24

RESPONSE OF ARTERIOLE-VENULE PAIRS TO ENDOTHELIN-1 IN THE CREMASTER MUSCLE OF LEPTIN DEFICIENT MICE

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Leptin has been shown to up-regulate endothelin-1 production. We investigated the responses to endothelin-1 (ET) in leptin deficient diabetic mice (db/db or ob/ob) in comparison to C57BL/6J genetic background mice in the absence or presence of L-NNA, a nitric oxide synthase inhibitor. The cremaster muscle of anesthetized (50 mg/kg Nembutal) mice (n=63) was prepared for intravital microscopy. Arteriole (A)/ venule (V) pairs were chosen for study. Baseline arteriole diameters in C57s (A 27.2±14.16µm, V 35.8 ±16.1µm) were larger than in db/dbs (A 17.4±6.7µm, V 25.8±14.9µm) but not different from ob/obs (A 22.5±10.5 µm, V 29.1 ± 12.7 µm). Vasodilatory tone tested by adenosine (10-4M) was not significantly different in A or V of each strain. However, vasoconstrictor tone (Phenylephrine, 10-4M) was decreased in both A and V of ob/ob and, to a greater extent, db/db compared to C57. ET-1 dose dependent constriction (logM EC50: C57 -11.9±29.6 A, -11.7±2.8 V; db/db -11.7±4.4 A, -10.0 V; ob/ob -9.4 A, -9.0 V) was not compromised in either db/db or ob/ob mice. LNNA pretreatment in C57 appeared to suppress constriction in A (logM EC50, -11.5±1.1) and blocked constriction in V (logM EC50, -12.1±1.7E+06). Endothelial dysfunction may result in attenuated vasoconstriction. However, constriction to ET-1 is not compromised in leptin deficient. NIH DK68401.

16.25

ENDOTHELIAL CELLS-DERIVED ENDOTHELIN-1 REGULATES PROTEINURIA IN DIABETIC MICE

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Recent study has reported the important role of endothelin-1 (ET-1) in diabetic nephropathy. However, correlation of ET-1 and glomerular filtration barrier function was remained unclear. Here we hypothesize that endothelial cell-derived

ET-1 regulates proteinuria in diabetic mice. We developed type 1 diabetes model in vascular endothelial cells-specific endothelin-1 knockout (VEETKO) mice, and its wild type (WT) littermates. Diabetes increased urinary protein excretion in WT mice after 8 and 24 weeks duration of hyperglycemia, which is prevented in VEETKO mice (170±23.19 vs. 30.12±10.09 mg/dl, respectively, $p<0.01$, n=6 each). This is associated with the decreasing of nephrin and podocin expression – slit diaphragm proteins – in WT, but not in VEETKO glomeruli. The increasing of ET-1 induced by diabetes also caused mesangial matrix expansion and fibrosis in WT glomeruli, which are associated with upregulation of ICAM-1 and MCP-1. These inflammatory cytokines further promotes macrophage recruitment and glomerulosclerosis in WT, but not in VEETKO mice. Taken together, we observed that endothelial cells-derived ET-1 plays a role in regulating proteinuria by preservation of podocyte function and amelioration of glomerulosclerosis in diabetic mice. Targeting endothelial cells-derived ET-1 might beneficial to prevent diabetic-induced chronic kidney disease (CKD).

16.26

ENDOTHELIN-1 INDUCES PULMONARY SMOOTH MUSCLE CELL MIGRATION BY STIMULATING THE ET-A RECEPTOR AND THE ERK1/2 PATHWAY

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Pulmonary arterial hypertension (PAH) is a devastating disease of elevated precapillary pulmonary vascular resistance. A central pathologic feature is intimal hyperplasia in arterioles, involving proliferation and migration of smooth muscle cells (SMC). The endothelin-1 (ET-1) system plays a well-established role in the pathogenesis of PAH and ET-1 receptor antagonists (ERA) are an important treatment for PAH. In contrast, ERAs are ineffective for systemic hypertension and left heart failure. We hypothesized that ET-1 plays a unique role in the pulmonary circulation by inducing migration of pulmonary SMCs. Using a trans-well assay, we observed ET-1 induced migration of pulmonary smooth muscle cells (PAC) that was blocked by an ET-A receptor antagonist (BQ-123) but not by an ET-B blocker (BQ-788). Consistent with others' findings, ET-1 had no effect on systemic (rat aortic, RASM) SMC migration. Flow cytometry showed that PAC and RASM express the same amount of ET-A receptor, excluding receptor density as an explanation for the difference in response to ET-1. Blocking the ERK1/2 pathway abrogated ET-1-induced PAC migration. By Western blot, ET-1 stimulated phosphorylation of ERK1/2 in PAC, but not in RASM. We conclude that ET-1 induces pulmonary SMC migration because the ET-A receptor is coupled to downstream signaling machinery in pulmonary, but not systemic, SMCs. This finding may help explain why ERAs are effective in the treatment of PAH but not of systemic hypertension.

