ALDOSTERONE AND ENAC IN HEALTH AND DISEASE: THE KIDNEY AND BEYOND

PROGRAM BOOK

american physiological society

ESTES PARK, COLO.
OCTOBER 2–6, 2019

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american physiological society

> “APS meetings [...] are the best way to keep up with what is going on in my field.”
>
> —Eleanor Lederer, MD, APS Member
> University of Louisville

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American Physiological Society (APS) meetings bring together researchers, educators and students from around the world to share the most recent advances and breakthroughs in physiology and related disciplines. APS members enjoy discounted registration rates. Take advantage of your benefits at the-aps.org/benefits.

the-aps.org

2019 APS Aldosterone and ENaC in Health and Disease: The Kidney and Beyond
October 2 – 6, 2019
The Stanley Hotel
Estes Park, Colorado

Conference Organizing Committee:

Iris Jaffe *Co-chair*	Daniela Rotin *Co-chair*	*Peter Snyder* *Co-chair*
Thomas Kleyman Alicia McDonough	David Pearce	Olivier Staub

Acknowledgements:

The conference organizers and the American Physiological Society and gratefully recognize the generous financial support from the following:

Sponsors:

American Heart Association, Council on the Kidney in Cardiovascular Disease*	National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)**	relypsa A Vifor Pharma Group Company

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San Diego

Abstract Submission

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american physiological society

New look.
New website.
What's next for APS?

NEW LOOK

This year we revealed a new brand for the Society, including new themes and messaging. The new brand presents a dynamic image that represents the process of scientific discovery itself, how idea leads to idea and discovery leads to discovery.

NEW WEBSITE

Our new state-of-the-art website reflects APS' commitment to provide our members and community with exceptional experiences.

MYAPS FEATURE NOW LIVE

Our new website now features the MyAPS dashboard where you can update your membership information, stay in touch with your fellow members and view the latest Society news.

THE PHYSIOLOGIST MAGAZINE

Our new member magazine has a journalistic style that dives deeper into what our members do, the conversations they're having in the lab and the interesting stories and experiences that set our membership apart from the rest.

FUNCTION

Function, a new, high-profile journal that will provide a home for physiology-focused papers that might otherwise have been published in other top-tier, high-impact journals, is in development and scheduled to launch in 2020.

APS ANNUAL MEETING 2023

Recently announced, the APS Council unanimously voted to part ways with Experimental Biology following the 2022 meeting. Join us as we begin the process of building a new, world-class APS Annual Meeting.

MORE INITIATIVES

Look out for more on developing Society initiatives geared toward strengthening our membership, our community and the discipline.

"We will increasingly be putting the spotlight on you, our members, on your work, and on the ways in which you are changing the world. So stay tuned for more exciting changes."

Scott Steen, CAE, FASAE
Executive Director of the American Physiological Society

2019 APS Aldosterone and ENaC in Health and Disease: The Kidney and Beyond
October 2–6, 2019
The Stanley Hotel
Estes Park, CO
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General Information

Location:

The 2019 APS Aldosterone and ENaC in Health and Disease: The Kidney and Beyond is held at The Stanley Hotel, 333 E Wonderview Ave, Estes Park, CO 80517. Phone: (970) 577-4000

Meeting Registration Hours:

Wed., October 2.....3:00 p.m.–8:30 p.m.
Thurs., October 3.....7:00 a.m.–9:00 p.m.
Fri., October 4.....7:00 a.m.–9:00 p.m.
Sat., October 5.....7:00 a.m.–6:00 p.m.
Sun., October 6.....7:00 a.m.–10:30 a.m.

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. Non-member students who register on-site 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 PhD degree in physiology or related field within five years of the conference start date, as attested to by their 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.**

Included in your Registration:

Your registration to this meeting includes entry into all oral and poster scientific sessions; all meals, welcome reception, morning and afternoon breaks, awards banquet during the conference; and a program book which serves at the conference proceedings.

Registration is nontransferable. You must pay the entire fee regardless of the number of sessions/events you attend. Guests of attendees are not permitted in the oral scientific sessions, poster sessions, opening reception, meals, conference breaks or social events.

Press Registration:

Press badges will be issued at the Conference Registration Desk to working members of the 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.

Photograph/Video Recording:

Photo or video capture of any scientific presentation, whether an oral or poster presentation in whole or in part, is expressly prohibited. Recording or taking photography of another person without their explicit permission is prohibited.

Individuals observed photographing or videotaping any presentation, in whole or in part, will be asked to leave the conference immediately, forfeiting the registration fee.

Social Media Policy:

APS encourages the use of social media during our conferences and conferences as a way of connecting with other attendees and expanding the reach of science being presented. If you plan on using social media to discuss the conference, please use the official APS conference hashtag, #PhysiologyConf.

Sharing of specific research presentations, poster or slides is prohibited without the express permission of the presenter.

Code of Conduct:

APS is committed to providing a safe, productive and welcoming environment for all conference participants and staff. All participants including, but not limited to, attendees, speakers, volunteers, APS staff, hotel staff, service providers and others are expected to abide by the APS Conference Code of Conduct which maintains that all individuals should:

- Be treated with respect and consideration, valuing a diversity of views and opinions;
- Be considerate, respectful and collaborative;
- Communicate openly and with respect, critiquing ideas rather than individuals; avoid personal attacks;
- Be mindful of your surroundings and fellow participants; and,
- Be respectful of the rules and the conference venue.

Contact the APS staff at the Conference Registration Desk if you notice a dangerous situation or someone in distress or in violation of this Code of Conduct.

Additional information on APS' Conference Policies can be found on our website: https://www.the-aps.org/conferences-awards/conferences/conference-events/control-of-renal-function/Code-of-Conduct?SSO=Y

Program Objective:

The goal of this conference is to bring together clinical and basic researchers from around the world with an interest in ENaC (and related transporters) and aldosterone (and the MR), to move forward the understanding of the basic biology of these molecules, their role in renal function and hypertension, and the emerging role in other tissues and diseases. This conference will bring together aldosterone and ENaC experts in basic, clinical, and translational research to:

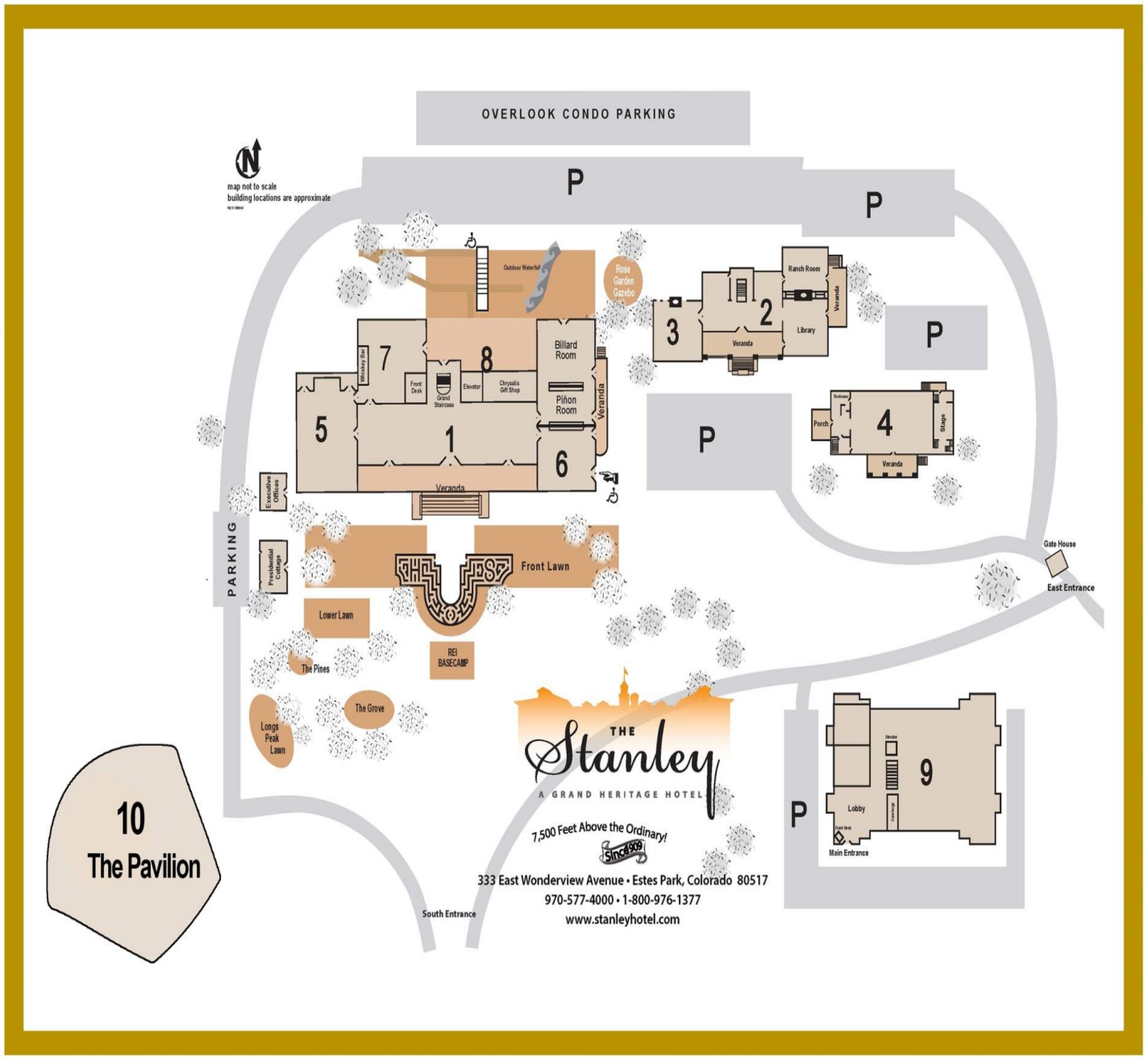
- Present and discuss the latest research in the field and outline the future direction of that work.
- Facilitate participation and career development for trainees, early career investigators, and underrepresented groups. This will allow for the enhanced opportunities to present their new work, and also benefit from exposure to the research and thinking of leaders in the field.
- Create an environment in which clinical and basic scientists in the field can exchange ideas with diverse perspectives; novel ideas and approaches to these fields which can initiate collaboration in the investigation of aldosterone/MR, ENaC, and related areas.

2019 APS Aldosterone and ENaC in Health and Disease: The Kidney and Beyond
October 2 – 6, 2019
The Stanley Hotel
Estes Park, Colorado

	Wednesday October 2	Thursday October 3	Friday October 4	Saturday October 5	Sunday October 6
6:30 a.m. - 9:30 p.m.		Registration	Registration	Registration	
6:30 a.m. - 10:00 a.m.					Registration
7:00 a.m. - 8:00 a.m.		Breakfast	Breakfast	Breakfast	Breakfast
8:00 a.m. - 10:00 a.m.		**Symposium 1A:** Aldosterone and mineralocorticoid receptors in the kidney and hypertension	**Symposium 3A:** Signaling and regulation of ENaC and other epithelial channels/trans-porters I	**Symposium 5A:** MR in the vasculature	**Symposium 6A**: Normal and pathogenic regulation of aldosterone biosynthesis
10:00 a.m. - 10:15 a.m.					Break
10:00 a.m. - 10:30 a.m.		Break	Break	Break	
10:15 a.m. - 12:00 p.m.					**Symposium 6B:** Integrated regulation of renal ion transport
10:30 a.m. - 12:00 p.m.		**Symposium 1B:** Metabolic and sex differences in aldosterone responses	**Symposium 3B:** Signaling and regulation of ENaC and other epithelial channels/trans-porters II	**Symposium 5B:** MR structure and role in the heart and lungs	
12:00 p.m. - 1:00 p.m.		Lunch	Lunch	Lunch	Box lunches & departure
1:00 p.m. - 3:00 p.m.		**Symposium 2A:** Structure function of ENaC and related transporters	**Symposium 4A:** ENaC biogenesis and trafficking		

	Wednesday October 2	Thursday October 3	Friday October 4	Saturday October 5	Sunday October 6
1:30 p.m. - 2:30 p.m.				**Clinical Plenary Lecture:** The role of non-steroidal MR antagonists and new potassium binders for the treatment of cardiovascular disease	
3:00 p.m. - 3:30 p.m.		Break			
3:00 p.m. – 5:00 p.m.			**APS Career Development Workshop/Hike**		
3:00 p.m. - 10:00 p.m.	Registration				
3:30 p.m. - 5:00 p.m.		**Symposium 2B:** ENaC function and regulation in tissues			
6:00 p.m. - 7:00 p.m.	Dinner	Dinner	Dinner		
6:00 p.m. - 9:00 PM				**APS Banquet and Awards Presentation**	
7:00 p.m. - 9:30 p.m.		**Poster Session 1**	**Poster Session 2**		
7:30 p.m. - 8:30 p.m.	**Keynote Lecture:** Kidney organoids				
8:30 p.m. - 10:00 p.m.	Welcome Reception				

