Integrating the Life Sciences from Molecule to Organism

The Physiologist

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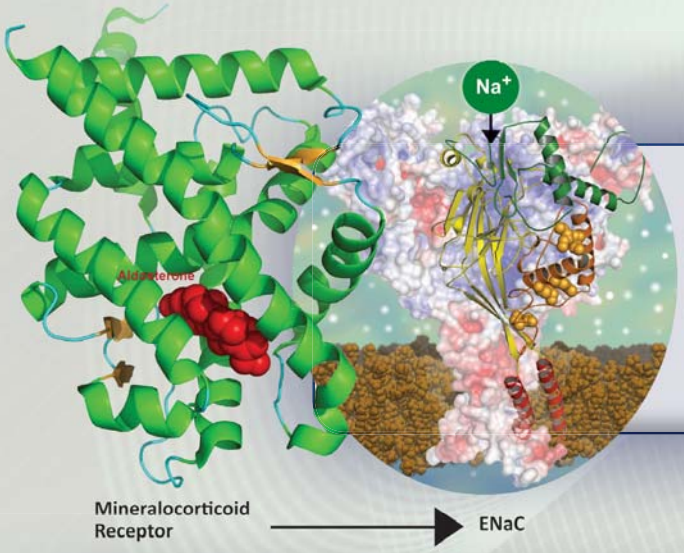

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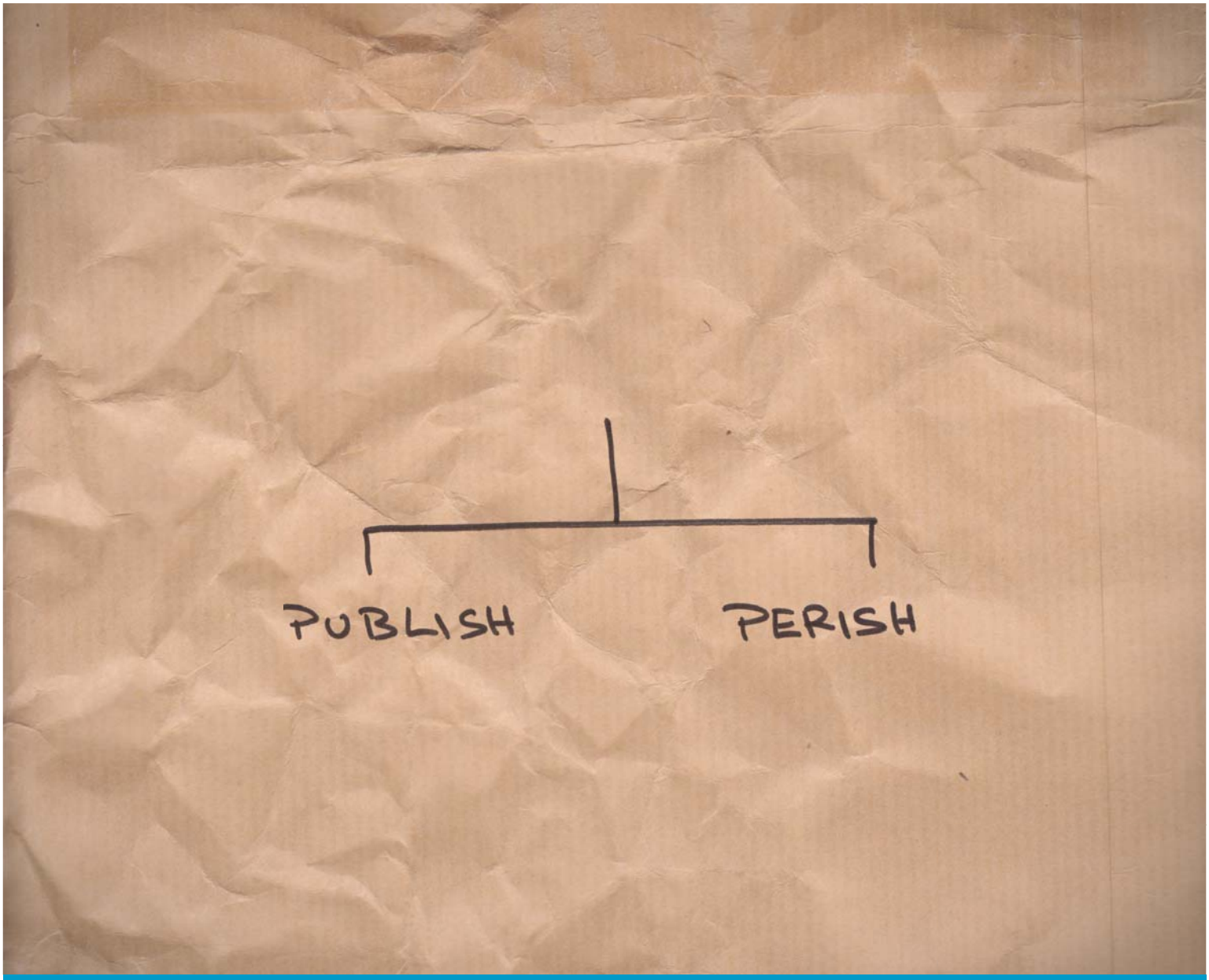

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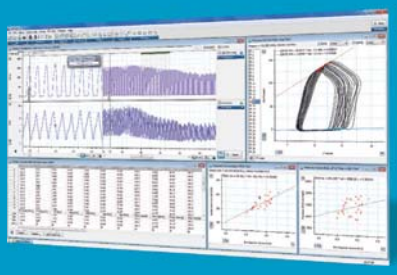

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Physiology: Evolving, Not Declining

Dee U. Silverthorn
Integrative Biology, Univ. of Texas

In April 2011 *The Scientist* magazine published an opinion piece by Richard Naftalin, a British physiologist, titled "The Decline of Physiology" (20). Despite the foreboding title and accompanying photograph of a dissected grass frog, the article dealt largely with the decline in physiology teaching in British medical schools: fewer lecture hours, the loss of animal-based laboratory classes, and replacement of practical laboratories with demonstrations, computer-aided learning programs and problem-based learning (PBL) sessions that focused on human medicine to the exclusion of fundamental basic science. An expanded version of the article (actually the original essay, which was too long for *The Scientist*) was subsequently published in *Physiology News*, The Physiological Society newsletter, under the title "The decline of physiology teaching in British universities" (19; R. Naftalin, personal communication). The two articles were then followed by a debate at the July 2011 meeting of The Physiological Society on the statement, "Physiology as a separate discipline is no longer relevant to modern science" (24). Naftalin's article in *The Scientist* struck a chord with physiologists around the world, and evoked a lively discussion on the International Union of Physiological Societies (IUPS) Teaching listserve and a session on "decline" at the September 2011 Federations of Asian and Oceanian Physiological Societies (FAOPS) meeting in Taiwan.

But is Physiology really declining? Concern about "the future of Physiology" is a theme that has been repeated many times and has been written about at regular intervals over the last 40 years (5, 8, 10, 11, 16, 28). The reality is that as research tools for investigating physiological mechanisms shifted from whole animal and tissue studies to cellular and molecular levels of organization, attention moved away from the traditional scientific disciplines, and a chasm developed between researchers and physiology educators. Departments of physiology disappeared, subsumed into departments of basic biomedical sciences for budgetary reasons or renamed, with titles such as "Molecular Physiology and Biophysics" or "Cellular and Molecular Physiology," to be more attractive to contemporary researchers. Even more alarming in terms of the pipeline for physiologists of the future, physiology began to disappear from the undergraduate biology curriculum.

Is Physiology a discipline that needs to be preserved? In answer to the debate statement, "Physiology as a separate discipline is no longer relevant to modern science," I would contend that physiology will never be irrelevant as long as there is life on earth. Physiology is arguably the most egalitarian of the biological disciplines. It is integrative, spanning all levels of organization, from biochemistry and cell biology to the physiological adaptations that allow species to occupy particular ecological niches. Physiology embraces physics and mathematics and engineering and

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Institute for Integrative Physiology: Resurrection of Physiology at the University of Chicago

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The University of Chicago is one of the oldest US universities founded in 1890 by the American Baptist Education Society and oil magnate John D. Rockefeller. The first president William Rainey Harper, hoped for a university that combined an American-style undergraduate liberal arts college with a German-style graduate research university. Soon after the formation of the university, a physiology department was established in 1892. From its inception the efforts were focused on "general physiology". As the university's fortunes grew, so did those of the physiology department. They were able to recruit distinguished faculty including the German born physiologist Jacques Loeb, who came as an assistant professor of "physiology and experimental biology" in 1892 and rose to the rank of Professor of physiology in 1899. Loeb left for Berkeley in 1902 where he became Chair and then he moved to the Rockefeller Institute for Medical Research in 1910 to Chair a department. Loeb was the founder of the Journal of General Physiology. Another famous (more correctly infamous) faculty member was Alexis Carrel who worked at the University of Chicago and the Rockefeller Institute for Medical Research. He helped to develop new vascular sutures and performed ground breaking research in organ transplantation and surgery. In 1912 he was awarded the Nobel prize in Physiology and Medicine for this work. Unfortunately he is also well known as a Nazi collaborator and being an advocate of eugenics in league with the Vichy government. Another notable Chicago faculty member at that time was Frank R. Lilly, who received his degree in zoology at The University of Chicago and then joined the faculty in 1900. He became Chair of Zoology in 1910. Lilly was a key contributor to the Woods Hole Marine Biological laboratory, soon after it was founded, and was the director of the institution from 1908-1939. Woods Hole became a haven for physiologists for the next 100 years.

The heyday for physiology at The University of Chicago arrived with Anton Julius Carlson ("Ajax") who joined the faculty in 1904 after receiving his doctorate (in physiology) from Stanford. Carlson was the Chair of the department from 1916 to 1940. Carlson had an impact on a variety of different areas of physiology. He was undoubtedly one of the preeminent physiologist of his day. Carlson was the President of the American Physiological Society from 1923 to 1925. In 1929 he was elected as foreign member of the Royal Swedish Academy of Sciences. In 1941 Carlson was featured on the cover of Time magazine. He became president of the AAAS in 1944. In 1953, Carlson received the first American Humanist Association's "Humanist of the Year" award for his contributions to education and scientific research. He received the American Medical Association's Service Gold Medal the same year. During his tenure, physiology teaching was paramount and Chicago was a prolific center that trained a large number of physiologists (the university itself is knows as "the teacher of teachers"). Demonstrations were a key part of his training program. Carlson had a flair for the dramatic. Today, some of his demonstrations would be considered quite dangerous. For instance, Carlson once fasted for fifteen days with a balloon crammed into his stomach to measure contractions. His experiment led to significant insights into gastric function. As a faculty member, Carlson defended empirical research. As Time magazine wrote: "That consuming interest in vitality has carried him on a lifelong voyage of discovery—through the heart, stomach, alimentary tract, liver, ductless glands, lymphatic and nervous systems". They also wrote that he was "One of the most colorful characters in the history of American physiology". Although he retired in 1940, he remained professor emeritus. Carlson died in 1956.

A little later, in 1947, George Marmont invented a chamber and circuit that allowed for "space clamp"; a technique that permitted sections of membrane along an axon to be kept at a uniform potential. He used this technique to clamp the current of neurons. Using the idea of the space clamp, Kenneth S. Cole introduced the voltage clamp to the world, which was used soon afterwards by Hodgkin and Huxley in their pioneering work. Both Cole and Marmont were associated with the University of Chicago for periods of time and Marmont with the department of Physiology.

Ralph W. Gerard spent most of his professional career in the physiology department at the University of Chicago. He was an early neurophysiologist who made important discoveries related to the nervous system. Gilbert N. Ling received his Ph.D. in 1948 from the physiology department at the University of Chicago and remained there as a post-doctoral fellow for the following two years. Intracellular recording became possible with the invention of the glass microelectrode by Ling and Gerard in 1948, which was soon used by Alan Hodgkin in his "classic" experiments on squid axon. It is difficult to overstate the importance of the invention of glass micrpelectrode.

This "glory era" was followed by a long decline similar to many other departments of physiology around the country. The department committed "suicide" in 1970 (Harry Fozzard personal communication) and by 1973 there were just 2 faculty members left. These two scientists merged with those from Pharmacology to make the Department of Pharmacological and Physiological Sciences. In the late 1980's The University of Chicago tried to resuscitate physiology by creating the Committee on Cell Physiology under the leadership of Harry Fozzard. The Committee on Cell Physiology established a dynamic graduate program and was responsible for recruiting physiologists, albeit into different departments. But as is so often the case, when Fozzard stepped down as head of the Committee it fell on hard times and died slowly and the department was formally disbanded in 2007.

The revolutionary advances in cell and molecular biology as well as in

genetic engineering in the past half-a-century have provided spectacular insights into our understanding of structure and function of biological systems at the molecular, cellular and subcellular levels. Integrative physiology embraces the concepts of cell/molecular physiology, biochemistry and applies these concepts and experimental approaches to understand the function at the level of whole animal or organ. Realizing its importance, Everett Vokes, then the Interim Dean and Kenneth Polonsky, the current Dean of the University of Chicago established the Institute for Integrative Physiology (IIP) in the Biological Sciences Division as of October 2010. Nanduri R. Prabhakar was appointed as its Inaugural Director. The Institute has four divisions: a) Autonomic Physiology (Head: Daniel McGehee); b) Membrane Physiology (Head: Francisco Bezanilla); c) Epithelial Physiology (Head: Jerrold R. Turner); d) Oxygen Physiology (Head: Ganesh K. Kumar). Currently, IIP has 30 faculty members including 15 senior and 15 junior members (http://integrativephysiology.uchicago.edu/ and is in the process of recruiting additional faculty.

An Inaugural Symposium of IIP was held at the University of Chicago on May 26th 2011. Four plenary speakers were invited including Solomon H. Snyder (the Johns Hopkins University School of Medicine), Antonio Scarpa (National Institutes of Health), Gregg L. Semenza (the Johns Hopkins University School of Medicine) and Martin Frank. (American Physiological Society). Snyder gave a talk entitled "Novel Neural Messengers" and Scarpa spoke on "Challenges and opportunities of Peer review". Semenza gave a talk on "Regulation of oxygen homeostasis" and Frank presented an overview on "APS-Advancing the discipline of physiology". The symposium was attended by more than 100 students and faculty members.

Nanduri Prabhakar with Plenary Speakers of the Inaugural Symposium of IIP: Antonio Scarpa, Solomomn H. Snyder, Nanduri Prabhakar, Martin Frank, and Gregg L. Semenza.

Financial restraints posed by the current economy is forcing many universities to merge basic science departments and as a consequence losing the identity and importance of basic sciences, especially Physiology as a discipline. Creating Institutes devoted to Physiology such as that created at the University of Chicago might be a way to keep the identity of physiology and retain its importance in making fundamental discoveries related to the future of medicine. ❖

Acknowledgement: I want to thank Aaron P.Fox and Harry Fozzard for providing me with the information on the history of physiology at the University of Chicago.

Martin Frank presenting APS pin to Nanduri Prabhakar.

APS Bylaw Changes

ARTICLE III. Membership

SECTION 1. The Society shall consist of regular, honorary, affiliate, emeritus, graduate student, undergraduate student, and sustaining associate members.

SECTION 2. *Regular Members*. Any person who has conducted and published meritorious original research in physiology, and who is presently engaged in physiological work, shall be eligible for proposal for regular membership in the Society.

SECTION 3. *Honorary Members*. Distinguished scientists of any country who have contributed to the advance of physiology shall be eligible for proposal as honorary members of the Society.

SECTION 4. *Affiliate Members*. Persons who are interested in fostering the mission and aims of the American Physiological Society but do not have evidence of scholarly work in the physiological sciences shall be eligible for proposal for affiliate membership in the Society provided they are residents of The Americas.

SECTION 5. *Emeritus Members*. A regular member may apply to Council for transfer to emeritus membership if that person (1) has reached the age of 65 and is retired from regular employment or (2) has been forced to retire from regular employment because of illness or disability and (3) has been a Regular member in good standing for a minimum of 10 years. An emeritus member may be restored to regular membership status on request to Council.

SECTION 6.a. *Graduate Student Members*. Any doctoral or masters program student who is actively engaged in physiological work ~~as attested to by two regular members of the Society~~ shall be eligible for proposal for graduate student membership. No individual may remain in this category for more than five years, without reapplying.

SECTION 6. b. *Undergraduate Student* Members. Any matriculated undergraduate student, as demonstrated by submission of verification of student status, who has an interest in physiology is eligible for undergraduate student membership. No individual may remain in this category for more than five years.

SECTION 7. *Sustaining Associates*. Individuals and organizations who have an interest in the advancement of biological investigation may be invited by the President, with approval of Council, to become sustaining associates.

SECTION 8. ~~Nominations~~ *Evaluation for Membership*. ~~Two regular members of the Society must nominate a person for regular or affiliate membership on APS membership application forms.~~

~~a. The Membership Committee shall assess the qualifications of potential regular members and recommend nominations to Council.~~

~~b~~a. ~~Nominations~~ Applications for ~~affiliate and student~~ regular, affiliate and student membership~~membership~~ shall be reviewed by the Executive Director. If the ~~nominees~~ applicants meet the criteria established by Council, they will be accepted immediately and so notified. The Executive Director will inform Council of the names of new ~~affiliate and student~~ members.

~~SECTION 9. Election of Members. Election of regular and honorary members shall be by vote of members of Council. A two thirds majority of the members present and voting shall be necessary for election.~~

SECTION ~~10~~9. *Voting*. Only regular members shall be voting members. ~~Honorary, affiliate, and emeritus members shall have the privilege of attending Business Meetings of the Society but shall have no vote.~~

SECTION ~~11~~10. *Expulsion of Members*. The Society reserves the right to revoke the membership of a member found guilty of scientific misconduct.

ARTICLE VII. Financial

SECTION 1. Operating Fund Purpose. The Operating Fund is used to provide sufficient cash to meet daily and ongoing financial obligations of APS. The Operating Fund will contain sufficient cash to cover current expenditures. Investments in the Operating Fund are limited to cash and fixed income instruments.

SECTION 2. Short-Term Fund Purpose. The Short-Term Fund is used to meet unanticipated expenditures that exceed the Operating Fund's reserves. Investments in ~~The~~ the Short-Term Fund ~~is to contain approximately 50% of the value of the Operating Fund~~ are limited to cash and fixed income instruments.

The Society's Bylaws specify:

Article IV. Officers. Section 4.

a. *Nomination of Officers.* Nominations for President Elect and for members of Council will be made by ballot, on forms provided by the Executive Director, before September 30 of each Year. ~~Each member may nominate no more than one candidate for each office.~~ If a member wishes to nominate the same person for President Elect and for Councillor he/she must nominate that individual for each position.

b. *Nominating Committee.* The Nominating Committee shall consist of the immediate Past President, who will serve as Chairperson, and each member of the Section Advisory Committee. The Chairpersons of the Joint Program Committee and Publications Committee shall serve as ex officio members. The Nominating Committee shall select a slate from candidates nominated by the Society membership. The slate presented for vote shall be such that no more than one of the nine Councillors shall be from a single institution and no more than two of the nine shall have a primary affiliation from the same section. The Nominating Committee shall make two nominations for the office of President Elect and six nominations for Councillor.

c. *Election of Officers*. Election of the President Elect and members of Council shall be made by ballot, on forms provided by the Executive Director, prior to the Spring Business Meeting. Each voting member must indicate on the ballot his or her choice of the candidate for office. The candidate(s) receiving the most votes shall be elected. In case of a tie vote, the decision shall be made by lot. Ballots will be counted according to the Election Plan. The results of the election will be announced at the Spring Meeting of the Society and the newly elected officers shall take office at the close of the Spring Meeting of Council. ❖

expresses them in the context of living organisms. As Ralph W. Gerard observed in his 1958 essay (12): "Physiology is not a science or a profession, but a point of view." If there is unique value to this point of view, we need to work to sustain the identity of Physiology.

Physiologists bring an integrative perspective to research that most reductionist scientists lack. In his recent essay for *Physiology* (15), Michael Joyner presented strong arguments for the fundamental importance of understanding physiology in the development of treatments for disease. Physiology has always been the basis of medicine, and the role of physiology and pathophysiology in medical education and clinical practice is appreciated by practicing physicians. Each year the Association of American Medical Colleges (AAMC) surveys graduating physicians about their medical education experience. One question in the 2011 survey (2) asked graduates how well the different basic sciences had prepared them for clinical work, on a scale of 1=poor to 4=excellent. Physiology (3.4) and pathophysiology (3.5) ranked the highest, along with introduction to clinical medicine (3.5) and gross anatomy (3.4). In 2002, the last year graduates were asked about the importance of a premedical physiology class in preparing them for medical school, the ranking for undergraduate physiology was 3.5, with biochemistry second at 3.2 (1). The battle for recognition that physiologists fight, therefore, is one in which the opposition comes from our colleagues who do not appreciate the integrative and translational nature of Physiology and from our own failure to promote Physiology adequately. To paraphrase Rodney Dangerfield, Physiology gets no respect.

If Physiology as a discipline is to prosper and thrive as we move through the 21st century, it must evolve and find new directions. In introductory biology classes, when we discuss the properties of living organisms, students learn the phrase "adapt or die"—individuals adapt and species evolve. Over the last 30 years, individual physiologists have been adapting to the changing academic scene. They are now found not only in departments of physiology, but in clinical departments such as cardiology and nephrology, or partnered with pharmacologists. Comparative physiologists hold positions in colleges, universities, and schools of veterinary medicine. As individuals, most of us have found ways to keep our research funded and a niche in which we can survive. But how can we ensure that in another 50 years there will be scientists who identify themselves as physiologists? For Physiology to survive, it's time for the discipline to evolve, and that requires the united effort of individual physiologists.

Perhaps we can take a lesson on how to adapt and survive from friend of mine who was an academic psychiatrist at Univ. of Texas Medical Branch in Galveston. Rudy Roden was a 17-year-old medical student in Eastern Europe when the Nazis invaded. He and his family were detained and sent to a concentration camp. Not long after their arrival, they were lined up and asked their occupations. Rudy watched as the group was divided, with some people going to work gangs, while women, children, and professionals were hustled off to what he later learned were the death chambers. When asked his occupation, Rudy said, "I'm a baker," and he was sent to work in the kitchens. When he was transferred to a new camp, he noticed construction going on, so he said he was a bricklayer. Rudy survived four different camps by being observant and making himself into whatever was needed. After liberation from Auschwitz, he immigrated to Canada and became first a family physician, then a psychiatrist. The lesson he taught me was that to survive, you have to take an active role in your future. Look around and see what is needed, what needs to be done, then figure out how you can be the one to fill that need. This is how physiology needs to adapt and evolve.

Taking a page from strategic planning meetings, I decided to do a SWOT analysis of the strengths, weaknesses, opportunities, and threats to physiolo-

Table 1 - Key Concepts in Biology.

Scientific Foundations for Future Physicians (Howard Hughes Medical Institute and the Association of American Medical Colleges) (14)	**Vision and Change** (National Science Foundation and American Association for the Advancement of Science) (23)	**The 2010 Advanced Placement Biology Curriculum** (College Board) (9)
Structure/function from molecules to organisms	Structure and function (anatomy and physiology)	Relationship of structure to function
Physical and chemical principles applied to living systems	Pathways and transformations of energy and matter	Energy transfer
Biomolecules and their functions	Information flow, exchange and storage	Continuity and change
Organisms sense and control their internal environment and respond to external change.	Systems	Regulation ("a state of dynamic balance")
Evolution as an organizing principle	Evolution	Evolution

gy in the United States today. What follows is my personal view. I have no answers, only ideas, but perhaps these comments can serve as the starting point for future discussions.

Strengths of Physiology: The integrative nature of Physiology is probably our biggest strength. Physiology is fundamental to the study of living organisms, be they humans, other animals, plants or microbes. In the past two years, three different national reports on biology education identified physiology as one of the core concepts in biology (Table 1). One problem, however, is that physiology was not explicitly named in any of the reports. It appears disguised, along with anatomy, as "structure and function," or as "systems" (23), "regulation—a state of dynamic balance" (9) or in statements such as "Organisms sense and control their internal environment and respond to external change" (14).

In the case of the *Scientific Foundations for Future Physicians* (SFFP) report (14), the omission of discipline names was intentional. I was privileged to be a member of the SFFP committee and because our intent was to promote innovative and interdisciplinary curricula, we carefully avoided discipline- and course-associated terms such as biochemistry and physics. Instead we substituted descriptive terms such as "biochemical concepts" and "physical principles."

The lack of specific identification of physiology also exists in public materials on the MR5, the pending revision of the AAMC's Medical College Admission Test (MCAT) (http://www.aamc.org/initiatives/mr5). The committee recommendations for content mention "biochemistry concepts" and "cellular/molecular biology topics," but organismal and integrative biology topics are missing from that list. On the other hand, a table in the MR5 Fall Update (distributed by email but not available on the AAMC website at the time this was written) uses the following description of the revised test section called *Biological and Biochemical Foundations of Living Systems*:

Processes that are unique to living organisms including growing and reproducing, maintaining a constant internal environment, acquiring materials and energy, sensing and responding to environmental changes, and adapting. This section of the exam will test the extent to which examinees know how cells and organ systems within an organism act independently and in concert to accomplish these processes. It will also test the extent to which examinees can reason about these processes at various levels of biological organization within a living system.

That sounds like Physiology to me.

Weaknesses of Physiology

Physiology has a public relations problem. The word "physiology" has fallen out of favor, as shown by the words that replaced it in the three reports discussed above. Physiology is a classic science and, therefore, viewed as no longer on the cutting edge. Many nonscientists have no idea what *physiology* is. The word and field have lost prestige among contemporary scientists. The press shortens the title "Nobel Prize in Physiology or Medicine" to simply "Nobel Prize in Medicine," even when the work being recognized was done in Physiology. It is time for an extreme image makeover.

A second important weakness is the perception of physiology as a static subject taught primarily to future healthcare professionals. There is a disconnect between the exciting science we do in the research laboratory and what colleagues think we teach. In addition, as Richard Naftalin pointed out in his essays (19, 20), student laboratories are disappearing. At too many academic institutions, both medical and undergraduate, time and resources for student laboratories are among the first items to be cut. Financial considerations and pressure from animal rights organizations have severely curtailed the use of vertebrate animals in student experiments.

A third weakness is the loss of physiology teaching hours in the curriculum, both in medical schools and undergraduate institutions. Many American medical schools have moved to organ-system-based integrated curricula, where physiology shares time with anatomy, biochemistry, and other basic sciences. Even institutions that retain discipline-based courses have seen their basic science contact hours decreased to make room for more clinical work in the initial years of medical school. Integration of basic sciences into the clinical curriculum has not been as successful, unfortunately.

At the undergraduate level, the close association between physiology and medicine has worked to physiology's detriment. This first became obvious with the National Academies of Science publication *Biology 2010* in 2003 (22). The distinguished biologists who were asked to write this report did not include physiology in their suggested core curricula, relegating it to an upper division elective course. The committee did not appreciate the integrative nature of physiology, and some of the topics that they indicated as belonging to physics and engineering, such as "the blood circulatory system and its control [and] fluid dynamics" are topics that physiologists teach every day. (For additional examples, see (25).)

Today, the standard introductory biology curriculum at many undergraduate institutions focuses on cellular and molecular biology in one semester, and ecology and evolution in the second. Organismal biology often gets short shrift, to some extent because teaching plant and animal physiology is outside the competencies and comfort zone of instructors whose research is focused at the extremes of levels of organization. There is a curious dissonance in this because the majority of students starting as biology majors like biology because they became interested in "critters" or because they plan a career in health professions. How can we expect students to become interested in Physiology if their first exposure to the subject comes in their junior or senior year of college? By that time, students are already planning their graduate work or taking the MCAT to apply for medical school.

Indicative of the exclusion of Physiology in the introductory biology curriculum is an entire series of introductory biology textbooks that come in two versions: one simply called some variation of "Biology," and the second called "Biology with Physiology." (See, for example, (3, 4).) What has happened that there are biologists who think that biology exists without physiology? Even introductory biology textbooks for biology majors avoid using the word "physiology." For example, one of the best-selling books (27) lists physiology in the index with one page reference, to the page where physiology is defined at the beginning of a section called "Structure and Function."

Some of the problem at undergraduate institutions lies with the decreasing numbers of biologists who identify themselves as physiologists. In one discussion of the premedical competencies

from the SFFP report, the dean of a small liberal arts college asked, "Do these changes mean that I'm going to have to hire a physiologist?" As amazing as it seems, there are schools teaching biology without a physiologist on the faculty or Physiology in the curriculum. Both curriculum and faculty research interests are focused on the extremes of the levels of organization: cellular and molecular biology, or ecology and evolution. Somehow the integrative middle, the physiology of living organisms, has disappeared.

Opportunities for Physiology and threats: Is there any hope for Physiology? Yes, but it requires action. If we simply maintain the status quo, Physiology as an identifiable discipline of biology will continue to decline in prestige, recognition, and inclusion. There are many changes taking place in science, medicine, and education, and to take advantage of these changes, physiologists need to be creative and innovative about their future roles in academia.

Undergraduate Education

One of the biggest opportunities for physiology in the US is the movement to competency-based education, both in academic medicine and undergraduate colleges. At the undergraduate level, the SFFP, *Vision and Change*, and *Advanced Placement Biology* reports all identified physiological principles among the core concepts in biology. Those of us who teach in undergraduate institutions must work to see that physiology, identified as such, achieves parity in the introductory biology curriculum. If physiology is an integral part of the introductory curriculum, particularly at larger schools, the textbook problem will resolve itself. Publishers are responsive to the marketplace.

One way to ensure the inclusion of more physiology in the undergraduate curriculum is for medical schools to add competency in physiology to their entrance requirements. For most medical schools, this will mean listing a physiology course alongside the required hours in chemistry, physics, and frequently, biochemistry. At the undergraduate level, a physiology requirement could provide instructors the opportunity to create an interdisciplinary curriculum by collaborating with colleagues in physics or chemistry. For example, instead of a traditional physics course for the premedical students, why not develop a two-semester course with laboratory in physiology and biophysics, one that teaches the biologically relevant principles of physics in the context of living organisms? That strategy would allow students to gain competency in physiology without adding additional courses to the required premedical course load.

Notice that I said "living organisms" and did not specify "in the context of humans" for the physiology and biophysics course. If physiology is to make inroads into the undergraduate curriculum, we must become more inclusive, and collaborate more with our comparative physiology colleagues. The American Physiological Society (APS) is not always the primary professional society for comparative physiologists, many of whom belong to the Society for Comparative and Integrative Biology (SICB), with its section devoted to Comparative Biochemistry and Physiology. Some APS comparative physiologists also belong to SICB, and we must encourage those APS members to coordinate efforts across the two societies. The relatively recent name change of the APS Comparative Physiology Section to Comparative and Evolutionary Physiology shows that some physiologists are already aligning themselves with evolutionary biologists. Many smaller schools have only one or two physiologists on their faculties, and finding support from disciplines outside physiology will be essential to effect change.

At the undergraduate level, the fundamentals of physiology need not be taught in a human or mammalian physiology class. Core concepts, such as homeostasis, control systems, fluid flow, gas exchange and so on can just as easily be taught and tested using other vertebrate and invertebrate animals and even plants. We use some of these organisms as models in our research because of evolutionary conservation of physiological mechanisms, so why not use them in our teaching as well? Being more inclusive and less human-centric would appeal to those biology faculty who feel their role is to teach future biologists, not future physicians. Another area for physiologists to explore is collaboration is with the growing undergraduate interest in computational biology, biomedical engineering, and systems biology. One undergraduate competency from the SFFP report was quantitative skills, and the quantitative aspects of Physiology lend themselves to systems modeling and analysis of functional networks in systems biology courses.

In addition, broadening the undergraduate physiology competency beyond human or mammalian physiology would expand opportunities for MCAT writers to create questions that test for understanding of core concepts and students' ability to transfer knowledge learned in one system to an unfamiliar one. Currently, commercial MCAT preparation classes use the knowledge that the MCAT tests mostly mammalian physiology to help students memorize content. If suddenly MCAT questions asked about sucrose transport across plant cell membranes or countercurrent mechanisms in fish gills, the MCAT courses would have to focus more on problem solving and core concepts that are fundamental to biology—a change that would undoubtedly appeal to non-biomedical biologists.

The large and growing numbers of students interested in careers in the health professions provide physiologists with a new opportunity in the undergraduate arena: the development of baccalaureate programs in biomedical sciences or undergraduate majors in physiology, such as the one that was developed at the Univ. of Arizona's College of Medicine (13). Politically, these majors are likely to be seen as a threat by traditional biology departments, but one solution would be collaboration between the undergraduate and medical colleges, or between departments of exercise science and biology (6).

Medical Education

I have been proposing to colleagues for a number of years that medical schools add physiology as an entry requirement, and the biggest resistance to this idea has come from medical school physiologists themselves. Their argument has been that if students take a physiology course before medical school, then medical school administrators will cut curriculum hours for Physiology even more. I contend that instead, this would provide an opportunity for the medical physiology faculty to integrate more into the clinical curriculum, to teach more complex and clinically relevant physiology, and to add more pathophysiology to the curriculum. Pathophysiology is a standard course in medical schools outside of North America, and it is standard in

the American nursing curriculum. If students all came to medical school with fundamental physiological principles as baseline knowledge, then the faculty could start their teaching at a higher level and build on the foundation instead of teaching to the lowest common denominator.

One of the threats to medical physiology has been the loss of instructional hours in the curricula. This could and should be viewed as an opportunity instead—an opportunity to revisit how we teach. At many medical schools, attendance in the basic science lectures is often low unless required. Students are saying by their absence that they do not need the faculty to learn the information being conveyed during the lecture. Why not eliminate the duplication and do something else during the lecture time, something that is more difficult for students to learn on their own? For example, give students the course learning objectives and a set of resources, which could include online videos of faculty lecturing and a set of objective questions on the content. To ensure that students learn the material before the lecture, create a simple assessment, such as an online, open-book quiz or a set of questions administered at the beginning of the lecture. Then spend the lecture time having the students work on problems. All too often our students think they understand material they have studied, only to find that when asked to apply their understanding to a clinical correlation or other problem, they are unable to make the transfer. By giving students an ungraded opportunity to practice problem solving, with an expert present to help them through the difficult concepts, they develop better problem-solving skills and retain the information longer. If the problems have clinical relevance, students are more interested in the material and less likely to view the content as simply another barrier to be crossed before they can get down to the business of learning to be a physician.

A good example of how medical students are memorizing basic science content rather than developing conceptual understanding appeared several years ago in a presentation on the proposed revision to the US Medical Licensing Examination (USMLE). A representative from the USMLE showed a graph question from the Step 1 test that illustrated the effect of substrate concentration on the rate of an enzymatic reaction. There were two lines on the graph, one for the control condition and one showing the effect of an inhibitor. Students were asked what principle the second line illustrated. About 75% of the examinees answered the question correctly. The identical question appeared on the Step 2 tests taken by the same cohort. This time only about 25% of the examinees were able to answer the question. If these students knew how to read a graph and had basic problem-solving skills, they should have been able to answer the question correctly the second time simply by reading the axes and interpreting the graph. Instead, they had apparently memorized kinetics graphs while studying for Step 1, then promptly erased that information from memory when they reached the clinics.

To ensure that physiology remains prominent in medical education, physiologists need to volunteer for the USMLE question-writing committees and for the Liaison Committee on Medical Education (LCME) teams that assess medical education programs in the United States. Although these service activities are time-consuming, they are essential for maintaining visibility. Similarly, physiologists who are active in research must be willing to serve on National Institutes for Health (NIH) Study Sections or on review panels for the National Science Foundation.

Laboratory Education

The role of student laboratories in medical curricula has been declining for many years and is not likely to see a revival for reasons of space, money, and scarcity of trained personnel for teaching in this setting. But laboratory teaching is still a major part of the undergraduate curriculum, and the integrative nature of physiology provides numerous opportunities for creating innovative laboratory curricula. As Naftalin pointed out in his essays, experiments using live animals teach students in ways that computer simulations and paper-based case studies cannot. The threat from the animal rights movement is not going away. But many of the physiological mechanisms that are traditionally taught using rats or frogs can be demonstrated equally well with invertebrate models (17, 26).

For example, in our physiology laboratories we have substituted the earthworm crop-gizzard for rat intestinal smooth muscle, crayfish heart and nerve for the frog heart and sciatic nerve, and cricket Malpighian tubule for fish kidney transport. These organisms are easily obtained, inexpensive, and do not require protocols filed with an Institutional Animal Care and Use Committee. Most are commercially raised or collected for food or bait. The dissections are relatively simple, yet still test students' fine motor skills. The same physiological and biochemical mechanisms that exist in humans occur, with slight variations, in these organisms. And instead of doing a single demonstration-type experiment, students can ask questions and carry out experiments with enough replicates to do statistical analysis of the data.

All of the calls for reform in medical and undergraduate education emphasize developing skills in the process of science: formulating hypotheses, designing experiments, collecting and analyzing quantitative data, and writing concisely. Practical, hands-on laboratories that require students to ask questions and design and execute their experiments are an excellent way to develop these skills. The experiments do not need to be complex or equipment-intensive because the process is more important than the question. Thinking about laboratories as a medium for training future scientists rather than as simply a place to observe phenomena provides opportunities for physiologists to design experiments that can be used from pre-college classes through professional training. Even medical school demonstration laboratories, such as recording electrocardiograms and blood pressure, can be tweaked to include an element of inquiry. For example, after students have learned to monitor blood pressure and pulse, send them to the literature to learn about the Valsalva maneuver and how it is used clinically to assess autonomic function (18). Then ask them to explain the underlying physiology and design an experiment to test for the reflex in their fellow students—a simple demonstration turned into an inquiry investigation.

Although student laboratories can achieve many of their goals without vertebrate animal experimentation, there continues to be need in both the pharmaceutical industry and academic research for scientists who are trained in mammalian whole animal studies. As researchers shift from describing cellular and molecular mechanisms to

understanding their role in complex systems, these scientists will need to be trained in Physiology and physiological research methods. Here is an opportunity for physiologists to provide the bridge to clinical applications. Institutions with strong animal research programs can develop training and research opportunities, such the Michigan State University (MSU) summer course, "Integrative and Organ Systems Pharmacology Short Course" (http://www.phmtox.msu.edu/sc/index.html), and the MSU In Vivo Pharmacology Facility (http://www.vprgs.msu.edu/node/1390). The directors of graduate training programs in Physiology and Pharmacology are actively working to coordinate graduate training, and these efforts should be encouraged and expanded.

Graduate Education

Another opportunity that should be exploited is the chance to expand graduate programs by collaborating with groups whose research has an integrative or quantitative perspective, such biomedical engineers or the pharmacologists just described. Exercise physiologists are often found in settings outside the medical schools, with separate graduate training programs. For Physiology to regain its stature, it will also be necessary to integrate successfully with scientists whose focus is in more reductionist areas of cellular and molecular biology and molecular genetics.

One glimmer of hope that this may be easier to achieve than we have thought is an October 7, 2011 editorial in *Science* titled "Genomics Is Not Enough" (7). In it, Aravinda Chakravarti, from the McKusick-Nathans Institute of Genetic Medicine at the Johns Hopkins Univ. of Medicine, points out that although we have associated specific genes with certain diseases, knowing the genes and their products is not sufficient to develop appropriate treatments. In his words, "Genome biology now needs to move to cell biology and physiology (systems biology) to understand how genetic perturbations lead to . . . disease." After so many decades of taking a back seat to the reductionist disciplines, it appears that physiology may again be moving to the cutting edge of biomedical research. We as physiologists need to be ready to take advantage of the opportunities this will present.

Health Professions Education

Physiologists also need to think beyond the training of MDs and PhDs to look at what we can do in one of the largest and fastest growing areas of physiology teaching—the nursing and other health professional programs, such as physical therapy, physician assistant, and optometry. Many of these are now graduate programs that, unlike medical and dental programs, require students to have an undergraduate course in human physiology with laboratory and another in human anatomy with laboratory before entry. Often these courses are taught in community colleges and two-year schools as a two-semester sequence of combined anatomy and physiology (A&P), with the first semester focusing on anatomy and the second on physiology. Faculty teaching these classes are being required by accrediting bodies to demonstrate graduate training in A&P, yet PhD programs in the two disciplines usually do not overlap.

Perhaps it is time for the medical basic science faculty to collaborate and create Master's degree programs that would train students to become excellent A&P teachers. These programs could include combined anatomy and physiology tracks, or physiology and anatomy separately. One indication that these new graduate programs would be well received is the success of the HAPS Institute, an outreach effort by the Human Anatomy & Physiology Society that provides short courses in selected A&P topics for graduate credit.

We need to be sure that physiologists are involved in the growing trend of programs that teach graduate students and postdoctoral students how to become excellent educators (See, for example, (21)). As long as we have people who need healthcare, there will be demand for instructors to teach physiology. Physiology trainees in academic medical settings should consider broadening their skills and acquiring experience teaching gross anatomy and histology to expand their future job prospects.

Conclusion

Science is not static, and if Physiology is to evolve in the future, it is time for physiologists to step up, look at what the needs are, and be creative about adapting Physiology to fill those needs. It will not be easy. Just as it has been difficult to change academic culture in terms of recognizing education as a scholarly activity, it may be difficult to change the perception of Physiology in established players in the academic scientific community. But we have an opportunity to affect scientists in the pipeline, and there are many opportunities for expanding the scope of Physiology across the curriculum. The threats to successful implementation will come mostly from those close to us, the faculty in related disciplines who see a stronger Physiology as threatening to their own interests. It is up to us as physiologists to show them that collaboration can be a win-win situation for all concerned when scientists and students come to appreciate the integration of function across all levels of biological organization.

Acknowledgements

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References

1. Association of American Medical Colleges. 2002 Medical School Graduation Questionnaire: All School Report. https://www.aamc.org/download/90070/data/gq-2002.pdf [October 2011].

2. Association of American Medical Colleges. Medical School Graduation Questionnaire: 2011 All Schools Summary Report. https://www.aamc.org/download/263712/data/gq-2011.pdf [October 2011].

3. Audesirk G, Audesirk T, Byers BE. Biology: Life on Earth 9/E. San Francisco: Benjamin Cummings, 2011.

4. Audesirk G, Audesirk T, Byers BE. Biology: Life on Earth with Physiology 9/E. San Francisco: Benjamin Cummings, 2011.

5. Blaustein MP. The New Golden Age of Physiology: Perspective on the Future of Physiology. *The Physiologist* 30(4): 49, 1987.

6. Carroll RG, Matyas ML, Atwater AE, Doze V, Faircloth R, Finkenstadt P, Goodman B, Henriksen EJ, Horwitz B, Looft-Wilson R, Madsen B, Mody J, Pelaez N, Pressley TA. APS undergraduate brainstorming summit report. *Adv Physiol Educ* 31: 380-386, 2007.

7. Chakravarti A. Genomics is not enough. *Science* 334: 15, 2011.

8. Chien, S. The Future of APS and Physiology. *The Physiologist* 34(1): 1, 1991.

9. College Board. (2010) College

Board AP Biology Course Description. http://apcentral.collegeboard.com/apc/public/repository/ap-biology-course-description.pdf [November 2010]

10. Forster RE. Prejudices on the future of physiology. *The Physiologist* 10(4): 373, 1967.

11. Frank M, Matyas M. The status of physiologists and physiology at U.S. medical schools. *The Physiologist* 45(4): 179, 2002.

12. Gerard RW. Mirror to Physiology: A Self-Survey of Physiological Science. Washington D.C.: American Physiological Society, 1958.

13. Henriksen EJ, Atwater AE, Delamere NA, Dantzler WH. The physiology undergraduate major in the University of Arizona College of Medicine: past, present, and future. *Advan Physiol Edu* 35: 103-109, 2011.

14. Howard Hughes Medical Institute and the Association of American Medical Colleges. (2009) Scientific Foundations for Future Physicians. http://www.aamc.org/scientificfoundations [July 2009]

15. Joyner MJ. Why physiology matters in medicine. *Physiology* 26: 72-75, 2011.

16. Knobil, E. What's past is prologue (The Tempest, Act II, Scene I). A "white paper" on the future of physiology and the role of the American Physiological Society in it. *The Physiologist* 33(6): 161, 1990.

17. McNeal AP, Silverthorn DU, Stratton DB. Involving students in experimental design: three approaches. *Advan Physiol Edu* 275: S28-S34, 1998.

18. Michael J, Silverthorn DU, Galey WR, Carroll RG. (2009) Effects of Valsalva maneuver on cardiovascular function. http://www.apsarchive.org/resource.cfm?submissionID=2456 [August 2011]

19. Naftalin RJ. The decline of physiology teaching in British Universities. *Physiol News* 83: 8-11, 2011.

20. Naftalin RJ. (2011) The decline of physiology. *The Scientist,* http://www.the-scientist.com/news/print/58122/ [April 2011]

21. National Postdoctoral Association (2009) Postdoctoral teaching fellowships. http://www.nationalpostdoc.org/careers/career-planning-resources/186-postdoctoral-teaching-fellowships [October, 2011]

22. National Research Council of the National Academies, Committee on Undergraduate Biology Education to Prepare Research Scientists for the 21st Century, Board on Life Sciences, Division on Earth and Life Studies. BIO 2010: Transforming Undergraduate Education for Future Research Biologists. Washington, D.C.: The National Academies Press, 2003.

23. National Science Foundation and American Association for the Advancement of Science. (2011) Vision and Change: A Call to Action. http://visionandchange.org/finalreport [August 2011]

24. Physiological Society. Is Physiology Dead? A debate at the July 2011 Oxford meeting of the Physiological Society. (2011) http://www.physiology2011.org/programme/oxford_debate.html [September 2011].

25. Silverthorn DU. Restoring physiology to the undergraduate biology curriculum: A call for action. *Advan Physiol Edu* 27: 91-96, 2003.

26. Silverthorn DU. Active transport in Malpighian tubules. In: Tested Studies for Laboratory Teaching, Proceedings of the 16th Workshop/Conference of the Association for Biology Laboratory Education. Volume, pp. 141-153, 1995.

27. Urry LA, Cain ML, Wasserman SA, Minorsky PV, Jackson RB. Campbell Biology 9/E. San Francisco: Benjamin Cummings, 2011

28. Zucker IH. Through the looking glass: The future of physiology. *The Physiologist* 51(2): 49, 2008. ❖

Chapter News

Tennessee Physiological Society Meeting 2011

Approximately 50 students, fellows and faculty assembled at the Quillen College of Medicine at East Tennessee State University in Johnson City for the third annual meeting of the TPS. In addition to the host institution, attendees represented the Univ. of Tennessee Health Science Center, Memphis, Meharry Medical College and Vanderbilt Medical School. The two-day meeting featured the research of graduate students and postdoctoral fellows presented in a morning symposium and afternoon poster session.

Some TPS2011 attendees assembled for a group photo after the keynote lecture and prior to the business meeting.

The meeting began on the evening of Thursday, October 13 with a welcome reception followed by an opening lecture by David Williams, Professor of Surgery at Quillen COM. William's talk was titled "Macrophage Scavenger Receptor A (SR-A): It's Not Just About Cholesterol Anymore!" and set the tone for a very lively and interactive meeting. As much of William's research relates to the function of the innate immune system, his lectures was especially timely in light of the recent awarding of the Nobel Prize in Physiology or Medicine for discoveries in innate immunity. The Friday morning symposium featured six graduate students and two postdoctoral fellows presenting their research to a receptive audience. Discussions of student research continued over lunch and onto the early afternoon poster session. A total of 22 posters were displayed in the foyer outside the main lecture halls

for Quillen medical classes. A panel of TPS members evaluated student poster presentations and selected two students for TPS travel awards to be used for the EB2012 meeting in April. These students and their poster titles are:

Exogenous Ubiquitin Inhibits Chronic ß-Adrenergic Receptor--Stimulated Cardiac Myocyte Apoptosis and Myocardial Remodeling.

Christopher R. Daniels, Cerrone R. Foster, Sana Yakoob, Mahipal Singh, Krishna Singh. Department of Physiology, Quillen College of Medicine, JHQ Veterans Affairs Medical Center East Tennessee State Univ., Johnson City.

Novel KCC3 mouse models to study ACCPN, a degenerative neuropathy disorder.

Jinlong Ding, José Ponce-Coria, and Eric Delpire. Department of Anesthesiology, Vanderbilt Univ. School of Medicine, Nashville, TN.

Judges remarked that the quality of all presentations was so good it was hard to choose just two for the travel awards. Next year the Society hopes to make additional travel awards and this was discussed at the business meeting later in the day.

Coincident with the afternoon poster session were two breakout sessions. One session led by a representative of AD Instruments, presented the latest hardware and software available for basic research and for teaching physiology to undergraduate and graduate students and new products for medical physiology courses. A second breakout session featured a discussion and information exchange among faculty from Meharry and Quillen interested in Phun Week activities.

Following the poster and breakout sessions, attendees reconvened for the APS Invited Keynote Lecture delivered by William Aird, Director of Vascular Biology Research, Harvard Medical School. Aird's lecture was titled "Vascular bed-specific regulation of the von Willebrand factor gene." In his talk, Aird covered a broad range of topics, from the genetic mechanisms underlying phenotypic diversity and plasticity of endothelial cells to the evolutionary origins of the endothelial cell system in vertebrates. To illustrate this latter point, Aird described his studies of endothelial structure and function in hagfish, organisms that diverged from the major vertebrate lineages over 500 million years ago.

A brief business meeting followed the keynote lecture. ZhongMao Guo, Meharry Medical College, was introduced as the next TPS president and plans were announced for election of the 2013 president. The TPS2012 meeting will be held at Meharry. Members were asked to reach out and recruit additional members, especially physiologists at four- year colleges and universities in Tennessee. Recipients of the Peter Lauf and Norma Adragna student travel awards for EB2012 were announced (see above). These travel awards are possible because of a generous donation from Drs. Peter Lauf and Norma Adragna to establish the award fund. As the TPS is now a 501(c)(3) organization making donations to TPS tax deductable, plans to seek additional donors and gifts to the student travel and awards fund were discussed.

The TPS2011 meeting was made possible by support from the Quillen College of Medicine Department of Physiology and the Office of the Dean of Medicine. In addition, office staff of Physiology managed local arrangements including scheduling lecture halls, poster session space, and food and beverages served throughout the meeting (reception, breakfast, lunch, coffee breaks, etc.). Corporate sponsorships were received from Kent Scientific, AD Instruments, and Fisher Scientific. The American Physiological Society provided funding to support the keynote speaker and student awards. ❖

Recipients of Peter Lauf and Norma Adragna TPS2011 travel grants to EB2012 are Chris Daniels of Quillen College of Medicine (center left) and Jinlong Ding of Vanderbilt Medical School (center right). The awards were announced and recognition certificates presented by Evangeline Motley--Johnson of Meharry Medical College (left) and keynote speaker William Aird of Harvard Medical School (right).

Graduate student and postdoctoral fellow presenters in the morning symposium session are pictured. From left to right are José Ponce--Coria, Emmanuel Okoro, Jinlong Ding, David Gibbons, Toh Gang, Rachel Lippert, Brandon Panaro, session moderator Cerrone Foster and TPS president Tom Ecay.

American Physiological Society
Professional Skills Training
2012 Course Calendar

January
Writing and Reviewing for Scientific Journals
4-Day Live Course

February
Presenting a Scientific Poster
7-Day Online Course

March
Networking at a Scientific Meeting
4-Day Online Course

April
Experimental Biology
APS 125th Anniversary

May
Interviewing for an Academic Position
10-Day Online Course

June
Writing and Reviewing for Scientific Journals
6-Week Online Course

July
Writing and Reviewing for Scientific Journals
6-Week Online Course

August
Writing and Reviewing for Scientific Journals
6-Week Online Course

September
Interviewing for an Industry Position
TBA

October
Abstract Writing for Scientific Meetings
4-Day Online Course

November
Creating a Poster for a Scientific Meeting
7-Day Online Course

December
Find PST on Facebook:
facebook.com/APS.PST
Watch for the 2013 Calendar

Course availability dependent on enrollment.

Visit our Website for More Information and Deadlines:
http://www.the-aps.org/education/profskills/

PHYSIOLOGY IN PERSPECTIVE: THE WALTER B. CANNON AWARD LECTURE (SUPPORTED BY SUCAMPO AG)

L. Gabriel Navar
Tulane Univ., HSC

"The Wisdom of the Body Revisited: Tribute to Walter B. Cannon and his Concept of Homeostasis as applied to Pathophysiology of Hypertension"

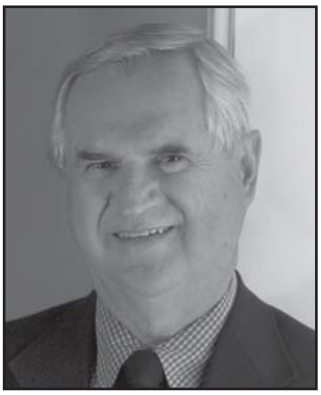
SATURDAY, APRIL 21, 5:45 PM

HENRY PICKERING BOWDITCH AWARD LECTURE

Mingyu Liang
Medical College of Wisconsin

"MicroRNAs and Systems Molecular Medicine"

SUNDAY, APRIL 22, 6:00 PM

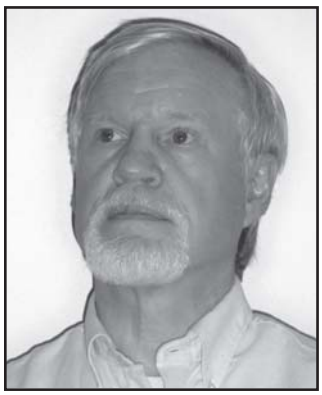


CLAUDE BERNARD DISTINGUISHED LECTURESHIP OF THE TEACHING OF PHYSIOLOGY SECTION

William Galey
Howard Hughes Medical Institute

"Reflections on the Teaching and Learning of Science in the Late 20th and Early 21st Centuries"

SUNDAY, APRIL 22, 10:30 AM



HUGH DAVSON DISTINGUISHED LECTURESHIP OF THE CELL AND MOLECULAR PHYSIOLOGY SECTION

Mark Knepper
NHLBI, NIH

"After the Interlude: Cell-level Systems Biology in the 21st Century"

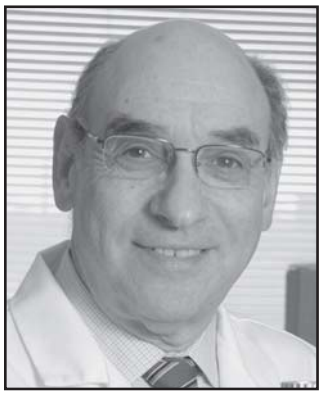
SUNDAY, APRIL 22, 10:30 AM

ERNEST H. STARLING DISTINGUISHED LECTURESHIP OF THE WATER AND ELECTROLYTE HOMEOSTASIS SECTION

Kathryn Sandberg
Georgetown Univ.

"The Female Paradox: Resistance and Vulnerability in Hypertension and Renal Vascular Disease"

SUNDAY, APRIL 22, 3:30 PM



CARL LUDWIG DISTINGUISHED LECTURESHIP OF THE NEURAL CONTROL AND AUTONOMIC REGULATION SECTION

Irving Zucker
Univ. of Nebraska Med. Ctr.

"Sympathetic Nerve Activity in Heart Failure: A Critical Role for Central Angiotensin II Receptors"

MONDAY, APRIL 23, 8:00 AM

SOLOMON A. BERSON DISTINGUISHED LECTURESHIP OF THE ENDOCRINOLOGY AND METABOLISM SECTION

Michael Schwartz
Univ. of Washington

"Understanding Obesity as a Disorder of Energy Homeostasis"

MONDAY, APRIL 23, 10:30 AM

EDWARD F. ADOLPH DISTINGUISHED LECTURESHIP OF THE ENVIRONMENTAL AND EXERCISE PHYSIOLOGY SECTION

Laurie Goodyear
Harvard Medical School

"Exercise Only Affects Muscle? Fat Chance

MONDAY, APRIL 23, 2:00 PM

Joseph Erlanger Distinguished Lectureship of the Central Nervous System Section

Stephen Woods
Univ. of Cincinnati

"Peptides, Food Intake and Body Weight: Problems of Interpretation"

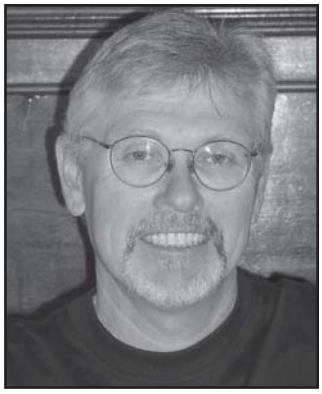
Monday, April 23, 3:30 pm

Carl W. Gottschalk Distinguished Lectureship of the Renal Section

Michael J. Caplan
Yale Univ., School of Medicine

"Playing in Traffic: Sorting and Signaling in Renal Epithelial Cells"

Monday, April 23, 3:30 pm

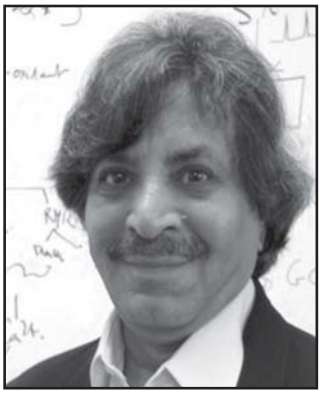


August Krogh Distinguished Lectureship of the Comparative & Evolutionary Physiology Section

James Hicks
Univ. of California, Irvine

"Tales from the Heart: A Comparative and Evolutionary Perspective of the Vertebrate Circulatory System"

Tuesday, April 24, 8:00 am



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

Nanduri Prabhakar
Univ. of Chicago

"Sensing Hypoxia: Physiology, Genetics and Epigenetics

Tuesday, April 24, 10:30 am

Robert M. Berne Distinguished Lectureship of the Cardiovascular Section

Rhian M. Touyz
Ottawa Hospital Research Institute

"Molecular Mechanisms of Hypertension: Redox Signalling, Lipid Rafts and TRPMs

Tuesday, April 24, 2:00 pm

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

Hamid Said
Univ. of California

"Mechanism and Regulation of Intestinal Absorption of Water-Soluble Vitamins: Cellular and Molecular Aspects"

Tuesday, April 24, 3:30 pm

Two APS Members Awarded the 2011 ASBMB STEM SEED Grant for PhUn Week Activities

In June 2011, the American Society for Biochemistry and Molecular Biology (ASBMB) announced a grant opportunity for awards of up to $2,000 to support the development of a K-12 Science, Technology, Engineering, and Math (STEM) outreach program and/or partnership. APS members Patricia A. Halpin (Univ. of New Hampshire, Manchester) and David Holtzclaw (Nebraska) were selected as two of ten outreach awardees. The following highlights Holtzclaw's framework for using his experience with the APS outreach program, Physiology Understanding Week (PhUn Week), in successfully receiving the competitive ASBMB award.

How did you first get involved with PhUn Week?

I first got involved with PhUn Week in 2007 in response to an advertisement I saw at Experimental Biology that year. I was new to the world of physiology and APS and wanted to get more involved. I thought PhUn Week would be ideal way to get more involved with APS because it required very little effort on my part, the APS staff do all the heavy lifting. All I had to do was fill out a form and call some local teachers to schedule a time. The APS and PhUn Week websites had all the information I needed and a list of over 50 proven activities I could choose from. What could be easier?!

From your experience, what kind of science teaching works in the classroom and with your teacher partners?

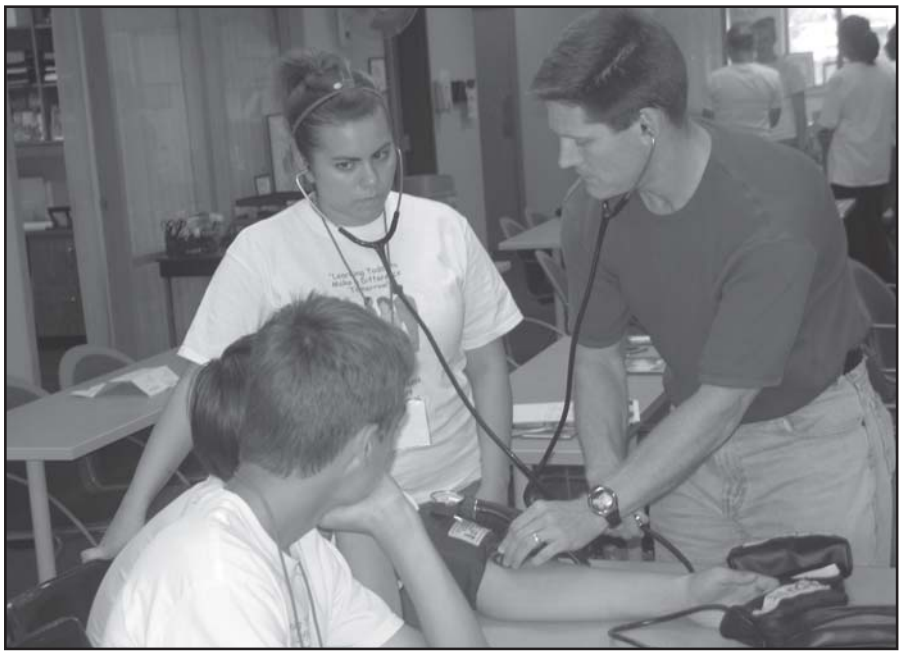
For younger children (K-6th grade), you definitely need to plan activities that are hands-on, short (less than 30 minutes), and simple. Don't even THINK about lecturing. Give the students very simple instructions in less than five minutes and get out of the way and let them go to work. Make sure you do the experiment or activity before going to the classroom to ensure everything is working and that there isn't anything you are missing to complete the activity. Since younger children are often easily distracted, you should check-in with the students every five minutes or so to keep them on task. For K-6 graders, the experimental data or observation should be easy and the desired outcome or learning objective of the activity should be extremely obvious. K-6th grade students actually have very little science in their curriculum, so don't assume that the students will have much background knowledge like the difference between an acid and base.

For older students (7-12th grade), you still want to have hands-on, engaging activities, but you can be a bit more challenging. I've found it works best to actually given them less instruction, just give the students the objective, supplies, and let them come up with the experiment. They will struggle for a while and will want you to tell them what to do next or how to proceed—but don't—let them come up with the experiment and procedures. They can be pretty creative and insightful. Also, have the students work in groups of three to five students if possible. If the groups get much bigger, than some of the students tend to try and blend into the walls. As with younger students, you need to check-in with the students every five to 10 minutes to keep them on task. It is best to constantly reinforce the main points or objectives of the activity. Finally, avoid experiments that have them doing repetitive, mindless activities like pipetting or counting cells;, this is a BIG turn off.

For the K-12 teachers, try and be as flexible as you can. Often, teachers will want you to come in and make a presentation when they are covering that topic in their curriculum. So don't be offended if they ask you to come next month or next semester when they are covering the human body and not geology. Also, appreciate how much effort the teachers have to make to get you into the classroom. Often, they have to have approval of their science department chair, principal or even district specialist. Finally, don't "blame" the teacher if the students are rowdy, or unable to perform the activity.

What will you do with the grant funding you received?

One thing I've noticed in almost all of the K-12 science classrooms I've been in is the lack of independent, inquiry-based learning on a day-by-day basis. Some of the science labs have excellent equipment (some don't, but that's another article), but for the most part, the equipment is locked up and students are only allowed to access it under supervision or when that particular piece of equipment is needed for a specific, curriculum-defined activity. There is almost no spontaneity or



APS member David Holtzclaw at a PhUn Week event with students from Omaha Public School system.

curiosity, which is often when good science occurs. Students rarely get the chance to take lab equipment off the shelf, let alone home to tinker with. I wanted to provide K-12 classrooms with equipment that students could use to engage in inquiry-based learning. Something the students could "check out," take home, and "play with" to learn on their own. Unfortunately, $2,000 doesn't go too far in terms of lab equipment. So I decided on buying about 200 "nice" pedometers for three different schools. Pedometers are cheap equipment, easy to use, durable, and provide a fair amount of data (steps taken, calories burned, BMI, distance walked, even elevation changes). Students can use pedometers for numerous experiments and activities covering topics such as physiology, nutrition, ATP, molecular biology, math, even engineering and surveying! So, I will spend two to three classroom periods at each school distributing the pedometers and having the students come up with their own experiments that will address a hypothesis they develop using the pedometers. The schools will get to keep the pedometers and the students will hopefully continue to use them for future activities.

Why do you invest your time to do this voluntary outreach to your local school?

I really don't understand this question. The question to me is "why do we NOT invest our time in our local schools?" In a time of decreased local and state funding of primary education, teachers being under fire for everything from poor test scores to state budget pitfalls, fewer and fewer students going into STEM fields, increased reliance on technology in the workplace, aging population, and low science literacy among the general U.S population, are you REALLY asking me why I volunteer in K-12 classrooms?

The reason more physiologists don't get involved in K-12 outreach activities is that they simply don't think these activities (and many other legitimate service opportunities) will help them get published or funded. This is simply not true. There are now at least 20 peer-reviewed journals (*Advances* being one of them) that will accept manuscripts focusing on K-12 outreach activities (see http://homepages.wmich.edu/~rudged/journals.html for a partial list of these journals). Numerous private organizations, state, and federal agencies (yes, including NIH) now provides programmatic grant opportunities for scientific outreach endeavors including the NIH **S**cience **E**ducation **P**artnership **A**ward (SEPA) Program (http://www.ncrrsepa.org/).

If that isn't good enough, let me put it another way that might offend some of you, but at least think about it: what will be a bigger impact to science; your 9th paper on ion channel X response to drug/condition Y that only 12 people will read or turning on a child to science? Yes, that kid may grow up to be an insurance agent, but an insurance agent, who may not understand physiology, but knows physiology is important and will vote against the state ballot initiative to cut funding for higher education or limit the use of animals for medical research. Since 2007, I've talked to about 3,000 K-12 students. Assuming I reached only 1% (30 kids), then that's probably 30 kids that will become your graduate students and postdocs.

It's fun, helps to fine tune your teaching and communication skills, kids love it, teachers, parents, and schools love it, and, for at least a few minutes, it'll make you glad you went into science. What more do you want! ❖

APS Leads Teachers to New Physiology Frontiers in Online Program

The first Frontiers in Physiology Online Professional Development Fellows completed their fellowship year in October. Twenty-six middle and high school teachers from across the nation began this online course in December and progressed through the online professional development lessons for nine months. This variation of the original Frontiers in Physiology Program was made available with a supplement to APS' NCRR Science Education Partnership Award (SEPA) grant. Teachers participated in reading, sharing of resources, experimental design, poster sessions, discussion boards, lesson development, peer reviews, production of Bench-to-Bedside Primers, and pre- and post- fellowship content surveys and physiology tests. Overall teachers from 13 states completed this rigorous professional development course, learning not only about physiology but about the best ways to help their students learn science via the scientific method.

The teachers completing the program include:

Katie Anderson
Dakota Middle School, Rapid City, SD
Myra Arnone
Redmond HS, Redmond, WA
Daniel Bartsch
Billings Senior HS, Billings, MT
Rebecca Block
Tulsa School of Arts and Sci., Tulsa, OK
Wanda Bryant
Detroit Public Schools, Detroit, MI
Regina Cowan
Detroit Public Schools, Detroit, MI
Greg Dierson
Great Plains Lutheran Sch., Watertown, SD
Mary Eldridge-Sandbo
Des-Lacs-Burlington HS, Des Lacs, ND
Myriaha Felker
Cabell Midland HS, Ona, WV
Charles Galarza
El Paso Indep. School District, El Paso, TX
Ellen Gant
Dakota Valley HS, North Souix City, SD
Denise Gipson
Jefferson HS, Shenandoah, WV
Mary Haus
Los Osos HS, Rancho, Cucamonga, CA
Kelly Hennessey
A.C. Davis HS, Yakima, WA
Cora James
Haskell HS, Haskell, OK
Nancy Keller
Heritage HS, Vancouver, WA
Tami Kepshire
Portage HS, Portage, IN
Carla McFadden
Okanogan School District, Okanogan, WA
Jannette Moehlman
Dakota MS, Rapid City, SD
Melanie Shaver
West McDowell Jr. HS, Marion, NC
Tara Veazey
West Virginia Univ. HSTA, Charleston, WV
Darrell Walker
Elizabeth City MS, Elizabeth City, NC
Pete Whipple
Central Valley School District,
Spokane Valley, WA
Debora Wines
Central Catholic HS, Billings, MT
William Wilson
Clover Park HS, Lakewood, WA
Daniel Zielaski
Phelps ACE SHS, DC Public,
Washington, DC

APS Promotes Physiology to Biology Educators at National Convention

The APS highlighted physiology for K-12 biology teachers with a featured speaker, workshop, exhibit booth, and a poster presentation at the National Association of Biology Teachers (NABT) 2011 Professional Development Conference in Anaheim, CA in October. The annual national conference attracts middle and high school teachers, as well as two- and four-year college faculty from across the nation.

APS Council member Patricia E. Molina, Louisiana State Univ. Health Sciences Center, New Orleans, LA was the APS sponsored speaker. Molina presented her work on new findings of how alcohol and drugs of abuse can impact the course of HIV disease progression. In a hands-on workshop led by Margaret Shain, Project Coordinator, Mel Limson, Education Office K-12 Programs, and a team of APS Research Teachers, including Stephen Biscotte (Roanoke, VA) and Peggy Deichstetter (West Chicago, IL), teachers explored kidney function while completing several of the modules in *Kidney Under Pressure,* an APS inquiry unit. Melinda Lowy, Higher Education Programs Coordinator, showcased APS Education Programs and teacher fellowships at the exhibit booth through-out the three-day conference.

During the K-12 Outreach Symposium of education programs across the nation, Limson and Shain promoted the APS K-12 Frontiers in Physiology with a poster, "If students are to EXPERIENCE science as a way of KNOWING, Teachers MUST experience science first." The theme of this year's symposium was "marketing your program." ❖

Margaret Shain, Education Office, discusses the Frontiers in Physiology program with a teacher during the poster session.

Teachers work through modules of the "Kidney Under Pressure" inquiry unit at the NABT Conference workshop.