16.27

ENDOTHELIN-1 AND ANGIOTENSIN II-INDUCED PKB PHOSPHORYLATION IS DEPENDENT ON INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR AND C-SRC ACTIVATION IN VASCULAR SMOOTH MUSCLE CELLS

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We have demonstrated earlier that insulin-like growth factor-1 receptor (IGF-1R) plays a role in tranducing the effect of H_2O_2, leading to protein kinase B (PKB) phosphorylation. Since vasoactive peptides elicit their responses through generation of reactive oxygen species (ROS), including H_2O_2, we investigated whether IGF-1R transactivation plays a similar role in endothelin-1 (ET-1) and angiotensin II (Ang II)-induced PKB phosphorylation and hypertrophic responses in VSMC. AG-1024, a specific inhibitor of IGF-1R protein tyrosine kinase (PTK), attenuated both ET-1 and Ang II-induced PKB phosphorylation in a dose-dependent manner. ET-1 and Ang II treatment also induced the phosphorylation of tyrosine residues in the autophosphorylation sites of IGF-1R, which was blocked by AG-1024. In addition, both ET-1 and Ang II evoked tyrosine phosphorylation of c-Src, a non-receptor PTK, and pharmacological inhibition of c-Src PTK activity by PP-2, a specific inhibitor of Src-family tyrosine kinase, significantly reduced PKB phosphorylation as well as tyrosine phosphorylation of IGF-1R induced by the two vasoactive peptides. Furthermore, protein and DNA synthesis enhanced by ET-1 and Ang II were also attenuated by AG-1024 and PP-2. In conclusion, these data suggest that IGF-1R and c-Src PTK play a critical role in mediating PKB phosphorylation as well as hypertrophic and proliferative responses induced by ET-1 and Ang II in A-10 VSMC. (Supported by the Canadian Institutes of Health Research).

16.28

IMPAIRED CAVERNOSAL RESPONSE TO ET-1 IN GOTO-KAKIZAKI TYPE II DIABETIC RATS

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Introduction: Diabetes is a risk factor for erectile dysfunction (ED) and ET-1 is involved in vascular dysfunction observed in diabetic conditions. The penile smooth muscle cells not only respond to, but synthesize ET-1. We hypothesized that Goto-Kakizaki (GK) rats, a non-obese model of diabetes, display cavernosal overactivation of the ET-1 system as a contributing mechanism for ED. **Methods and Results**: Male 10 and 18 week-old Wistar (Control) and GK rats were used. Contractile responses to ET-1 were decreased in cavernosal strips from GK rats at 10 (Control: 4.96±0.58 vs GK: 1.47±0.29; mN, maximal response) and 18 weeks (Control: 2.32±0.38 vs GK: 1.63±0.14; mN, maximal response) compared to control. Gene expression of prepro-ET-1 was decreased in cavernosal strips from GK rats at 10 weeks (GK: 0.25±0.03; fold of change compared to control). However at 18 weeks prepro-ET-1 expression was increased (GK: 1.93±0.16; fold of change compared to control), as well as ET_A receptor (GK: 1.41±0.08; fold of change compared to control) expression. No change was observed in cavernosal ET_A receptor expression between control and GK animals at 10 weeks. ET_B receptor expression was decreased in cavernosal strips from GK rats at both 10 (GK: 0.58±0.08; fold of change compared to control) and 18 weeks (GK: 0.66±0.06; fold of change compared to control). **Conclusion:** ET-1 and ET_A receptors are regulated in a time-dependent manner in the cavernosum of GK rats along the progression of the diabetic condition. Interestingly, the decreased contractile response to ET-1 must represent a counter-regulatory mechanism to prevent further increase in cavernosal smooth muscle cells contractility.