1. Stanley Hotel Lobby
2. Lodge Lobby
3. Manor Hall/ Table
4. Concert Hall
5. MacGregor Ball Room
6. Music Room
7. Cascades/Whiskey Bar
8. Cascades Patio Bar
9. Aspire
10. The Pavilion



WEDNESDAY, OCTOBER 2, 2019

6:00 p.m. – 7:00 p.m. — Dinner
Pavilion, 1st Floor

7:30 p.m. – 8:30 p.m. — **1.0** **Keynote Lecture**
Pavilion Theater, 1st Floor
Chair: **Daniela Rotin**, *Hospital for Sick Children and Univ. of Toronto*

1.1 Human kidney organoids
Joseph Bonventre, *Brigham and Women's Hospital and Harvard Medical School*

8:30 p.m. – 10:00 p.m. — Welcome Reception
Pavilion, 1st Floor

THURSDAY, OCTOBER 3, 2019

7:00 a.m. – 8:00 a.m. — Breakfast
Pavilion, 1st floor

8:00 a.m. – 10:00 a.m. — **2.0** **Symposium 1A: Aldosterone and mineralocorticoid receptors in the kidney and hypertension**
Pavilion Theater, 1st Floor
Chairs: **David Calhoun**, *Univ. of Alabama Birmingham*
Celso Gomez Sanchez, *Univ. of Mississippi*

8:00 a.m. – 8:30 a.m. — **2.1** Aldosterone-independent activation of mineralocorticoid receptor in salt-sensitive hypertension and glomerular diseases
Toshiro Fujita, *The Univ. of Tokyo*

8:30 a.m. – 9:00 a.m. — **2.2** The expanding spectrum of primary aldosteronism
Anand Vaidya, *Brigham and Women's Hospital and Harvard Medical School*

9:00 a.m. – 9:15 a.m. — **2.3** The intercalated cell mineralocorticoid receptor regulates pendrin diretly and regulates principal cell ENaC indirectly over a wide range in serum K+
Susan Wall, *Emory Univ.*

9:15 a.m. – 9:30 a.m. — **2.4** Kir5.1-mediated changes in renin-angiotensin-aldosterone system balance in salt sensitive hypertension
Anna Manis, *Medical College of Wisconsin*

9:30 a.m. – 9:45 a.m. — **2.5** A proof-of-principle study of sodium loading in prehypertension
J. Brian Byrd, *Univ. of Michigan Medical School*

9:45 a.m. – 10:30 a.m. — Coffee Break
Pavilion, 1st Floor

10:30 a.m. – 12:00 p.m. **3.0** **Symposium 1B: Metabolic and sex differences in aldosterone responses**
Pavilion Theater, 1st Floor
Chairs: **Massi Caprio**, *IRCCS*
James Luther, *Vanderbilt Univ.*

10:30 a.m. – 11:00 a.m. **3.1** Biological sex-specific differences in the aldosterone responses to angiotensin II in humans and rodents
Jose Romero, *Brigham and Women's Hospital and Harvard Medical School*

11:00 a.m. – 11:30 a.m. **3.2** The leptin-aldosterone-mineralocorticoid receptor axis: a major contributor to cardiovascular disease in obese females
Eric J. Belin de Chantemele, *Medical College of Georgia at Augusta Univ.*

11:30 a.m. – 11:45 a.m. **3.3** Hypersensitivity of renal ENaC to aldosterone is a sex-specific determinant of blood pressure control in females
Mykola Mamenko, *Augusta Univ.*

11:45 a.m. – 12:00 p.m. **3.4** Female mice exhibit higher increases in aldosterone synthase expression and aldosterone production than males in response to low salt diet
Jessica Faulkner, *Medical College of Georgia at Augusta Univ.*

12:00 p.m. – 1:00 p.m. Lunch
Pavilion, 1st Floor

1:00 p.m. – 3:00 p.m. **4.0** **Symposium 2A: Structure function of ENaC and related transporters**
Pavilion Theater, 1st Floor
Chairs: **Peter Snyder**, *Univ. of Iowa*
Bernard Rossier, *Univ. of Lausanne*

1:00 p.m. – 1:30 p.m. **4.1** ENaC structure by cryo-electron microscopy
Isabelle Baconguis, *Oregon Health and Science Univ.*

1:30 p.m. – 2:00 p.m. **4.2** ENaC gating regulation by biliary factors
Ossama Kashlan, *Univ. of Pittsburgh*

2:00 p.m. – 2:15 p.m. **4.3** ENaC subunit N-glycans have different roles for the ability of the channel to respond to shear force
Martin Fronius, *Univ. of Otago*

2:15 p.m. – 2:30 p.m. **4.4** N-methyl-D-aspartate (NMDA) receptor interacts with ENaC to induce renal vasodilation in the connecting tubule.
Cesar Romero, *Emory Univ. School of Medicine*

2:30 p.m. – 2:45 p.m. **4.5** Proton block of the epithelial sodium channel
Daniel Collier, *Univ. of Tennessee Health Science Center*

2:45 p.m. – 3:30 p.m. Coffee Break
Pavilion, 1st Floor

3:30 p.m. – 5:10 p.m. **5.0** **Symposium 2B: ENaC function and regulation in tissues**
Pavilion Theater, 1st Floor
Chairs: **Larry Palmer**, *Cornell Univ.*
Edith Hummler, *Univ. of Lausanne*

3:30 p.m. – 4:00 p.m. **5.1** Regulation of ENaC by extracellular Na
Thomas Kleyman, *Univ. of Pittsburgh*

4:00 p.m. – 4:30 p.m. **5.2** ENaC regulation by aldosterone and proteases
Christoph Korbmacher, *Friedrich-Alexander Univ. Erlangen-Nürnberg (FAU)*

4:30 p.m. – 4:50 p.m. **5.3** Potassium sensing in the distal nephron
David Penton Ribas, *Univ. of Zurich*

4:50 p.m. – 5:10 p.m. **5.4** The hypertension pandemic: an evolutionary perspective
Bernard Rossier, *Univ. of Lausanne*

6:00 p.m. – 7:00 p.m. Dinner
Pavilion, 1st Floor

7:00 p.m. – 9:30 p.m. **6.0** **Poster Session 1**
Pavilion, 2nd Floor

Board #

1 **6.1** Ubiquitination of ENaC subunits in vivo
Lawrence Palmer, Gustavo Frindt, Lei Yang, Weill-Cornell Medical College; Weill-Cornell Medical College; Weill-Cornell Medical College

3 **6.2** Modulation of ENaC mediated sodium transport by the basolateral Kir4.1/Kir5.1 channels
Oleg Palygin, Elena Isaeva, Alexey Shalygin, Ruslan Bohovyk, Mykhailo Fedoriuk, Christine Klemens, Ashraf El-Meanawy, Jerod Denton, Alexander Staruschenko, Medical College of Wisconsin; Medical College of Wisconsin; Medical College of Wisconsin; Medical College of Wisconsin; Medical College of Wisconsin; Medical College of Wisconsin; Medical College of Wisconsin; Vanderbilt Univ. Medical Center; Medical College of Wisconsin

5 **6.3** Regulation of ENaC by paraoxonase 3
Shujie Shi, Nicolas Montalbetti, Xueqi Wang, Stephanie Mutchler, Allison Marciszyn, Marcelo Carattino, Thomas Kleyman, Univ. of Pittsburgh; Univ. of Pittsburgh; Univ. of Pittsburgh; Univ. of Pittsburgh; Univ. of Pittsburgh; Univ. of Pittsburgh; Univ. of Pittsburgh

Board #		
7	**6.4**	Is the membrane-bound serine protease Tmprss3 implicated in ENaC-mediated sodium absorption? **Deepika Vijayan, Thibaud Peyrollaz, Simona Frateschi, Edith Hummler**, Univ. of Lausanne; Univ. of Lausanne; Univ. of Lausanne; Univ. of Lausanne
9	**6.5**	Regulation of endothelial sodium channel (EnNaC) by mTOR and SGK-1 signaling. **Michael Hill, Yan Yang, Guanghong Jia, Annayya Aroor, James Sowers**, Univ. of Missouri; Univ. of Missouri; Univ. of Missouri; Univ. of Missouri; Univ. of Missouri
11	**6.6**	What is new about ENaC regulation by the serine protease CAP1/Prss8 in kidney? **Asma Mechakra, Yannick Jaeger, Chloe Sergi, Thibaud Peyrollaz, Anne-Marie Merillat, Ivan Gautschi, Stephan Kellenberger, Simona Frateschi, Edith Hummler**, Univ. of Lausanne; Univ. of Lausanne; Univ. of Lausanne; Univ. of Lausanne; Univ. of Lausanne; Univ. of Lausanne; Univ. of Lausanne; Univ. of Lausanne; Univ. of Lausanne
13	**6.7**	Renal denervation improves sodium excretion in rats with chronic heart failure: effects on ENaC and angiotensin II-aldosterone actions **Hong Zheng, Xuefei Liu, Kenichi Katsurada, Kaushik Patel**, Univ. of South Dakota; Univ. of South Dakota; Univ. of Nebraska Medical Center; Univ. of Nebraska Medical Center
15	**6.8**	Withdrawn
17	**6.9**	Sprinonolactone treatment reverses arterial ENaC expression and improves arterial responsiveness in hypertensive Cyp1a1-Ren2 rats **Martin Fronius, Sama Mugloo, Zoe Ashley, Ivan Sammut, Catherine Leader, Rob Walker, Fiona McDonald**, Univ. of Otago; Univ. of Otago; Univ. of Otago; Univ. of Otago; Univ. of Otago; Univ. of Otago; Univ. of Otago
19	**6.10**	High salt diet and aldosterone: focus on CaSR-RAS on plasma and urinary outcomes in salt-sensitive rats. **Charles Okechukwu, Emmanuel Awumey**, North Carolina Central Univ.; North Carolina Central Univ.
21	**6.11**	Aldosterone-independent regulation of ENaC and Na and K excretion **Lei Yang, Gustavo Frindt , Lawrence Palmer**, Weill Cornell Medical College; Weill Cornell Medical College; Weill Cornell Medical College
23	**6.12**	Mineralocorticoid receptor antagonism in acute hyperkalemia: is aldosterone a must-have? **Frederico Fazan, Eduardo Colombari**, Federal Univ. of São Paulo; UNESP/FOAr

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25	**6.13**	Probiotics effectively restore the function and structure of damaged kidney in gout **Rostyslav Bubnov, Mykola Spivak**, Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine; Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine
27	**6.14**	Sex-dependent protection from high fat diet-induced metabolic disease in mice lacking normal levels of TrpC6 and/or ASIC2 and βENaC proteins **Heather Drummond, Zachary Mitchell, Emily Hildebrandt, David Stec**, Univ. of Mississippi Medical Center; Univ. of Mississippi Medical Center; Univ. of Mississippi Medical Center; Univ. of Mississippi Medical Center
29	**6.15**	Effects of dietary potassium restriction and K+ loading on blood pressure and renal tubular Na+ transport **Cary Boyd-Shiwarski, Rebecca Beacham, Claire Weaver, Daniel Shiwarski, Kelly Connolly, Allison Marciszyn, Arohan Subramanya**, Univ. of Pittsburgh; Univ. of Pittsburgh; Univ. of Pittsburgh; Carnegie Mellon Univ.; Univ. of Pittsburgh; Univ. of Pittsburgh; Univ. of Pittsburgh
31	**6.16**	PIP2 regulates ENaC through multiple electrostatic interactions **Crystal Archer, Chase Carver, Benjamin Enslow, Yinghua Chen, James Stockand**, Univ. of Texas Health San Antonio; Univ. of Texas Health San Antonio; Univ. of Texas Health San Antonio; Case Western Reserve Univ.; Univ. of Texas Health San Antonio
33	**6.17**	Effects of dietary habits on hypertension among medical students **Suyog Mhamankar**, Spartan Health Science Univ.
35	**6.18**	A mechanism to reduce cysts in autosomal dominant polycystic kidney disease by inducing fluid absorption via CFTR, NHE3 and ENaC **Liudmila Cebotaru, Muraly Yanda**, Johns Hopkins Univ.; Johns Hopkins Univ.