APS Archive of Teaching Resources Thanks its 2011 Reviewers

The APS Archive of Teaching Resources would like to thank the following members for serving as reviewers for the 2011 Summer Review Cycle: Robert Augustyniak, Maureen Basha, Melissa Bates, Gregory Brower, Barbara Goodman, Erin Keen-Rhinehart, Jae-Woo Lee, Robin Looft-Wilson, Julie Rennison, Michael Ryan, Thomas Schmidt, and Dexter Speck.

Thanks to their efforts, fourteen education-related items were reviewed and accepted into the Archive. These items, along with over 4,900 other teaching and education resources, may be viewed for free at www.apsarchive.org:

Active Learning in a Large Classroom for Teaching Physiology: Acid-Base Cases *John Dietz*

Alveolar Gas (Computer Program for Mac or PC) *A.P. (Pete) Shepherd*

Anatomy 199 Basic Human Anatomy Course Syllabus *Jennifer Burgoon*

BI 206 Anatomy and Physiology I Course Syllabus with Lab Supplement *Jennifer Burgoon*

BI 207 Anatomy and Physiology II Course Syllabus with Lab Supplement *Jennifer Burgoon*

Glucose Tolerance Test for Endocrine Labs *Karen Sweazea*

Hypertension (Case Discussion) *Charles Preuss and John Dietz*

Research Compliance: An Overview (Powerpoint Presentation) *Sue Barman and Eric Deprie*

Teaching Physiology with the Marble Game *Peter Nelson*

Willie's Acid-Base Box (Teaching Method) *John Dietz*

Working Knowledge of Hormones: Function (Figure) *Thomas Pressley*

Working Knowledge of Hormones: Pathology (Figure) *Thomas Pressley*

Working Knowledge of Hormones: Regulation (Figure) *Thomas Pressley*

Working Knowledge of Hormones: Synthesis (Figure) *Thomas Pressley*

Do you have a great teaching tool or activity that you would like to share with your colleagues? Submit your item to the Archive by December 30th to be included in the Winter Review Cycle. Visit http://www.apsarchive.org or e-mail the Archive at archive@the-aps.org for more information. ❖

APS Undergraduate Summer Research Fellows Finish Research

The American Physiological Society's Undergraduate Summer Research Fellows (UGSRF) have completed their 10 weeks of research. They are working on writing abstracts to present their findings at Experimental Biology 2012 in San Diego, CA, April 21-25.

Below is a list of UGSRFs, research hosts, and research projects on which the students will be reporting during EB.

The UGSRF program is sponsored by the APS Career Opportunities in Physiology Committee and funded by the APS Council. The program was established in 2000, making this the 11th year of the program.

These 24 fellowships are to support full-time undergraduate students to work in the laboratory of an established investigator. The intent of this program is to excite and encourage students to pursue a career as a basic or clinical research scientist. Faculty sponsors/advisors must be active members of the APS in good standing but do not have to be US residents.

These Fellowships provide a $4,000 summer stipend to the student (10 weeks of support), a $300 grant to the faculty sponsor/advisor, and up to $1,300 to the student so that he/she may attend and present their data at the Experimental Biology meeting the following year.

Applications for the 2012 class of UGSRFs are due February 1, 2012. ❖

Student	Research Host	Research Topic
Nicholas Clute-Reinig Pomona College, CA	Virginia L. Brooks Oregon Health Sci. Univ.	The Role of Neuropeptide Y in the Baroreflex Between the Arcuate and Paraventricular Nuclei of the Hypothalamus
Julia H. Crowley College of William & Mary, VA	M. Brennan Harris College of William & Mary, VA	Effects of SIRT1 knockdown on eNOS activity in endothelial cells and vascular rings
Jeff Engle Univ. of Iowa	Kevin Campbell Univ. of Iowa	Contraction-induced EC uncoupling in dystroglycan deficient mice
Robert Fidelibus Ohio State Univ.	Maqsood A. Chotani Research Inst., Nationwide Children's Hosp.	A Decoy Peptide Approach to Inhibit Mobilization of Vascular á2C-Adrenoceptors
Jennifer Frielle Gettysburg College, PA	Sean D. Stocker Penn State Univ.	Intra-Arcuate Injection of Anti-Insulin Affibody Blocks the Sympathoexcitatory Effect of Insulin
Michael F. Gowen Univ. of Pittsburgh, PA	Bill J. Yates Univ. of Pittsburgh, PA	The Rostral Ventrolateral Medulla's Responsibility for the Patterning of Regional Blood Flow
Maria Gulas Appalachian State Univ., NC	Scott R. Collier Appalachian State Univ. NC	The Effects of Play-based Physical Activity on Metabolic Signatures in Adolescents
Kaitlyn Kennard Ursinus College, PA	Beth A. Bailey Ursinus College, PA	Sarcomere Shortening in Pregnancy Induced Hypertrophy
Tiffany Kuo Univ. of California, Davis	Anne A. Knowlton Univ. of California, Davis	Determining if HSP60 is on the Surface of Exosome
Mali Lavi Tel Aviv Univ., Israel	Noga Kronfeld-Schor Tel Aviv Univ., Israel	Effects of Agomelatine on Seasonal Affective Disorder (SAD) of a Diurnal Rodent, the Fat Sand Rat (Psammomys obesu)
Anfei Li Cornell Univ., NY	Robin L. Davisson Cornell Univ., NY	The Effect of Angiotensin-II Type 1A Receptor Expression in the Central Nervous System on Angiotensin-II Mediated Hypertension
Erin E. McClure Juniata College, PA	Gregory L. Stahl Brigham & Women's Hosp., MA	Mannose-binding Lectin Levels Correlate With Enhanced Whole Blood Aggregation and Thrombin-like Activity in Humans
Edward J. McKenna Univ. of Wisconsin-Madison	William G. Schrage Univ. of Wisconsin-Madison	Cerebral Vascular Function in Obesity and Metabolic Syndrome
Stephanie M. Mutchler College of William & Mary, VA	Robin C. Looft-Wilson. College of William & Mary, VA	Methylation Patterns in the Vascular System Due to Exercise Training
Bonnie K. Patchen Williams College, MA	Steven J. Swoap Williams College, MA	The Role of mTOR Signaling in Skeletal Muscle Adaptations to Endurance Exercise

Student	Research Host	Research Topic
Irina Popovich Univ. of California, Merced	Rudy M. Ortiz Univ. of California, Merced	Improved Cardiac Insulin Sensitivity with AT1 Blockade in Insulin Resistant Rat Model
Darcy J. Porter Univ. of Kentucky	Francisco H. Andrade Univ. of Kentucky	Myosin Heavy Chain Isoforms in Rat Extraocular Muscle Fibers
Margaret Robotham Barnard College, NY	Rae Silver Barnard College, NY	The Effect of Time and Day and Mode of Administration on Methamphetamine-Induced Neural Activation in Mice
Louis A. Ruiz Univ. of Puerto Rico	Sabzali Javadov Univ. of Puerto Rico	SIRT3 Expression and Mitochondrial Permeability Transition Pore Opening Relationship in H9c2 Cells
Amy Shiah Univ. of California, San Diego	Michael C. Hogan Univ. of California, San Diego	Effect of Hypoxia on Contractility in Single Skeletal Muscle Fibers at Physiological Temperatures
Casey A. Toombs Univ. of New England, ME	Markus Frederich Univ. of New England, ME	Anoxia and Hypoxia Tolerance of the Two Color Morphs of Carcinus maenas
Asmae Toumi McGill Univ., Quebec, CA	Laurie J. Goodyear Harvard Medical School, MA	The Function of Skeletal Muscle SNARK (Sucrose nonfermenting AMPK Related Kinase) on Glucose homeostasis
Rebekah I. Webster Radford Univ., VA	Mark A. Cline Radford Univ., VA	Central Mechanisms of LPLRFamide's Anorectic Effect in Rat
Brian P. Wynia South Dakota State Univ.	Richard D. Minshall Univ. of Illinois, Chicago	Caveolin-1 Regulation of Mouse Lung Epithelial Progenitor Cell Differentiation and Morphogenesis

Plan Now to Attend APS Career Development Sessions at EB2012

Trainee Symposium: E-Media Tools for the Professional Scientist

(sponsored by the Trainee Advisory Committee)
Organizers: Jennifer Bomberger and Erica Dale-Nagle
Wednesday, April 25; 10:30 AM-12:30 PM

Using e-media to Find Funding Opportunities
Christopher Dant (Dartmouth Univ.)
E-medial Tools for Teaching
Aaron Bunker (Morningside College)
Social Media in the Life Sciences
Kristy Meyer (Sigma-Aldrich)
Ethical Implications of Social Media
Natalie Brown (Happy Place Marketing)

Mentoring Symposium: Conflict Resolution: How to Keep Everyone Happy!

Organizers: Roy L. Sutliff and Angela J. Grippo
Wednesday, April 25; 8:00-10:00 AM

Squashing Squabbles
Patrick O. Smith (Univ. of Mississippi Medical Center)
Disagree and Advance Your Career: Nonviolent Approaches for Dealing With Your Superior
Barbara A. Horwitz (Univ. of California, Davis)
Overcoming Laboratory Disagreements From a Trainee Perspective
Jennifer Sasser (Univ. of Florida)

Careers Symposium: Do I Need Another Degree?

Organizers: John D. Imig and Sonya D. Coaxum
Monday, April 23, 10:30 AM-12:30 PM

Benefits and Opportunities in Science for a JD Degree
Blair Taylor, (San Diego, CA)
Opportunities in Academia for Scientists With an MBA degree
Matthew Kluger, (George Mason Univ.)
Physiology and Public Health: What Can an MPH Degree Do?
Robert Carter III, (US Army)
Public Policy and Career Opportunities in Physiology
Sarah K. England (Univ. of Iowa)

The Physiologist
Vol. 54, No. 6, 2011

7th International Symposium on Aldosterone and the ENaC/Degenerin Family of Ion Channels: Molecular Mechanisms and Pathophysiology

Asilomar Conference Grounds, Pacific Grove, CA
September 18—22, 2011

The 2011 APS Conference—7th International Symposium on Aldosterone and the ENaC/Degenerin Family of Ion Channels: Molecular Mechanisms and Pathophysiology, was held at the rustic and picturesque setting of the Asilomar Conference Grounds that is located in Pacific Grove, CA. Attendees were not only treated to the spectacular views of the rugged coastline and the Pacific Ocean, but also to the various nature trails, natural wildlife and the beautiful weather during the five day event.

The Organizing Committee was chaired by Thomas Kleyman, Univ. of Pittsburgh and Co-Chair, David Pearce, Univ. of California, San Francisco. In addition, Marcelo Carattino, Univ. of Pittsburgh; Aniko Fejes-Toth, Dartmouth Medical School; Toshiro Fujita, Univ. of Tokyo, Japan; Catherine Fuller, Univ. of Alabama at Birmingham; John Funder, Prince Henry's Institute of Medical Research, Australia; Edith Hummler, Univ. of Lausanne, Switzerland; Olivier Staub, Univ. of Lausanne, Switzerland and Gordon Williams, Harvard Medical School made up the rest of the organizing committee and were instrumental in helping set up a successful conference program. 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.

Rudy Ortiz, Univ. of California, Merced discusses his abstract with Catherine Fuller, Univ. of Alabama at Birmingham during a poster session.

Conference attendees listening to a lecture during the conference.

The conference was attended by 149 total registrants: 31% of registrants were represented by trainees, including 24 postdoctoral and 22 students. Twenty-four (16%) attendees identified themselves as APS members, and 28 (19%) registered as nonmembers, invited chairs and speakers made up 51 (34%) attendees. Table 1 shows the

Table 1. Registration Statistics

Registrant Type	Number of Attendees (%)
APS Member	24 (16%)
Nonmember	28 (19%)
Postdoctoral	24 (16%)
Student	22 (15%)
Invited Chairs/Speaker	51 (34%)
Total	**149**

Abstract-based Travel Award Winners: (L-R) Catherine Fuller, APSCC Representative, Amanda Rickard, Daniel Collier, Conference Organizers Thomas Kleyman and David Pearce, Nourdine Faresse and Ankit Patel.

breakdown of the different registration types. This conference also attracted a large group of registrants from outside the United States. Of the 149 registrants: 68 (46%) represented countries from Argentina, Australia, Brazil, Canada, Chile, Denmark, France, Germany, Ireland, Israel, Italy, Japan, New Zealand, Spain, Sweden, Switzerland and the United Kingdom.

The conference program consisted of one key note lecture, two plenary lectures and eight symposia on a wide variety of topics related to ENaC and related transporters, mineralocorticoid and glucocorticoid receptors and aldosterone. The audience was encouraged to share their ideas and thoughts with the speakers at the end of their talks which often prompted a very animated discussion period. During the conference there were four oral presentation sessions which allowed a selected number of registrants the opportunity to present their abstract in an oral presentation format. In addition to the symposia and oral presentation sessions there were two well-attended poster sessions and a Career Workshop designed to engage and encourage students and postdoctoral fellows in writing and data skills. 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 delicious desserts and beverages while enjoying the beautiful weather and scenery. Furthermore, the conference schedule allowed for some free time during the afternoon so that registrants could get together and explore the surrounding area.

A total of 123 abstracts were submitted for the conference. Of these, 59 abstracts were programmed as poster presentations. Another 21 abstracts were selected for an oral presentation. The remaining 43 abstracts submitted for the conference were by invited speakers. Of the abstracts submitted for the conference, 41 (29%) were submitted by a female first author; 69 (56%) were submitted from institutions outside of the United States, including a total of 47 abstracts from Europe, 11 abstracts from Japan, five abstracts from Australia and New Zealand, five from the South American countries of Argentina, Brazil and Chile, as well one abstract respectively from Canada, Israel and Mexico,

On the last evening of the conference Kleyman and Pearce presented travel awards to two postdoctoral fellows and two 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: Daniel Collier, Univ. of Iowa, Nourdine Faresse; Univ. of Lausanne, Switzerland; Ankit Patel, Weill Medical College of Cornell Univ. and Amanda Rickard, Prince Henry's Institute of Medical Research, Melbourne, Australia.

In addition the conference organizers provided an honorable mention to the runner ups in the abstract travel award competition. The following individuals were presented with a certificate: Yehoshua Enuka, Ariel Univ. Center, Israel; Daria Ilatovskya, Medical College of Wisconsin; Caroline Ronzaud, Univ. of Lausanne, Switzerland; and Shuje Shi, Univ. of Pittsburgh.

The American Physiological Society and the Organizing Committee gratefully acknowledge the financial support provided though generous educational grants from Amgen, NIH, National Institutes of Diabetes and Digestive and Kidney Diseases (DK93327-01), Daiichi-Sankyo Company, Ltd., Dainippon Sumitomo Pharmaceuticals Company, Ltd., Otsuka and Kent Scientific, Inc. ❖

Travel Award Runner-ups: (L-R) Catherine Fuller, APSCC Representative, David Pearce, Conference Organizer, Shuje Shi, Daria Ilatovskya, Caroline Ronzaud, Thomas Kleyman, Conference Organizer and Yehoshua Enuka.

Physiology of Cardiovascular Disease: Gender Disparities

Univ. of Mississippi Medical Center, Jackson, MS
October 12-14, 2011

The 2011 APS Conference on Physiology of Cardiovascular Disease: Gender Disparities was held in Jackson, MS. The conference took place over two days at the Univ. of Mississippi Medical Center. The university campus offered an excellent conference facility in a collegiate atmosphere. The Organizing Committee included Chair, Jane Reckelhoff, Vice-Chair, Michael Ryan, Barbara Alexander and Christine Maric-Bilkan all from the Univ. of Mississippi Medical Center, as well as C. Noel Bairey Merz, Cedars-Sinai Medical Center and Meir Steiner, McMaster Univ., Canada. The committee organized a program that included symposia, a plenary lecture, oral presentations for students and postdoctoral fellows, interactive poster sessions, a career session and social networking opportunities that made the conference a valuable experience for those who attended.

The conference was attended by 127 total registrants, of whom 29% of registrants were represented by young scientists, including 18 postdoctoral and 19 students. Forty-five (35%) attendees identified themselves as APS members, and 16 (13%) registered as nonmembers, invited chairs and speakers made up the remaining 29 (23%) 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 127 registrants, 11 (9%) represented countries from Argentina, Australia, Brazil and Canada.

The conference program consisted of one plenary lecture and eight 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 seven oral presentation sessions that were dedicated to the postdoctoral fellows and students attending the conference. During the conference, Jennifer Sasser chaired a workshop on Career in Physiology that gave young scientists the opportunity to gain information and valuable career skills. 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 hors d'oeuvres and beverages, as well as listening to Jacksonian style music. There were two afternoon poster sessions where scientists presented their work and discussed their findings with other attendees. In addition, the conference program included two dinner events where conference awards were presented. Conference attendees were also entertained by a special guest who joined them for dinner on the last night of the conference.

A total of 81 abstracts were submit-

Conference attendees enjoying the Opening Reception at the Historic King Edward Hotel.

Table 1. Registration Statistics

Registrant Type	Number of Attendees (%)
APS Member	45 (35%)
Nonmember	16 (13%)
Postdoctoral	18 (14%)
Student	19 (15%)
Invited Chairs/Speaker	29 (23%)
Total	**127**

APS President, Joey Granger, Conference Organizer, Jane Reckelhoff and APS Executive Director, Martin Frank pose with special guest, "Elvis Pressley," during the closing dinner event at the Fairview Inn.

APS President, Joey Granger, Conference Organizer, Jane Reckelhoff and APS Executive Director, Martin Frank present awards to the winners of the APS Abstract-based Travel Award.

ted for the conference. Fifty-seven of these abstracts were programmed as poster presentations. The remaining 24 abstracts were submitted by invited speakers. Of the abstracts submitted for the conference, 58 (72%) were submitted by a female first author; 10 (12%) were submitted from institutions outside of the United States, including four abstracts from Canada, three from Argentina, two from Brazil and one from Australia.

On Friday evening, Reckelhoff hosted the Banquet and Awards Presentation Dinner. Attendees gathered at the historic Fairview Inn located in downtown Jackson for dinner, wine, and conversation. During the event, four 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: Linda Gallo, Univ. of Melbourne, Australia, Jennifer Stuart, Harvard School of Public Health, Gina Yosten, St. Louis Univ. and Margaret Zimmerman, Georgia Health Sciences University.

APS President, Joey Granger, Conference Organizer, Jane Reckelhoff and APS Executive Director, Martin Frank present the NIDDK Award Winners Krystal Brinson, Mark Cunningham and Ashlee Tipton with certificates.

In addition Krystal Brinson, Georgia Health Sciences Univ.; Mark Cunningham, Univ. of Florida, Gainesville; Victoria McIntosh, Wayne State Univ.; and, Ashlee Tipton, Georgia Health Sciences 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: Christine Maric-Bilkan, Univ. of Mississippi Medical Center; David Harrison, Vanderbilt Univ.; Rita Tostes, Univ. of Sao Paulo, Brazil and Norma Ojeda-Gammara, Univ. of Mississippi Medical Center 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 the Women's Health Research Center at the Univ. of Mississippi Medical Center, Faculty Scholarship Exchange Program at the Univ. of Mississippi Medical Center, Council for High Blood Pressure, American Heart Association, Isis Cardiovascular Network, Society for Women's Health Research and NIH, National Institutes of Diabetes and Digestive and Kidney Diseases. ❖

**Conference photos courtesy of Dr. Barbara Alexander and Andy Chen of the Univ. of Mississippi Medical Center.*

New Regular Members

***transferred from student membership**

Khosrow Adeli
Hosp. for Sick Children, Toronto, Ontario
Robin Altman
Univ. of California, Davis
Frank A. Anania
Emory Univ. Sch. Med., GA
Cristina Arranz
Univ. De Buenos Aires, Argentina
Cenk Aydin
US Environ. Protection Agency, NC
Jasmohan Singh Bajaj
Virginia Commonwealth Univ.
Anthony J. Baker
Univ. of California, San Francisco
Frédéric Becq
Univ. of Poitiers, France
Vipa Bernhardt
Texas Health Presbyterian Hospital
Edward J. Catapane
Medgar Evers College, Brooklyn, NY
Xinnian Chen
Univ. of Connecticut, Storrs
Kristine Deleon
Univ. of Texas HSC, San Antonio
Ingrid Eftedal
Norwegian Univ. of Sci. Tech. Med.
Stefan Everling
Univ. of Western Ontario, Canada
Michael J. Falvo
VA NJ Health Care System, NJ
Ilana Sharon Fortgang
Tulane Univ. Sch. Med., LA
Erin Leigh Glynn*
Duke Univ., NC
John Richard Gray
Univ. of Saskatchewan, Canada
Emma Heart
Marine Biological Laboratory, MA
Julio Santos Hilario-Vargas*
National Univ. of Trujillo, Peru
Peter Andrew Hosick
Univ. of Mississippi Med. Ctr.
Ramon Imenez-Moreno
Wake Forest Univ., NC
Roozbeth Kiani*
Stanford Univ., CA
Jing Li
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Mikhail Yuryevich Lipin
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Dhavalkumar B. Patel
Washington Hosp. Ctr., Washington, DC
Vincent Pialoux
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Wilton Remigio
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Haifei Shi
Miami Univ., OH
Lawrence Snyder
Washington Univ., MO
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Lilly Research Laboratories, IN
Sharmila Venugopal
Univ. of California, Los Angeles
Matam Vijay-Kumar
Georgia State Univ.
Xiaonan Wang
Emory Univ., GA
Erika Boerman Westcott*
Univ. of Missouri, Columbia

New Undergraduate Member

Justin M. Anderson
Morningside College, IA

Recently Deceased Member

Louis-Paul Dugal
St. Nicholas, Canada

New Graduate Student Members

Murtala Bello-Abubakar
Univ. of Sains, Malaysia
Brandon H. Cherry
Univ. of North Texas HSC
Namjik Cho
Univ. of Florida
Gladys Chompre
Ponce Sch. Med., Puerto Rico
Brittany C. Collins
Univ. of South Carolina
Clark Cotton
Univ. of Wyoming
Troy Cross
Griffith Univ., Australia
Peter Cseplo
Univ. of Pecs, Med. Sch., Hungary
April Darrow
Univ. of Hawaii
Todd Dart
Texas A&M HSC
Jason Davis
Georgia Health Sci. Univ.
Michelle Eggen
Univ. of Nebraska Med. Ctr.
Brianne Ellis
Univ. of Mississippi Med. Ctr.
Juan Estrada
Univ. of North Texas HSC
Trent Evans
Univ. of Colorado
Katelynn E. Faulk
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Kelsey Fisher-Wellman
East Carolina Univ.
Gruangchao J. Fu
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Leticia Gonzalez
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David M. Gundermann
Univ. of Texas Med. Branch
Jenny Gustafsson
Univ. of Gothenburg, Sweden
Liberty Hamilton
Univ. of California, Berkeley
Mary Kristina Hamilton
Univ. of California, Davis
Michael Hendel
Cleveland Clinic Foundation, OH
Siomara Hernandez
Ponce Sch. Med. and Health Sci., Puerto Rico
Marie Hoffman
Univ. of Wisconsin, Madison
Arve Jorgensen
Norwegian Univ. Sci. & Tech.
Jeremy Thomas Keen
Kansas State Univ.
Zac Kohl
Univ. of North Texas HSC
Alisha Lacewell
Univ. of Oregon
Xiangdong Li
Kansas State Univ.

Ziyang Liu
Ball State Univ., IN
Tanner McNamara
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Ball State Univ., IN
Venkateshwar Mutyam
Univ. of Dayton, OH
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Univ. of Puerto Rico
Samelia Okpodu
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Traci Parry
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Sagi Perel
Univ. of Pittsburgh
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Inger Susanne Stallmann-Jorgensen
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Achini Kushanthi Vidanapathirana
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Angela Vinturache
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Christopher Wolff
Ball State Univ., IN
Qiong Wu
Univ. of North Texas HSC
Leah M. Wuescher
Univ. of Toledo College of Med.
Rosita Zakeri
Mayo Clinic, NY

Meetings & Conferences
of the American Physiological Society

Experimental Biology 2012
San Diego, California • April 21-25, 2012

2012 APS Conference:
Autonomic Regulation of Cardiovascular Function in Health and Disease
Omaha, Nebraska • July 7-10, 2012

2012 APS Intersociety Meeting:
The Integrative Biology of Exercise VI
Westminster, Colorado • October 10-13, 2012

The American Physiological Society, Meetings Department
Phone: 301.634.7967 • Fax: 301.634.7264 • E-mail: meetings@the-aps.org
11-8-11

APS Comments on the Future of the Biomedical Workforce

On August 17, 2011, a working group established by the Advisory Committee to the NIH director issued a request for information (RFI) on the future of the biomedical workforce. The goal of the working group is to gather information and develop a model of "a sustainable, diverse, and productive U.S. biomedical research workforce." The RFI posed a number of questions, available online at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-11-106.html. Excerpts of the APS response follow.

The continued success of the American biomedical research enterprise depends on developing a sustainable model for educating a well-trained and diverse workforce that is prepared to meet the country's need for individuals with scientific training. At the outset it must be emphasized that the workforce decisions made now will have very long-term consequences and must be considered with a long-term perspective on needs. Decisions must not be overly influenced by current scientific trends because history has shown that trends change significantly with time. We thus advocate a balanced approach that recognizes the continued importance of research at all levels from molecular to organismal. Below we offer our comments on some of the areas outlined in the RFI.

The balance between supply, including the number of domestic and foreign trained PhDs and post-docs, and demand, i.e. post-training career opportunities.

The APS recognizes the need to balance the number of trainees entering the education pipeline with the post-training career opportunities available. Absolutely fundamental to such a discussion will the long-term future financial health of the NIH. However, we also wish to caution that determining the optimal number is complex and imposing strict limitations could lead to unintended consequences, particularly in the long term, which could negatively affect our international competitiveness in biomedical research. One approach as outlined below, would be to change the nature of the PhD training process, which could provide added flexibility and will be more readily adaptable to shifts in demand for qualified biomedical researchers.

Consideration should also be given to ensuring balance between disciplines. Some fields of research may have too many students entering training programs for the available number of post-graduate career opportunities, while others have too few students entering the pipeline.

Characteristics of PhD training in biomedical research

Graduate training (at the pre- and post-doctoral levels) should be focused on trainees developing the skills and knowledge required to solve our nation's biomedical and health problems and become productive members of the biomedical workforce. Trainees should be provided with career development resources designed to match the needs of the marketplace.

Currently PhD training programs do an excellent job of preparing students for careers in academic science. But biomedicine needs a broader diversity of professionals to be successful, and PhD programs are not designed to prepare students for these careers. One way to achieve this goal would be to alter pre-doctoral graduate training such that the first two years provide a broad base of knowledge and skills, including program curriculum, research experience and career development. Career development should include the skills necessary to run a successful research program including business skills, project and lab management, as well as resources to prepare students for careers in fields beyond academic bench research. This two year training program may culminate in the award of a Master of Science degree, followed by entry into a PhD program. Given the prior two years of training we would anticipate that the PhD program would be more focused on research with reduced emphasis on course work.

Clearly, new mechanisms of support will need to be developed for the Master's degree portion of training, including scholarships, loans and teaching assistantships; such funding mechanisms will be especially important to ensure that under served minority groups continue to enter the biomedical research work force. Once a student moves to the PhD program we would anticipate that federal support would be available from training grants and other similar mechanisms.

We anticipate that this approach will provide graduate students an additional set of basic skills to expand their career opportunities, as well as result in a more flexible pipeline for biomedical research by effectively increasing the scientific workforce while at the same time providing for increased career opportunities.

Characteristics of clinician-research training

Physician scientists have a critical role to play in advancing basic, translational and clinical research. In particular physician scientists play a unique role in facilitating the understanding of the clinical relevance of basic research as well as making major contributions to both basic and translational research. Physician scientists should be encouraged to enter and remain in the biomedical research workforce, and consideration should be given to the specific impediments which have caused a decrease in this segment of the investigator pool. We encourage the working group to thoroughly survey the factors that contribute to physician scientists' success in developing a career in research, and conversely those factors that cause them to leave the research workforce.

Issues related to the attractiveness of biomedical research careers (e.g. salary, working conditions, availability of research funding)

Maintaining and increasing the attractiveness of research careers should be a central goal of any efforts to reform the current training model. Limited research funding, reduced success rates, fewer available tenure-track academic jobs, increased length of training, and requirements to support a large percentage of their salary from grants have all combined to reduce the attractiveness of research careers. In the long-term this will discourage the most qualified students from pursuing research careers. Therefore, we strongly urge the NIH to examine the potential impact of any proposed changes in support for trainees to try to minimize any long-term negative impact on our international competitiveness in biomedical research.

The effect of changes in NIH policies on investigators, grantee institutions and the broader research enterprise.

As noted earlier, changes to the current training model will have far-reaching impacts on the research enterprise. Therefore prior to implementation of any changes, we strongly urge the NIH to model and consider the immediate and long-term impacts of any changes as well as obtain input from all groups that will be affected by these changes, including investigators, the institutional community and industrial partners.

Full text of the APS response is available online at http://www.the-aps.org/pa/policy/nih/APSResponseNOT-OD-11-106.pdf.

Science Policy Committee Members Advocate for Research Funding

On September 20th, 2011, 12 members of the APS Science Policy Committee (SPC) went to Capitol Hill as part of the annual committee meeting in the Washington, DC area. SPC members met with staff in the offices of nine Members of Congress, including two members of the Joint Committee on Deficit Reduction. APS members emphasized the importance of maintaining federal funding for research, citing the health and economic benefits that result from investing in research. Advocacy for federally funded research is particularly important in the face of current economic challenges. Congressional staff were generally supportive of funding at the National Institutes of Health, National Science Foundation, VA and NASA, but also cited the efforts to rein in federal spending as an impediment to budget increases. APS and the SPC will continue efforts to encourage support for research funding. ❖

Members of the Science Policy Committee meet with Representative Gregg Harper (R-MS). From left to right: Carrie Northcott, Michael Brands, Representative Harper, Robert Hester and John Chatham.

Make Plans Now to Attend the Refresher Course at EB2012

2012 Refresher Course on Endocrinology

Sponsored by APS Education Committee

Organizers: Clintoria Williams, and Michael Ryan

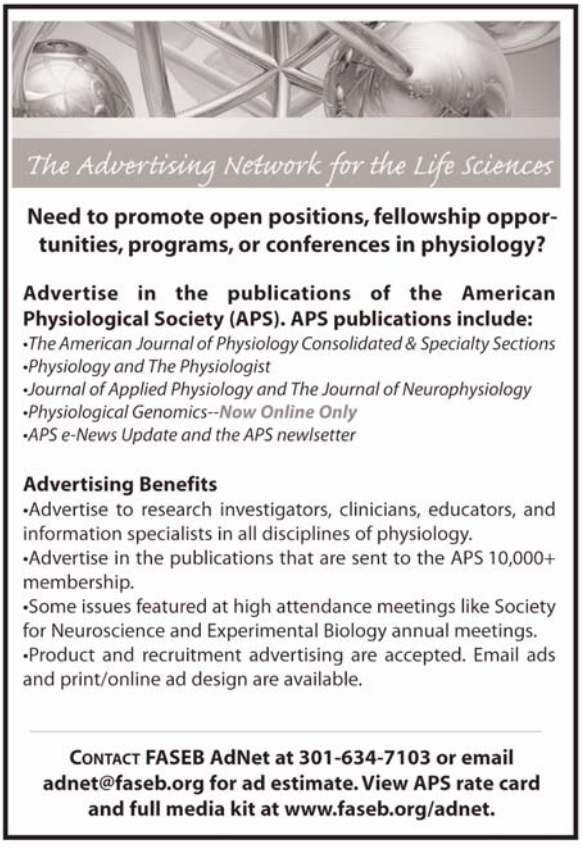
Saturday, April 21; 8:00 AM-12:00 PM

Brain-Gut Interactions
Shanthi Srinivasan, (Emory Univ.)

Adipocyte-Islet Interactions
TBD

Islet-Brain Interactions
Stephan C. Woods, (Univ. of Cincinnati)

Mechanisms of Current Drug Therapies in Diabetes
Peter M. Thule, (Emory Univ./Atlanta VA Medical Center)



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Summer Research Programs

Roy L. Sutliff
Emory Univ./Atlanta VA Medical Center

Students interested in pursuing an MS or PhD in biomedical sciences, such as physiology, need to get as much research experience as they can during their undergraduate career. This research experience will not only help students clarify whether they want to pursue a career in research, but also will help them to be more marketable in today's competitive environment for graduate school. Undergraduate research experience also can help students develop a passion for research; it conveys awareness that the student knows what they are getting into—characteristics that are highly sought by admissions committees. Often undergraduate universities do not have the necessary infrastructure to provide the necessary research experience. Fortunately, there are numerous universities and organizations (professional societies, non-profit organizations, government agencies, etc.) with excellent biomedical research programs that offer summer programs that can provide this crucial research experience.

Roy L. Sutliff

What Is a Summer Research Program?

Summer research programs provide opportunities for undergraduate students to work in a research laboratory for eight to ten weeks. The programs typically enable the student to work closely with faculty members and other researchers on a project that is of interest to the undergraduate student. Students often have the opportunity to participate in departmental and institutional seminar series and symposia. Students are given stipends and in many cases, assistance with housing and travel to gain this valuable research experience. Applications are generally due between January 1 and March 15 for the summer programs. Students may be asked to provide their GPA, an essay describing their research experience/ interests, and one or more letters of recommendation to apply to the programs. Each program varies as to what it offers and requires so it is imperative to review programs carefully.

How Do I Find Out About the Programs?

There are a number of ways that interested students can find out about summer research programs. First, students should consult with professors that teach undergraduate courses that are related to their research interests. These professors often have ties to the summer programs through previous students or collaborations.

Second, interested students should watch for flyers or email notices that are sent out by the schools or organizations offering summer research programs.

Finally, the internet is an excellent source of information concerning summer research opportunities. Major sponsors of the summer research experiences include the National Science Foundation, which provides support for summer programs through their Research Experiences for Undergraduates program, and the Howard Hughes Medical Institute, which supports numerous programs throughout the country. Opportunities can be found by searching at the NSF website (http://www.nsf.gov/crssprgm/reu/reu_search.cfm) and the Howard Hughes Medical Institute website (http://www.hhmi.org/grants/reports/scienceopp/main).

There are also programs that are sponsored by individual societies, such as the American Physiological Society (http://www.the-aps.org/education/ugsrf/index.htm), which is one program that is open to undergraduate students worldwide. These societies are often constituents of the Federation of American Societies for Experimental Biology (FASEB) and may have programs that target students interested in a particular field of research. A listing of these constituent societies can be found at http://www.faseb.org/Who-We-Are/Constituent-Societies.aspx.

Another website that allows students to search for summer research opportunities by both research interest and geography can be found at http://www.pathwaystoscience.org/programs.asp?descriptorhub=SummerResearch_Summer%20Research%20Opportunity. Students can also identify fellowships by setting up automated search agents. Students can join websites, such as the Community of Science (http://www.cos.org/), and customize funding alerts or search directly under COS Funding Opportunities. Alternatively, students can set up Google alerts for keywords such as "summer research" or "undergraduate fellowship."

In addition, APS has a list of national and international summer research

Roy L. Sutliff, is Associate Professor of Medicine in the Division of Pulmonary, Allergy and Critical Care Medicine at Emory Univ. School of Medicine. He received his BSin biochemistry from Temple University, and PhD in pharmacology from Allegheny Univ. He completed postdoctoral work at the Univ. of Cincinnati, and then moved to Emory in 2001. He has served as the director of recruiting for the MSP program at Emory and is active in Emory Graduate School Recruiting. Research in his laboratory focuses on examining the interaction between endothelial cells and vascular smooth muscle cells and how certain pathophysiological conditions impair vascular function. The three areas that are most heavily studied are 1) the mechanisms of pulmonary vascular disease and accelerated atherosclerosis in HIV infection; 2) the role of PPARγ and its subsequent effects on NADPH oxidases in regulating endothelial function in pulmonary hypertension and diabetes and 3) the cardiovascular effects of alcohol. Dr. Sutliff is a member of the APS Women in Physiology Committee.

programs on the website (http://www.the-aps.org/education/ugsrf/SumResLINKs.htm).

Students are encouraged to apply to as many programs as feasible to increase their odds of being accepted into a summer research program.

How Can I Get the Most Out of My Summer Research Experience?

As important as it is to get into the program, it is even more crucial that students use the time in the laboratory wisely. Students who come into the program having done some research on the area that they will be working in generally have the most productive experiences. Students should work with their research host to identify the research question on which they want to work, locate background research articles to read, and discuss the project before the research experience begins.

In addition, there will likely be a number of regulatory processes that must be completed before beginning work in the laboratory, such as trainings in lab safety and working with animals. These trainings are often given online and having them completed before the start date can facilitate beginning work in the lab. Eight to ten weeks will go very fast, and the more that can be done to get into the laboratory as quickly as possible will enhance the research experience.

Once in the laboratory, it is important that the student understand all aspects of their project. Students planning to go on to graduate school will likely be asked about the research experience. They can expect to be asked questions about what they did, why they did it, and how it advanced the field. Students should discuss these questions with members of the laboratory and may want to take notes so that the answers are familiar when they are interviewed for graduate school months later.

Students should also use this opportunity to convey excitement about a research career to their research mentor. A strong letter of recommendation from the PI of the laboratory positively influences the review of a graduate program application.

Often, summer programs require the preparation of a poster or presentation at the end of the summer. Students should discuss the possibility of using this poster to create an abstract for presentation at a national scientific meeting. The summer program or principal investigator may be able to subsidize travel expenses for the presentation. Again, presenting a poster at any meeting requires students to be able to articulate the research question, present the results and discuss how those results fit into the big picture and possible next steps.

Additionally, students should discuss the possibility of authorship on a manuscript with the principal investigator soon after arriving in the lab. Manuscript authorship is highly valued by graduate admission committees and is an excellent addition to any application.

Summary

A summer research experience is an excellent opportunity for undergraduate students to gain valuable research experience. With a little bit of planning, students can become familiar with the research area in which they are interested and begin to make connections with experts in the field. Time spent discussing goals for the project can yield peer-reviewed abstracts and publications.

As a result, an application to graduate school can be greatly improved by a summer research experience, and students can have more options to consider as they embark on graduate training.

To comment on this article, go to: http://www.the-aps.org/careers/careers1/mentor/summer-research-programs.htm.

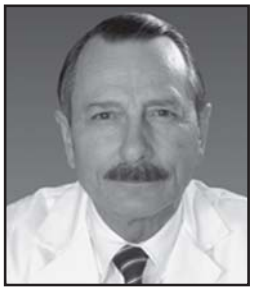
Acknowledgments

Special thanks to Dr. Catherine Quiñones, Associate Director of the Center for Science Education at Emory University, for her advice and assistance with this article. ❖

People & Places

Brinster and Chien Receive National Medal of Science

President Obama named seven eminent researchers as recipients of the National Medal of Science, the highest honors bestowed by the United States government on scientists, engineers, and inventors. The recipients received their awards at a White House ceremony in October.



Ralph L. Brinster

"Each of these extraordinary scientists, engineers, and inventors is guided by a passion for innovation, a fearlessness even as they explore the very frontiers of human knowledge, and a desire to make the world a better place," President Obama said. "Their ingenuity inspires us all to reach higher and try harder, no matter how difficult the challenges we face."

Shu Chien

The National Medal of Science was created by statute in 1959 and is administered for the White House by the National Science Foundation. Awarded annually, the Medal recognizes individuals who have made outstanding contributions to science and engineering. Nominees are selected by a committee of Presidential appointees based on their extraordinary knowledge in and contributions to chemistry, engineering, computing, mathematics, and the biological, behavioral/social, and physical sciences.

APS Members receiving this year's awards include: Ralph L. Brinster, Univ. of Pennsylvania: "*For his fundamental contributions to the development and use of transgenic mice. His research has provided experimental foundations and inspiration for progress in germline genetic modification in a range of species, which has*

generated a revolution in biology, medicine, and agriculture"; and Shu Chien, Univ. of California, San Diego: "*For pioneering work in cardiovascular physiology and bioengineering, which has had tremendous impact in the fields of microcirculation, blood rheology and mechanotransduction in human health and disease.*"

Hiroko Nishimura Receives Irvine Page & Alva Bradley Lifetime Achievement Award

APS member Hiroko Nishimura has been selected to receive The Irvine Page & Alva Bradley Lifetime Achievement Award. The Award is presented each year to an individual who has had a lifetime of outstanding achievements in the field of hypertension and has served as a role model through service, research and teaching. Nishimura has made many original contributions to the understanding of the neurohumoral mechanisms regulating blood pressure and kidney function, and the pathophysiology of diseases such as hypertension and atherosclerosis.

Nishimura is a Professor at Niigata Univ. Graduate School of Medical and Dental Sciences, Niigata, Japan. She is also Professor Emeritus of the Department of Physiology at the Univ. of Tennessee Health Science Center.

The Lifetime Achievement Award is named in honor of Dr. Irvine Page and Alva Bradley, who played a prominent role in establishing the National Foundation for High Blood Pressure Research in 1945. The foundation became the Council for High Blood Pressure Research of the American Heart Association in 1949.

Greg Fink, left, and Rhian Touyz, right, present Hiroko Nishimura, center, the Irvine Page & Alva Bradley Lifetime Achievement Award.

APS Congratulates Garcia and Hökfelt on Their IOM Election

Two APS members were part of the class of 65 new members and five foreign associates elected to the Institute of Medicine (IOM). Joe G.N. Garcia, vice president for health affairs, vice chancellor for research, and Earl M. Bane Professor of Medicine, Pharmacology, and Bioengineering, at the Univ. of Illinois, Chicago was elected as a new member and Tomas Hökfelt, professor of histology and cell biology, department of neuroscience, Karolinska Institutet, Stockholm was elected as a foreign associate. 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.

Jennifer Melinda Bomberger is now Assistant Professor in the Department of Microbiology and Molecular Genetics at the Univ. of Pittsburgh School of Medicine, Pittsburgh, PA. Prior to this move, Bomberger was a Postdoctoral Fellow in the Department of Microbiology and Immunology at Dartmouth University, Hanover, NH.

Ryan Pelis is now an Assistant Professor of the Department of Pharmacology, Dalhousie Univ., Halifax, NS, Canada. Previously, Pelis worked in the Transplational Sciences Department of Novartis Pharmaceuticals Corp., East Hanover, NJ.

Sumanth D. Prabhu is currently a Professor/Director at the Univ. of Alabama, Birmingham, Division of Cardiovascular Disease. Prior to this position, Prabhu was an Associate Professor at the Univ. of Louisville, KY.

Dennis K. Stone has moved and is now Chief Scientific Officer at the Remeditix Ventures in Dallas, TX. Previously, Stone was a Professor in the Office for Technology Department, Univ. of Texas Southwestern Medical Center, Dallas, TX.

Itaru Yazawa is presently an Assistant Professor in the Department of Anatomy at Showa Univ. School of Medicine, Tokyo Japan. Prior to this position, Yazawa was at NINDS, National Institutes of Health, Department of Developmental Neurobiology, Section of Lab Neural Control, Bethesda, MD.

Teresa Zimmers is now an Associate Professor in the Department of Surgery and Cancer Biology, Thomas Jefferson Univ., Kimmel Cancer Center, Philadelphia, PA. Prior to this move, Zimmers was an Assistant Professor in the Department of Surgery, Univ. of Miami, Millers School of Medicine, Miami, FL. ❖

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Medical Neurobiology

Peggy Mason
New York, USA: Oxford Press, 2011,
655 pp.
ISBN 978-0-19-533997

How to educate contemporary medical students about the structure and function of the human nervous system is guaranteed to elicit fervent debate between basic scientists, clinicians and students. In her introductory textbook *Medical Neurobiology*, Professor Peggy Mason turned to conversations with medical students for, in her words, an epiphany on bridging the demands between time, relevance and depth of material. While it is generally recognized that the last two to three decades has seen an explosion in our understanding of neurobiology, not every new medical student has an undergraduate neurobiology background. To this end, Mason designed *Medical Neurobiology* not as a neurology reference text with overwhelming detail, but as a means to introduce the relevance of neurobiology to almost any specialization in medicine.

While Mason acknowledges numerous collaborators, this text is a single-author product. As a result the text benefits from consistency in organization of the material and Mason's relaxed writing style. The text is organized into seven major sections that are organized around functional systems rather than a topographical, caudal to rostral progression through the CNS. Illustrations are a combination of original and republished figures and generally complement the topic under discussion.

Mason begins her overview of the central nervous system (Section 1) with a clinical presentation of the devastating aftermath of a rare pontine lesion resulting in locked-in syndrome. Mason uses that example to provide the medical student with a patient's perspective of how neurobiology, ranging from homeostasis to sensory-motor functions to mentation, plays a critical role in normal and abnormal function.

Section 2 presents the fundamental principles of excitable cells in a traditional style that will be familiar to most lecturers, though there are numerous text-boxes presenting clinically-relevant examples not commonly found in introductory texts. Mason frequently employs these text-boxes throughout the book in order to highlight clinical examples. In doing so, Mason organizes brief clinical vignettes and important "take-home" messages in a way that is easily accessible while continuously reinforcing the interrelationship between neurobiology and the practice of medicine.

In section 3 Mason's text begins to diverge from traditional formats. Specifically, discussion of the spinal cord begins with a presentation of dorsal columns, spinothalamic and corticospinal tracts. It is only afterward that more fundamental spinal neuroanatomy is introduced. Understandably, these major pathways are of considerable diagnostic value to clinicians and they do merit detailed presentation. The remaining spinal tracts are not introduced until later chapters in the sensory or motor sections on an "as needed" basis. While this style could prove vexing to the lecturer assigning readings for the typical one or two lectures on the spinal cord, there is an underlying efficiency to this organization since the most essential spinal tracts are featured early, then reinforced often throughout the remainder of the text.

Having presented the spinal cord as a conduit between body and brain, the chapter focusing on cranial nerves receives attention next as an important window into the CNS. The chapter presents not only the administration and interpretation of a cranial nerve exam, but also emphasizes to students the patient vernacular that necessitates the exam. This continues the organizational theme which is centered upon diagnostic criteria or relevance. Afterward, the remainder of the section follows the neuraxis beginning with the medulla and culminating in the forebrain.

While components of sensory processing are introduced throughout Section 3, it is in Section 4 that the sensory systems deemed most essential to humans and to clinical practice are organized. Following an overview of fundamental principles common to all sensory processing, the remaining chapters are largely devoted to vision, audition, somatosensation (Mason places considerable emphasis on somatosensory modalities that often compel individuals to seek medical attention) and the vestibular system. Mason admits to giving other sensations, such as olfaction and gustation, short shrift. It is slightly perplexing that, given the clinically-relevant examples Mason provides throughout the text, so little attention is paid to senses involved in feeding. Arguably, these senses play a part in patient over- and under-nutrition which routinely challenge physicians.

In Section 5 (Motor Control) Mason presents a concise overview demonstrating the interdependent roles of sensory, homeostatic and cognitive functions that shape the motor hierarchy. The remainder of Motor Control is organized from a bottom-up perspective in order to illustrate to students that increasing layers of complexity (and refinement) are necessary for smooth, purposeful movement. Again, Mason builds upon material introduced in earlier chapters to provide separate, detailed, discussions regarding the role of motor units as the building block of purposeful movement, spinal reflexes involved in gait, and on through to supraspinal motor control and modulation. While the cortical initiation of movement is included in an overview of the hierarchy, is not specifically addressed in the Section, having been introduced previously.

In must be noted that throughout the text, Mason often uses metaphorical examples and analogies to explain neurobiological concepts; sometimes to mixed effect. Specifically, there are times when a metaphor succinctly illustrates a concept in a real world perspective while others become overly lengthy. For example, the presentation of action selection by the basal ganglia cumulatively occupies over one full page of text, but does not particularly clarify this topic further.

Autonomic control of digestive processes is one clinically-relevant area that should receive greater attention in the text; if not as a unified chapter, then within Section 6 (Homeostatic Systems). Digestive complaints are common in an aging population and gastrointestinal motility disorders across all age ranges routinely elicit visits to primary care and specialist physicians. Understanding regarding the role of the brain-gut axis as a crucial element in digestive health has matured in recent years and many patients with gastrointestinal disorders are recognized to have impairments in CNS to gut function. It seems that CNS control of digestion and nutrient homeostasis would logically complement Mason's clinical oriented framework within the text.

Throughout the text, and particularly within the summary presented in

Section 7, Mason presents numerous clinical examples in a more personalized style. Some of these compelling examples are in the customary form of anonymous case presentations (central itch following a bout of herpes zoster), formerly anonymous cases (patient HM), while others (such as the examples of a locked-in Jean-Dominique Bauby and the profoundly amnestic Clive Wearing) provide intimate examples which contemporary students can explore further by virtue of extensive online documentation and video.

Overall, this text provides an interesting and comprehensive survey of neurobiology as it pertains to medical practice and does so without hopelessly overwhelming the majority of students yet still challenge the future neurologist. Course instructors could, and should, supplement the text with additional details. Basic scientists may well appreciate the wealth of clinical examples that support the finer details of important functional lectures. By the end of the typical 50-60 hour medical neurobiology course, Mason's text succeeds in providing an answer to that age-old question asked by medical students on the first day of class "…is this relevant?"

Yes, it is. As Mason demonstrates, it is frequently neurobiology that brings patients to the clinician. ❖

Gregory M. Holmes
Penn State Univ.

Book Received

Endothelin in Renal Physiology and Disease
Edited by M. Barton and D. E. Kohan
Karger Publishers, 2011, 226 pp, 35 fig., 9 in color, 3 tab, hard cover, $268.00.
ISBN: 978-3-8055-9794-4

Positions Available

Faculty Positions

Assistant Professor in Develop-mental Neurobiology: The Department of Biology at the Univ. of Vermont is seeking applications for a tenure track Assistant Professor position in Developmental Neurobiology, beginning in the fall of 2012. The successful candidate will have training in cellular, molecular, and developmental neurobiology. Candidates applying functional genomics approaches to questions in neural development are especially encouraged to apply. All applicants are expected to: 1) hold a PhD degree and have two or more years of postdoctoral experience; 2) develop a competitively funded program; 3) teach undergraduate courses from among general biology, physiology as well as in an area of the candidate's expertise; and 4) teach, mentor and advise undergraduate and graduate students. Candidates must apply online at http://www.uvmjobs.com. Search for the position using department name (Biology) only. Include a curriculum vitae, two representative publications, a statement of research focus, a statement of teaching interests, and the contact information of three (3) references. The reference providers will be emailed information to upload their letters. Review of applications will begin on September 15, 2011 and will continue until the position is filled. Questions may be directed to: Rona.Delay@uvm.edu. The Univ. of Vermont recently identified three "Spires of Excellence" in which it will strategically focus institutional investments and growth over the next several years (see: http://www.uvm.edu/~tri/). One of these spires is Neuroscience, Behavior and Health; candidates whose research, scholarship, and/or creative work interests align or intersect with this spire are especially encouraged to apply. The Univ. is especially interested in candidates who can contribute to the diversity and excellence of the academic community through their research, teaching, and/or service. Applicants are requested to include in their cover letter information about how they will further this goal. The Univ. of Vermont is an Affirmative Action/Equal Opportunity employer. The Depart-ment is committed to increasing faculty diversity and welcomes applications from women and underrepresented ethnic, racial and cultural groups and from people with disabilities.

Assistant Professor: Applications are sought for a tenure-track position at the rank of assistant professor, who will focus on neurophysiological mechanisms underlying obesity and/or regulation of physical activity, energy balance and/or metabolism. The successful candidate will be expected to participate actively in a multidisciplinary Univ. of Iowa Obesity Initiative (OI), encompassing research and education. New faculty will complement the University's existing expertise to form the core of this ambitious initiative. Participation in the OI will be an important component in performance evaluations. Outstanding research space with state-of-the- art shared instrumentation is available. Successful applicants must show clear potential to secure external research funding and be interested in teaching in a growing undergraduate major. All applicants must have a relevant doctoral degree and productive post-doctoral research experience. Faculty in the Department of Health and Human Physiology collaborate with scientists throughout the Univ. including those in the College of Engineering and in the adjacent Carver College of Medicine. Start-up support will be highly competitive with other institutions and ample laboratory space is available. The Department and the College of Liberal Arts and Sciences are strongly committed to gender and ethnic diversity; the strategic plans of the Univ. and College reflect this commitment. Women and minorities are especially encouraged to apply. The Univ. of Iowa is a large public university in a friendly, culturally diverse community. The Univ. of Iowa is an affirmative action/equal opportunity employer. Please apply online at: http://jobs.uiowa.edu/faculty/view/5998. Please contact Ms. Joyce Murphy (joyce-murphy@uiowa.edu) for additional information.

Assistant Professor, Department of Biology: The Department of Biology at Lafayette College invites applications for a tenure-track position at the Assistant

Professor level beginning August 2012. We seek a broadly trained physiologist committed to combining teaching and research at a small liberal arts college. Teaching responsibilities include participation in the lecture portion of General Biology, a one-semester course in physiology with lab, an advanced course in the applicant's area of interest, and contribution to the Common Course of Study. **Qualifications:** Applicants should have a PhD, teaching experience, a strong commitment to undergraduate education and a willingness to contribute to an integrative Biology curriculum. Postdoctoral experience is desirable. Successful candidates will be expected to establish an independent, active research program that will provide opportunities for undergraduate participation. The department is especially interested in applicants with broad and diverse experiences, training, and scholarly interests who can contribute to enriching diversity in the curriculum and enhance the vibrant learning community at Lafayette College. All application materials (curriculum vitae, a statement of teaching philosophy that includes interest and/or experience in teaching a diverse student body, a statement of research interests readable by non-specialists, and three letters of recommendation) should be submitted online at the job opportunities link at http://biology.lafayette.edu. Applications will be reviewed beginning October 17, 2011 and will be accepted until the position is filled. Questions regarding this position should be addressed to the search chair, Laurie Caslake, at caslakel@lafayette.edu or by calling 610-330-5462. Lafayette College is committed to creating a diverse community: one that is inclusive and responsive, and is supportive of each and all of its faculty, students, and staff. All members of the College community share a responsibility for creating, maintaining, and developing a learning environment in which difference is valued, equity is sought, and inclusiveness is practiced. Lafayette College is an equal opportunity employer and encourages applications from women and minorities.

Assistant Professor: The Division of Cardiovascular Diseases (http://www2.kumc.edu/internalmedicine/cv/) and the Cardiovascular Research Institute (CVRI) at the Univ. of Kansas Medical Center, are seeking candidates for a tenure track or research track faculty position at the Assistant Professor level. The successful candidate will have training in cardiovascular experimentation and research emphasis preferably in stem cell biology, cardiac repair, and related areas. The candidate will be expected to secure extramural funding and lead research efforts in cardiac repair with stem cells. Candidates with PhD, MD, or MD/PhD degrees will be considered. The candidate will likely spend 70-90% of his/her time to research activities with variable commitments to teaching and service activities. The recruitment package will be commensurate with qualification and experience. Applications should include a curriculum vitae, and an outline of research and funding goals. Interested candidates should apply online at http://jobs.kumc.edu (search for position J0085053). The University of Kansas Medical Center is an Equal Opportunity and Affirmative Action Employer.

Assistant/Associate Professor of Inflammatory Diseases: The Department of Biomedical Sciences & Pathobiology in the Virginia-Maryland Regional College of Veterinary Medicine has an opening for a faculty position in Inflammatory Diseases available. This tenure-track position may be filled at the Assistant or Associate Professor Rank, depending on the experience and qualifications of the successful candidate. The primary responsibility will be to establish and maintain a high-quality research program, with secondary responsibilities for teaching graduate-level courses, and possibly in the DVM program. Candidates with a documented record of research accomplishments (publications and extramural grant support) will be preferred. Candidates with experience in inflammatory experimental models are especially encouraged to apply. Inflammatory models could include (but are not limited to): inflammation induced by infectious or non-infectious causes, aging, cardiovascular or joint diseases. We are seeking candidates with research interests that fit into one or more of our three main research focus areas of the college: 1) Inflammatory/Regulatory Disorders (e.g. Cancer); 2) Infectious Diseases, and 3) Regenerative Medicine (e.g., tissue repair). Work at the interface of these focus areas, as well as that with a translational medicine emphasis, is desired. Opportunities to develop independent and collaborative research programs exist both within the college and with other life sciences-oriented departments, colleges, and institutes on campus. Opportunities for instructional participation are available at the graduate level and can be tailored to the candidates' interests and expertise. Excellent opportunities also exist to mentor and train PhD students. This is a research-intensive nine-month tenure-track position and is part of active recruiting for multiple faculty positions that includes physiologists/stem cell biologist, immunologist, clinical pathologist in the college, all of which provide a vibrant environment to collaborate. Interested applicants must apply online at http://www.hr.vt.edu/employment/, job posting 0110865. Review of applicants will begin November 01, 2011. Website: http://www.vetmed.vt.edu/. Contact Dr. S. Ansar Ahmed by phone 540-231-4652 or by Email at ansrahmd@vt.edu for individuals with disabilities desiring accommodations in the application process or needing this material in alternate format. Required qualifications are PhD in an area of Biomedical Sciences (Molecular cell biology of cytokines, disease modeling, and translational medicine experience preferred). Candidates with a DVM are encouraged to apply.

Assistant Professor: Biology, full-time, tenure-track position. Physiologist. Teach human anatomy and physiology (including cadaver lab), introductory biology, and elective courses in area of specialization. Candidates are expected to establish and maintain an active research program with undergraduates. PhD in a biological science required with broad training in an area related to physiology desired. Application must include a statement of teaching philosophy and evidence of potential for conducting and directing undergraduate research. Dr. Joel B. Hagen, Chair, Department of Biology, Box 6931. Email: biology2@radford.edu. All new hires to Radford Univ. will be subject to E-Verify beginning June 1, 2011. E-Verify is administered by the US Department of Homeland Security, USCIS-Verification Division and the Social Security Administration and allows participat-

ing employers to electronically verify employment eligibility. Review of applications will being immediately, and continue until the position is filled. Radford University is an EO/AA employer committed to diversity.

Assistant Professor: The Human & Evolutionary Biology (HEB) section of the Department of Biological Sciences in the Dana and David Dornsife College of Letters, Arts and Sciences of the Univ. of Southern California invites applications for a tenure-track assistant professor of biological sciences in the area of endocrinology & metabolism for an anticipated start date of Fall 2012. We seek an individual with an interest in neural-metabolic interactions, metabolic diseases, and/or broadly related areas. Applicants should hold a doctoral degree, with demonstrated research experience and excellence. The successful candidate is expected to contribute to undergraduate and graduate teaching, and to develop an active externally funded research program. Further information about the HEB program and its faculty can be found at http://dornsife.usc.edu/bisc/heb/home. Applicants should submit a cover letter, curriculum vitae, research plan, and three letters of reference to elsie@usc.edu or, if electronic submission is impossible, to Elsie Reyes, Senior Administrator at Human & Evolutionary Biology Search Committee, Department of Biological Sciences, 3560 Watt Way, PED 107, Univ. of Southern California, Los Angeles, CA 90089-0652. In order to be considered for this position, applicants are also required to submit an electronic USC application; follow this job link or paste in a browser: https://jobs.usc.edu/applicants/Central?quickFind=61382. Consideration of complete applications will begin November 1, 2011. For more information, contact Casey Donovan (Donovan@usc.edu), search committee chair. USC strongly values diversity and is committed to equal opportunity in employment. Women and men, and members of all racial and ethnic groups, are encouraged to apply.

Tenure Track Faculty Position: The Univ. of Illinois at Chicago, College of Medicine, Center for Cardiovascular Research (CCVR) seeks outstanding faculty candidates with expertise in key areas of cardiovascular science, with an emphasis on, but not limited to cardiac mitochondrial biology, metabolism, and non-coding RNA. Consideration will be given to applicants at all ranks (Assistant, Associate, Full Professors). Successful candidates are expected to lead comprehensive and innovative research programs that focus on heart failure, diabetes, obesity, metabolic syndrome and hypertension. A commitment to excellence in teaching is also required. Minimum degree requirements are a PhD or MD degree with three or more years of postdoctoral training. The UIC College of Medicine is located within the Illinois Medical District on the near west side of Chicago, adjacent to a vibrant neighborhood of shops and restaurants and easy access within minutes to the lakefront, downtown, and the many cultural amenities of the nation's third largest city. Applicants will submit a letter of interest, stating a research plan, a curriculum vitae and names of at least three references. Please go to the following to apply: https://jobs.uic.edu/default.cfm?page=job&jobID=13483.[AA/EOE]

Faculty Appointment: Full-time, Department of Biological Sciences, Anatomy and Physiology (Tenure Track): Supervisor: Department Chair. Qualifications: PhD with an emphasis in anatomy and/or physiology. Primary interest and ability in teaching undergraduate students; a record of successful college teaching is preferred. Candidate must be committed to the liberal arts educational mission and evangelical Christian orientation of the Univ. and demonstrate commitment to excellence in undergraduate teaching, mentoring students, and scholarship. Candidate must demonstrate the ability to contribute to Bethel's anti-racism efforts and cross-cultural understanding. Responsibilities: Teach the equivalent of a full seven-course load per year. Participation in teaching courses in the introductory biology curriculum, sophomore-level anatomy, physiology and pathophysiology/pharmacology courses and upper-level biology courses appropriate to the candidate's qualifications. Strong interest and ability to participate in a research program involving undergraduates. Participation and evidence of ongoing professional and/or scholarly development. General faculty responsibilities will include student advising, and department and committee assignments. Compensation: Salary and rank commensurate with qualifications and experience. Position Open: Fall 2012. Application Process: http://www.bethel.edu/human-resources/employment-cas.html. Bethel University is a leader in Christ-centered higher education with approximately 6,400 students from 47 states and 45 countries enrolled in undergraduate, graduate, seminary, and adult education programs. Based in St. Paul, Minn., with additional seminary locations on both coasts, Bethel offers rigorous bachelors and advanced degrees in nearly 100 relevant fields. Programs are taught by renowned faculty within a distinctly evangelical Christian framework, equipping women and men for culturally sensitive leadership, scholarship, and service around the world.

Postdoctoral Positions

Postdoctoral Training in Cardiovascular and Renal Physiology: The Center for Excellence in Cardiovascular-Renal Research (CECR) at the Univ. of Mississippi Medical Center is currently recruiting postdoctoral fellows to train in cardiovascular and renal research. The Center is an interdisciplinary research program that involves a wide range of basic and clinical research, including genetic, molecular, and cellular mechanisms, and integrative physiology of cardiovascular and renal disease in a variety of animal models. The center currently has over 65 members actively involved in biomedical research. Areas of research emphasis include: renal and molecular mechanisms of hypertension, obesity and metabolic diseases, fetal programming of cardiovascular diseases, gender differences in cardiovascular-renal disease, microcirculation, immune mechanisms in cardiovascular and renal disease, pregnancy and preeclampsia, vascular biology, genetics of hypertension and cardiovascular diseases, stroke, endothelial biology, angiogenesis and neuro-endocrine control of the circulation. Members of the Center receive extramural support from a variety of sources, such as the National Institutes of Health and the American Heart Association. The CECR is also funded by a NHLBI Institutional Training Grant entitled "Hypertension and Cardiorenal Diseases Research Training Program." Applicants must

have a PhD, MD, or comparable degree. Please send curriculum vitae, a statement of research interests and the names of three references to: Joey P. Granger, PhD, Director, Center for Excellence in Cardiovascular-Renal Research at the Univ. of Mississippi Medical Center at jgranger@umc.edu.

Postdoctoral Scientist Position: Applications are invited to fill a postdoctoral scientist position in cardiac electrophysiology and metabolism at The George Washington Univ. in Washington DC. The prospective postdoctoral scientist will work on a NIH-funded research project to study cardiac arrhythmias that originate from alterations in coronary flow. A combination of organ and cellular-based approaches will be used to study how metabolic disturbances resulting from local changes in the microcirculation may contribute to the formation of spontaneous propagating potentials. Studies will be conducted in living tissue using both confocal microscopy and fast fluorescence imaging to measure transmembrane potentials, intracellular calcium transients, and levels of mitochondrial NADH. Additionally, the postdoctoral scientist will have the opportunity to work as a member of an energetic multidisciplinary team of scientists comprised of faculty from the GWU School of Engineering and Applied Science, the GWU School of Medicine and Health Sciences, and the GW Medical Faculty Associates. A starting date may be as early as January 2012. Applicants will be reviewed until the position is filled. Minimum qualifications: The position requires a doctoral degree in an appropriate field of bioengineering, biomedical engineering, biophysics, biochemistry, physiology, pharmacology, or a related scientific field. The ideal candidate should have a strong background in cardiac physiology, physiological signal acquisition, and data analysis. The candidate should have excellent skills in written and oral English. Salary will be based on the NIH scale that is commensurate with background and experience. The George Washington University is an equal opportunity employer. Interested candidates should *Email* a short statement of interest, updated curriculum vitae, and contact information for at least three references to Matthew Kay, DSc., The George Washington Univ., 801 22nd Street NW, Phillips Hall Suite 619, Washington, DC 20052. Email: phymwk@gwu.edu. ❖

Current Calls for Papers

Physiological Genomics

Mitochondrial Metabolism

NextGen Sequencing Technology-Based Dissection of Physiological Systems

Functional Analysis of Sequence Variation

Technology Development for Physiological Genomics

Advances in Physiology Education

Teaching and Learning of Professional Ethics

American Journal of Physiology—Renal Physiology

Biology of the Central Cilium and Cystic Diseases of the Kidney
(Submission deadline: January 1, 2012)

Programming Normal Renal Development and Modeling Disease Pathogenesis
(Submission deadline: January 1, 2012)

Special Joint Call for Papers: Integrative Aspects of Renal Endocrinology
(Submission deadline: January 1, 2012)

Aldosterone and Epithelial Na^+ Channels
(Submission deadline: July 1, 2012)

Mathematical Modeling of Renal Function
(Submission deadline: April 1, 2012)

American Journal of Physiology—Endocrinology and Metabolism

Special Joint Call for Papers: Integrative Aspects of Renal Endocrinology
(Submission deadline: January 31, 2012)

American Journal of Physiology—Gastrointestinal and Liver Physiology

Physiology and GI Cancer

Intestinal Stem Cells in GI Physiology and Disease

Innovative and Emerging Technologies in GI Physiology and Disease

American Journal of Physiology-Regulatory, Integrative and Comparative Physiology

Integrative and Translational Physiology: Inflammation and Immunity in Organ System Physiology
(Submission deadline: April 30, 2012)

Integrative and Translational Physiology: Integrative Aspects of Energy Homeostasis and Metabolic Diseases
(Submission deadline: April 30, 2012)

Journal of Applied Physiology

Call for Commentaries on Point:Counterpoint Debates and Viewpoint Articles

Imaging Lung Physiology
(Submission deadline: January 1, 2012)

For a complete list of current Calls for Papers, visit *The Physiologist* website.

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The Wine Wizard

Peter Wagner

Hi all! I had a great time teaching in Australia followed by research on the Tibetan Plateau, but all wineless, hence my silence. Now it is time for a reality check and the important stuff, i.e., tasting wine. To ease back into the saddle I thought to first skewer the seven varieties of Charles Shaw wines for you this month. You know, Two-buck-chuck from Trader Joe. Yes, they are still $1.99 in California. But I could not leave you with such a paltry offering, so there are some real wines to go with them:

Two-buck-chuck

2009 Pinot Grigio, California, $2. Actually recognizable PG! Stone fruit and some citrus on the nose and palate. A bit simple, but clean (i.e., no sulfur). It is a bit thin, but for $2 I would serve this to a large crowd; actually worth a try. It has some residual sugar (RS).

2009 Chardonnay, California $2. What can I say? Sort of recognizable, simple, light oak, lighter fruit, clean, barely OK to sip cold (it has RS) on a hot day when you run out of water. I don't think I could get away with serving this at a party, at least not until everyone was already hosed.

2009 Sauvignon Blanc, California $2. The only words to describe it are not allowed in this column. Dirty, generic, dull, almost sweet, I actually poured it down the drain.

2009 White Zinfandel, California $2. Sweet cherry Kool-aid. This has to be drunk ice cold. It is actually clean, but the sweetness is overpowering, and it needs more acid for balance.

2010 Merlot, California $2. One has no idea what one is tasting here. Sweet red berry of no character; thin and slightly dirty, short, almost fails the definition of wine.

2010 Cabernet, California $2. One has no idea what one is tasting here. Sweet red berry of no character; thin and slightly dirty, short, almost fails the definition of wine.

2010 Shiraz, California $2. One has no idea what one is tasting here. Sweet red berry of no character; thin and slightly dirty, short, almost fails the definition of wine. Then add sulfur and a bitter finish……

Peter Wagner

At least I got 7 bottles of wine for $14.

White wines

2009 Vina Robles White Blend, Paso Robles, CA $11. This is 42% Vermentino (2nd vowel is an e not an i), 29% Verdelho, 26% Viognier, and 3% Sauvignon Blanc. With at least three, maybe four, countries represented this defines enodiplomacy, which might just be considered when nuclear discussions break down. Especially as this unqiue blend is actually good stuff. The nose is complex with pear, peach and citrus. The palate is rich and viscous with excellent acidity and a hint of sweetness. There is peach, pear, apricot and lemon at various points through the palate. Very clean, very good length.

2010 Wither Hills Sauvignon Blanc, New Zealand $9. This is outstanding wine at a great price. Very varietal and clean, with grassy, herbal lime/passionfruit and gooseberry notes on both nose and palate. Acid is medium – less than in most NZSB's, and this makes it very sippable. Rich, viscous and long, everything you want. Almost has RS (see above). And yes, I bought some. Really. Dinner party quality, but drink it young.

2009 Christom Pinot Gris Willamette Valley, Oregon $15. This is a classy wine. The nose is forward with lots of stone fruit (peach, apricot) and a similar palate that is beautifully balanced, viscous, long, clean, rich and with strong acid and no sweetness.

Red wines

2007 Columbia Crest Merlot "Grand Estates", Columbia Valley, WA $7. This wine does not taste old, but ya gotta love American oak (dill, sweet coconut) which dominates the wine at the start. Leave the glass to sit and the oak softens a bit. Good black cherry fruit, and some interesting green olive hints. Not a bad party wine given the price.