16.29

EXERCISE DECREASES OXIDATIVE STRESS IN MICEOVEREXPRESSING HUMAN ENDOTHELIN-1 IN THE ENDOTHELIUM

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Erythropoietin (EPO) therapy has been shown to increase blood pressure in chronic renal failure patients and in animal models. EPO-induced hypertension is blunted by endothelin (ET) A receptor blocker treatment. Chronic exercise prevents or reduces development of cardiovascular diseases such as diabetes and hypertension. However, it is unknown whether exercise prevents EPO-induced hypertension. We have developed transgenic mice with endothelium-restricted human preproendothelin (eET)-1 overexpression, which exhibit vascular dysfunction, remodeling and inflammation in the absence of elevation of blood pressure. To determine whether exercise prevents EPO-induced hypertension, 8-10 week old male eET-1 mice treated or not with EPO (100 U/kg, s.c., 3 times/week) were subjected to a program of chronic swimming (1 h/d) for 8 weeks, or were maintained in sedentary condition (n=6). EPO treatment increased systolic blood pressure by 20%, which was prevented by exercise. NADPH oxidase activity increased 2-fold ($P<0.01$) in the aorta of both eET-1 and EPO-treated eET-1 mice. This increase was reduced by exercise by 30 % ($P<0.05$). Similar results were observed in the heart. These findings suggest that swimming reduces oxidative stress in ET-1 mice treated or not with EPO and blunts EPO-induced elevation of blood pressure. Funded by CIHR grant 37917.

17.0: ENDOTHELIN-1 IN INFLAMMATORY DISEASES AND PAIN

17.2

SEXUALLY DIMORPHIC NOCICEOTIVE PRIMING BY ENDOTHELIN: INVOLVEMENT OF THE ENDOTHELIN B. RECEPTOR

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Effective treatment of painful vaso-occlusive episodes in sickle cell disease is limited by a lack of understanding of the mechanisms of vaso-occlusive pain. To examine the mechanisms by which early painful vaso-occlusive events leads to pain sensitization, our lab has developed a model in which endothelin-1 (ET-1) exposure in postnatal day 7 rats results in nociceptive priming when followed by a second exposure to ET-1 on postnatal day 11. ET-1-induced priming was sexually dimorphic with males developing systemic sensitization and females a localized de-sensitization. Systemic sensitization in males was accompanied by down-regulation of the endothelin B receptor in left hindpaw and forepaw, and right hindpaw. In contrast, localized de-sensitization in females was accompanied by a local up-regulation of the endothelin B receptor. ET-1 induced nociceptive priming was attenuated by pre-treatment with the opioid analgesic morphine at time of ET-1 first exposure. In addition, morphine prevented ET-1 induced alterations in endothelin B receptor expression. These findings suggest that even a single acute vaso-occlusive episode early in development can result in systemic changes in expression of the endothelin B receptor and pain sensitization. Furthermore, these findings highlight the need to include sex as a variable in studies of pain in infants and children. This study was funding by National Institutes of Health grant DA023593.

17.3

MECHANISMS UNDERLYING BENEFICIAL EFFECTS OF ATRASENTAN IN MURINE MODELS OF COLITIS

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This study aimed to clarify the roles of endothelins (ETs) and mechanisms associated to ETA/ETB receptors in experimental colitis induced by intracolonic TNBS (1.5 mg in 0.1 ml) administration or by dextran sodium sulfate (DSS, 3% solution) ingestion. Balb/c mice received either vehicle, Atrasentan (Atra, ETA receptor antagonist, 10 mg/kg once daily, i.v.), A-192621 (ETB receptor antagonist, 20 mg/kg once daily, i.v.) or dexamethasone (Dex, 1 mg/kg twice daily, s.c.). At 72 h after TNBS, mice displayed high mortality and weight loss. Atra or Dex enhanced survival, but only Atra (but not A-192621) restored body weight. Atra and Dex reduced colonic macroscopic and microscopic damage and MPO activity of TNBS-treated mice, and abolished the increases in colonic IL-1β, MIP-2 and KC levels induced by TNBS. Dex blocked reductions of colonic IL-10 and IL-13 levels caused by TNBS, but Atra only restored IL-10. Both treatments inhibited TNBS-induced increases in colonic E-selectin and β2-integrin immunostaining. Real time RT-PCR showed that colonic ET-1 mRNA decreased at 24 h and ET-2 mRNA decreased at 24 and 72 h, but increased at 48 h after TNBS. ETA and ETB receptor mRNA was increased at all 3 time points. Atra reduced body weight loss, disease activity index and macroscopic damage of DSS-treated mice. These results show that Atra treatment is highly effective in reversing colonic injury and inflammation in both murine models of IBD. Financial Support: CAPES, CNPq, PRONEX, FAPESC.