FRIDAY, OCTOBER 4, 2019

7:00 a.m. – 8:00 a.m.		Breakfast Pavilion, 1st floor
8:00 a.m. – 10:00 a.m.	**7.0**	**Symposium 3A: Signaling and regulation of ENaC and other epithelial channels/transporters I** **Pavilion Theater, 1st Floor** **Chair: Doug Eaton**, *Emory Univ.*
8:00 a.m. – 8:30 a.m.	**7.1**	ENaC regulation by the mTORC2-SGK1 signaling module **David Pearce**, *Univ Calif San Francisco*
8:30 a.m. – 9:00 a.m.	**7.2**	Role of microRNAs in aldosterone signaling and ENaC regulation **Michael Butterworth**, *Univ. of Pittsburgh*

9:00 a.m. – 9:30 a.m.	**7.3**	AMPK regulates ENaC via a tripartite inhibitory complex **Kenneth R. Hallows**, *Univ. of Southern California*
9:30 a.m. – 9:45 a.m.	**7.4**	Elevated sodium activates the NLRP3 inflammasome in antigen presenting cells through an ENaC-dependent mechanism **Ashley Pitzer**, *Vanderbilt Univ. Medical Center*
9:45 a.m. – 10:00 a.m.	**7.5**	Postprandial effects on ENaC-mediated sodium absorption **Christine A. Klemens**, *Medical College of Wisconsin*
10:00 a.m. – 10:30 a.m.		Coffee Break Pavilion, 1st Floor
10:30 a.m. – 12:00 p.m.	**8.0**	**Symposium 3B: Signaling and regulation of ENaC and other epithelial channels/transporters II** **Pavilion Theater, 1st Floor** **Chairs: Alexander Staruschenko**, *Medical College of Wisconsin* **Jim Stockand**, *Univ. of Texas, San Antonio*
10:30 a.m. – 11:00 a.m.	**8.1**	WNK kinases and cell volume regulation **Arohan Subramanya**, *Univ. of Pittsburgh*
11:00 a.m. – 11:30 a.m.	**8.2**	From SPLUNC1 to SPX-101, novel peptidomimetics to treat sodium hyperabsorption in the CF lung **Robert Tarran**, *Univ. of North Carolina*
11:30 a.m. – 11:45 p.m.	**8.3**	Direct effect of potassium on ENaC regulation and potassium secretion in collecting duct cells: role of mTORC2/SGK1 signaling **Bidisha Saha**, *Univ. of California, San Francisco*
11:45 a.m. – 12:00 p.m.	**8.4**	High mobility group box-1 protein regulates lung epithelial sodium channel activity via the receptor for advanced glycation end products **Garett J. Grant**, *Univ. of Utah*
12:00 p.m. – 1:00 p.m.		Lunch Pavilion, 1st Floor
1:00 p.m. – 3:00 p.m.	**9.0**	**Symposium 4A: ENaC biogenesis and trafficking** **Pavilion Theater, 1st Floor** **Chair: Daniela Rotin**, *Hospital for Sick Children and Univ. of Toronto*
1:00 p.m. – 1:30 p.m.	**9.1**	Novel mechanisms of diuretic resistance revealed by single cell analysis **Vivek Bhalla**, *Stanford Univ. School of Medicine*
1:30 p.m. – 2:00 p.m.	**9.2**	Regulation of renal ion transport by ubiquitylation and phosphorylation networks **Olivier Staub**, *Univ. of Lausanne*

2:00 p.m. – 2:30 p.m.	**9.3**	Regulation of ENaC by the ER lumenal, molecular chaperone, GRP170 **Teresa Buck**, *Univ. of Pittsburgh*
2:30 p.m. – 2:45 p.m.	**9.4**	A conserved region in the N-terminus of $^{\alpha}$-ENaC regulates proteolytic processing during anterograde transport **Pradeep Kota**, *Univ. of North Carolina at Chapel Hill*
3:00 p.m. – 5:00 p.m.	**10.0**	**Career development workshop/hike** The purpose of the workshop/hike is to provide career advice from senior investigators to junior faculty, postdoctoral fellows and students on areas including grant writing, academic promotion, manuscript writing, mentorship and other faculty activities.
6:00 p.m. – 7:00 p.m.		Dinner Pavilion, 1st Floor
7:00 p.m. – 9:30 p.m.	**11.0**	**Poster Session 2** **Pavilion, 2nd Floor**

Board #		
2	**11.1**	Myeloid mineralocorticoid receptor contributes to lung inflammation and vascular remodeling in experimental pulmonary hypertension **Guanming Qi, Rod R. Warburton, Krishna C. Penumatsa, Mary E. Moss, Joshua Man, Qing Lu, Seung Kyum Kim, Nicholas S. Hill, Iris Z. Jaffe, Ioana R. Preston**, Tufts Medical Center; Tufts Medical Center; Tufts Medical Center; Tufts Medical Center; Tufts Medical Center; Tufts Medical Center; Tufts Medical Center; Tufts Medical Center; Tufts Medical Center; Tufts Medical Center
4	**11.2**	Endothelial mineralocorticoid receptor deletion protects female mice from obesity-induced endothelial dysfunction and modulates vascular TRPV4 expression and function **Lauren Biwer, Brigett Carvajal, Qing Lu, Iris Jaffe**, Tufts Medical Center; Tufts Medical Center; Tufts Medical Center; Tufts Medical Center
6	**11.3**	Long extracellular beta strand in ENaC gating **Shaohu Sheng, Jingxin Chen, Xueqi Wang, Thomas Kleyman**, Univ. of Pittsburgh; Univ. of Pittsburgh; Univ. of Pittsburgh; Univ. of Pittsburgh
8	**11.4**	ENaC-α agonism can induce endothelial dysfunction in control female mice only independently of endothelial mineralocorticoid receptors **Jessica Faulkner, Simone Kennard, Iris Jaffe, Rudolf Lucas, Eric Belin de Chantemèle**, Medical College of Georgia at Augusta Univ.; Medical College of Georgia at Augusta Univ.; Tufts Medical Center; Medical College of Georgia at Augusta Univ.; Medical College of Georgia at Augusta Univ.
10	**11.5**	Effect of amiloride on epithelial sodium channel among adult Nigerian subjects **Simiat Elias, Olusoga Sofola, Smith Jaja**, Lagos State Univ. College of Medicine; College of Medicine, Univ. of Lagos; College of Medicine, Univ. of Lagos

Board #

12 **11.6** Epithelial sodium channel modulation by acid and anions in salt taste
Alan Ryan, Daniel Collier, Peter Snyder, Univ. of Iowa; Univ. of Tennessee Health Science Center; Univ. of Iowa

14 **11.7** The stimulation of the basolateral Kir4.1/Kir5.1 in distal convoluted tubule is involved in augmenting NCC activity of Nedd4-2-deficient mice
Peng Wu, Xiaotong Su, Zhong-Xiuzi Gao, Olivier Staub, Wenhui Wang, Daohong Lin, New York Medical College; Oregon Health & Science Univ.; New York Medical College; Univ. of Lausanne; New York Medical College; New York Medical College

16 **11.8** MLP regulates ENaC activity in renal distal convoluted tubule cells
Chang Song, Janet Le, Qiang Yue, Monisha Mistry, Otor Al-Khalili, Brandi Wynne, Douglas Eaton, Emory Univ. School of Medicine; Univ. of Texas; Emory Univ. School of Medicine; Emory Univ. School of Medicine; Emory Univ. School of Medicine; Emory Univ. School of Medicine; Emory Univ. School of Medicine

18 **11.9** The differential effects of aldosterone and high salt on kidney injury
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20 **11.10** Characterization of mice with a hypomorphic ENaC γ subunit
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24 **11.12** Epithelial Na+ channel activation by cleavage coevolved with the terrestrial migration of vertebrates
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28	**11.14**	Angiotensin and aldosterone receptor antagonism attenuates angiotensin ii-induced hypertension in Sprague Dawley rats **Stacy Tletlepantzi**, Univ. of California, Merced
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32	**11.16**	Primary aldosteronism decreases insulin secretion and increases insulin clearance in humans **James Luther, Gillian Murray, Adina Turcu, Nian Hui, Chang Yu, Dungeng Peng, Gail Adler**, Vanderbilt Univ. Medical Center; Brigham and Women's Hospital; Univ. of Michigan; Vanderbilt Univ.; Vanderbilt Univ.; Vanderbilt Univ. Medical Center; Brigham and Women's Hospital
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36	**11.18**	Age and sex-specific reference ranges are needed for the aldosterone/renin ratio **Jun Yang, Pravik Solanki, Stella May Gwini, Peter J Fuller, Jimmy Shen, James CG Doery, Kay Weng Choy,** Dept. of Endocrinology, Monash Health, Clayton; Dept. of Medicine, Monash University, Clayton; University Hospital Geelong, Barwon Health, Geelong; Hudson Institute of Medical Research, Clayton, Victoria, Australia

SATURDAY, OCTOBER 5, 2019

7:00 a.m. – 8:00 a.m.		Breakfast Pavilion, 1st floor
8:00 a.m. – 10:00 a.m.	**12.0**	**Symposium 5A: MR in the vasculature** **Pavilion Theater, 1st Floor** **Chairs: Frederic Jaisser**, *INSERM* **Iris Jaffe**, *Tufts Medical Center*
8:00 a.m. – 8:30 a.m.	**12.1**	Sex-specific mechanisms of resistance vessel endothelial dysfunction in obesity **Ana Davel**, *Univ. of Campinas*
8:30 a.m. – 9:00 a.m.	**12.2**	Sex differences in the role of the smooth muscle cell mineralocorticoid receptor in cardiovascular aging **Jennifer DuPont**, *Tufts Medical Center*
9:00 a.m. - 9:15 a.m.	**12.3**	Role of the myeloid mineralocorticoid receptor in vascular inflammation in atherosclerosis **Joshua Man**, *Tufts Medical Center*

9:15 a.m. – 9:30 a.m.	**12.4**	Aldosterone and angiotensin II increase aortic stiffness and endothelial dysfunction via an action of oxidative stress on the endothelial sodium channel **James Sowers**, *Univ. of Missouri*
9:30 a.m. – 9:45 a.m.	**12.5**	The novel non-steroidal MR antagonist finerenone improves metabolic parameters via ATGL-mediated lipolysis of brown adipose tissue in high-fat diet fed mice **Vincenzo Marzolla**, *IRCCS San Raffaele Pisana*
9:45 a.m. – 10:30 a.m.		Coffee Break Pavilion, 1st Floor
10:30 a.m. – 12:00 p.m.	**13.0**	**Symposium 5B: MR structure and role in the heart and lungs** **Pavilion Theater, 1st Floor** **Chairs: Gail Adler**, *Brigham and Women's Hospital* **Shawn Bender**, *Univ. of Missouri*
10:30 a.m. – 11:00 a.m.	**13.1**	Enhanced endothelium epithelial sodium channel signaling prompts left ventricular diastolic dysfunction in obese female mice **Guanghong Jia**, *Univ. of Missouri School of Medicine*
11:00 a.m. – 11:30 a.m.	**13.2**	Structural determinants of activation of the mineralocorticoid receptor: an evolutionary perspective **Peter Fuller**, *Hudson Institute of Medical Research*
11:30 a.m. – 11:45 a.m.	**13.3**	The quaternary structure of the mineralocorticoid receptor depends on ligand and DNA binding **Diego Alvarez de la Rosa**, *Universidad de La Laguna*
11:45 a.m. – 12:00 p.m.	**13.4**	Vascular cell-specific roles of mineralocorticoid receptors in pulmonary hypertension **Ioana Preston**, *Tufts Medical Center*
12:00 p.m. – 1:00 p.m.		Lunch Pavilion, 1st Floor
1:30 p.m. – 2:30 p.m.	**14.0**	**Clinical Plenary Lecture** **Pavilion Theater, 1st Floor** **Chair: Maria-Christina Zennaro, *Paris Cardiovascular Research Center***
1:30 p.m. – 2:30 p.m.	**14.1**	The role of non-steroidal MR antagonists and new potassium binders for the treatment of cardiovascular disease **Bertram Pitt**, *Univ. of Michigan*
2:30 p.m. – 6:00 p.m.		**Free Time**