Now three very good wines:

2009 Seghesio Zinfandel, Sonoma county, CA $18. While their "lowest tier" zin, it is just as good this year as in past years. Has American oak on the nose and palate, but the fruit is clean and prominent with dark berries and plums. Medium tannin, very clean, balanced and with good length. It is not an over-extracted wine at all, and has elegance.

2009 Bogle Petite Sirah, CA $8.50. Great value for the price, no question. The nose has blueberries and dark cherries. The palate has very rich, ripe dark fruit flavors which easily cope with the medium high tannins. This is a big, solid, extracted wine. Interesting element of sage and honey can be identified. There is a freshness to the wine. Needs good red meat, to be sure.

2008 Penley Cabernet Sauvignon "Phoenix" Coonawarra, South Australia $16. In spite of the meteoric rise in value of the Oz dollar to now equal the US$ (a year ago it was perhaps $1.50 Oz to the US$), this is affordable stuff of high quality and typical Coonawarra style (elegant, restrained, balanced, yet with full fruit, not too alcoholic, not too oaky, just the right acidity), with some green olive character. There is signature eucalyptus as well which is common in Oz wines and blends well. Most wines of this quality would be twice the price.

Enjoy! ❖

2012

January 27-29
5th European Neurological Conference on Clinical Practices Neurovascular and Neurodegenerative Diseases, Park inn By Radisson, Krakow, Poland. *Information:* Shirley Dinenson, Conference Secretariat. Tel.: +41 22 5330 948; Fax: +41 22 5802 953; Email: sdinenson@paragon-conventions.com; Internet: http://www.enccp.net/.

February 2-5
Up Close and Personalized, International Congress on Personalized Medicine, Florence, Italy. *Information:* Ilana Berkowitz, Conference Secretariat. 18 Avenue Louis-Casai, 1209 Geneva, Switzerland. Tel.: +41 22 5330 948; Fax: +41 22 5802 953; Email: ilanab@paragon-conventions.com; Internet: http://www.upcp.org/.

February 26-29
The 11th International Dead Sea Symposium on Cardiac Arrhythmias and Device Therapy (IDSS), Jerusalem, Israel. *Information:* Anat Regev, 18 Avenue Louis-Casai, 1209 Geneva, Switzerland. Tel.: +41 22 5330 948; Fax: +41 22 5802 953; Email: idss@idss-ep.com; Internet: http://www.idss-ep.com/.

March 27-29
Researchers, Teachers, Learners – We're All in it Together! Charles Darwin House, London. *Information:* Talja Dempster, Charles Darwin House, 12 Roger Street, London WC1N 2JU. Tel.: +44 (0) 2076852605; Fax: +44 (0) 2076852601; Email: T.Dempster@sebiology.org; Internet: http://www.sebiology.org/meetings/EPASymposium/home.html.

April 16-17
The 59th Annual Conference on the Israel Heart Society in Association with the Israel Society of Cardiothoracic Surgery, Tel Aviv, Israel. *Information:* Anat Regev, 19 Hayetzira Street, Ramat Gan, 52118. Tel.: +972-3-5767716; Fax: +972-3-5767716; Email: seretariat@israelheart.com; Internet: http://www.israelheart.com/en/.

May 13-15
The International Conference on Integrative Medicine, Jerusalem, Israel. *Information:* Ravit Levy, 19 Hayetzira street, Ramat Gan 52118, Israel. Tel: +972-3-5767750; Fax: +972-3-5767750; Email: rlevy@paragon-conventions.com; Internet: http://www.mediconvention.com/.

May 17-20
The 2nd Global Congress for Consensus in Pediatrics and Child Health, Moscow, Russia. *Information:* Meital Nave Fridenzon, Paragon Conventions, 18 Avenue Louis-Casai, 1209 Geneva, Switzerland. Tel.: +41 22 5330 948; Fax: +41 22 5802 953; Email: cip@cipediatrics.org; Internet: http://www.cipediatrics.org/.

May 18-23
2012 American Thoracic Society International Conference, San Francisco, California. *Information:* ATS International Conference Department. Tel.: 212-315-8652; Email: conference@thoracic.org; Internet: http://conference.thoracic.org.

May 29-June 2
59th ACSM Annual Meeting and 3rd World Congress on Exercise in Medicine, San Francisco, CA. *Information:* http://acsmannualmeeting.org/educational-highlights/2012-session-submission/.

June 23 – 27
Woodstock 2012, Abbazia di Spineto, Tuscany, Italy. *Information:* Talja Dempster, Charles Darwin House, 12 Roger Street, London WC1N 2JU. Tel.: +44 (0) 2076852605; Fax: +44 (0) 2076852601; Email: T.Dempster@sebiology.org; Internet: http://www.sebiology.org/meetings/Woodstock/home.html.

June 26-29
4th International Congress on Cell Membranes and Oxidative Stress Focus on Calcium Signaling and TRP Channels, Isparta, Turkey. *Information:* Internet: http://www.cmos.org.tr/2012.

June 29–July 2
Society for Experimental Biology Salzburg 2012, Salzburg Congress Centre, Salzburg, Austria. *Information:* Talja Dempster, Charles Darwin House, 12 Roger Street, London WC1N 2JU. Tel.: +44 (0) 2076852605; Fax: +44 (0) 2076852601; Email: T.Dempster@sebiology.org; Internet: http://www.sebiology.org/meetings/Salzburg2012/Salzburg.html.

August 18-22
The 30th World Congress of Biomedical Laboratory Science, Berlin, Germany. *Information:* Ilana Berkowitz, Conference Secretariat. 18 Avenue Louis-Casai, 1209 Geneva, Switzerland. Tel.: +41 22 5330 948; Fax: +41 22 5802 953; Email: secretariat@ifbls-dvta2012.com; Internet: http://www.ifbls-dvta2012.com/.

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.

October 22-25
2nd International Neural Regeneration Symposium (INRS2012), Shenyang, China. *Information:* Internet: http://www.crter.org/E_Journal/e-images/meeting1.jpg.

October 18-21
Pan American Heart Failure Congress (PAHF 2012), Panama City, Panama. *Information:* Mrs. Tali Ogorek, Conference Secretariat, Paragon-Conventions, 18 Avenue Louis-Casai, 1209 Geneva, Switzerland. Tel.: 41 22 5330 948; Fax: 41 22 5802 953; Email: secretariat@pahfcongress.com; Internet: http://www.pahfcongress.com.

October 28-30
45th Annual Meeting of the Society for Leukocyte Biology, Maui, Hawaii. *Information:* Society Management Services, 9650 Rockville Pike, Bethesda, MD. Tel.: 301-634-7814; Fax: 301-634-7455; Email: slb@faseb.org; Internet: http://www.leukocytebiology.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 _____	#2 Sponsor Name _____
Mailing Address _____	Mailing Address _____
_____	_____
_____	_____
Phone _____	Phone _____
Fax _____	Fax _____
E-mail _____	E-mail _____
Sponsor Signature* _____	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
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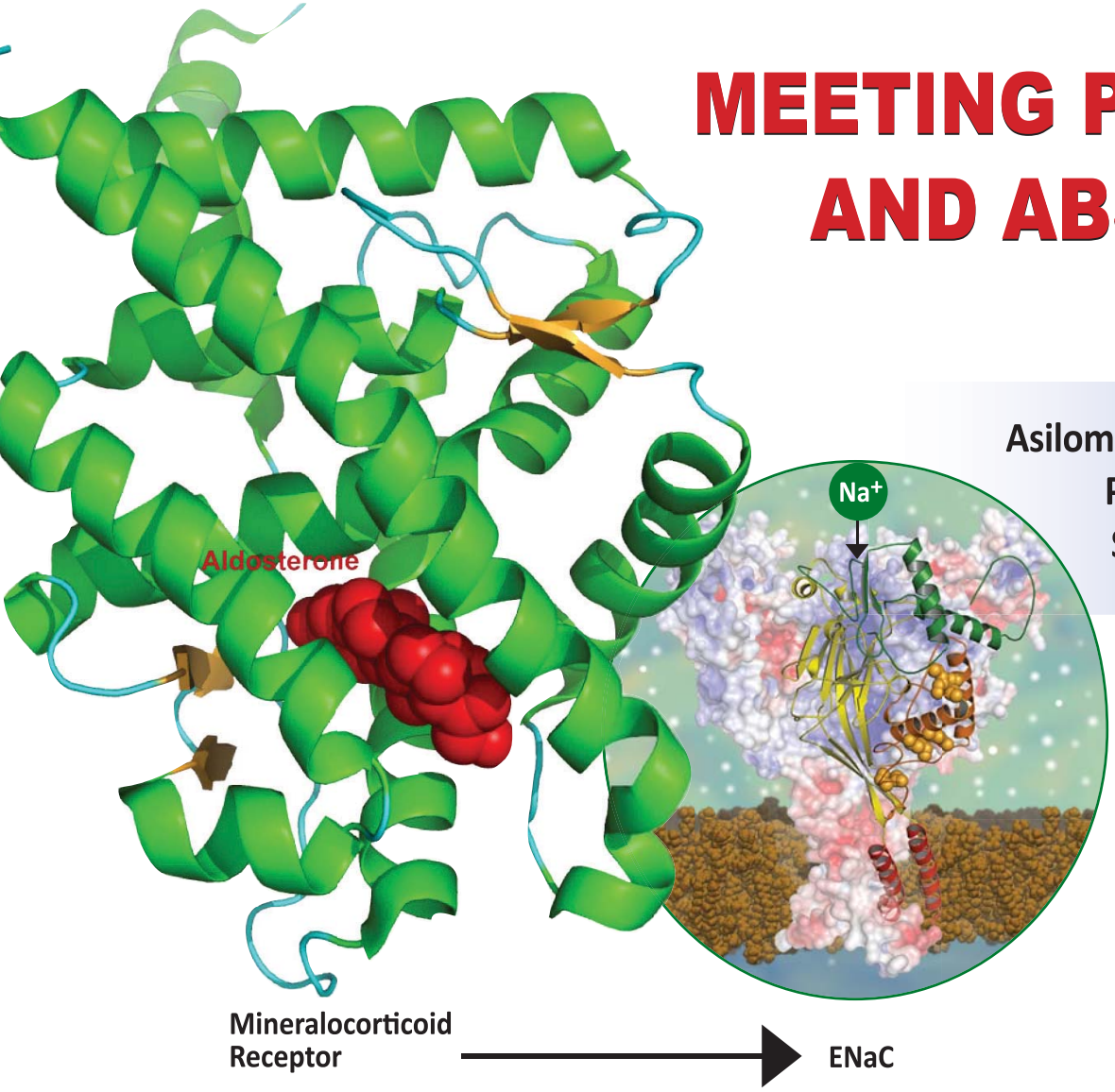
2011 American Physiological Society Conference

7th International Symposium on Aldosterone and the ENaC/Degenerin Family of Ion Channels: Molecular Mechanisms and Pathophysiology



MEETING PROGRAM AND ABSTRACTS

Asilomar Conference Grounds
Pacific Grove, California
September 18-22, 2011

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2011 APS Conference
7th International Symposium on Aldosterone and the ENaC/Degenerin Family of Ion Channels: Molecular Mechanisms and Pathophysiology

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2011 APS Conference
7th International Symposium on Aldosterone and the ENaC/Degenerin Family of Ion Channels: Molecular Mechanisms and Pathophysiology
September 18—22, 2011,
Asilomar Conference Grounds, Pacific Grove, CA

Sunday September 18, 2011	Monday September 19, 2011	Tuesday September 20, 2011	Wednesday September 21, 2011	Thursday September 22, 2011
3:00 PM **Registration Opens** 6:00—7:00 PM **Dinner** 7:30—8:30 PM Keynote Lecture: **Aldosterone and Hereditary Hypertension** **R. Lifton,** Yale Univ. Sch. of Med. 8:30—10:00 PM **Opening and Welcome Reception** *Seascape Room*	7:00—8:00 AM **Breakfast** 8:00 AM—12:00 Noon Symposia I: **Structure and Function of ENaC and Related Transporters** Participants: **L. Schild,** (Chair), Univ. of Lausanne, Switzerland **L. Palmer**, Weil Med. Coll. **J. Loffing**, Univ. of Zurich, Switzerland **S. Sheng,** Univ. of Pittsburgh **M. Carattino,** Univ. of Pittsburgh **S. Kellenberger,** Univ. of Lausanne, Switzerland **A. Staruschenko,** Med. Coll. of Wisconsin **Y. Marunaka,** Kyoto Prefectual Univ., Japan **K. Tomita,** Kumamoto Univ., Japan **E. Hummler**, Univ. of Lausanne, Switzerland 10:00—10:30 AM **Coffee Break** 12:00 Noon—1:00 PM **Lunch** 1:00—5:30 PM Symposia I (continued): **O. Kashlan,** Univ. of Pittsburgh **P. Snyder**, Univ. of Iowa Symposia II: **Structure and Function of Mineralocorticoid and Glucocorticoid Receptors** Participants: **P. Fuller** (Chair), Prince Henry's Inst. of Med. Res., Australia **M. Young**, Prince Henry's Inst. of Med. Res., Australia **M. Lombes**, INSERM, Paris, France **F. Jaisser**, Univ. Pierre et Marie Curie, France **C. Gomez-Sanchez,** Univ. of Mississippi **I. Jaffe**, Tufts Univ. Sch. of Med. **J. Fagart,** INSERM, Paris, France 2:30—3:00 PM **Coffee Break** 6:00—7:00 PM **Dinner** 7:00—9:30 PM **Poster Presentations**	7:00—8:00 AM **Breakfast** 8:00 AM—12:00 Noon Symposia III: **Regulation of ENaC Biogenesis, Trafficking and Gating** Participants: **D. Eaton** (Chair), Emory Univ. **R. Soundararajan**, Univ. of California, San Francisco **J. Stockand**, Univ. of Texas, San Antonio **M. Butterworth,** Univ. of Pittsburgh **K. Hallows**, Univ. of Pittsburgh **J. Stutts,** Univ. of North Carolina **O. Staub,** Univ. of Lausanne, Switzerland **D. Lagnaz,** Univ. of Lausanne, Switzerland **C. Korbmacher**, Friedrich-Alexander Univ., Germany 10:00—10:30 AM Coffee Break 12:00 Noon—1:00 PM **Lunch** 1:00—4:00 PM Symposia III (continued): **D. Rotin,** Hosp. for Sick Children, Toronto, Canada **V. Bhalla**, Stanford Univ. Med. Sch. Symposia IV: **Aldosterone: Synthesis, Crosstalk and Non-epithelial Actions** Participants: **B. Rainey** (Chair), Georgia Hlth. Sci. Univ. **H. Okamura**, Univ. of Kyoto, Japan **G. Adler**, Harvard Med. Sch. **C. Grossmann**, Univ. of Halle-Wittenberg, Germany 4:20—4:40 PM **Coffee Break** 4:40—5:30 PM Plenary Lecture: **Role of Aldosterone and Mineralocorticoid Receptor in Kidney Disease and the Metabolic Syndrome** **T. Fujita,** Univ. of Tokyo, Japan 6:00—7:00 PM **Dinner** 7:00—9:30 PM **Poster Presentations**	7:00—8:00 AM **Breakfast** 8:00—8:45 AM Symposia V: **Remembering J. D. Horisberger and D. J. Benos** Participants: **L. Schild,** Univ. of Lausanne, Switzerland **C. Fuller,** Univ. of Alabama at Birmingham 8:45 AM—12:00 Noon **Abstract-based Presentations** 10:00—10:30 AM **Coffee Break** 12:00 Noon—1:00 PM **Lunch** 1:00—3:00 PM **Free Time** 3:00—4;00 PM Career Session: **The *Ins* and *Outs* of Authorship** Presented by: **T. Schmidt,** Univ. of Iowa 4:00—4:50 PM Symposia VI: **Congestive Heart Failure: The Intertwined Roles of Water and Salt** Participants: **B. Pitt,** Univ. of Michigan **T. Berl,** Univ. of Colorado, Denver 5:00—6:00 PM Plenary Lecture: **ASIC Structure and Function** **M. Welsh,** Univ. of Iowa and HHMI 6:00—8:30 PM **Dinner and Awards Presentation** *Seascape Room*	7:00—8:00 AM **Breakfast** 8:00—10:00 AM Symposia VII: **ENaC Pathophysiology** Participants: **B. Rossier** (Chair), Univ. of Lausanne, Switzerland **H. Drummond**, Univ. of Mississippi **R. Tarran**, Univ. of North Carolina **C. Fuller,** Univ. of Alabama at Birmingham **R. Boucher**, Univ. of North Carolina **O. Skott,** Univ. of Southern Denmark, Denmark 10:00—10:30 AM **Coffee Break** 10:30 AM—12:00 Noon Symposium VIII: **Aldosterone Pathophysiology** Participants: **E. Davies** (Chair), Univ. of Glasgow, UK **M-C. Zennaro**, INSERM, Paris, France **B. Sechi,** Univ. of Udine, Italy 12:00 Noon—1:00 PM **Lunch**

GENERAL INFORMATION

Location:

The 2011 APS Conference: 7th International Symposium on Aldosterone and the ENaC/Degenerin Family of Ion Channels: Molecular Mechanisms and Pathophysiology, will be held September 18—22, 2011, at the Asilomar Conference Grounds, 800 Asilomar Ave., Pacific Grove, CA 93950, telephone (831) 642-4222, FAX: (831) 642-4261.

On-Site Registration Information:

On-site registration will be available at the conference for badge pick-up, receipts, program distribution and conference information. The registration desk will be open daily in the Chapel Meeting room. If you are staying at an off-site hotel you will need to purchase meal tickets in order to dine with the other conference registrants. Meal tickets can be purchased at the front desk of the Heart Social Hall, located on the Asilomar Conference Grounds.

On-site Registration Hours:

Sunday, September 18……3:00—9:00PM
Monday, September 19.....7:00AM—6:00 PM
Tuesday, September 20……..7:00 AM—6:00 PM
Wednesday, September 21….7:00 AM—6:00 PM
Thursday, September 22…..7:00—10:30 AM

On-Site Registration Fees:

On-site registration for this conference will not be available.

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 Thomas Schmidt, 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:

A major goal of this conference will be to bring together clinical and basic researchers from all over the globe with an interest in ENaC (and related transporters) and aldosterone, particularly as they pertain to renal and cardiovascular disorders, including hypertension. Hence, the scientific themes are a blend of clinical, basic and translational research, which allow a diverse community to break down obstacles to communication and engage in crosstalk, which would not otherwise be possible. By focusing on ENaC and aldosterone in the context of the renal and cardiovascular systems, the meeting provides a window into basic and clinical questions, which are fundamental to human biology in health and disease.

The conference program combines presentations from leading authorities with presentations from young investigators pursuing research on ENaC and aldosterone. Ample time for poster sessions will allow for in-depth discussions, and special abstract-based oral sessions will highlight ongoing research of students, fellows, and young scientists.

Target Audience:

This meeting is intended to bring together world leaders in clinical, translational and basic research in aldosterone and ENaC, *and* related areas for a multi-day intensive retreat to present and discuss the latest research in the field.

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

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SUNDAY, SEPTEMBER 18, 2011

Keynote Lecture

1.0 **PLENARY LECTURE**
Sun., 7:30-8:30 PM, Chapel Room.

7:30 PM **1.1** Opening Comments. **Thomas R. Kleyman.** *Univ. of Pittsburgh* and **David Pearce.** *Univ. of California, San Francisco.*

7:40 PM **1.2** Aldosterone and Heredity Hypertension. **Richard Lifton.** *Yale Univ. Sch. of Med.*

MONDAY, SEPTEMBER 19, 2011

Symposia I

2.0 **STRUCTURE AND FUNCTION OF ENaC AND RELATED TRANSPORTERS**
Mon., 8:00 AM-12:00 Noon, Chapel Room.

Chair: **Laurent Schild,** *Univ. of Lausanne, Switzerland.*

8:00 AM **2.1** ENaC Structure/function Overview. **Laurent Schild.** *Univ. of Lausanne, Switzerland.*

8:20 AM **2.2** Regulation of ENaC Expression in Rat Kidney. **Larry Palmer.** *Weill Med. Coll. of Cornell Univ.*

8:40 AM **2.3** ENaC Regulation in the Connecting Tubule. **Johannes Loffing.** *Univ. of Zurich, Switzerland.*

9:00 AM **2.4** Structural Insights into the Regulation of ENaCs by External Na. **Shaohu Sheng.** *Univ. of Pittsburgh.*

9:20 AM **2.5** Structural Transitions Associated with the Gating of ASIC1a. **Marcelo Carattino.** *Univ. of Pittsburgh.*

9:40 AM **2.6** Mechanisms of pH-Dependant Gating of ASICs. **Stephen Kellenberger.** *Univ. of Lausanne, Switzerland.*

10:00 AM Break.

10:30 AM **2.7** Mechanisms and Physiological Importance of ENaC Regulation by Growth Factors and Small GTPases. **Alexander Staruschenko.** *Med. Coll. of Wisconsin.*

10:50 AM **2.8** Hypotonicity-induced Upregulation of β- and γ-ENaC Expression Through Suppression of ERK by Inducing MKP-1. **Yoshinori Marunaka.** *Kyoto Prefectual Univ. Japan.*

11:10 AM **2.9** A Synthetic Serine Protease Inhibitor Camostat Mesilate Inhibited the Proteolytic Activation of γENaC in the Kidney of Aldosterone-infused Rats. **Kimio Tomita.** *Kumamoto Univ. Japan.*

11:30 AM **2.10** Lessons Learned from Knockout Studies. **Edith Hummler.** *Univ. of Lausanne, Switzerland.*

1:00 PM **2.11** Conformational Trapping of the Closed State of ENaC. **Ossama Kashlan.** *Univ. of Pittsburgh.*

1:20 PM **2.12** The Extracellular Domain of ENaC is a Sensor of the Extracellular Milieu. **Peter Snyder.** *Univ. of Iowa.*

1:40 PM **2.13** The ER Lumenal Chaperone, Lhs1/GRP170, Plays a Unique Role in the Biogenesis of the Epithelial Sodium Channel. **Teresa Buck.** *Univ. of Pittsburgh.*

1:52 PM **2.14** Regulation of the Epithelial Na^+ Channel by Intracellular Na^+: Mechanism and Time Course. **Ankit Patel.** *Weill Med. Coll. of Cornell Univ.*

2:04 PM **2.15** Revisiting the Subunit Oligomerization of ENaC. **Rosanna De Nuccio.** *Univ. of Lausanne, Switzerland.*

2:16 PM **2.16** Acidic Residues in the Extracellular Domain of Human α-, β-, and γENaC Contribute to H^+ Regulation of Channel Activity. **Daniel Collier.** *Univ. of Iowa.*

2:30 PM Break

Symposia II

3.0 **STRUCTURE AND FUNCTION OF MINERALOCORTICOID AND GLUCOCORTICOID RECEPTORS**
Mon., 3:00-5:30 PM, Chapel Room.

Chair: **Peter Fuller,** *Prince Henry's Inst. of Med. Res. Melbourne, Australia.*

3:00 PM **3.1** Structure-Function Relationships in the Mineralocorticoid Receptor and Interactions with Novel Transcriptional Coregulators. **Peter Fuller.** *Prince Henry's Inst. of Med. Res. Melbourne, Australia.*

3:20 PM **3.2** Physiologic Roles of MR Revealed by Tissue Selective Knockouts. **Morag Young.** *Prince Henry's Inst. of Med. Res. Melbourne, Australia.*

3:40 PM **3.3** Epithelial Sodium Channel (ENaC) is a Key Mediator of Growth Hormone-Induced Sodium Retention: Pathophysiology of Volume Expansion in Acromegalic Patients. **Marc Lombes.** *INSERM, Paris, France.*

4:00 PM **3.4** Molecular Mechanisms of Mineralocorticoid Receptor Function in Heart. **Frederic Jaisser.** *INSERM, Paris, France.*

4:20 PM **3.5** Subcellular Distribution of the Mineralocorticoid Receptor. **Celso Gomez-Sanchez.** *Univ. of Mississippi, Jackson.*

4:40 PM **3.6** Vascular Mineralocorticoid Receptors Mediate Aldosterone-Dependant Vascular Injury. **Iris Jaffe.** *Tufts Univ. Sch. of Med.*

5:00 PM **3.7** Structural Characterization of Mineralocorticoid Receptor Inactivation. **Jerome Fagart.** *INSERM, Paris, France.*

Poster Session I

4.0 ENaC STRUCTURE AND REGULATION

Mon., 7:00-9:30 PM, Chapel Room.

Board #

1 **4.1** Renal Nedd4-2 is Crucial for NCC Regulation and Ca Balance. **C. Ronzaud, J. Loffing and O. Staub.** *Univ. of Lausanne, Switzerland and Zurich Univ. Switzerland.*

2 **4.2** Sodium Selectivity of Amiloride-sensitive Currents in Inner Ear Epithelial Cells. **D. Marcus, M. Yamazaki, K.X. Kim, T. Wu, S. R. Pondugula and D. G. Harbidge.** *Kansas State Univ.*

3 **4.3** Aldosterone Induces Accumulation of an αENaC Immunereactive Peptide in Proteosomes of Distal Renal Tubules. **M. U. Cheema, J. Nielsen, R. A. Fenton and J. Praetorius.** *Aarhus Univ. Denmark.*

4 **4.4** A New Invertebrate Model for the Study of the Epithelial Sodium Channel (ENaC). **W. Clauss, S. Krumm and S. Goebel-Lauth.** *Justus-Liebig-Univ. Germany.*

5 **4.5** xShroom1 Regulates the Number of ENaC Channels Inserted in the Membrane of Oocytes from *Xenopus laevis.* **B. Kotsias, Y. Assef, M. Ozu, G. Marino and L. Galizia.** *Inst. Inv. Médicas A. Lanari, Univ. of Buenos Aires, IDIM-CONICET, Buenos Aires, Argentina.*

6 **4.6** Proteolytic Channel Activation by Plasmin Involves Two Distinct Cleavage Sites in the γ-Subunit of Human ENaC. **S. Haerteis, M. Krappitz, A. Diakov, R. Rauh and C. Korbmacher.** *Univ. Erlangen-Nürnberg, Germany.*

7 **4.7** Does Insulin Regulate ENaC? **G. Frindt and L. Palmer.** *Weill-Cornell Medical Coll.*

8 **4.8** Cleavage of Endogenous γENaC and Elevated Abundance of αENaC is Associated with Increased Na^+ Transport in Response to Apical Fluid Volume Expansion in Human H441 Airway Epithelial Cells. **D. Baines, I. Selvanathar and C. Tan.** *St George's, Univ. of London, UK.*

9 **4.9** Aldosterone-Independant Regulation of ENaC in ADX Mice. **E. Mironova, V. Bugay and J. Stockand.** *UTHSCSA.*

10 **4.10** Collecting Duct-Specific Endothelin B Receptor Knockout Increases ENaC Activity. **V. Bugay, E. Mironova, D. Kohan and J. Stockand.** *UTHSCSA and Univ. of Utah.*

11 **4.11** Disease Causing Mutations Affect ENaC Gating. **V. Kucher, N. Boiko, O. Pochynyuk and J. D. Stockand.** *UTHSCSA and Univ. of Texas Hlth. Sci., Houston.*

Board #

12 **4.12** The PPK1 Channel of Drosophila Sensory Neurons. **N. Boiko, B. A. Eaton and J.D. Stockand.** *UTHSCSA.*

13 **4.13** Subunit Oligomerization of Human ASIC1a in *Xenopus laevis* Oocytes. **D. Huser, M. van Bemmelen, A. Maquelin and L. Schild.** *Univ. of Lausanne, Switzerland.*

14 **4.14** Acetylation Modulates αENaC Degradation. **P. Butler and P. M. Snyder.** *Univ. of Iowa.*

15 **4.15** CU^{2+} is a Inhibitor of Human Epithelial NA^+ Channels. **J. Chen, M. M. Myerburg, C. J. Passero, K. L. Winarski and S. Sheng.** *Univ. of Pittsburgh.*

16 **4.16** Epithelial Sodium Channel (ENaC) Activity and Gating is Modulated by Palmitoylation of Both the β and γ Subunits. **G. M. Mueller, Z. Wang, N. Sheng, M. Tolino, T. R. Kleyman and R. Hughey.** *Univ. of Pittsburgh.*

17 **4.17** TMPRSS4 Activates the Epithelial Sodium Channel by Cleaving the Gamma Subunit Distal to the Furin Cleavage Site. **R. Hughey, C. J. Passero, G. M. Mueller, M. M. Myerburg and T. R. Kleyman.** *Univ. of Pittsburgh.*

18 **4.18** Functional Characterization of the Permeation Pathway of ASIC1a. **M. Carattino, M. Della Vecchia and L. Tolino.** *Univ. of Pittsburgh.*

19 **4.19** Mechanistic Basis for Specific Activation of SGK1 by mTOR. **M. Lu, J. Wang, H. Ives and D. Pearce.** *Univ. of California, San Francisco.*

20 **4.20** *In vivo* and *in vitro* Inhibition of the Membrane-bound Serine Protease CAP1/Prss8 by Serpins. **G. Crisante, S. Frateschi, C. Sergi, A-M. Mérillat, B. Rossier and E. Hummler.** *Univ. of Lausanne, Switzerland.*

21 **4.21** The Cystic Fibrosis Transmembrane Conductance Regulator Inhibits Proteolytic Stimulation of ENaC. **M. Gentzsch, Y. Dang, H. Dang and M. J. Stutts.** *Univ. of North Carolina.*

22 **4.22** Generation of Mice Deficient for the Channel Activating Protease 2/Tmprss4. **A. Keppner, D. Andreasen, A-M. Mérillat, B. Rossier and E. Hummler.** *Univ. of Lausanne, Switzerland.*

23 **4.23** Role of the ESCRT Protein Tsg101 in the Turnover of the Epithelial Na+ Channel in the Kidney. **J. Vitagliano and O. Staub.** *Univ. of Lausanne, Switzerland.*

24 **4.24** A New Method for Isolation and Culture of Principal Cells for Studies of ENaC Regulation. **M. Labarca, W. Dong, P. K. Adishesha, R. B. McClellan, J. B. Stokes and V. Bhalla.** *Stanford Univ. and Univ. of Iowa.*

Board #

25 **4.25** Regulation of Na+ Homeostasis by the Deubiquitylating Enzyme Usp2 *in vivo*. **D. Pouly, A. Debonneville, N. Faresse, D. Ruffieux-Daidié, M. Maillard and O. Staub.** *Univ. of Lausanne, Switzerland; Lausanne Univ. Hosp., Switzerland.*

26 **4.26** Withdrawn.

27 **4.27** Epithelium Sodium Channel (ENaC) Delta Subunit and its Functional Expression in Human Respiratory Epithelial Cells. **E. Schwagerus, S. T. Buckley, H. Fischer, B. Illek and C. Ehrhardt.** *Trinity College Dublin, Ireland and Children's Hosp. Oakland Res. Inst., California.*

28 **4.28** Rac1-Mediated Nadph Oxidase Production of O_2^- Regulates Lung ENaC. **M. Helms.** *Emory Univ.*

29 **4.29** WNK4 Inhibition of ENaC is Independent from Nedd4-2 Mediate ENaC Ubiquitination. **L. Yu, H. Cai, D. X. Wang, A. Alli, Q. Yue, O. Al-Khalili, P. Snyder and D. Eaton.** *Emory Univ. and Univ. of Iowa.*

30 **4.30** MARCKS Regulates ENaC by Reversibly Sequestering Phosphatidylinositol Phosphates. **A. Alli, H. Bao, O. Al-Khalili, L. Yu, Y. Aldrugh, A. Alli, S. Aldrugh and D. Eaton.** *Emory Univ.*

31 **4.31** Controlling the ENaC with Light. **M. Schoenberger, M. Althaus, M. Fronius, W. Clauss and D. Trauner.** *Ludwig Maximilians Univ., Munich, Germany and Justus-Liebig Univ., Giessen, Germany.*

TUESDAY, SEPTEMBER 20, 2011

Symposia III

5.0 REGULATION OF ENaC BIOGENESIS, TRAFFICKING AND GATING

Tues., 8:00 AM-12:00 Noon, Chapel Room.

Chair: **Douglas Eaton,** *Emory Univ.*

8:00 AM **5.1** Overview of ENaC Regulation. **Douglas Eaton.** *Emory Univ.*

8:20 AM **5.2** CNK3 and the ENaC Regulatory Complex. **Rama Soundararajan.** *Univ. of California, San Francisco.*

8:40 AM **5.3** Role of Purinergic Signaling Regulating ENaC Activity. **James Stockand.** *UTHSCSA.*

9:00 AM **5.4** MicroRNAs: Novel ENaC Regulators. **Michael Butterworth.** *Univ. of Pittsburgh.*

9:20 AM **5.5** Interplay Between Kinases, Nedd4-2 and ENaC. **Kenneth Hallows.** *Univ. of Pittsburgh.*

9:40 AM **5.6** P2Y2-R Regulation of ENaC-mediated Na^+ Absorption in Airway Epithelia. **Jack Stutts.** *Univ. of North Carolina, Chapel Hill.*

10:00 AM Break

10:30 AM **5.7** Ubiquitylation-deubiquitylation Cycles in the Control of Membrane Protein Stability and Trafficking. **Olivier Staub.** *Univ. of Lausanne, Switzerland.*

10:50 AM **5.8** Regulation of NCC by the Aldosterone-SGK1-NEDD4-2 Pathway. **Dagmara Lagnaz.** *Univ. of Lausanne, Switzerland.*

11:10 AM **5.9** ENaC Regulation by Proteases. **Christoph Korbmacher.** *Univ. Erlangen-Nürnberg, Germany.*

1:00 PM **5.10** Deletion of the Ubiquitin Ligase Nedd4L in Lung Epithelia Causes Cystic Fibrosis-like Disease. **Daniela Rotin.** *Hosp. for Sick Children, Toronto, Canada.*

1:20 PM **5.11** Regulation of Ubiquitin Ligase Activity and Phosphorylation by SGK1. **Vivek Bhalla.** *Stanford Univ.*

1:40 PM **5.12** Aldosterone-independent Regulation of ENaC by Salt Intake. **Oleh Pochynyuk.** *Univ. Texas Hlth. Sci. Ctr., Houston.*

1:52 PM **5.13** Rab-GAP Regulation of Epithelial Sodium Channel (ENaC) Forward Trafficking in Response to Aldosterone and Vasopressin. **Xiubin Liang.** *Univ. of Pittsburgh.*

2:04 PM **5.14** The Role of mTOR and SGK1 in Mediating Aldosterone Regulation of ENaC in vivo. **Atif Kidwai.** *Univ. of California, San Francisco.*

2:30 PM Break

Symposia IV

6.0 ALDOSTERONE: SYNTHESIS CROSSTALK AND NON-EPITHELIAL ACTIONS

Tues., 3:00-4:30 PM, Chapel Room.

Chair: **William Rainey,** *Georgia Hlth. Sci. Univ.*

3:00 PM **6.1** Regulation of Aldosterone Production Through Expression of Aldosterone Synthase (CYP11B2). **William Rainey.** *Georgia Hlth. Sci. Univ.*

3:20 PM **6.2** Clock and Renin Angiotensin Aldosterone System in Adrenal Gland and Kidney. **Hitoshi Okamura.** *Kyoto Univ., Japan.*

3:40 PM **6.3** Role of Nitric Oxide and Caveolin in the Vascular Actions of Aldosterone and the Mineralocorticoid Receptor *in vivo*. **Gail Adler.** *Harvard Med. Sch.*

4:00 PM **6.4** Compartment-Specific Mineralocorticoid Receptor Signaling. **Claudia Grossmann.** *Univ. Halle-Wittenberg, Germany.*

4:20 PM Break

Plenary Lecture

7.0 PLENARY LECTURE

Tues., 4:30-5:30 PM, Chapel Room.

4:30 PM **7.1** Aberrant RAC1-MR Pathway in Salt-Sensitive Hypertension and Metabolic Syndrome. **Toshio Fujita.** *Univ. of Tokyo, Japan.*

Poster Session II

8.0 ALDOSTERONE AND ENaC

Tues., 7:00-9:00 PM, Chapel Room.

Board #

1 **8.1** Cholera Toxin Enhances Sodium Absorption Across Cultured Human Mammary Gland Epithelia: Novel Mechanisms of Regulation ENaC Function in Mammary Gland. **Q. Wang and B. Schultz.** *Kansas State Univ.*

2 **8.2** Thiol-reactive Compounds from Garlic Inhibit the Epithelial Sodium Channel (ENaC) – A Possible Mechanism for Lowering Blood Pressure? **M. Althaus, P. Krumm, T. Giraldez, D. Alvarez de la Rosa, W. Clauss and M. Fronius.** *Justus-Liebig Univ. of Giessen, Germany; Univ. Hosp. NS Candelaria (HUNSC), Spain and Univ. de La Laguna, Spain.*

3 **8.3** The δ1 and δ2 ENaC Subunits Form Mechanosensitive Channels when Coexpressed with β and γ Subunits. **M. Fronius, M. Althaus, M. Assmann, M. Bednarz and W. Clauss.** *Justus-Liebig-Univ., Giessen, Germany.*

4 **8.4** Exercise Training Improves ENaC-mediated Sodium Regulation in Rats with Chronic Heart Failure. **H. Zheng, X. Liu, N. Sharma and K. Patel.** *Univ. of Nebraska Med. Ctr.*

5 **8.5** Dependence of ENaC Recycling Rate on the Total Amount of Recycled Channels. **A. Taruno and Y. Marunaka.** *Kyoto Prefectural Univ. of Med., Japan.*

6 **8.6** Effects of Cytochrome P450 Metabolites of Arachidonic Acid on ENaC. **D. Ilatovskaya, T. Pavlov, V. Levchenko, R. J. Roman and A. Staruschenko.** *Med. Coll. of Wisconsin and Univ. Mississippi Med Ctr.*

7 **8.7** Key Role for the Cortical Actin Binding Protein Cortactin in Regulation of ENaC by the Actin Cytoskeleton. **D. Ilatovskaya, T. Pavlov, V. Levchenko, Y. Negulyaev and A. Staruschenko.** *Med. Coll. of Wisconsin and Inst. of Cytology RAS, St. Petersburg, Russian Fed.*

8 **8.8** Mechanisms of ENaC Regulation by Insulin. **T. Pavlov, V. Levchenko, C. Ecelbarger and A. Staruschenko.** *Med. Coll. of Wisconsin and Georgetown Univ.*

9 **8.9** Nephron-specific Gene Inactivation of α and γ ENaC in Adult Mouse: Distinct Effects on Na+ and K+ Balance Hood Leads to a Severe Disturbance of Na+ and K+ Balance. **R. Perrier, E. Boscardin, R. Koesters, B. C. Rossier and E. Hummler.** *Univ. of Lausanne, Switzerland and Univ. of Heidelberg, Germany.*

Board #

10 **8.10** Modulation of ENaC-mediated Na^+ Absorption by Th2-dependent Airway Inflammation in Mice. **J. Duerr, P. Anagnostopoulou, L. Dai, J. Schatterny, S. Hirtz and M. A. Mall.** *Univ. of Heidelberg, Germany.*

11 **8.11** Withdrawn.

12 **8.12** Dissection of the Aldosterone and Glucocorticoid-dependent Pathway Implicated in Sodium Retention in the Rat. **V. Ponce de Leon, J. Canonica and E. Hummler.** *Univ. of Lausanne, Switzerland.*

13 **8.13** Involvement of p38-mediated Endocytosis in Aldosterone-stimulated Na^+ Reabsorption in Renal Epithelial A6 Cells. **N. Niisato, M. Ohta and Y. Marunaka.** *Kyoto Prefectural Univ. of Med., Japan.*

14 **8.14** The Renal Na+-Cl- Co-transporter is Regulated by the Aldosterone-SGK1-Nedd4-2 Pathway. **D. Lagnaz, J. P. Arroyo, C. Ronzaud, G. Gamba and O. Staub.** *Univ. of Lausanne, Switzerland and Inst. Natl. de Ciencias Medicas & Nutrition, Mexico City, Mexico.*

15 **8.15** A Salt Losing Phenotype of the Sgk1 Inducible Kidney Specific Knock-out Mouse. **N. Faresse, A. Ismailji, A. Naray-Fejes-Toth and O. Staub.** *Univ. of Lausanne, Switzerland and Dartmouth Med. Sch.*

16 **8.16** The Role of Renal Mineralocorticoid Versus Glucocorticoid Receptor in Oedematous Diseases. **J. Canonica, V. Ponce de Leon, F. Frey and E. Hummler.** *Univ. of Lausanne, Switzerland and Univ. of Bern, Switzerland.*

17 **8.17** Identification of Proteins Regulated by 24-hour Aldosterone Treatment in Murine Late Distal Convoluted Tubules and Connecting Tubules. **J. Praetorius, U. B. Jensen, R. A. Fenton, H. A. Praetorius, J. D. Hoffert, T. Pisitkun, M. A. Knepper and T. B Jensen.** *Aarhus Univ. Denmark and NIH, NHLBI.*

18 **8.18** Modulation of the Epithelial Sodium Channel (ENaC) Activity by Norepinephrine in Cultured Collecting Duct Cells is Partially Mediated by α_2-Adrenoceptors. **M. Mansley, M. Bertog and C. Korbmacher.** *Friedrich-Alexander-Univ. Erlangen-Nürnberg, Germany.*

19 **8.19** Deregulation of ENaC, Respiratory Distress and Perinatal Lethality in Nedd4-2 Deficient Mice. **N. Boase, G. Rychkov, S. Townley, A. Voss and S. Kumar.** *SA Pathology, Australia,Univ. of Adelaide, Australia and Walter & Eliza Hall Inst. Med. Res. Melbourne, Australia.*

20 **8.20** Identification of Permissive Insertion Sites for Generating Functional Fluorescent Mineralocorticoid Receptors. **D. Alvarez de la Rosa, C. Aguilar, I. Hernandez-Diaz, F. Lorenzo-**

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Diaz and T. Giraldez. *Univ. of La Laguna, Spain, Hosp. Univ. Ntra. Sra. de Candelaria, Spain.*

21 **8.21** Potassium Diet, Hypertension, and Remodeling of the Distal Nephron. **H. Ehmke, M. Müller, L. Schulte, A. Seniuk and H. Vitzthum.** *Univ. Med. Ct., Hamburg, Germany.*

22 **8.22** Aldosterone Independent Activations of MR-Sgk1-ENaC and Tubular Renin Angiotensin Systems in Sodium Sensitive Hypertension in Mice. **H. Yamana, T. Ishigami, S. Minegishi and S. Umemura.** *Yokohama City Univ. Grad. Sch. of Med., Japan.*

23 **8.23** Angiotensin Receptor Activation Contributes to Impaired Renal Insulin Receptor Phosphorylation, Increased γ ENaC Cleavage and Volume-dependent Hypertension in Insulin Resistant OLETF Rats. **S. Balayan, R. Rodriguez, J. Viscarra, D. Nakano, A. Nishiyama, M. S. Awayda and R. M. Ortiz.** *Univ. of California, Merced, Kagawa Univ. Japan and State Univ. of New York at Buffalo.*

24 **8.24** Physiological Modulation of Urinary Prostasin in Normotensives Individuals. **A. Castagna, L. Chiecchi, F. Pizzolo, K. Kitamura, R. Raffaelli, M. Gunasekaran, G. L. Salvagno, P. Guarini and O. Olivieri.** *Univ. of Verona, Italy and Univ. Kumamoto, Japan.*

25 **8.25** Extra Cellular Potassium Modulated Aldosterone Secretion in Relation to Hypertensive States. **M. Egfjord, R. Dreier and J. Hofman-Bang.** *Rigshospitalet, Copenhagen, Denmark.*

26 **8.26** Differences Among Renin-angiotensin System Blockade for the Target Organ Damage and Angiotensin II Induced SCN5A-Nedd4L Activation with Mineralocorticoid Receptor Transactivaion in the Model of Sodium Sensitive Hypertension. **S. Minegishi, T. Ishigami, H. Yamana and S. Umemura.** *Yokohama City Univ. Grad. Sch. of Med., Japan.*

27 **8.27** Aldosterone Deficiency During Pregnancy in Mice does not Lead to Preeclampsia but Results in Placenta Dysfunction, Reduced Litter Size, and Smaller Pups. **A. Todkar, M. Di Chiara, D. Loffing-Cueni, C. Bettoni, N. Makhanova, O. Smithies, J. Loffing and C. Wagner.** *Univ. of Zurich, Switzerland and Univ. of North Carolina.*

28 **8.28** Recovery of Endothelium-dependent Vasodilation by Acute Inhibition of Epithelial Sodium Channel (ENaC) in Rats made Hypertensive by Angiotensin II. **M. Boric, N. Soto, M. Beltran, L. Leon, E. Sepulveda, M. Marquez and X. Figueroa.** *P. Univ. Catolica de Chile, Santiago,Chile.*

29 **8.29** Functional Assessment of the Epithelial Sodium Channel (ENaC) in the Rat Heart. **M. Boric, R. Varas, E. Sepulveda-Kattan, F. Venegas, F. Pérez and L. Michea.** *P. Univ. Catolica de Chile, Santiago, Chile; Univ. de Chile, Santiago, Chile.*

30 **8.30** αENaC Polymorphisms Alter ENaC Current. **U. C. Emerenini and P. Snyder.** *Univ. of Iowa.*

WEDNESDAY, SEPTEMBER 21, 2011

Symposia V

9.0 REMEMBERING J. D. HORISBERGER AND D. J. BENOS

Wednes., 8:00-9:00 AM, Chapel Room.

8:00 AM **9.1** Remembering Jean-Daniel Horisberger. **Laurent Schild.** *Univ. of Lausanne, Switzerland.*

8:20 AM **9.2** Remembering D. J. Benos. **Catherine Fuller.** *Univ. of Alabama at Birmingham.*

Oral Presentations I

10.0 ABSTRACT-BASED PRESENTATIONS

Wednes., 8:45 AM-12:00 Noon, Chapel Room.

Co-Chairs: **Thomas Kleyman,** *Univ. of Pittsburgh* and **David Pearce,** *Univ. of California, San Francisco.*

8:45 AM **10.1** Three COMMD Family Proteins are Located in Collecting Duct Cells and Regulate ENaC. **Fiona McDonald Cowles.** *Univ. of Otago, Dunedin, New Zealand.*

8:56 AM **10.2** A Model of Partnership Co-opted by Tsg101 and USP2-45 for the Negative Feedback Loop of the Mineralocorticoid Receptor Pathway. **Nourdine Faresse.** *Univ. of Lausanne, Switzerland.*

9:08 AM **10.3** Analysis of Two Spontaneous Mouse and Rat CAP1/Prss8 Mutations. **Simona Frateschi.** *Univ. of Lausanne, Switzerland.*

9:20 AM **10.4** Wrist Domain of the Epithelial Sodium Channel Modulates Channel Gating. **Shujie Shi.** *Univ. of Pittsburgh.*

9:32 AM **10.5** Mechanism of Interaction and Inhibition of ENaC Activity by SPLUNC1 Peptides. **Carey Hobbs.** *Univ. of North Carolina, Chapel Hill.*

9:43 AM **10.6** Steroid Regulation of the ENaC Recycling Pathway: A Proteomic Analysis. **Robert Edinger.** *Univ. of Pittsburgh.*

9:54 AM Break

10:25 AM **10.7** Possible Vascular ENaC Inhibition by Amiloride in Young Overweight Prehypertensives. **Yanbin Dong.** *Georgia Hlth. Sci. Univ.*

10:36 AM **10.8** Polyclonal Antibodies Against Epithelial Sodium Channel (ENaC) Subunits Reveal Distinct Patterns of ENaC Expression and Subcellular Localization in Human Tissues. **Yehoshua Enuka.** *Ariel Univ. Ctr., Israel.*

10:47 AM **10.9** Energetic and Structural Basis for Activation of ENaC by CAP-3. **Pradeep Kota.** *Univ. of North Carolina, Chapel Hill.*

10:58 AM **10.10** Aldosterone is Dispensable for Renal but not for Colonic Regulation of Potassium Homeostasis. **Abhijeet Todkar.** *Univ. of Zurich, Switzerland.*

11:09 AM **10.11** Cross-talk of the Small GTPase Rac1 and Mineralocorticoid Receptor Cascades in Cardiorenal Disease. **Miki Nagase.** *Univ. of Tokyo Grad. Sch. of Med. Japan.*

11:20 AM **10.12** A Novel Redox-sensitive E3-Ubiquitin Ligase Regulates Surface Expression of Epithelial Sodium Channels in Alveolar Epithelial Cells. **Amrita Kumar.** *Emory Univ.*

11:32 AM **10.13** NADPH Oxidase and ENaC. **My Helms.** *Emory Univ.*

11:45 AM **10.14** ENaC Regulation by Cell Surface Associated SGK1. **Alan Chun-Yao Pao.** *Stanford Univ.*

Career Workshop

11.0 **THE *INS* AND *OUTS* OF AUTHORSHIP**

Wednes., 3:00-4:00 PM, Chapel Room.

Chair: **Thomas Schmidt,** *Univ. of Iowa.*

3:00 PM **11.1** The *Ins* and *Outs* of Authorship. **Thomas Schmidt.** *Univ. of Iowa.*

Symposia VI

12.0 **CONGESTIVE HEART FAILURE: THE INTERTWINED ROLES OF WATER AND SALT**

Wednes., 4:00-4:50 PM, Chapel Room.

Chair: **David Pearce,** *Univ. of California, San Francisco.*

4:00 PM **12.1** Potential Future Role of Mineralocorticoid Receptor Blockade in Patients with Heart Failure. **Bertram Pitt.** *Univ. of Michigan.*

4:25 PM **12.2** Role of Vasopressin in the Water Retention in Congestive Heart Failure- Pathogenesis and Treatment. **Tomas Berl.** *Univ. of Colorado, Denver.*

Plenary Lecture

13.0 **PLENARY LECTURE**

Wednes., 5:00-6:00 PM, Chapel Room.

5:00 PM **13.1** ASIC Structure and Function. **Michael Welsh.** *Univ. of Iowa, HHMI.*

THURSDAY, SEPTEMBER 22, 2011

Symposia VII

14.0 **ENaC PATHOPHYSIOLOGY**

Thurs., 8:00-10:00 AM, Chapel Room.

Chair: **Bernard C. Rossier,** *Univ. of Lausanne, Switzerland.*

8:00 AM **14.1** Evolution of ENaC and Na,K-ATPase as Limiting Factors of Aldosterone Action. **Bernard C. Rossier.** *Univ. of Lausanne, Switzerland.*

8:20 AM **14.2** The Role of βENaC in Renal Vascular Function. **Heather Drummond.** *Univ. of Mississippi Med. Ctr. Jackson.*

8:40 AM **14.3** Regulation of ENaC and Airway Surface Liquid Volume by SPLUNC1. **Robert Tarran.** *Univ. of North Carolina, Chapel Hill.*

9:00 AM **14.4** Expression and Function of a Cross Clade ASIC/ENaC Channel in Glioblastoma. **Catherine Fuller.** *Univ. of Alabama at Birmingham.*

9:20 AM **14.5** Role of ENaC in the Regulation of Airway Surface Liquid Volume and Pathogenesis of Lung Disease. **Richard Boucher.** *Univ. of North Carolina.*

9:40 AM **14.6** Plasmin, ENaC and Nephrotic Syndrome. **Ole Skøtt.** *Univ. of Southern Denmark, Odense, Denmark.*

10:00 AM Break

Symposia VIII

15.0 **ALDOSTERONE PATHOPHYSIOLOGY**

Thurs., 10:30 AM-12:00 Noon, Chapel Room.

Chair: **Eleanor Davies,** *Univ. of Glasgow, UK.*

10:30 AM **15.1** The Ups and Downs of Aldosterone Biosynthesis: Regulation of Aldosterone Synthase Expression by MicroRNAs. **Eleanor Davies.** *Univ. of Glasgow, UK.*

10:50 AM **15.2** Mineralocorticoid Receptor Mutations in Human Disease. **Maria-Christina Zennaro.** *INSERM, Paris, France.*

11:10 AM **15.3** End-organ Damage in Primary Aldosteronism: Response to Treatment and Comparison with Essential Hypertension. **Leonardo Sechi.** *Univ. of Udine, Italy.*

11:30 AM **15.4** Cardiomyocyte MR Signaling is Essential for DOC/Salt-mediated Cardiac Fibrosis and Blood Pressure Regulation. **Amanda Rickard.** *Prince Henry's Inst. of Med. Res. Melbourne, Australia.*

11:42 AM **15.5** Aldosterone Producing Adenoma Formation Involves Expression of Stem/progenitor Cell Markers. **Sheerazed Boulkroun.** *INSERM, Paris, France.*

2011 APS Conference
7th International Symposium on Aldosterone and the ENaC/Degenerin Family of Ion Channels: Molecular Mechanisms and Pathophysiology

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3.0 Structure and Function of Mineralocorticoid and Glucocorticoid Receptors……...14

4.0 ENaC Structure and Regulation……...15

5.0 Regulation of ENaC Biogenesis, Trafficking and Gating……19

6.0 Aldosterone: Synthesis Crosstalk and Non-Epithelial Actions……21

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2.0: STRUCTURE AND FUNCTION OF ENaC AND RELATED TRANSPORTERS

2.2

REGULATION OF ENAC EXPRESSION IN RAT KIDNEY

Lawrence Palmer[1], Gustavo Frindt[1]
[1]Physiology & Biophysics, Weill-Cornell Med. Coll., 1300 York Ave., New York, NY, 10065.

The activity of epithelial Na channels (ENaC) in the apical membrane of the CCD and CNT is strongly and inversely dependent on dietary Na, an effect mediated at least in part by the mineralocorticoid aldosterone. The surface expression of cleaved forms of the γENaC subunit was the biochemical parameter best correlated with channel function. Even under conditions of high-salt intake, with no measurable channel activity, significant expression of βENaC and uncleaved γENaC were observed at the surface of the cell. We further examined the nature of this expression using the deglycosylating enzymes PNGaseF and endoH. Cleaved forms of γENaC at the surface were sensitive to PNGaseF but not to endoH, indicating that these forms were fully processed on their way to the surface. In contrast, the full-length form of γENaC was sensitive to endoH, suggesting that it was incompletely processed. In salt-replete rats, much of the βENaC at the surface was also endoH sensitive. However in salt-depleted animals much of the βENaC became endoH resistant. The increase in endoH-resistant βENaC at the cell surface correlated well with that of cleaved, endoH-resistant γENaC. We conclude that ENaC can reach the surface in both mature and immature forms, possibly involving two routes to the apical membrane. The mature form of the protein represents the fully active channel, and its delivery to the membrane is stimulated by Na depletion. NIH DK59659. Reference: Frindt, G, Ergonul, Z. and Palmer, L.G Surface expression of epithelial Na channel protein in rat kidney. J. Gen. Physiol. 131:617-627 (2008).

2.3

ENaC REGULATION IN THE CONNECTING TUBULE

Johannes Loffing[1]
[1]Inst. of Anatomy, Univ. of Zurich, Winterthurerstrasse 190, Zurich, 8057, Switzerland.

The renal connecting tubule (CNT) is part of the aldosterone-sensitive distal nephron (ASDN) and connects the late distal convoluted tubule (DCT2) with the collecting duct (CD). A variety of recent ion transport, electrophysiological and morphological studies on various animal models indicated that the CNT is of particular importance for the regulation of renal Na^+ and K^+ excretion and hence for the long-term control of extracellular fluid and ion homeostasis. Na^+ reabsorption in the CNT depends on the activity of the apical amiloride-sensitive epithelial sodium channel (ENaC), which does also provide the electrochemical driving force for K^+ secretion via luminal K^+ channels such as ROMK. Given its crucial role for renal Na^+ and K^+ transport, ENaC is the main target for hormones (e.g. aldosterone, angiotensin II) and non-hormonal factors (e.g. tubular flow), which adjust transepithelial Na^+ and K^+ transport along this segment to the homeostatic needs. The presentation will highlight the structural and functional properties of the CNT that contribute to its high Na^+ and K^+ transport capacity. Moreover, some of the cellular and molecular mechanisms that contribute to ENaC regulation along the CNT *in vivo* will be discussed. Reference: Loffing J, Korbmacher C: Regulated sodium transport in the renal connecting tubule (CNT) via the epithelial sodium channel (ENaC). Pflugers Arch. 458: 111-35 (2009).

2.4

STRUCTURAL INSIGHTS INTO THE REGULATION OF ENaCS BY EXTERNAL NA

Shaohu Sheng[1]
[1]Medicine, Univ. of Pittsburgh, S929 Scaife Hall, 3550 Terrace St., Pittsburgh, PA, 15261.

High concentrations of extracellular Na^+ reduce ENaC Po via a mechanism referred to as Na^+ self-inhibition. Many extracellular factors including cations, anions, proteases, temperature and small molecules regulate ENaC Po, in part, by modulating the Na^+ self-inhibition response. The *Xenopus* oocyte expression system has provided a convenient tool to characterize and probe the structural basis of Na^+ self-inhibition. The crystal structure of an ENaC/DEG member, ASIC1, also provides an invaluable tool to interpret results from mutagenesis studies and to develop hypotheses that drive future studies. The structural basis of Na^+ self-inhibition remains unclear. However, site-directed mutagenesis studies have implicated several extracellular structures in Na^+ self-inhibition, including the finger, thumb and palm subdomains, as well as the pore domains. While all cloned ENaCs exhibit Na^+ self-inhibition, the three ENaC subunits appear to have distinct roles in the process with the γ subunit being the most important. We speculate that Na^+ self-inhibition serves as a central mechanism mediating the effects of specific channel regulators. Elucidating the allosteric mechanism of Na^+ self-inhibition may provide clues for development of novel therapeutic agents. (Support: NIH R01 ES014701, R01 DK054354, and P30 DK079307). Reference: Winarski KL, Sheng N, Chen J, Kleyman TR, Sheng S. Extracellular allosteric regulatory subdomain within the gamma subunit of the epithelial Na^+ channel. J Biol Chem. 2010; 285: 26088-96.

2.5

STRUCTURAL TRANSITIONS ASSOCIATED WITH THE GATING OF ASIC1a

Marcelo Carattino[1]
[1]Dept. of Med., Univ. of Pittsburgh, 3550 Terrace St., S828 Scaife Hall, Pittsburgh, PA, 15261.

Acid-sensing ion channels are trimeric proton-gated cation selective channels expressed in the nervous system. Proton binding to the extracellular region of these channels triggers activation and subsequently desensitization. The ectodomain is linked to the pore forming transmembrane helices by short loops. We previously found that the loop preceding the second transmembrane (TM2) domain of ASIC1a experiences a conformational rearrangement following extracellular acidification that is associated with activation. Residues in the TM2 helices define the ion conductive pathway. We have now found that MTS reagents directly activate quiescent G428C channels in the absence of a change in extracellular pH. Gly428 is located in the outer vestibule of the pore one helical turn above the desensitization gate. Mutant cycle analysis suggested that steric repulsion between the MTSET-modified Cys at position 428 and Tyr424 in the loop preceding the TM2 domain initiates a conformational change that triggers pore opening. Moreover, Y424C-G428C migrate under non-reducing conditions as a homo-trimer stabilized by intersubunit disulfide bonds. These channels have parameters of activation and desensitization similar to wild type channels, suggesting that intersubunit 424-428 disulfide bonds do not restrict conformational changes associated with activation and desensitization. Our studies suggest that a simultaneous rotation of the TM2 helices in the clockwise direction (from the extracellular view) mediates pore opening. References: Passero CJ, Okumura S, Carattino MD. Conformational changes associated with proton-dependent gating of ASIC1a. J Biol Chem 284:36473-81, 2009. Tolino LA, Okumura S, Kashlan OB, Carattino MD. Insights into the mechanism of pore opening of acid-sensing ion channel 1A. J Biol Chem 286: 16297-307, 2011.

2.6

MECHANISMS OF pH-DEPENDENT GATING OF ASICS

Stephan Kellenberger[1]
[1]Dept. of Pharmacology and Toxicology, Univ. of Lausanne, Rue du Bugnon 27, Lausanne, CH-1005, Switzerland.

ASICs are activated by a lowering of the extracellular pH. Initial analysis of the crystal structure suggested an acidic pocket in the thumb domain as a candidate pH sensor. In order to determine whether other parts of the channel protein also contribute to pH sensing in ASIC1a we have applied a systematic approach. We calculated the pK_a of all extracellular His, Glu and Asp residues using a Poisson-Boltzmann continuum approach based on the ASIC 3D structure. The role of residues with a pK_a in the pH range of ASIC gating was then assessed by site-directed mutagenesis and functional analysis. The localization of putative pH-sensing residues suggests that pH changes control ASIC gating by protonation / deprotonation of many residues per subunit in different channel domains, thus that protons act differently from larger ligands which bind to a low number of distinct binding sites. Interestingly, many of the putative pH-sensing residues participate in both activation and inactivation. Analysis of the function of residues in the palm domain close to the central vertical axis of the channel allowed for prediction of conformational changes of this region during gating. Based on this work and on studies by other groups we conclude that different domains contribute to pH-dependent gating of ASICs, with the thumb and the lower palm domains playing likely the most important roles. The parts of the protein that link the pH-sensing domains to the channel gate are also critical for ASIC function and may be additional potential drug target sites. (Swiss NF grant 310030_135542).

2.7

MECHANISMS AND PHYSIOLOGICAL IMPORTANCE OF ENaC REGULATION BY GROWTH FACTORS AND SMALL GTPASES

Alexander Staruschenko[1]
[1]Physiology, Med. Coll. of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI, 53226.

Long term control of blood pressure involves Na^+ homeostasis through the precise regulation of ENaC in the ASDN. EGF and its related EGF-family members bind to ErbB receptors and act as signaling factors responsible for renal development, physiology and pathophysiology. Under physiological conditions, ErbB receptors play an important role in the regulation of renal hemodynamics and electrolyte handling by the kidney, while in different pathophysiological states ErbB activation may mediate either beneficial or detrimental effects on the kidney. Stimulation of ErbB receptors activates an intracellular cascade involving small GTPases, particularly Rac1. Small G proteins and their regulatory proteins contribute to the pathology of renal and cardiovascular diseases. We demonstrate that ENaC is regulated by EGF and Rac1, possibly through a convergent mechanism. Dahl salt-sensitive (SS) rats used in these studies develop severe hypertension on high-salt diet. We provide data indicating that ENaC contributes to the development of hypertension in the SS rat strain. Furthermore, our data reveal that EGF concentration is reduced in the SS rats, which we propose would enhance ENaC activity. We hypothesize that EGF acting through Rac1 is important for physiologic control of renal sodium handling through regulation of ENaC. REFERENCES: 1) Karpushev A., Levchenko V., Ilatovskaya D., Pavlov T., Staruschenko A. (2011) Novel role of Rac1/WAVE signaling mechanism in regulation of the epithelial Na^+ channel (ENaC). *Hypertension* 57: 996-1002. 2) Levchenko V., Zheleznova N., Pavlov T.S., Vandewalle A., Wilson P.D. Staruschenko A. (2010). EGF and its related growth factors mediate sodium transport in mpkCCD_{c14} cells via ErbB2 (neu/HER-2) receptor. *J Cell Physiol* 223(1): 252-259.

2.8

HYPOTONICITY-INDUCED UPREGULATION OF β- AND γ-ENaC EXPRESSION THROUGH SUPPRESSION OF ERK BY INDUCING MKP-1

Yoshinori Marunaka[1], Naomi Niisato[1], Mariko Ohta[1]
[1]Dept. of Molecular Cell Physiology, Kyoto Prefectural Univ. of Med., Kamigyo-ku, Kyoto, 602-8566, Japan. [1]Japan Inst. for Food Edu. and Hlth, St. Agnes' Univ., Kyoto 602-8013, Japan.

We studied a physiological role of ERK in stimulatory action of hypotonicity on ENaC-mediated Na^+ reabsorption in renal epithelial A6 cells, and obtained the following observations: 1) Hypotonicity dephosphorylated ERK after transient phosphorylation; 2) PD98059 (a MEK inhibitor) dephosphorylating ERK enhanced the stimulatory action of hypotonicity; 3) Hypotonicity increased expression of MKP-1 mRNA by activating p38, while inhibition of MKP-1 by NSC95397 (an MKP-1 inhibitor) suppressed the dephosphorylation of ERK; 4) Inhibition of p38 suppressed MKP-1 induction, preventing hypotonicity from dephosphorylating ERK; 5) NSC95397 suppressed the stimulatory action of hypotonicity on mRNA expression of both β- and γ-ENaC and ENaC-mediated Na^+ reabsorption; 6) PD98059 enhanced mRNA expression of β- and γ-ENaC even under an isotonic condition, but did not stimulate Na^+ reabsorption under an isotonic condition, suggesting that the ERK inactivation under an isotonic condition is not an enough signal stimulating Na^+ reabsorption, although ERK inactivation enhances β- and γ-ENaC mRNA expression. These observations suggest that hypotonicity has at least two signaling pathways stimulating Na^+ reabsorption: 1) ERK activity suppression inducing β- and γ-ENaC mRNA expression via MKP-1 induction, and 2) promotion of the newly synthesized ENaC translocation to the apical membrane. Supported by JSPS Scientific

Research (20390060), The Salt Science Research Foundation (1035), Research Conference for Cell Function, and Fuji Foundation for Protein Research.

2.9

A SYNTHETIC SERINE PROTEASE INHIBITOR CAMOSTAT MESILATE INHIBITED THE PROTEOLYTIC ACTIVATION OF γENaC IN THE KIDNEY OF ALDOSTERONE-INFUSED RATS

Kimio Tomita[1]
[1]Dept.. of Nephrology, Kumamoto Univ., Honjo 1-1-1, Kumamoto, 860-8556, Japan.

ENaC consists of α, β, and γ subunits, and the activation of ENaC is mainly regulated by aldosterone in living body. It was reported that aldosterone induced a molecular weight shift of γENaC from 85 to 70 kDa, and recently this shift has been considered as the result of proteolytic cleavages by serine proteases and necessary for the activation of ENaC from the in vitro experiment. But detail mechanisms about the cleavage of γENaC in vivo are still unclear. In order to study the role of serine proteases in this cleavage in vivo, we administrated a synthetic serine protease inhibitor camostat mesilate to aldosterone-infused rats. Camostat decreased 70kDa form of γENaC and produced the new about 75kDa form with increase of urinary Na/K ratio, suggesting that camostat inhibited one site of the dual cleavages of γENaC and suppressed the activation of ENaC. Prostasin is one candidate serine protease involved in the cleavage of γENaC in these model rats, because prostasin was shown to cleave this subunit in vitro and its excretion into urine was increased by aldosterone. Camostat inhibited protease activity, activating processing and urinary secretion of prostasin. These results suggest that prostasin is one important serine protease in the pathogenesis of aldosterone-induced salt sensitive hypertension. A synthetic serine protease inhibitor, camostat mesilate, would be a new strategy in the treatment of salt-sensitive hypertension in human. REFERENCE: Narikiyo T., et al. Regulation of prostasin by aldosterone in the kidney. J. Clin. Invest.,109:401-408, 2002.

2.10

LESSONS LEARNED FROM KNOCKOUT STUDIES

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The amiloride sensitive epithelial Na+ channel (ENaC) is an important modulator of Na+ homeostasis, and thereby plays a critical role in regulating blood pressure, renal function and fluid balance in the lung. ENaC distribution is widespread and has been found in a variety of epithelia, including sweat glands, epidermis and taste cells. Mice lacking ENaC expression die soon after birth due to failure to clear the lungs of liquid, but also show severe skin dehydration. Furthermore, these animals present metabolic acidosis with lower blood pH and low bicarbonate concentrations, suggesting a metabolic component added to the probable respiratory acidosis. Tissue-specific and inducible knockout mice are valuable tools to validate and to define the importance of pathways and proteins that are implicated in ENaC-mediated Na+ transport and blood pressure regulation. This approach is also valid to test candidate genes that have been identified but tested so far only in cell culture systems, like some channel activating proteases. We recently identified CAP1/Prss8 as upstream activator of the protease-activated receptor 2 that may well contribute to the regulation of Na absorption in the kidney. Transgenic mice will help to define their role in the kidney and other tissue and organs. They serve as mammalian models of human diseases and later on to validate drug targets. References : Frateschi S, Camerer E, Crisante G, Rieser S, Membrez M, Charles R-P, Beermann F, Stehle J-C, Breiden B, Sandhoff K, Rotman S, Haftek M, Wilson A, Ryser S, Steinhoff M, Coughlin S., Hummler E. PAR2 absence completely rescues inflammation and ichthyosis caused by altered CAP1/Prss8 expression in mouse skin. Nature Comm. 2011, 2 ; 161.

2.11

CONFORMATIONAL TRAPPING OF THE CLOSED STATE OF ENaC

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Atypical among ion channels, the epithelial Na+ channel (ENaC) is proteolytically processed during biogenesis. ENaC is assembled from three homologous subunits, and it is the alpha and gamma subunits which are susceptible to proteolysis. Cleavage at two sites in either subunit releases an inhibitory tract and activates the channel. Peptides corresponding to either released tract are inhibitory. Our recent mutagenesis and double mutant cycle data implicated sites at the finger-thumb domain interface in inhibitory peptide binding. Based on these data and on homology to ASIC1, we built a model of the alpha subunit of ENaC. Our model predicts that specific sites of the channel are in close proximity to either end of the bound inhibitory peptide. We hypothesized that we could covalently bind the peptide to the channel after introducing Cys at sites predicted to be in close proximity in the bound state on both the channel and the peptide. We observed length specific crosslinks between specific sites in the finger and thumb, with one end of the inhibitory peptide. We also hypothesized that a crosslink between sites in the channel may trap ENaC in an open or closed conformation, if the sites involved move relative to one another during gating. We found that crosslinks between specific sites in the finger and thumb domains of the alpha subunit constrain the channel in a closed conformation. Our data suggest that movement at a finger-thumb interface has an important role in modulating channel activity. Supported by DK065161 and DK078734.