17.4

CO-EXPRESSION OF ETB IN KERATINOCYTES ENABLES THE COUPLING OF ETA RECEPTORS TO INTRACELLULAR CALCIUM RELEASE

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Endothelin-1 (ET-1) is a pain mediator produced by keratinocytes (KCs). ET-1 is elevated in skin by injury/inflammation and causes pain through the activation of ETA (and, perhaps, ETB) receptors in cutaneous tissues. A coincident analgesic action of ET-1 is mediated only by ETB receptors, on KCs, releasing β-endorphin and thereby activating μ-opioid receptors on pain fibers. However, the cascade of intracellular events in the response of KCs to ET-1 remains largely unknown. To clarify these pathways we used Ca imaging on a variety of cultured cell lines. We noticed that the ability of ET-1 to elicit an elevation of [Ca]in (ΔCa) is always correlated with the dual presence of ETA and ETB mRNA, as detected by RT-PCR. In these cells 30nM ET-1 elicits ΔCa independently of extracellular calcium, implying a pathway involving phospholipase C (PLC). Furthermore, this response is blocked by 100nM BQ123, an ETA-selective antagonist, but not by 200nM BQ788, an ETB-selective antagonist. Desensitization of ETB by exposure to 100nM IRL-1620, an ETB agonist, did not disrupt the ΔCa response to ET-1. To further test the hypothesis that co-expression of both receptor types is required for ΔCa, we transfected ETB into HaCaT and Pam212 cells, respectively human and mouse KC cell lines; both lack endogenous ETB. In these transfected cells 30nM ET-1 elicited ΔCa, whereas in untransfected cells it did not. It thus appears that the presence of ETB enables the activation of PLC by ETA.

17.5

ENDOTHELIN SYSTEM PLAYS ROLE IN BK POLYOMA VIRUS INFECTION OF RENAL EPITHELIAL CELLS

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In recent years, nephritis induced by BK virus (BKV), a non-enveloped double-stranded DNA polyomavirus, has become a severe problem after renal transplantation. The comprehensive immunohistological analysis of endothelin (ET) system in different allograft pathologies documented endothelin receptor A (ETRA) and endothelin receptor B (ETRB) expression and suggested the important role of the ET system in different types of human renal allograft damage. We aimed to use ET receptor antagonists to study the involvement of ET system in BKV infection of human proximal tubular epithelial cells (HRPTEC). HRPTEC were pre-incubated with either 100 nM BQ123 (antagonist of ETRA), 100 nM BQ788 (antagonist of ETRB) or combination of two antagonists for 1 hour prior to co-incubation with BKV. The percentage of infected cells and the cellular levels of BKV large T antigen expression were significantly decreased in cells treated either with BQ788, or with combination of BQ788 and BQ123, but not in cells treatment with BQ123 alone. Our data suggest that antagonist of ETRB BQ788 represses BKV infection in HRPTEC. How exactly ET system is involved in regulation of BKV infection after renal transplantation remains to be determined. Since ET antagonists are used for treatment of primary pulmonary hypertension, and ET blockade usually ameliorate kidney pathology, our findings set the stage for realistic consideration of the use of ET receptor antagonists in treating BKV-related kidney pathologies.

17.6

DISTRIBUTION OF ENDOTHELIN-1 AND ET-A/B RECEPTORS IN HEMORRHOIDS

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Background and aim: Endothelin-1 (ET-1) plays an important role in the regulation of vascular tone. ET-A receptor stimulation causes vasoconstriction whereas ET-B receptor stimulation may produce either vasoconstriction or vasodilation. Little is known regarding ET-1-related regulation of tone in the dilated vascular spaces found in hemorrhoids. The aim of this study was to determine the distribution of ET-1 and ET-A/B receptors in hemorrhoids. Methods: Under local ethics committee approval and patients' informed consent, 14 samples of grade III or IV hemorrhoidal tissue and 6 samples of normal rectal mucosa and submucosa were obtained. The distribution of ET-1 and its (ET-A/B) receptors were studied by immunohistochemistry. Results: Endothelium-dependent ET-B receptor staining was uniformly observed in both the hemorrhoids (14/14) and rectal tissues (6/6). ET-B immunostaining in hemorrhoids was paralleled by that of endothelial nitric oxide synthase. ET-A receptor immunostaining was observed in 3/14 hemorrhoidal tissue and 1/6 in the endothelial cells of the rectal tissue. ET-1 immunostaining was observed in 2/14 hemorrhoidal endothelial tissues but in none of the rectal tissues. Conclusions: This study demonstrates an endothelium-dependent ET-B receptor immunostaining of both hemorrhoidal vascular channels and rectal submucosal vessels suggesting there may be a potential use of ET-B receptor antagonists to reduce the ET-B receptor-mediated dilatation in hemorrhoids.

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