6:00 p.m. – 9:00 p.m. **Banquet and Awards Presentation**
Pavilion, 1st Floor

SUNDAY, OCTOBER 6, 2019

7:00 a.m. – 8:00 a.m. Breakfast
Pavilion, 1st floor

8:00 a.m. – 10:00 a.m. **15.0** **Symposium 6A: Normal and pathogenic regulation of aldosterone biosynthesis**
Pavilion Theater, 1st Floor
Chairs: **Eleanor Davies**, *Univ. of Glasgow*
Jun Yang, *Monash Univ.*

8:00 a.m. – 8:30 a.m. **15.1** Development of an inducible mouse model of aldosteronism
William Rainey, *Univ. of Michigan*

8:30 a.m. – 9:00 a.m. **15.2** A gain of function mutation in CLCN2 chloride channel gene causes primary aldosteronism
Fabio Fernandes-Rosa, *INSERM U970*

9:00 a.m. – 9:15 a.m. **15.3** Circulating microRNAs as diagnostic biomarkers of primary aldosteronism
Eleanor Davies, *Univ. of Glasgow*

9:15 a.m. – 9:30 a.m. **15.4** The retinoic acid receptor a contributes to the development of primary aldosteronism by regulating adrenal cortex structure through interactions with Wnt and Vegfa signaling
Sheerazed Boulkroun, *INSERM U970*

9:30 a.m. – 10:15 a.m. Coffee Break
Pavilion, 1st Floor

10:15 a.m. – 12:00 p.m. **16.0** **Symposium 6B: Integrated regulation of renal ion transport**
Pavilion Theater, 1st Floor
Chairs: **David Ellison**, *Oregon Health Science Univ.*
Johannes Loffing, *Univ. of Zurich*

10:15 a.m. – 10:45 a.m. **16.1** The role of ENaC in hyperoxia-induced preterm lung injury
My Helms, *Univ. of Utah*

10:45 a.m. – 11:15 a.m. **16.2** Regulation of renal ion transport and blood pressure by the CRL3-WNK-SPAK pathway
James McCormick, *Oregon Health and Science Univ.*

11:15 a.m. – 11:45 a.m. **16.3** WNK regulation of ion transport in the malpighian tubule
Aylin Rodan, *Univ. of Utah*

11:45 a.m. – 12:00 p.m.	**16.4**	Interleukin 6 activation of the epithelial sodium channel in the distal convoluted tubule and cortical collecting duct via Rac1 **Oishi Paul**, *Emory Univ.*
12:00 p.m.		Boxed lunches available upon departure Pavilion, 1st floor

2019 APS Aldosterone and ENaC in Health and Disease: The Kidney and Beyond
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1: KEYNOTE LECTURE:

1.1

Human kidney organoids

Joseph Bonventre[1]

[1]Renal Division, Brigham and Women's Hospital and Dept. of Medicine, Harvard Medical School

This human kidney differentiation system has spawned a rapidly moving field of kidney biology with potential for transformative impact on models of disease and drug development. We have developed an efficient, chemically defined protocol for differentiating human embryonic stem (ES) cells and induced pluripotent stem cells (iPSCs) into multipotent nephron progenitor cells (NPCs) that can form nephron-like structures. By recapitulating metanephric kidney development in vitro we generate SIX2+SALL1+WT1+PAX2+ NPCs with 75-90% efficiency within 8-9 days of initiation of differentiation. The NPCs form PAX8+LHX1+ renal vesicles that self-organize into nephron structures. NPCs form kidney organoids containing epithelial nephron-like structures expressing markers of podocytes, proximal tubules, loops of Henle and distal nephrons in an organized, continuous arrangement that resembles the nephron *in vivo*. The organoids express genes of many transporters seen in adult metanephric-derived kidney. Stromal cells are also generated with the presence of PDGFRβ+ (pericyte), CD31+endomucin+ (endothelial cell), or α-SMA+ (myofibroblast) interstitial cells. The entire procedure is performed with completely defined conditions without the need for embryonic spinal cord. Organoids can be used to study kidney development, mechanisms of disease processes nephrotoxicity of drugs. Epithelial toxins and pro-inflammatory agents, such as IL-1β and TGFα can cause increased numbers of stromal cells with characteristics of myofibroblasts and deposition of collagen. Kidney organoids are effective tools to study other genetic and non-genetic disorders of the kidney. As an example, organoids derived from iPSCs generated from individuals with autosomal dominant or recessive polycystic kidney disease can be induced to form cysts while organoids derived from subjects without cystic disease do not. The convergence of "personalized" kidney organoids with genome editing (e.g. CRISPR-Cas 9 approaches) and single cell sequencing technology holds great promise to result in better insight to disease, better human cell disease models, more predictive toxicology, and potentially "clinical trials in a dish". There is a great deal of interest in functional assays but most success at this point is limited to demonstration of secretory ability of the proximal tubule and improvement in these assays are ongoing. Another area of research is enrichment of nephron structures, such as podocytes, proximal or distal tubule cells which is of particular interest for further studies of these segments or retrieval of particular cell types from the organoids. Placing organoids under the kidney capsule has been found to enhance differentiation and vascularization and connections with the host circulation.
As the organoid field develops there is increasing opportunity at the boundary of biology and engineering to control and modify differentiation and maturation and also analysis of function. Construction of cellular microfabricated hybrid devices are guided by anatomical and functional microenvironmental relationships that characterize the kidney. For example printed channels can be lined with epithelial cells or endothelial cells to mimic the tubule and it's adjacent vasculature. Challenges are many in this "organ on chip" approach. One key feature which must be recapitulated by the cell-matrix and microfluidic environmental conditions in the maintenance of viable

functional differentiated cells in vitro. This has been challenging as isolated cells ex vivo have a strong tendency to undergo de-differentiation and a metabolic shift from oxidative to glycolytic metabolism.
In conclusion, human kidney organoids represent an important addition to our armamentarium to better understand kidney physiological and pathophysiological processes and to bring new therapeutic agents to growing number of individuals with kidney disease.

2: SYMPOSIUM 1A: ALDOSTERONE AND MINERALOCORTICOID RECEPTORS IN THE KIDNEY AND HYPERTENSION

2.1

Aldosterone-independent activation of mineralocorticoid receptor in salt-sensitive hypertension and glomerular diseases

Toshiro Fujita[1]
[1]Research Center for Academic Sciences & Technology, The Univ. of Tokyo

Obesity is associated with increased salt-sensitivity of blood pressure (BP). Obesity-induced salt-sensitive hypertension is caused by hyperaldosteronism that is due to adipocyte-derived aldosterone releasing factors. Moreover, high salt diet aggravates BP elevation and cardio-renal injury with the inadequate suppression of plasma aldosterone, but treatment of the mineralocorticoid receptor (MR) antagonist eplerenone improved these abnormalities. Indeed, MR is activated by aldosterone excess and high salt, but aldosterone-independent MR activation is involved in salt-sensitive hypertension and glomerular diseases, despite normal, even low, circulating aldosterone concentration. 1) Cortisol and aldosterone, in vitro, equally bind to MR, but 11β -HSD2 in the kidney converts cortisol to cortisone, an inactive metabolite; aldosterone, but not cortisol, serves as a sole ligand of MR in the kidney. Recently, we found that deletion of 11β -HSD2 in aldosterone-sensitive distal nephron activate ENaC through MR activation induced by corticosterone excess. Resultantly, hypokalemia and alkalosis activate NCC and pendrin, respectively, resulting in salt-sensitive hypertension through increased NaCl reabsorption (Hypertension 2017). 11β -HSD2 is also present in placenta, and placental 11β -HSD2 block the penetration of high cortisol from pregnant mother into fetus. But, malnutrition during pregnancy downregulates placental 11β -HSD2 activity, leading to cortisol excess in fetus, and in turn, excessive cortisol during the fetal period causes prenatal programmed salt-sensitive hypertension in adult life. We also clarified epigenetic mechanism of prenatal programmed hypertension (JCI-Insight 2018). 2) Previously, we found that Rac1, a member of RhoGTPases, activate MR, aldosterone-independently (Nat Med 2008). In salt-loaded Dahl S hypertensive rats, treatments of Rac1 inhibitor, as well as eplerenone, decreased BP and albuminuria, associated with downregulation of Sgk1, a downstream molecule of MR signaling, indicating that salt-induced activation of Rac1-MR pathway contribute to salt-sensitive hypertension and glomerular injury (JCI 2011). Moreover, podocyte-specific RhoGDI-KO mice showed albuminuria and podocte damage by Rac1 activation, but both podocyte injury and albuminuria were ameliorated by eplerenone. Consistently, Hildebrandt et al. found steroid-resistant congenital nephrotic syndrome in patients with RhoGDI mutation, associated with activation of Rac1-MR pathway (JCI 2013). We recently established the diabetic nephropathy-model mice showing massive albuminuria with intense staining of active Rac1 in podocytes and upregulation of Sgk1. But, the Rac1 inhibitor and MR antagonist decreased albuminuria.
Moreover, we tried to have the RCT to evaluate the effect of eplerenone on urinary albumin excretion in hypertensive CKD patients receiving RAS blockades. Eplerenone decreased urinary albumin excretion, in an aldosterone-independent manner (Lancet Endocrinol & Diabetes 2017). Both BP and albuminuria increased during 1-year treatment in the placebo group with higher salt intake, despite receiving RAS blockades, but not in the lowest salt group, suggesting salt-induced resistance to RAS blockades. But, salt-induced increase in BP and albuminuria disappeared in eplerenone-treated patients. This result

supports our hypothesis that progression of albuminuria and BP elevation on the high salt diet may be mediated by salt-induced activation of Rac1-MR pathway, aldosterone-independently.

2.2

The expanding spectrum of primary aldosteronism

Anand Vaidya[1]
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Primary aldosteronism is characterized by autonomous aldosterone production that is independent of renin and angiotensin II and sodium status. For decades, the traditional approaches to defining and diagnosing primary aldosteronism have been relatively constant and generally focused on detecting severe and classical presentations of the disease. However, accruing evidence indicates that the prevalence of primary aldosteronism is much greater than previously recognized, and that milder and non-classical forms or primary aldosteronism that impart heightened cardiovascular risk may be common and unrecognized. This presentation will review the physiologic approach to considering primary aldosteronism and will summarize evidence from human studies that have characterized a broad severity spectrum of primary aldosteronism, ranging from mild to overt, that correlates with cardiovascular risk. The expanding spectrum of primary aldosteronism may have important implications for understanding the pathogenesis and treatment of hypertension and cardiovascular disease.

2.3

The intercalated cell mineralocorticoid receptor regulates pendrin diretly and regulates principal cell ENaC indirectly over a wide range in serum K^+

Susan Wall[1], Truyen Pham[1], Yanhua Wang[1], Cesar Romero[1], Qiang Yue[1], Chao Chen[2], Monika Thumova[1], Douglas Eaton[1], Yoskaly Lazo-Fernandez[1], Jill Verlander[2]
[1]Medicine, Emory Univ.; [2]Medicine, Univ. of Florida

Background: Aldosterone activates the intercalated cell (IC) mineralocorticoid receptor (MR), which is enhanced during hypokalemia. Whether the intercalated cell MR directly regulates the intercalated cell Cl-/HCO3- exchanger, pendrin, K+'s role in this response, and the receptor's impact on IC and principal cell function in the cortical collecting duct (CCD) are unresolved. Methods: We measured CCD Cl- absorption, transepithelial voltage (VT), ENaC channel activity, plus pendrin abundance and subcellular distribution in wild type and IC-specific MR null mice. Results: In CCDs from aldosterone-treated mice, IC MR ablation reduced Cl- absorption as well as ENaC channel activity and ENaC open probability despite principal cell MR expression in the KO mice. Since Cl- absorption in CCDs of aldosterone-treated mice occurs in large part through pendrin-mediated, Cl- absorption, we asked if pendrin protein abundance and/or pendrin's relative abundance in the region of the apical membrane is lower in kidneys from aldosterone-treated IC MR KO relative to wild type mice. We observed that pendrin total protein abundance and pendrin's relative abundance in the region of the apical plasma membrane are lower in kidneys from IC MR null relative to wild type mice following treatment models associated with high circulating aldosterone concentration. To determine if the IC MR directly regulates pendrin and the impact of serum K+ on this response, we made use of the fact that occasional intercalated cells from the IC MR null mice express the MR. Thus, we measured pendrin label intensity and subcellular distribution in MR (+) and (-) intercalated cells taken from the same IC MR KO mice. Under

conditions associated with high serum aldosterone, pendrin's relative abundance in the apical membrane region and pendrin abundance/cell were lower in the MR (-) than the MR (+) intercalated cells, whether serum K+ was high or low. Moreover, application of aldosterone in vitro increased pendrin label intensity in cultured CCDs. Conclusions: In treatment models associated with high circulating aldosterone, IC MR ablation reduces CCD Cl- absorption and indirectly reduces principal cell ENaC abundance and function. Aldosterone and the IC MR directly regulate pendrin. IC MR gene ablation reduces pendrin protein abundance and pendrin's relative abundance in the apical membrane region over a wide range in serum K+.