2.12

THE EXTRACELLULAR DOMAIN OF ENaC IS A SENSOR OF THE EXTRACELLULAR MILIEU

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The epithelial Na^+ channel (ENaC) functions as a pathway for Na^+ absorption across epithelia of the kidney and lung. In order to maintain homeostasis, ENaC activity must vary over a wide range. With volume depletion, ENaC activity increases to maximize Na^+ reabsorption. Conversely, ENaC activity is reduced in response to volume excess. Under these conditions, ENaC is exposed to extreme changes in extracellular ion concentrations. For example, Na^+ and Cl^- can range from 1-150 mM. pH can vary from 4.5-8 in response to metabolic acidosis and alkalosis as well as with changes in diet and volume status. Extracellular Na^+ is known to inhibit ENaC through a process known as Na^+ self-inhibition. We found that protons increase ENaC activity by reducing Na^+ self-inhibition. This occurs through the titration of residues in the extracellular domains of β- and γENaC. Extracellular Cl^- inhibits ENaC over the physiologically relevant concentration range (half-maximal at 30 mM). This occurs through binding of Cl^- to sites located at the interfaces between α- and βENaC and between β- and γENaC. At the α-β interface, Cl^- inhibits ENaC through enhanced Na^+ self-inhibition, whereas Cl^- inhibits ENaC through a Na^+-independent mechanism at the β-γ interface. Together, the data support a model in which the large highly structured extracellular domain functions as a sensor to modulate ENaC activity to respond to extreme changes in its environment. Support: HL072256 (NIH) to PMS and 10PRE2610282 (AHA) to DMC.

2.13

THE ER LUMENAL CHAPERONE, LHS1/GRP170, PLAYS A UNIQUE ROLE IN THE BIOGENESIS OF THE EPITHELIAL SODIUM CHANNEL

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The Epithelial Sodium Channel (ENaC) is composed of three homologous subunits – α, β, and γ – that assemble in the endoplasmic reticulum (ER) to form the mature ENaC channel. However, heterotrimer formation is highly inefficient, thus targeting the channel subunits for Endoplasmic Reticulum Associated Degradation (ERAD). To characterize the mechanism of ENaC degradation, a yeast ENaC expression system was developed. Using this system, we found that Jem1 and Scj1, two ER lumenal Hsp40 chaperones, play an essential role in ENaC degradation. Expression of the human Hsp40 homologs similarly increased ENaC degradation in a Xenopus oocyte expression system. Jem1 and Scj1 facilitate the ATP hydrolysis of the ER lumenal Hsp70 chaperone, BiP, although surprisingly BiP does not play a role in ENaC degradation. Therefore, we hypothesized that another ER chaperone with an Hsp70-like domain, Lhs1, may be involved. We found that Lhs1 is involved in αENaC subunit degradation, but not the degradation of the β- or γENaC subunits. Surprisingly, Lhs1 ATPase activity is dispensable for ENaC degradation. This result further supports our conclusion that the BiP cochaperones, Jem1, Scj1 and Lhs1, are acting in a BiP independent fashion. Preliminary data indicate the human Lhs1 homolog, GRP170, also affects ENaC biogenesis in oocytes. In conclusion, we have identified a new class of ER chaperones as novel effectors of αENaC biogenesis. Funded in part by K01DK090195 to T.M.B. and DK79307 to T.R.K and J.L.B.

2.14

REGULATION OF THE EPITHELIAL NA+ CHANNEL BY INTRACELLULAR NA^+: MECHANISM AND TIME COURSE

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Feedback inhibition of the epithelial Na^+ channel (ENaC) by cell Na^+ occurs by regulating both channel surface expression and open probability. We investigated the time course and mechanism of this regulation using two-electrode voltage clamp to measure ENaC activity and biotin labeling to examine cell surface expression. Incubation of rENaC-expressing *Xenopus* oocytes in high-Na^+ buffer decreased ENaC currents by 70% over 80 minutes but had little effect on oocytes expressing Liddle's mutant (βR564X). Western blots showed little to no change in cell-surface γ ENaC of WT or βR564X with 1-1.5 hour high-Na^+ incubation. Overnight incubation in high-Na^+ caused a significant decrease in ENaC currents and cell-surface γ ENaC in both WT- and βR564X-expressing oocytes. There was no change in cell surface expression of β ENaC during 1-1.5 hour or overnight high Na^+ incubation suggesting that cell surface expression of the β and γ subunits of ENaC are differentially regulated. In the presence of Brefeldin A, high-Na^+ incubation decreased ENaC currents by 82% over 8 hours, indicating that cell Na^+ stimulates retrieval of active channels from the surface. Decreased surface expression of cleaved γ ENaC but not total γ or β ENaC suggests that cleaved γ ENaC is preferentially retrieved with high-Na^+ incubation. This work was funded by NIH grants DK278847 and DK59659 and NIH MSTP grant GM07739 (to Ankit Patel).

2.15

REVISITING THE SUBUNIT OLIGOMERIZATION OF ENaC

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The functional epithelial sodium channel (ENaC) is a heteromultimeric channel formed by three homologous α, β and γ subunits. Several evidences support a tetrameric structure of ENaC made of 2α, 1β and 1γ subunits (*Firsov D et al. 1998; Anantharam A. et al. 2007*). Recently the crystal structure, obtained from a homolog of ENaC, the acid-sensing ion channel ASIC1a, shows a homotrimeric channel (*Gonzales EB et al.2009*). The discrepancy between ENaC and ASIC1 stoichiometry motivated us to revisit the subunit oligomerization of ENaC. In Xenopus oocytes a unique population of functional His(6)ENaC channels made of αβγ subunits is expressed at the cell surface. *On Western blot, the ENaC subunits show typical* post-translation modifications associated with a functional ENaC channel. Copurification on *Ni-NTA* agarose of differentially tagged ENaC subunits show that two different α subunits copurified with β and γ subunits, indicating that ENaC is formed by more than one α subunit. No evidence could be found for ENaC complex made of more than 1β or 1γ subunit. The surface biotinylation of ENaC channel confirmed that the purified ENaC channel made of two different α-subunits. Finally the purified ENaC complex elutes on Sephadex G200 column in a fraction corresponding to a higher molecular weight of 350 KDa, than expected for a trimer. Our data suggest that ENaC is a heteromultimer made of 2 different α subunits, 1β, and 1γ subunits and its apparent molecular weight support a tetramer.

2.16

ACIDIC RESIDUES IN THE EXTRACELLULAR DOMAIN OF HUMAN α-, β-, AND γENaC CONTRIBUTE TO H^+ REGULATION OF CHANNEL ACTIVITY

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A growing body of evidence suggests that the extracellular domain of ENaC functions as a sensor that fine tunes channel activity in response to changes in the extracellular environment. We previously demonstrated that H^+ have dual effects on ENaC activity; they increase ENaC activity by decreasing Na^+ self-inhibition, and inhibit ENaC activity by increasing Cl^- inhibition. In the current work, we investigated the mechanisms by which H^+ stimulate ENaC. We found that rat ENaC is less sensitive to H^+ than human ENaC, an effect mediated by the γ subunit. Our strategy was to mutate acidic residues in the extracellular domain of human γENaC that are not conserved in rat γENaC. We expressed the mutant γENaC cDNAs (with wild type α- and βENaC) in *Xenopus* oocytes and tested the effect of pH changes on amiloride-sensitive Na^+ current (by TEVC at -60 mV). We identified a group of 7 residues in the extracellular domain of γENaC (D164, Q165, D166, E292, E335, H439, and E455) that, when individually mutated to Ala, decreased H^+ activation of ENaC. Intriguingly, mutating the residues equivalent to γE455 in αENaC (αK477) and βENaC (βE446) increased and decreased the response to acidic pH, respectively. Combining these seven mutations in γENaC with βE446A generated a channel that was not activated by acidic pH. The data demonstrate that residues in human β- and γENaC are required for regulation by pH. Supported by NIH HL072256 (PMS) and AHA 10PRE2610282 (DMC).

3.0: STRUCTURE AND FUNCTION OF MINERALOCORTICOID AND GLUCOCORTICOID RECEPTORS

3.1
STRUCTURE-FUNCTION RELATIONSHIPS IN THE MINER-ALOCORTICOID RECEPTOR AND INTERACTIONS WITH NOVEL TRAN-SCRIPTIONAL COREGULATORS

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The mineralocorticoid receptor (MR) differs from the other steroid receptors in that it responds to two physiological ligands, aldosterone and cortisol. In epithelial tissues, aldosterone selectivity is determined by the activity of 11β-hydroxysteroid dehydrogenase type II. In other tissues, including the heart and CNS, cortisol is the primary ligand for the MR; in some tissues cortisol is an antagonist. To understand the structural determinants of tissue and ligand-specific MR activation we have focused on interactions of the ligand-binding domain (LBD) with ligand, with the N-terminal domain and with putative co-regulatory molecules. Both agonist and antagonist binding has been characterised using chimeras between the human (h)MR LBD and both the glucocorticoid receptor and the zebra fish (z)MR together with molecular modelling. An interaction between the N-terminus and C-terminus/LBD (N/C-interaction) observed in the MR is aldosterone-dependent but is unexpectedly antagonised by cortisol and DOC in the hMR but not the zMR. Nuclear receptor mediated transactivation is critically dependent on, and modulated by, co-regulatory molecules. Yeast-2-hybrid screens with the MR LBD have identified proteins which interact in the presence of either aldosterone or cortisol but not both. These have been confirmed as coactivators of the full-length hMR in a transactivation assay. The successful identification of ligand-specific interactions of the MR may provide the basis for the development of novel MR ligands with tissue specificity. Support: NHMRC 1002559.

3.2
PHYSIOLOGIC ROLES OF MR REVEALED BY TISSUE SELECTIVE KNOCKOUTS

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To dissect the pathological role of the mineralocorticoid receptor (MR) in specific cell types of the cardiovascular system in the development of cardiovascular disease, we have generated transgenic mice containing tissue selective deletion of the MR. Comparison of responses in transgenic and wild type mice to models of cardiovascular disease have revealed novel roles for the MR in several different cell types of the heart and vasculature. We have shown that MR activation in monocytes/macrophages is required for cardiac tissue remodelling in DOC/salt and L-NAME(nitric oxide deficient)/salt models of disease. However, recruitment of macrophages to the heart remains unchanged in these models. Instead, the proinflammatory and tissue-reparative phenotype of the macrophages is altered suggesting a central role for macrophage MR in these disease models. Interestingly, systolic blood pressure increases in these models are also limited, although the degree of protection is model-dependent. More recent studies in cardiomyocyte MR null mice show that the cardiomyocyte MR plays an in-dependent role in the tissue remodelling process in addition to an important role in chemoattractant signalling for inflammatory cell types. These studies, taken together with data from endothelial cell MR null mice that will be presented, demonstrate a wide range of responses to MR signalling in the heart that are cell-type dependent. The differential expression of the specificity-conferring enzyme 11β HSD2 amongst these cell types suggests further layers of complexity in MR signalling by corticosteroid hormones in physiology and disease. Selective modu-lators of the MR that are specifically directed towards non-epithelial cardio-vascular tissue would increase tissue protection without the side effects associated with renal MR blockade. Moreover, activation of the MR by other ligands would argue for the use of MR antagonists in renal and cardiovascular protection even when plasma aldosterone is normal.

3.3
EPITHELIAL SODIUM CHANNEL (ENaC) IS A KEY MEDIATOR OF GROWTH HORMONE (GH)-INDUCED SODIUM RETENTION: PATHOPHYSIOLOGY OF VOLUME EXPANSION IN ACRO-MEGALIC PATIENTS

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GH excess in acromegalic patients is associated with volume expansion and hypertension but the underlying mechanisms remain unclear. We provided experimental evidence that GH exerts a direct stimulatory effect on ENaC-dependent transport (Kamenisky, Endocrinology 2008). Renal metabolic studies performed on acromegalic rats revealed a decreased natriuretic response to furosemide compared to controls, whereas amiloride induced increased natriuresis. We showed an enhanced cleavage of αsubunit of ENaC and an increased abundance of γENaC in GC rats. The presence of functional GH receptors coupled to JAK/STAT and ERK activation was demonstrated in cortical collecting duct KC3AC1 cells. GH-stimulated Na reabsorption, inhibited by GH antagonist, was associated with a GH-induced αsubunit expression. Next, natriuretic and kaliuretic responses to diuretics were compared in patients before and after acromegaly treatment (Clinicaltrials NTC00531908) (Kamenisky JCEM 2011). Na/Kratio was more increased by amiloride in patients before than after acromegaly treatment (13.9 *vs* 6.3), while uNa/Kratio after furosemide was lower in untreated patients (5.2 *vs* 7.1). Amiloride-sensitive nasal potential was also significantly higher before acromegaly treatment (5.8 *vs* 4.2 mV). Our findings suggest enhanced renal and extrarenal ENaC activity in acromegaly and provide first evidence that GH stimulates ENaC-mediated Na transport, contributing to soft-tissue swelling and high blood pressure in acromegalic patients. Support: Inserm, Univ Paris-Sud, APHP (CRC06062).

3.4
MOLECULAR MECHANISMS OF MINERALOCORTICOID RE-CEPTOR FUNCTION IN HEART

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Several large clinical studies have demonstrated the important benefit of mineralocorticoid receptor (MR) antagonists in patients with left ventricular dysfunction after myocardial infarction or with heart failure. The traditional view of the action of aldosterone restricted to sodium reabsorption in epithelial tissues must be updated. Experimental studies have demonstrated that chronically increased aldosterone levels in plasma or in target tissues induce cardiac fibrosis, inflammation and vascular remodeling. MR activation also modulates cardiac electrical activity and leads to ventricular and atrial arrhythmias. Aldosterone is generally considered as the main ligand of MR. However, this is a matter of debate especially in the heart. Complexity arises from the glucocorticoids with plasma concentrations much higher than those of aldosterone, the structural similarity of these two hormones and of their receptors, and the possible binding of glucocorticoids on the aldosterone receptor since the hydroxysteroid dehydrogenase type 2 is not expressed in cardiomyocytes. However this hypothesis cannot explain the well-described activating effect of aldo on electrophysiological remodeling in vivo. Thus, there must be other selectivity mechanism(s) to allow specific MR activation by aldosterone in the absence of 11HSD2 activity. Diverse experimental models and strains of transgenic mice have allowed to dissect the effects of aldosterone in the heart. Taken together experimental and clinical data clearly highlight the dele-terious cardiovascular effects of MR stimulation.

3.5
SUBCELLULAR DISTRIBUTION OF THE MINERALOCORTICOID RECEPTOR

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The mineralocorticoid receptor (MR) is a ligand-activated transcription factor in the steroid receptor superfamily. In the unbound state it is primarily in the cytosol; upon binding it enters the nucleus and binds hormone response elements to activate gene transcription. Translocation between the cytosol and nucleus involves binding to multiple chaperonins. The MR also mediates rapid non-genomic actions. A panel of monoclonal antibodies against different epitopes of the MR has been developed. We performed subcellular fractionation by differential centrifugation and density gradient purification and detection by western blot of M1 cells expressing the MR and of rat heart and hippocampus and used different antibodies to detect the MR. The MR was found in the cytosol, nuclei, mitochondria and microsomes. Using a combination of biotin labeling, sucrose gradient centrifugation and cationized silica, we demonstrated MR in the plasma membrane in association with caveolin 1. MR were also demonstrated by immunoelectron microscopy in mitochondria of rat kidney. (NIH HL27255 and VA) Ref: Gomez-Sanchez, C.E., de Rodriguez, A.F., Romero, D.G., Estess, J., Warden, M.P., Gomez-Sanchez, M.T., and Gomez-Sanchez, E.P. 2006. Development of a panel of monoclonal antibodies against the mineralocorticoid receptor. Endocrinology 147:1343-1348. Galigniana, M.D., Erlejman, A.G., Monte, M., Gomez-Sanchez, C., and Piwien-Pilipuk, G. 2010. The hsp90-FKBP52 Complex Links the Mineralocorticoid Receptor to Motor Proteins and Persists Bound to the Receptor in Early Nuclear Events. Mol Cell Biol 30:1285-1298.

3.6
VASCULAR MINERALOCORTICOID RECEPTORS MEDIATE ALDOSTERONE-DEPENDENT VASCULAR INJURY

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Aldosterone(aldo) regulates blood pressure(bp) by activating renal mineralocorticoid receptors (MR). In clinical trials MR antagonists decrease mortality and vascular ischemia out of proportion to modest decreases in bp, suggesting direct vascular protective effects of MR antagonists. We have found functional MR in human vascular smooth muscle cells (VSMC) and hypothesized that VSMC MR regulates genes that promote vascular injury. Using gene expression profiling, we identified the mitogenic factor, placental growth factor (PGF), as a vascular MR target gene. In mouse vessels with endothelial damage and in human atherosclerotic vessels, aldo further enhances expression of PGF and it's receptor, Flt1. Moreover, treatment of diseased human vessels with MR antagonist, decreases vascular PGF expression. In the mouse carotid injury model, aldo-stimulated SMC proliferation and fibrosis observed in WT mice is inhibited in PGF KO mice. We recently developed a mouse model with

inducible, SMC-specific, deletion of MR to test the specific role of SMC-MR in vascular physiology in vivo. Deficiency in SMC-MR prevented aldo-mediated SMC proliferation and fibrosis following injury. These findings demonstrate that SMC-MR mediates aldo-stimulated vascular injury, perhaps via regulation of PGF transcription, suggesting novel mechanisms for the protective effects of MR antagonists in clinical trials and supporting the SMC-MR/PGF/Flt1 pathway as a novel target to prevent or treat hypertension, atherosclerosis and other common vascular diseases in humans.(NIH HL095590 & AHA GIA0855920D). REFERENCES: Jaffe and Mendelsohn.Circ Res 2005. Functional MR in human coronary SMC. Jaffe et al., JCI 2010. PGF regulation by vascular MR and role in aldo-dependent vascular injury.

3.7

STRUCTURAL CHARACTERIZATION OF MINERALOCORTICOID RECEPTOR INACTIVATION

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Aldosterone regulates sodium homeostasis and controls blood pressure. It acts via its binding to the mineralocorticoid receptor (MR), a transcription factor that modulates the expression of target genes by recruiting specific coregulators. MR antagonists used in the treatment of hypertension and heart failure are steroids (spironolactone, canrenone, eplerenone) characterized by a C17 lactonic ring. Their antagonistic potency and selectivity are highly depending on their substituents. Recently, dihydropyridine or pyrazoline derivatives were identified as potent and selective MR antagonists. In order to establish clear structure activity relationships among MR antagonists, we developed a structural approach. We took advantage of the stabilizing effect of the S810L mutation to solve the structure of the ligand-binding domain (LBD) of MRS810L complexed with spironolactone. C17-substituted spirolactones and BR-4628, a dihydropyridine derivative identified in a pharmacological screening, were then docked within this structure. The structure of the MRS810L LBD complexed with spironolactone showed that its C7-substituent is well accommodated. Docking of spirolactones revealed that their C7-substituent is either nicely (RU26752) or badly (mexrenone and eplerenone) accommodated, explaining the hierarchy in their antagonist potency. Docking of BR-4628, validated by using a mutagenesis approach, allowed identifying two residues which confer to BR-4628 its selectivity for MR. Moreover, we showed that BR-4628 protrudes from the LBD in the direction of the H12 helix, impairing it to adopt the agonist orientation and to allow transcriptional coregulators recruitment. Altogether, these findings suggest that BR-4826 represents the prototype of a new class of potent and selective MR antagonist devoid of any partial agonist activity with the potential for a broad therapeutic use.

4.0: ENaC STRUCTURE AND REGULATION

4.1

RENAL NEDD4-2 IS CRUCIAL FOR NCC REGULATION AND Ca BALANCE

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To maintain proper Na/K balance and blood pressure (BP), aldosterone acts in the kidney by preventing ENaC degradation by the ubiquitin ligase Nedd4-2 (N4-2). To determine the role of renal N4-2 in mediating salt-sensitive hypertension observed in N4-2 total knockout (KO) mice, inducible renal tubule-specific N4-2 KO mice were generated using the TetOn/CreLoxP systems. Pax8/LC1 mice, allowing tetracycline-inducible Cre-mediated recombination in renal tubules, were bred with N4-2fl/fl mice to obtain mutants (N4-2fl/fl/Pax8/LC1) and controls (N4-2fl/fl/Pax8 or N4-2fl/fl/LC1). N4-2 was completely lost in the different renal tubular segments in doxycycline-treated mutants. Plasma aldosterone was increased under standard and high-Na diets in mutants. Under challenging conditions, mutants displayed normal Na/K balance and BP, but increased water consumption, diluted urine, and elevated urine Ca excretion. b/gENaC, NCC and ROMK protein were increased, but aENaC protein and mRNA were decreased, suggesting decreased ENaC activity to compensate increased NCC, and thus prevent hypertension. All these data show 1) the direct effect of N4-2 on b/gENaC in vivo, 2) a compensatory regulation on aENaC, and 3) the importance of N4-2 for controlling NCC and Ca absorption. The mechanisms behind this regulation remain to be elucidated. Work funded by the Leducq Foundation Transatlantic Network on Hypertension.

4.2

SODIUM SELECTIVITY OF AMILORIDE-SENSITIVE CURRENTS IN INNER EAR EPITHELIAL CELLS

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Luminal Na is maintained at 1 mM in the cochlea and 10 mM in the vestibular labyrinth, while K is >150 mM. Epithelial cells of both Reissner's membrane (RM) in the cochlea and the semi-circular canal duct (SCCD) in the vestibular labyrinth absorb Na via amiloride-sensitive mecha-nisms and absorption is thought to contribute to the homeostasis of luminal volume and neces-sary to maintain sensory function. The most commonly-observed drug target is the epithelial Na channel (ENaC), comprised of the three subunits α- β- and γ-ENaC. However, other amiloride-sensitive cation channels have also been observed. We determined the molecular and functional expression of candidate cation channels with gene array, RT-PCR, whole-cell patch clamp and short circuit current recordings. Expression analysis of RM detected no acid-sensing ion channels (ASIC1a, ASIC2a, ASIC2b, ASIC3) nor cyclic-nucleotide gated channels (CNGA3, CNGA1, CNGA2, CNGA4, CNGB3). By contrast, α- β- and γ-ENaC were all present in both RM and SCCD. Homomeric □-ENaC is reportedly poorly selective for Na. We found the selec-tivity of the currents of both RM and SCCD epithelial cells to be Na >> K and the permeability to Li > Na in RM.These results are consistent with the amiloride-sensitive absorptive flux of RM and SCCD mediated by highly Na-selective αβγ-ENaC. These epithelia therefore absorb only Na via the amiloride-sensitive pathway and do not provide a parasensory K efflux. Sup-ported by NIH grants R01-DC000212 and P20-RR017686.

4.3

ALDOSTERONE INDUCES ACCUMULATION OF AN αENaC IMMUNE-REACTIVE PEPTIDE IN PROTEOSOMES OF DISTAL RENAL TUBULES

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The epithelial sodium channel, ENaC, is a protein complex formed by α-, β-, and γ- subunits. Renal ENaC plays a crucial role in Na+ homeostasis and extracellular fluid volume control. Regulation of ENaC is multifaceted and includes differential protein expression of the individual subunits and redistribution of ENaC containing vesicles to and from the apical plasma membrane. Using an antibody against the NH2-terminus of αENaC, we previously demonstrated increased apical αENaC expression in connecting tubules and collecting duct in rats receiving 50 μg aldosterone/kg body weight/24 hrs for 7 days as compared to vehicle treated controls. In this study, using an antibody against the extracellular loop of αENaC there was marked labeling of spherical intracellular structures in connecting tubules of the aldosterone treated rats with no labeling in untreated animals. Double-labeling immunofluorescence analysis revealed low level of colocalization of the αENaC immunoreactivity with cathepsin D (lysosomes), less with early endosomes (EEA1) and minimal with recycling endosomes (rab11). By contrast, the structures colocalize to a high degree with protesomes specifically in distal convoluted and connecting tubules. The results suggest that aldosterone infusion is accompanied by an increased endocytosis of anti-αENaC reactive peptide, which eventually ends up in proteosome for proteolytic degradation.

4.4

A NEW INVERTEBRATE MODEL FOR THE STUDY OF THE EPITHELIAL SODIUM CHANNEL (ENaC)

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The archetype of the epithelial sodium channel (ENaC) is already present in nematodes and evolved during evolution to a highly specific channel, crucial for the regulation of salt and water homeostasis. Although the ENaC-molecule and its function have been studied in many invertebrate and vertebrate tissues, there is to date no suitable model for the study of an amiloride-sensitive ENaC occurring in early invertebrates, i.e. close to the beginning of its evolutionary modification. Here we present a new invertebrate model for such studies. Dissected segments of earthworm integument were mounted in modified Ussing chambers and perfused with pond water (PW) or earthworm ringer solution (ERS) on the apical side. Transepithelial voltage, resistance and virtual short circuit current were monitored under current clamp conditions. The integument has a high transepithelial resistance (9-11 kOhm cm2), and an ultrahigh paracellular resistance (24 MOhm cm2). This very tight epithelium shows only under PW-conditions a pronounced and fast inhibition of the current by low doses of amiloride and its analogues phenamil and benzamil. As the Ki and the sequence of this inhibition is similar to the ENaC blocker-response in mammalians, we conclude that the earthworm Lumbricus has already expressed highly evolved ENaCs in the apical membrane of the integument, and possesses a very fast up- and down regulation of apical Na channels according to the outside salt concentration.

4.5

xShroom1 REGULATES THE NUMBER OF ENAC CHANNELS INSERTED IN THE MEMBRANE OF OOCYTES FROM *Xenopus laevis*

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Shroom is a family of proteins linked to the actin cytoskeleton. We studied its effect upon the currents through ENaC channels. Oocytes (*X. laevis)* were injected with α, β, and γ mENaC and xShroom1 sense or antisense oligonucleotides and amiloride-sensitive Na^+ currents (INa_{amil}) were measured. We observed a strong reduction in INa_{amil} in oocytes co-injected with the xShroom1 antisense, thus, the inward conductances (G_{inward}) (-160 to 0 mV) were 36± 12 μS and 1.80 ±.50 μS with xShroom1 sense and antisense respectively (n=18). The same results were obtained in oocytes expressing a DEG mutant β-mENaC subunit (β-S518K) which has a Po of nearly 1. The G_{inward} were 65 ± 10 μS and 1.80 ± 2.0 μS for oocytes injected with xShroom1 sense or xShroom1 antisense (n=16). Addition of low (20 ng/ml) concentration of trypsin which activates the membrane-resident ENaC channels led to a slow increase in INa_{amil} in oocytes with xShroom1 sense (n=22). Trypsin had no effect on the currents generated by most of the oocytes coinjected with ENaC and xShroom1 antisense. The same results were obtained with high trypsin concentration (2 μg/ml, 2.5 min). In addition, fluorescence positive staining of plasma membrane in the oocytes expressing α,β and γ mENaC and xShroom1 sense were observed but not in oocytes coinjected with ENaC and xShroom1 antisense oligonucleotides. These data are consistent with the idea that the reduced INa_{amil} in oocytes with blocked expression of xShroom1 is most probably due to a lack of functional ENaC channels in the plasma membrane. Acknowledgements: ENaC cDNAs were provided by Dr M. Carattino (Pittsburgh, Pa) and the set for oocytes was a gift of Dr C. Peracchia (Rochester, NY).

4.6

PROTEOLYTIC CHANNEL ACTIVATION BY PLASMIN INVOLVES TWO DISTINCT CLEAVAGE SITES IN THE γ-SUBUNIT OF HUMAN ENaC

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Proteolytic processing of the epithelial sodium channel (ENaC) is essential for channel activation [1]. Recently, we reported that plasmin proteolytically activates ENaC known to be important for sodium absorption in the distal renal tubule [2]. In mouse γENaC a putative plasmin cleavage site has been described [3] and for rat ENaC an indirect stimulatory effect of plasmin via

prostasin has been proposed [4]. The aim of this study was to identify cleavage sites in human γENaC that are functionally important for channel activation by plasmin. Sequence comparison of human and mouse ENaC suggested a putative plasmin cleavage site in human γENaC (K189). To study its functional relevance we generated a γK189A mutant by site-directed mutagenesis and expressed wild-type and αβγK189A-ENaC in *Xenopus laevis* oocytes. The γK189A mutation reduced but did not abolish the stimulatory effect of plasmin (10 μg/ml) on ENaC. In contrast, mutating a putative prostasin site (γRKRK178AAAA) had no apparent effect on the stimulatory response to plasmin. Interestingly, the combination of both mutations (γRKRK178AAAA;K189A) abolished the stimulatory effect of plasmin. We conclude that channel cleavage at a putative plasmin site and at a putative prostasin cleavage site is involved in mediating proteolytic activation of human ENaC by plasmin.This work was supported by the Interdisziplinäres Zentrum für Klinische Forschung (IZKF) and by the ELAN program of the University of Erlangen-Nürnberg. Reference: Rossier BC and Stutts MJ (2009). Annu Rev Physiol 71, 361-79.Svenningsen et al. (2009). J Am Soc Nephrol 20, 299-310. Passero et al. (2008). J Biol Chem 283, 36586-91.Svenningsen et al. (2009). Am J Physiol Regul Integr Comp Physiol 297, R1733-41.

4.7

DOES INSULIN REGULATE ENaC?

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Insulin increases Na transport in vitro in amphibian epithelia via ENaC. Data on the hormone action on this transport in mammalian epithelia are scant and controversial. We tested the effect of insulin (2 nM, 30-60 min) on principal cells of isolated split-open rat CCD using whole-cell current measurements. Insulin addition to the superfusate of the tubules dissected from control animals did not induce the appearance of amiloride-sensitive Na current, while in high-K fed animals the currents were slightly smaller in the presence of insulin (142±34) vs. controls (184±70 pA/cell). However, the hormone enhanced Na-pump current (ouabain-sensitive) from 18±3 to 31±3 pA/cell in control and from 74±9 to 126±11 pA/cell in high-K fed animals. It also more than doubled ROMK (TPNQ-sensitive) K currents in control CCD from 324±44 to 698±82 pA/cell, although it did not affect this current in tubules from K-loaded rats. Efforts to demonstrate an effect of insulin on Na excretion rats in vivo were unsuccessful. In summary, although the hormone does activate the Na pump and apical K channels, we find no evidence for up-regulation of ENaC by insulin in the mammalian CCD.

4.8

CLEAVAGE OF ENDOGENOUS γENaC AND ELEVATED ABUNDANCE OF αENaC IS ASSOCIATED WITH INCREASED Na^+ TRANSPORT IN RESPONSE TO APICAL FLUID VOLUME EXPANSION IN HUMAN H441 AIRWAY EPITHELIAL CELLS

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We have correlated the functional response to apical fluid volume expansion with abundance and cleavage of endogenous α and γENaC proteins in the apical membrane of H441 airway epithelial cells cultured at air liquid interface (ALI). When placed in fluid-filled Ussing chambers, monolayers cultured at ALI rapidly elevated I_{sc} ($t_{1/2}$ = 2.3 mins). The increase in I_{sc} was not altered by apical addition of trypsin but was abolished by inhibitors of serine proteases (aprotinin) and furin (decRVKRcmk). These treatments also increased the IC_{50} amiloride indicating that highly Na^+-selective ENaC channels were inhibited. Apical fluid, 5–500 μl for 1 hour in culture, increased the spontaneous starting I_{sc} in a dose dependent manner. Maximal fluid-induced I_{sc} in the Ussing chamber was unchanged. Apical fluid expansion increased the abundance of 63-65kDa αENaC proteins in the apical membrane. However, this could not be attributed to increased cleavage as protease inhibitors had no effect on the ratio of cleaved to non-cleaved (90 kDa) αENaC proteins. Instead, fluid expansion increased αENaC abundance in the membrane. In contrast, function correlated well with cleavage of γENaC by furin and extracellular proteases. Cleavage of γENaC was also associated with increased retrieval from the membrane and processing via the proteosome. Thus, the response to apical fluid volume expansion in H441 cells involves cleavage of γENaC, and changes in α and γENaC protein abundance at the apical membrane. Funded by BBSRC & Wellcome Trust.

4.9

ALDOSTERONE-INDEPENDENT REGULATION OF ENaC IN ADX MICE

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The corticosteroid hormone aldosterone is thought to be the primary regulator of ENaC activity. This mineralocorticoid increases ENaC activity in response to dietary Na+ restriction in cor-rection of falling circulating volume. Our recent findings suggest that complementary systems function in parallel with aldosterone to modulate ENaC activity, including AVP and purinergic signaling localized to the distal nephron. To explore further the relation of aldosterone with this other regulation and to test the primacy of aldosterone in regulation of ENaC activity, we used mice with bilateral *adrenalectomy*. The activity of ENaC in the murine aldosterone-sensitive distal nephron (ASDN) was assayed with patch-clamp electrophysiology in freshly isolated split-open tubules. In normal mice, ENaC activity is inversely related to Na+ intake. This relation is thought to be a manifestation, at least in part, of changes in aldosterone levels. Consistent with this, treatment with the mineralocorticoid DOCA reversed the effects of a high Na+ diet re-turning ENaC activity to normal levels. Unexpectedly, ENaC in the ASDN had robust activity in Adx mice even in the presence of low adrenal corticosteroid levels, as measured with HPLC. Moreover, the sensitivity of ENaC activity to changes in Na+-intake in Adx mice was reversed with channels having greater activity in the presence of higher Na+ intake. A consequence is that ENaC is more active in Adx mice compared to wild type mice under any Na+ feeding. ENaC activity in Adx mice is likely controlled primarily by AVP responding mostly to changes in plasma tonicity and to a lesser degree volume status. These results demonstrate that in complement to corticosteroids other hormones play an important role in setting ENaC activity, and that ENaC can be present and functional in the ASDN in the absence of adrenal corti-costeroids.

4.10

COLLECTING DUCT-SPECIFIC ENDOTHELIN B RECEPTOR KNOCKOUT INCREASES ENaC ACTIVITY

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Collecting duct (CD)-derived endothelin-1 (ET-1) acting via endothelin B (ETB) receptors favors Na+ excretion. Compromise of ET-1 signaling or ETB receptors in the CD cause Na+ retention and increase blood pressure. Activity of ENaC is limiting for Na+ reabsorption in the CD. To test for ETB receptor regulation of ENaC, we combined patch clamp electrophysiology with CD-specific knockout of endothelin receptors. We also tested how ET-1 signaling via specific endothelin receptors influences ENaC activity under differing dietary Na+ regimens. ET-1 significantly decreased ENaC activity in CD isolated from wild type (wt) and CD ETA KO mice but not CD ETB KO and CD ETA/B KO mice. ENaC activity was inversely related to dietary Na+ intake. ENaC activity in CD ETB KO and CD ETA/B KO mice tended to be elevated under all dietary Na+ regimens compared to wt and CD ETA KO mice, reaching significance with high (2%) Na+ feeding. In CD ETB KO and ETA/B KO mice ENaC activity is less responsive to changes in dietary Na+ than wt being inappropriately elevated in the presence of high Na+. These results show that the bulk of ET-1 inhibition of ENaC activity is mediated by the ETB receptor. In addition, they could explain the Na+ retention and elevated blood pressure observed in CD ET-1 KO, CD ETB KO and CD ETA/B KO mice consistent with ENaC regulation by ET-1 via ETB receptors contributing to the antihypertensive and natriuretic effects of the local endothelin system in the mammalian CD.

4.11

DISEASE CAUSING MUTATIONS AFFECT ENaC GATING

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The activity of the epithelial Na channel (ENaC) is a critical determinant of systemic Na levels and thus, effective circulating volume and blood pressure. Gain- and loss-of-function mutations in ENaC affect renal Na handling to increase and decrease, respectively, blood pressure. The majority of disease-causing mutations are believed to influence channel expression level. Those that influence the biophysical properties of channels within the plasma membrane, though, are also capable of causing disease, and should be informative about structure-function relations within the channel protein. Here, we identify one gain-of-function Liddle's and three loss-of-function PHA-I mutations in ENaC that lead to changes in single channel properties. The N530S Liddle's mutation in the γ-subunit of ENaC substitutes a conserved (in ENaC) Arg that is one position downstream of the *Deg* site. Channels containing γ-ENaC harboring the N530S substitution have increased activity and open probability. Channels containing the PHA-I mutation, G37S in β-ENaC, have decreased at physiological potentials. This critical Gly is in an absolutely conserved HG motif in the cytosolic NH_2-terminal of the protein key to gating. Another PHA-I mutation, the KYS106-108->N substitution in γ-ENaC, also markedly decreases current likely by decreasing open probability. The PHA-I mutation, S562P in γ-ENaC, results in complete loss of function. S562 occupies a critical position in the selectivity filter of the channel pore possibly being involved in coordination of the conductive ion during permeation. Thus, this mutation is likely to affect either gating or permeation. Additional study of these and related mutations is expected to reveal important structure-function relations in ENaC/Deg channels.

4.12

THE PPK1 CHANNEL OF DROSOPHILA SENSORY NEURONS

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PPK (Pickpocket) channels are ENaC/Deg homologs expressed in *Drosophila melanogaster* sensory neurons. These channels are believed to be involved in mechanosensory transduction, but their exact function has not been determined. Using a primary neuronal culture from *Drosophila* embryos and whole cell patch-clamping, we identified two acid-sensing currents in sensory multidendritic (*md*) neurons. One current is robust, Na^+ and K^+-selective and activated by pronounced acidification. Amiloride, a nonspecific antagonist of ENaC/Deg channels, reduces this pH-sensitive current in a dose-dependent manner. A distinct sodium current that is transient becomes apparent upon relief from inhibition following removal of amiloride after extracellular acidification. This transient pH-sensitive current is absent in PPK1-/- flies and rescued by expression of a PPK1 transgene identifying it as the PPK1 current. Initial analysis of repetitive firing in wild type and PPK1-/- *md* neurons finds no difference in activation threshold. Phenotype assays of PPK1-/- flies will clarify the physiological role of PPK1 channels. Transient expression of a PPK1 gain-of-function mutant, S551F, in *Drosophila* eyes causes degeneration of eye bristle organization and thus, should be useful in identifying enhancers and suppressors of PPK1 activity, which are expected to clarify the function of these channels in neurons.

4.13

SUBUNIT OLIGOMERIZATION OF HUMAN ASIC1a IN *XENOPUS LAEVIS* OOCYTES

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The crystal structure of the chicken Acid Sensing Ion Channel 1 (ASIC1) shows a homotrimeric channel. Previous results of our and other labs support a different oligomerization of ENaC/Degenerin family members. We have revisited the oligomeric state of the functional human ASIC1a channel expressed in Xenopus oocytes. We have used sulfhydryl cross-linkers or oxidizing agents targeting the intracellular cysteines at the C-terminus to stabilize the ASIC1 functional complex at the cell surface. The impact of these cross-linkers on the ASIC1 activity was assessed with voltage-clamp technique either in the two-electrodes or the cut-open oocyte

configuration. After ASIC1a biotinylation and purification, western blot analysis shows that cross-linkers induce a shift in the molecular weight of ASIC1a from monomeric ~70kDa, to higher molecular weights complexes of approximatively 150, 210 and 300kDa. This subunit cross-link depends on cysteines in the C-terminus. We confirmed by size exclusion chromatography the presence of a hASIC1a channel complex of a mass corresponding approximatively to 300kDa. We conclude that in Xenopus oocytes sulfhydryl cross-linkers or oxidizing reagents stabilize a ASIC1a complex of 300kDa that cannot simply account for a homotrimeric channel. The C-terminus of ASIC1a subunits may be important for the subunit-subunit interactions for the ASIC1a oligomerization.This work was supported by a grant from the SNF.

4.14

ACETYLATION MODULATES αENaC DEGRADATION

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The epithelial sodium channel (ENaC), a heterotrimer of homologous α, β, and γ subunits, forms a pathway for Na^+ absorption across epithelia in the renal collecting duct and lung. Defects in ENaC trafficking in the endocytic pathway by Nedd4-2 cause genetic forms of hypertension, including Liddle's syndrome. Less is known about ENaC assembly and trafficking in the biosynthetic pathway. We tested the hypothesis that ENaC trafficking is modulated by acetylation. To test this hypothesis, we expressed αENaC in HEK 293 cells. To increase protein acetylation, we treated the cells for 24h with the HDAC inhibitor trichostatin A (TSA). We found that TSA increased αENaC abundance (immunoblot). This resulted in increased expression of αENaC at the cell surface, as detected by biotinylation. TSA also increased αENaC abundance in mouse collecting duct cells (mpkCCD). Overexpression of αENaC with specific HDAC cDNAs had the opposite effect, decreasing αENaC abundance. To investigate the underlying mechanism, we quantitated ENaC stability using a cycloheximide chase assay. We found that TSA dramatically slowed the rate of αENaC degradation. We conclude that acetylation modulates αENaC trafficking to the cell surface through an increase in αENaC stability. This mechanism may play an important role in ENaC assembly and trafficking in the biosynthetic pathway.

4.15

CU^{2+} IS A INHIBITOR OF HUMAN EPITHELIAL NA^+ CHANNELS

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Activity of the epithelial Na^+ channel (ENaC) is regulated by various extracellular factors. In this study, we examined the effect of extracellular Cu^{2+} on human ENaC expressed in *Xenopus* oocytes and investigated its mechanism. External Cu^{2+} inhibited human αβγ ENaC with an estimated IC_{50} of 0.3 μM. The inhibition developed slowly with a time constant of 32 s at 10 μM. The slow time course, a lack of change in the current-voltage relationship and an ineffective protection by ENaC pore blocker amiloride were consistent with an allosteric inhibition. Cu^{2+} did not significantly inhibit mouse αβγ ENaC in oocytes. The α and β hENaC subunits were found primarily responsible for the inhibitory effect of Cu^{2+} on human ENaC based on experiments with mixed human and mouse ENaC subunits. The inhibition of hENaC by Cu^{2+} was pH de-pendent. Mutations of multiple His residues within extracellular domains significantly reduced the inhibition of human ENaC by Cu^{2+}. We identified αH468 as a putative Cu^{2+} binding site at the subunit interface between thumb subdomain of αhENaC and palm subdomain of another counterclockwise subunit (viewed from above). The inhibition by Cu^{2+} was not dependent on an existence of Na^+ self-inhibition, suggestive of a unique mechanism.We conclude that extra-cellular Cu^{2+} is a high affinity inhibitor of human ENaC and binds to sites within the extracellular domains including a subunit interface. (NIH R01 ES014701 and P30 DK079307).

4.16

EPITHELIAL SODIUM CHANNEL (ENaC) ACTIVITY AND GATING IS MODULATED BY PALMITOYLATION OF BOTH THE BETA AND GAMMA SUBUNITS

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Activity of ENaC is modulated by regulation of its membrane trafficking, binding of extracellular CTandmetals such as Na^+, and cytoplasmic interactions with inositol phospholipids. We recently reported that αβγENaC activity is modulated by palmitoylation of cytoplasmic Cys (Mueller et al., 2010 J Biol Chem 285, 30453-62). Using fatty acid-exchange chemistry, we found that β and γ, but not α, are modified with palmitate. Analyses of mutant ENaCs revealed that two Cys in β were palmitoylated (βCys43 and βCys557). *Xenopus* oocytes expressing ENaC with mutant βC43A,C557A had significantly reduced whole cell currents, enhanced Na^+ self-inhibition, and reduced single channel P_o when compared with wild-type ENaC, while membrane trafficking, proteolytic processing and surface levels were unchanged. We now report that mutation of either of the two cytoplasmic γ subunit Cys residues (γC33A or γC41A) inhibits ENaC activity by a similar mechanism, although single channel P_o was reduced for only one mutation. As Cys palmitoylation is reversible and regulates ENaC activity, we are screening the 23 mouse palmitoyltransferases to identify those that modulate ENaC activity when co-expressed with wild-type but not ENaCs with key Cys mutated. To date, four transferases that are expressed in kidney and lung enhance ENaC activity. We conclude that ENaC gating is modulated by palmitoylation of both its beta and gamma subunits. (NIH DK65161, DK079307).

4.17

TMPRSS4 ACTIVATES THE EPITHELIAL SODIUM CHANNEL BY CLEAVING THE GAMMA SUBUNIT DISTAL TO THE FURIN CLEAVAGE SITE

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The epithelial sodium channel (ENaC) is activated by a unique mechanism whereby inhibitory peptides are released by proteolytic cleavage within the extracellular loops of two of its three subunits. While cleavage by furin within the biosynthetic pathway releases one inhibitory tract from the α subunit and moderately activates ENaC, full activation through release of a second inhibitory tract from the γ subunit requires cleavage once by furin and then at a distal site by a second protease such as prostasin, plasmin or elastase. We now report that co-expression of mouse TMPRSS4 with mouse ENaC in *Xenopus oocytes* was associated with a two- to three-fold increase in channel activity and production of a unique ~70 kDa C-terminal fragment of the γ subunit, similar to the ~70 kDa γ fragment we previously observed with prostasin-dependent channel activation. Channel activation by TMPRSS4 and production of the ~70 kDa fragment were partially blocked by mutation of the prostasin cleavage site (γRKRK186QQQ). Complete inhibition of TMPRSS4 activation of ENaC and γ subunit cleavage was observed when three basic residues between the furin and prostasin cleavage sites were mutated (γK173Q, γK175Q and γR177Q) in addition to γRKRK186QQQ. We conclude that TMPRSS4 fully activates ENaC by cleaving basic residues within the tract γK173-K186 and thereby releasing a previously defined key inhibitory tract encompassing γR158-F168, from the γ subunit. (NIH DK065161, DK079307, DK080574, and HL087932).

4.18

FUNCTIONAL CHARACTERIZATION OF THE PERMEATION PATHWAY OF ASIC1a

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Acid-sensing ion channels (ASICs) are cation selective channels that undergo activation and desensitization in response to extracellular acidification. The pore of ASIC1 has an hourglass-like shape with residues in the second transmembrane (TM2) helices forming the ion permeation pathway. To functionally define the localization of the closed gate of ASIC1a, we mutated residues in the TM2 helix to cysteine and investigated the reactivity of these mutant channels toward MTSET in the closed and open states. Our studies indicate that I426C channels are modified by MTSET in the closed state, A427C and G428C channels are modified in both closed and open states, while L429C and G431C channels are modified only in the open state. Our results are consistent with the presence of an extracellular vestibule and gate, as suggested by the limited reactivity toward MTSET in the closed state of residues distal to Gly428. To define residues in the TM2 segment that contribute to cation selectivity we investigate the Na/K permeability ratio of channels bearing single substitutions in the tract 426 to 450. The capability to discriminate between Na and K was significantly diminished in G434A, G438C, L439C, G442C, A443C and L446C channels. We conclude that multiple residues in the inner pore of ASIC1a contribute to cation selectivity.

4.19

MECHANISTIC BASIS FOR SPECIFIC ACTIVATION OF SGK1 BY mTOR

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The serum- and glucocorticoid-induced kinase 1 (SGK1) plays an important role in hormone regulation of ENaC-dependent Na+ transport. We have previously reported that the mTOR complex-2 (mTORC2) activates ENaC by phosphorylating SGK1. Here we identify mSIN1 as the mTORC2 component that mediates interaction with SGK1, and demonstrate that this interaction is required for SGK1 phosphorylation and ENaC activation. We used the yeast two-hybrid system coupled with random mutagenesis to identify a mutant mSIN1 that does not interact with SGK1. Expression of this mutant does not restore SGK1 phosphorylation to wild-type levels in mSIN1-deficient murine embryo fibroblasts. Furthermore, in kidney epithelial cells, the mSIN1 mutant has a dominant-negative effect on SGK1 phosphorylation and on SGK1-dependent ENaC-mediated Na+ transport. Interestingly, the role of mSIN1 to recruit SGK1 to mTOR appears to be specific for SGK1: although mSIN1 is essential for phosphorylation of another mTORC2 substrate, Akt, it does not interact with Akt and its ability to phosphorylate and activate Akt is unaffected by the point mutation that abrogates interaction with SGK1. These data support the conclusion that mTOR, which regulates a wide array of cellular processes, uses distinct strategies to phosphorylate its various substrates, and suggest a mechanism for specific regulation of ENaC-mediated Na+ transport without inadvertent effects on unrelated cellular processes.

4.20

IN VIVO AND IN VITRO INHIBITION OF THE MEMBRANE-BOUND SERINE PROTEASE CAP1/Prss8 BY SERPINS

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Serine proteases are involved in the regulation of many biological processes like e.g., blood coagulation, wound healing, digestion, immune response and channel activation. This requires a tight regulation that may be achieved by specific serine protease inhibitors (serpins), and any alteration of this balance may lead to diseases. CAP1/Prss8 was the first of several membrane-bound serine proteases found to activate ENaC. In our present study, we used the *in vitro Xenopus* oocyte expression system and tested the inhibitory effect of potential CAP1/Prss8 inhibitors on CAP1/Prss8-induced ENaC currents. Thereby, we identified a serpin that was able to block ENaC activation by CAP1/Prss8 *in vitro*. To verify its inhibitory effect *in vivo*, we generated mice transgenic for this serpin and crossed those with mice transgenic for CAP1/Prss8 in the skin that exhibit a scaly skin phenotype, an increased epidermal thickness and an excessive water loss through the skin. Strikingly, in double transgenic mice, this phenotype can be prevented strongly suggesting that the effects through CAP1/Prss8 over-expression is blocked by this inhibitor. In conclusion, we identified an inhibitor of CAP1/Prss8 that may well be implycated in the regulation of CAP1/Prss8 activity in various organs. This was supported by the Swiss National Science Foundation (Grant 3100AO-102125/1 to Edith Hummler).

4.21

THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR INHIBITS PROTEOLYTIC STIMULATION OF ENaC

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The epithelial sodium channel (ENaC) and cystic fibrosis transmembrane conductance regulator (CFTR) are required for ion absorption and secretion at the apical membrane of epithelia in airways and other tissues. In airway epithelia, inhibition of ENaC by CFTR prevents excessive sodium absorption. In cystic fibrosis, the absence of functional CFTR results in hyperactive ENaC channels and dehydration of human airway surface. Limited proteolysis of ENaC's extracellular domains regulates its open probability and we have demonstrated that CFTR markedly impedes proteolytic processing of ENaC. Furthermore, co-immunoprecipitation experiments revealed that several domains of CFTR, including the R-domain, interact with ENaC subunits. Proteolytic cleavage of both α- and γ-ENaC by channel activating protease 3 was drastically diminished in the presence of CFTR. To verify a role of CFTR in ENaC proteolysis in human airway epithelia, we analyzed the proteolytic state of ENaC in normal and cystic fibrosis airway epithelial cultures that lack functional CFTR. Strikingly, in primary cystic fibrosis airway epithelial cells that were homozygous for ΔF508 CFTR the amount of proteolytically cleaved α- and γ-ENaC was significantly increased when compared to normal cultures. These observations suggest that CFTR protects ENaC from proteolytic processing by proteases in airway epithelia. Supported by the NIH [5R01HL080561, 5P01HL034322].

4.22

GENERATION OF MICE DEFICIENT FOR THE CHANNEL ACTIVATING PROTEASE 2/Tmprss4

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Channel activating protease 2 (CAP2/Tmprss4), a member of the family of membrane-bound serine proteases, has been identified as a channel-activating protease of ENaC-mediated sodium current. We have previously shown that ENaC activation *in vitro* requires and intact CAP2 HDS catalytic triad. To study the effect of CAP2 *in* vivo, we generated mutant mice for CAP2/Tmprss4, by using a replacement type vector that targets the histidine and aspartate of the catalytic triad. This targeting vector has been electroporated into mouse 129SVEV ES cells, and correctly targeted clones were injected into blastocysts. Germline chimeras have been generated and $CAP2^{loxneo/+}$ mice were born. Following breeding with Flp-mice and Nestin-CRE deleter mice, mice with $CAP2^{lox}$ and $CAP2^{\Delta}$ alleles have been obtained. Currently, these mice are interbred to generate floxed $CAP2^{lox/lox}$ and $CAP2^{\Delta/\Delta}$ mice. If they survive to adulthood, these mice will be analysed with respect to their capacity to induce ENaC-mediated sodium currents in kidney, colon and lung. This work is supported by the Swiss National Science Foundation (Grant 3100A0-102125/1 to E. Hummler).

4.23

ROLE OF THE ESCRT PROTEIN Tsg101 IN THE TURNOVER OF THE EPITHELIAL Na^+ CHANNEL IN THE KIDNEY

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Kidneys are the main regulator of salt homeostasis and blood pressure. In the distal region of the tubule active Na-transport is finely tuned. This transport is regulated by various hormonal pathways including aldosterone that regulates the reabsorption at the level of the ASDN, comprising the late DCT, the CNT and the CCD. In the ASDN, the amiloride-sensitive epithelial Na-channel (ENaC) plays a major role in Na-homeostasis, as evidenced by gain-of function mutations in the genes encoding ENaC, causing Liddle's syndrome, a severe form of salt-sensitive hypertension. In this disease, regulation of ENaC is compromised due to mutations that delete or mutate a PY-motif in ENaC. Such mutations interfere with Nedd4-2-dependent ubiquitylation of ENaC, leading to reduced endocytosis of the channel, and consequently to increased channel activity at the cell surface. After endocytosis ENaC is targeted to the lysosome and rapidly degraded. Similarly to other ubiquitylated and endocytosed plasma membrane proteins (such as the EGFR), it is likely that the multi-protein complex system ESCRT is involved. To investigate the involvement of this system we tested the role of one of the ESCRT proteins, Tsg101. Here we show that Tsg101 interacts endogenously with ENaC and that the disruption of Tsg101 in renal epithelial cells increases the total and cell surface pool of ENaC, thus implying Tsg101 and consequently the ESCRT system in ENaC degradation by the endosomal/lysosomal system.

4.24

A NEW METHOD FOR ISOLATION AND CULTURE OF PRINCIPAL CELLS FOR STUDIES OF ENaC REGULATION

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The connecting tubules and collecting ducts are composed mainly of two cell types: (1) principal cells (PCs), for regulating Na^+ and K^+ homeostasis; and (2) intercalated cells, for regulating acid-base balance. The main carrier of Na^+ in PCs is ENaC, a channel composed of three homologous subunits (α, β and γ), which is highly regulated in health and disease. After its synthesis and assembly, ENaC is inserted in the apical membrane where it reabsorbs Na^+ in response to various stimuli. We currently lack adequate models to study ENaC regulation in PCs in physiologic and pathophysiologic states. We have developed an *ex vivo* model of murine PCs to perform biochemical, cytological, and electrophysiological assays. PCs were isolated from adult mouse kidney by DBA lectin-coated magnetic beads. These cells achieve high-resistance (1000-1500 $\Omega \cdot cm^2$) after 10-12 days of growth on semi-permeable supports, exhibit an aldo-sterone response, show predominantly amiloride-sensitive electrogenic sodium transport, retain expression of markers of principal cells (Calbindin, ROMK-1, SGK1, GiLZ, MR), and show markedly diminished expression of markers from other nephron segments (SGLT2, UTA-2). This method can be used to investigate differences between wild-type and transgenic mouse models. Funded by an ASN Career Development Award (VB) and NIH R03DK83613 (VB).

4.25

REGULATION OF Na^+ HOMEOSTASIS BY THE DEUBIQUITYLATING ENZYME USP2 *IN VIVO*

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Na+ homeostasis maintenance is a key process for renal blood pressure regulation in mammals. Na+ reabsorption occur all along the renal tubule and about 15% is left under the hormonal control of Aldosterone in the Aldosterone-sensitive Distal Nephron (ASDN) through the Thiazide-sensitive Na+-Cl- Cotransporter (NCC) and the Amiloride-sensitive Epithelial Na+ Channel (ENaC). Both have been shown by our lab and collaborators to be regulated by ubiquitin mediated degradation via the SGK1-NEDD4-2 pathway. The deubiquitylating enzyme Usp2-45 was identified as an aldosterone-induced gene and in vitro studies showed that it enhances ENaC cell surface expression and activity. Moreover, USP2-45 interacts with the ubiquitin ligase NEDD4-2 and ENaC. Altogether, these data make USP2-45 a positive regulator of ENaC by counteracting its down-regulation by NEDD4-2.We address here the implication of Usp2 in Na+ homeostasis in vivo by taking advantage of a Usp2-knockout mouse model. We challenged these animals by on their adaptation to dietary switch from Normal Sodium (0.17% Na+; NSD) to either Low Sodium (>0.01% Na+; LSD) or High Sodium (3.2% Na+; HSD) Diets. We report here that the Usp2-KO mice adapt perfectly to Na+ dietary changes, display normal plasma aldosterone levels, comparable expression levels of áENaC, NCC, SGK1 and NEDD4-2 and show no variation in blood pressure under LSD or HSD, suggesting that compensatory mechanisms have taken place in these animals.

4.26 Withdrawn.

4.27

EPITHELIUM SODIUM CHANNEL (ENaC) DELTA SUBUNIT AND ITS FUNCTIONAL EXPRESSION IN HUMAN RESPIRATORY EPITHELIAL CELLS

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The α-, β- and γ-subunits of ENaC play a significant role in ion and fluid homoeostasis in the lung, however, the function of the fourth homologous ENaC subunit, delta (δ-ENaC) remains largely unknown. Here, we studied δ-ENaC expression and its contribution to ion transport function in human respiratory epithelial cells *in vitro*. Expression of the δ-ENaC subunit was investigated in human respiratory epithelial cell lines (A549, H441, Calu-3 and 16HBE14o-) and in primary cultures of alveolar epithelial type II cells on gene and protein levels. Pharmacological effects of δ-ENaC modulators (Evans blue, capsazepine and icilin) were investigated in H441 and Calu-3 cell monolayers in Ussing chamber studies. Messenger RNA transcripts encoding δ-ENaC were detected in all investigated cell types. PCR data were confirmed by Western blot and confocal laser scanning microscopy. The reported modulators of δ-ENaC function resulted in concentration-dependent blockade of Na^+ current in H441 cell monolayers. Intriguingly, in Calu-3 cell monolayers only capsazepine showed an inhibitory effect, whereas Evans blue and icilin stimulated currents, which were likely Cl^- currents. Our data indicate that δ-ENaC is expressed in all investigated cell types. The fact that reported modulators showed pronounced pharmacological effects suggests a physiological role for δ-ENaC in human respiratory epithelial cells. However, further studies need to determine the specificity of the observed pharmacological effects. ES is funded by an IRSCET postgraduate scholarship.

4.28

Rac1-MEDIATED Nadph OXIDASE PRODUCTION OF O_2 -REGULATES LUNG ENaC

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Our lab's goal is to achieve a better understanding of how epithelial sodium channel (ENaC) is regulated in the lung by reactive oxygen species (ROS). Using single channel patch clamp analysis, we initially found that sequestering O_2^- with tetramethylpiperidine-N-oxyl (TEMPO) immediately inhibits ENaC open probability (Po) from 0.10±0.03 to 0.03±0.01 (n=15, P<0.005). Conversely, increasing ROS, with either a combination of xanthine oxidase and hypoxanthine reagents, or superoxide dismutase inhibitor, prevents nitric oxide inhibition of ENaC and increases channel activity. These findings show that ROS play an important role in normal ENaC activity, and as such, warrant better understanding of the physiological regulators ROS production in the lung. Using standard immunohistochemistry, qPCR, and western blot techniques, we established that alveoli express several members of the Nox family of Nadph oxidases that release O_2^- into the cytoplasm or luminal space, and that the catalytic domain of Nox2 co-IPs with α-ENaC. The important implication is that Nox-mediated ROS production plays an important role in maintaining normal ENaC activity in the lung. Indeed, we and others have shown that there is substantial cross-talk between Nox and ENaC regulation by regulatory proteins 14-3-3 and small G protein Rac1. We show that inhibiting Nox, using 1μM Rac1 inhibitor, NSC23766, significantly decreased both ROS production and ENaC activity (from 1.16±0.27 to 0.38±0.10) in alveolar type 1 cells. Using small animal X-ray and multispectral imaging, we confirm that inhibition of Rac1 *in vivo*, indeed lowers O_2^- levels in freely breathing (12 week old) mouse lung, and leads to alveolar flooding, compared to control (saline instilled) animals.

4.29

WNK4 INHIBITION OF ENaC IS INDEPENDENT FROM Nedd4-2 MEDIATE ENaC UBIQUITINATION

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Epithelial sodium channels (ENaC) in the distal nephron play key role in regulating Na^+ reabsorption. A serine-threonine protein kinase, WNK4, expressed in this region inhibits Na^+ and K^+ reabsorption by reducing delivery of NCC to and enhancing ROMK channel retrieval from the apical membrane. WNK4 regulation ENaC in expression systems has also been described, but these results are controversial. We investigated the effect of WNK4 on endogenously expressed ENaC in A6 cells, both trans-epithelial current and single channel recordings show that WNK4 inhibits ENaC activity. Further analysis channel number in a patch showed that WNK4 reduces channel number but has no effect on channel open probability. Western blots of apical and total ENaC provide additional evidence that WNK4 reduces apical as well as total ENaC expression. In exploring the mechanism of WNK4-inhibition of ENaC, we found that WNK4 enhances ENaC endocytosis. We also examined whether WNK4 enhances Nedd4-2 mediated ENaC retrieval and found no additive effect. The magnitude of forskolin-stimulated increases in trans-epithelial current in WNK4 or Nedd4-2 expressing cells is similar, but the $t_{1/2}$ of forskolin stimulation is slower in Nedd4-2 expressing cells. Lastly, we performed co-immuno precipitation experiment on ENaC and WNK4 and found that even Liddle's mutated ENaC is associated with WNK4. Our results demonstrate that WNK4 inhibits endogenously expressed ENaC by enhancing channel internalization, but this inhibition is independent of Nedd4-2-mediated ENaC ubiquitination.

4.30

MARCKS REGULATES ENaC BY REVERSIBLY SEQUESTERING PHOSPHATIDYLINOSITOL PHOSPHATES

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Phosphatidylinositol phosphates (PIPs) stimulate epithelial sodium channels (ENaC) in the apical membrane of Na transporting epithelia. Neither PIPs nor ENaC are abundantly expressed, therefore, we hypothesize that MARCKS (Myristoylated Alanine-rich C-Kinase Substrate) is a chaperone molecule required to promote association of PIPs with ENaC. PIP strip binding assays showed that recombinant MARCKS binds strongly to various PIPs, but mutated MARCKS protein binds with less affinity. Confocal microscopy showed colocalization of MARCKS and PIP2 at the apical membrane and the translocation of MARCKS to the cytoplasm after an ionomycin-induced increase in intracellular calcium. Lipid raft fractionation assays and Western blot analysis showed a redistribution of MARCKS from low density lipid raft associated fractions to higher density non-lipid raft fractions after PMA treatment. Transepithelial current decreased significantly in a dose and time dependent manner after PMA treatment. Co-immunoprecipitation and direct overlay binding assays showed MARCKS and ENaC associate in the presence of PIPs. FRET studies confirmed MARCKS and ENaC are in close proximity allowing MARCKS to release PIPs in the vicinity of ENaC. Single-channel recordings showed that removal of calmodulin-mediated inhibition of MARCKS increased ENaC activity. These finding suggest a molecular mechanism for the PIP-dependent regulation of ENaC by MARCKS.

4.31

CONTROLLING THE ENaC WITH LIGHT

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The sodium selective non-voltage gated epithelial sodium channel (ENaC) is widely expressed with varying subunit composition in both the periphery and the central nervous system (CNS). In the periphery, ENaC is involved in water and sodium homeostasis. In the CNS however, ENaC function is far from being well understood and new tools are needed to decipher the role of ENaC isoforms in neuronal systems with high spatiotemporal precision. Our laboratory is interested in controlling ion channels and other transmembrane receptors with light. To this end, we have developed photoswitchable agonists and blockers that have been used to control, among others, voltage gated potassium channels and ionotropic glutamate receptors in cell culture and in vivo. We now show that this concept can be extended to ENaC as well. Based on the well known structure activity relationships of amiloride, a widely used ENaC blocker, we are synthesizing light-switchable derivatives. Promising candidates are being tested using two-electrode recordings from *Xenopus* oocytes expressing ENaC. Preliminary results have demonstrated a light-dependent block of amiloride sensitive currents. Future work will aim at further improving the photosensitive ligand and assessing its ability to reversibly block ENaC with different subunit composition and related ion channels. These tools could be used to investigate the role of ENaC isoforms in neurons with optical methods. This work was supported by CIPSM and ERC. M.S. was supported by the German National Academic Foundation.

5.0: REGULATION OF ENaC BIOGENESIS, TRAFFICKING AND GATING

5.1

OVERVIEW OF ENaC REGULATION

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Amiloride-sensitive Na transport via ENaC is a hallmark of Na^+-transporting epithelia where it plays a key role in regulation of total body salt and fluid balance. The magnitude of ENaC-mediated Na^+ transport across epithelial cells depends on the average open probability of the channels, P_o, and the number of channels, N, on the apical surface. The number of channels in the apical membrane, in turn, depends upon a balance between the rate of ENaC insertion and the rate of removal from the apical membrane. Most of the primary genetic defects in ENaC activity associated with abnormal blood pressure change ENaC trafficking and change the number of functional channels in renal cells. The C-terminus of all three homologous subunits, α, β,and γ, is intracellular and contains a proline-rich motif (PPxY). Mutations or deletion of this PPxY motif in the βand γ subunits prevent the binding of a specific ubiquitin ligase, Nedd4-2, to the channel, thereby, impeding ubiquitin conjugation of the channel subunits. The rate of ENaC degradation is controlled by the rate of Nedd4-2-mediated ENaC ubiquitination. Controlling the degradation rate is important enough to have multiple, redundant pathways to control Nedd4-2 and ENaC ubiquitination. On the other hand, ENaC P_o can be regulated by specific binding of phosphatidylinositides to ENaC. Stimulation of phospholipase C by either purinergic P2Y receptors or EGF receptors reduces membrane PI(4,5)P2, and decreases ENaC activity. The mechanism for colocalization of phosphatidylinositides with ENaC requires specific protein binding partners. Supported by NIH R37 DK037963.

5.2

CNK3 AND THE ENaC REGULATORY COMPLEX

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Members of the Connector Enhancer of KSR (CNK) family of proteins possess multiple protein interaction domains, and have been proposed to function as scaffolds possibly assisting various interactions in a multiple signaling cascade. Mammalian CNK3 is an aldosterone-induced protein that is essential for the activity of the epithelial sodium channel (ENaC). In the present study we show that CNK3 is associated with ENaC at the cell surface and that it interacts with the components of the previously described ENaC regulatory complex (ERC). The PDZ domain in CNK3 appears to be crucial for its association with SGK1 and GILZ1, and for its stimulation of ENaC cell surface expression in HEK293T cells. We further demonstrate that the PDZ domain in CNK3 is required for aldosterone-controlled ENaC-mediated Na^+ transport in $mpkCCD_{c14}$ kidney epithelial cells. These results strongly suggest that CNK3 is essential for the proper assembly of ENaC-regulatory factors thus facilitating appropriate aldosterone signaling to stimulate Na^+ reabsorption via ENaC (NIH Grants DK078679, DK056695 and DK085101).

5.3

ROLE OF PURINERGIC SIGNALING REGULATING ENaC ACTIVITY

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Purinergic tone in the aldosterone-sensitive distal nephron (ASDN), in complement with the renin-angiotensin-aldosterone system, sets ENaC activity enabling appropriate responses to changes in Na+ intake. Loss of local control of ENaC by inhibitory purinergic signaling disrupts renal Na+ excretion increasing blood pressure. Release of ATP from the ASDN increases in response to increases in Na+ intake. Connexin 30 (Cx30) serves as a conduit for ATP release in the ASDN. Metabotropic P2Y2 receptors carry the bulk of inhibitory purinergic signaling to ENaC. Mice engineered to lack the P2Y2 receptor or Cx30 have inappropriately elevated ENaC activity, decreased Na+ excretion and increased blood pressure. These findings demonstrate that modulation of ENaC activity in the ASDN by a local purinergic control system is essential for the proper control of blood pressure. Support: R01 DK087460 to JDS. References: Pochynyuk, O, Bugaj, V, Rieg, T, Insel, PA, Mironova, E, Vallon, V and Stockand, JD. Paracrine regulation of the epithelial Na+ channel in the mammalian collecting duct by purinergic P2Y2 receptor tone. JBC 283:36599-36607, 2008. Pochynyuk, O, Reig, T, Bugaj, V, Schroth, J, Fridman, A, Boss, G, Insel, PA, Stockand, JD and Vallon, V. Dietary Na+ inhibits the open probability of the epithelial Na+ channel in the kidney by enhancing apical P2Y2 receptor tone. FASEB J. 24:2056-2065, 2010. Stockand, JD, Mironova, E, Bugaj, V, Reig, T, Insel, PA, Vallon, V, Peti-Peterdi, J, and Pochynyuk, O. Purinergic inhibition of ENaC produces aldosterone escape. JASN 21:1903-1911, 2010. Mironova, E, Peti-Peterdi, J, Bugaj, V and Stockand, JD. Diminished paracrine regulation of the epithelial Na+ channel by purinergic signaling in mice lacking connexin 30. JBC 286:1054-1060, 2011. These studies identify the underlying mechanism and physiological consequences of local control of ENaC by purinergic signaling intrinsic to the ASDN.

5.4

MICRORNAS: NOVEL ENaC REGULATORS

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Aldostrone (aldo) is known to increase ENaC expression and apical membrane density. This is achieved in part by changing the expression of regulatory proteins responsible for delivering and maintaining ENaC at the cell surface. Proteomic profiling to identify changes in aldo-induced proteins reveal a larger set of regulated proteins than would be predicted from the direct action of the mineralocorticoid receptor. MicroRNAs (miRs) are short non-coding nucleotides that pair to mRNA of protein coding genes to direct their post-transcriptional repression. miRs are potential candidates that could facilitate aldo's diverse genomic action and may account for the breadth of protein regulation observed following aldo stimulation. We profiled the expression of all known miRs in a mouse CCD cell line before and after aldo stimulation using microarrays (50nM, 24hrs n=5). Several miRNAs were significantly up- or down-regulated by aldo. Repression of the most significantly altered miRs (mmu-miR-335-3p; 290-5p and 1983) was confirmed by qPCR. We selectively reduced the expression of these miRs in the absence of aldo and observed a significant increase in ENaC-mediated short-circuit current over control cells (73.9±4.5% increase, n=32). By using an in silico prediction of mRNA targets we profiled the aldo-induced expression of selected proteins by RT-PCR and demonstrated a significant increase in expression of several novel targets. The data indicate that miRs are aldo regulated and that miRs may alter the expression of novel aldo-induced proteins to regulate ENaC.