2.4

Kir5.1-mediated changes in renin-angiotensin-aldosterone system balance in salt sensitive hypertension

Anna Manis[1], Oleg Palygin[1], Sherif Khedr[1], Vladislav Levchenko[1], Matthew Hodges[1], Alexander Staruschenko[1]

[1]Physiology, Medical College of Wisconsin

Kir5.1 (encoded by Kcnj16 gene) is an inwardly rectifying K+ channel (Kir) subunit which is highly expressed in the aldosterone-sensitive distal nephron, where it forms a functional heterotetramer with Kir4.1. Our previous studies revealed that a knockout of Kcnj16 in the Dahl salt-sensitive rat (SSKcnj16-/-) resulted in hypotension, salt wasting tubulopathy, and hypokalemia, which was exacerbated and led to mortality within a few days when SSKcnj16-/- rats were fed a high salt diet. Moreover, we found that accompanying the 4% NaCl challenge with increased dietary potassium (1.41% K+) or administering the ENaC blocker benzamil (but not a furosemide or HCTZ) prevented all occurrences of salt-induced mortality. However, specific mechanisms pertaining to cardiorenal abnormalities in SSKcnj16-/- rats and the interaction of Kir4.1/Kir5.1-mediated potassium transport with the renin-angiotensin-aldosterone system (RAAS) remain unclear. The objective of this study was to evaluate the role of Kir5.1 in RAAS regulation. We used a mass spectrometry-based method to quantify plasma aldosterone and angiotensin metabolites in SSKcnj16-/- rats and SSWT control rats. We found that SSKcnj16-/- rats had substantial increases in all RAAS hormones compared to SSWT rats. A high salt diet magnified the observed increases in angiotensin and its metabolites but caused a significant attenuation in aldosterone preceding deaths. Furthermore, blocking aldosterone action in SSKcnj16-/- rats with spironolactone did not prevent these salt-induced deaths, but itself caused 100% mortality within 48 hours of administration, even during low salt treatment. Supplementation of the diet with high K+ showed strong attenuation in the RAAS response to high salt and was protective against mortality resulting from aldosterone-mediated mechanisms. Administration of angiotensin receptor blocker, Losartan, and ACE inhibitor, Captopril, did not impact survival. These studies reveal that the deletion of Kir5.1 resulted in marked RAAS modulation and the resulting amplification in aldosterone (but not angiotensin) signaling is required for survival without this channel, revealing disparate effects of angiotensin and aldosterone pathways on Kir4.1/Kir5.1-mediated control of K+ balance.

2.5

A proof-of-principle study of sodium loading in prehypertension

J. Brian Byrd[1,2], Brian Bazzell[1], William Rainey[3], Richard Auchus[1], Davide Zocco[4], Marco Bruttini[5], Scott Hummel[1,6]

[1]Internal Medicine, Univ. of Michigan Medical School; [2]Internal Medicine, Univ. of Michigan Comprehensive Hypertension Center; [3]Molecular and Integrative Physiology, Univ. of Michigan Medical School; [4]Research and Development, Exosomics Siena; [5]Life Sciences, Univ. Degli Studi di Siena; [6]Section of Cardiology, Ann Arbor Veterans Affairs Medical Center

Background: mRNA shielded by extracellular vesicles and perhaps other carriers such as proteins is found in urine supernatant. This mRNA might encode information about renal and cardiorenal pathophysiology, including hypertension. However, whether the mRNA

transcriptome found in urine supernatant reflects that of renal tissues and whether changes in renal physiology are detectable using US-mRNA has not been known. Like other nuclear hormone receptors, the mineralocorticoid receptor (MR) is a ligand-activated transcription factor. Objective: The first objective was to use RNA-Seq data to compare human urinary extracellular vesicles mRNA content to human renal cortex. The second objective was to characterize the effect of low-sodium diet and subsequent sodium-loading on transcripts regulated by mineralocorticoid receptor activation assayed in the supernatant of human urine. Methods: Using publicly available data, we compared the transcriptomes of a healthy male volunteer's human urinary extracellular vesicles and deceased males' human renal cortex. To avoid similarities attributable to ubiquitously expressed genes, we separately analyzed ubiquitously expressed and highly kidney-enriched genes. To determine whether mRNA in human urine supernatant reflects changes in renal gene expression, we assayed cell-depleted urine for transcription factor activity of MR using per-amplified probe-based quantitative polymerase chain reaction. The urine was collected from prehypertensive individuals (n=18) after 4 days on low-sodium diet to stimulate MR activity and again after suppression of MR activity via sodium infusion. All work was conducted in accordance with the guidelines for experimental procedures set forth in the Declaration of Helsinki, and all study participants gave informed consent. Results: In comparing mRNA in human urine supernatant and human kidney cortex, expression of 55 highly kidney-enriched genes correlated strongly (rs=0.82) while 8,457 ubiquitously expressed genes correlated moderately (rs=0.63). Standard renin-angiotensin-aldosterone system phenotyping confirmed the expected response to sodium loading. Assays were designed for genes expected to be regulated by MR activation and genes expected not to be regulated in this way, with more than one assay per gene in view of the possibility that the mRNA would be fragmented. Gene expression inferred from cycle threshold for assays for MR-regulated targets (SCNN1A, SCNN1G, TSC22D3) changed after sodium loading, and MR-regulated targets (SCNN1A, SCNN1G, SGK1, and TSC22D3) gene expression correlated significantly with serum aldosterone and inversely with urinary sodium excretion. Conclusions: RNA-sequencing of urinary extracellular vesicles shows concordance with human kidney. Perturbation in human endocrine signaling (MR activation) was accompanied by changes in mRNA in urine supernatant. Our findings could be useful for individualizing pharmacological therapy in patients with disorders of mineralocorticoid signaling, such as resistant hypertension. More generally, these insights could inform discovery of novel non-invasive biomarkers of disordered renal and cardiorenal physiology. Sources of Funding Dr Byrd was supported by National Institutes of Health grant K23HL128909 and the Michigan MTRAC Kickstart Award. Disclosures Drs Byrd, Rainey, and Auchus are inventors of a provisional patent related to detecting mineralocorticoid receptor activation. Dr Zocco is an employee of a company, Exosomics Siena SpA, that develops sample preps in the field of liquid biopsy. He is an inventor of patents owned by this company. This work was published in Circulation: Genomic and Precision Medicine in September 2018 and is submitted to this meeting because the presenter thinks it will interest the audience and they may not have seen the work yet. The full manuscript is available on an open access basic at: https://www.ahajournals.org/doi/full/10.1161/CIRCGEN.118.002213

3: SYMPOSIUM 1B: METABOLIC AND SEX DIFFERENCES IN ALDOSTERONE RESPONSES

3.1

Biological sex-specific differences in the aldosterone responses to angiotensin II in humans and rodents

Jose Romero[1], Gail Adler[1], Gordon Williams[1], Luminita Pojoga[1]
[1]Medicine, Brigham and Women's Hospital and Harvard Medical School

There is abundant evidence showing that in humans and rodents, chronic activation of the mineralocorticoid receptor (MR) and disordered levels of angiotensin II (AngII) and aldosterone lead to increased blood pressure (BP) and cardiovascular and renal damage. However, the impact of biological sex on the renin–angiotensin–aldosterone system (RAAS) is unclear. We hypothesized that sex would modify the interaction between aldosterone responses to salt intake and AngII. To test this hypothesis, in over 1500 subjects from the well-controlled Hypertensive Pathotype cohort, we compared the responses of women and men to chronic (BP response to dietary salt) and acute (aldosterone responses to AngII infusion) manipulations. Women had a 30% higher salt sensitivity of BP than men (P<0.0005) regardless of age and a 15% greater aldosterone response to AngII on both restricted and liberal salt diets (P<0.005). The means of aldosterone responses to AngII on a liberal salt diets were grouped according to quartiles of age. The aldosterone responses decrease with age in both sexes (linear regression analyses in women P<0.001 and men P<0.005) and was higher in women than men from the lowest quartile of age and reached similar levels in women and men at the highest quartile. Furthermore, we observed that aldosterone responses to AngII on a restricted salt diet correlated with estradiol levels in women <51 years old (P=0.033, coefficient=0.154). We assessed potential mechanisms for this sex effect by comparing and contrasting aldosterone responses to AngII and potassium from ex vivo rat zona glomerulosa cells. We observed greater aldosterone production in female than male zona glomerulosa cells basally and in response to both AngII and potassium (P<0.0001). We then studied a rat model of aldosterone-mediated cardiovascular disease induced by increased AngII and low NO. In this model, circulating aldosterone levels (P<0.01), myocardial damage (P<0.001), and proteinuria (P<0.05) were greater in female than male rats despite having similar BP responses. Thus, increased aldosterone production likely contributes to sex differences in cardiovascular disease, suggesting that women may be more responsive to mineralocorticoid receptor blockade than men. (This work was supported, in part, by the National Institute of Health, National Heart, Lung and Blood Institute Grants R01HL096518 and R01HL114765) Hypertension. 2018; 71:1083-1090. DOI: 10.1161/HYPERTENSIONAHA.117.11087.

3.2

The leptin-aldosterone-mineralocorticoid receptor axis: a major contributor to cardiovascular disease in obese females

Eric J. Belin de Chantemele[1]
[1]Vascular Biology Center and Dept. of Medicine (Cardiology), Medical College of Georgia at Augusta Univ.

While female sex steroids protect premenopausal women from cardiovascular disease, recent clinical evidence indicates that obesity, which is more prevalent and severe in women compared to men, ablates the protective effects of female sex hormones and predisposes young women to cardiovascular disease. However, the mechanisms whereby obesity promotes vascular disease and hypertension in females remain ill-defined. Excess production in the adipocyte-derived hormone leptin contributes to hypertension in males via overactivation of the sympathetic nervous system. Despite higher leptin levels than males, obese premenopausal women do not exhibit sympatho-activation but show inappropriately high aldosterone levels which positively correlate with the level of adiposity and blood pressure. However, the origin of these inappropriately high aldosterone levels as well

as the potential contribution of aldosterone to obesity-related cardiovascular disease remained to be determined. My group tacked these questions and reported that leptin as well as leptin receptor deficiency or leptin receptor blockade ablated obesity-mediated increases in adrenal aldosterone synthase (CYP11B2) expression and aldosterone production. Concomitantly, we showed that increases in leptin signaling in female mice or adrenocortical cells in culture elevated adrenal CYP11B2 expression and aldosterone production. With these data, we demonstrated for the first time that leptin is a direct regulator of aldosterone production. In parallel, we showed that both reduction in leptin signaling and inhibition in aldosterone action via mineralocorticoid receptor (MR) blockade abolished obesity-associated endothelial dysfunction, hypertension and cardiac fibrosis. The effects of aldosterone blockade were specific to females, which led to the proposal that obesity triggers cardiovascular disease via sex-specific mechanisms. We extended our findings by reporting that endothelial cell MR contribute to predispose females to obesity-induced cardiovascular disease. We showed that female mice and human express more endothelial MR than males and that selective deletion of endothelial MR protects female mice from leptin-induced endothelial dysfunction. Finally, we reported that progesterone positively regulates endothelial MR expression providing an explanation for the sex-difference in endothelial MR expression. All together our findings suggest that progesterone-dependent up-regulation of endothelial MR receptor and leptin-mediated aldosterone production are two parallel mechanisms whereby obesity ablates the cardiovascular protective effects of female sex hormones.

3.3

Hypersensitivity of renal ENaC to aldosterone is a sex-specific determinant of blood pressure control in females

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Excessive renal Na+ retention is a common pathogenic determinant of hypertension. Final adjustments of sodium transport in the kidney occur in the aldosterone sensitive distal nephron through regulation of the Epithelial Na+ Channel (ENaC). Both males and females employ aldosterone to stimulate ENaC-dependent Na+ reabsorption and maintain their hydromineral homeostasis. Recent studies suggest the existence of profound sex disparities pertaining to aldosterone-induced regulation of ENaC function during hypertension. Specifically, aldosterone antagonism with mineralocorticoid receptor (MR) inhibitors is insufficient to reduce excessive ENaC activity in the kidney and fails to effectively decrease blood pressure (BP) in hypertensive males. At the same time, inability to suppress circulating aldosterone levels has been associated with elevated BP in females that is successfully treated with MR antagonists. The contribution of aldosterone-driven ENaC-mediated renal sodium reabsorption to the increased BP observed in the hypertensive females is unclear. The objective of this study was to test if enhanced sensitivity of renal ENaC to aldosterone is a sex-specific determinant of excessive Na+ retention and elevated BP in females. We employed a rat model of Ang II dependent hypertension. Sprague Dawley rats of both sexes were infused with a slow-pressor dose of Ang II (400 ng·kg−1·min−1) via subcutaneously implanted mini-osmotic pumps for 14 days. This resulted in a graduate increase of BP reaching a steady state after 6-9 days of infusion. To assess the contribution of aldosterone-dependent mechanisms to the observed increase in BP, a group of Ang II infused males and females received aldosterone antagonist spironolactone (30 mg·kg−1·day−1) in the drinking water. Where necessary, non-hypertensive rats were used as controls. Ang II infusion markedly elevated circulating

aldosterone levels in rats of both sexes. However, tail cuff plethysmography demonstrated that treatment with spironolactone significantly decreased BP only in hypertensive females, from 205± 4 mmHg to 178± 3 mmHg (BP in spironolactone untreated and treated males was 217± 2 and 215± 5 mmHg respectively). Metabolic cage studies showed that spironolactone markedly improved renal capacity to excrete sodium at lower BP in Ang II infused females. Patch-clamp recordings of single-channel ENaC currents from freshly isolated split-opened collecting ducts showed that spironolactone was markedly more effective at inhibiting ENaC activity in Ang II infused females. The activity of the channel returned to basal levels, observed in non-hypertensive female controls. MR inhibition primarily diminished the abundance of functional ENaCs on the membrane and only moderately decreased the channel's open probability. In contrast, aldosterone antagonism was only partially effective in Ang II infused males. After treatment with spironolactone ENaC activity remained at least two times higher than in normotensive male rats. RT-qPCR analysis revealed that MR expression is significantly higher in the kidneys of Ang II infused females, when compared to males. Semi-quantitative western blotting demonstrated that spironolactone significantly increased the activity of Nedd4-2 in the kidneys extracted from hypertensive females, pointing to the facilitated ENaC internalization. Treatment with spironolactone did not affect the abundance of other distal nephron sodium transporters, NCC and NKCC2, and did not result in elevation of plasma K+ levels in hypertensives of either sex. Thus, in contrast to males, aldosterone is a primary regulator of ENaC activity in hypertensive females. MR inhibition effectively reduces high BP in females through improvement of pressure-natriuresis. This makes MR antagonism a promising therapeutic approach to combat hypertension in women.

3.4

Female mice exhibit higher increases in aldosterone synthase expression and aldosterone production than males in response to low-salt diet

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It is well established that the renin-angiotensin-aldosterone system responds to changes in dietary salt consumption to promote either salt retention or excretion. However, the response of aldosterone production to changes in dietary salt may be sex specific. Our previous published work indicates that aldosterone levels and adrenal aldosterone synthase (CYP11B2) expression are not suppressed in female mice in response to a high-salt diet, in contrast to males, consistent with similar findings in humans. This failure of high-salt diet to suppress aldosterone in female mice in our previous study was associated with an increase in blood pressure and development of endothelial dysfunction, which was ablated by mineralocorticoid receptor antagonism. We therefore hypothesized that high aldosterone levels induced by a low-salt diet will result in endothelial dysfunction in female mice. Wild-type male and female mice were fed either a normal-salt (0.4%NaCl) or a low-salt (0.05% NaCl) diet for either 14 or 28 days. Plasma aldosterone levels were increased in female mice to a greater extent than males following both 14 days (Δ increase from same-sex normal-salt 125± 63 pg/ml male low-salt vs 236± 68 pg/ml female low-salt, *P<0.05) and 28 days (Δ increase from same-sex normal-salt of 96± 42 pg/ml male low-salt vs 246± 78 pg/ml female low-salt, P=0.05) of low-salt diet. In correlation, adrenal CYP11B2 protein expression was unchanged following 14-day feeding in male mice on low-salt diet (1.10± 0.15 normal-salt vs 1.21± 0.14 low-salt ratio to β -actin) but modestly increased in females on low-salt (1.10± 0.11

normal-salt vs 1.68± 0.28 low-salt ratio to β -actin, P=0.06). CYP11B2 protein expression was similarly unchanged with low-salt diet in males following 28 days of feeding (0.62± 0.23 normal-salt vs 0.53± 0.17 low-salt ratio to β -actin) whereas low-salt diet significantly increased CYP11B2 expression in females (0.91± 0.07 normal salt vs 1.26± 0.16 ratio to β -actin, *P<0.05). No difference in adrenal renin, angiotensin II type 1 receptor or angiotensinogen mRNA expression was observed between male or female mice on normal- or low-salt diet. In addition, no differences were observed between male or female mice on low-salt diet in acetylcholine (endothelium-dependent) or sodium nitroprusside (endothelium-independent) aorta relaxation responses nor phenylephrine- or KCl-induced contraction. However, female mice on high-salt diet displayed an increased constriction response to serotonin in response to low-salt diet, whereas no such alteration was observed in male mice on low-salt diet (2-way ANOVA with repeated measures, *P<0.05). Collectively, these data indicate that dietary sodium intake is a more potent regulator of aldosterone production in female mice than in male mice which may promote serotonin-mediated constriction in females. Given that studies are currently emerging that indicate that females are more sensitive to the blood pressure lowering effects of mineralocorticoid receptor antagonists than males, the contribution of dietary salt to aldosterone levels in females may play a role in cardiovascular health in women both on high- and low-salt diets.

4: SYMPOSIUM 2A: STRUCTURE FUNCTION OF ENAC AND RELATED TRANSPORTERS

4.1

ENaC structure by cryo-electron microscopy

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The epithelial sodium channel (ENaC), a member of the ENaC/DEG superfamily, regulates Na+ and water homeostasis. Structures of a relative of ENaC, acid-sensing ion channels (ASICs), have shown that the members of the ENaC/DEG superfamily are trimers with large extracellular domains and narrow transmembrane domains. While ASICs can form physiologically relevant homomeric and heteromeric ion channels, ENaCs assemble as heterotrimeric channels that harbor protease-sensitive domains critical for gating the channel. The structure of human ENaC in the uncleaved state determined by single-particle cryo-electron microscopy reveals that ENaC assembles with a 1:1:1 stoichiometry of α:β :γ subunits arranged in a counter-clockwise manner. Like ASIC, the shape of each subunit is reminiscent of a hand holding a ball. Surrounding the ball-domain are the palm, finger, and thumb domains. The finger domain of each subunit forges interaction with knuckle domain of the adjacent subunit. Wedged between the finger and thumb domains is the elusive protease-sensitive inhibitory domain, not found in ASICs, and is poised to regulate conformational changes of the ‘finger’ and ‘thumb,’ domains that play critical roles in gating the ion channel.

4.2

ENaC gating regulation by biliary factors

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The epithelial sodium channel (ENaC) mediates Na+ transport in several epithelia, including the aldosterone-sensitive distal nephron, distal colon, and biliary epithelium. Numerous factors

regulate the activity of the channel, including several extracellular ligands and membrane-resident lipids. Bile acids are abundant in the biliary tree and intestinal tract, and can be elevated in the urine of patients with advanced liver disease. Using Xenopus oocytes and a cultured mouse cortical collecting duct cell line, we found that bile acids regulate ENaC gating. Regulation was dependent on specific bile acid functional groups, rather than on their biophysical properties. Enhanced regulation by taurine-conjugated bile acids, which are membrane impermeant, suggest that bile acids regulate the channel by binding sites accessible from the extracellular side of the channel. Activating bile acids had greater effects on ENaC mutants with a very low open probability, and no effect on fully active trypsin-treated channels, consistent with regulation of ENaC gating. Modeling of the voltage-dependence of regulation suggest at least two binding sites for taurocholic acid, one of which is voltage-sensitive. To test whether bile acid regulation of ENaC in the kidney could contribute to the pathophysiology of advanced liver disease, we tested the ability of urines collected from advanced liver disease patients to regulate ENaC expressed in oocytes. Urine pH and Na+ concentrations were adjusted to match control buffers, and buffers were supplemented with mannitol to control for osmolarity. We found that urines from some advanced liver disease patients activated amiloride-sensitive currents in ENaC expressing oocytes. Activation was attenuated when urines were treated with cholestyramine, a bile acid sequestering resin. Our data suggest that bile acids regulate ENaC in the biliary tract under physiological conditions, and may regulate ENaC in the nephron under pathophysiological conditions.
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4.3

ENaC subunit N-glycans have different roles for the ability of the channel to respond to shear force

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Background: Post-translational modification of ENaC in form of N-glycans was identified to be important for channel maturation and trafficking. The role of individual N-glycans for the ability of the channel to sense shear force is unknown. We hypothesized that ENaC N-glycans are important for shear force sensing by providing a connection to the extracellular matrix. Material and Methods: Human αβ γ ENaC subunits were expressed in Xenopus oocytes and transmembrane currents were recorded by two-electrode voltage clamp under no flow conditions and after application of a fluid stream generating ~0.2 dynes/cm2 of shear force (SF). ENaC–mediated current was determined with amiloride (10 µM). To assess the role of N-glycans asparagines within glycosylation consensus motifs of each subunit were individually replaced against alanines by site-directed mutagenesis. The normalized SF responses of channels lacking asparagines was compared with wild type (wt) ENaC. Results: In α ENaC two asparagines were identified that – if replaced individually – reduced the normalized SF response by about 40% in comparison to wt ENaC. Combined replacement of both asparagines reduced the SF response by ~80 % indicating additive effects. In β ENaC two asparagines were identified that, if replaced individually, resulted in channels that provided a stronger response to SF compared with the wt channel. Replacement of N-glycans in γ ENaC did not influenced the normalized current response to SF. Conclusion: Results indicate that N-glycans of α and β ENaC have different/antagonistic roles for the ability of the channel to respond to SF. N-glycans of α ENaC may facilitate a connection to the extracellular matrix since their removal impairs the ability to respond to SF. N-glycans of β ENaC seem to have a different role. It may be hypothesized,

that N-glycans of ENaC are tethers that facilitate specific interactions with either the extracellular matrix or adjacent channel subunits.

4.4

N-methyl-D-aspartate (NMDA) receptor interacts with ENaC to induce renal vasodilation in the connecting tubule

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Introduction: N-methyl-D-aspartate (NMDA) receptors are expressed throughout the nephron, including distal nephron principal cells (PC), and induce renal vasodilation through an unknown mechanism. These receptors are activated by glutamate and glycine, and mediate the vasodilation that follows a high protein diet, which is an indicator of renal functional reserve commonly used in clinical practice. Epithelial Na+ channel (ENaC), the primary mechanism of sodium absorption in the connecting tubule (CNT) and cortical collecting duct (CCD) mediates CNT-glomerular feedback (CNTGF), a vasodilator feedback mechanism producing afferent arteriole (AfA) vasodilatation following high distal delivery of sodium. We hypothesized that NMDA receptors activation stimulate ENaC, which increases CNTGF when amino acids reach the distal nephron. Methods: To determine the role of NMDA receptors in ENaC channel activity and ENaC-mediated CNTGF we examined the effect of NMDA agonists and antagonists on ENaC-mediated current in mpkCCD monolayers and ENaC channel activity in principal cell apical membrane patches from split-open mouse CCDs. To determine the effect of NMDA receptor on CNTGF-mediated AfA dilation, we measured glycine plus glutamate-induced CNTGF-mediated AfA dilation in the presence or absence of an NMDA receptor antagonist (MK801), using a double-perfusion method of the AfA and CNT ex vivo in rabbits and stop-flow pressure (SFP) technique in vivo in rats. Results: NMDA receptor activation increased, whereas NMDA blockade reduced, amiloride-sensitive current in mpkCCD monolayers. In split open native mouse CCDs, NMDA activation increased channel activity, NPo ($p=0.04$). In pre-constricted AfA, in vitro glycine plus glutamate application to the CNT lumen increased AfA dilation ($p<0.001$). This amino-acid-induced dilation was blocked with ENaC inhibitors (benzamil) and blunted with the NMDA receptor blocker MK-801. Conclusions: NMDA receptor activation increased both amiloride-sensitive current in principal cell monolayers, as well as ENaC channel activity in native, mouse split open CCDs. NMDA receptor activation increased amino acid-induced renal vasodilation by increasing ENaC-dependent CNTGF responses in the CNT. These data explain the physiological basis of renal functional reserve test and provide a potential signaling pathway, which could be targeted to prevent the renal damage associated with a high protein diet.