5.5

INTERPLAY BETWEEN KINASES, Nedd4-2 AND ENaC

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ENaC function is highly regulated by hormones and other intra- and extracellular stimuli and plays a critical role in the regulation of total-body volume and airway fluid balance. A central locus for ENaC regulation is the E3 ubiquitin ligase Nedd4-2. This regulation involves Nedd4-2 binding to and ubiquitylating ENaC, processes that appear to be regulated by a growing number of kinases. Classically, these include kinases that phosphorylate either Nedd4-2 (e.g., SGK1, an aldosterone-induced protein, and PKA, stimulated by vasopressin) or ENaC (e.g., ERK and CK2) and thereby modulate the cellular ENaC-Nedd4-2 interaction. We have recently identified additional stress-dependent kinases that can phosphorylate Nedd4-2 and regulate ENaC apical membrane expression. The metabolic sensor AMP-activated protein kinase (AMPK) inhibits ENaC by promoting β-ENaC association with Nedd4-2 and appears to play a significant role in the acute ENaC inhibitory response to metabolic depletion. The IκB kinase (IKK)/NF-κB inflammatory pathway biphasically regulates ENaC, with acute IKKβ-dependent ENaC activation via Nedd4-2 phosphorylation at an overlapping PKA/SGK1 site and chronic ENaC inhibition via NF-κB-dependent down-regulation of transcriptional events. Finally, we have identified by mass spectrometry additional Nedd4-2 phosphorylation sites targeted by JNK1 and possibly other kinases, which enhance Nedd4-2 catalytic activity. In summary, Nedd4-2 has emerged as a key intersection point for integrating and coordinating the kinase-dependent regulation of ENaC and other epithelial transport proteins by various cellular pathways. (Supported by NIH/NIDDK).

5.6

P2Y2-R REGULATION OF ENaC-MEDIATED Na^+ ABSORPTION IN AIRWAY EPITHELIA

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Failure of Na^+ absorption by airway epithelia to be restrained contributes to excessive Na^+ absorption, underhydration of airway surface liquid (ASL) and airway disease. ATP in ASL inhibits ENaC through apical $P2Y_2$-R. $P2Y_2$-R couple through Gq/11 to phospholipase C, which hydrolyzes PIP_2 at the inner leaflet of the plasma membrane. ENaC open probability (P_O) is stimulated by exposure to anionic membrane phosphoinositides, which utilize basic residues contained within ENaC cytosolic domains. Therefore, we considered that $P2Y_2$-R mediated inhibition of ENaC was likely due to decreased P_O. ENaC current in Ussing chambers was inhibited by up to 80% within minutes by 1-10 μM luminal UTP. Surprisingly, this marked inhibition of ENaC mediated current recovered very little following removal of UTP. We find that MG132, an inhibitor of ubiquitin mediated endocytosis, and U0126, and inhibitor of ERK kinase, additively block ~70% of UTP mediated inhibition of ENaC. Accordingly, $P2Y_2$-R may limit Na^+ absorption by reducing the density of ENaC at the cell surface (supported by PO1-HL034322). Tarran R, Trout L, Donaldson SH, Boucher RC. Soluble mediators determine airway surface liquid volume in normal and cystic fibrosis superficial airway epithelia. J Gen Physiol. 2006 May;127(5):591-604. Garcia-Caballero A, Rasmussen JE, Gaillard E, Watson MJ, Olsen JC, Donaldson SH, Stutts MJ, Tarran R. SPLUNC1 regulates airway surface liquid volume by protecting ENaC from proteolytic cleavage. Proc Natl Acad Sci U S A. 2009 Jul 7;106(27):11412-7).

5.7

UBIQUITYLATION-DEUBIQUITYLATION CYCLES IN THE CONTROL OF MEMBRANE PROTEIN STABILITY AND TRAFFICKING

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Ubiquitylation, the post-translational modification of target proteins with ubiquitin polypeptides, is a major mechanism of cellular regulation. It allows not only the labelling of proteins for degradation, but can affect functions as different as protein traffic, DNA repair, localization or control of enzymatic activity. Ubiquitylation is the result of an enzymatic cascade, including E1, E2 enzymes and E3 ubiquitin-protein ligases and is a reversible process involving deubiquitylating enzymes. The possibility to label proteins with either monoubiquitins or different types of polyubiquitin chains makes it a very versatile process. Since the discovery that the ubiquitin-protein ligase Nedd4-2 binds to and ubiquitylates ENaC it has been well established that ubiquitylation plays a major role in the control of sodium homeostasis in the kidney, and that it is highly regulated by numerous pathways, including different kinases and/or deubiquitylating enzymes. Recent data on the role of ubiquitylation/deubiquitylation at different levels of Na+ transport regulation will be discussed. Rotin D and Staub O. Role of the ubiquitin system in regulating ion transport. Pflugers Arch 461: 1-21, 2010.

5.8

REGULATION OF NCC BY THE ALDOSTERONE-SGK1-NEDD4-2 PATHWAY

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Aldosterone is a key regulator of the epithelial sodium channel (ENaC) through the activation of the serum glucocorticoid kinase 1 (Sgk1) and the subsequent inhibition of the E3-ubiquitin ligase Nedd4-2 which is known to downregulate ENaC. However the posttranslational mechanism through which aldosterone regulates the Na^+:Cl^- cotransporter (NCC) is unknown. Due to the co-expression of the aldosterone transducing apparatus and NCC in the second portion of the distal convoluted tubule (DCT 2) we explored the relationship between the Nedd4-2 – Sgk1 pathway and NCC. Using HEK 293 cells and *Xenopus* oocytes we have shown that NCC interacts with Nedd4-2 and decreases NCC functional activity, in a kinase dependent fashion. The presence of Sgk1 abrogated both the interaction between Nedd4-2 and NCC in HEK cells as well as NCC functional activity in oocytes in a kinase dependent effect. Sgk1 has been shown to regulate Nedd4-2 by phosphorylating serine 328 and to a lesser extent serine 222. Unlike ENaC the Nedd4-2 S328A mutant was still able to inhibit NCC and inhibited by Sgk1. The Nedd4-2 S222A mutant increased the Nedd4-2 mediated inhibition in the presence of Sgk1 and the Nedd4-2 S222A, S328A mutant was no longer recoverable with Sgk1. In addition, an inducible nephron specific Nedd4-2 KO mouse model shows increased NCC protein with no change in NCC mRNA. These results point to the Aldosterone-Sgk1-Nedd4-2 pathway as a regulator of NCC.

5.9

ENaC REGULATION BY PROTEASES

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Regional differences in ENaC regulation exist along the aldosterone-sensitive distal nephron (1). A unique feature of ENaC is its proteolytic activation which involves cleavage of the α- and γ-subunits. In humans, cleavage of δ-ENaC may also contribute to channel activation (2). Trypsin, a prototypical serine protease, can activate ENaC in microdissected mouse distal nephron. This indicates that ENaC activation by extracellular proteases may be relevant in native tissue. However, the physiologically relevant proteases remain to be determined. In nephrotic syndrome filtered plasminogen is converted to plasmin by tubular urokinase. Plasmin can activate ENaC which may contribute to sodium retention in nephrotic syndrome. Furthermore, ENaC may be a modifier gene in patients with cystic fibrosis (CF). Interestingly, a recently described ENaC mutation mimics proteolytic channel activation and may contribute to the pathophysiology of atypical CF (3). Supported by DFG (SFB 423).References:1. Loffing J & Korbmacher C (2009). Regulated sodium transport in the renal connecting tubule (CNT) via the epithelial sodium channel (ENaC). *Pflügers Arch.* 458, 111-35. 2. Haerteis S, Krueger B, Korbmacher C, Rauh R (2009). The δ-subunit of the epithelial sodium channel (ENaC) enhances channel activity and alters proteolytic ENaC activation. *J Biol Chem.* 284, 29024-40. 3. Rauh R, Diakov A, Tzschoppe A, Korbmacher J, Azad AK, Cuppens H, Cassiman JJ, Dötsch J, Sticht H, Korbmacher C (2010). A mutation of the epithelial sodium channel associated with atypical cystic fibrosis increases channel open probability and reduces Na^+ self inhibition. *J Physiol.* 588, 1211-25.

5.10

DELETION OF THE UBIQUITIN LIGASE NEDD4L IN LUNG EPITHELIA CAUSES CYSTIC FIBROSIS-LIKE DISEASE

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Cystic Fibrosis is caused by impaired ion transport due to mutated CFTR, accompanied by elevated activity of the amiloride-sensitive Epithelial Na^+ Channel (ENaC) in the airways, intestine and other tissues. We and others have previously demonstrated that the ubiquitin ligase Nedd4L (Nedd4-2) inhibits ENaC by promoting its endocytosis from the cell surface. Here we show that knockout of Nedd4L specifically in lung epithelia (Surfactant Protein C (SPC)-expressing Type II and Clara cells) causes cystic fibrosis-like lung disease, with airway mucus obstruction, goblet cell hyperplasia, massive inflammation, fibrosis and death by 3-weeks of age. These effects of Nedd4L loss are likely caused by enhanced ENaC function, as reflected by increased ENaC protein levels, increased lung dryness at birth, amiloride-sensitive dehydration of lung explants, and elevated ENaC currents in primary alveolar-Type II cells harvested from the knockout lungs and analyzed by patch clamp recordings. Moreover, the lung defects were rescued with administration of amiloride into the lungs of young knockout pups via nasal instillation. Our results therefore suggest that the ubiquitin ligase Nedd4L can suppress the onset of cystic fibrosis symptoms by inhibiting ENaC in lung epithelia. We are now studying the function of Nedd4L in the intestine by specific knockout of Nedd4L in intestinal epithelium. Some of these recent studies will be presented as well. Funding: CIHR and CCFF.

5.11

REGULATION OF UBIQUITIN LIGASE ACTIVITY AND PHOSPHORYLATION BY SGK1

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Regulation of epithelial Na^+ channel (ENaC)-mediated transport in the distal nephron is a critical determinant of blood pressure in humans. Nedd4-2, a member of the Homology to the E6-associated protein C Terminus (HECT) family of E3 ubiquitin ligases, is a physiologically important inhibitor of ENaC. Site-specific phosphorylation has been uncovered as a general mechanism to regulate ubiquitin ligases to either increase or decrease their activity. Aldosterone stimulates ENaC through several pathways but importantly, via induction of serum-and-glucocorticoid kinase 1 (SGK1). SGK1 phosphorylates Nedd4-2 and induces interaction with 14-3-3 proteins which in turn, decrease Nedd4-2-mediated inhibition of ENaC. SGK1 augments cell surface alpha-ENaC and increases ENaC-mediated sodium transport through phosphorylation of specific Nedd4-2 residues, deemed 'minor' 14-3-3 interaction sites, which are necessary and sufficient to stimulate ENaC. These 'minor' sites are phosphorylated by aldosterone in kidney epithelia, and bind to dimeric 14-3-3 proteins to inhibit Nedd4-2. Only two isoforms (beta- and epsilon) of the large, redundant family of 14-3-3 proteins are regulated by aldosterone, and have been shown to inhibit endogenous Nedd4-2 in distal nephron cells. Other kinases including Akt, protein kinase A (PKA), and Ikappa kinase-β (IκK-β) use a 14-3-3 dependent mechanism to phosphorylate and inhibit Nedd4-2 at different sites to stimulate ENaC. Thus, aldosterone utilizes precise aspects of a general pathway to stimulate ENaC-mediated transport in tight epithelia. (NIH K081DK071648; NKF Young Investigator Grant).

5.12

ALDOSTERONE-INDEPENDENT REGULATION OF ENaC BY SALT INTAKE

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Sodium handling in the distal nephron is under control by RAAS with aldosterone increases ENaC activity to drive sodium reabsorption and to compensate for volume depletion. However, the apparent aldosterone paradox exists which allows the kidney to effectively conserve Na+ with no apparent K+ wasting during changes in systemic salt intake. Here, we used patch clamp electrophysiology in freshly-isolated split-opened murine distal nephrons to test if ENaC can be regulated by systemic salt intake independently of aldosterone. We found that spironolactone treatment, while decreasing ENaC membrane levels, did not prevent regulation of ENaC open probability by salt intake. This raises a possibility that other components of RAAS, such as Ang II, contribute to regulation of ENaC by salt intake. Indeed, we found that Ang II in the range from 5 to 500 nM acutely and reversibly increases ENaC Po with 5 nM of Ang II having only a subtle effect. This stimulatory effect was abolished when AT1 receptors were inhibited with losartan.We next probed if Ang II stimulates ENaC activity in the presence of saturated aldosterone levels. The stimulatory effect of Ang II on ENaC was preserved although blunted in DOCA-treated animals. Moreover, we found that Angiotensin-converting enzyme (ACE) plays an important role in cleaving locally produced kinins, such as Bradykinin (BK), to further stimulate ENaC activity. ACE inhibition augmented the inhibitory action of BK on ENaC and caused marked natriuresis in wild type but not in mice lacking BK receptors. We concluded that activation of Ang II cascade complements the well-described aldosterone signaling to ENaC. This allows effective ENaC activation during salt restriction providing a mechanism for aldosterone paradox.

5.13

RAB-GAP REGULATION OF EPITHELIAL SODIUM CHANNEL (ENaC) FORWARD TRAFFICKING IN RESPONSE TO ALDOSTERONE AND VASOPRESSIN

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The Rab-GAP, AS160 (TBC1D4), is an Akt/SGK1 substrate that stabilizes ENaC in a regulated intracellular apical recycling compartment under basal conditions. Its phosphorylation by aldosterone/SGK1 induces 14-3-3 protein binding, suppresses its GAP activity, augments its expression level and permits ENaC trafficking to the apical membrane to augment Na absorption *(Liang et al. Mol Biol Cell. 2010).* Whether AS160 or a related Rab-GAP modulates ENaC trafficking in response to vasopressin is not known. Among the ~50 Rab-GAPs in humans, TBC1D1 is AS160s closest structural relative. D4 and D1 were expressed in mpkCCD epithelia, and they were present in 14-3-3 affinity capture eluates. Unlike D4, D1 is phosphorylated in vivo by PKA; forskolin promoted D1 phosphorylation at T596, but not at PKA site S237. To probe for potential phosphorylation-dependent 14-3-3 interactions in response to regulators, we IPed D4 and D1 and blotted with a pan-14-3-3 antibody. Aldosterone increased D4 expression and its interaction with 14-3-3, whereas forskolin stimulation had minimal effect. TBC1D1-14-3-3 interactions were primarily regulated by forskolin/PKA stimulation, and 1 hr treatment with forskolin increased D1 expression. These findings suggest that TBC1D1 is a target for PKA-mediated phosporylation and that this Rab-GAP plays a role in vasopressin-stimulated ENaC apical trafficking, similar to AS160s role in the response to aldosterone. [Supported by NIH 1K01DK090118 and DK054814].

5.14

THE ROLE OF mTOR AND SGK1 IN MEDIATING ALDOSTERONE REGULATION OF ENaC *IN VIVO*

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SGK1 is a key mediator of aldosterone activation of ENaC in the kidney tubules. The protein kinase mammalian target of rapamycin, mTOR, has been shown in cultured cells and *in vitro* to phosphorylate SGK1, and stimulate ENaC. To elucidate mTOR actions *in vivo,* we examined the effects of mTOR inhibition on urine electroylytes and volume in mice. Six adult C57BL6 mice were monitored in balance cages for six hours after intraperitoneal injection with either vehicle or the mTOR inhibitor, PP242 (30mg/kg; n = 10), in a cross-over fashion, allowing one week of washout between experiments. We found that urine volume (ul) was increased by 56% (609 +/- 55 vs 953 +/- 152 ul p = 0.048), sodium (mmol/L) was increased by 79% (19.1 +/- 3.51 vs 53.4 +/- p = 0.034), total sodium excreted (mmol) was increased by 167% (11.7 +/- 2.46 vs 42.9 +/- 11.1 p = 0.014). The Urine sodium to potassium ratio showed a 62% increase (0.20 +/- 0.03 vs 0.53 +/- 0.13 p = 0.026). No change was noted in urinary potassium level (p= 0.45) or total creatinine excreted (p =0.23), but there was an increase in total potassium excreted (mmol) (56.7 +/- 7.2 vs 91.7 +/- 15.0 p =0.05) as well as significant increase in urinary glucose (mg/dl) (31 +/- 2.29 vs 153 +/- 45 p=0.014). The increase in urine volume and Na, and specifically the Na/K ratio points to an inhibition of ENaC, possibly due to decreased SGK1 phosphorylation. The lack of decrease in urine potassium may reflect a coincident independent effect of mTOR inhibition to increase urinary glucose. Together with cell culture and in vitro data, these data strongly suggest that the PI3-kinase-mTOR-SGK1 signaling network plays a physiologically important role in ENaC regulation in the kidney tubules. (NIH DK085101 NIH DK056695).

6.0: ALDOSTERONE: SYNTHESIS CROSSTALK AND NON-EPITHELIAL ACTIONS

6.1

REGULATION OF ALDOSTERONE PRODUCTION THROUGH EXPRESSION OF ALDOSTERONE SYNTHASE (CYP11B2)

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The capacity of the adrenal to produce aldosterone is controlled by the expression of the enzyme aldosterone synthase (CYP11B2).CYP11B2 is responsible for the conversion of deoxycorticosterone to aldosterone and underphysiologic conditions is expressed in the adrenal zona glomerulosa under tight controlof circulating angiotensin II (Ang II) and serum potassium (K^+). Our research indicates that Ang II and K^+share activation ofadrenal cell calcium signaling and this pathway is the primarily mechanism regulating aldosterone production and CYP11B2 expression. This process involves activation of calmodulin (CaM) and CaM kinases followed byphosphorylation of transcription factors (the CREB/ATF family) and increasedtranscription factor expression (the NGFI-B family) thatactivate CYP11B2 gene transcription. CYP11B2 expression also appears to play a critical role in the dysregulation of aldosterone seen in patients with primary aldosteronism (PA). In PA caused byaldosterone-producingadenomas theadrenal continues to express CYP11B2 even under low-renin conditions. We and others have shown that multiple factors contribute to the dysregulation of CYP11B2 expression including ectopic G-protein coupled receptors and alterations in ion channels that impact intracellular signaling.

6.2

CLOCK AND RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN ADRENAL GLAND AND KIDNEY

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We recently identified a new type of 3β-HSD (or 3-β-hydroxysteroid dehydrogenase/Δ-5-4 isomerase) in zona glomerulosa (ZG) cells of the adrenal gland. This enzyme, called Hsd3b6 in mice, is regulated by the circadian clock and is over-expressed in arrhythmic *Cryptochrome-1/Cryptochrome-2* double knockout (*Cry*-null) mice. Due to the strong elevated levels of this enzyme, *Cry*-null mice suffer from hyperaldosteronism, and exhibit salt-sensitive hypertension. In human, we characterized HSD3B1, the homologue of mouse HSD3B6, as the ZG-specific 3BHSD enzyme, and we are investigating the molecular link between the clock and the renin-angiotensin-aldosterone system in the regulation of HSD3B1. The circadian clock oscillatory system is functional in the aldosterone sensitive distal nephron (ASDN), which we described using real-time PCR combined with laser capture microdissection (LCM). Moreover, low-amplitudecircadian expression profiles of epithelial sodium channel (ENaC) and Na^+/K^+-ATPase were observed in the ASDN. Clock-controlled oscillations of adrenal and kidney cells metabolism will likely contribute to the daily homeostasis and dynamic changes of fluid and electrolytes balance. REFERENCES: Doi, M., Takahashi, Y., Komatsu, R., Yamazaki, F., Yamada, H., Haraguchi, S., Emoto, N., Okuno, Y., Tsujimoto, G., Kanematsu, A., Ogawa, O., Todo, T., Tsutsui, K., van der Horst, G.T.J. & Okamura, H. 2010. Salt-sensitive hypertension in circadian clock-deficient mice involves dysregulated adrenal Hsd3b6. Nature Med., 16, 67-74.

6.4

COMPARTMENT-SPECIFIC MINERALOCORTICOID RECEPTOR SIGNALING

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The mineralocorticoid receptor (MR) classically acts as an aldosterone-dependent transcription factor at hormone-response-elements (GRE) that it shares with its closest relative, the glucocorticoid receptor (GR). Besides contributing to water, electrolyte and blood pressure homeostasis, the MR can also elicit pathophysiological effects in the renocardiovascular system, including inflammation and fibrosis. Because these effects are not mediated by the GR, additional signaling mechanisms for the MR have been postulated. Recent findings revealed interactions of MR with signaling molecules of different cellular compartments and a cross-talk between non-genomic and genomic MR effects. The additional MR-signaling components include plasma membrane receptors like the epidermal growth factor receptor as well as cytosolic components like calcineurin. As pathophysiological consequences of these interactions an enhanced secretion of extracellular matrix components and a reduction in glucose-6-phosphate dehydrogenase expression have been documented. In the nucleus classical GRE-dependent signaling takes place but further hormone-responsive-elements have been described as well. Overall, these findings suggest compartment-specific signaling of the MR embedded in an in-tricate signaling network. (DFG: GR 3415/1-2, GE 905/14-1). Grossmann C, Wuttke M, Ruhs S, Seiferth A, Mildenberger S, Rabe S, Schwerdt G, Gekle M. Mineralocorticoid receptor inhibits CREB signaling by calcineurin activation. FASEB J. 2010 Jun; 24(6):2010-9.

7.0: PLENARY LECTURE

7.1

ABERRANT RAC1-MR PATHWAY IN SALT-SENSITIVE HYPERTENSION AND METABOLIC SYNDROME

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Obese persons with metabolic syndrome often have associated with salt-sensitive hypertension, Proteinuria and cardiac dysfunction, and the serum aldosterone level in one-third of metabolic syndrome patients is clearly elevated. Salt loading aggravates the proteinuria and induces cardiac diastolic dysfunction because of inadequate suppression of serum aldosterone level. Indeed, aldosterone excess and and a high-salt diet exert an unfavorable synergistic action on cardiovascular damage. In Dahl salt-sensitive (S) rats, however, despite appropriate suppression of serum aldosterone with a high-salt diet, salt loading paradoxically activated renal MR signaling, and hypertension and renal injury was markedly prevented by MR antagonists. Accordingly, we discovered an alternative pathway of MR activation in which Rac1, a small GTP-binding protein, activates MR (Nat Med 2008). Salt loading changed Rac1 activity in opposite directions: Rac1 upregulation in Dahl-S rats and downregulation in Dahl salt-resiatant (R) rats. Despite the suppression of serum aldosterone, salt-loaded Dahl-S rats showed increased MR signaling in the kidneys, suggesting the key role of Rac1 in modulating salt susceptibility. Moreover, several metabolic syndrome-related factors such as IL-6 induce Rac1 activation. Treatment of Rac1 inhibitors decreased blood pressure and attenuated renal damages in both salt-loaded Dahl-S rats and obese hypertensive rats. Thus, both salt and obesity activate Rac1 and cause MR activation. Abnormal activation of Rac1-MR pathway plays a key role in salt-sensitive hypertension and metabolic syndrome. Reference: Shibata S, Nagse M, Yoshida S, Kawarazaki W, sKurihara H, Tanaka H, Miyoshi J, Takai Y, Fujita T. Modification of mineralocorticoid receptor function by Rac1 GTPase: implication in proteinuric kidney disease. Nat Med 14: 1370-1376, 2008.

8.0: ALDOSTERONE AND ENaC

8.1

CHOLERA TOXIN ENHANCES SODIUM ABSORPTION ACROSS CULTURED HUMAN MAMMARY GLAND EPITHELIA: NOVEL MECHANISMS OF REGULATION ENAC FUNCTION IN MAMMARY GLAND

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Cellular mechanisms to account for the low Na^+ concentration in human milk are poorly defined. MCF10A cells, derived from human mammary epithelium and grown on permeable supports, exhibited amiloride- and benzamil-sensitive ion transport (short circuit current, I_{sc}), suggesting activity of the epithelial Na^+ channel, ENaC. When cultured in the presence of cholera toxin (Ctx), MCF10A cells exhibited greater amiloride sensitive I_{sc} at all time points tested (2h to 7d), an effect that was not reduced with Ctx washout for 12 hours. Similarly, the amiloride sensitive I_{sc} remains elevated by Ctx in the presence of inhibitors for PKA (H-89), PI3K (LY294002) and protein trafficking (brefeldin A). Additionally, the Ctx B subunit, alone, did not replicate these effects. RT-PCR and western blot analysis showed no significant increase in either the mRNA or protein expression for α, β, or, γ-ENaC subunits. Likewise, Nedd4-2 abundance was not changed. Biotinylation analysis showed that Ctx increased β and γ-ENaC expression in the apical membrane. These results demonstrate that human mammary epithelia express ENaC, which can account for low milk Na^+ concentration, and that Ctx enhances ENaC localization in the apical membrane. This mechanism of Ctx-elevated ENaC function may provide clues regarding mechanisms in human mammary gland epithelia that regulate Na^+ transport via ENaC, which will likely have implications for epithelia throughout the body. [NIH P20-RR017686 & KS Ag Exp Stn support].

8.2

THIOL-REACTIVE COMPOUNDS FROM GARLIC INHIBIT THE EPITHELIAL SODIUM CHANNEL (ENaC) – A POSSIBLE MECHA-NISM FOR LOWERING BLOOD PRESSURE?

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Garlic is well known as a natural remedy with beneficial effects against high blood pressure. However, the mechanisms how garlic exerts its hypotensive effects are poorly understood. The regulation of blood pressure in the kidney is linked to transepithelial sodium reabsorption from primary urine and particularly the activity of epithelial sodium channels (ENaC). Therefore we questioned whether there might be any impact of compounds from garlic on ENaCs. Human ENaCs, consisting of the α, β and γ subunits, were heterologously expressed in *Xenopus* oocytes. Transmembrane currents (I_M) were recorded by the two-electrode voltage-clamp technique. Garlic extract (GE) was made from 5 g of fresh garlic in 10 ml of oocyte ringer solution on ice for 1 hour and further diluted with oocyte ringer. The application of GE dose-dependently decreased the I_M of ENaC expressing oocytes, peaking at 80 % inhibition with the highest concentration (1 %). The effect was not apparent on water-injected control oocytes. The decrease of I_M due to GE was not reversible and was fully sensitive to the ENaC inhibitor amiloride. In the presence of saturating concentrations of L-cysteine (20 mM), the effect of garlic was blocked. In sum, these data indicate that thiol-reactive compounds from GE irreversibly inhibit ENaCs. Decreasing sodium reabsorption in the kidney epithelium might represent a mechanism for diuresis, natriuresis and consequently hypotension as attributed to the health benefits of garlic.

8.3

THE δ1 AND δ2 ENaC SUBUNITS FORM MECHANOSENSITIVE CHANNELS WHEN COEXPRESSED WITH β AND γ SUBUNITS

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Four different types of ENaC subunits have been identified (α,β,γ and δ) and it is known that channels consisting of the α, β and γ subunits are activated by shear force (SF). However, little is known concerning the effect of SF on δβγ ENaC. Two isoforms of human δENaC (δ1and δ2) were heterologously expressed together with β and γ in Xenopus oocytes. Transmembrane currents were recorded by the two-electrode-voltage-clamp technique. SF was applied with a laminar fluid stream. Both δ1βγ and δ2βγ were activated by SF and there was no difference between the two isoforms. The SF effect was pH sensitive: Acidic pH values (pH 4-6) increased ENaC activity and decreased the effect of SF. With alkaline pH values (pH 8-10) opposite results were observed: Alkaline pH inhibited ENaC activity, but increased the SF effect. Half-maximal SF activation was determined at pH 7.9 for δ1 and at pH 8.3 for δ2 comprising channels. These observations indicate that SF increases the open probability of δβγENaCs. Preincubation with trypsin, known to activate ENaCs by proteolytic cleavage, attenuated the SF effect of δβγ ENaCs. In summary, δβγ ENaCs are activated by SF. The extracellular loops, which are accessible for protons and proteases, are likely involved in mechanical gating of ENaC. This indicates that, similar to αβγ ENaC, SF increases the open probability of δβγ ENaCs rather then increasing the number of active channels.

8.4

EXERCISE TRAINING IMPROVES ENaC-MEDIATED SODIUM REGULATION IN RATS WITH CHRONIC HEART FAILURE

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One hallmark of chronic heart failure (CHF) is sodium and fluid retention. One of the key elements involved in renal sodium retention is activation of epithelial sodium channels (ENaC) in the collecting tubule by aldosterone and vasopressin. Previously, we have shown that in rats with CHF there is increased abundances of α-, β- and γ-ENaC subunits and a significant corresponding increase in ENaC activity in the kidneys. The goal of the present study was to determine the impact of exercise training (ExT) on the ENaC-mediated sodium regulation in rats with CHF. The experiments were conducted in left coronary ligation rats with CHF, ExT (treadmill run for 3-4 weeks) significantly reduced diuretic (Δ45% CHF-ExT compared to CHF-sedentary) and natriuretic (Δ43%) responses to ENaC inhibitor benzamil. Exercise training also significantly reduced the protein expression of α- (Δ55%), β- (Δ40%) and γ-ENaC (Δ22%) subunits in the cortex of CHF rats. To further determine one possible mechanism related to the activation of ENaC, we found that the levels of urinary serine proteases including furin, prostasin and plasmin were dramatically increased in the rats with CHF by using immnunoblotting. In conclusion, ExT may improve sodium regulation in CHF by alteration of the ENaC pathway.

8.5

DEPENDENCE OF ENaC RECYCLING RATE ON THE TOTAL AMOUNT OF RECYCLED CHANNELS

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Trafficking is one of primary mechanisms of ENaC regulation. In the present study, we investigated if the recycling rates of ENaCs depend on the total amount of recycled ENaCs. To clarify this point, we established a novel method estimating the total amount of recycled ENaCs and ENaC recycling rates by using benzamil (a specific blocker of ENaC) for functional labeling of recycled ENaCs in renal epithelial A6 cells. Applying this method, we studied if the rates of insertion and endocytosis of ENaCs to and from the apical membrane of A6 cell monolayers are affected by a brefeldin A (5 μg/mL, 1 h)-caused decrease in total amount of ENaCs. We obtained the following observations: 1) both insertion and endocytosis rates of ENaCs are increased by a decrease in the total amount of ENaCs, and 2) the increase in the insertion rate is larger than that in the endocytosis one. This larger increase in the insertion rate than the endocytosis one caused by the decrease in the total amount of ENaCs plays an important role in preventing Na^+ transport from drastic diminution due to a decrease in the total amount of ENaCs. This newly established analysis using blocker-labeled ENaCs in the present study enables us to investigate the recycling of endogenously expressed ENaCs. Supported by JSPS Fellows (20·5712) and Scientific Research (20390060) from JSPS, and The Salt Science Research Foundation (1035), Research Conference for Cell Function, and Fuji Foundation for Protein Research.

8.6

EFFECTS OF CYTOCHROME P450 METABOLITES OF ARACHIDONIC ACID ON ENaC

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Previous studies have indicated that arachidonic acid (AA) and its metabolite 11,12-EET but not other regioisomers of EETs inhibit ENaC activity in the collecting duct. The goal of this study was to investigate the endogenous metabolism of AA in mpkCCD_{c14} cells and the effects of the metabolites formed on ENaC activity. LC/MS analysis of the mpkCCD_{c14} cells indicated that these cells produce prostaglandins, 8,9-EET, 11,12-EET, 14,15-EET, 5-HETE, 12/8-HETE, and 15-HETE, but not 20-HETE. Patch clamp experiments revealed that 8,9-EET, 14,15-EET and 11,12-EET all decrease ENaC activity. Neither 5-HETE nor 12-HETE and 15-HETE had any effect on ENaC activity. Diclofenac and ibuprofen, inhibitors of COX, decreased transepithelial Na^+ transport in mpkCCD_{c14} cells. Inhibition of epoxygenases with MS-PPOH activated ENaC-mediated sodium transport when cells were pretreated with AA and diclofenac. Co-expression of CYP2C8 but not CYP4A10 with ENaC in CHO cells significantly decreased ENaC activity in whole-cell experiments, whereas 11,12-EET mimicked this effect. Biotinylation assay and single channel analysis revealed that long-term treatment with 11,12-EET and overexpression of CYP2C8 decrease the number of channels in the plasma membrane. In contrast, the acute inhibitory effects are mediated by a decrease in the P_o. We conclude that 11,12-EET, 8,9-EET and 14,15-EET are all endogenously formed eicosanoids that modulate ENaC activity in the collecting duct. Supported by AHA, ADA and NIH.

8.7

KEY ROLE FOR THE CORTICAL ACTIN BINDING PROTEIN CORTACTIN IN REGULATION OF ENaC BY THE ACTIN CYTOSKELETON

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ENaC activity is mediated, at least partially, by the actin cytoskeleton. The goal of this study was to ascertain the mechanism of ENaC regulation by the actin filaments. Here we demonstrate for the first time that cortactin plays a key role in ENaC regulation by F-actin. IHC and Western blotting revealed that cortactin is expressed in the kidney cortex and kidney epithelial cells, and is localized to the cortical collecting ducts. Coexpression of cortactin with ENaC in CHO cells decreases ENaC activity. Knockdown of cortactin in mpkCCD_{c14} cells results in increased ENaC activity and sodium reabsorption. As shown by biotinylation and single-channel analysis, cortactin reduces ENaC P_o. Co-IP analysis reveals interaction between cortactin and ENaC. To clarify the mechanism of the cortactin's effect we assayed various cortactin mutants, but only a mutant unable to bind Arp2/3 complex did not affect ENaC activity. Interestingly, ENaC overexpression with the SH3 domain of cortactin responsible for dynamin binding also decreases ENaC current. Inhibitor of the Arp2/3 CK-0944666 precludes cortactin effects. Depolymerization of the actin microfilaments and inhibition of the Arp2/3 complex do not result in the loss of association between ENaC and cortactin. Thus, these results indicate that cortactin is

functionally important for ENaC activity and Arp2/3 complex is involved into this mechanism. REFERENCE: Ilatovskaya et al., *FASEB J.* (2011) PMID: 21536685.

8.8

MECHANISMS OF ENaC REGULATION BY INSULIN

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Insulin is recognized as a powerful regulator of ENaC in the aldosterone-sensitive distal nephron. To study mechanisms of ENaC regulation by insulin, we generated insulin receptor knockout (IR-KO) mice targeted specifically to the collecting duct principal cells using Cre-lox mediated recombination. Mice with loxP sites flanking the IR gene were crossed with mice possessing Cre-recombinase driven by the AQP2-promoter. After one week of sodium-deficient diet the IR-KO mice demonstrated significantly lower ENaC activity compared to their wild type littermates as was demonstrated by cell attached patch clamp measurements in freshly isolated split opened collecting duct. Acute insulin application in such experiments revealed that loss of insulin reception prevented increase of ENaC activity which was observed in wild type mice. Immunohistochemical and western-blot assays demonstrated that total abundance of all three ENaC subunits in the kidney cortex were not different between WT and IR-KO mice. Thus, these results suggest that insulin via IR increases ENaC activity affecting the channel open probability (P_o). To further determine mechanism of insulin's action on ENaC, we used immortalized $mpkCCD_{cl4}$ principal cells. Insulin rapidly increased amiloride-sensitive transepithelial flux in $mpkCCD_{cl4}$ cells with the EC_{50}=12.2±1.7nM. Pretreatment of the $mpkCCD_{cl4}$ cells with PI3-kinase or mTOR inhibitors LY294002 or PP242, respectively precluded the effect of insulin. Thus, we propose that insulin is a key regulator of ENaC activity and its effects are mediated via PI3-kinase and mTOR signaling. Funding: ADA 1-10-BS-168, AHA 10POST4140109, NIH DK082507.

8.9

NEPHRON-SPECIFIC GENE INACTIVATION OF α AND γ ENaC IN ADULT MOUSE: DISTINCT EFFECTS ON Na^+ AND K^+ BALANCE HOOD LEADS TO A SEVERE DISTURBANCE OF Na^+ AND K^+ BALANCE

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Constitutive and ubiquitous gene activation of α or γENaC subunit leads to perinatal lethality caracterized by a severe lung phenotype (α) and renal PHA-1 phenotype (α and γ). The aim of this work is to study the importance of ENaC-mediated Na+ absorption and K+ secretion by deleting either α or γ ENaC along the aldosterone sensitive distal nephron (ASDN) of adult animals. Thus, genetically engineered mice lacking α or γENaC subunit in the whole nephron have been developed using a tetracycline-inducible Cre system (Pax8-rTta/LC1) active along the entire nephron, except the glomeruli. Upon normal salt diet and following five days of doxycycline treatment, 4 week -old α and γENaC nephron-specific knockout mice kept loosing body weight (- 10% and -18%, respectively), whereas control mice continued to gain weight normally (+8 and +4%, respectively). The αENaC KO mice develop a severe and lethal PHA-1 phenotype and are hyponatremic and severely hyperkalemic (7-8 mM) with daily cumulative loss of urinary Na+ and gain of urinary K+. Whereas, surprisingly, γENaC KO mice are normonatremic but severely hyperkalemic (7-8 mM) with no significant daily cumulative loss of urinary Na+ but a dramatic cumulative gain of urinary K+. This unexpected dissociation between Na+ and K+ transport suggest that K+ secretion is no longer tightly coupled to Na+ reabsorption along the entire (or some part) of the ASDN. This work is supported by the Leducq Foundation.

8.10

MODULATION OF ENaC-MEDIATED Na^+ ABSORPTION BY TH2-DEPENDENT AIRWAY INFLAMMATION IN MICE

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Airway epithelium is a central effector tissue in allergic inflammation (AI) and Th2-driven epithelial responses, such as mucus hypersecretion, contribute to airflow obstruction in allergic airway disease. Previous in vitro studies demonstrated that Th2 cytokines act as potent modulators of ENaC mRNA expression and function, but the in vivo effects of AI on ENaC-mediated Na^+ and fluid absorption across airway epithelia have not been studied. In this study, we induced AI in airways by i.t. instillation of Aspergillus fumigatus extract or the Th2 cytokine IL-13 in mice and determined effects on ion transport in native tracheal and bronchial tissue in perfused micro-Ussing chambers. We demonstrate that AI inhibited ENaC-mediated Na^+ absorption and enhanced basal and Ca^{2+}-activated Cl^- secretion producing a pro-secretory epithelial ion transport phenotype in mouse airways. Allergen-induced inhibition of Na^+ absorption was associated with reduced transcript levels of α-, β- and γENaC, and was largely abrogated in mice lacking Stat6, a transcription factor critical for IL-13 signaling. We conclude that AI of airways inhibits ENaC mRNA expression and ENaC-mediated Na^+ and fluid absorption in a Stat6-dependent manner in vivo. These results suggest that Th2-mediated inhibition of ENaC, together with enhanced Cl^- secretion, may improve airway surface hydration and clearance of hypersecreted mucus in allergic airway diseases such as asthma. Supported by: DFG MA 2081/3-2 and MA 2081/4-1

8.11 Withdrawn

8.12

DISSECTION OF THE ALDOSTERONE AND GLUCOCORTICOID-DEPENDENT PATHWAY IMPLICATED IN SODIUM RETENTION IN THE RAT

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The glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) are major players in the regulation of salt homeostasis. Our project aims to understand the role of the aldosterone- and glucocorticoid-dependent pathways in the control of sodium retention in the rat by generating GR and MR mutants. Due to its physiological characteristics and ease of manipulation, the rat presents an ideal model for cardiovascular diseases. For the past decades, the lack of gene targeting technologies in the rat was a major setback in experimental genetics. A few years ago, a new efficient technology for gene targeting in mammals emerged, based on recombinant nucleases called Zinc Finger Nucleases (ZFNs). ZFNs generate double stranded breaks (DSB) in the target sequence, which are amended by the endogenous DNA repair system. This amending sometimes generates insertions or deletions which later lead to a nonsense-mediated mRNA decay thereby generating a «knock-out» of the targeted gene. By introducing a donor plasmid with the desired mutation, the cell will integrate the mutation by homologous recombination. To engineer these zinc fingers, we use two methods: first, «OPEN» for Oligomerized Pool Engineering and second, «CoDA» for Context-Dependent Assembly offered by the ZFN Consortium (www.zincfingers.org). The selected ZFNs will be tested in vivo in a rat cell line as well as by injection in fertilized oocytes in order to generate knock-out and knock-in GR and MR mutant rats.

8.13

INVOLVEMENT OF p38-MEDIATED ENDOCYTOSIS IN ALDOSTERONE-STIMULATED Na^+ REABSORPTION IN RENAL EPITHELIAL A6 CELLS

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Aldosterone (ALD) is an important hormone to regulate renal Na^+ reabsorption for controlling ECF volume and blood pressure. In this study, we found ALD activated p38 and investigated a role of p38 in the regulatory mechanism of Na^+ reabsorption stimulated by ALD. To clarify the involvement of p38 in stimulatory action of ALD on Na^+ reabsorption, we examined effects of p38 inhibitor on ALD-stimulated benzamil-sensitive Isc (I_{Na}) and conductance (G_{Na}). Pretreatment with SB202190 (a specific p38 inhibitor: SB) significantly diminished ALD-stimulated Na^+ reabsorption by decreasing G_{Na}. We next clarified a role of p38 in the surface expression of ENaC through exocytic and endocytic pathways. Pretreatment with MG132 (a proteasome inhibitor) mostly recovered the SB-induced reduction of I_{Na}, although MG132 slightly increased the I_{Na} in the absence of SB. In contrast, brefeldin A (an inhibitor of protein translocation from ER to Golgi) showed further an inhibitory effect on the SB-decreased I_{Na}. Moreover, addition of SB caused rapid reduction of the ALD-stimulated Na^+ reabsorption and the reduction rate of Na^+ reabsorption in ALD-treated cells was larger than that in untreated cells. This suggests that the p38-mediated signal pathway suppresses the endocytic/proteasome-dependent ENaC degradation contributing to the stimulation of Na^+ reabsorption by ALD. Supported by JSPS (19590212, 20390060), SSRF (0837, 1035) and FFPR.

8.14

THE RENAL NA+-CL- CO-TRANSPORTER IS REGULATED BY THE ALDOSTERONE-SGK1-NEDD4-2 PATHWAY

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Aldosterone stimulates Na+ reabsorption in the aldosterone-sensitive distal nephron (ASDN), via the Na+-Cl- co-transporter (NCC) in the distal convoluted tubule (DCT), and the epithelial Na+ channel (ENaC) in the late DCT, connecting tubule and collecting duct. Aldosterone increases NCC protein expression by an unknown post-translational mechanism. The ubiquitin-protein ligase Nedd4-2 (N4-2) is expressed along the ASDN and regulates ENaC: under aldosterone induction, the serum/glucocorticoid-regulated kinase SGK1 phosphorylates N4-2 on S328, thus preventing the N4-2/ENaC interaction, ubiquitylation and degradation of the channel. Here, we present evidence that N4-2 regulates NCC. In transfected HEK293 cells, N4-2 co-immunoprecipitates with NCC and stimulates NCC ubiquitylation at the cell surface. In Xenopus laevis oocytes, co-expression of NCC with N4-2 strongly decreases NCC activity. This inhibition is prevented by SGK1 in a kinase-dependent manner. Moreover, we show that NCC expression is up-regulated in inducible renal tubule-specific N4-2 knockout mice. Interestingly, in contrast to ENaC, N4-2-mediated NCC inhibition is independent of a PY motif in NCC. Moreover the double mutation S328 and S222 to alanine enhances N4-2 activity and abolishes SGK1-dependent inhibition. These results indicate that NCC expression and activity is controlled by a regulatory pathway involving SGK1 and Nedd4-2.

8.15

A SALT LOSING PHENOTYPE OF THE SGK1 INDUCIBLE KIDNEY SPECIFIC KNOCK-OUT MOUSE

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The serum- and glucocorticoid-regulated kinase (Sgk1) is induced by mineralocorticoids and, in turn, upregulates the renal epithelial Na channel (ENaC) activity. Total inactivation of Sgk1 has been associated with transient urinary Na wasting on low-sodium diet, and the aldosterone-mediated ENaC channel activation unchanged in the collecting duct. Since Sgk1 is ubiquitously expressed, we aimed to study the role of renal Sgk1 and generated an inducible kidney-specific KO mouse. We took advantage of the previously described TetOn/CreLoxP system, in which the rtTA is under the control of the Pax8 promotor, allowing inducible inactivation of the floxed Sgk1 gene in the renal tubules (Sgk1fl/fl/Pax8/LC1 mice). We found that under standard Na diet, the renal water and Na¬/K excretion had a tendency to be higher in doxycycline treated Sgk1 mutant mice as compared to control mice. The impaired ability of Sgk1 mutant mice to retain Na increased significantly at low salt diet (LSD) despite higher plasma aldosterone levels.

At LSD the Sgk1 mutant mice are also hyperkaliuric and loose body weight. This phenotype is accompanied by a decrease of Nedd4-2 phosphorylation and a decrease in the expression of the Na,Cl-cotransporter NCC and at a lower extent of á-ENaC. These results indicate that Sgk1 is involved in the modulation of renal Na/K excretion via the regulation of the expression of Na transporters in the distal part of the nephron.

8.16

THE ROLE OF RENAL MINERALOCORTICOID VERSUS GLUCOCORTICOID RECEPTOR IN OEDEMATOUS DISEASES

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Proper kidney function is a prerequisite for healthy life. In the aldosterone-sensitive distal nephron (ASDN), the control of sodium balance, fluid homeostasis and blood pressure are regulated by transepithelial sodium transport. This fine tuning is achieved by aldosterone which binds and activates MR and thus triggers the signaling cascade. Moreover, several largely unknown aldosterone-independent mechanisms including cross-talks with other pathways may contribute to water and electrolyte homeostasis in the normal and diseased state. Oedematous diseases, such as liver cirrhosis, nephrotic syndrome and cardiac failure are associated with extracellular fluid expansion and hydro-electrolytic disorders. In order to acquire novel insights of the adrenal corticoid-dependent and independent mechanisms that control water, sodium and potassium handling under physiological and pathophysiological conditions, the role of MR- versus glucocorticoid receptor (GR)-dependent and independent mechanisms will be analyzed in these diseases. Using floxed GR and MR mice, we generated kidney-specific MR and GR knockout mice and oedematous diseases will be induced. These mice will then be analyzed with respect to their gene profiling to identify target genes. This work is supported by the NCCR (National Center of Competence in Research) Kidney.CH (Kidney Control of Homeostasis).

8.17

IDENTIFICATION OF PROTEINS REGULATED BY 24-HOUR ALDOSTERONE TREATMENT IN MURINE LATE DISTAL CONVOLUTED TUBULES AND CONNECTING TUBULES

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Proteomic studies of late distal convoluted tubule (DCT2) and connecting tubule (CNT) have so far been hindered by insufficient yield and purity of manually isolated tubular cells. Our goal was to study the short-term effects of elevated plasma aldosterone on the protein expression profile in DCT2 and CNT. Suspensions of single kidney cells were obtained by incubating kidneys sliceswith 22 µg/mlproteinase K and 1mg/ml collagenase II and subsequently with trypsin in Ca^{2+}-free/EGTA buffer. Fluorescence activated sorting of cells from mice endogenously expressing enhanced green fluorescent protein in DCT2 and CNT yielded 1.5 million cells per mouse with over 70% purity (FACSAriaIII, BD Biosciences). Protein samples from 10 mice were pooled to obtain 100µg protein samples and tagged with 8-plex isobaric tags (iTRAQ). Strong cation exchange yielded 18 fractions for liquid chromatography mass spectrometry/mass spectrometry (Eksigent nanoflow LC system and LTQ Orbitrap Velos mass spectrometer, Thermo Scientific). The resulting spectra were matched to proteins by the SEQUEST search algorithm, which identified 3933 peptides corresponding to 508 unique proteins. Preliminary analysis suggests changes on abundance in 10% of these proteins after 24-hour aldosterone treatment. Among the induced proteins, fructose-1,6-biphosphatase, pyruvat dehydrogenase E1, and pyruvatkinase isozyme M2 are all involved in the carbohydrate metabolism, suggesting an increased capacity for energy production.

8.18

MODULATION OF THE EPITHELIAL SODIUM CHANNEL (ENaC) ACTIVITY BY NOREPINEPHRINE IN CULTURED COLLECTING DUCT CELLS IS PARTIALLY MEDIATED BY α_2-ADRENOCEPTORS

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ENaC activity can be modulated by a variety of hormones and factors [1]. Renal sympathetic innervation may contribute to the regulation of tubular Na^+ transport but its precise role remains unclear. A recent clinical study has demonstrated that renal sympathetic denervation significantly reduced blood pressure in patients with resistant hypertension [2]. Therefore, we investigated the effects of the sympathetic transmitter norepinephrine on ENaC-mediated transepithelial Na^+ transport in $mCCD_{cl}$ cells, a model of renal collecting duct principal cells [3]. ENaC activity in $mCCD_{cl}$ cells was quantified using Ussing chambers to record equivalent short circuit current (I_{SC}). Addition of norepinephrine (10µM) to the basolateral bath produced a complex response involving a rapid transient peak in I_{SC}, followed by an inhibition (15min) below baseline and a subsequent increase in I_{SC} above baseline over 2.5h. The brief decline and late increase in I_{SC} were amiloride-sensitive indicating that altered ENaC activity is responsible for these effects. Addition of the α_2-adrenoceptor antagonist yohimbine (1µM) partially prevented the transient peak and the late increase in I_{SC}. These results indicate that ENaC activity in $mCCD_{cl}$ cells can be modulated by norepinephrine and is at least partially mediated by basolateral α_2-adrenoceptors.This work was supported by the Bayerische Forschungsstiftung.1. Loffing J and Korbmacher C (2009). *Pflug Arch Eur J Phy* 458, 111-135. 2. Krum H et al. (2009). *Lancet* 373, 1275-1281.3. Gaeggeler HP et al. (2005). *J Am Soc Nephrol* 16, 878-891.

8.19

DEREGULATION OF ENaC, RESPIRATORY DISTRESS AND PERINATAL LETHALITY IN Nedd4-2 DEFICIENT MICE

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In vitro studies show that the E3 ubiquitin ligase Nedd4-2 ubiquitinates ENaC to down-regulate its cell surface expression. To examine the *in vivo* role of Nedd4-2 in the regulation of ENaC, we generated Nedd4-2 deficient mice that show a complete loss of Nedd4-2 expression. Nedd4-2 deficient animals develop normally but most die just prior to/after birth. We show that the absence of Nedd4-2 in mice leads to increased ENaC expression and activity in embryonic lung which results in premature lung fluid clearance, a failure to inflate the lungs and subsequent respiratory distress[a]. Approximately 5% of *Nedd4-2*$^{-/-}$ animals survive up to 22 days, and these animals also show increased ENaC expression and develop lethal sterile inflammation of the lung, presumably due to the drying of the lung epithelia[a]. Thus, our studies provide *in vivo* evidence that Nedd4-2 is essential for the correct regulation of ENaC expression and lung function.This work was funded by the National Health and Medical Research Council of Australia.[a] Boase *et al.* (2011) Nat Commun. **2**:287.

8.20

IDENTIFICATION OF PERMISSIVE INSERTION SITES FOR GENERATING FUNCTIONAL FLUORESCENT MINERALOCORTI-COID RECEPTORS

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The mineralocorticoid receptor (MR), a member of the nuclear receptor superfamily of transcription factors, mediates aldosterone modulation of Na^+ reabsorption in tight epithelia. In addition, it is increasingly apparent that MR is involved in a variety of other functions in non-epithelial tissues. In spite of the important pathophysiological and pharmacological roles of this receptor, many important questions about its cellular biology and functionality remain unanswered. A major challenge in the study of MR is the unavailability of fully functional fluorescent derivatives of the receptor. In this study we have created a library of MR mutants with insertions of the yellow fluorescent protein (YFP) in various internal locations in the receptor using a random-insertion transposon-based technique. Screening of this library using a transactivation assay allowed us to identify several fluorescent constructs that retain functionality. Detailed characterization of one of these construct showed that aldosterone affinity, hormone-induced nuclear translocation, DNA binding and the induction of non-genomic pathways are all indistinguishable from the wild-type receptor. This new tool will be useful in studying the cell biology of MR. Funded by Ministerio de Ciencia e Innovación (MICINN, Spain), grants BFU2010-16225 and Consolider CSD2008-00005 to D.A.R.

8.21

POTASSIUM DIET, HYPERTENSION, AND REMODELING OF THE DISTAL NEPHRON

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Epidemiological and clinical data show that potassium depletion is associated with an elevated arterial blood pressure and a higher prevalence of hypertension. To directly investigate the effects of changes in potassium intake on blood pressure and the signaling network in the distal nephron, we fed sodium replete C57BL6 mice either a low (0.03%), intermediate (0.93%), or high potassium (5%) diet for 10 days. Both the low and high potassium diet increased blood pressure, reduced sodium clearance, and suppressed renin gene expression, but only the high potassium diet increased circulating aldosterone. Analysis of the mRNA expression by real time PCR revealed an increased expression of WNK4, SPAK, and NCC during the low potassium diet. In contrast, high potassium intake increased SGK1, ENaCß, and the KS-WNK1/L-WNK1 ratio. The renal expression of ACE was highest during low potassium intake, and an inhibition of AT-1 receptors by losartan completely abolished the increased blood pressure induced by potassium depletion. These data demonstrate that both potassium depletion as well as potassium loading increase blood pressure and promote sodium retention in sodium replete mice. The functional and expression data suggest that two distinct forms of remodeling of the distal nephron contribute to these effects: a Gordon syndrom type remodeling during potassium depletion and a Conn syndrome type remodeling during potassium loading.

8.22

ALDOSTERONE INDEPENDENT ACTIVATIONS OF MR-Sgk1-ENaC AND TUBULAR RENIN ANGIOTENSIN SYSTEMS IN SODIUM SENSITIVE HYPERTENSION IN MICE

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Objectives: To elucidate potential interactions between tubular renin angiotensin systems and MR-sgk1-ENaC in urinary epithelium. Methods: Total sixteen C57Bl6/J mice were divided into four groups; (1)normal-salt diet containing 0.5%NaCl,(2)high-salt diet containing 8%NaCl,(3) high-salt diet with perindopril (2mg/kg/day,po),(4) high-salt diet with olmesartan(3.5mg/kg/day,po), respectively. At day 14, mice were sacrificed and blood samples were collected by cardiac punctures for measurements of plasma aldosterone concentrations. Subsequently, kidneys were removed for protein extractions, total RNA extractions and histological analyses. Results: The subjects with high sodium diet shows blood pressure elevation with enhanced ENaC, MR, sgk1 expressions for qPCR, western blotting and histological examinations without elevations of plasma aldosterone concentrations. Both ACEI and ARB effectively lowered blood pressure with enhancement of Nedd4L expressions especially in ACEI treatments. Conclusions: Tubular renin angiotensin systems and ENaCs interplay significantly for developing sodium sensitive hypertension.

8.23

ANGIOTENSIN RECEPTOR ACTIVATION CONTRIBUTES TO IMPAIRED RENAL INSULIN RECEPTOR PHOSPHORYLATION, INCREASED γ ENaC CLEAVAGE AND VOLUME-DEPENDENT HYPERTENSION IN INSULIN RESISTANT OLETF RATS

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Epithelial Na+ Channels (ENaC) are regulated by the insulin signaling pathway in cultured cells. However, the in vivo regulation of ENaC by insulin receptor (IR) activation and the interaction between insulin and aldosterone signaling on ENaC is not well described. To address the hypothesis that IR activation compensates for angiotensin receptor blocker (ARB)-induced decrease in aldosterone, we studied three groups of rats: 1) normotensive LETO (control strain), 2) untreated, hypertensive, insulin resistant OLETF and 3) OLETF + ARB (10 mg olmesartan/kg/d x 6 wk; OLETF ARB). Insulin resistance is associated with a 29% increase in SBP (LETO: 113 ± 4 mmHg vs OLETF: 146 ± 2 mmHg), which was effectively reduced by ARB (107 ± 2 mmHg) and associated with a reciprocal 166% increase in UNa+V. Plasma aldosterone and insulin decreased 18% and increased 95%, respectively in OLETF. Both plasma aldosterone and insulin decreased 29% and 42%, respectively with ARB. Phosphorylation of IR increased 43% with ARB suggesting that AT1 activation contributes to impaired insulin signaling in the kidney. Cleaved α- and γ-ENaC subunits positively correlated with decreased plasma aldosterone suggesting that α and γ cleavage (and presumably channel activation) is associated with aldosterone in insulin resistance. Increased α- and γ-ENaC corresponded with decreased UNa+V indicating that, along with impaired IR activation, cleavage in vivo contributes to UNa+V regulation and SBP during insulin resistance.

8.24

PHYSIOLOGICAL MODULATION OF URINARY PROSTASIN IN NORMOTENSIVES INDIVIDUALS

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The GPI–anchored serin-protease Prostasin activates the Epithelial Na Channel and it is secreted in extracellular fluids (including urine). We aimed at the evaluation of prostasin concentration and its modulation in urine of healthy subjects (normotensive individuals of similar age and in women during the menstrual cycle and after oral contraceptive therapy-OC) by a new ELISA assay, looking for possible relationships of prostasin concentration with natriuresis. Moreover we characterized by western immunoblotting this protein also in urinary exosome fractions, extracted using a nanomembrane concentrator, and at different day time points. Urinary prostasin presented a wide range of values with no difference by gender and was positively correlated with Aldosterone to Renin ratio; it increased linearly up to the limit of about 200 mmol U-Na concentration whereas it decreased for more elevated U-Na concentration. In fertile women no significant changes of prostasin concentration were observed during menstrual phases whereas after OC therapy urinary prostasin increased to a relevant extent. In conclusion urinary prostasin is physiologically modulated by natriuresis in normotensive individuals and it is present in exosomes. Grant from the Italian Ministry for University and Scientific Research.

8.25

EXTRA CELLULAR POTASSIUM MODULATED ALDOSTERONE SECRETION IN RELATION TO HYPERTENSIVE STATES

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Aldosterone (A) secretion is mainly stimulated by angiotensin II (aII) and extra cellular potassium (Ko). However, in an environment with a concomitant high intake of sodium and potassium, a regulatory mechanism to reduce the Ko induced A secretion is needed to prevent hypertension. The aim of this study was to investigate such a possible mechanism. Studies were done in the human adrenocortical cell line H295R. Radioligand binding assays were developed to measure the specific binding of ^{3}H-oubain and ^{125}I-aII to the cells at different Ko. ^{3}H-oubain binding: After 24 h of stimulation with basal Ko of 3mM, a saturating binding with a Kd value of 328nM was found, while +15mM Ko resulted in a markedly lower and non saturating binding. ^{125}I-angiotensin II binding: Both after 24 h of stimulation with Ko of 3 and +15mM a saturating binding was observed. However, +15mM Ko resulted in a 60% reduction of Kd from 9.7 to 3.9nM and a 50% reduction of Bmax when compared to basal Ko. Conclusion**:** Stimulation of H295R cells for 24 h with a high Ko of +15mM decreased the specific binding of ^{3}H-oubain and ^{125}I-angiotensin II, reflecting a Ko induced impairment of the Na/K-ATPase and desensitization of the response to aII in human zona glomerulosa cells. Both indicated a down regulation of A secretion after sustained exposure to high Ko, which could be the explanation of why Ko induced hypertension can be avoided and of the blood pressure lowering effect of a potassium rich (DASH) diet.

8.26

DIFFERENCES AMONG RENIN-ANGIOTENSIN SYSTEM BLOCKADE FOR THE TARGET ORGAN DAMAGE AND ANGIOTENSIN II INDUCED SCN5A-NEDD4L ACTIVATION WITH MINERALOCORTICOID RECEPTOR TRANS-ACTIVAION IN THE MODEL OF SODIUM SENSITIVE HYPERTENSION

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Background: Both ENaC and SCN5A have a PY motif in their cytoplasmic C terminal. Through the binding with Nedd4L via PY motif of their C-terminals, the channels cellsurface expressions are regulated. DESIGN and METHODS: We performed in vitro experiments using H9C2 cardiomyocytes.Next, total twenty male 10 weeks old C57Bl6/J mice were divided into five groups according; normal salt diet, high salt (8%NaCl) diet, high salt diet with perindopril, high salt diet with olmesartan, and high salt diet with eplerenone, respectively. Hearts were removed at day 14 for subsequent analyses for SCN5A, Nedd4L and MR. RESULTS: We discovered significant AII induced transcriptional activation in both Nedd4L and SCN5A for AII treated H9C2 cell lines. ARB treatments downregulated the expressions at low concentrations, but upregulated at high concentrations, paradoxically. Both eplerenone and PD123319 partially but salarasin suppressed completely this AII induced transcriptional changes. Quantitative pcr analyses for mice heart revealed that the expressions of SCN5A and MR gene were enhanced according to high salt diet and suppressed significantly by ACEI and SAB, but not by ARB. Conclusions: The management of cardiovascular consequences for subjects with essential hypertension is an essential requirement for the disease. The Na channel-Nedd4L-proteasome system might be the important converging molecular system.

8.27

ALDOSTERONE DEFICIENCY DURING PREGNANCY IN MICE DOES NOT LEAD TO PREECLAMPSIA BUT RESULTS IN PLACENTA DYSFUNCTION, REDUCED LITTER SIZE, AND SMALLER PUPS

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Preeclampsia is a syndrome with severe hypertension and proteinuria during pregnancy. Clinical studies pointed to a compromised aldosterone-synthase activity in some of the preeclamptic women. We used aldosterone-synthase knock-out (KO) mice to address the role of aldosterone during pregnancy. Two types of breedings were used: wild type (WT) males mating with KO females and KO males mating with WT females. KO female mice were neither hypertensive nor proteinuric throughout the entire gestation period but became hypotensive at the end of pregnancy. Litter sizes, bodyweights of pups and weights of placentas were significantly smaller in KO than in WT female mice. Moreover, KO female mice revealed many small haemorrhagic placentas indicating prenatal death of pups. Feeding a high salt diet (5% NaCl) during pregnancy improved litter size and body weights of the pups in KO female mice. Thus, aldosterone deficiency in pregnant mice does not lead to preeclampsia but may impair placental function and intrauterine pup growth and survival. A high dietary NaCl intake appears to improve placental function.Supported by ZHIP and SNF.

8.28

RECOVERY OF ENDOTHELIUM-DEPENDENT VASODILATION BY ACUTE INHIBITION OF EPITHELIAL SODIUM CHANNEL (ENaC) IN RATS MADE HYPERTENSIVE BY ANGIOTENSIN II (AII)

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Inhibitors of the mineralo corticoid receptor improve the outcome in different models of hypertension, independently of its effects on renal function. Among possible targets, we hypothesized that in hypertension, ENaC presence may be enhanced in vascular endothelium; reducing endothelium-dependent vasodilation, probably by modifying the Na/Ca exchanger (NCX) function and NOS signaling. To test this, hypertension was induced in SD rats (100g) by infusing AII (120 ng/Kg/min, osmotic pump). After 2 weeks, AII-treated rats presented higher arterial content of α-ENaC and NCX, as compared with sham operated controls. Endothelial function was assessed in phenyleprhrine-contracted thoracic aortic rings. Acetylcholine (ACh)-induced relaxation was reduced in aortas of AII-treated rats, whereas isoproterenol-induced relaxation was similar to control. Blockade of ENaC with benzamil (1μM), or amiloride (300nM) fully restored relaxation to ACh, but did not modify endothelium-independent relaxation to isoproterenol. The effect of ENaC inhibitors on ACh-induced relaxation was abolished by NOS blockade (L-NA 100μM). In contrast, in vessels of control rats, ENaC blockade did not modify the responses to ACh, or isoproterenol. We conclude that activation of the renin-angiotensin-aldosterone system increases ENaC endothelial activity, and ENaC blockade improves endothelium-dependent relaxation in AII-induced hypertensive rats. Grants Fondecyt 1090757, Anillos ACT71.

8.29

FUNCTIONAL ASSESSMENT OF THE EPITHELIAL SODIUM CHANNEL (ENaC) IN THE RAT HEART

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We studied the functional expression of the epithelial sodium channel (ENaC) in the rat heart, a potential novel way of sodium uptake in cardiac cells. Studies were performed in isolated adult rat ventricular myocytes using ENaC blockers benzamil and amiloride. Patch clamp studies (whole cell configuration) demonstrated the presence of currents sensitive to amiloride (0.2-1.0 μM). According to ENaC channels characteristics, inversion potential for amiloride sensitive current was +8.1 ± 2.1mV (n=6). Furthermore, both amiloride and benzamil decreased calcium transients and diastolic calcium levels, as measured with Fura-2 in isolated cardiomyocytes under electrical stimulation. In isolated adult rat hearts, left ventricle maximal pressure and contraction velocity ((dP/dt)max) were reduced (16.6±3.9% and 12.5±3.8% of basal value respectively, n=4, P<0.05) after benzamil treatment. ENaC blockade with benzamil (1μM), or 0.3 μM amiloride significantly reduced the inotropic response to isoproterenol (0.1nM-1μM). On the contrary, ENaC blockade with either inhibitor did not affect the preload-contractility

response. These results are consistent with the expression of functional ENaC channels in ventricular myocytes of rat heart that contribute to maintain intracellular sodium and calcium levels and normal contractility. Grants Fondecyt 1090757 Anillos Act71.

8.30

αENaC POLYMORPHISMS ALTER ENaC CURRENT

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The epithelial sodium channel ENaC, a heterotrimer of homologous α, β, and γ subunits, is found at the apical surface of epithelia where it facilitates sodium absorption. It therefore plays a pivotal role in the maintenance of salt and extracellular fluid balance. Gain-of-function mutations in β- and γENaC cause Liddle's syndrome, a severe form of salt-sensitive hypertension, making ENaC a candidate gene for the pathogenesis of essential hypertension. Less is known about the role that αENaC sequence variations play in the regulation of ENaC function. We hypothesized that single nucleotide polymorphisms in αENaC alter ENaC function and play a role in the pathogenesis of hypertension.To test this hypothesis, we coexpressed human αENaC (wild type or polymorphic) with wild type β- and γENaC in Xenopus oocytes. We measure amiloride-sensitive ENaC current using two-electrode voltage clamp (oocytes) recordings. We identified α-ENaC polymorphisms in the SNP database and tested the effects of 12 polymorphisms on ENaC current. Compared to wild type ENaC, one polymorphism (A334T) decreased amiloride-sensitive current in oocytes. We speculate that this polymorphism may protect from salt sensitive hypertension.

9.0: REMEMBERING J. D. HORISBERGER AND D. J. BENOS

9.1

REMEMBERING JEAN-DANIEL HORISBERGER

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Jean-Daniel Horisberger died on April 1st 2009. He studied medicine in Lausanne, then he began a PhD thesis in the Departement of Pharmacology under the mentorship of Jacques Diezi. He published seminal papers on the in vivo action of aldosterone that are still cited as the best experimental basis for studying the control of sodium and potassium balance by mineralocorticoid hormones2. In 1981 Jean-Daniel temporarily completed his medical training, before committing himself definitively to research when he moved to the Department of Physiology at Yale for a post-doctoral fellowship with Gerhard Giebisch. There he focused his attention on potassium balance and again published very important papers on potassium transport in isolated perfused amphibian collecting tubule, using sophisticated electrophysiological approaches. Back in Lausanne, Jean-Daniel was at the intersection of all major projects and successes of the Department during the last three decades. His contribution was essential for the cloning of ENaC; then he discovered the activation of ENaC by proteases such as trypsin3. In parallel he pursued an outstanding research on the structure and function of the Na/K-ATPase, providing a detailed understanding of the gating mechanism that controls the access of potassium or sodium from the extracellular solution to their binding sites4. His science was characterized by the highest scientific and ethical standards, and remains an example for us and for our students. 2Horisberger, J., and Diezi, J. (1983) Am J Physiol 245, F89-F99. 3Chraibi, A. et al. (1998) J Gen Physiol 111, 127-138. 4Li, C. et al.(2005) Proc Natl Acad Sci U S A 102, 12706-12711.

10.0: ABSTRACT-BASED PRESENTATIONS

10.1

THREE COMMD FAMILY PROTEINS ARE LOCATED IN COLLECTING DUCT CELLS AND REGULATE ENaC

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Copper Metabolism Murr1 Domain-containing protein 1 (COMMD1) reduces amiloride-sensitive short circuit current (I_{sc}-amil) in epithelial cells expressing ENaC. To investigate whether other COMMD proteins also regulate ENaC we used co-immunoprecipitation and showed that COMMD1-10 interact with ENaC. COMMD1, 3, 9 were widely expressed in kidney including collecting duct cells and showed some colocalization with ENaC. COMMD1, 3, 9 all reduced cell surface expression of ENaC, and COMMD1, 3, 9 inhibited I_{sc}-amil in ENaC-expressing FRT cells. COMMD proteins share a conserved C-terminal COMM domain, and N-terminal domains that are not conserved. The N-terminal domain of COMMD9 was solved by X-ray crystallography. The N-terminal domains of COMMD1 and COMMD9 share a common and unique fold, indicating they are homologous, despite the lack of sequence similarity, but the surface features are different, suggesting N-terminal domains may encode specificity. We found that both N- and C-terminal COMMD9 domains interacted with ENaC and inhibited I_{sc}-amil, unlike COMMD1 as only its COMM domain interacted and inhibited current. Thus COMMD family members may differ in their interaction with other proteins and therefore in their regulatory properties. Animal experiments were approved by the University of Otago Animal Ethics Committee. Funding: Marsden Fund Council, and University of Otago (to YFL) and Department of Physiology (to MS/YK) MSc/PhD stipend/scholarships.