4.5

Proton block of the epithelial sodium channel

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The Epithelial Sodium Channel (ENaC) is exposed to wide variations in extracellular pH. In the kidney, pH can range from 9 to 5. In the tongue, many of the foods and beverages we ingest can be highly acidic (pH < 3). In previous work, we found that H+ in these ranges stimulate ENaC by altering its gating. Here we observed that H+ can also have an inhibitory effect on ENaC that is independent of channel gating. In Xenopus oocytes, once ENaC was fully activated (e.g. DEG mutation, trypsin, pH 2.25), addition of H+ to the extracellular solution reduced ENaC current. Inhibition occurred over a pH range of 6-2 ($IC50^{\sim}$ 3.5). H+ inhibition was voltage-dependent, suggesting that H+ might block the ENaC pore. To test whether H+ could permeate ENaC, we replaced Na+ in the extracellular bathing solution with H+. Under these conditions, we observed an inward H+ current that was blocked by amiloride. Mutation of pore residues altered H+ permeability relative

to Na+ and Li+; γS540C enhanced H+ permeation, whereas γS542L reduced H+ permeation. An additional residue within the inner vestibule also contributed to H+ permeation (β D546). Together, the data support a model in which H+ blocks ENaC Na+ current by competition for binding sites within the channel pore. We speculate that this may play a role in modulating sour and salt taste sensation.

5: SYMPOSIUM 2B: ENAC FUNCTION AND REGULATION IN TISSUES

5.1

Regulation of ENaC by extracellular Na

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ENaC activity is regulated by number of extracellular factors, including specific ions, proteases and shear stress. Intracellular factors, including acidic phospholipids and channel modifications by ubiquitination, palmitoylation and phosphorylation also affect ENaC activity. Many of these factors likely work in a coordinated manner to regulate ENaC open probability. Among the factors that affect channel open probability is extracellular Na, which binds to specific extracellular site(s) and transitions the channel to a low open probability state. Our group and others have identified a growing number of rare human ENaC variants (nsSNVs) that alter ENaC expression in heterologous expression systems. Many of these variants are in the extracellular regions of ENaC subunits and affect the inhibitory response of extracellular Na. The contributions of these gain- or loss-of-function variants to human physiologic parameters, including blood pressure, largely remains to be determined. We are using mouse models to explore the function roles of ENaC-specific variants that modify the inhibitory response of extracellular Na.

5.2

ENaC regulation by aldosterone and proteases

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The epithelial sodium channel (ENaC) constitutes the rate-limiting step for sodium absorption in the aldosterone-sensitive distal nephron (ASDN), which comprises the late distal convoluted tubule (DCT2), the connecting tubule (CNT) and the entire collecting duct. It is well known that ENaC activity in the cortical collecting duct (CCD) is tightly regulated by aldosterone which stimulates channel activity by activating the mineralocorticoid receptor (MR). In contrast, ENaC activity in the early part of the ASDN appears to be independent of aldosterone [1] but is likely to contribute to blood pressure control [2]. To test the hypothesis that ENaC function in the early part of the ASDN is aldosterone-independent but dependent on the presence of MR, we used doxycycline-inducible nephron-specific MR-deficient (MR KO) mice [3]. Using whole-cell recordings, ENaC function was assessed in nephron fragments from the transition zone of DCT2/CNT or CNT/CCD. As expected, ENaC activity was detectable in CNT/CCD cells of control mice but absent or barely detectable in the majority of CNT/CCD cells from MR KO mice. Importantly, ENaC currents in DCT2/CNT were greatly reduced in MR KO mice compared to ENaC currents measured in DCT2/CNT of control mice. A characteristic feature of ENaC is its regulation by proteases [4]. To explore a possible link between the stimulatory effect of aldosterone and proteolytic ENaC activation, we used the mCCDcl1 cell line derived from microdissected mouse CCD. ENaC-mediated sodium transport was assessed by recording the amiloride-sensitive equivalent short circuit current (ISC-Ami) in Ussing chambers [5]. Exposure of the mCCDcl1 cells to 3 nM aldosterone for 2.5 h stimulated ISC-Ami about 2.5-fold. Apical application of chymotrypsin had no stimulatory effect on ISC-Ami with or without aldosterone pretreatment. This suggests that in mCCDcl1 cells ENaC is fully activated by endogenous

proteases in the presence and absence of aldosterone. To inhibit these endogenous proteases, we used an intracellularly acting convertase inhibitor (furin inhibitor-1) and an extracellularly acting serine protease inhibitor (nafamostat). Pretreatment of mCCDcl1 cells with furin inhibitor-1 in combination with nafamostat essentially prevented ENaC stimulation by aldosterone. Subsequent application of chymotrypsin rapidly and completely rescued the stimulatory effect of aldosterone in mCCDcl1 cells treated with protease inhibitors. We conclude that in CCD cells stimulation of ENaC activity by aldosterone requires proteolytic channel activation by endogenous proteases. Interestingly, ENaC activity in DCT2/CNT is aldosterone-independent, but largely reduced in the absence of MR. Further studies are needed to elucidate the complex regulatory interplay of aldosterone and proteases to modify renal ENaC activity in a site-specific manner. [1] Nesterov et al. 2012, Am J Physiol 303:F1289–F1299 [2] Nesterov et al. 2016, Hypertension 67:1256-62 [3] Canonica et al. 2016, Pflügers Arch 468:895–908 [4] Loffing and Korbmacher. 2009, Pflügers Arch 458:111-35 [5] Mansley et al. 2018, Pflügers Arch 470: 295-304 Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) with project numbers 387509280 – SFB 1350 and KO 1057/10-1

5.3

Potassium sensing in the distal nephron

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The basolateral K+ channel KCNJ10, is expressed in the renal distal convoluted tubule (DCT) and controls the activity of the thiazide-sensitive NaCl cotransporter (NCC). Loss-of-function mutations of KCNJ10 cause EAST/SeSAME syndrome with salt wasting and hypokalemia. KCNJ10 is also expressed in the principal cells of the collecting system (CS); however, its role in this segment has not been studied in detail. To address this question, we generated the mouse model AQP2cre:Kcnj10flox/flox with a deletion of Kcnj10 specifically in the CS (CS-Kcnj10-KO). CS-Kcnj10-KO mice responded normally to standard and high K+ diet. However, CS-Kcnj10-KO exhibited a higher kaliuresis and lower plasma K+ than control mice when treated with thiazide diuretics. Likewise, CS-Kcnj10-KO displayed an inadequately high kaliuresis and renal Na+ retention upon dietary K+ restriction. In this condition, CS-Kcnj10-KO mice became hypokalemic due to an insufficient downregulation of the epithelial Na+ channel (ENaC) and the renal outer medullary K+ channel (ROMK) in the CS. Consistently, the phenotype of CS-Kcnj10-KO was ameliorated by either pharmacological inhibition of ENaC or by genetic inactivation of ROMK in the CS. In conclusion, KCNJ10 in the CS contributes to the renal control of K+ homeostasis by regulating ENaC and ROMK. Impaired KCNJ10 function in the CS predisposes for thiazide- and low K+ diet-induced hypokalemia and likely contributes to the pathophysiology of renal K+ loss in EAST/SeSAME syndrome.

5.4

The hypertension pandemic: an evolutionary perspective

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The hypertension pandemic has become the most critical and expensive public health problem. How and why a pandemic? Recent progresses in genetics and population genetics, genomics, comparative genomics helped by new sequencing methods and bioinformatics tools pave the way to a better understanding of hypertension using a evolutionary based approach. I will discuss the importance of the renin-angiotensin-aldosterone system (RAAS) for the control of blood pressure, focusing on the evolution of the system and its critical importance for adaptation of vertebrates to a terrestrial and dry environment. The evolution of blood pressure control during the evolution of primates, hominins, and humans will be

discussed, together with the role of common genetic factors and the possible causes of the current hypertension pandemic in the light of evolutionary medicine.

6: POSTER SESSION 1

6.1

Ubiquitination of ENaC subunits *in vivo*

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Ubiquitination is thought to control ENaC trafficking in renal epithelial cells and may mediate hormonal regulation of the channels. We assessed ENaC subunit ubiquitination (ub-ENaC) in rat kidney using affinity beads (Cytoskeleton Inc) to bind ubiquitinated proteins. Kidneys were homogenized in 0.5M urea and 0.5% SDS to solubilize membrane proteins. Ubiquitinated proteins were captured and subsequently eluted from the affinity beads, separated using SDS-PAGE and assessed with anti-ENaC antibodies. Ub-αENaC was observed as a series of proteins of apparent MW 40-70 kDa, consistent with addition of variable numbers of ubiquitin molecules to the N-terminal cleaved fragment (30 kDa) of the subunit. No ubiquinated form of the full-length protein was observed. Ub-γENaC subunit was also detected. This species ran at the same apparent MW as the fully cleaved C-terminal fragment of the subunit (~60 kDa). This suggests that the N-terminus of γENaC is ubiquitinated and remains attached to the C-terminal moiety until treatment with reducing agent. No ubiquitinated form of the full-length protein was observed. No significant Ub-β ENaC was detected. No protein was detected with control beads coated with probes lacking the affinity site. These results are consistent with in vitro data indicating ubiquitination of the α and γ but not β subunits (Staub et al EMBO J 16:6325, 1997). To see how ubiquitination changed with hormone status we fed rats either control or low-Na diet for 7 days prior to kidney harvest. Na depletion increased the amount of ub- αENaC and ub-γENaC (by 3-5 fold). This suggests that ubiquitination occurs after complete processing of the subunits, contributing to retrieval and/or disposal of channels expressed at the cell surface. The enhanced ubiquitination may reflect increased amounts of fully processed ENaC under these conditions resulting from stimulation of forward ENaC trafficking by elevated aldosterone. It does not support the idea that diminished ubiquitination is a major factor in hormonal regulation of the channel.

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6.2

Modulation of ENaC mediated sodium transport by the basolateral $K_{ir}4.1/K_{ir}5.1$ channels

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Despite highly relevant clinical and translational evidence supporting benefits of high-potassium diet, there is a substantial lag in our understanding of underlying molecular mechanisms. Furthermore, there is a critical need in the identification of pharmacological tools for manipulating potassium homeostasis. Inwardly rectifying (Kir) channels, specifically Kir4.1 and Kir5.1 (encoded by Kcnj10 and Kcnj16 genes, respectively) play a dominant role in determining blood potassium level and serve as potassium sensors in the distal nephron. In addition to Na+-Cl− cotransporter (NCC) in the distal convoluted tubules (DCT), Kir4.1/Kir5.1 channels might control Na+ transport through the epithelial Na+ channel (ENaC) in the cortical collecting ducts (CCD) and potentially serve as a new compelling pharmacological target for the blood pressure management. Here we have tested several potential Kir inhibitors on the activity of Kir4.1/Kir5.1 and ENaC channels. Single channel analysis of either Kir4.1 or Kir4.1/Kir5.1 overexpressed in CHO cells revealed that Kir4.1 channel shows a high sensitivity to fluoxetine (100 μM) with no or moderate block by amitriptyline (100 μM). In

contrast, amitriptyline inhibits Kir4.1/Kir5.1 heteromeric channel activity with no response to fluoxetine, as we and others previously have shown. Further, to test native properties of basolateral channels in the kidney, we isolated rat (N=5) and human (N=4) CCDs for the electrophysiological assessment of the basolateral potassium activity and pharmacological sensitivity. Our experiments revealed the presence of Kir4.1/Kir5.1 channels (48 and 54 pS for rat and human, respectively) and pharmacological blockade of single channels activity by amitriptyline. Moreover, using recently described inhibitor VU0134992 (Kharade et al., Mol Pharmacol, 2018; IC50 are 0.97 and 9 µM for Kir4.1 or Kir4.1/Kir5.1, respectively), we demonstrated that potassium conductance in the basolateral membrane of CCD is predominantly mediated by the activation of Kir4.1/Kir5.1 (blocked by 30 µM VU0134992). Furthermore, using short-circuit current measurements in polarized epithelial mCCDcl1 cells, we showed that application of amitriptyline (100 µM), but not fluoxetine, resulted in the inhibition of ENaC-mediated sodium current. Control experiments in CHO cells overexpressed with ENaC subunits showed that application of amitriptyline did not cause any direct modulation on sodium conductance, confirming that the effects of amitriptyline on ENaC are mediated through inhibition of Kir4.1/Kir5.1 channel. In conclusion, Kir4.1/Kir5.1 is the predominant channel in human, and rat CCD and its inhibition by amitriptyline or VU0134992 strongly modulate electrolyte transport and indirectly block sodium conductance through apical ENaC channel. Funding: NHLBI R35 HL135749, T32 HL134643 CVC A.O. Smith Fellowship and MCW CVC Michael H. Keelan Research Foundation Grant