10.2

A MODEL OF PARTNERSHIP CO-OPTED BY TSG101 AND USP2-45 FOR THE NEGATIVE FEEDBACK LOOP OF THE MINERALOCORTICOID RECEPTOR PATHWAY

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After activation of a signalling pathway and cell response, mechanisms often take place to downregulate prolonged response and avoid tissues damages (cancers, inflammations…). In the context of aldosterone/MR signalling, it was shown that prolonged exposure to aldosterone decreased MR expression via the proteasome, but the mechanisms of this feedback regulation remained unknown. We were therefore interested in elucidating mechanisms involving MR signaling termination. We first observed that MR was mono-ubiquitylated at the basal state and that this modification was removed after aldosterone treatment. As for other nuclear receptor, we found that MR interacted with Tsg101 at the basal state and this association was disrupted after aldosterone treatment. We found that Tsg101 can stabilize MR probably by maintaining its mono-ubiquitylation. Because USP2-45 is an aldosterone induced deubiquitylating enzyme, we wondered if USP2-45 was involved in the deubiquitylation of the receptor. We found that USP2-45 interacted with MR, removed its mono-ubiquitylation and decreased its expression via the proteasome. Our data imply a mechanism in which MR is mono-ubiquitylated at the basal state and protected by Tsg101. Aldosterone treatment stimulates USP2-45 expression, which interacts with MR and deubiquitylates the receptor. The removal of the mono-ubiquitin destabilizes the MR/Tsg101 interaction and induces MR degradation via the proteasome by a so far unknown mechanism. These results reveal the existence of a functional network involving USP2-45 and Tsg101 into a negative feedback loop of the MR pathway that mediates the degradation of MR in response to aldosterone.

10.3

ANALYSIS OF TWO SPONTANEOUS MOUSE AND RAT CAP1/PRSS8 MUTATIONS

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CAP1/Prss8 is a membrane-bound serine protease able to activate the epithelial sodium channel ENaC1. We revealed a critical role for this serine protease in skin homeostasis and recently identified CAP1/Prss8 as activator of the protease-activated receptor 2 (PAR2)2,3. The mouse fr ('frizzy') and rat frCR ('hairless') mutants exhibit skin abnormalities that were proposed to be caused by point and deletion mutations in the CAP1/Prss8 gene, respectively4. Since CAP1/Prss8 knock-out mice are embryonic lethal, fr and frCR animals emerged as suitable models to investigate the role of CAP1/Prss8 in the whole organism and its implication in sodium homeostasis. Electrophysiological measurements of ENaC-mediated sodium current in Xenopus oocytes expressing the CAP1/Prss8 variants as well as in vivo amiloride-sensitive rectal potential difference measurements revealed that both variants present a significant reduction in the basal activity of ENaC, indicating a diminution of CAP1/Prss8 function possibly due to a decrease in protein stability. Thereby, we provide functional and molecular evidence that these variants affect CAP1/Prss8 function and might thus contribute to the phenotypes. This work was supported by the Swiss National Science Foundation (Grant 3100A0-102125/1 to E. Hummler) and the NCCR (National Centre of Competence in Research) funds. References: 1Rossier et al. 2009; 2Leyvraz et al. 2005; 3Frateschi et al. 2011; 4Spacek et al. 2010.

10.4

WRIST DOMAIN OF THE EPITHELIAL SODIUM CHANNEL MODULATES CHANNEL GATING

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The epithelial sodium channel (ENaC) is a key component in the regulation of extracellular fluid volume and blood pressure, primarily by mediating Na^+ uptake in the distal nephron. External factors may affect ENaC activity by inducing conformational changes within the extracellular regions of ENaC that are transmitted to the gate. The wrist domain refers to two small linkers connecting the extracellular domain and the two membrane spanning domains where the channel pore resides. We employed site-directed mutagenesis combined with two-electrode voltage clamp to investigate the role of the wrist domain of αENaC (αF135 to αP138; and αS568 to αM571) in channel gating in response to extracellular stimuli. We observed that Na^+ self-inhibition response was largely inhibited by Cys mutation of αP138 or αS568, likely reflecting increases in channel open probability. Channels αP138CS568Cβγ exhibited reduced Na^+ self-inhibition that was indistinguishable from αP138Cβγ or αS568Cβγ. In contrast, Cys mutations of αY137 and αS568 elicited an additive effect on the Na^+ self-inhibition response. The reducing agent DTT did not alter the effects of double Cys mutations on Na^+ self-inhibition. In addition, αS568Cβγ exhibited an enhanced flow response with a slower activation rate, suggesting a role in modulating channel gating in reaction to shear stress. Taken together, our data are consistent with the hypothesis that external stimuli induce movement within the extracellular regions of ENaC subunits that are transmitted, via the wrist domain, to the channel's gate.

10.5

MECHANISM OF INTERACTION AND INHIBITION OF ENaC ACTIVITY BY SPLUNC1 PEPTIDES

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The epithelial Na+ channel (ENaC) is responsible for Na+ absorption in ion-transporting epithelia, including the kidney, colon and lung. Upregulation of ENaC results in Na+ hyperabsorption, mucus dehydration and stasis in cystic fibrosis (CF) lungs. Conversely, loss of ENaC function can result in pseudohypoaldosteronism which has increased mucus hydration. Activation of ENaC depends on proteolytic cleavage. The short palate lung and nasal epithelial clone (SPLUNC1) protein has recently been discovered by our lab to inhibit the activation of ENaC. This inhibition is achieved through the binding of SPLUNC1 to ENaC, preventing cleavage.

The active site of SPLUNC1 was determined to be within an 18 amino acid region located near the N-terminus. We synthesized a peptide corresponding to this region, S18, and its inhibitory mechanism is being determined. Through binding studies with individual ENaC subunits we determined that S18 interacts with only the β-ENaC subunit. This interaction is being further explored using β-ENaC truncants to determine the specific site of interaction between S18 and β-ENaC. Addition of this peptide to CF cultures blocked Na+ hyperabsorption for 72 hrs, even in the presence of neutrophil elastase, suggesting that this peptide may have therapeutic potential. Understanding how S18 inhibits ENaC activation is key to the development of therapeutic peptides for the treatment of CF patients.

10.6

STEROID REGULATION OF THE ENAC RECYCLING PATHWAY: A PROTEOMIC ANALYSIS

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ENaC recycles to the apical membrane in kidney cortical collecting duct cells through sequential endosomal compartments. We have demonstrated ENaC flow through early endosomal antigen (EEA1) and Rab11 positive compartments. The size of the ENaC recycling compartment is regulated by ENaC expression which diminishes in the absence of steroids (SD) and is reconstituted by steroid repletion (SR). We propose that steroids regulate specific components of the recycling pathway in addition to ENaC and have used an unbiased proteomic approach to examine the relative proteome of EEA1 and Rab11 endosomes under conditions of SD and SR. Using two different approaches (iTRAQ and DIGE), the proteome of each compartment was identified. Several proteins were recognized as up-regulated in SR cells and have been confirmed by Western Blot. One major protein induced is Annexin 2 (Anx2). Anx2 has been implicated in both exocytic and endocytic pathways and has been described to form a functional complex with both CFTR and ASIC1a. Anx2 colocalizes with Rab11 endosomes and is more abundant in these endosomes with the addition of aldosterone. Knockdown of Anx2 results in a decrease of basal and forskolin induced ENaC activity. These results suggest that Anx2 localizes to recycling endosomes upon addition of aldosterone and facilitates the recycling of ENaC. (Supported by NIH).

10.7

POSSIBLE VASCULAR ENaC INHIBITION BY AMILORIDE IN YOUNG OVERWEIGHT PREHYPERTENSIVES

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Background: Recent data demonstrate the presence of the epithelial sodium channel (ENaC) in endothelial and smooth muscle cells. However, the function of vascular ENaC remains unknown in humans. Therefore, we aimed to determine the effects of ENaC inhibition on vascular function and structure in prehypertensives. Methods: Young black and white overweight prehypertensives (n=13; 19-35 yrs; body mass index: 29.5±1.6 kg/m2; 46% female; 46% blacks) were enrolled and received amiloride 10mg/day. Blood pressure (BP), brachial artery flow-mediated dilation (FMD), and pulse wave velocity (PWV) were evaluated at baseline, 2 weeks and 4 weeks. Results: After 4 weeks of amiloride treatment, but not 2 weeks, a significant reduction was observed in both systolic (4.0±1.6 mmHg) and diastolic BP (3.7±1.2 mmHg). However, compared to baseline (5.1±1.0%), FMD was increased as early as at 2 weeks (6.4±1.2%, p=0.045), and further increased at 4 weeks (8.2±1.3%, p=0.024). On the other hand, compared to baseline (8.4±0.7 m/sec), carotid-radial PWV was decreased as early as at 2 weeks (7.8±0.6 m/sec, p=0.054), and further decreased at 4 weeks (7.6±0.5 m/sec, p=0.012). These tests were adjusted for gender, race, and systolic/diastolic BP. Conclusion: Amiloride appears to improve vascular function and structure in prehypertensives. The effects may be independent of blood pressure reduction, suggesting the inhibition of vascular ENaC. This study was funded by the Medical College of Georgia, GHSU.

10.8

POLYCLONAL ANTIBODIES AGAINST EPITHELIAL SODIUM CHANNEL (ENaC) SUBUNITS REVEAL DISTINCT PATTERNS OF ENAC EXPRESSION AND SUBCELLULAR LOCALIZATION IN HUMAN TISSUES

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Most antibodies currently available against ENaC have been generated against synthetic peptides and do not allow visualization of ENaC in cells expressing relatively low levels of the channel. To increase the sensitivity of immunofluorescent imaging we generated polyclonal antibodies against ENaC subunits. For this purpose we produced cDNA constructs to express human ENaC subunits in E. coli. We purified proteins expressed in E. coli and injected them to rabbits for production of polyclonal antisera. The specificity of the rabbit antisera was confirmed using Western blots of human tissues. To determine whether anti α-ENaC antibodies distinguish be-tween α-ENaC and β-ENaC we expressed each separately in insect SF9 cells. In these cells, anti-α-ENaC specifically recognized α-ENaC but showed very low cross-reactivity with expressed β-ENaC. For immunohistochemical analysis, tissue samples including human kidney, lung, and samples of tissue along the female reproductive tract were fixated in formalin, frozen and then cryo-sectioned. The sections were reacted with the primary antibody followed by secondary fluorescent antibody. Three dimensional confocal microscopy of the sections revealed that while in some segments ENaC is uniformly expressed in epithelial cells, in others ENaC is expressed in subpopulations of epithelial cells. The high resolution of imaging also permitted us to distinguish association of ENaC with significant subcellular structures.

10.9

ENERGETIC AND STRUCTURAL BASIS FOR ACTIVATION OF ENaC BY CAP-3

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Limited proteolysis by endopeptidases is a ubiquitous phenomenon underlying regulation and activation of many enzymes and other proteins synthesized as inactive precursors. Serine proteases are among the largest families of endopeptidases involved in many cellular processes like wound healing and immune response. Heteromeric α,β,γ-epithelial sodium channels (ENaC) implicated in diseases like cystic fibrosis and Liddle's syndrome, are irreversibly stimulated by type II transmembrane serine proteases (TTSP) and furin-like convertases. Despite identification of protease cleavage sites, the basis for enhanced susceptibility of α,γ-ENaC to proteases remains elusive. Here, we elucidate the energetic and structural bases for activation of ENaC by the TTSP CAP3. We find a region near the γ-ENaC furin site that is previously unidentified as a critical cleavage site for CAP3-mediated stimulation. CAP3 also mediates cleavage of ENaC at basic residues downstream of the furin site. Our results indicate that surface proteases alone are sufficient to fully activate ENaC, and explain how ENaC in presence of surface-active proteases appears refractory to soluble proteases. Our results support a model where proteases prime ENaC for activation by cleaving at the furin site, and downstream cleavage is accomplished by membrane surface proteases or extracellular soluble proteases. In future, we will examine whether the accessibility of the CAP3 cleavage sites in ENaC is modulated by intracellular signals, extracellular conditions and proximity to CFTR.

10.10

ALDOSTERONE IS DISPENSABLE FOR RENAL BUT NOT FOR COLONIC REGULATION OF POTASSIUM HOMEOSTASIS

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Aldosterone (aldo) stimulates potassium (K) excretion in kidney and colon and is hence thought to be crucial for K homeostasis. To define the role of aldo, we studied aldo-synthase knock-out (KO) mice lacking any aldo. KO mice are normokalemic and tolerate dietary K loading (2% K for 2 days) without any signs of illness. Urinary K excretion rises and plasma K levels remain in the normal range. Consistently, K diet-induced activation of the renal secretory K channel ROMK and of the epithelial sodium channel ENaC, providing the electrochemical driving force for K excretion, is maintained in kidneys of KO mice. This aldosterone-independent renal response requires angiotensin II (ANG II) as inhibition of the ANGII receptor AT1R diminishes urinary K excretion and causes severe hyperkalemia in KO but not in wildtype (WT) mice. In contrast to the kidney, the colon of KO mice does not respond to K-loading as indicated by measuring distal colon Na and K channel activites in Ussing-type chambers at day 2 and 4 of K-loading. Thus, aldo is dispensable for the renal but not for the colonic regulation of K homeostasis. In the kidney, ANG II appears to compensate for the loss of aldo. Supported by ZHIP and SNF (Kidney.CH).

10.11

CROSS-TALK OF THE SMALL GTPASE RAC1 AND MINERALOCORTICOID RECEPTOR CASCADES IN CARDIO-RENAL DISEASE

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Overactivation of the mineralocorticoid receptor (MR) signaling is implicated in cardiovascular and renal disease. We previously reported that the small GTPase Rac1 activates MR in a ligand-independent manner, and that this Rac1-mediated MR activation contributes to the progression of chronic kidney disease. In the present study, we investigated whether oxidative stress augments MR signal transduction and Rac1 is involved in the process. Oxidant stress was induced in rat cultured cardiomyocytes by buthionine sulfoximine (BSO), an inhibitor of glutathione synthesis. BSO depleted intracellular glutathione, and increased reactive oxygen species. BSO significantly enhanced the MR-dependent luciferase activity both with and without ligand stimulation. These effects were inhibited by antioxidant N-acetylcysteine. The ligand independency of BSO action was indicated using a mutant MR which does not bind ligands. We next examined whether Rac1 mediates this MR transactivation by BSO. BSO increased active, GTP-bound Rac1 in a redox-dependent manner. Moreover, Rac inhibition suppressed the enhancing effect of BSO. MR transcriptional activation by BSO was accompanied by enhanced nuclear accumulation of MR, which was reduced by Rac inhibition. We conclude that alteration of redox state modulates MR-dependent transcriptional activity via Rac1. This redox-sensitive, ligand-independent MR activation may contribute to the mechanism by which oxidant stress promotes cardiovascular disease.

10.12

A NOVEL REDOX-SENSITIVE E3-UBIQUITIN LIGASE REGULATES SURFACE EXPRESSION OF EPITHELIAL SODIUM CHANNELS IN ALVEOLAR EPITHELIAL CELLS

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We recently reported that reactive oxygen species (ROS) such as superoxides positively regulate

under normal or pathological conditions remain unclear. It is, however, clear that ROS can regulate the activity of an E3 ubiquitin ligase, E3-SCF, which consists of the skip, cullin and the F-box proteins. The activity of this E3-ligase complex is in turn regulated by another posttranslational event which requires neddylation of the cullin subunit. We have shown that ROS directly inhibits neddylation of the cullin subunit, and thereby inhibits E3-SCF enzymatic activity. If ENaC interacts with this E3-SCF ligase complex, then this may well be a novel mechanism by which ROS can regulate ubiqutin-mediated degradation of ENaC. Using standard immunoprecipitation techniques, we show for the first time that ENaC interacts with proteins associated with the E3-SCF ubiquitin ligase complex in type 1 and type 2 cells. Single channel patch clamp analysis confirm that MLN4924, a specific inhibitor of the neddylation pathway, significantly increased ENaC activity; ENaC NP_0 values increased from 0.07 ± 0.03 to 0.80 ± 0.16 in type 2 cells treated with 10-100 nM MLN4924 for 60 mins ($P<0.05$; n=8). Likewise, our preliminary studies in type 1 cells show that Na current increases by ~80% after 60 min MLN4924 treatment (n=4). These increases in sodium channel activity indeed correlate with significant increases in surface expression of ENaC following MLN4924 treatment in type1 and type2 cells. Together, these findings provide mechanistic insight into how alveolar cells could regulate ENaC activity in response to oxidative stress.

12.0: CONGESTIVE HEART FAILURE: THE INTERTWINED ROLES OF WATER AND SALT

12.1

POTENTIAL FUTURE ROLE OF MINERALOCORTICOID RECEPTOR BLOCKADE (MRB) IN PATIENTS WITH HEART FAILURE (HF)

Bertram Pitt MD

MRBs reduce mortality in patients with severe heart failure (HF) with a reduced left ventricular ejection fraction (REF) (RALES) ; patients with HFREF and mild symptoms (EMPHASIS-HF) and in patients with HFREF post myocardial infarction (EPHESUS) . There are however opportunities to further reduce mortality in patients with HF. Current therapy has not been successful in reducing mortality in patients with HF and a preserved ejection fraction (PEF). There is however evidence suggesting a role for MRBs. The effectiveness of MRBs in HFPEF to reduce mortality may depend not only on its effectiveness in reversing the underlying myocardial and vascular pathophsiology but also on its effectiveness in treating the comorbid conditions occurring in these patients such as sleep apnea, chronic kidney disease (CKD), the metabolic syndrome, and atherosclerosis. MRBs may have a role in of each of these conditions. This hypothesis is currently being tested in the NHLBI sponsored TOPCAT trial. MRBs may also have a role by preventing myocardial and vascular fibrosis in the prevention of HF. Acute decompensated HF (ADHF) is another condition in which current therapy has failed to reduce mortality. The role of MRBs in patients with ADHF has however been limited by the risk of hyperkalemia (HK). New strategies to prevent and to treat HK using the potassium binding polymer RLY5016 as well as new non steroidal MRBs, which in preclinical studies have been shown to be relatively cardio selective and to have a better urinary Na+/K+ ratio than spironolatone or eplerenone , hold the promise of safely using MRBs in patients admitted for AFDHF.

14.0: ENaC PATHOPHYSIOLOGY

14.1

EVOLUTION OF ENaC AND Na, K-ATPase AS LIMITING FACTORS OF ALDOSTERONE ACTION

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The kidney of vertebrates plays a major role in the homeostasis of the extracellular fluid. Despite large changes in salt intake, the kidney is able to maintain the extracellular osmolarity and volume within very narrow margins. During the evolution of vertebrates, aldosterone played a critical physiological role about 300 million years ago with the emergence of amphibia, the first vertebrates to adapt to a terrestrial environment. Comparative studies of physiology, biochemistry, and molecular biology have helped in delineating the most significant steps involved. In the present study we have focused on the evolution of ENaC and Na,K-ATPase, which are the key effectors and limiting factors of the aldosterone response in the mammalian distal nephron. By searching for homologs in various eukaryotes, from unicellular eukaryotes ("protists") to multicellular metazoan, we provide here a novel view of how mammalian aldosterone-dependent control of sodium homeostasis, blood volume and blood pressure might have evolved. We propose that Na,K-ATPase emergence, together with ENaC/Degenerin, is linked to the development of multicellularity in the Metazoan kingdom. The establishment of multicellularity and the associated extracellular compartment ("internal milieu") precedes the emergence of other key elements of the aldosterone signaling pathway. REFERENCE: Studer, R.A., Person, E., Robinson-Rechavi, M. & Rossier, B.C. Evolution of the epithelial sodium channel and the sodium pump as limiting factors of aldosterone action on sodium transport (2011) Physiol Genomics. Published ahead of print.

14.2

THE ROLE OF BENAC IN RENAL VASCULAR FUNCTION

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Our laboratory has considered certain Epithelial Na^+ Channel (ENaC) proteins may act as sensors of vascular stretch because of their strong evolutionary link to mechanotransduction in the nematode, *C. elegans*. Studies from our lab demonstrate vascular smooth muscle cell βENaC protein plays an important role as a mediator of pressure-induced vasoconstriction (myogenic constriction) in the kidney. Silencing of endogenous βENaC (siRNA, genetically modified mice) inhibits myogenic constriction in renal interlobar arteries and afferent arterioles, the primary site of development of vascular resistance in the kidney. Mice with reduced levels of βENaC (βENaC m/m) have a delayed correction of whole kidney RBF within the first 5 sec following a step increase in perfusion pressure, the time frame in which the myogenic mechanism (~5 sec) is responsible for correcting RBF. These findings suggest βENaC mediates myogenic constriction and plays an important role in control of RBF. βENaC m/m mice have increased renal expression of inflammatory and remodeling markers, such as macrophage infiltration, IL1-β, IL6, TNFα, collagen III and TGFβ, suggesting loss of myogenic control is associated with mild renal injury. Moreover, βENaC m/m mice increased mean arterial blood pressure (MAP, 120 ± 3 vs. 105 ± 2 mm Hg, $p=0.016$), as measured using radio telemetry. Our findings suggest βENaC is an important mediator of renal myogenic constriction in-vivo and loss of the myogenic mechanism is associated with mild signs of renal injury and increased MAP. (Work supported by NHLBI 086996 and 51971).

14.3

REGULATION OF ENaC AND AIRWAY SURFACE LIQUID VOLUME BY SPLUNC1

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Human bronchial epithelial cultures (HBECs) utilize ENaC to regulate Na^+ absorption and maintain airway surface liquid homeostasis (ASL). In contrast, cystic fibrosis (CF) epithelia fail to regulate Na^+ absorption leading to ASL depletion. We identified SPLUNC1 as a potential soluble regulator of ENaC in NL airways: SPLUNC1 co-immunoprecipitates with ENaC, decreases its proteolytic activation, and decreases ENaC surface expression. Furthermore, NL HBECs with knocked down SPLUNC1 lost the ability to restrain Na^+ absorption, suggesting that SPLUNC1 is an important soluble regulator of ENaC. We have recently identified the inhibitory region of SPLUNC1, and a peptide derived from this region robustly inhibits CF ASL volume hyperabsorption. Surprisingly, despite the efficacy of this peptide, full length SPLUNC1 fails to regulate ENaC in CF airways and knockdown of SPLUNC1 was without effect. Thus, we speculate that regions of SPLUNC1 adjacent to the inhibitory site prevent SPLUNC1-ENaC interactions in CF airways, and that this interaction is abolished when the inhibitory site is administered to CF airways alone. Thus, we propose that SPLUNC1 is an important regulator of ENaC which fails to function in CF airways. In addition, our results make it clear that the effectiveness of slowing ENaC proteolytic stimulation depends on the presence and function of CFTR. These results suggest that restoring SPLUNC1 inhibition of ENaC may have therapeutic benefits for the treatment of CF lung disease. Funded by the NIH and the CFF.

14.4

EXPRESSION AND FUNCTION OF A CROSS CLADE ASIC/ENaC CHANNEL IN GLIOBLASTOMA

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High grade glioma cells express an amiloride-sensitive cation conductance that is not present in normal astrocytes or in lower grade gliomas. This current is also inhibited by Psalmotoxin 1 (PcTx1), suggesting that at least one component of the channel is ASIC1. Knockdown of ASIC1, ahENaC or ghENaC abolished the glioma cation current. However, knocking down dhENaC was without effect. Furthermore, ASIC1 co-immunoprecipitated with both a- and ghENaC, but not with dhENaC, consistent with an ASIC1/ahENaC/ghENaC channel. Two of the hallmarks of a glioblastoma are its ability to grow to very large size and to migrate long distances, establishing multiple foci. We have recently found that benzamil and PcTx1, slow down both proliferation and migration of glioma cells. Cell cycle analysis showed that these inhibitors arrested cells at the G0/G1 checkpoint and caused an increase in expression of the cyclin kinase inhibitors p21cip and p27kip. Benzamil and PcTx1 also decreased expression of pERK1/2, a regulator essential for cell migration. Similar results were obtained when external Na+ was replaced by NMDG+ or when ASIC1 was knocked down. These results suggest that expression of this conductance is critical for migration and proliferation of glioma cells. Support: NIH Grant DK037206. Reference: Kapoor, N., Bartoszewski, R., Qadri, Y.J., Bebok, Z., Bubien, J.K., Fuller, C.M., and Benos, D.J. Knockdown of ASIC1 and epithelial sodium channel subunits inhibits glioblastoma whole-cell current and migration. J. Biol. Chem. 284:24526-24541, 2009.

14.5

ROLE OF ENaC IN THE REGULATION OF AIRWAY SURFACE LIQUID VOLUME AND PATHOGENESIS OF LUNG DISEASE

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ENaC is limiting for absorption of salt and water across epithelia including the kidney, the colon and the lung. In vitro studies in airway epithelia from patients with cystic fibrosis (CF) demonstrated that mutations in the CFTR gene result in increased ENaC-mediated Na^+ absorption and airway surface liquid (ASL) volume depletion indicating that this mechanism may be involved in initiating chronic lung disease in CF. To elucidate the role of increased ENaC activity in the in vivo pathogenesis of lung disease, we generated transgenic mouse models with airway-specific overexpression of α-, β-, and γENaC subunits. In these ENaC transgenic mice, we demonstrated that overexpression of βENaC is sufficient to increase ENaC activity in airway epithelia in vivo, and that elevated airway Na^+ absorption causes ASL volume depletion, reduced mucus clearance and a spontaneous CF-like lung disease characterized by airway mucus obstruction and chronic airway inflammation. Comparative studies in βENaC-transgenic mice and Liddle mice indicate that selective overexpression of βENaC, but not expression of Liddle gain-of-function βENaC, causes a shift in subunit stoichiometry and a subpopulation of αβENaC channels that are constitutively active and thus impair physiological ASL regulation. Hence, βENaC-transgenic mice also provide a useful tool for preclinical evaluation of therapeutic strategies designed to inhibit dysregulated ENaC and improve airway surface hydration. Taken together, the results driven from these studies in mouse models support a critical role of ENaC-mediated ASL depletion in the in vivo pathogenesis and as a therapeutic target of dehydration-

induced lung diseases such as CF. Supported by: EU MEXT-013666-2004, DFG MA2081/3-3 and DFG MA2081/4-1.

14.6

PLASMIN, ENaC AND NEPHROTIC SYNDROME

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We found that nephrotic urine from the rat PAN-model and from patients activate the epithelial sodium channel (ENaC) in model systems – mainly mouse collecting duct M1 cells The activation depended on serine protease activity, which was identified by MALDI-TOF mass spectrometry to be plasmin. Consistent with this, purified plasmin activated ENaC currents and inhibitors of plasmin abolished urinary protease activity and the ability of nephrotic urine to activate ENaC. The activation by plasmin involved cleavage of an inhibitory peptide from the ENaC gamma subunit ectodomain. Plasmin bound to the surface of the M-1 cells, and this binding depended on a GPI-anchored protein, which was identified by plasmin biotin-label transfer to be prostasin. Removal of GPI-anchored proteins inhibited stimulation of ENaC only at low plasmin concentrations (1-4 µg/ml). Consistent with this, knockdown of prostasin blocked plasmin-stimulated ENaC activity as measured by the whole-cell patch clamp tech-nique. In nephrotic urine plasmin was likely to be converted from filtered plasminogen by tubular urokinase-type plasminogen activator (uPA). Thus, the uPA-inhbitor amiloride blocked production of plasmin in nephrotic rat urine in vivo. In conclusion, a defective glomerular filtration barrier allows passage of proteolytic enzymes that activate ENaC directly, or indirectly. This mechanism provides a coupling between proteinuria and sodium retention, and is currently under investigation in proteinuric patient groups.

15.0: ALDOSTERONE PATHOPHYSIOLOGY

15.1

THE UPS AND DOWNS OF ALDOSTERONE BIOSYNTHESIS: REGULATION OF ALDOSTERONE SYNTHASE EXPRESSION BY MICRORNAS

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Aldosterone is a key cardiovascular hormone and increased levels are known to result in a number of cardiovascular disorders including hypertension. Aldosterone production is catalysed by aldosterone synthase which is encoded by the CYP11B2 gene and dysregulation of CYP11B2 gene expression is thought to play a role in the pathogenesis of hyperaldosteronism. CYP11B2 gene expression is regulated primarily by transcription factors. However, microRNAs which bind to the 3'UTR of target mRNA and reduce gene expression have recently emerged as important regulatory factors. In this study, have investigated the role of miRNAs in the regulation of human CYP112 gene expression. Knockdown of Dicer1, which is fundamental for miRNA maturation, in adrenal cells caused a significant increase in CYP11B2 mRNA and aldosterone production. Bioinformatic data and microarray analysis of human adrenal tissue identified 16 miRNAs that are expressed in the human adrenal gland and are predicted to bind to CYP11B2. Using multiple experimental approaches including 3'CYP11B2/reporter gene constructs, manipulation of intracellular miRNA concentrations, measurement of CYP11B2 gene expression and aldosterone production, we have shown that a number of miRNAs (e.g mir-24) exhibit effects that are consistent with canonical CYP11B2 miRNA binding and repression. Interestingly, we have observed a differential expression pattern of miRNAs in aldosterone producing adenoma tissue compared to normal adrenal control tissue. Given the pivotal role of the CYP11B2 gene in adrenal pathophysiology and hypertension, such regulation may prove to be a novel therapeutic target. (Funded by grants from the BHF and MRC).

15.2

MINERALOCORTICOID RECEPTOR MUTATIONS IN HUMAN DISEASE

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Sodium handling is highly variable between individuals and genetic factors are involved in the development of hypertension. Rare mutations of the mineralocorticoid receptor (MR) are responsible for Mendelian disorders of renal salt handling associated with high or low BP. Heterozygous loss of function mutations of the MR lead to type I pseudohypoaldosteronism (PHA1), while the rare activating mutation p.Ser810Leu leads to juvenile hypertension exacerbated by pregnancy. Recent studies showed associations between more common genetic variations in the MR and blood pressure. We have recently shown that the MR c.-2G>C (rs2070951) variant is associated with differential expression of the MR in vitro; importantly, in vivo, this SNP influences circulating levels of plasma aldosterone and renin and blood pressure. In contrast, the amino acid changing SNP in exon 2 MR I180V (rs5522) affects the cortisol-dependent transcriptional properties of the MR in vitro and modulates the stress responsiveness, rather than affecting electrolyte homeostasis. Finally, we have recently reported the first case of a newborn with a severe recessive form of PHA1 caused by two heterozygous MR nonsense mutations. Analysis of MR expression and residual MR function indicated that the phenotype resulted from partial haploinsufficiency. This exceptional case demonstrates that minimal residual activity of MR is compatible with life. It also suggests that rare hypomorphic NR3C2 alleles may be more common than expected from the prevalence of detected PHA1 cases and may affect renal salt handling and blood pressure in the general population. REFERENCES: Hubert EL, et al. Mineralocorticoid receptor mutations and a severe recessive pseudohypoaldosteronism type 1. J Am Soc Nephrol, in press (2011).

15.3

END-ORGAN DAMAGE IN PRIMARY ALDOSTERONISM: RESPONSE TO TREATMENT AND COMPARISON WITH ESSENTIAL HYPERTENSION

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Primary aldosteronism (PA) is a common form of secondary hypertension. Recent evidence has suggested that PA is associated with a variety of cardiac, vascular, and renal sequelae that reflect the capability of inappropriately elevated aldosterone to induce tissue damage over that induced by hypertension itself. This evidence has been obtained from experiments conducted in hypertensive animal models and studies involving patients with PA. Preclinical studies have also indicated that aldosterone causes cardiovascular and renal tissue damage only in the context of inappropriate salt status. It has been suggested that untoward effects of high-salt intake are dependent on activation of mineralocorticoid receptors (MRs) that might result from increased oxidative stress and changes in the intracellular redox potential. Adrenalectomy or treatment with MR antagonists are the accepted options for treating an aldosterone-producing adenoma (APA) or idiopathic adrenal hyperplasia (IHA), respectively. Treatments are effective in correcting hypertension and hypokalemia, and currently available information on their capability to prevent cardiovascular events and deterioration of renal function indicates that surgery and medical treatment are equally beneficial in the long term. Reference: Sechi LA et al. Long-term renal outcomes in patients with primary aldosteronism. JAMA 2006;295:2638–2645. Catena C et al. Cardiovascular outcomes in patients with primary aldosteronism after treatment. Arch Intern Med 2008;168:80–85.

15.4

CARDIOMYOCYTE MR SIGNALING IS ESSENTIAL FOR DOC/SALT-MEDIATED CARDIAC FIBROSIS AND BLOOD PRESSURE REGULATION

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Inappropriate mineralocorticoid receptor (MR) signaling in the cardiovascular system is associated with inflammation and remodeling. However the mechanisms responsible and the contribution of the different cells of the myocardium remain unknown. We investigated the role of cardiomyocyte MR in an in vivo model of DOC/salt-induced pathology. Myocyte MR-null mice (KO) were examined after 8 days or 8 weeks of DOC/salt administration and compared to control mice (WT). At 8 days loss of MR signaling in myocytes did not alter DOC/salt-induced macrophage recruitment, whereas it limited early collagen deposition. Microarray revealed a novel gene expression profile in untreated KO mice compared to WT. Loss of MR from myocytes prevented the DOC/salt-induced increase in NOX2, CCR-5 and PER2 mRNA. At 8 weeks, loss of myocyte MR prevented the DOC/salt-induced increase in infiltrating macrophages and T-cells, collagen deposition and systolic blood pressure. KO mice also showed a novel gene expression profile at 8 weeks. Compared to WT mice, the DOC/salt-induced increase in CCR5, CD14 and CD81 mRNA was prevented in KO mice, whereas the DOC/salt-induced increases in fibronectin, CTGF, COL-3, MCP-1 and DCN were unaffected. These findings suggest a direct role for myocyte MR signaling in DOC/salt-induced tissue remodelling, the secondary inflammatory phase and blood pressure regulation. Moreover, changes in basal gene expression profiles in untreated KO mice, suggest a physiological role for endogenous glucocorticoid-occupied MR complexes.

15.5

ALDOSTERONE PRODUCING ADENOMA FORMATION INVOLVES EXPRESSION OF STEM/PROGENITOR CELL MARKERS

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Aldosterone producing adenoma (APA) is the most common form of surgically curable hypertension. To understand mechanisms involved in APA formation, we have investigated expression of molecules linked to adrenal stem/precursor cells [β-catenin, Sonic hedgehog (Shh), CD56] in 6 control adrenal glands and 14 adrenals with APA, and compared their expression with that of specific markers of zona glomerulosa (ZG) [CYP11B2, Disabled 2 (Dab2)]. Both Dab2 and CD56 were expressed in ZG. However, while Dab2 associates uniquely with differentiated ZG cells and its expression is lost when cells transdifferentiate to zona fasciculata (ZF)-cells, CD56 was also expressed in ZF and in aldosterone producing cell clusters, confirming that these structures possess an intermediate phenotype between ZG and ZF cells. Shh was barely detectable in few cells located to the outer part of the ZG in the control adrenal; in contrast, its expression was detected in the entire APA and dramatically increased in the hyperplastic peritumoral ZG. Transcriptome profiling revealed differential expression of components of Shh signalling pathway in a subgroup of APA. Similarly, Wnt/βcatenin signalling was activated in the majority of APA, as well as in the entire peritumoral adrenal cortex. Our data suggest that both APA and adjacent ZG present characteristics of stem/precursor cells; the re-expression of genes involved in fetal adrenal development could underlie excessive ZG cell proliferation and APA formation.

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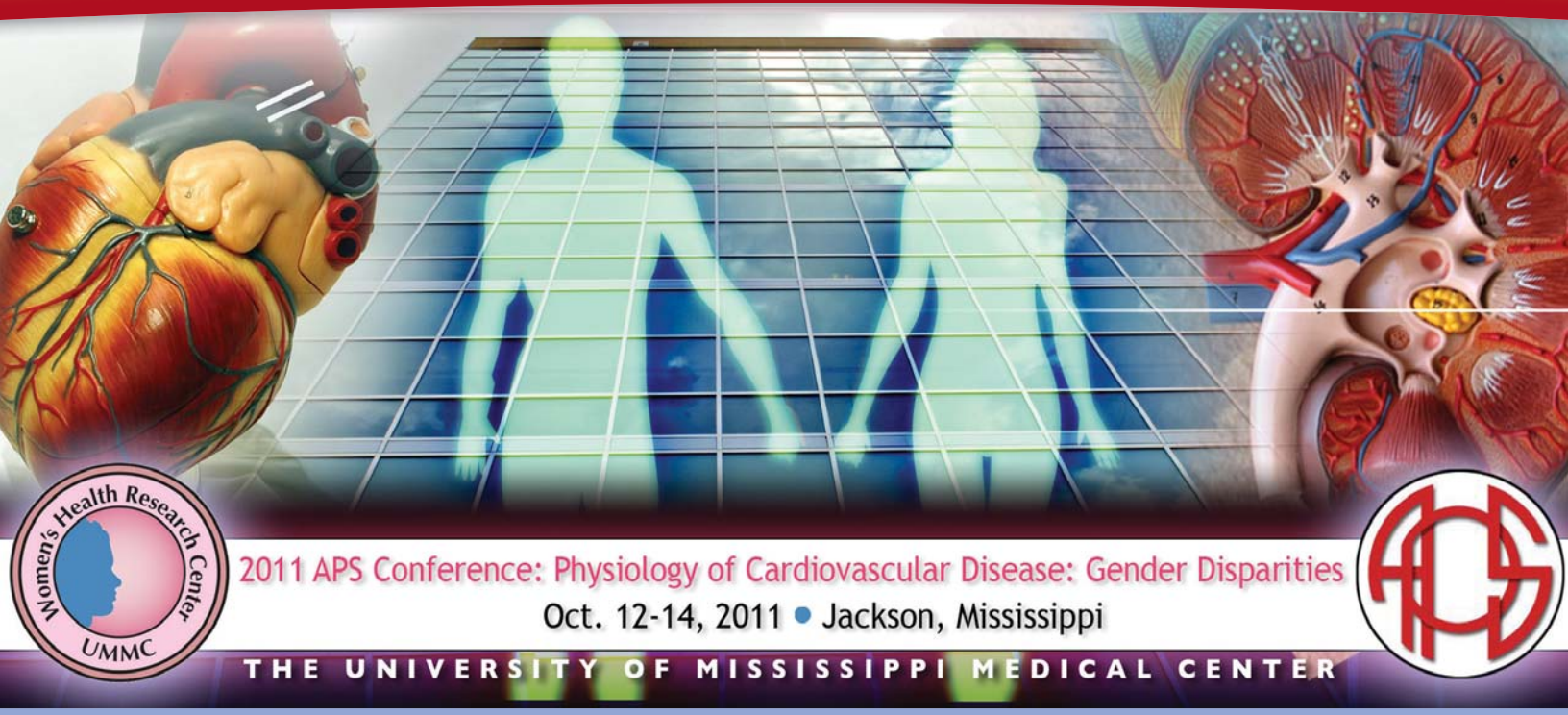
2011 American Physiological Society Conference



Physiology of Cardiovascular Disease: Gender Disparities

MEETING PROGRAM AND ABSTRACTS

University of Mississippi Medical Center
Jackson, Mississippi
October 12-14, 2011

www.the-aps.org/gender2011

2011 APS Conference
Physiology of Cardiovascular Disease: Gender Disparities

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Women's Health Research Center, University of Mississippi Medical Center
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2011 APS Conference

Physiology of Cardiovascular Disease: Gender Disparities

October 12—14, 2011, University of Mississippi Medical Ctr., Jackson, Mississippi

Wednesday, October 12	Thursday, October 13	Friday, October 14
7:00 – 9:00 PM **Opening Reception** Historic King Edward Hotel	7:00 AM **Breakfast/Registration** 8:00 – 8:40 AM **Plenary Lecture: From Stem Cells and Cadaveric Matric to Engineered Organs** **Doris Taylor,** Univ. of Minnesota 8:40 –9:40 AM Symposia I: **Aging and CVD** **Heddwen Brooks**, Univ. of Arizona **Pamela Ouyang,** Johns Hopkins Univ. **Rhian Touyz,** Univ. of Ottawa, Canada 9:40—10:00 AM Break 10:00—11:00 AM Symposia II: **Gender Disparities in Renal Disease** **Sharon Elliot,** Univ. of Miami **Vesna Garovic,** Mayo Clinic **Michal Schwartzman,** New York Med. Coll. 11:00 AM—11:50 AM Selected Abstract Oral Presentations 11:50 AM—1:00 PM Lunch and Poster Session I 1:00—2:00 PM Symposia III: **Diabetes, Obesity and Cardiovascular Disease** **Willis Samson,** St. Louis Univ. Sch. of Med. **David Parkes,** Amylin Pharmaceuticals, Inc. **John Hall,** Univ. of Mississippi Med. Ctr. 2:00—2:40 PM Selected Abstract Oral Presentations 2:40—3:00 PM Break 3:00—4:00 PM Symposia IV: **Neuro Mechanisms and Depression in Cardiovascular Disease** **Virginia Brooks,** Oregon Hlth. and Science Univ. **Meir Steiner,** McMaster Univ., Canada **Nabil Alkayed,** Oregon Hlth, and Science Univ. 4:00—4:50 PM Selected Abstract Oral Presentations 5:00—6:00 PM Career Session: **Careers in Physiology Trainee Session** **Jennifer Sasser,** Univ. of Florida 6 :30—10:00 PM **Dinner** Historic King Edward Hotel	7:00 AM **Breakfast/Registration** 8:00—9:00 AM Symposia V: **Gender Disparities in Cardiology** **C. Noel Bairey Merz,** Cedars-Sinai Med. Ctr. **Janet Rich-Edwards,** Harvard Univ. **Nanette Wenger,** Emory Univ. 9:00—9:40 AM Selected Abstract Oral Presentations 9:40—10:00 AM Break 10:00—11:00 AM Symposia VI: **Cardiovascular Disease and Inflammation** **David Harrison,** Vanderbilt Uiv. **R. Ansar Ahmed,** Virginia Tech. **Jennifer Sullivan,** Med. Coll. of Georgia 11 :00—11:50 AM Selected Abstract Oral Presentations 11:50 AM—1:00 PM Lunch and Poster Session II 1:00—2:00 PM Symposia VII: **Gender Differences in Vascular Function** **Marilyn Cipolla,** Univ. of Vermont **Christopher Minson,** Univ. of Oregon **Sandra Davidge,** Univ. of Alberta, Canada 2:00—2:40 PM Selected Abstract Oral Presentations 2:40—3:00 PM Break 3:00—4:00 PM Symposia VIII: **Cardiovascular Disease and Fertility** **Sarah Berga,** Emory Univ. **Babbette LaMarca,** Univ. of Mississippi Med. Ctr. **S. Ananth Karumanchi,** Harvard Med. Sch. 4:00—5:00 PM Selected Abstract Oral Presentations 6 :30 PM **Closing Dinner and Awards Presentation** Fairview Inn

GENERAL INFORMATION

Location:

The 2011 APS Conference, Physiology of Cardiovascular Disease: Gender Disparities will be held October 12—14, 2011 at the University of Mississippi Medical Center, 2500 N. State Street, Jackson, MS 39216, telephone (601) 984-2103 FAX: (601) 984-2105

Onsite Registration Hours:

Thursday, October 13 ……7:00 AM—5:30 PM
Friday, October 14……..7:00 AM—5:00 PM

On-Site Registration Fees:

APS Member..... $350
Retired Member $250
Nonmember..... $400
Postdoctoral..... $250
Student $250

The registration fee includes entry into all scientific sessions, opening reception, lunches 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:

Career Workshop: This special session entitled: "Careers in Physiology Trainee Session" will be presented by Jennifer Sasser, University of Florida.

Program Objective:

The role that sex steroids and gender play in the physiology and pathophysiology of cardiovascular and renal disease (CVRD) is becoming an increasingly more important area of research. The program will be balanced to include both basic science and clinical studies, ranging from the gene to the whole animal or human. The global aspect of the conference is to gather a critical mass of scientists with interests and expertise in the role of sex steroids and/or the gender differences in the physiology of CVRD, and to promote an exchange of ideas to foster collaboration that will further advance this important line of scientific investigation. In addition, this conference will be to increase the awareness of sex disparities in CVRD that need to be understood in order to ultimately improve clinical outcomes for men and women and promote individualized health care.

Target Audience:

The intended audience for this conference includes all levels of researchers working in the field of gender disparities in cardiovascular disease. Furthermore, this conference will provide a diverse program that covers many of the organ systems in which sex steroids and gender have been shown to be important in cardiovascular diseases.

Daily Shuttle Bus Schedule

Thursday, October 13, 2011:
Departs the Historic King Edward Hotel at 7:00 AM and 7:30 AM

Departs UMMC at 5:00 PM, 5:30 PM and 6:00 PM for the Historic King Edward Hotel.

Friday, October 14, 2011:
Departs the Historic King Edward Hotel at 7:00 AM and 7:30 AM

Departs UMMC at 5:00 PM, 5:30 PM for the Historic King Edward Hotel.

Departs the Historic King Edward Hotel at 6:15 PM for the Fairview Inn.

THURSDAY, OCTOBER 13, 2011

Plenary Lecture

1.0 PLENARY LECTURE

Thurs., 8:00-8:40 AM, A/B.

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

8:00 AM **1.1** Sex Cells and Matrix: Cardiac Regeneration in 2011. **Doris Taylor.** *Univ. of Minnesota.*

Symposia I

2.0 AGING AND CVD

Thurs., 8:40-9:40 AM, A/B.

Co-Chairs: **Rudy Ortiz,** *Univ. of California, Merced.*
Virginia Huxley, *Univ. of Missouri, Columbia.*

8:40 AM **2.1** Diabetes and Metabolic Syndrome: Progression Across the Perimenopause Transition. **Heddwen Brooks.** *Univ. of Arizona.*

9:00 AM **2.2** Early Menopause and Cardiovascular Disease. **Pamela Ouyang.** *Johns Hopkins Univ.*

9:20 AM **2.3** Cardiovascular Remodelling, Hypertension and Sex Hormones in Follitropin Receptor Knockout (FORKO) Mice. **Rhian Touyz.** *Univ. of Ottawa, Canada.*

9:40 AM Break

Symposia II

3.0 GENDER DISPARITIES IN RENAL DISEASE

Thurs., 10:00 AM-12:00 Noon, A/B.

Co-Chairs: **Kathryn Sandberg,** *Georgetown Univ.*
Jing Li, *Univ. of Mississippi Med. Ctr.*

10:00 AM **3.1** Sex Differences in Renal Injury: Role of Podocytes. **Sharon Elliot.** *Univ. of Miami.*

10:20 AM **3.2** Podocyturia as an Early Predictive Marker of Pre-eclampsia. **Vesna Garovic.** *Mayo Clinic.*

10:40 AM **3.3** Role of Androgens and 20-HETE in Renal Disease. **Michal Schwartzman.** *New York Med. Coll.*

11:00 AM **3.4** No Sexual Dimorphism in Development of Kidney Damage in the Aging Fischer-344 Rat Kidney. **Jennifer Sasser.** *Univ. of Florida, Gainesville.* **(4.1).**

11:10 AM **3.5** Sex Steroids and Renal Sodium Transport in Mice Consuming 1% and 4% Salt Diet. **Al Rouch.** *Oklahoma State Univ. Ctr. for Hlth. Sci.* **(4.2).**

11:20 AM **3.6** Aldosterone Escape is Influenced by Sex Chromosomal Complement in Mice. **Carolyn Ecelbarger.** *Georgetown Univ.* **(4.3).**

11:30 AM **3.7** GPR30 Agonist G-1 Restores Megalin Expression and Reduces Proteinuria in Salt-sensitive mRen2.Lewis Females. **Sarah Lindsey.** *Wake Forest Univ. Sch. of Med.* **(4.4).**

11:40 AM **3.8** Collecting Duct-derived Renin Exhibits Sex Differences During Normal Salt and High Salt Diets in Sprague-Dawley Rats. **Vicky Rands.** *Tulane Univ.* **(4.5).**

Poster Session I

4.0 POSTER SESSION I

Thurs., 11:50 AM-1:00 PM, C/D

Board #

1 **4.1** No Sexual Dimorphism in Development of Kidney Damage in the Aging Fischer-344 Rat Kidney. **J. Sasser, O. Akinsiku, N. Moningka, K. Jerzewski, A. LeBlanc, L. Kang, A. Sindler, J. Muller-Delp and C. Baylis.** *Univ of Florida, Gainesville and West Virginia Univ.*

2 **4.2** Sex Steroids and Renal Sodium Transport in Mice Consuming 1% and 4% Salt Diet. **A. Rouch, K. Curtis, L. Fan and L. Kudo.** *Oklahoma State Univ. Ctr. for Hlth. Sci.*

3 **4.3** Aldosterone Escape is Influenced by Sex Chromosomal Complement in Mice. **C. Ecelbarger, R.M. Garikepati, H. Ji, A. Arnold, K. Sandberg and L. Li.** *Georgetown Univ. and UCLA.*

4 **4.4** GPR30 Agonist G-1 Restores Megalin Expression and Reduces Proteinuria in Salt-sensitive mRen2.Lewis Females. **S. Lindsey, L. Yamaleyeva and M. Chappell.** *Wake Forest Univ. Sch. of Med.*

5 **4.5** Collecting Duct-derived Renin Exhibits Sex Differences During Normal Salt and High Salt Diets in Sprague-Dawley Rats. **V. Rands, D. Seth and M. Prieto.** *Tulane Univ.*

6 **4.6** Association between Menopause, Obesity and Cognitive Impairment. **J. Zilberman, M. Del Sueldo, G. Cerezo, S. Castellino, E. Theiler and A. Vicario.** *Certus Res. Grp. Buenos Aires, Argentina and Municipalidad de Villa María, Cordoba, Argentina.*

7 **4.7** GPR30 Activation Increases ACE2 Expression in Diabetic Ovariectomized mRen2.Lewis Females. **H. El-Bassossy, S. Lindsey, L. Yamaleyeva and M. Chappell.** *Wake Forest Univ. Sch. of Med.*

8 **4.8** Androgen Receptor and Estrogen Receptor alpha Expression after Combined Dihydrotestosterone Supplementation and Aromatase Inhibition in the male streptozotocin-induced diabetic rat. **M. Manigrasso, R. Sawyer, Z. Hutchens, E. Flynn and C. Maric.** *Univ. of Mississippi Med. Ctr.*

9 **4.9** The Effects of Streptozotocin-induced Diabetes and Gender on the Rat Aortic Endothelial Function. **X. Han, L. Anderson and R. Rahimian.** *Univ. of the Pacific.*

10 **4.10** Withdrawn.

Board #

11 **4.11** Central Blockade of Angiotensin(1-7) or Angiotensin II Receptor Type 2 (AT_2) Enhances Aldosterone/Salt-induced Increases in Blood Pressure in Female Rats. **B. Xue, Z. Zhang, F. Guo, M. Hay and A. Johnson.** *Univ. of Iowa and Univ. of Arizona.*

12 **4.12** Sex Difference in the ß-Adrenergic Contractile Response- Roles of Adenylyl Cyclase and Phosphodiesterase. **V. McIntosh and R. Lasley.** *Wayne State Univ. Sch. of Med.*

13 **4.13** GPR30 is Involved in the Regulation of the KCa1.1 Channel Current in a Gender Specific Population of Myelinated Vagal Afferents. **J. Schild and B. Li.** *Indiana Univ., Perdue Univ., Indianapolis.*

14 **4.14** Angiotensin Peptides and FMD: Does Sex Matter? **Y. Shah, M. Zimmerman, B. Berry, T. Poore, J. Sullivan and R. Harris.** *Georgia Hlth. Sci. Univ.*

15 **4.15** Atherogenic Index of Plasma: A Significant Indicator for the Onset of Atherosclerosis during Menopause in Hypatensive Females of South East Nigeria. **J. Igweh.** *Delta State Univ., Abraka, Nigeria.*

16 **4.16** Fetal Programming of Hypertension Induced by Zinc Restriction in Fetal Life: Gender Differences in Early Effects on Kidney. **C. Arranz, A. Sofía, V. Luciana, A. Costa and A. Tomat.** *Univ. of Buenos Aires, Argentina.*

17 **4.17** Gender Differences in Renal Oxidative Stress Enzymes in Dahl SS rats: Effects of Salt and Ovariectomy. **D. Romero, J. Reckelhoff and L. Yanes.** *Univ. of Mississippi Med. Ctr.*

18 **4.18** Sex Hormones Modulate Responses to Oxidative Stress in Renal Proximal Tubule Cells. **I. Arany, J. Clark, D. Reed, G. Booz and L. Juncos.** *Unive. of Mississippi Med. Ctr.*

19 **4.19** Exaggerated Angiotensin II-Induced Hypertension in Male rats Exposed to Early Life Stress Depends on Testosterone Levels. **A. Loria, D. M. Pollock and J. S. Pollock.** *Georgia Hlth. Sci. Univ.*

20 **4.20** Androgen Induces the Expression of CYP4F2, A Major 20-HETE Producing Enzyme in Human, via Activation of the Androgen Receptor. **V. Garcia, Y. Liu, C.-C. Wu and M. L. Schwartzman.** *New York Med. Coll.*

21 **4.21** Profile of Antioxidant and Pro-oxidant Enzymes and Markers in the Kidney Cortex During Rat Pregnancy. **M. Cunningham, J. Sasser and C. Baylis.** *Univ. of Florida, Gainesville.*

22 **4.22** Testosterone Induces ROS Generation by Modulating Mitochondrial Activity. **R. Lopes, A. Chignalia, M. H.Carvalho, Z. Fortes, F. Laurindo, R. Touyz and R. Tostes.** *Univ. of São Paulo, Brazil, Heart Inst., São Paulo, Brazil and Univ. of Ottawa, Canada.*

Board #

23 **4.23** Perinatal Exposure to an SSRI Antidepressant Programs Sex Difference in Adult Cardiovascular Risk in Rodent Offspring. **T. Glenn, S. Intapad, F. L. Tull, R. C. S. Lin, B. Alexander and I. A. Paul.** *Univ. of Mississippi Med. Ctr.*

24 **4.24** Sex Differences in the Susceptibility to Ischemic Renal Injury in a Rat Model of Low Birth Weight. **N. Ojeda, J. Dasinger and B. Alexander.** *Univ. of Mississippi Med. Ctr.*

25 **4.25** Sex Differences in ACE Modulates Ang 1-7 Levels in Normotensive WKY Rats. **K. Bhatia, M. Zimmerman and J. Sullivan.** *Georgia Hlth. Sci. Univ.*

26 **4.26** A Role for Ethnic Disparity: Endothelin-1 Secretion by Human Placentas from Pregnancies Complicated by Preeclampsia? **K. Wallace, K. Frazier, W. Bennett, J. Martin and B. LaMarca.** *Univ. of Mississippi Med. Ctr.*

27 **4.27** Differential Programming of Endothelin Receptor Expression Contributes to Sex Differences in Adult Blood Pressure Regulation in Intrauterine Growth Restricted Offspring. **S. Intapad, F. L. Tull, J. H. Dasinger, N. Ojeda and B. Alexander.** *Univ. of Mississippi Med. Ctr.*

28 **4.28** Influence of Female Sex Hormones and Salt in Essential and Salt-Sensitive Hypertension. **K. Brinson and J. Sullivan.** *Georgia Hlth. Sci. Univ.*

29 **4.29** Heme Oxygenase-1 as a Potential Therapeutic Agent for the Treatment of Preeclampsia. **E. George, K. Cockrell, M. Arany, M. Storm, D. Stec and J. Granger.** *Univ. of Mississippi Med. Ctr.*

30 **4.30** Dietary Genistein Induces Sex-dependent Cardiovascular Effects in Mice. **L. Al-Nakkash, J. Martin, L. Batia, S. Lynch, C. Hamrick, D. Petty, D. Lucy, A. Peterson, L. Rubin and T. Broderick.** *Midwestern Univ. and Univ. of Missouri, Columbia.*

Don't forget to join your colleagues for the poster sessions held daily in room C/D—lunch is included!

Symposia III

5.0 **DIABETES, OBESITY AND CARDIOVASCULAR DISEASE**

Thurs., 1:00-2:40 PM, A/B.

Co-Chairs: **Mark Chappell,** *Wake Forest Univ. Sch. of Med.*

Meredith Hay, *Univ. of Ariziona.*

1:00 PM **5.1** Novel Pancreatic Peptides Control Glucose Homeostasis and Appetite. **Willis (Rick) Samson.** *St. Louis Univ.*

1:20 PM **5.2** Translational Cardiovascular Benefits of Exenatide-Preclinical and Clinical Evidence. **David Parkes.** *Amylin Pharma., Inc.*

1:40 PM **5.3** Pathophysiology of Hypertension in Obesity/metabolic Syndrome. **John Hall.** *Univ. of Mississippi Med. Ctr.*

2:00 PM **5.4** Association Between Menopause, Obesity and Cognitive Impairment. **Judith Zilberman.** *Human Hlth. Commission, Buenos Aires, Argentina.* **(4.6).**

2:10 PM **5.5** GPR30 Activation Increases ACE2 Expression in Diabetic Ovariectomized mRen2.-Lewis Females. **Mark Chappell.** *Wake Forest Univ. Sch. of Med.* **(4.7).**

2:20 PM **5.6** Androgen Receptor and Estrogen Receptor alpha α Expression after Combined Dihydrotestosterone Supplementation and Aromatase inhibition in the male streptozotocin-induced Diabetic rat. **Michaele Manigrasso.** *Univ. of Mississippi Med. Ctr.* **(4.8).**

2:30 PM **5.7** The Effects of Streptozotocin-induced Diabetes and Gender on the Rat Aortic Endothelial Function. **Xiaoyuan Han.** *Univ. of the Pacific.* **(4.9).**

2:40 PM Break.

Symposia IV

6.0 NEURO MECHANISMS AND DEPRESSION IN CARDIOVASCULAR DISEASE

Thurs., 3:00-4:50 PM, A/B.

Co-Chairs: **Carolyn Ecelbarger,** *Georgetown Univ.*
Margaret Zimmerman, *Georgia Hlth. Sci. Univ.*

3:00 PM **6.1** Baroreflex Function in Females: Changes with the Reproductive Cycle and Pregnancy. **Virginia Brooks.** *Oregon Hlth. and Sci.Univ.*

3:20 PM **6.2** Sex Differences in Depression and Cardiovascular Disease. **Meir Steiner.** *McMaster Univ., Canada.*

3:40 PM **6.3** Mechanism of the Sex Difference in Endothelial Dysfunction after Stroke. **Nabil Alkayed.** *Oregon Hlth. and Sci.Univ.*

4:00 PM **6.4** Heme Oxygenase-1 as a Potential agent for the Treatment of Pre-eclampsia. **Eric George.** *Univ. of Mississippi Med. Ctr.* **(4.29).**

4:10 PM **6.5** Central Blockade of Angiotensin (1-7) or Angiotensin II Receptor Type 2 (AT_2) Enhances Aldosterone/Salt-induced Increases in Blood Pressure in Female Rats. **Baojian Xue.** *Univ. of Iowa.* **(4.11).**

4:20 PM **6.6** Sex Difference in the β-Adrenergic Contractile Response Roles of Adenylyl Cyclase and Phosphodiesterase. **Victoria McIntosh.** *Wayne State Univ.Sch. of Med.* **(4.12).**

4:30 PM **6.7** GPR30 is Involved in the Regulation of the KCa1.1 Channel Current in a Gender Specific Population of Myelinated Vagal Afferents. **John Schild.** *Indiana Univ-Purdue Univ., Indianapolis.* **(4.13).**

4:40 PM **6.8** Angiotensin Peptides and FMD: Does Sex Matter? **Ryan Harris.** *Georgia Hlth. Sci. Univ.* **(4.14).**

Career Session

7.0 CAREER SESSION

Thurs., 5:00-6:00 PM, A/B.

5:00 PM **7.1** Careers in Physiology Trainee Session. **Jennifer Sasser.** *Univ. of Florida, Gainesville.*

Dinner on Thursday, October 13th will be held at the Historic King Edward Hotel at 6:30 PM

FRIDAY, OCTOBER 14, 2011

Symposia V

8.0 GENDER DISPARITIES IN CARDIOLOGY

Fri., 8:00-9:40 AM, A/B.

Co-Chairs: **Christine Carter,** *Society for Women's Hlth. Res.*
Suttira Intapad, *Univ. of Mississippi Med. Ctr.*

8:00 AM **8.1** Women and Ischemic Heart Disease. **C. Noel Bairey Merz.** *Cedars-Sinai Med. Ctr., Los Angeles.*

8:20 AM **8.2** Pregnancy Complications Predict Increased Risk of Cardiovascular Disease in Women: Is this Useful to Know? **Janet W. Rich-Edwards.** *Harvard Univ.*

8:40 AM **8.3** The Feminine Face of Heart Disease: What Do We Know About Angina in Women? **Nanette Wenger.** *Emory Univ.*

9:00 AM **8.4** Dietary Genistein Induces Sex-dependent Cardiovascular Effects in Mice. **Layla Al-Nakkash.** *Midwestern Univ.* **(4.30).**

9:10 AM **8.5** Antioxidant Tempol Exacerbates Pressor Response to Stress in Multiparous Rats. **Susan Jacobs-Kaufman.** *Univ. of Alberta, Canada.* **(10.2).**

9:20 AM **8.6** Estrogen Receptor Beta-Mediated Anti-Inflammatory Effect of Dihydrotestosterone During Cytokine-Induced Inflammation in Human Brain Vascular Smooth Muscle Cells. **Kristen Zuloaga.** *Univ. of Arizona Coll. of Med.* **(10.3).**

9:30 AM **8.7** Hypertensive Disorders During Pregnancy Predict Cardiovascular Events: Can we Trust Maternal Recall of Pregnancy Complications? **Jennifer Stuart.** *Harvard Sch. of Public Hlth.* **(10.4).**

9:40 AM Break.

Symposia VI

9.0 CARDIOVASCULAR DISEASE AND INFLAMMATION

Fri., 10:00-11:50 AM, A/B

Co-Chairs: **Kate Denton,** *Monash Univ., Ausutralia.*
Analia Loria, *Georgia Hlth. Sci. Univ.*

10:00 AM **9.1** Inflammation, Immunity and Hypertension. **David Harrison.** *Vanderbilt. Univ.*

10:20 AM **9.2** Unequal Immune Capabilities Between Males and Females: Implications for Health and Autoimmune diseases. **R. Ansar Ahmed.** *Virginia Tech.*

10:40 AM **9.3** Sex Differences in Inflammatory Mediators. **Jennifer Sullivan.** *Georgia Hlth. Sci. Univ.*

11:00 AM **9.4** Inflammation-Induced TLR4 Expression and Reactive Oxygen Species are Attenuated by Dihydrotestosterone in Human Primary Vascular Smooth Muscle Cells. **Rayna Gonzales.** *Univ. of Arizona Coll. of Med.* **(10.5).**

11:10 AM **9.5** Bone Marrow-derived Angiogenic Progenitor Cells are Dysfunctional in Chronic Ang II Infusion Rat Model of Hypertension. **Mohan Raizada.** *Univ. of Florida, Gainesville.* **(10.1).**

11:20 AM **9.6** Testosterone Induces Leukocyte Migration by COX2 and NADPH Oxidase-dependent Pathways. **Rheurre Lopes.** *Univ. of São Paulo, Brazil.* **(10.7).**

11:30 AM **9.7** Sex-Dependent Immune-Protection with Minocycline after Experimental Embolic Stroke. **Irina Sazonova.** *Georgia Hlth. Sci. Univ.* **(10.8).**

11:40 AM **9.8** Estrogen Promotes Cardiac Stem Cell Paracrine Action and thus Facilitates CSC-mediated Protection Following Hypoxia. **Meijing Wang.** *Indiana Univ. Sch. of Med.* **(10.9).**

Poster Session II

10.0 POSTER SESSION II

Fri., 11:50 AM-1:00 PM, C/D

Board #

1 **10.1** Bone Marrow-derived Angiogenic Progenitor Cells are Dysfunctional in Chronic Ang II Infusion Rat Model of Hypertension. **J. Y. Jun, J. Zubcevic, A. Afzal, G. Lamont, J. Marulanda, J. Mocco and M. Raizada.** *Univ. of Florida, Gainesville.*

2 **10.2** Antioxidant Tempol Exacerbates Pressor Response to Stress in Multiparous Rats. **S. Jacobs-Kaufman and J. Levasseur.** *Univ. of Alberta, Canada.*

3 **10.3** Estrogen Receptor Beta-Mediated Anti-Inflammatory Effect of Dihydrotestosterone During Cytokine-Induced Inflammation in Human Brain Vascular Smooth Muscle Cells. **K. Zuloaga, R. Handa and R. Gonzales.** *Univ. of Arizona Coll. of Med., Phoenix.*

4 **10.4** Hypertensive Disorders During Pregnancy Predict Cardiovascular Events: Can we Trust Maternal Recall of Pregnancy Complications? **J. Stuart, C. N. Bairey Merz, S. Berga, V. Miller, P. Ouyang, L. Shaw, C. Shufelt, M. Steiner, N. Wenger and J. Rich-Edwards.** *Harvard Sch. of Public Hlth., Cedars-Sinai Hosp., Emory Univ., Mayo Clinic, Johns Hopkins Univ., McMaster Univ., Canada and Brigham & Women's Hosp.*

5 **10.5** Inflammation-Induced TLR4 Expression and Reactive Oxygen Species are Attenuated by Dihydrotestosterone in Human Primary Vascular Smooth Muscle Cells. **R. Gonzales, R. Techapinyawat, D. O'Connor and K. Zuloaga.** *Univ. of Arizona Coll. of Med.,Phoenix.*

6 **10.6** Withdrawn.

7 **10.7** Testosterone Induces Leukocyte Migration by COX2 and NADPH Oxidase-dependent Pathways. **A. Chignalia, M. Oliveira, V. Debbas, F. Laurindo, M.H. Carvalho, R. Touyz, Z. Fortes and R. Tostes.** *Heart Inst., São Paulo, Brazil; Inst. of Biomed. Sci., São Paulo, Brazil, Univ. of Ottawa, Canada, Med. Sch. of Ribeirao Preto, Univ. of São Paulo, Brazil.*

8 **10.8** Sex-Dependent Immune-Protection with Minocycline after Experimental Embolic Stroke. **I. Sazonova, N. Hoda and D. Hess.** *Georgia Hlth. Sci. Univ.*

9 **10.9** Estrogen Promotes Cardiac Stem Cell Paracrine Action and thus Facilitates CSC-mediated Protection Following Hypoxia. **M. Wang, H. Gu, M. Manukyan and C. Huang.** *Indiana Univ. Sch. Med.*

10 **10.10** Estrogen Specific Regulation of Endothelial Homocysteine and Vascular Function. **A. Huang, G. Kaley and D. Sun.** *New York Med. Coll.*

11 **10.11** 20-HETE-Induced Vascular Remodeling in the Model of Androgen-Induced Hypertension. **Y. Ding, C-C. Wu, V. Garcia and M. Schwartzman.** *New York Med. Coll.*

12 **10.12** Sex Difference in Vascular Response to Atrial Natiuretic Peptide in Spontaneously Hypertensive Rats. **C. Arranz, M. Romero, G. Bouchet, L. Savignano, C. Caniffi, R. Elesgaray and M.A. Costa.** *Univ. of Buenos Aires, Argentina.*

Board #

13 **10.13** Sex Differences in Downstream TGF-beta Signaling in the Arteries of Spontaneously Hypertensive Rats. **A. Tipton and J. Sullivan.** *Georgia Hlth. Sci. Univ.*

14 **10.14** Gender Differences in the Intergenerational Transmission of Hypertension Associated with Uteroplacental Insufficiency in Rats. **L. Gallo, K. Moritz, M. Tran, L. Cullen-McEwen, K. Denton and M. Wlodek.** *Univ. of Melbourne, Australia, Univ. of Queensland, Australia and Monash Univ., Australia.*

15 **10.15** Exposure of Neonatal Female, but not Male Mice to Testosterone Promotes Angiotensin II-Induced Abdominal Aortic Aneurysms. **X. Zhang, S. Thatcher, D. Rateri, A. Daugherty and L. Cassis.** *University of Kentucky.*

16 **10.16** ACE2 Deficiency is Associated with Impaired Gestational Weight Gain and Fetal Growth Restriction. **L. Yamaleyeva, M. Bharadwaj, W. Strawn, M. Chappell, L. Groban, K. Atkins, C. Horta, L. Firmes, L. Farah, S. Gurly and K. B. Brosnihan.** *Wake Forest Sch. of Med., Duke Univ. and Durham Vet. Affairs Med. Ctr.*

17 **10.17** Seeking the Mechanisms of Action for Intravenous Dexamethasone to Benefit Patients with HELLP Syndrome: the SMASH Study. **M. Owens, J. Martin Jr., K. Tam Tam, K. Wallace and B. LaMarca.** *Univ. of Mississippi Med. Ctr.*

18 **10.18** A Novel Reproductive Hormone, Cospeptin. **G. Yosten, C. Bryant and W. Samson.** *St. Louis Univ.*

19 **10.19** At the Heart of the Matter. **C. Carter and N. Wenger.** *Society for Women's Hlth. Res., Washington, D.C. and Emory Univ.*

20 **10.20** Hypertension in Mice with the Chronic Inflammatory Disease Systemic Lupus Erythematosus is Not Salt-Sensitive. **K. Mathis, M. Venegas-Pont, C. Masterson, K. Wasson and M. Ryan.** *Univ. of Mississippi Med. Ctr.*

21 **10.21** Female SHR Maintain Higher Levels of Ang (1-7) Through Enhanced Levels of Renal Cortical ACE 2 Activity During Chronic Ang II Infusion. **M. Zimmerman, K. Bhatia and J. Sullivan.** *Georgia Hlth. Sci. Univ.*

22 **10.22** Exercise Training Before and During Pregnancy Improves Endothelial Function and Stimulates Cytoprotective and Antioxidant Pathways in the Pregnant Rat. **J. Gilbert, C. Banek and A. Bauer.** *Univ. of Oregon and Univ. of Minnesota Med. Sch.*

23 **10.23** Relationships Between Body Mass Category and Systolic Blood Pressure in Rural Adolescents. **R. Ortiz, R. Rodriguez, A. Aleman and S. Weffer.** *Univ. of California, Merced and California State Univ. Stanislaus.*

Board #

24 **10.24** Angiotensin II Increases Aromatase and Androgen Receptor Expression in Coronary Vascular Smooth Muscle Cells but not Cardiac Myofibroblasts. **T. Hale, M. Georgi, L. Biwer and R. Gonzales.** *Univ. of Arizona.*

25 **10.25** Exercise Increases Soluble Vascular Endothelial Growth Factor Receptor-1 in the Circulation of Adult Women. **J.-W. Gu, K. Makey, E. Chinchar, M. Huang, J. Robinson and L. Miele.** *Univ. of Mississippi Med. Ctr.*

26 **10.26** Interactions Between the Estrogen Receptor and Heme Oxygenase Contribute to the Regulation of Blood Pressure in Female Rats. **A. Soljancic, K. Chandrashekar, A. Lopez-Ruiz, J. F. Reckelhoff, R. Liu and L. A. Juncos.** *Univ. of Mississippi Med. Ctr.*

27 **10.27** Estrogen Protects Against the Development of Hypertension During Systemic Lupus Erythematosus. **E. Gilbert, M. Venegas-Pont and M. Ryan.** *Univ. of Mississippi Med. Ctr.*

28 **10.28** Sex Reporting is Lacking in Cardiovascular Studies using Cell Cultures. **K. E. Taylor, C. Vallejo-Giraldo, N. Schaible, R. Zakeri and V. M. Miller.** *Mayo Clinic.*

29 **10.29** Estrogen Receptor (ERα) Modulates Heme Oxygenase in Response to Hypertension and Renal Injury in Females. **K. Chandrashekar, A. Lopez Ruiz, R. Liu, J. F. Reckelhoff and L. Juncos.** *Univ. of Mississippi Med. Ctr.*

30 **10.30** Higher PDE-5 Expression in Pregnant Rats with Reduced Uterine Perfusion Pressure. **A. Palei, E. George, K. Cockrell, M. Arany and J. Granger.** *Univ. of Mississippi Med. Ctr.*

Symposia VII

11.0 GENDER DIFFERENCES IN VASCULAR FUNCTION

Fri., 1:00-2:40 PM, A/B.

Co-Chairs: **Virginia M. Miller,** *Mayo Clinic.*
Rita Tostes, *Univ. of São Paulo, Brazil.*

1.00 PM **11.1** Cerebral Vascular Function in Pregnancy and Pre-eclampsia. **Marilyn Cipolla.** *Univ. of Vermont.*

1:20 PM **11.2** Sex Hormones and Endothelial Function in Humans. **Christopher Minson.** *Univ. of Oregon.*

1:40 PM **11.3** Neuronal Nitric Oxide Synthase in the Endothelium is a Novel Regulator of Estrogen Signaling. **Sandra L. Davidge.** *Univ. of Alberta, Canada.*

2:00 PM **11.4** Hypertension in Mice with the Chronic Inflammatory Disease Systemic Lupus Erythematosus us not Salt-sensitive. **Keisa Mathis.** *Univ. of Mississippi Med. Ctr.* **(10.20).**

DAILY SCHEDULE

2:10 PM **11.5** 20-HETE-Induced Vascular Remodeling in the Model of Androgen-Induced Hypertension. **Yan Ding.** *New York Med. Coll.* **(10.11).**

2:20 PM **11.6** Sex Difference in Vascular Response to Atrial Natiuretic Peptide in Spontaneously Hypertensive Rats. **Cristina Arranz.** *Univ. of Buenos Aires, Argentina.* **(10.12).**

2:30 PM **11.7** Sex Differences in Downstream TGF-beta Signaling in the Arteries of Spontaneously Hypertensive Rats. **Ashlee Tipton.** *Georgia Hlth. Sci. Univ.* **(10.13).**

2:40 PM Break.

Symposia VIII

12.0 CARDIOVASCULAR DISEASE AND FERTILITY

Fri., 3:00-5:10 PM, A/B

Co-Chairs: **Jeffrey Gilbert,** *Univ. of Oregon.*
Mark Cunningham, *Univ. of Florida, Gainesville.*

3:00 PM **12.1** PCOS, Stress-Induced Anovulation, and CVD. **Sarah Berga.** *Emory Univ.*

3:20 PM **12.2** Hypertension in Response to Placental Ischemia: Role of Agonistic Autoantibodies to the Angiotensin II Type I Receptor. **Babbette LaMarca.** *Univ. of Mississippi Med. Ctr.*

3:40 PM **12.3** Angiogenic Factors in Women's Health. **Anath Karumanchi.** *Harvard Med. Sch.*

4:00 PM **12.4** Gender Differences in the Intergenerational Transmission of Hypertension Associated with Uteroplacental Insufficiency in Rats. **Linda Gallo.** *Univ. of Melbourne, Australia.* **(10.14).**

4:10 PM **12.5** Exposure of Neonatal Female, but not Male Mice to Testosterone Promotes Angiotensin II-Induced Abdominal Aortic Aneurysms. **Xuan Zhang.** *Univ. of Kentucky.* **(10.15).**

4:20 PM **12.6** ACE2 Deficiency is Associated with Impaired Gestational Weight Gain and Fetal Growth Restriction. **Liliya Yamaleyeva.** *Wake Forest Sch. of Med.* **(10.16).**

4:30 PM **12.7** Seeking the Mechanisms of Action for Intravenous Dexamethasone to Benefit Patients with HELLP Syndrome: the SMASH Study. **Michelle Owens.** *Univ. of Mississippi Med. Ctr.* **(10.17).**

4:40 PM **12.8** A Novel Reproductive Hormone, Cospeptin. **Gina Yosten.** *St. Louis Univ.* **(10.18).**

4:50 PM **12.9** Estrogen Specific Regulation of Endothelial Homocysteine and Vascular Function. **An Huang.** *New York Med. Coll.* **(10.10).**

Dinner on Friday, October 14th will be held at the Fairview Inn at 6:30 PM

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

Women's Health Research Center at the University of Mississippi Medical Center

Faculty Scholarship Exchange Program at the University of Mississippi Medical Center

Council of High Blood Pressure, American Heart Association

Council on Clinical Cardiology, American Heart Association

Isis Cardiovascular Network

Society for Women's Health Research

NIH, National Institute of Diabetes and Digestive and Kidney Diseases

1.0 PLENARY LECTURE

1.1

SEX CELLS AND MATRIX: CARDIAC REGENERATION IN 2011

Doris Taylor[1]
[1]Ctr. for Cardiovascular Repair, Univ. of Minnesota, Minneapolis, 312 Church St. S.E., 7-105A NHH, Minneapolis, MN, 55455.

Sex differences in symptomatology, risk, and even the effectiveness of therapies exist. It's not surprising then to think about sex differences in response to cells, genes, molecules, and every aspect of cardiovascular repair. Evaluating these differences in cutting edge therapies as they emerge will be critical to developing effective therapies. One novel therapy that is bone marrow mononuclear cell delivery for coronary artery disease (atherosclerosis) and acute myocardial infarction. I will describe our pre-clinical studies and early clinical data on the composition and function of bone marrow in individuals with cardiovascular disease. I will also discuss our more recent data on beginning to engineer whole organs for treatment of endstage organ failure and how sex differences are present even at the level of the extra cellular matrix. It's no surprise that men and women differ. What is emerging as intriguing is that those differences exist at the level of cells, at the level of genes, and at the level of organs and tissues. Capitalizing on those differences should allow us to create personalized healthcare solutions at a level previously unimaginable.

2.0 AGING AND CVD

2.1

DIABETES AND METABOLIC SYNDROME: PROGRESSION ACROSS THE PERIMENOPAUSE TRANSITION

Heddwen Brooks[1], Melissa Romero-Aleshire[1], Maggie Diamond-Stanic[1], Patricia Hoyer[1]
[1]Physiology, Univ. of Arizona, MRB, Tucson, AZ, 85718.

The 4-vinylcyclohexene diepoxide (VCD) model of menopause progresses gradually through perimenopause to post-menopause and preserves postmenpausal ovarian production of androgens. Studies in the VCD-model of menopause have shown that loss of ovarian function leads to the rapid development of the metabolic syndrome and diabetic kidney disease. To model metabolic syndrome, both control and VCD-treated mice were fed a high-fat diet. Menopausal mice on the high-fat diet demonstrated greater weight gain, higher circulating insulin levels, and had increased insulin resistance relative to cycling mice on the high-fat diet. On a standard diet, menopausal mice also had impaired glucose tolerance which improved with estrogen re-placement. When treated with streptozotocin (STZ) to induce diabetes, induction of diabetes post-ovarian failure resulted in higher blood glucose levels than induction in peri-menopause. Renal damage was accelerated in post-menopause along with an increase in macrophage infil-tration and glomerular hypertrophy. The VCD model of menopause provides a model for the impact of the menopause transition on diabetic kidney disease and the metabolic syndrome (NIH RO1 DK073611, RO1 AG021948) References: Romero-Aleshire, M.J., Diamond-Stanic, M.K., Hasty, A.H., Hoyer, P.B., Brooks, H.L. 2009. Loss of ovarian function in the VCD mouse-model of menopause leads to insulin resistance and a rapid progression into the metabolic syndrome. *AJP Reg* **297**, 587–592. Diamond-Stanic, M.K., Romero-Aleshire, M.J., Hoyer, P.B., Greer, K., Hoying, J.B., Brooks, H.L. 2011. Midkine, a heparin-binding protein, is increased in the diabetic mouse kidney postmenopause. *AJP Renal* **300**, F139-46.

2.2

EARLY MENOPAUSE AND CARDIOVASCULAR DISEASE

Pamela Ouyang[1]
[1]Medicine, Johns Hopkins Univ., JH Bayview Med. Ctr., 4940 Eastern Ave, 301 Mason Lord Dr., Ste. 2400, Baltimore, MD, 21224.

Cardiovascular disease (CVD) is the leading cause of death in women. Current risk stratification tools have limitations in identifying younger women at moderate to high risk for CVD. Aspects of reproductive health or disorders may add additional predictive information in women. Studies have shown an increase in coronary heart disease (CHD) mortality in women with early menopause (< 45 years) compared to women with average menopausal age (49 years or more). The Nurses' Health Study reported that early menopause was associated with increased risk for myocardial infarction. These studies have included mostly Caucasian women. The MultiEthnic Study of Atherosclerosis enrolled women of white, African-American, Hispanic and Chinese ethnicity, who were age 45 to 84 yrs and free of clinical atherosclerotic disease. This longitudinal study evaluates factors related to progression of subclinical disease. There were 693 women with self-reported early menopause (at age <46 yr) and 1816 women without early menopause, with mean followup of 57 months. We evaluated the association between early menopause and incident CHD (definite or probable MI, resuscitated cardiac arrest, and definite CHD death) and stroke (fatal and nonfatal). Early menopause is an independent predictor of CHD (HR 2.08) and stroke (HR 2.19) even after adjustment for traditional CVD risk factors. Support: NHLBI contracts N01-HC-95159 through N01-HC-95169, K23-HL-87114. Reference: Hu F, Grodstein F, Hennekens C, et al. Age at natural menopause and risk of cardiovascular disease. Arch Intern Med 1999;159:1061-6.

2.3

CARDIOVASCULAR REMODELLING, HYPERTENSION AND SEX HORMONES IN FOLLITROPIN RECEPTOR KNOCKOUT (FORKO) MICE

Rhian Touyz[1]
[1]Kidney Res. Ctr., Ottawa Hosp. Res. Inst., Univ. of Ottawa, 451 Smyth Rd., Rm. 2513, Ottawa, ON, K1H8M5, Canada.

In physiological conditions, regulated production of reactive oxygen species (ROS) plays an important role in signal transduction and cellular function. In pathological conditions oxidative stress (increased ROS) contributes to oxidative damage, implicated in hypertension and end-organ damage. Oxidative stress also plays a role in gender-related differences in cardiovascular disease and in menopause-associated hypertension. This has been attributed to increased ROS generation related to elevated androgen and/or to decreased estrogen levels. Estrogen deficiency may also contribute to reduced antioxidant capacity, which further exacerbates oxidative stress. Of the many antioxidants, including catalase, peroxidase and glutathione, thioredoxin (Trx) is one of the most abundant. Thioredoxin-interactingprotein (Txnip) is an endogenous Trx inhibitor. Independent of its anti-oxidant properties, Trx also functions as a signalling molecule by modulating kinases involved in cell growth/apoptosis. In particular ASK-1, which is pro-apoptotic, is inhibited by Trx. Changes in Trx status have been implicated in various cardiovascular pathologies, including atherosclerosis and hypertension. We demonstrated that the Trx system is dowregulated in FORKO mice, a model of menopause-associated hypertension. In particular Ang II-induced cardiovascular hypertrophy/fibrosis is associated with reduced Trx activety and upregulation of Trx-sensitive ASK-1/caspase signaling. These data suggested that in estrogen-deficient states, protective actions of Trx are blunted. Such phenomena may contribute to redox-sensitive cardio-vascular remodeling and target-organ damage, important in menopause-associated hypertension. (J Hypertens.2007;25:1263;Am J Physiol.2008;295:H1481; Hypertension.2009;54:427).

3.0 GENDER DISPARITIES IN RENAL DISEASE

3.1

SEX DIFFERENCES IN RENAL INJURY: ROLE OF PODOCYTES

Sharon Elliot[1], Paola Catanuto[1]
[1]Surgery, Miller Sch. of Med., Univ. of Miami, 1600 NW 10th Ave., R104, RMSB Rm. 1038B, Miami, Fl, 33136.

Estrogens modulate the development and progression of chronic kidney diseases (CKD) not related to diabetes. Clinical studies have demonstrated that the severity and rate of progression of renal damage tends to be greater among men,compared with women. Experimental studies also support the notion that female sex is protective and male sex permissive, for the development of CKD in non-diabetics, through the opposing actions of estrogens and testosterone. While multiple experimental studies have suggested that 17β-estradiol (E_2) treatment may protect the glomerulus against injury, most studies have focused on mesangial cells. Recently, our laboratory has studied podocytes, the cell type whose role may include in-itiation of progressive diabetic renal disease and other kidney diseases. We showed that E_2 treatment ameloriated type 2 diabetic glomerular disease part by preventing deleterious signaling and increasing estrogen receptor β expression in podocytes. We have now found that E_2 administration to diabetic female mice stabilizes podocyte F actin through a decrease in Hsp-25 activation and an increase in Rac1 expression. In addition, E_2 treatment increases AKT activation and decreases caspase expression. These data suggest that E_2 treatment may prevent podocyte loss and effacement by preserving the cytoskeleton and decreasing apoptosis. Support: NIH AG017170-12. References: Catanuto P, Doublier S, Fornoni A,Lupia E, Berho M, Striker GE, Xia X, Karl M, Elliot SJ. 17β-estradiol and Tamoxifen upregulate estrogen receptor β and regulate podocyte signaling pathways in a model of type 2 diabetes. Kidney Int. 75:1194-201, 2009.

3.2

PODOCYTURIA AS AN EARLY PREDICTIVE MARKER OF PRE-ECLAMPSIA

Vesna Garovic[1]
[1]Nephrology and Hypertension, Mayo Clinic, 200 First St. SW, Rochester, MN, 55905.

Preeclampsia is a syndrome of hypertension and proteinuria that occurs after 20 weeks gestation. Recent work shows that podocyturia, the shedding of live podocytes in the urine, is present at the time of delivery in preeclamptic patients. We aim to test whether podocyturia is predictive of preeclampsia and whether it can differentiate between preeclampsia and other hypertensive disorders of pregnancy. We prospectively enrolled 122 patients at first obstetric presentation. Urine samples were obtained at presentation, second trimester, delivery, and 4-6 weeks post-partum. Urine sediment was cultured for 24 hours to select for viable cells. Podocytes were then identified on the basis of podocin staining. The presence or absence of podocyturia was then correlated with the later development of preeclampsia or high risk pregnancy, including gestational hypertension, gestational diabetes, and twin pregnancy. At delivery, podocyturia was consistently present in all 10 women with preeclampsia and absent from the 19 women with high risk pregnancy disorders. None of the 93 women with normal pregnancies developed podocyturia at delivery. At mid-gestation, all 10 women who later developed preeclampsia had podocyturia. In addition the women with high risk pregnancy disorders who did not develop preeclampsia and those with normal pregnancy did not develop podocyturia at mid-gestation. Podocyturia may be helpful in the diagnosis of preeclampsia at delivery and in differentiating women with preeclampsia from other high risk pregnancy disorders, including gestational hypertension. At mid-gestation, the presence of podocyturia may identify women at risk of developing preeclampsia later in pregnancy. Garovic VD, Wagner SJ, Turner ST, et al. Urinary podocyte excretion as a marker for preeclampsia. *Am J Obstet Gynecol.* 2007 Apr; 196 (4): 320.e1-320.e7.

3.3

ROLE OF ANDROGENS AND 20-HETE IN RENAL DISEASE

Michal Schwartzman[1]
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Androgen plays an important role in blood pressure regulation. Epidemiological studies have shown that men have a higher prevalence for developing hypertension than aged-matched premenopausal women. Postmenopausal women and women with polycystic ovary syndrome, both of which have increased endogenous androgen production, have elevated risks for hypertension suggesting that androgen may contribute to its development. Recent studies demonstrated that 20-hydroxyeicosatetraenoic acid (20-HETE), the cytochrome P450-derived ω-hydroxylated arachidonic acid metabolite, mediates the hyper-tension seen in rodents treated with androgen. It plays a complex role in blood pressure regulation. In kidney tubules, 20-HETE decreases blood pressure by promoting natriuresis, while in the microvasculature it has a pressor effect. 20-HETE sensitizes the smooth muscle cells to constrictor stimuli and contributes to myogenic, mitogenic and angiogenic responses. It also acts on the endothelium to promote endothelial dysfunction and activation. Recently, we demonstrated that 20-HETE induces endothelial ACE thus setting forth a potential feed forward mechanism via activation of the RAS. The role of 20-HETE in androgen-induced hypertension and vascular dysfunction including endothelial activation and vascular remodeling in the renal microcirculation will be discussed. (NIH HL034300; HL HL097402). Reckelhoff, J.F. Gender differences in the regulation of blood pressure. Hypertension 2001;37:1199-208. Singh, H., and Schwartzman, M.L. Renal vascular cytochrome P450-derived eicosanoids in androgen-induced hypertension. Pharmacol Rep 2008;60:29-3.

4.0 POSTER SESSION I

4.1

NO SEXUAL DIMORPHISM IN DEVELOPMENT OF KIDNEY DAMAGE IN THE AGING FISCHER-344 (F344) RAT KIDNEY

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The aging kidney exhibits slowly developing kidney injury and females (F) are usually protected com-pared to males (M), in association with maintained renal nitric oxide (NO). Here, we compared intact and ovariectomized (Ovx) young (Y; 6mo) and old (O; 24m) F rats with Y and O intact M and measured kidney injury and protein abundance of endothelial NO synthase (eNOS). There was no difference in age-dependent kidney damage between Y or O intact M and F F344 (Table). YM had *greater* kidney cortex eNOS abundance than YF, and this persisted in OM vs OF. Ovx had no impact on kidney injury and *increased* eNOS protein in the OF. Conclusion: The kidney damage expressed in the aging F344 is fairly mild, develops similarly in M and F and is not related to loss of eNOS. This is in contrast to the aging Sprague-Dawley M rat (Erdely et al, 2003) where kidney damage is exacerbated and eNOS is lost compared to the OF. The lack of effect of Ovx on injury in OF suggests that in F344 ovarian hormones do not influence these aspects of kidney aging.

	% Damaged glomeruli	Glomerular Sclerosis Index (GSI, 1-4 scale)	Casts (#)	eNOS
YM	2.1±0.6	0.03±0.01	0.3±0.2	1.9±0.4 +
OM	12.7±2.3*	0.27±0.06*	10.0±2.9*	2.6±0.5 +
YF	2.0±0.2	0.02±0.01	0.0±0.0	1.0±0.1
OF	17.0±2.0*	0.38±0.05*	14.0±4.9*	0.9±0.2
YF-OVX	2.7±0.3	0.04±0.01	0.1±0.1	1.5±0.2
OF-OVX	17.4±5.6*	0.41±0.16*	12.7±5.6*	2.4±0.3 +

*p<0.05 vs respective Y, + p<0.05 vs F of same age.

4.2

SEX STEROIDS AND RENAL SODIUM TRANSPORT IN MICE CONSUMING 1% AND 4% SALT DIET

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The purpose of this study was to determine the effects of sex steroids on the renal excretion of Na^+ ($U_{Na}\cdot V$) and mRNA expression of renal Na^+ transporters in 10-week old CD-1 mice consuming 1% and 4% salt diets. Female mice were divided into three groups (n=4) – normal (N), ovariectomized implanted with a placebo pellet (OP), and ovariectomized implanted with a 2.5 mg estrogen pellet (OE). Mice were in metabolic cages and consumed 1% salt diet for the first 10 days, 4% salt for the next 10 days, and 1% salt for the final 10 days. Average $U_{Na}\cdot V$ (μEq/day ± se) for the first 10 days were (N, OP, OE, respectively): 457 ± 39, 460 ± 30, 221 ± 13*, and for the 10 days on 4% salt were: 1952 ± 129, 1967 ± 95, 1056 ± 72* (*p<.0001 compared to N & OP). In the final 10 days OE mice also had lower $U_{Na}\cdot V$. Using real-time, qt-PCR and customized PCR arrays from SABiosciences (Frederick, MD), we com-pared relative expression of various renal Na^+ transporters from whole kidney tissue taken from the OP and OE groups at the end of the study. OE showed higher expression of NCC, α and γ subunits of ENaC, Na^+-glucose cotransporter, and α -1 subunit of Na^+/K^+ ATPase. Relative expression of the angiotension II type 1a receptor was lower in the OE group. We conclude that continuous estrogen administration yields significant renal Na^+ absorption via proximal and distal Na^+ transporters which could affect mean arterial blood pressure. The respective study in males is underway.

4.3

ALDOSTERONE ESCAPE IS INFLUENCED BY SEX CHROMOSOMAL COMPLEMENT IN MICE

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Differences in sensitivity to aldosterone may play a role in blood pressure (BP) disparity between males and females. In order to evaluate the role of the sex chromosomal complement (SCC) in this response we used our unique mouse model in which Sry (male sex-determining gene) was translocated from the Y chromosome to an autosome. Mice of 4 distinct genotypes: 1) XX-F, 2) XY-F 3) XX-M, and 4) XY-M were gonadectomized to remove masking effects of sex steroids. On a low NaCl diet (0.085%), mice were implanted with osmotic minipumps to infuse aldosterone and later switched to a high-NaCl (5%) diet. By day 2 of high-NaCl diet, mice of the XX SCC demonstrated a significantly more robust aldo-sterone escape, as evident by higher urine sodium excretion (mmol Na+/40 g·bw/d): 3.5 ± 0.9 (XX-F), 1.5 ± 0.2 (XY-F), 2.1 ± 0.3 (XX-M), and 1.5 ± 0.4 (XY-M), p = 0.028 for SCC. This significantly in-creased excretion of sodium in the XX SCC was maintained on day 3 of high-NaCl diet (p = 0.031 for genotype). Interestingly, potassium excretion was also significantly increased in the XX SCC on day 3 (p = 0.045). These results suggest either: 1) XX SCC are more aldosterone sensitive, leading to early sodi-um retention necessitating greater pressure natriuresis or 2) XX SCC have a greater efficiency of escape mechanisms relative to XY. Studies to evaluate BP in this model are currently ongoing. Overall, these studies highlight important differences due to SCC in renal sodium handling in response to high-NaCl diet with aldosterone infusion. This findings may be particularly relevant in the absence of sex steroids, e.g., postmenopausally.

4.4

GPR30 AGONIST G-1 RESTORES MEGALIN EXPRESSION AND REDUCES PROTEINURIA IN SALT-SENSITIVE MREN2.LEWIS FEMALES

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Female mRen2.Lewis (mRen2) rats maintained on high salt (HS, 4% sodium) exhibit markedly higher systolic blood pressure and significant end organ injury in the heart, vasculature and kidney. We previously reported that chronic treatment with the GPR30 agonist G-1 improved renal hypertrophy and creatinine clearance while reducing proteinuria and oxidative stress in HS females. The current study assessed renal GPR30 expression and its relationship with megalin, an endocytic receptor for filtered proteins. In mRen2 females fed HS for 10 weeks, cortical GPR30 protein increased approximately 6-fold (0.6 ± 0.3 vs. 4.0 ± 0.8; P<0.01) compared to the normal salt (NS) group; however, cortical ERα isoforms (66, 46, and 36 kDa) were not changed. Immunofluorescent studies revealed co-localization of GPR30 and megalin on the luminal surface of proximal tubules. HS reduced megalin by 58% (P<0.01, n=4-5) in comparison to the NS group while G-1 treatment restored its expression (P<0.05 vs. HS), as well as reduced tubulointerstial oxidative stress (4-HNE staining). Moreover, megalin was negatively as-sociated with both proteinuria (r = -0.75, P<0.005) and 8-isoprostane excretion (r = -0.64, P<0.05). We conclude that the GPR30-induced renoprotective effects may involve restoration of megalin-mediated protein reabsorption through attenuation of oxidative stress within the proximal tubules of the salt-sensi-tive female mRen2 rat. Funding: NIH HL56973, HL51952, HL103974; AHA 0825515.

4.5

COLLECTING DUCT-DERIVED RENIN EXHIBITS SEX DIFFERENCES DURING NORMAL SALT AND HIGH SALT DIETS IN SPRAGUE-DAWLEY RATS

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In contrast to the inhibitory effect of high salt (HS) on renin from the juxtaglomerular cells, renin from the collecting duct (CD renin) is not suppressed in male rats fed HS. To determine if CD renin exhibits sex disparity in response to HS, we measured the inner medullary renin expression, content and excretion in male (M) and female (F) Sprague-Dawley rats fed normal salt (NS) and HS (8%NaCl) diet for 14 days. SBP were similar between sexes and unchanged by HS. Under NS, CD renin mRNA levels were higher in males compared to females (M: 1.0±0.4 vs F: 0.2 ±0.1, AU, p<0.05); however, in response to HS, these levels increased only in females (M HS: 1.3±0.1 vs. F HS: 2.5±0.5 AU). Protein levels were similar to mRNA except in HS F where increased prorenin but not renin levels were found. Males had significantly more medulla renin content at baseline (M: 9217 ± 1393 vs. F: 2608 ± 356 ug/hr/g Ang I); however, HS increased content in females but not males (F HS: 3439 ± 217 ug/g/hr Ang I). The renin urinary levels were higher in male rats (M 2.6±0.7 vs. F 0.2±0.04 X 10^{-5} EUE/day) and increased in both sexes with HS (M HS 9.5±2.0 vs. F HS 2.3±0.6 X 10^{-5} EUE/day). In basal conditions, CD renin synthesis and intratubular secretion are higher in males. Although CD renin expression is more responsive to HS in

females, the greater urinary renin activity in males, suggest greater capability to form intratubular Ang I and ultimately Ang II, in males. Tulane-BIRCWH (K12HD043451).

4.6

ASSOCIATION BETWEEN MENOPAUSE, OBESITY AND COGNITIVE IMPAIRMENT

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Background: Obesity (Ob) has been associated with cognitive decline (CD). The protective role of estrogen on cognition is controversial. Aims: To evaluate the association between CD and Ob in menopausal women (MW). Methods: We included 678 women ≥ 18years (y) who participated in the Cardiovascular Prevention Program "Healthy Heart 2" (Villa María, Córdoba, Argentina). Design: cross-sectional and observational study. Multistage sampling stratified (sex and age) and call voluntary. The validated survey was used. Anthropometric data were recorded. Definition: MW as amenorrhea ≥ 1 y, Ob as waist circumference (WC) ≥ 88 cm or body mass index (BMI) ≥30. Cognition assessment: Minimal Cognitive Examination (MCE) that includes: Mini-Mental Statement Examination (MMSE) (global cognition), Clock Drawing Test (executive function) and Boston abbreviated test (memory). Results: The average age 59.8 ± 9.4 y. The MW prevalence was 44.3% (n=300) and Ob 52.6% (n=158). BMI was positively correlated with: MMSE (coef. Pearson (r) 0.09, p 0.019) and MCE (r 0.21, p 0.03). There was equal correlation between WC and MMSE (r 0.04, p 0.02) and MCE (r 0.10, p 0.03). Conclusions: The prevalence of Ob in MW was higher, both overall and abdominal. There was positive correlation between Ob and better cognition. The result was attributable to the hypothesis that increased production peripheral estrogen (adipose tissue) is protective.

4.7

GPR30 ACTIVATION INCREASES ACE2 EXPRESSION IN DIABETIC OVARIECTOMIZED MREN2.LEWIS FEMALES

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We previously reported that activation of the estrogen receptor GPR30 by the selective ligand G-1 reduced proteinuria and angiotensinogen excretion in estrogen-depleted (OVX), diabetic mRen2.Lewis (mRen2) rat. The present study evaluated the influence of diabetes on ACE2 and neprilysin (NEP) expression, two key enzymes involved in Ang II and Ang-(1-7) metabolism, within the kidneys of intact and OVX-mRen2. Diabetes was induced with a single dose of streptozotocin (STZ 65 mg/kg; ip) for 4 weeks without insulin replacement and kidneys removed for analysis of GPR30, ACE2 and NEP expression. Tubular expression of GPR30 increased significantly in the intact STZ mRen2 and was correlated with an increase in ACE2 protein expression ($r=0.65$, $p<0.05$). Both GPR30 and ACE2 were localized to the apical region of proximal tubules. NEP expression was significantly increased in intact STZ mRen2 ($p<0.05$) but did not correlate with GPR30 expression. In contrast, estrogen-depletion alone increased GPR30 expression, and diabetes had no further influence on the receptor or peptidase expression. In STZ OVX-mRen2, the GPR30 agonist G-1 significantly increased ACE2 expression ($p<0.01$) but did not affect NEP. In summary, GPR30 is upregulated in intact STZ mRen2 females that is associated with increased ACE2 expression. We conclude that the renoprotective effects of estrogen in the diabetic kidney may involve GPR30-dependent activation of ACE2. Support: HL56973, HL51952, HL103974, AHA0825515.

4.8

ANDROGEN RECEPTOR (AR) AND ESTROGEN RECEPTOR ALPHA (ERA) EXPRESSION AFTER COMBINED DIHYDROTESTOSTERONE (DHT) SUPPLEMENTATION AND AROMATASE INHIBITION IN THE MALE STREPTOZOTOCIN (STZ)-INDUCED DIABETIC RAT

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Our previous data showed that STZ-diabetic males have increased expression of ERα and decreased expression of AR correlating with renal injury. We also showed that combined therapy of DHT supplementation and estradiol synthesis inhibition is renoprotective. The aim of this study was to examine if this renoprotection was mediated via restoring ERα and AR expression. The study was performed in 12 week old male non-diabetic (ND), STZ-induced diabetic (D) and STZ-diabetic treated with DHT supplementation and anastrozole (Dta) for 12 weeks. Urine albumin excretion (UAE) was determined by ELISA. Glomerulosclerosis (GSI) and tubulointerstitial fibrosis (TIFI) indices were graded using a semiquantitative scoring method. Protein expression were determined by Western blot and normalized to total β-actin expression. Our data suggest that combined treatment of DHT and anastrozole reduced diabetes-associated increases in UAE, GSI, and TIFI. Furthermore, diabetes was associated with reduced AR and ERα protein expression in the renal cortex. Combined treatment with DHT and anastrozole re-stored both receptor protein expressions to levels observed in ND animals. These data suggest that one of the mechanisms by which DHT supplementation and anastrozole mediate their renoprotective effects is by restoring the expression of ERα and ARs in the male STZ-induced diabetic rat.

Group	UAE (mg/day)	TIFI (AU)	GSI (AU)	AR (ROD)	ERα (ROD)
ND	2.6±0.7*	0.4±0.1*	0.6±0.1*	1.7±0.1*	1.4±0.1*
D	47.3±9.8	1.7±0.2	1.7±0.1	0.9±0.1	0.9±0.1
Dta	7.7±1.3*	0.7±0.1*	0.8±0.1*	1.8±0.1*	1.2±0.1*

Avg±SEM; *P<0.05 vs. D; #P<0.05 vs. Dta

4.9

THE EFFECTS OF STREPTOZOTOCIN-INDUCED DIABETES AND GENDER ON THE RAT AORTIC ENDOTHELIAL FUNCTION

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Little is known of the interaction between diabetes and gender in the vasculature. The objective of this study was to investigate whether there are gender differences in rat aortic endothelial function in streptozotocin (STZ, 65mg/kg, iv)-induced diabetes. Endothelium-dependent vasodilation (EDV) to acetylcholine (ACh; 10^{-8} to 10^{-5}M) was measured in aortic rings precontracted with phenylephrine (PE; 2 μM) before and after pretreatment with indomethacin (indo; 10μM), a cyclooxygenase (COX) inhibitor. Constrictor response curves (CRC) to PE (10^{-8} to 10^{-5}M) were also generated before and after incubation with L-NAME (200μM), an endothelial nitric oxide synthase (eNOS) inhibitor. Furthermore, eNOS mRNA expression was measured using real-time PCR. STZ-induced diabetes impaired EDV in both genders, however the extent of impairment was more pronounced in females. Similarly, eNOS mRNA expression was reduced in aorta of diabetic rats, but the extent of decrease was greater in diabetic females. Furthermore, diabetic females were characterized by an increased sensitivity to ACh after indo compared with other groups. Incubation of aortic rings with L-NAME potentiated PE responses in all groups. However, aorta from control females showed a greater potentiation of the PE after NOS inhibition than those in other groups. These data suggests the predisposition of female rat aorta to vascular injury in diabetes, possibly via altered NO production and COX metabolites (Supported by NIH/NIDCR).

4.10 Withdrawn.

4.11

CENTRAL BLOCKADE OF ANGIOTENSIN(1-7) OR ANGIOTENSIN II RECEPTOR TYPE 2 (AT_2) ENHANCES ALDOSTERONE/SALT-INDUCED INCREASES IN BLOOD PRESSURE (BP) IN FEMALE RATS

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In comparison to male rats, females are protected against ANG II- and Aldo-induced hypertension (HT). However, the mechanism underlying this protective effect is not well-understood. Recent studies show brain-selective overexpression of ACE2 or AT_2R attenuates ANG II-induced HT in male mice. ACE2- and AT_2R- expression in the kidney is also higher in female rats than males, and may account for attenuated BP induced by systemic ANG II in females. Our study tested if central blockade of ACE2 or AT_2R enhances Aldo/salt-induced increase in BP in female rats. Systemic infusion of Aldo (0.75 μg/h, 4 weeks) into females with 1% salt as a sole drinking fluid resulted in a slight increase in BP (Δ7.5±1.2 mmHg). But females receiving this same treatment along with icv infusions of ANG (1-7) receptor antagonist (A-779) or AT_2R antagonist (PD123,319) showed significantly augmented pressor effects (Δ20.4±1.8 and Δ18.9±4.6 mmHg, respectively). RT-PCR analysis of brain revealed a significant increase in mRNA expression of renin (1.4-fold), Aldo synthase (1.5-fold), 11-β-hydroxylase (2.9-fold), ACE2 (1.5-fold) and AT_2R (2.5-fold). Yet neither AT_1R nor ACE1 in LT or PVN changed in Aldo/salt-treated females compared to basal conditions. Our results suggest an antihypertensive arm of the brain RAS (ACE2/ANG (1-7)/Mas/AT_2R) may play an important compensatory role in development of Aldo/salt induced HT in females. (HL-14388, HL-98207, DK-66086, and MH-80241).

4.12

SEX DIFFERENCE IN THE B-ADRENERGIC CONTRACTILE RESPONSE ROLES OF ADENYLYL CYCLASE AND PHOSPHODIESTERASE

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Female hearts exhibit a blunted contractile response to β-adrenergic receptor (β-AR) stimulation as com-pared to male hearts. The roles of adenylyl cyclase (AC), phosphodiesterase (PDE) and cAMP signaling in generating these sex-dependent differences were examined in dose response studies in isolated perfused hearts from male and female mice using AC agonist forskolin, PDE inhibitor isobutylmethylxanthine (IBMX), and a non-hydrolyzable form of cAMP, CPT-cAMP. Females showed a modestly lower contractile response to forskolin as compared to males at 5 μM (left ventricular systolic pressure in % change from baseline, 62±5 vs. 77±2), but there were no sex differences in the contractile responses to IBMX or CPT-cAMP. Additionally, there were no sex differences in the expression of the β_1-AR, PDE4D, $G\alpha_s$ or $G\alpha_{i-2}$, determined using western blotting. Paradoxically, expression of adenylyl cyclase V/VI in ventricular membranes is 78% greater in females then males. The re-

duced contractile re-sponse to forskolin seen in females is not due to lower AC expression, and is likely only a minor com-ponent in generating sex differences in the β-AR response. A possible explanation for the paradoxical observation of greater AC expression but reduced contractile responses to forskolin could be sex differences in regulation of AC activity by protein kinases. Future studies will address sex differences in the complex regulation of the β-AR contractile response by PDEs and protein kinases. This work was sup-ported by National Heart, Lung, and Blood Institute Grant, R01HL066132 (Robert D. Lasley).

4.13

GPR30 IS INVOLVED IN THE REGULATION OF THE KCA1.1 CHANNEL CURRENT IN A GENDER SPECIFIC POPULATION OF MYELINATED VAGAL AFFERENTS

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Autonomic nervous system control of cardiovascular function is sexually dimorphic. Our lab has a long standing interest in cardiovagal afferent function and recently showed that that female rats have a unique and functionally distinct class of low threshold myelinated (Ah-type) aortic baroreceptor neurons (ABN) that is rarely observed in age-matched males. Furthermore, we have shown that the sensitivity and excitability of Ah-type ABN are markedly reduced in an ovariectomized rat model (OVX). Here, we test two hypotheses: 1) regulation of BK-type KCa (KCa1.1) ion channels underlies the loss of excitability in these gender specific afferents, and 2) a GPR30 dependent pathway is involved in the regulation of KCa1.1 channels. Voltage and current clamp protocols were carried out using the patch clamp technique on vagal neurons (VGN) from aged matched female (NF, n = 10) and OVX (n = 10). The whole cell KCa1.1 (iberiotoxin sensitive) current in OVX was ~50% greater than that measured in NF. Companion current clamp studies in OVX revealed that repetitive discharge in Ah-type VGN was abolished as a re-sult of the KCa1.1 current increase. Application of G-1, a selective agonist of the G-protein coupled estrogen receptor GPR30, restored the excitability of Ah-type afferents to near control levels. Application of G-1 to voltage clamped VGN reduced the whole cell KCa1.1 current in Ah-type neurons from OVX to levels measured in NF. Collectively, these studies implicate that the GPR30 is involved in the regulation of KCa1.1 channels in myelinated Ah-type VGN. We contend that this gender specific population of myelinated vagal afferents may provide, at least in part, a neurophysiological explanation for the sexual dimorphism in neurocirculatory control. NIH HL081819 and HL072012.

4.14

ANGIOTENSIN PEPTIDES AND FMD: DOES SEX MATTER?

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The prevalence of cardiovascular disease is greater in men than in women, and although the mechanism responsible is unknown, sex differences in Angiotensin (Ang) peptides have been suggested to con-tribute. In fact, evidence in rats indicates Ang(1-7), a vasodilatory peptide, is higher in females when com-pared to males. In humans, the flow-mediated dilation (FMD) test represents a bioassay of NO-de-pendent vasodilation and is a predictor of future cardiovascular disease. This study sought to test the hy-potheses that 1) Ang(1-7) is higher in women compared to men, and 2) concentrations of Ang(1-7) are associated with FMD. A total of 29 subjects participated in this study; FMD was obtained on 11 (4 men; 7 women). Following an overnight fast, FMD was performed and blood samples were collected. Plasma concentrations of Ang(1-7) were significantly higher in women compared to men (43±5 vs. 32±4 pg/mL; p=0.04). Although FMD (6.0±0.6 vs. 6.8±0.8%) and plasma AngII concentrations (81±19 vs. 68±14 pg/mL) were similar between groups, there was a trend (p=0.26) for a lower Ang II/Ang (1-7) ratio in women compared to men (2±1 vs. 3±1). Additionally, FMD was positively associated with Ang(1-7) (r=0.602;p=0.04) and inversely proportional to AngII (r=-0.626;p=0.03) and AngII/Ang(1-7) ratio (r=-0.599;p=0.04). For the first time, we have observed differences in plasma Ang(1-7) con-centrations between men and women and have identified a link between FMD and circulating Angi-otensin peptides. These data may provide insight into the cardiovascular disease risk disparity between sexes.

4.15

ATHEROGENIC INDEX OF PLASMA: A SIGNIFICANT INDICATOR FOR THE ONSET OF ATHEROSCLEROSIS DURING MENOPAUSE IN HYPATENSIVE FEMALES OF SOUTH EAST NIGERIA

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Hypertension and menopause are independent risk factors for dyslipidaemia. In normotensive subjects, menopause causes significant alteration in lipid levels that may require medical intervention. It is thus expected that this alteration will become more relevant in hypertensive menopausal subjects. In our environment, diverse dietary, socioeconomic, and geographical variables might alter the expected pattern of deraignment. This is a comparative, cross sectional cohort study involving 116 hypertensive females (67 premenopausal and 49 postmenopausal) consecutively recruited over a two year period. Serum total cholesterol and its sub fractions, high density lipoprotein (HDL), low density lipoprotein (LDL). Very low density lipoprotein (VLDL), and triglyceride, (TG) were estimated using standardized enzymatic and mathematical methods. Significant differences were analysed with student T –test using SPSS version 11 computer software. There were no statistically significant differences in total cholesterol, HDL, LDL, VLDL and HDL/LDL ratio between the two groups (P> 0.05). However there were statistically significant differences in triglycerides and the atherogenic index of plasma (P<0.05). Simultaneous occurrence of menopause and hypertension leads to alteration in lipid profile that favours the use of triglyceride based indices (instead of HDL/LDL ratio) in determining the risk of developing atherosclerosis. Funding: This project was self sponsored.

4.16

FETAL PROGRAMMING OF HYPERTENSION INDUCED BY ZINC RESTRICTION IN FETAL LIFE: GENDER DIFFERENCES IN EARLY EFFECTS ON KIDNEY

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We have shown that moderate zinc deficiency during fetal and postnatal growth induced hypertension and renal diseases in adults. **Aim:** To evaluate renal oxide synthase (NOS) activity and oxidative stress at 21 days of life and sex differences in the response to fetal and lactation zinc deficiency. **Methods:** It was determined renal NOS activity with L-(U14C)-arginine (pmol/min□g tissue) and protein abundance by western blot (% density/beta actin), renal thiobarbituric acid-reactive substances (TBARS, nmol/mg prot), glutathione concentration (GLUT, mg/mg prot,), superoxide dismutase (SOD, U/mg prot), catalase (CAT, pmol/mg prot) and glutathione peroxidase activity (GPx, pmol/min/mg prot) in 21 days female (f) and male (m) offspring of Wistar rats exposed from pregnancy up to weaning: low(L,8 ppm) or control(C,30 ppm) zinc diet (n=6). **Results:** Renal NOS activity was decreased in Bm (237±5) and Bf (245±7) compared with Cm (302±5) and Cf (317±11) respectively. It was not associated with lower ex-pression of eNOS, nNOS, iNOS protein. *p<0.01vs Cm; † p<0.01vs Cf. Kidney morphological alterations observed in this model may be associated with nitric oxide system and oxidative stress alterations. Lower renal NOS activety is not due to a decrease in protein expression so we suggest that other mechanisms may be involved, like alteration in NOS zinc cluster, cofactors or the oxidative stress. The impairment of antioxidant enzymes due to this deficiency is more evident in female than in male rats.

	Cm	Lm	Cf	Lf
GLUT	7,2±0.6	6,2±0.5	7,5±0.9	6,8±0.3
TBARS	0,13 ± 0,02	0,35 ± 0,09*	0,14 ± 0,01	0,5 ± 0,07†
SOD	13,2±0.9	12,2±0.9	19,1±1.0*	12,1±0.9 †
CAT	6,1±0.8	5,6±0.5	10,3±0.3*	4,1±0.7†
GPx	252 ± 27	166±10*	264±26	177±17†

4.17

GENDER DIFFERENCES IN RENAL OXIDATIVE STRESS ENZYMES IN DAHL SS RATS: EFFECTS OF SALT AND OVARIECTOMY

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Experimental and clinical data have failed to show a definitive link between oxidative stress and cardiovascular diseases. Contradictory clinical studies showed either a protective or a lack of effect of antioxidants in cardiovascular diseases. We had previously shown that male SHR rats have higher blood pressure (BP) and oxidative stress than females. Antioxidant treatment decrease BP in male but not female SHR rats. We hypothesize that there is a gender difference in renal oxidative stress enzymes expression under low (LS) or high (HS) salt in Dahl salt sensitive (SS) rats and that difference is due to sex hormones. Male, female and ovariectomized (OVX) SS rats (n= 6/group) were maintained on LS phy-toestrogen-free diet (0.28% NaCl) until 17 wk of age. Then, rats were placed on LS or HS diet (8% NaCl) for 4 wks. Renal cortex and medulla oxidative stress enzyme mRNA expression was quantified by qPCR. Males had higher levels of Nox4 and gp91phox compared to females on LS and HS diets. OVX rats had higher levels of Nox4 and gp91phox on LS but did not differ on HS. p47phox levels were higher in males on LS and did not change with HS, OVX rats had a similar pattern to male rats. EC-SOD levels were significantly higher in females compared to males, and OVX abolished that difference. Our data suggest that female rats may be unresponsive to oxidative stress secondary to high levels of renal SOD expression and that estradiol and/or progesterone may be involved in this protective mechanism.

4.18

SEX HORMONES MODULATE RESPONSES TO OXIDATIVE STRESS IN RENAL PROXIMAL TUBULE CELLS

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Women experience lower incidence of acute renal injury (AKI) and progression to chronic kidney disease (CKD) than men. This gender-associated dimorphism may be related to protective effects of estrogens and/or adverse effects of testosterone. We proposed that the oxidant-sensor adaptor protein p66shc may aggravate while the activated signal transducer and activator of transcription 3 (STAT3) may attenuate AKI and consequent renal fibrosis. The aim of this study was to ascertain the effects of sex

hormones on these factors in cultured renal proximal tubule cells. Dihydrotestosterone (DHT) treatment induced the p66shc promoter and exacerbated endogenous as well as H_2O_2-induced ROS production and con-sequent injury. DHT also augmented TGFβ1-mediated induction of the promoter of the profibrotic αSMA gene and attenuated IL-6-induced tyrosine phosphorylation of STAT3. In contrast, 17β-estradiol (E2) treatment augmented tyrosine phosphorylation of STAT3 but inhibited TGFβ1-mediated induction of the αSMA promoter. Thus, sex hormones may differentially regulate expression/activity of signaling molecules involved in survival or injury that may substantiate gender-specific responses to injury in the kidney. Funding: AHA GIA (10GRNT3790019, I. Arany), R01HL088101-04 and R01HL088101-02S1 (G.W. Booz) and DK073401 (L.A. Juncos).

	H_2O_2 (pmol/mg)	eNOS	Nitro-tyrosine	p22phox	ecSOD	mnSOD	CuZn SOD
V	29±4	100±13	100±4	100±9	100±7	100±14	100±25
EP	25±1	102±12	84±6	66±8	80±15	44±6	103±21
MP	25±3	88±12	98±9	97±13	68±7	81±26	110±28
LP	35±3	70±9	48±4*	93±4	57±9	79±24	126±19
PP	30±7	109±8	69±6*	84±3	81±6	113±41	92±16

4.19

EXAGGERATED ANGIOTENSIN II-INDUCED HYPERTENSION IN MALE RATS EXPOSED TO EARLY LIFE STRESS DEPENDS ON TESTOSTERONE LEVELS

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Previously, we reported that male rats exposed to maternal separation (MS), a model of early life stress, display enhanced sensitivity to angiotensin (Ang) II infusion (65 ng/day, 2 weeks) compared to non-MS control (C) male rats. This enhanced AngII-induced hypertension was also present in female MS rats, but significantly attenuated. The aim of this study was to investigate the role of sex hormones underlying the sensitivity to AngII. MS was performed in male and female WKY rats 3 hrs/day from day 2 to 14 of life. At the age of 12 weeks in baseline conditions, plasma testosterone (Ts) was similar in C and MS male rats (2456±377 and 2270±436 ng/ml, respectively) as well as in C and MS female rats (51±5 and 52±3 pg/ml). Also, estrogen (E2) was similar in C and MS male rats (4.0±0.5 and 3.4±0.5 ng/ml) as well as in C and MS female rats (14.2±1.8 and 13.3±2.5 pg/ml. In female C and MS rats, 14 days of AngII infusion reduced Ts (37±3 and 44±2 pg/ml) and E2 (6±1 and 5±1 pg/ml) similarly. However, in AngII-infused male rats, Ts was higher in MS than in C rats (1000±187 vs. 579±63 ng/ml, $p<0.05$). E2 was below detection in MS compared to C male rats. In castrated male rats, AngII infusion increased blood pressure in C and MS rats similarly (129±6mmHg and 125±6 mmHg) in contrast to the enhanced AngII-induced hypertension observed in non-castrated MS rats. These data suggest that Ts plays an important role in the mechanism underlying exaggerated AngII-induced hypertension due to early life stress.

4.20

ANDROGEN INDUCES THE EXPRESSION OF CYP4F2, A MAJOR 20-HETE PRODUCING ENZYME IN HUMAN, VIA ACTIVATION OF THE ANDROGEN RECEPTOR

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20-Hydroxyeicosatetraenoic acid (20-HETE) is a cytochrome P450 (CYP) 4-derived arachidonic acid metabolite and a vasoconstrictor found in the renal, cerebral and hepatic microcirculation. Increasing vascular 20-HETE has been associated with smooth muscle contractions, endothelial dysfunction and activation and hypertension. Androgen has been shown to induce cyp4a12 and CYP4A8 expression in mice and rats, respectively, resulting in 20-HETE-mediated hypertension. It remains to be determined whether androgen induces CYP ω-hydroxylase in humans and whether it contributes to gender differ-ences in hypertension. To study this paradigm in an in vitro system, we used a well-established androgen-responsive human prostate cancer cell line, LNCaP, which has the capability of producing 20-HETE (570±140 pg/mg). 20-HETE synthesis in LNCaP cells pretreated with charcoal stripped medium followed by androgen replacement with 5α-dihydrotestosterone (DHT) increased by 65% when com-pared to vehicle-treated cells. CYP4F2 and CYP4F3 mRNA levels increased by 2.3- and 2-fold higher in DHT-treated LNCaP as compared to vehicle, while CYP4A11 and CYP4A22 mRNA levels de-creased by 70-80%. The increase in CYP4F2 and CYP4F3 was attenuated by co-treatment with Flutamide. To further examine whether this association exists in non cancer cells, primary human coro-nary vascular smooth cells were treated with DHT±Flutamide. DHT increased CYP4F2 mRNA levels by 2-fold and was attenuated with Flutamide.

4.21

PROFILE OF ANTIOXIDANT AND PRO-OXIDANT ENZYMES AND MARKERS IN THE KIDNEY CORTEX (KC) DURING RAT PREGNANCY

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The increased metabolic demand of pregnancy may result in oxidative stress (OS), a risk factor for pregnancy complications. In the pregnant rat, increases in renal Na reabsorption occur by midterm leading to increased KC oxygen consumption. Here, we examined enzymes and markers of OS in early (E), mid (M) and late (L) pregnancy (P) (days 3, 12, 20) compared to virgins (V). Protein abundance of endothelial nitric oxide synthase (eNOS), the NADPH oxidase subunit p22phox, and extracellular (EC), cytosolic (CuZn), and mitochondrial (Mn) superoxide dismutase (SOD) as well as hydrogen peroxide (H_2O_2) and nitrotyrosine (NT) levels were measured in KC. The only variable altered by P was a reduction in NT at LP vs V (by one- way ANOVA; Table). This fall in NT was maintained at 4 days post partum (PP) vs V (t-test; Table). Conclusion: Despite increased renal metabolism during P, there is no change in KC antioxidant SODs or eNOS, pro-oxidant p22phox or H_2O_2 levels. The fall in NT by LP, which is maintained PP, suggests a fall in KC OS in normal P. This needs to be confirmed with other OS markers, and the mechanisms remain to be determined. *$p<0.05$ vs V. Protein abundance as integrated optical density normalized to total protein loaded and internal standard.

4.22

TESTOSTERONE INDUCES ROS GENERATION BY MODULATING MITOCHONDRIAL ACTIVITY

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The mechanisms whereby testosterone (TEST) induces its effects on the vascular system may involve generation of reactive oxygen species (ROS). We hypothesized that TEST regulates mitochondria-driven ROS production and activity. Cultured mesenteric vascular smooth muscle cells were incubated (30min) with flutamide (Flu, 10^{-5}M/L, androgen receptor antagonist), CCCP (10^{-6}M/L, mitochondrial uncoupling agent) or apocynin (Apo, $3x10^{-5}$M/L, NADPH oxidase nonspecific inhibitor) and then stimulated with TEST (10^{-7}M/L, 1.5 to 24hs). ROS generation was measured by HPLC, lucigenin-enhanced chemiluminescence and fluorescence microscopy. Expression of p47phox and nitrotyrosine-containing proteins were evaluated by immuneblotting. Lipid peroxidation was determined by the presence of TBARS and mitochondrial activity by the MTT assay. TEST increased ($p<0.05$) ROS generation (2 fold, 2h; n =4), lipid peroxidation (2h; 1 fold) and nitrotyrosine-containing proteins expression (6h, 1 fold). These effects were inhibited by Flu and Apo. TEST increased ($p<0.05$) p47phox expression and its translocation to the membrane (2h; n=6) as well as mitochondrial activity (2h; 1.36 *vs*. 1.19). TEST-induced ROS generation was inhibited by CCCP (2h; n=4). TEST induces ROS gener-ation through mitochondria and NADPH oxidase-related mechanisms. TEST may contribute to oxidative stress in cardiovascular diseases. FAPESP/CNPq.

4.23

PERINATAL EXPOSURE TO AN SSRI ANTIDEPRESSANT PROGRAMS SEX DIFFERENCE IN ADULT CARDIOVASCULAR RISK IN RODENT OFFSPRING

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Use of selective serotonin reuptake inhibitors (SSRIs) to manage perinatal maternal mood disorders can impact child neurobehavioral development. Poor nutrition during early life programs increased cardiovascular (CV) risk; yet, whether SSRIs program later CV risk is unknown. We previously demonstrated that early life SSRIs in the rat results in sex-specific alterations in neurobehavioral development, and organization and function of monoaminergic neurons. Using this model we now report that Citalopram-HBr (CTM, 20 mg/kg/day, PND 8-21) programs increased renal expression of aquaporin 2, suppression of water intake, and decreased urine volume and urinary excretion of sodium ($P<0.05$, CTM vs. saline) indicating sodium and fluid balance may be impaired. Mean arterial pressure (MAP) is elevated in male CTM-exposed rats relative to male saline (132±2 vs. 115±8 mmHg; $P<0.05$, CTM vs. saline, respectively). However, MAP is not elevated in adult female CTM compared to female saline (123±3 vs. 133±6 mmHg; CTM vs. saline, respectively) indicating that sex differences in adult blood pressure are programmed by early life SSRIs. Together, these data suggest that perinatal SSRI exposure exerts a per-vasive sex-dependent effect on developmental programming that includes not only neurobehavioral sequelae, but also significant increases in cardiovascular risk. RR017701 (IAP), MH084194 (RCSL) and HL074927 (BTA).

4.24

SEX DIFFERENCES IN THE SUSCEPTIBILITY TO ISCHEMIC RENAL INJURY IN A RAT MODEL OF LOW BIRTH WEIGHT

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Epidemiological and experimental studies reported that females showed less renal injury induced by ischemia-reperfusion (**I-R**). Previously, we reported that males low birth weight (**LBW**) rats are more susceptible to ischemic renal injury than normal birth weight (**NBW**) rats. However, is not clear whether females LBW rats present the same susceptibility to renal injury. Thus, the aim of this study was to investigate whether LBW female rats are protected against the susceptibility to ischemic renal

injury. Systemic and renal hemodynamic parameters were determined in male and female NBW and LBW adult rats after mild renal I-R (15 minutes of ischemia). Renal superoxide production and histological structures were also assessed. Mild renal I-R did not alter renal parameters, in male or female NBW rats as well as in female LBW rats. However, male LBW rats show significant changes compatibles with renal injury, (Table I). Thus, these findings suggest that LBW female rats are protected against the susceptibility to renal injury induced by mild ischemia.

Parameters/Rat ID	Male-NBW	Male-LBW	Female-NBW	Female-LBW
MAP (mmHg)	125±2	160±1*	117±5	114±6
GFR (ml/min/g K)	0.9±0.1	0.3±0.1*	0.8±0.1	0.7±0.2
Basal Superoxide (RLU)	1692±128	4223±190*	1342±135	1242±189
NADPH-ox derivate superoxide	22454±414	48371±397*	18454±292	16432±356
Renal injury Score	0.5±0.1	2.8±0.7*	0.5±0.2	0.6±0.3

*P<0.05 vs. All other groups.

4.25

SEX DIFFERENCES IN ACE MODULATES ANG 1-7 LEVELS IN NORMOTENSIVE WKY RATS

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The goal of this study was to determine 1) if there is a sex difference in Ang (1-7) levels in the renal cortex of normotensive rats, and 2) to measure the activity of the major enzymes that regulate Ang (1-7) formation. 12 week old male and female WKY were studied; the renal cortex was isolated to measure Ang II, Ang (1-7), ACE and ACE2 activities. Female WKY had greater Ang II (336±36 vs. 261± 32 pg/g cortex, p<0.05) and Ang (1-7) levels (343±109 vs. 214±108 ng/g cortex, p<0.05) compared to males. ACE enzymatic activity was also significantly greater in females vs. males (318±44 vs. 77±5 RFU/mg protein, p<0.05), however, ACE2 activity was comparable between the sexes. We hypothesized that greater ACE activity in females contributed to greater Ang (1-7) levels. Additional rats were treated with the ACE inhibitor enalapril (10mg/kg/day, 14 days). Enalapril abolished ACE activity in males (-21±8 RFU/mg protein, p<0.05 vs. control), and significantly decreased ACE activity in females (104±40 RFU/mg protein, p<0.05 vs. control). This was associated with a significant decrease in Ang II and Ang (1-7) levels in males (Ang II: 95±16 ng/g cortex; Ang (1-7): 155±12 pg/g cortex, p<0.05 vs. control) and females (Ang II: 155± 46 ng/g cortex, Ang (1-7): 154±33 pg/g cortex, p<0.05 vs. control). In summary, enalapril resulted in a larger decrease in Ang II levels in males than females, while females had a greater decrease in Ang (1-7).

4.26

A ROLE FOR ETHNIC DISPARITY: ENDOTHELIN-1 SECRETION BY HUMAN PLACENTAS FROM PREGNANCIES COMPLICATED BY PRE-ECLAMPSIA?

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It has been shown that preeclamptic women have high levels of circulating endothelin-1 (ET-1) during the later stages of pregnancy. We examined activation of placental and vascular ET-1 from women with preeclampsia (PE) compared to controls. Placentas were collected from 40 women (20 normotensive, 20 PE), mRNA preproendothelin (PPET) was measured and placental secretion of ET-1 was determined from cultured placental explants. In addition, human umbilical vein endothelial cells (EC) were exposed to conditioned media from placental explants to assess endothelial cell activation, measured by ET-1 se-cretion and PPET mRNA. Placental explants from women with PE secreted significantly more ET-1 (82.10+12.76 pg/ml; p<0.05) (p<0.0005) compared to normotensive women (30.88+4.39 pg/ml). ECs exposed to placental explants from AA women with PE secreted significantly more ET-1 compared to CA women with PE (20.53+.77 vs 14.43+.64 pg/ml; p<0.05). Both placental (12.35+.27 vs 10.55+.47 pg/ml in nonPE) and EC (9.18+.25 vs 7.70+.39 pg/ml in nonPE) PPET mRNA was significantly in-creased (p<0.05, p<0.05 respectively) with PE compared to normals. We conclude that ET-1 secretion and production is increased in women with PE compared to NP. Furthermore we conclude that endothelial dysfunction in response to factors released from the placenta is more prevalent among AA com-munity and may play a distinguishing role in the development of hypertension.

4.27

DIFFERENTIAL PROGRAMMING OF ENDOTHELIN RECEPTOR EXPRESSION CONTRIBUTES TO SEX DIFFERENCES IN ADULT BLOOD PRESSURE REGULATION IN INTRAUTERINE GROWTH RESTRICTED OFFSPRING

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Endothelin (ET) and its receptor subtypes play a key role in control of blood pressure in an age- and sex-specific manner. Intrauterine growth restriction (IUGR) programs hypertension and enhanced sensitivity to acute angiotensin II (ANG II) in male rats. Selective ET_AR blockade (ABT-627) at the dose of 10 mg/kg/min attenuates the acute ANG II pressor response in male control rats and abolishes the enhanced pressor response to acute ANG II in adult male IUGR rats. Yet, this same dose of ET_AR blockade does not reduce the increase in MAP in response to acute ANG II in female control or female IUGR. Thus, the goal of this study was determine whether IUGR programs sex-specific expression of the ET receptor subtypes. Protein expression of ET_AR was significantly increased in the kidney of male IUGR relative to male control (cortex: 1.6 fold and medulla: 1.5 fold, $P<0.05$). Expression of ET_BR was also significantly increased in male IUGR (cortex: 3.5 fold and medulla: 2.7 fold; $P<0.05$). In contrast, the expression of ET_AR did not differ in the kidney cortex and medullar of female IUGR and female control rats; but, protein expression of ET_BR was significantly reduced in the medulla of female IUGR relative to female control (0.5 fold; $P<0.05$). Thus, this study indicates differential programming of ET receptor expression and suggests that sex-specific differences in the ET system may contribute to contribute to acute ANG II sensitivity.

4.28

INFLUENCE OF FEMALE SEX HORMONES AND SALT IN ESSENTIAL AND SALT-SENSITIVE HYPERTENSION

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Literature suggests that female sex hormones may protect against the development of salt sensitivity. The goal of this study was to determine the effect of female sex hormones on blood pressure (BP) in models of essential and salt-sensitive hypertension. 13 week old intact and ovariectomized (OVX) female spontaneously hypertensive rats (SHR) and Dahl-salt sensitive (Dahl-SS) rats were placed on phytoestrogen-free normal salt (NS; 0.4% salt) or high salt diet (HS; 4% salt) for 2 weeks and BP was monitored by telemetry. HS increased BP in both intact and OVX SHR (baseline: 138±1 vs. 133±1; week 2: 145±1 vs. 141±1 mmHg, respectively, p<0.05). Intact Dahl-SS had lower BP than OVX at baseline (126±1 vs. 139±2 mmHg, respectively, p<0.05). HS diet increased BP in both Dahl-SS groups, however, the increase in BP was significantly greater in OVX than intact by the end of 2 weeks of HS (150±2 vs. 180±3 mmHg, respectively, p<0.05). Since female sex hormones with 2 weeks of HS did not alter BP in female SHR additional studies were performed to determine if 8 weeks of HS would alter BP. Intact and OVX SHR had comparable BP at baseline (131±1 vs. 130±1 mmHg, respectively). SHR BP was increased after 8 weeks of HS in both groups (week 8: 147±1 vs. 146±1 mmHg, respectively, p<0.05). These data suggest that female sex hormones are important in BP regulation and protecting against elevations in BP in Dahl-SS rats but not SHR. Funding by AHA.

4.29

HEME OXYGENASE-1 AS A POTENTIAL THERAPEUTIC AGENT FOR THE TREATMENT OF PREECLAMPSIA

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Preeclampsia is a gestational hypertensive disorder which affects ~5-10% of pregnancies in the U.S., and is a leading cause of maternal and fetal morbidity. The only definitive intervention currently available is the delivery of the placenta. The etiology of the disease is rooted in impaired vascular remodeling during placental development, leading to placental hypoperfusion, and resulting hypoxia and ischemia. The placenta produces factors which lead to the maternal syndrome, namely anti-angiogenic agents like the VEGF antagonist sFlt-1, inflammatory factors, and reactive oxygen species. Recently we have investigated the potential of the protein heme oxygenase-1 (HO-1) as a therapeutic intervention in preeclampsia. In rodents, HO-1 induction markedly attenuated placental ischemia-induced hypertension through normalization of angiogenic balance and reduction of placental superoxide. We further demonstrated that HO-1 induction significantly inhibits hypoxia-driven sFlt-1 production in placental villi. Subsequent studies in sFlt-1 infused rats have indicated that HO-1 can normalize angiogenic balance independently of its ability to suppress sFlt-1 expression and that the terminal effector of its action in both molecules is to reduce vascular endothelin-1. Overall, these studies indicate that the HO-1 system is a potential target for the treatment of preeclampsia. This work was supported by NIH grants HL105324-01, HL108618-01, HL51971, HL088421, and HL088421-S1.

4.30

DIETARY GENISTEIN INDUCES SEX-DEPENDENT CARDIOVASCULAR EFFECTS IN MICE

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Despite the purported beneficial cardiovascular health effects of the phytoestrogen genistein, the effects of dietary exposure to genistein and an insight into its underlying mechanism(s) of action remain vague. The objective of this study was to examine the effects of a genistein-containing diet (600 mg/kg food, 600G) and a genistein-free diet (0 mg/kg, 0G) on cardiovascular parameters in C57B1/6J male and female mice. Mice were fed the diets for a period of 1 month, at which time tissues and serum were collected. Blood pressures were compared at the start and end of the study. In males,

600G increased serum levels of insulin (2.9±0.5 ng/dL, n=6), compared to 0G controls (1.8±0.4 ng/dL, n=6, P<0.05) and decreased glucose (177.5±15.9 mg/dL, n=6), compared to 0G controls (242.4±22.5mg/dL, P<0.05). No effects in females. In males, 600G decreased body weight (by ~2.7 g, n=6, P<0.05), with no change in females. In females, 600G (n=11) decreased systolic blood pressure (107.1±3.5 mmHg), pulse pressure (27.7±1.6 mmHg) and cardiac work (18.9±1.2 mmHg beats/min), compared to 0G (115.9±1.7 mmHg, 33.41±1.9 mmHg and 22.78±1.61 mmHg beats/min respectively, n=15, P<0.05). Aortic contractility was increased with 600G in both males and female isolated intact aorta (P<0.05). These data suggest that a one month diet with 600G has disparate beneficial effects on cardiovascular parameters in males and female mice. *Josh Martin was supported by the Midwestern University Summer Fellowship Program. Layla Al-Nakkash was supported by NIH (1R15DK071625-01A2).*

5.0 OBESITY AND CARDIOVASCULAR DISEASE

5.1
NOVEL PANCREATIC PEPTIDES CONTROL GLUCOSE HOMEOSTASIS AND APPETITE

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While the incidence of diabetes in the North American population does not display a chromosomal sex bias, a major cause of insulin resistance, obesity, does. Traditionally the effect of obesity on insulin sensitivity is studied at the level of glucose transport and insulin signaling. The causes of obesity are more diverse including diet, exercise, and partitioning of fuel sources. We have studied two of those potential causes: appetite regulation and the insulin response to ingested carbohydrates and focused on novel islet factors that may co-ordinate both. Nesfatin-1 is produced in beta cells of the pancreas and acts there to increase the insulin response to glucose. It is also produced widely in brain where it exerts potent, physiologically relevant anorexigenic actions. Neuronostatin, on the other hand is produced in delta cells of the pancreas and acts in a paracrine fashion on the glucagon-producing alpha cells. In an as yet to be determined mechanism those intra-islet effects of neuronostatin result in decreased insulin response to glucose. While the physiologic relevance of this action is not known, whole animal studies have con-firmed the in vitro findings. Like nesfatin-1, neuronostatin also potently inhibits food intake, acting via the central melanocortin system. Both nesfatin-1 and neuronostatin act in brain to alter autonomic function, suggesting a third mechanism by which these peptides might influence the appearance of increased obesity risk in females, versus males.

5.2
TRANSLATIONAL CARDIOVASCULAR BENEFITS OF EXENATIDE-PRECLINICAL AND CLINICAL EVIDENCE

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Exenatide (Ex) is a naturally-occurring GLP-1 receptor agonist that exhibits antidiabetic effects in type 2 diabetic patients via glucose-dependent insulinotropism, slowing of gastric emptying, glucagon suppression and reduced food intake. Animal studies support a role for GLP-1 and Ex in improving cardiac function, hemodynamics and survival. We have shown that Ex can reverse corticosterone-induced hypertension independent of changes in body weight in rats. Furthermore, we have recently reported that the Ex analog, AC3174, significantly improved animal survival in both DSS hypertensive rats, and in MI-induced CHF rats. Marked improvements in cardiac function/remodeling, hypertension, insulin sensitivity and renal function were evident following AC3174 treatment. These findings are now translating into the clinic in patients with Type 2 diabetes. In humans, Ex lowers systolic blood pressure in type 2 patients with mild to moderate obesity and blood pressure reductions were correlated with the degree of elevation at baseline. A recent database analysis of ~39,000 patients reported that Ex treatment was associated with a lower risk of CV events and hospitalizations than treatment with other glucose-lowering therapies. Ongoing five-year CV outcome studies with Ex examining the incidence of major adverse CV events will validate the relevance of these short term (6-12 month) improvements in CV function. Over-all, these described benefits of Ex may have important clinical implications regarding therapeutic choices for patients with type 2 diabetes and associated CV co-morbidities.

6.0 NEURO MECHANISMS AND DEPRESSION IN CARDIOVASCULAR DISEASE

6.1
BAROREFLEX FUNCTION IN FEMALES: CHANGES WITH THE REPRODUCTIVE CYCLE AND PREGNANCY

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In females, baroreflex function fluctuates with the reproductive cycle, with baroreflex sensitivity or gain (BRG) reaching its peak when gonadal steroids are elevated. The estrogen surge likely mediates the increase in BRG, since ovariectomy in rats abolishes the variations in BRG induced by the estrous cycle, while estrogen increases BRG. In contrast, normal pregnancy markedly impairs the baroreflex. Two aspects of the sigmoidal baroreflex relationships between arterial pressure and heart rate or sympathetic nerve activity are attenuated: the maximal level of heart rate or sympathetic activity when arterial pressure is lowered and the maximal slope of the most linear segment of the curve, an index of BRG. Increased brain levels of the neurosteroid, 3-alpha-hydroxy-dihydroprogesterone, contribute to the decreased baroreflex maximums. Recent data suggest that the decrement in BRG is mediated by insulin resistance and decreases in brain insulin. These findings include that 1) the decreases in insulin sensitivity and BRG are correlated during pregnancy; 2) treatment of pregnant animals with the insulin sensitizing drug, rosiglitazone, improves insulin sensitivity and BRG; 3) brain insulin levels are decreased in pregnant animals; and 4) intracerebroventricular infusion of insulin normalizes BRG in pregnant rats. In further experiments, the arcuate nucleus was identified as the brain site at which insulin increases BRG. Thus, during pregnancy, decrements in the levels or action of insulin in the arcuate nucleus may contribute to the impaired BRG. Review: Brooks VL, Dampney RAL & CM Heesch. Pregnancy and the endocrine regulation of the baroreceptor reflex. *Am.J.Physiol.* 299: R439-R451, 2010.

6.2
SEX DIFFERENCES IN DEPRESSION AND CARDIOVASCULAR DISEASE

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It has been long established that depression is an independent risk factor for cardiovascular disease (CVD) and that CVD is a risk factor for depression (Grippo & Johnson 2009). Three lines of evidence have been proposed to date in an effort to explain the comorbidity of CVD and mood, as well as anxiety disorders: (1) the epidemiological evidence for a causal role of depression in the evolution and progression of CVD; (2) the biological evidence for the plausibility of an etiologic role of depression in CVD; and (3) both depression and CVD are manifestations of a common underlying pathophysiological process (Rudisch & Nemeroff 2003). The goal of this presentation is to briefly review and synthesize the evidence for the above proposed explanations with a special focus on the roles of serotonin, platelets and the immune system. Sex and gender differences in both depression/anxiety and CVD and the relevance of these differences as they pertain to women will be emphasized. References: Grippo, A.J. & Johnson, A.K. 2009. Stress, depression and cardiovascular dysregulation: a review of neurobiological mechanisms and the integration of research from preclinical disease models. Stress 12, 1–21. Rudisch, B. & Nemeroff, C.B. 2003. Epidemiology of comorbid coronary artery disease and depression. Biol Psychiatry 54, 227–240.

6.3
MECHANISM OF THE SEX DIFFERENCE IN ENDOTHELIAL DYSFUNCTION AFTER STROKE

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We evaluated cerebrovascular eicosanoid signaling as a mechanism underlying the sexually dimorphic response to cerebral ischemia. Young adult female mice sustained smaller infarcts after middle cerebral artery occlusion (MCAO) compared to age-matched males. The difference in infarct size was associated with lower expression and activity of soluble epoxide hydrolase (sEH) in cerebral microvessels, and was abolished by sEH gene deletion and pharmacological inhibition. sEH is a key enzyme in the metabolic conversion and inactivation of neuroprotective and vasodilator eicosanoids called epoxyeicosatrienoic acids (EETs). Accordingly, EETs levels were higher in wild-type (WT) females vs. males and in sEH knockout (KO) vs. WT males. Protection in WT female vs. male and in sEHKO vs. WT male mice was associated with higher cerebrovascular perfusion. Similarly, transgenic (Tg) overexpression of P450 epoxygenase under the endothelial Tie2 promoter was protective against ischemic brain injury in male but not female mice, with higher cerebrovascular perfusion in Tg vs. WT male mice. Endothelium-dependent vasodilation was attenuated after MCAO in WT male mice and mice with endothelial-specific overexpression of sEH. Post-ischemic cerebrovascular endothelial dysfunction in male mice was rescued by sEH deletion in sEHKO mice. We conclude that the sex difference in ischemic brain injury after MCAO in mice is in part linked to higher endothelium-derived EETs and improved post-ischemic endo-thelial function in females. (R01 NS44313 & R01NS070837). Davis CM, Siler DA, Alkayed NJ. Endothelium-derived hyperpolarizing factor in the brain: influence of sex, vessel size and disease state. Womens Health (Lond Engl). 2011;7:293-303.

8.0 GENDER DISPARITIES IN CARDIOLOGY

8.1
WOMEN AND ISCHEMIC HEART DISEASE

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Sex differences in coronary heart disease (CHD) demonstrates that prevalence, symptom manifestation, and pathophysiology for CHD varies between women and men. Given the lower burden of obstructive coronary artery disease (CAD) and preserved systolic function in women contrasted by higher rates of myocardial ischemia and higher near-term mortality compared to men, the term *ischemic heart disease* (IHD) as more appropriate for this discussion specific to women, rather than CAD or CHD. This paradoxical sex difference where women have lower rates of anatomical cardiac disease but worsened symptoms, ischemia and outcomes appears to be linked to a sex-specific pathophysiology of coronary reactivity which includes microvascular dysfunction. For women with obstructive CAD, near-term risk (i.e., in-hospital through 30 days) is elevated for females compared to men, and while longer-term risk

management strategies are equally effective, women are less likely to receive guidelines-indicated therapies. For women with evidence of ischemia but no obstructive CAD, anti-anginal and anti-ischemic therapies can ameliorate symptoms, improve endothelial function, and quality of life, however trials to evaluate impact on adverse cardiac events are needed. Ongoing research using proposed models for application of emerging knowledge to clinical practice, as well as new hypotheses, such as sex-differences in stem cell therapy, are being tested. Continued research is indicated to devise therapeutic regimens to improve symptom burden and reduce risk in women with stable and unstable IHD symptoms.

8.2

PREGNANCY COMPLICATIONS PREDICT INCREASED RISK OF CARDIOVASCULAR DISEASE IN WOMEN: IS THIS USEFUL TO KNOW?

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More than 20% of women who have born children have experienced at least one common pregnancy complication associated with increased risk of cardiovascular disease in maturity: preterm delivery, hypertensive pregnancy, gestational diabetes, or fetal growth restriction. To date, most of the evidence linking pregnancy complications with CVD events has been derived from data linkage of statistical birth, hospital, and mortality registries. These registries lack information on CVD risk factors, such as family history and body mass index that may predate both pregnancy complications and CVD events. Most analyses have also lacked data to determine the extent to which pregnancy complications predict CVD events above and beyond traditionally measured risk factors such as hypertension, diabetes, and dyslipidemia. We will present data from the longitudinal national Nurses' Health Study II on the extent to which common pregnancy complications predict CVD events independent of known risk factors that have been recorded prospectively for 18 years in this cohort. We will discuss the extent to which a history of complex pregnancy may be useful to identify young women who might benefit from targeted screening and intervention to prevent future CVD events. Reference: Rich-Edwards JW, McElrath TF, Karumanchi A, Seely EW. (2010) Breathing life into the lifecourse approach: pregnancy history and cardiovascular disease in women. Hypertension; 56:331-4.

8.3

THE FEMININE FACE OF HEART DISEASE: WHAT DO WE KNOW ABOUT ANGINA IN WOMEN?

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Angina is the predominant initial and subsequent presenting symptom of coronary heart disease in women, in contrast to myocardial infarction and sudden death for men. Only recently appreciated are non-chest-pain symptoms of myocardial ischemia, termed anginal equivalents, including shortness of breath, fatigue and weakness, lightheadedness, diaphoresis, nausea, and indigestion; these are more common in women than in men, in older than in younger patients, and in diabetics than in non-diabetics. Myocardial ischemia in women and its symptomatic consequences may relate to macrovascular disease (obstruction of the epicardial coronary arteries) or microvascular disease; a combination of these 2 may be present. Despite a lessened severity of obstructive epicardial coronary artery disease at angiography, women have greater morbidity and mortality from angina. Treatment options include optimal medical therapy encompassing lifestyle and other risk factor interventions and pharmacotherapy, as well as myocardial revascularization. In summary, angina is highly prevalent in women, is multifactorial in etiology, and imparts considerable morbidity and lethality. It is suboptimally recognized and treated. Sex-specific basic and clinical research is warranted to enhance clinical outcomes. References: Wenger NK. Angina in Women. Curr Cardiol Rep 12:307-314, 2010.

9.0 CARDIOVASCULAR DISEASE AND INFLAMMATION

9.1

INFLAMMATION, IMMUNITY AND HYPERTENSION

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There is increasing evidence that inflammation, and in particular components of the adaptive immune response, contribute to hypertension. Stimuli such as angiotensin II, salt and emotional stress cause T cell activation and the accumulation of these cells in the perivascular tissues and the kidney. These cells release cytokines that contribute to NADPH oxidase activation, entry of other inflammatory cells, vasoconstriction and renal sodium absorption. We propose that these induce an inflammatory response that promotes a second wave of more severe and sustained hypertension. The nature of the mechanical trauma, the precise role of reactive oxygen species, the characteristics of the neoantigens and the ultimate effect of immune cells in hypertension still require substantial study, however these considerations raise the possibility of new therapeutic approaches to treat this common disease. References: Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, Goronzy J, Weyand C, Harrison DG. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J Exp Med*. 2007;204:2449-2460. Harrison DG, Guzik TJ, Lob HE, Madhur MS, Marvar PJ, Thabet SR, Vinh A, Weyand CM. Inflammation, immunity, and hypertension. *Hypertension*. 2011;57:132-140.

9.2

UNEQUAL IMMUNE CAPABILITIES BETWEEN MALES AND FEMALES: IMPLICATIONS FOR HEALTH AND AUTOIMMUNE DISEASES

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An intriguing long-standing experimental and clinical observation is that the immune system of females and males is different. There are innate sex differences in the levels of immunoglobulin (IgM), cytokines, and lymphocyte subsets, as well as in their ability to respond to antigens, infections and vaccines. Overall, the female immune system tends to be more reactive and responds better to antigens than their male counterparts. The provocative question is what is the biological relevance of sex differences in the immune system? Does the stronger immune defense capabilities in females may contribute to increased longevity of females? This female "immunological superiority" is not without consequences. The female immune system also reacts vigorously against it's own self-antigens. Consequently, a majority of autoimmune diseases occur predominately in females in both animal models as well as in humans. The precise underlying reasons for the physiological and pathological sex differences in immune system are a subject of intense investigations. Potentially, they can be distilled into three main possibilities: (i) sex hormonal, (ii) Sex Chromosomal, and (iii) epigenetic and environmental influences. Of these, perhaps the most studied is the effects of sex hormones on immune cells. These cells have receptors for sex hormones, which serve as ligand-activated transcription factors to differentially alter the functions of cells in health and disease. Males and females are likely to have dissimilar exposures to environmental agents and differentially respond to natural stresses. Therefore, it is imperative to include gender as a variable in all immune studies. Supported by NIH, Lupus Foundation of America, AARDA, IRC grants.

9.3

SEX DIFFERENCES IN INFLAMMATORY MEDIATORS

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Approximately 74 million Americans that have hypertension, yet less than two fifths achieve blood pressure control. One of the greatest challenges to increasing the percent of hypertensive patients with controlled blood pressure is lack of knowledge regarding the molecular mechanisms driving hypertension. There is a growing body of evidence to support a role for lymphocytes and inflammatory cytokines in the development and progression of hypertension in males. The role of inflammation in hypertension in females is unknown; however, women have a greater predisposition to develop autoimmune and inflammatory-based diseases compared to men. We have previously published that number of inflammatory cytokines are more highly expressed in urine and the mesenteric arterial bed of female spontaneously hypertensive rats (SHR) than in males. These data have lead us to hypothesize that hypertension in females is more dependent on an inflammatory responses than in males. Preliminary data using the lymphocyte inhibitor mycophenolate mofetil (MMF) supports this hypothesis, as female SHR have a greater decrease in blood pressure to MMF than males. Defining the role of inflammatory lymphocytes in both hypertensive males and females may lead to better blood pressure control in both sexes. References: Sullivan JC, Pardieck JL, Doran D, Zhang Y, She JX, Pollock JS. Greater fractalkine expression in mesenteric arteries of female spontaneously hypertensive rats compared to males. Am J Physiol 296:H1080-1088, 2009; Harrison DG, Guzik TJ, Lob HE, Madhur MS, Marvar PJ, Thabet SR, Vinh A, Weyand CM. Inflammation, immunity, and hypertension. Hypertension 57(2):132-40. 2011.

10.0 POSTER SESSION II

10.1

BONE MARROW-DERIVED ANGIOGENIC PROGENITOR CELLS ARE DYSFUNCTIONAL IN CHRONIC ANG II INFUSION RAT MODEL OF HYPERTENSION

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Introduction: Bone marrow (BM)-derived angiogenic progenitor cells (APCs) contribute to the repair of endothelial damage and thus play a key role in the maintenance of the vascular homeostasis. Therefore, their dysfunction has been implicated in the vascular pathophysiology of hypertension and cardiovascular disease. In view of this, we propose the following hypothesis: BM APCs are dysfunctional in the chronic rat model of angiotensin II (Ang II)-induced neurogenic hypertension and this is due to an imbalance in the vasoprotective and vasodeleterious axes of the renin-angiotensin system (RAS). **Methods**: SD rats infused with AngII (200ng/kg/min, *s.c.*) for 4, 6 and 12 weeks, and their age-matched SD controls were used as a rodent model for neurogenic hypertension. BM derived $CD90^+$ ($CD4/CD5/CD8^-$) cells were isolated from femur and tibia and their function was assessed by their ability to migrate and proliferate upon stimulation with growth factors. Additionally, the ratio of APC and inflammatory cells (ICs: $CD4.8^+$, $CD4.8^+/25^+$; $CD3^+/45^+$; $CD68^+$) in the blood and BM was determined by FACS. **Results**: Numbers of BM $CD90^+$ were decreased by 35% and 70% after 4- and 12- weeks of Ang II infusion. Similar decreases in their numbers were observed in the blood. This was associated with significant increases in BM and blood levels of ICs in hypertensive rats. Thus APC/IC ratio was decreased by ~70%. The ability of the BM $CD90^+$ cells to migrate in response to SDF was reduced by 70±5% at 6 weeks ($p<0.01$), and by 75±4% at 12 weeks ($p<0.01$). Furthermore,

proliferation of APCs was decreased by 40±3% at 6 weeks ($p<0.05$), and by 44±5% at 12 weeks ($p<0.05$) of Ang II infusion compared to control. **Conclusion**: These data demonstrate that the numbers of APCs in BM and the ratio of APC/IC in both blood and BM is significantly decreased in hypertension as a result of a decrease in APCs and concomitant increase in ICs. In addition, APCs in Ang II-induced hypertension are dysfunctional.

10.2
ANTIOXIDANT TEMPOL EXACERBATES PRESSOR RESPONSE TO STRESS IN MULTIPAROUS RATS

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Multiparous (MP) women are at increased risk for cardiovascular disease. We have previously shown that MP rats have a greater pressor response to stress than virgin rats, due to oxidative stress-induced endothelial dysfunction rather than to augmented sympathetic activation. We examined whether an antioxidant would normalize the response. MP rats and age-matched virgins were administered Tempol (1mM in drinking water, 8wks) and implanted with telemetric blood pressure and renal nerve activity devices. In response to stress (airjet 10 sec) there was a triphasic response: a small transient rise in blood pressure with return to baseline (0-1 sec), a marked transient pressor response with partial recovery (1.5-6.0 sec), and a slow decline (6.5-10 sec). Tempol augmented the second phase of the response in the MP rats (Tempol: 47.9±6.6 mmHg; Control 33.4±3.3 mmHg; $P<0.05$); this was associated with an attenuated reflex reduction in heart rate (Tempol: -22±10 bpm vs. Control: -118±36 bpm at 2.5 sec) and sympathetic nerve activity (Tempol: 4.8±0.4 spikes /sec vs. Control: 2.2±1.0 spikes/sec at 2.5 sec). No such phenomenon was observed in the virgin animals. We conclude that Tempol exacerbates the pressor response to stress in MP rats due to impaired reflex suppression of sympathetic outflow and heart rate. These data suggest that unanticipated central neural effects may limit the efficacy of antioxidants such as Tempol in reducing cardiovascular disease in at-risk individuals.

10.3
ESTROGEN RECEPTOR BETA-MEDIATED ANTI-INFLAMMATORY EFFECT OF DIHYDROTESTOSTERONE DURING CYTOKINE-INDUCED INFLAMMATION IN HUMAN BRAIN VASCULAR SMOOTH MUSCLE CELLS

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Vascular inflammation plays a key role in the etiology of cardiovascular disease, particularly stoke. Previous studies have demonstrated that sex steroids modulate vascular inflammation. Our recent studies show that, in human vascular smooth muscle cells, chronic treatment with the potent androgen dihydrotestosterone (DHT) decreases expression of the proinflammatory mediator cyclooxygenase-2 (COX-2) during cytokine-induced inflammation or hypoxia via an androgen receptor-independent mechanism. Since DHT can be converted to 3β-diol, an estrogen receptor (ER) β-specific agonist, we hypothesized that DHT would decrease IL-1β induced COX-2 expression in primary human brain vascular smooth muscle cells (HBVSMC) via conversion to 3β-diol and subsequent activation of ERβ. Expression of sex steroid receptors and metabolizing enzymes was confirmed in HBVSMC via quantitative PCR. Pre-treatment for 18h with either DHT (10nM) or its estrogenic metabolite 3β-diol (10nM) reduced IL-1β-in-duced increases in COX-2 expression. Pre-treatment with the AR antagonist bicalutamide (1μM) did not block the effect of DHT. Both the non-selective ER antagonist ICI 182,780 (1μM) and the selective ERβ -antagonist PHTPP (1μM) inhibited the effect of DHT. In conclusion, DHT appears to be protective against cerebrovascular inflammation via conversion to 3β-diol and subsequent activation of ERβ in human vascular smooth muscle cells. *Support: AHA RJG, AHA KLO, UA Sarver Heart Center Grant (KLO).*

10.4
HYPERTENSIVE DISORDERS DURING PREGNANCY PREDICT CARDIOVASCULAR EVENTS: CAN WE TRUST MATERNAL RECALL OF PREGNANCY COMPLICATIONS?

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Hypertensive disorders in pregnancy are risk factors for future maternal cardiovascular disease (CVD). Clinical assessment of history of pregnancy complication relies on maternal self-report, but the accuracy of recall is unclear. This review was conducted to assess the literature on maternal recall of pregnancy history, focusing on hypertensive disorders in pregnancy. Thirteen studies were identified with recall periods ranging from 48 hours to 30 years after delivery. Sensitivity was lower for gestational hypertension than for preeclampsia (17.0-85.0% vs. 51.6-87.0%). Sensitivity rose with increasing severity of preeclampsia. Specificity was high for all hypertensive disorders (90.9-100%). Positive predictive value was poor for preeclampsia (approximately 50% in 2 studies reporting). Determinants of recall accuracy included maternal education and parity. Qualitative research may be required to fully elucidate demographic and process factors resulting in poor recall. Future research should test recall of disorder subtypes, recurrent complications, and recall over time in the same population. The utility of other outcome variables for clinical identification of women whose pregnancy history puts them at increased risk of CVD should also be explored.

10.5
INFLAMMATION-INDUCED TLR4 EXPRESSION AND REACTIVE OXYGEN SPECIES ARE ATTENUATED BY DIHYDROTESTOSTERONE IN HUMAN PRIMARY VASCULAR SMOOTH MUSCLE CELLS

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Cyclooxygenase-2 (COX-2) plays a role in vascular inflammation and has been implicated as a source of reactive oxygen species (ROS). We have shown that the androgen, dihydrotestosterone (DHT), attenuates endotoxin and hypoxia with glucose deprivation (HGD)-induced COX-2 in human vascular smooth muscle (VSM) cells. In rats, androgens have been shown to be protective during cerebral ischemia and to decrease arteriole oxidative stress. Because toll-like receptor 4 (TLR4) has been implicated in injury-induced inflammation, we hypothesized that DHT alleviates inflammation by attenuating TLR4 expression and decreasing ROS during inflammation or hypoxia in human VSM. TLR4 expression was detected via immunocytochemistry and Western blot, and ROS production was measured via the indicator dye carboxy-H_2DCFDA in human VSM cells. TLR4 was detected in the cytosol and nucleus. Endotoxin, hypoxia, and HGD all increased TLR4 expression compared to controls. DHT inhibited endotoxin (LPS) and HGD-induced TLR4 expression. Furthermore, DHT's effect during OGD was androgen receptor independent. Cytokine (IL-1β) increased ROS compared to vehicle, and DHT attenuated this response. These findings demonstrate that androgens may provide protection against inflammation in VSM under a variety of pathophysiological conditions in part by decreasing TLR4 expression and ROS production. *Sarver Heart Center (RT & RG), AHA (RG & KO).*

10.6 Withdrawn.

10.7
TESTOSTERONE INDUCES LEUKOCYTE MIGRATION BY COX2 AND NADPH OXIDASE-DEPENDENT PATHWAYS

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Mechanisms whereby Testosterone (T) exerts vascular effects remain unclear but inflammatory processes (IP) and reactive oxygen species (ROS) may be crucial. We investigated whether T induces leukocyte migration (LM) via NADPH oxidase (NADPHox) or COX2 activation. Wistar rats were treated with vehicle, sodium salicylate (SS, COX inhibitor, 0.2g/Kg, 1h), flutamide (Flu, androgen receptor blocker, 10μM, 4h), apocynin (Apo, NADPHox nonspecific inhibitor, 30mM, 4h) or NS398 (NS, COX2 inhibitor, 1mM, 4h) and then received T (10^{-7}M, 24h, i.p.). T plasma levels were assessed by ELISA, LM and ROS formation [dihydroethidium fluorescence (DHE-F)] by intravital microscopy, expression of NADPHox subunits and COX2 by immunoblot and NADPHox activity in membrane-fraction DHE-F. T plasma levels increased ($n=10$, $p<0.05$) 1 fold. Flu, Apo or NS had no effect on T levels. T enhanced LM ($n=12$; 4.1±0.2 (number of migrated leukocytes/2.500μ^2 after 24h) *vs* 1.5±0.1, $p<0.05$), an effect blocked by Flu (1.9±0.3), SS (2.4±0.4), NS (0.9±0.2) and Apo (0.9±0.1) ($n=6$; $p<0.05$). T did not alter COX2 expression ($n=4$), but enhanced ROS formation, expression of p22phox, NOX1, NOX2, p47phox and NADPHox activity (0.5 fold, $p<0.05$, $n=6$). These effects were blocked by Flu, Apo and NS ($p<0.05$, $n=5$). Our data show that T induces LM via COX2- and NADPHox-dependent pathways in which COX2 may be upstream NADPHox. T may contribute to IP and oxidative stress associated with cardiovascular diseases. FAPESP/CNPq.

10.8
SEX-DEPENDENT IMMUNE-PROTECTION WITH MINOCYCLINE AFTER EXPERIMENTAL EMBOLIC STROKE

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Stroke is a sex dimorphic disease with males and post-menopausal females having worse outcomes than young females. Growing data suggest that stroke induces peripheral immunosuppression. Recent studies showed that minocycline provides neurovascular protection. However, the role of sex in minocycline neurovascular protection is still unclear. We tested the effects of minocycline on brain injury and the peripheral immune system in both sexes using an embolic stroke model that closely mimics ischemic stroke in humans. Methods: Stroke was initiated by injection of a thrombus to the right middle cerebral artery of male and ovariectomized female mice (C57BL/6, 24±4 weeks old). Minocycline was given intravenously at stroke onset. At 24 hours, the animal brains, spleens and blood hematology were analyzed. Results: Cerebral embolization induced the highly reproducible infarct in males (43±11%) and

females (35±12%) determined as percentage to the contralateral hemisphere. The brain injury caused peripheral immunosuppression that resulted in similar reduction of spleen size (P<0.01) and peripheral lymphocytes (P<0.01) in both genders. Minocycline reduced infarct size both in males (56%, P<0.01) and females (69%, P<0.001) and significantly reduced mortality in females (P<0.05). However, unlike in males, in females minocycline prevented spleen shrinking (7% reduction vs. 42%, P<0.0001) and reduced the depletion of peripheral lymphocytes (77% reduction vs. 93%, P<0.05). Conclusion: Our study observed sex disparities in modulation of the immune response by minocycline. Further studies of neuro-protective minocycline mechanisms are warranted.

10.9

ESTROGEN PROMOTES CARDIAC STEM CELL (CSC) PARACRINE ACTION AND THUS FACILITATES CSC-MEDIATED PROTECTION FOLLOWING HYPOXIA

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Our group has previously indicated that gender differences exist in stem cell (SC) growth factor production with higher levels in female SCs compared to males following toxic stimuli. In addition, estrogen has been shown to improve SC-mediated cardioprotection. However, there is no information regarding estrogen in modulation of cardiac stem cell (CSC) function. Therefore, the study *objective* here is to investigate the role of17β-estradiol (E2) in CSC production of protective factors, and in CSC-mediated paracrine protection. To study this, CSC were isolated from mouse hearts. Flow cytometry data revealed that CSC expressed Sca-1, CD29 and CD44 (all >90%), but little CD31, CD34 or CD45. Cardiac specific transcription factors NKX2.5, Gata4, MEF2c and Tbx5 were detected in CSC by using mRNA-qPCR. E2 did not induce VEGF and HGF secretion in CSC, whereas E2 increased CSC-derived SDF-1 in a dose dependent manner using ELISA. To elucidate E2-induced SDF-1 in CSC-mediated paracrine protection, conditioned medium (CM) from CSC or E2-treated CSC were applied to myocytes (H9c2) following hypoxia (1% O2) with or without AMD3100, a SDF-1 receptor inhibitor. We observed that E2-treated CSC CM exhibited greater protection in myocyte survival compared to non-treated CSC CM following hypoxia. However, this increased protection was impaired by using SDF-1 receptor blocker. Conclusion: E2-treated CSC enhanced SDF-1 production and thus further promoted myocyte survival in response to hypoxia.

10.10

ESTROGEN SPECIFIC REGULATION OF ENDOTHELIAL HOMOCYSTEINE AND VASCULAR FUNCTION

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Plasma level of homocysteine (Hcy) is inversely proportional to circulating level of estrogen that imparts a cardiovascular protective effect. The role of estrogen in vascular Hcy synthesis however, has not yet been explored. To test the hypothesis that intracellular production of Hcy contributes significantly to vascular function via an estrogen-dependent mechanism, female rats were ovariectomized (OV) and given estrogen replacement (OVE) for three weeks. Mesenteric arteries were isolated, cannulated and then continuously perfused with 30 µl of 1X Laemmli sample buffer with 2-mercaptoethanol for two minutes, followed by collecting endothelial (EC) lysates for the isolation of EC protein. Western blotting shows that vessels of OV rats exhibited greater endothelial Hcy level, associated with an upregulation of Hcy receptor, namely N-methyl-D-aspartase receptor (NMDAR), compared to vessels of OVE rats. The altered endothelial Hcy and NMDAR expression in OV vessels were paralleled by a reduced expression of cystathionine β-synthase (CBS), a key enzyme responsible for the Hcy metabolism in vasculatures, all of which was prevented by estrogen replacement therapy. Moreover, perfusate NO in vessels of OVE was greater than in those of OV rats. All of these results were independent of plasma Hcy, confirming that estrogen-specific modulation of vascular Hcy synthesis via the intracellular regulation of Hcy signaling potentiates endothelial NO synthesis and vascular function. (Supported by NIH HL070653 and HL43023).

10.11

20-HETE-INDUCED VASCULAR REMODELING IN THE MODEL OF ANDROGEN-INDUCED HYPERTENSION

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20-Hydroxyeicosatetraenoic acid (20-HETE) is a cytochrome P450-derived arachidonic acid metabolite and a vasoconstrictor. It has been shown to increase smooth muscle contractions and proliferation, stimulate endothelial dysfunction and activation and promotes hypertension. We examine if 20-HETE con-tributes to vascular remodeling in androgen-induced hypertension. SD rats were treated with 5a-dihydrotestosterone (DHT) or vehicle with and without a 20-HETE synthesis inhibitor (HET0016) for 14 days. Media-to-lumen ratio of renal interlobar arteries from DHT-treated rats was higher than vehicle-treated rats (0.21±0.01 vs 0.15±0.02). This was prevented by HET0016 (0.14±0.01). Interlobar arteries from rats treated with DHT had increased collagen type IV as compared to vehicle-treated rats. HET0016 attenuated the increase in collagen type IV. To assess whether 20-HETE induces vascular remodeling independent of blood pressure (BP) increase, reserpine (100 µg/kg) was administered to DHT- and vehicle-treated rats for 21 days. DHT increased BP (133.7±2.4 vs 101.4±0.4 mmHg). Reserpine prevented DHT-mediated BP elevation (104.2±1.3 mmHg). Media-to-lumen ratio of renal interlobar arteries increased in DHT-treated rats as compared to vehicle-treated rats (0.20±0.02 vs 0.16±0.01). Reserpine had no effect (0.19±0.02). These results suggest that DHT-mediated vascular remodeling is 20-HETE-dependent. Whether 20-HETE contributes to BP independent of vascular remodeling is still unclear.

10.12

SEX DIFFERENCE IN VASCULAR RESPONSE TO ATRIAL NATRIURETIC PEPTIDE IN SPONTANEOUSLY HYPERTENSIVE RATS

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The aim was to investigate vascular effects of chronic infusion with atrial natriuretic peptide (ANP) in spontaneously hypertensive rats (SHR), look for sex differences. Methods: 10 weeks-old male (M) and female (F) SHR were infused (14 days, osmotic pumps) with ANP (100 ng/hr.rat) or saline (S).Systolic blood pressure (SBP) and nitrites and nitrates excretion (NOx,nmol/min.100g) were determined. Morphological studies were performed in thoracic aorta sections stained with haematoxylin-eosin (media/lumen ratio,AML,µm/mm) and fibrosis signs with Sirius Red (ASR, score). Aortic NOS activity ^{14}C L-arginine, pmol/g.tissue.min) was determined. Results:

	SBP	NOx	NOS	AML	ASR
M S	183±4	2.4±0.2	264±8	74.1±2.3	3.2±0.2
M ANP	171±4*	3.6±0.3*	353±6*	65.7±1.9*	3.1±0.1*
F S	170±3*	3.2±0.2*	301±4*	65.6±2.5*	2.7±0.4*
F ANP	162±3#	4.3±0.4#	410±3#	62.9±3.6	1.5±0.3#

*p< 0,01 vs M S; #p<0,01 vs F S. Two-way ANOVA, Bonferroni's post-hoc test.

ANP treatment decreased SBP in rats of both sexes. Vascular early fibrosis was higher in M than F SHR rats. ANP chronic treatment induced an increase in NO system activity and modified aorta remodeling only in males rats, probably improving aortic wall properties. Funding source: CONICET-UBA, Argentina.

10.13

SEX DIFFERENCES IN DOWNSTREAM TGF-BETA SIGNALING IN THE ARTERIES OF SPONTANEOUSLY HYPERTENSIVE RATS

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Recent evidence suggests that the protein, TGF-β has a role in the pathogenesis of vascular damage often associated with hypertension. TGF-β is known to exert inflammatory affects by signaling through the Smads and MAPK pathway. Our lab recently showed that female SHR have higher urinary secretion of TGF-β compared to male SHR, therefore the goal of this study was to determine if there were sex differences in the downstream TGF-β signaling molecules within the vasculature. To test this hypothesis, arteries were isolated from 12-14 week old female and male SHR. TGF-β protein expression was measured in arteries by immunoblotting and expression was significantly higher in arteries from female SHR compared to the male SHR (0.92±0.35 vs 0.19±0.04 relative densitometry units (RDU), p<0.05). Next, we investigated whether sex differences in TGF-β signaling proteins were present in the vasculature of SHR. TGF-β receptor II protein expression was significantly less in arteries from female SHR compared to male SHR (3.4±0.9 vs 9.6±1.8 RDU, p<0.05). We also found that phospho-Erk1/2 ex-pression was less in arteries from female SHR in comparison to male SHR (10.2±1.9 vs 29.7±5.0 RDU, p<0.05), while phospho-Smad2/3 tended to be more highly expressed in arteries from female SHR (1.1±0.3 vs 0.4±0.1 RDU). These findings might implicate a sex difference in the contribution of inflammation to the maintenance of hypertension in SHR.

10.14

GENDER DIFFERENCES IN THE INTERGENERATIONAL TRANSMISSION OF HYPERTENSION ASSOCIATED WITH UTEROPLACENTAL INSUFFICIENCY IN RATS

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Intrauterine growth restriction increases risk of disease, particularly in male offspring, with recent evidence for transmission to subsequent generations. We determined whether growth restriction, nephron deficits, hypertension and renal dysfunction are transmitted to the next generation. Late gestation uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted, R) or sham surgery (Control, C) in WKY rats. At 4mo, Restricted and Control female offspring (F1) were mated with normal males. Second generation (F2) fetal weight and nephron number (E20) were determined. In a separate cohort, F2 male and female offspring were aged to 6mo for blood pressure (tail-cuff) and 24h renal excretion. A cohort of F2 males were aged to 12mo for renal function (^{3}H-inulin and ^{14}C-PAH clearance) measures. F2R male and female fetuses were smaller than F2C (P<0.05). F2R male fetuses had fewer nephrons (P<0.05); female analysis ongoing. At 6mo, F2R males had increased systolic blood pressure compared with F2C (+15mmHg; P<0.05), while not different amongst females. Renal excretions and function were not different between F2C and F2R, but males had greater protein excretion than females (P<0.05, >3-fold). We provide novel evidence for intergenerational transmission of fetal growth restriction (males and

females) and nephron deficits (confirmed in males), with elevated blood pressure developing only in males. Funding: Heart Foundation of Australia and March of Dimes.

10.15

EXPOSURE OF NEONATAL FEMALE, BUT NOT MALE MICE TO TESTOSTERONE PROMOTES ANGIOTENSIN II-INDUCED ABDOMINAL AORTIC ANEURYSMS

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Objective: We previously demonstrated that administration of testosterone to adult female mice promotes angiotensin II (AngII)-induced abdominal aortic aneurysms (AAAs). In this study, we quantified effects of testosterone administered to neonatal female versus male mice on adult susceptibility to AngII-induced AAAs. *Methods and Results*: Female Ldlr-/- mice were administered a single dose of testosterone (100 µg/mouse) or vehicle at 1 day of age. In separate studies, neonatal male Ldlr-/- mice were administered a higher dose (400 µg/mouse) of testosterone using the same study design. At 12 weeks of age, female and male mice were infused with AngII (1,000 and 750 ng/kg/min, respectively) for 28 days. In females, neo-natal testosterone administration increased the formation of AngII-induced AAAs (from 30 to 64%). Serum testosterone concentrations in adult females were not significantly influenced by neonatal testosterone administration. In contrast, administration of testosterone to neonatal males had no effect on AngII-induced AAAs (vehicle: 50%; testosterone: 62%). *Conclusions*: These results demonstrate that female mice respond to neonatal testosterone administration to markedly promote increased adult susceptibility to AAAs. This effect was independent of elevated serum testosterone concentrations in adult females. Future studies will define mechanisms for differing sensitivity of neonatal females compared to males in promotion of AngII-induced AAAs. Funding: NIH P01 HL080100.

10.16

ACE2 DEFICIENCY IS ASSOCIATED WITH IMPAIRED GESTATIONAL WEIGHT GAIN AND FETAL GROWTH RESTRICTION

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Angiotensin converting enzyme 2 (ACE2) is a key enzyme of the renin-angiotensin system that influences the relative expression of angiotensin (Ang) II and Ang-(1-7). Although ACE2 expression increases in normal pregnancy, the impact of ACE2 deficiency in pregnancy is unknown. Gestational body weight gain was lower in the ACE2 knockout (KO) mice compared to Wild type (C57Bl/6) (WT) mice (30.3 ± 4.7 vs 38.2 ± 1.0 g, $p<0.001$) at day 18 of gestation. Fetal weight (0.94 ± 0.1 vs 1.24 ± 0.01 g, $p<0.01$) and length (19.6 ± 0.2 vs 22.2 ± 0.2 mm, $p<0.001$) were less in KO. Mean blood pressure (MBP) was higher in virgin KO as compared to virgin WT (86 ± 2 vs 76 ± 1 mmHg, $p<0.01$) and did not change with pregnancy in either WT or KO. Cardiac output significantly increased with pregnancy (20.8 ± 1.6 vs 25.3 ± 2.7 ml/min (WT) and 20.2 ± 1.3 vs 25.3 ± 2.8 ml/min (KO), $p<0.05$). Plasma Ang-(1-7) was reduced in pregnant KO. Placenta Ang II levels were higher in KO (52.9 ± 6.0 vs 22.0 ± 3.3 fmol/mg protein, $p<0.001$) and negatively correlated with maternal weight and total fetal weight. Renal Ang II levels were greater in KO vs WT virgin mice ($p<0.001$). There was no difference in angiotensin levels in KO vs WT fetal membranes, uterus, or heart. These results suggest that ACE2 deficiency and associated elevated placenta Ang II and reduced plasma Ang-(1-7) levels impact pregnancy by impairing gestational weight gain and restricting fetal growth.

10.17

SEEKING THE MECHANISMS OF ACTION FOR INTRAVENOUS DEXAMETHASONE TO BENEFIT PATIENTS WITH HELLP SYNDROME: THE SMASH STUDY

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Administration of dexamethasone to HELLP syndrome patients (10 mg IV every 12 hours) shortens disease course and reduces maternal morbidity associated with this severe form of preeclampsia. However, the pathophysiologic mechanisms involved with this intervention remain unclear. This study assessed the potential role of IV dexamethasone to restore the imbalance among anti-angiogenic and inflammatory factors known to be significantly elevated in women with HELLP syndrome. Women (N=11) diagnosed with HELLP syndrome, diagnosed as having at least two of the following parameters: 1) Platelet count 150,000/l, 2)Hepatic transaminase elevation 70 IU/L, 3)LDH 600 IU/L, who delivered at UMC were included in this IRB-approved study. Blood was obtained and analyzed at baseline and prior to third IV dose (24 hours) by ELISA (R&D Systems) for SFlt-1, Interleukin-6, and sEndoglin. Results reaching statistical significance of $P<0.05$ are reported. Twenty four hours post dexamethasone administration, sFlt-1 decreased by 50% from 4,681 + 822 pg/ml to 2,326 + 493 pg/ml ($P<0.002$), interleukin 6 decreased from 77 + 4 to 54 + 5 pg/ml ($P<0.005$), and sEndoglin decreased from 61 + 5 to 39 + 6 pg/ml ($P<0.006$). Therefore we conclude that one important mechanism of dexamethasone administration is to blunt the release of both anti-angiogenic and inflammatory factors suggested to play role in the patho-physiology of HELLP syndrome.

10.18

A NOVEL REPRODUCTIVE HORMONE, COSPEPTIN

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A bioinformatic search of the human genome for mRNAs encoding previously unidentified peptide sequences that are evolutionarily conserved and contain both a signal peptide for secretion and dibasic cleavage sites for liberation of small peptides, identified a previously undescribed peptide we named cospeptin. Mass spectrometry analysis revealed that endogenous cospeptin, isolated from rat hypothalamus, was likely a 20 amino acid, C-terminally amidated peptide. Immunoreactive cospeptin was detected in several tissues, most abundantly in hypothalamus. Labeled cospeptin exhibited high specific binding to pituitary and ovary, and i.p. injection of cospeptin led to the induction of early gene expression in pituitary gonadotrophs. In dispersed rat pituitary cells, cospeptin potentiated the action of GnRH to stimulate LH release and upregulated LH and FSH Beta, and GnRH receptor messages. Cycling female rats injected i.c.v. with siRNA directed against cospeptin on diestrous days 1 and 2, had reduced levels of cospeptin message in the hypothalamus, a delay in the appearance of the subsequent estrus, and a decrease in GnRH receptor message in the pituitary, suggesting that endogenous cospeptin "sensitizes" pituitary gonadotrophs by upregulating the transcription of the GnRH receptor gene. We have discovered a novel reproductive hormone, cospeptin, which acts in the pituitary to regulate expression of the GnRH receptor, and therefore reproductive hormone secretion.

10.19

AT THE HEART OF THE MATTER

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On June 21st, 2011 the Society of Women's Health Research (SWHR) and Women Heart in collaboration with leading experts in women's heart health released the 10Q Report on Capitol Hill. The 10Q re-port contains 10 unanswered questions to guide future research to improve detection, diagnosis and treatment for women at risk of or with cardiovascular disease (CVD). The 10 questions and subsequent recommendations for initiatives related to science, policy and education, were created by clinicians and basic scientists, collectively known as the 10Q Working Group. The 10Q Report recommends that researchers explore biological differences between women and men in the presentation, pathogenesis, diagnosis, and treatment of cardiovascular disease, in addition to the effects that reproductive history and psychosocial issues have on women's heart health. Policymakers are urged to increase funding for sex specific research and require sex-specific reporting of research studies, and clinicians and educational institutions are called upon to disseminate information about accurately recognizing symptoms of cardio-vascular disease in women. In addition to these challenges, there is a growing urgency for women and minorities to participate in clinical trials. Increasing women and minority participation in clinical studies will allow for valid analyses of sex differences in the presentation of and treatment effects of cardiovascular disease.

10.20

HYPERTENSION IN MICE WITH THE CHRONIC INFLAMMATORY DISEASE SYSTEMIC LUPUS ERYTHEMATOSUS IS NOT SALT-SENSITIVE

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Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disorder that primarily affects women during reproductive years. The prevalence of hypertension in patients with SLE is very high, reaching up to 74% depending on the cohort. Previous work of others shows a strong immune/inflammatory component to the development of salt-sensitive hypertension. Therefore, we tested whether blood pressure is salt-sensitive using an established mouse model of SLE (female NZBWF1 mice) with hypertension. Thirty-week old SLE and control mice (NZW) were fed 8% high salt (HS) diet or normal diet (0.4% salt) for 4 weeks. Plasma levels of dsDNA auto-antibody, a hallmark of SLE (measured by ELISA), was increased in SLE mice compared to controls (1e5±3e4 vs. 5e4±3e4 U/mL; all $p<0.05$). HS did not alter dsDNA auto-antibody in SLE (1e5±1e4) or control mice (6e4±2e4). Blood pressure (measured by arterial catheter in conscious mice) was increased in SLE mice compared to controls (130±3 vs. 117±1 mmHg). HS did not significantly alter blood pressure in SLE (136±3) or control mice (119±2). At 34 weeks, 43% of SLE mice fed HS diet showed positive albuminuria (>100 mg/dL measured by dipstick) compared with 33% of SLE mice fed normal salt diet. No control mice developed albuminuria throughout the study. In conclusion, these data suggest that in SLE blood pressure is not salt-sensitive. Supported by AHA Postdoctoral Fellowships 4350019 (KWM) and 2260874 (MVP), as well as NIH grants HL085907 (MJR), HL092284 (MJR), HL085907S1 (MJR), and HL051971 (UMMC-Physiology).

10.21

FEMALE SHR MAINTAIN HIGHER LEVELS OF ANG (1-7) THROUGH ENHANCED LEVELS OF RENAL CORTICAL ACE 2 ACTIVITY DURING CHRONIC ANG II INFUSION

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Our lab has previously published that female Spontaneously Hypertensive rats (SHR) have greater expression of Ang (1-7) in the renal cortex compared to males, both under control conditions and following Ang II infusion. The molecular mechanism(s) responsible, however, remain unknown. The goal of this study was to measure the enzymatic activity of the key enzymes involved in the generation of Ang (1-7) in control and Ang II-infused (200ng/kg/min, 14 days via osmotic minipump) 14-week-old male and female SHR. Results showed that angiotensin converting enzyme (ACE) 1 activity was not significantly altered by Ang II infusion in female (control: 34±6 vs. Ang II: 47±10 RFU/mg protein, N.S.) or male SHR (control: 37±5 to Ang II: 37±15 RFU/mg protein, N.S.). In contrast, ACE 2 activity was increased in Ang II treated female SHR compared to controls (14± 2 vs. 7±1 RFU/mg protein, respectively, $p<0.05$), with no effect of chronic Ang II on ACE 2 in males (control: 14±3 vs. Ang II: 14±3 RFU/mg protein $p<0.05$). Neprilysin activity was also measured, and significantly decreased in female SHR with Ang II (control: 300±7 vs. Ang II: 264±7 RFU/mg protein $p<0.05$), while males showed no significant change following treatment (control: 316±7 vs. Ang II: 315±7 RFU/mg protein $p<0.05$). Based on our data, we hypothesize that enhanced ACE2 activity in the presence of Ang II maintains greater Ang (1-7) levels in the renal cortex of female SHR as compared to males.

10.22

EXERCISE TRAINING BEFORE AND DURING PREGNANCY IMPROVES ENDOTHELIAL FUNCTION AND STIMULATES CYTOPROTECTIVE AND ANTIOXIDANT PATHWAYS IN THE PREGNANT RAT

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While exercise during pregnancy is generally recommended and thought to be beneficial to mother and fetus, the metabolic and cardiovascular adaptations to exercise during pregnancy remain poorly understood. We hypothesized that exercise training during pregnancy would increase angiogenic factors such as vascular endothelial growth factor (VEGF) and cytoprotective molecules such as heat shock proteins (HSPs) and improve vascular function. Female rats exercised voluntarily on an activity wheel for 6 weeks prior to and during pregnancy. Running time and distance were monitored weekly and samples collected on day 19 of pregnancy. Western blots expressed relative to β-actin, showed placental HSP 27 (3.7 ± 0.36 *vs.* 2.2 ± 0.38), 32 (0.14 ± 0.01 *vs.* 0.07 ± 0.01), 60 (2.2 ± 0.73 *vs.* 0.49 ± 0.08) and 90 (0.33 ± 0.09 *vs.* 0.11 ± 0.02) and plasma VEGF by ELISA (1046 ± 110 *vs.* 843 ± 54 pg/ml) were increased ($P<0.05$) in exercise trained rats compared to controls. Vascular endothelial function as determined by wire myography was improved ($P<0.05$) by exercise training compared to non-exercise controls. These data indicate chronic exercise training stimulates HSP and VEGF expression and this is associated with enhanced vascular function during late gestation. Increased expression of HSPs as a result of exercise could provide a preconditioning effect that may be protective against cellular damage in the placenta during pregnancies complicated with impaired placental perfusion and hypertension.

10.23

RELATIONSHIPS BETWEEN BODY MASS CATEGORY AND SYSTOLIC BLOOD PRESSURE IN RURAL ADOLESCENTS

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Ethnic/racial minorities appear to be more susceptible to the effects of elevated body mass on systolic blood pressure (SBP); however, this relationship is not well defined for adolescents from ethnic minority groups. To further examine this relationship changes in SBP as a function of body mass category (normal weight, overweight and obese) and as a function of blood pressure category (normal, pre-elevated and elevated blood pressure) were examined in adolescents (15 ± 0.1 yrs; n = 893 males, 842 females) of mixed ethnicities (Whites, Hispanics, Asian/Hmong and Blacks). Independent of ethnicity, mean SBP was 9% greater in males (124 ± 1 mm Hg) than females (113 ± 1 mm Hg). Mean BMI was positively and significantly correlated with mean SBP in all groups and genders regardless of how SBP was assessed (as either a function of body mass category or blood pressure category). When examining the BMI-SBP correlation as a function of body mass category, regression slopes were consistently less than 1; however, when examining the correlations as a function of blood pressure category, the slopes were greater than 2.5 suggesting that the impact of BMI on SBP is much greater when the relation is assessed as a function of blood pressure category. Odds ratio analysis reveals that obese males had 2- and 3.5-fold greater odds of developing pre-elevated and elevated SBP, respectively, than their normal weight cohorts. Similarly obese females had 4- and 9-fold greater odds of developing pre-elevated and elevated SBP, re-spectively. This suggests that obese females are at a higher risk for developing hypertension-related cardiovascular problems than obese males.

10.24

ANGIOTENSIN II INCREASES AROMATASE AND ANDROGEN RECEPTOR EXPRESSION IN CORONARY VASCULAR SMOOTH MUSCLE CELLS BUT NOT CARDIAC MYOFIBROBLASTS

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While many studies have focused on circulating systemic levels of sex steroids, there has been less appreciation for the local production and action of these hormones in the cardiovascular system during normal physiology and pathophysiology. Since sex steroids have been implicated in the outcome of cardiovascular disease, we investigated whether aromatase or androgen receptor (AR) expression was modified in human coronary vascular smooth muscle cells (HCVSM) and rat cardiac myofibroblasts (RCM) following stimulation with angiotensin II (AngII). Primary HCVSM (passage 8) and RCM (passage 0) were grown to 80% confluence and treated with AngII (24 hr; 10-7M). We demonstrate that AngII increased expression of both aromatase (77%) and androgen receptor (AR: 66%) in HCVSM, but not in RCMs. It has been shown that androgens may work in concert with AngII for some cardiovascular parameters, therefore additional experiments were performed in RCM with co-administration of testosterone (10nM). Testosterone did not have any impact on the effect of AngII on aromatase or AR expression in RCM. In conclusion, the presence of aromatase and AR in these two cell types suggests a possibility for circulating testosterone to act locally in an androgenic and/or estrogenic fashion. However the functional consequence of upregulation of both estrogenic (aromatase) and androgenic (AR) systems in HCVSM following AngII stimulation remains to be determined. Sarver Heart Center (TH) and AHA (RG).

10.25

EXERCISE INCREASES SOLUBLE VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-1 (SFLT-1) IN THE CIRCULATION OF ADULT WOMEN

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Physical inactivity increases the risk of several different cancers, including breast cancer. Soluble fms-like tyrosine kinase-1 (sFlt-1) inhibits the activities of VEGF and ovarian tumor growth. The present study tests the hypothesis that exercise can increase sFlt-1 in the circulation of adult women. 63 African American and Caucasian adult woman volunteers aged 18-44 were enrolled into a prospective exercise study. All the participants walked on a treadmill for 30 minutes at a moderate intensity (40-60% heart rate reserve), and oxygen consumption (VO2) was quantified by utilizing a metabolic cart. Blood samples were collected before and immediately after exercise. The plasma concentrations of sFlt-1, unbound VEGF, and endostatin were measured using ELISA. Plasma levels of sFlt-1 were 67.8±3.7 pg/ml immediately after exercise (30 minutes), significantly higher than basal levels of 54.5±3.3 pg/ml before exercise ($P<0.01$; n=63). There was no significant difference in plasma levels of endostatin before (92.4±4.4 ng/ml) and immediately after (93.8±4.4 ng/ml; P=0.8216) exercise. The basal plasma levels of unbound VEGF (21.5±4.3 pg/ml) ware similar to the plasma levels of VEGF (22.5±4.6 pg/ml; P=0.8652) immediately after exercise. Our study is the first to show that exercise in adult women significantly increases plasma levels of sFlt-1. Exercise-induced plasma levels of sFlt-1 in women could be an important clinical biomarker to explore the mechanisms of exercise training in reducing breast cancer progression.

10.26

INTERACTIONS BETWEEN THE ESTROGEN RECEPTOR (ER) AND HEME OXYGENASE (HO-1) CONTRIBUTE TO THE REGULATION OF BLOOD PRESSURE (BP) IN FEMALE RATS

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Estrogen protects females against the development of hypertension (HTN). While the mechanisms remain unclear, recent data suggests that ERα activation may induce HO-1, which in turn, may prevent HTN and protect against renal injury. We tested whether blocking the ERα in the presence of HO-1 inhibition induces HTN and renal damage. Female SD rats were randomized in 4 groups; 1) Vehicle (Veh), 2) HO-1 inhibition (SnPP, 30ug ip every 3 days), 3) ERα antagonist (Fulvestrant [FVT], 10mg/kg every 7 days), 4) FVT+SnPP. BP was monitored by tail cuff for 14 days. After 14 days, Cortical [C-RBF] and Outer Medullary Renal Blood Flow [OM-RBF] were measured, urine was collected to measure endo-thelin-1 (ET-1), and renal tissue was harvested to evaluate renal damage (KIM-1) and NOX activity (an index of oxidative stress). Treating the rats with either FVT or SnPP did not alter any of the measured parameters. However, blocking ERα and HO-1 simultaneously, raised BP, impaired renal hemodynamics (lower C-RBF and OM-RBF) and increased KIM-1, NOX activity and urinary ET-1.(*$p<0.05$ vs FVT or SnPP). Our data suggest that activation of either ERα or HO-1 protect against renal injury and HTN. However, if the activities of both are impaired, renal injury and HTN may ensue.

Group	BP (mmHg)	C-RBF (TPU)	OM-RBF (TPU)	NOX activity (RLU/ug)	KIM-1 (pg/ug)	ET-1 (pg/ml)
Veh	106±0.5	38.5±2.2	21±1	695±120	22±9	4.3±0.5
SnPP	109±1.8	36.4±1.4	20.5±1.2	891±121	41±12	6.7±0.1
FVT	107±0.6	37.8±1.5	21.3±0.9	758±110	31±7	5.4±0.2
FVT+SnPP	137±2.5*	31.2±1.9*	17.7±0.7*	1250±95*	98±6*	16.4±0.8*

10.27

ESTROGEN PROTECTS AGAINST THE DEVELOPMENT OF HYPER-TENSION DURING SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease of unknown etiology. Due to SLE's predilection for women during their childbearing years, estrogen is implicated as a contributor to SLE disease progression. Cardiovascular disease is a leading cause of mortality in women with SLE and the prevalence of hypertension, a major cardiovascular risk factor, is very high in these patients. Based on the presumed role for estrogens to promote SLE and the prevalent hypertension, we hypothesized that estrogens may promote hypertension during SLE. In the present study, we tested whether removal of estrogen ameliorates hypertension in a female mouse model of SLE (NZBWF1). An ovariectomy or sham control procedure was performed in 30 week old SLE and control (NZW/LacJ) mice. Four weeks after the surgery, mean arterial pressure (MAP in mmHg) was higher in SLE sham mice compared to control sham mice (138±2 vs. 118±3, n=9, $p<0.05$). Removal of estrogen by ovariectomy exacerbated the hypertension in SLE mice (152±5, n=5, $p<0.05$ vs. SLE sham) but had no effect in OVX controls (118, n=2). 33% of the SLE mice developed urinary albumin over the 4 weeks compared to 0% of the control mice. 100% of the OVX SLE mice developed albuminuria during this time. As expected, the characteristic anti-dsDNA antibodies were increased in SLE mice compared to controls. Ovariectomy did not significantly alter the production of autoantibodies. In conclusion, these data suggest estrogens play an important protective role against the development of hyper-tension and renal injury during SLE.

10.28

SEX REPORTING IS LACKING IN CARDIOVASCULAR STUDIES USING CELL CULTURES

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In 2009 between 22-42% of articles published in neuroscience and physiology journals failed to report the sex of the animals used in the study. Since every cell has a sex and sex chromosomes influence expression of proteins and molecular signaling, a survey was undertaken to ascertain sex reporting in cardiovascular studies utilizing cultured cells. Ten cardiovascular journals with high impact factors were selected (Circ Res, Cardiovasc Res, Circulation, JACC, Eur Heart J, J Mol Cell Cardiol, Am J Physiol Heart Circ Physiol, Arterioscler Thromb Vasc Biol, J Heart Lung Transplant and J Cardiovasc Pharmacol) and the first ten articles published in 2010 found using search terms "cultured" and "cells" in any order were reviewed. Studies using established cell lines were excluded. Of 90 articles meeting inclusion criteria, only 25 (28%) reported the sex of cells; none used only female cells, 7 used male and female cells and 18 used only male cells. Sex was reported in 7 of the 10 articles reviewed from Am J Physiol Heart Circ Physiol and sex was not reported in any of 10 articles reviewed from Cardiovasc Res. Given that expression of proteins and molecular signaling are influenced by the sex chromosomes, sex is a critical experimental variable which should be reported in published studies to uphold scientific excellence. (Supported in part by the Mayo Clinic Graduate School of Medicine).

10.29

ESTROGEN RECEPTOR (ERA) MODULATES HEME OXYGENASE (HO-1) IN RESPONSE TO HYPERTENSION (HTN) AND RENAL INJURY IN FEMALES

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Gender specific protection in females may be mediated through ERα. We have shown, females had higher baseline HO (a cytoprotective enzyme) levels than males and that ERα induced HO. We hypothesized that; ERα may induce HO-1 apropos to the magnitude of HTN and renal injury. To test this, female Sprague-Dawley rats were grouped into Control (CT), FVT (fulvestrant-ERα antagonist), CoPP (cobalt protoporphyrin-HO-1 inducer), CoPP+ FVT, SP-AngII, SP-AngII+ FVT, SP-AngII+ CoPP & Sp-AngII+ FVT+ COPP. Blood pressure (MAP) was measured and kidney tissue collected to assess function (PL.Creat), injury (KIM-1) and HO enzyme. ERα blockade blunted SP-AngII-induced HO-1 expression and HO activity, conse-quently worsening HTN and renal injury. Thus, ERα modulates HO, which may ac-count for gender differences in HTN and renal injury.

Group	**HO-1 Expression ng/ml**	**HO Activity nMol bil/mg**	**MAP mmHg**	**PL.Creat mg/dl**	**KIM-1 pg/ µg**
CT	1.4±0.2	1.1±0.04	89±2	0.5±0.06	22±9
FVT	0.95±0.04	1.3±0.03	89±1	0.6±0.09	31±7
CoPP	13.1±2	3.2±0.07	95±4	0.45±0.08	32±14
CoPP + FVT	10±0.4*β	2.8±0.03*β	110±2*β	0.59±0.01*β	94±5*β
SP-AngII	14.3±1.2*	3.4±0.4*	128±1*	0.7±0.02*	185±16*
SP-AngII+ FVT	7.7±1.1*#	2.7±0.1*#	152±3*#	1.1±0.07*#	425±28*#
Sp-AngII+ CoPP	39.7±2.1#	4.5±0.1#	98±4#	0.5±0.04#	210±30
SP-AngII + FVT+ CoPP	28.3±1.5 Kω*#	3.6±0.1 Kω*#	116±1Kω*#	0.7±0.01 Kω*#	259±34 ω*#

$p< 0.05$: * vs. CT; # vs SP-AngII; K vs SP-AngII+CoPP; ω vs. SP-AngII+FVT; β vs. CoPP.

10.30

HIGHER PDE-5 EXPRESSION IN PREGNANT RATS WITH REDUCED UTERINE PERFUSION PRESSURE

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Objective: Cyclic nucleotide phosphodiesterases (PDEs) are a family of enzymes with multiple regulatory properties and wide tissue distribution. Such activity includes cyclic guanosine monophosphate (cGMP) breakdown. The goals of this study were to investigate whether uterine and placental expressions of PDE-5 enzyme are increased in an animal model of preeclampsia, possibly causing decreases in cGMP. Methods: On day 19 of pregnancy, placental villous explants and uterine vessels were collected from Sprague-Dawley normal pregnant rats (NP, n=5) and pregnant rats that underwent reduction in uterine perfusion pressure surgeries (RUPP, n=5). Uterine and placenta PDE-5 levels were measured by Western Blotting, and cGMP concentrations by Enzyme Immunoassay. Results: PDE-5 immunoreactivity was present in NP and RUPP placental villous explants and uterine vessels. A dominant antibody-specific band was identified around 100 kd in both tissue samples, the same weigh found in lung. Although PDE-5 expression in uterine tissues was similar in NP and RUPP groups ($P>0.05$), its levels were higher (~ 1.5 fold) in placental tissues of RUPP compared with NP ($P<0.05$). We found no significant differences in cGMP concentrations between NP and RUPP groups ($P>0.05$). Conclusion: Our results suggest that PDE-5 play a role in the pathophysiology of preeclampsia, and its inhibition may be a potential therapeutic for the treatment of this disease. Funding source: NIH.

11.0 GENDER DIFFERENCES IN VASCULAR FUNCTION

11.1

CEREBRAL VASCULAR FUNCTION IN PREGNANCY AND PRE-ECLAMPSIA

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Pregnancy has a profound effect on the cerebral circulation, including selective outward remodeling of brain parenchymal arterioles that decreases cerebrovascular resistance and promotes edema during acute hypertension. The mechanism by which brain arterioles undergo remodeling during pregnancy appears to be due to activation of PPARγ via relaxin. In addition, cerebral arteries appear to be in a state of inflammation during normal pregnancy, including increased expression of pro-inflammatory cytokines that promotes greater sensitivity to the inflammatory effects of endotoxin. Hypertension also has a profound effect on the cerebral circulation that is altered by pregnancy. Pregnancy prevents and reverses hypertensive inward remodeling of cerebral arteries, an effect that leaves the brain vulnerable to high hydrostatic pressure during hypertension. Lastly, circulating factors produced during pregnancy and pre-eclampsia affect the brain and cerebral circulation. Serum from normal pregnant, but not nonpregnant rats, is hyperexcitable to neuronal tissue, causing evoked seizure potentials that are blocked by inhibition of TNF-α signaling. Because pregnant rats do not normally display seizure activity, these results suggest that the blood-brain barrier (BBB) adapts to high levels of seizure-provoking factors produced during pregnancy to limit their effects. During preeclampsia, however, circulating factors increase BBB permeability that could expose the brain to these damaging factors. Plasma from pre-eclamptic women increases BBB permeability through activation of VEGF receptors and increasing sensitivity to VEGF. (Supported by: RO1 NS045940, RO1 NS043316, PO1 HL095488, The NINDS Neural Environment Cluster NS045940-06S1, ARRA Supplement NS045940-05S1, The Preeclampsia Foundation, and The Georgio Pardi Foundation).

11.2

SEX HORMONES AND ENDOTHELIAL FUNCTION IN HUMANS

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The vascular endothelium is at the intersection of the blood and the blood vessel, and a disruption in its function is known to be a precursor to coronary heart disease. Sex

steroids can directly or indirectly alter endothelium-dependent vasodilation by affecting vasodilators such as NO or vasoconstrictors such as ET-1, although our knowledge is far from complete. In humans, the use of flow-mediated vasodilation (FMD) has emerged as a tool to assess endothelial function and is an independent, prognostic indicator of cardiovascular risk. In recent years, we have used FMD to evaluate how different types of estrogens and progestogens alter endothelial function in young, reproductive aged women. Although estrogen increases FMD, we have found that the type of progestin, route of administration, and dose relative to estrogen can alter FMD over the course of administration. Some progestins when taken orally, such as desogestrel and medroxyprogesterone acetate, antagonize estrogens positive effects on endothelial function, while others, such as drospirenone, do not. Recently, we found that estrogen via oral, vaginal, and transdermal treatment can improve endothelial function in women taking the intramuscular progestin-only injection Depo-Provera. A better understanding of how the sex steroids impact endothelial function is needed to help women reach the menopausal transition with as healthy a vasculature as possible. Support: NIH Grant HL 081671. References: Torgrimson, B.N., Meendering, J.R., Kaplan, P.F., and Minson, C.T. 2011. Depot-Medroxyprogesterone Acetate and Endothelial Function. Hypertension 57; 819-824.

11.3

NEURONAL NITRIC OXIDE SYNTHASE IN THE ENDOTHELIUM IS A NOVEL REGULATOR OF ESTROGEN SIGNALING

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Estrogen is a vasorelaxant, which increases nitric oxide (NO) generation. Traditionally, endothelial NO synthase (eNOS) was believed to be the primary source of NO. However, recent data suggest an important role for neuronal NOS (nNOS) on NO production in the endothelium; although little is known about the regulation and roles of this nNOS. We hypothesized that estrogen regulates vascular tone partly via endothelium-derived nNOS. Human umbilical vein endothelial cells were used to test whether acute (5 min) stimulation with 17beta-estradiol (E2) at 1 or 10 nmol/l affected nNOS activity. Small mesenteric arteries from male and female Sprague Dawley rats as well as ovariectomized and E2 replaced females were assessed on a wire myograph system. Relaxation responses to E2 (0.001-10 µmol/l) were conducted in the absence or presence of a selective nNOS inhibitor (L-NPA) or pan-NOS inhibitor (L-NAME). E2 rapidly increased ($p<0.001$) activating site phosphorylation of nNOS in isolated endothelial cells. Moreover, endothelial-dependent relaxation to E2 was mediated by nNOS in arteries from cycling females. Ovariectomy reduced nNOS contribution to relaxation. Interestingly, chronic E2 replacement, although enhanced acute vascular responses to E2, did not restore the nNOS contribution. Arteries from male animals had little response to E2 and were unaffected by NOS inhibition. Altogether, our data suggest that nNOS contributes to vascular regulation in females and vascular nNOS is a novel mechanism in E2 signaling.

12:0 CARDIOVASCULAR DISEASE AND FERTILITY

12.1

PCOS, STRESS-INDUCED ANOVULATION, AND CVD

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The 2 most common causes of anovulation are PCOS and functional hypothalamic amenorrhea (FHA) /stress-induced anovulation (SIA). The traditional conceptualization of PCOS emphasizes ovarian hyperandrogenism and insulin resistance (IR) as proximate determinants of metabolic syndrome, obesity, diabetes, and CVD. However, animal models demonstrate that consequences of IR vary with context. In a low fuel milieu, IR confers longevity and stress resistance. While PCOS predisposes to obesity, diabetes, and CVD in a fuel-replete milieu (Pierpont), it is not clear that this association holds in hypocaloric conditions. Indeed, since PCOS have greater athletic prowess (Rickenlund) and better fertility with advancing age call, PCOS may be adaptive in some settings. Further, by midlife, women without PCOS acquire comparable risk factors for CVD. Taken together, these data suggest the need for studies to determine if women with PCOS require different dietary, lifestyle, or pharmacologic interventions to reduce risk of CVD than women without PCOS. In contrast to women with PCOS, women with FHA / SIA are typically thin, active, and assumed to be at low risk of CVD. However, women with FHA / SIA display chronic hypercortisolism and hypoestrogenism (Berga 1989). Monkey models of FHA/SIA reveal increased CVD (Kaplan) and limited human data (Bairey-Merz) corroborate this conceptualization. Stress management may reduce CVD risk in FHA/SIA (Berga 2003). The mechanisms by which PCOS and FHA/SIA predispose to CVD may differ, but both groups appear be at increased risk for CVD. Physicians caring for women need to ascertain reproductive function, both past and present, to accurately assess CVD risk.

12.2

HYPERTENSION IN RESPONSE TO PLACENTAL ISCHEMIA: ROLE OF AGONISTIC AUTOANTIBODIES TO THE ANGIOTENSIN II TYPE I RECEPTOR

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Preeclampsia, (PE) new onset hypertension with proteinuria during pregnancy, is associated with increased reactive oxygen species, the vasoactive peptide ET-1, T and B lymphocytes and agonistic auto-antibodies to the angiotensin II type I receptor (AT1-AA). One important area of investigation for our laboratory has been to determine what role AT1-AA play in the pathophysiology associated with PE. To answer this question we have infused purified rat AT1-AA (1:50) into normal pregnant rats (NP) beginning at day 12 of gestation and on day 19 blood pressure was measured and tissues and blood collected for molecular analysis. We have consistently shown that chronic infusion of purified rat AT1-AA into NP rats increased AT1-AA from 0.68 „b 0.5 to 10.88 „b 1.1 chronotropic units ($P<0.001$ and blood pressure (MAP) 20 mmHg, from 99 + 1 to 119 + 2 mmHg ($P<0.001$). The hypertension was associated with significantly increased ET-1 in renal cortices (11-fold) and placental (4-fold), and approximately 2 to 3 fold increase in placental oxidative stress. To determine a role for endogenous AT1-AA to mediate hypertension during placental ischemia, B cells were depleted in RUPP rats, a rat model of PE. MAP and AT1-AA decreased significantly in B cell depleted RUPP rats compared to RUPP controls. Collectively, these data indicate an important role for AT1-AA stimulated in response to placental ischemia to cause hypertension during pregnancy.

12.3

CIRCULATING SFLT1 IN THE PATHOGENESIS OF PREECLAMPSIA

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Preeclampsia is a devastating medical complication of pregnancy associated with significant maternal and fetal morbidity and mortality. The prevailing hypothesis suggests that preeclampsia involves a placental factor which circulates to distal organs and exerts damage to the vasculature. We previously suggested that excess placental derived soluble fms-like tyrosine kinase 1 (sFlt-1 or sVEGFR-1), an alternatively spliced variant of VEGF receptor 1, mediates the signs and symptoms of preeclampsia, and elevated circulating levels are associated with clinical preeclampsia. However, the mechanisms of sFlt-1 (a matrix bound protein) release into the systemic circulation remains unclear. Since sFlt-1 expression is most prominent in the abnormal clusters of degenerating placental syncytiotrophoblasts (syncytial knots), we hypothesized that syncytiotrophoblast knots/microparticles that are released into maternal circulation may be one mechanism of sFlt1 release into the systemic circulation. We now present data that suggests that syncytiotrophoblast knots express sFlt1 mRNA and protein and is released into the maternal circulation thus contributing to the high circulating levels of sFlt1 in preeclampsia. We also provide evidence that shed syncitial knots/microparticles are metabolically active and can synthesize mRNA and protein thus allowing for transferring bioactive molecules like sFlt1 to distal vasculature.

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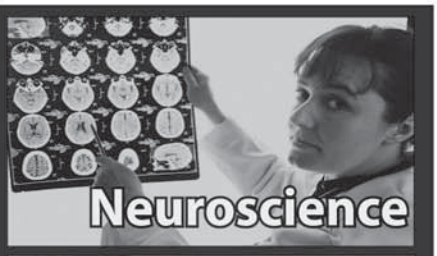

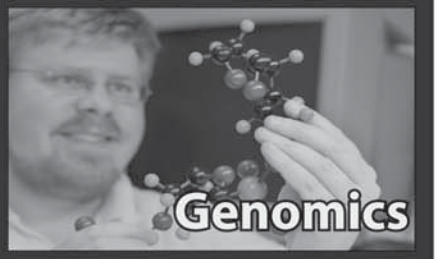

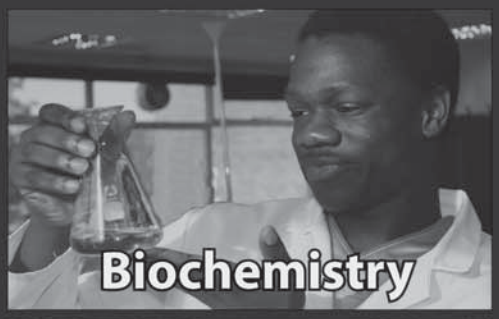

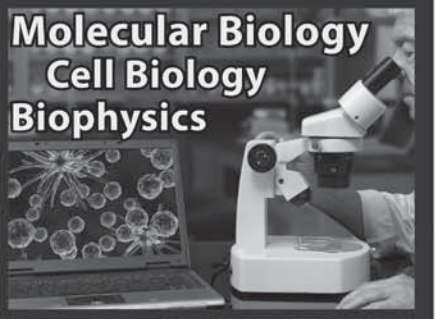

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