6.3

Regulation of ENaC by paraoxonase 3

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The epithelial sodium channel (ENaC) mediates the rate-limiting step of Na+ uptake across the apical membrane of specific epithelia. ENaC-dependent Na+ absorption in the kidney has important roles in regulating extracellular fluid volume, extracellular [K+] and blood pressure. ENaC functional expression is tightly regulated by multiple intracellular and exogenous factors, including molecular chaperones that are implicated in key steps during ENaC biogenesis. We've recently shown that ENaC was regulated by paraoxonase 2 (PON2), a mammalian orthologue of the C. elegans MEC-6. MEC-6 is an ER-resident chaperone that has an essential role in the proper assembly and surface expression of the touch-sensing MEC-4/MEC-10 channel. There are significant sequence and structural conservation between MEC-6 and mammalian PONs. However, whether the chaperone function is also conserved within this family remain undetermined. Like PON2, PON3 is expressed in principal cells of the distal nephron, where ENaC resides. We hypothesize that mammalian PONs function as molecular chaperones and PON3 regulates ENaC functional expression. We first examined the effect of PON3 KD on endogenous ENaC in mCCD cells, a mouse cortical collecting duct cell line. ENaC-mediated Na+ transport was determined with Ussing chambers recording. We observed that PON3 KD led to an enhances Na+ transport in mCCD cells, which was associated with increased ENaC subunits processing. We furtherly examined the mechanism by which PON3 regulates ENaC expression in two heterologous expression systems, fisher rat thyroid (FRT) cells and Xenopus oocytes. When co-expressed in FRT cells, PON3 co-immunoprecipitated with all three ENaC subunits, and the presence of PON3

reduced both the whole-cell and surface abundance of ENaC. In oocytes co-expressing PON3, ENaC-mediated inward Na+ currents were reduced, at least in part due to a reduced ENaC surface expression. In addition, chymotrypsin-mediated ENaC activation was not altered by PON3 in oocytes, suggesting that PON3 did not affect the channel open probability. Together, our data suggest that PON3 regulates ENaC expression via affecting its biogenesis and trafficking. Future studies will investigate the physiological roles of PON3 in regulating ENaC activity, blood pressure and extracellular [K+] using the Pon3 KO mice.

6.4

Is the membrane-bound serine protease Tmprss3 implicated in ENaC-mediated sodium absorption?

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Epithelial sodium channel (ENaC) plays a critical role in the maintenance of sodium homeostasis and regulating blood pressure. Previously, ENaC was shown to be activated and regulated by channel activating proteases (CAPs) like e.g., Tmprss3 in Xenopus laevis oocytes (Andreasen D. et al, 2006, Guipponi M. et al, 2002). In human, autosomal recessive missense mutations in the TMPRSS3 gene cause non-syndromic deafness characterized by hearing loss. These variants failed to undergo proteolytic cleavage and activate ENaC in-vitro (Guipponi M. et al, 2002, Fasquelle et al., 2011). In mice, Tmprss3 mRNA transcript expression was detected in the whole kidney lysate using qPCR (Guipponi M. et al, 2002) and localized in the endoplasmic reticulum membrane compartment (Fasquelle et al., 2011). However, nothing is known about its implication in the ENaC-mediated sodium absorption in the kidney. Therefore, in-vivo experiments were performed using B6;129S5-Tmprss3tm1Lex/Mmucd KO mice model (Tang T et al, 2010). Preliminary data from KO mice subjected to sodium-deficient diet do not support the hypothesis that Tmprss3 is an ENaC channel activating protease in the kidney, as the animals do not show significant weight loss, indicative for disturbed ENaC regulation. Further experiments are required and ongoing to determine its role in ENaC regulation and kidney function. References: 1. Guipponi, M. et al. The transmembrane serine protease (TMPRSS3) mutated in deafness DFNB8/10 activates the epithelial sodium channel (ENaC) in vitro. Human Molecular Genetics (2002). 2. Andreasen D et al. Activation of Epithelial Sodium Channels by Mouse Channel Activating Proteases (mCAP) Expressed in Xenopus Oocytes Requires Catalytic Activity of mCAP3 and mCAP2 but not mCAP. JASN (2006). 3. Tang T et al. A mouse knockout library for secreted and transmembrane proteins. Nat Biotechnol (2010). 4. Fasquelle et al. Tmprss3, a transmembrane serine protease deficient in human DFNB8/10 deafness, is critical for cochlear hair cell survival at the onset of hearing. J Biol Chem (2011).

6.5

Regulation of endothelial sodium channel (EnNaC) by mTOR and SGK-1 signaling

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Published studies provide strong support for a significant role for EnNaC in the increased vascular and cardiac stiffness that is associated with endothelial cell mineralocorticoid receptor (ECMR) activation in pathophysiological states such as diet induced-obesity and aldosterone excess (Sowers et al., 2019). Currently, our mechanistic understanding of the molecular and cellular pathways regulating ENaC function is largely derived from studies of epithelial cells, leaving us to assume that similar mechanisms are present in vascular ECs. Recent studies from our group show that ECMR activation by a western diet or aldosterone, stimulates mTOR2 and serum glucocorticoid regulated kinase 1 (SGK-1), pathways known to contribute to membrane localization of ENaC (Gleason et al.,

2015). Thus, using a pharmacological approach the present studies examined the role of mTOR2/SGK-1 signaling in the acute regulation of lung vascular EnNaC activity, using a whole cell patch clamp protocol. Cells were freshly isolated from mouse lung using a sequential two-step antibody (anti CD45 and CD31)-bound magnetic bead technique and cultured for 5-7 days at 37oC, 5%CO2. The presence of functional EnNaC was verified by a reduction in current in the presence of the Na+ channel inhibitor, amiloride (1 uM). SGK-1 inhibition with GSK-650394 (20 uM) caused a substantial decrease (approximately 40% inhibition at -80 mV) in whole cell Na+ current in lung ECs. Further, 30 mins exposure to the mTORC2 inhibitor, PP242 (1 uM), caused a reduction (approximately 53% inhibition at -80 mV) in Na+ current similar to that reported for renal epithelial cells (Gleason et al., 2015). In additional studies, aldosterone was found to increase mTORC2 expression in association with decreased levels of miR99a. Further, treatment with a miR99a mimic (20 nM) attenuated the effect of aldosterone on mTORC2 expression. Collectively, the data are consistent with vascular EnNaC conductance being, in part, regulated by mTORC/SGK-1 signaling. Future studies will be aimed at determining the relative contributions of cell surface expression and channel activity. (Support provided by NHLBI and VA) References Jia, G. et al. Epithelial sodium channel in aldosterone-induced endothelium stiffness and aortic dysfunction. Hypertension 72:731-738, 2018. Sowers, J.R. et al. Epithelial sodium channels in endothelial cells mediate diet-induced endothelium stiffness and impaired vascular relaxation in obese female mice. Metabolism 2019. Gleason, C.E. et al., mTORC2 regulates renal tubule sodium uptake by promoting ENaC activity. J. Clin. Invest. 125:117-128, 2015.

6.6

What is new about ENaC regulation by the serine protease CAP1/Prss8 in kidney?

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The membrane-bound serine protease CAP1/Prss8 (also called prostasin) has been identified as a regulator of ENaC, also called “channel-activating protease (CAP), thereby revealing a novel autocrine regulation of this sodium channel (Vallet et al., Nature, 1997). Using gene targeting experiments, we previously showed that CAP1/Prss8 is implicated in vivo in ENaC-mediated sodium absorption in lung (Planes et al., EMM, 2010) and colon (Malsure et al., JASN, 2014). Nothing is known yet about its function in kidney. We generated renal tubular-specific CAP1/Prss8 knockout mice using the Pax8-rtTA-LC1-Cre system as described by (Traykova-Brauch et al., Nat Med, 2008). Ko (CAP1/Prss8PaxLC1) and control (CAP1/Prss8Pax8, CAP1/Prss8LC1) mice on sodium restriction were analyzed with respect to their physiological and metabolic parameters. Surprisingly, preliminary studies show that ko mice do not show any salt-wasting phenotype, indicative for a disturbed ENaC activation by CAP1/Prss8. We are currently studying whether compensatory mechanisms take place, like e.g., through upregulation of other serine proteases (CAPs), or through other sodium transporting systems).

6.7

Renal denervation improves sodium excretion in rats with chronic heart failure: Effects on ENaC and angiotensin II-aldosterone actions

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Previously we have shown that increased expression of renal epithelial sodium channels (ENaC) may contribute to the renal sodium and

water retention observed during chronic heart failure (CHF). The goal of this study was to examine whether renal denervation (RDN) reduced Na+ retention through modulation of angiotensin II (ANG II)-aldosterone (ALDO) actions on renal ENaC in rats with CHF. CHF was produced by left coronary artery ligation in rats. Four weeks after ligation surgery, surgical bilateral RDN were performed. Seven days after RDN, renal norepinephrine (NE), ANG II and ALDO were measured by ELISA using renal tissue from four groups of rats (Sham, Sham+RDN, CHF, CHF+RDN, n=6/group). Renal ANG II AT1 receptors and ALDO receptors from renal cortex and medulla were also measured by immunoblotting (n=4/group). We found that; 1) RDN reduced the renal NE concentration to undetectable in both Sham and CHF rats, confirming RDN; 2) RDN significantly decreased ANG II levels but did not change ALDO levels in the renal cortex and medulla of rats with CHF; 3) RDN reduced renal ANG II AT1 receptor expressions but not ALDO receptor expressions in CHF rats. We also examine renal adrenoceptors (alpha1, alpha2, beta1, beta3) protein expressions in renal cortex and medulla in the same four groups of rats. The results showed that RDN reduced renal adrenoceptor protein levels in CHF rats, particular for alpha1 and beta1 adrenoceptors. As a functional test for ENaC activation, diuretic and natriuretic responses to ENaC inhibitor Benzamil were monitored in the same four groups of rats. There were significant increases in diuretic and natriuretic responses to ENaC inhibitor Benzamil in rats with CHF. RDN significantly reduced the diuretic and natriuretic responses to Benzamil in CHF rats. These results suggest that adrenergic and ANG II/ALDO systems are activated during CHF and that RDN appears to reduce Na+ retention through modulation of ANG II/ALDO actions on ENaC in the renal tubules of rats with CHF. (This work was supported by National Institutes of Health grants 1R01DK114663.)

6.8

Withdrawn

6.9

Sprinonolactone treatment reverses arterial ENaC expression and improves arterial responsiveness in hypertensive Cyp1a1-Ren2 rats

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Background: Vascular ENaC has been implicated in mediating endothelium-dependent vasodilation and myogenic vasoconstriction. The role of vascular ENaC in hypertension and whether it is a suitable target for antihypertensive medication remains elusive. We hypothesized that elevated vascular ENaC expression is associated with hypertension and a potential target for mineralocorticoid receptor antagonism. Material and Methods: The study was approved by the Univ.'s animal ethics committee. Male Cyp1a1-Ren2 rats were either kept on normal diet (normotensive) or an indole-3-carbinol substituted diet (hypertensive). After two weeks on I3C diet or normal diet, animals were also treated orally with 4.41 mg/day spironolactone (4 weeks). Conduit and resistance arteries were collected from these animals. Arteries were used for analysing ENaC subunit expression (mRNA and protein) and pressure myography (mesenteric). Results: The mRNA and protein level of α ENaC was upregulated in hypertensive aorta, carotid, femoral and mesenteric arteries. Amiloride increased acetylcholine (ACh)-induced vasodilation in normotensive carotid arteries but did not change the ACh response in hypertensive carotid arteries indicating a loss of ENaC function in hypertension. In contrast, in hypertensive mesenteric arteries amiloride elevated the ACh-response in hypertension indicating an elevated ENaC activity. Surprisingly, spironolactone did not change systolic arterial blood pressure in hypertensive animals. However, spironolactone normalized ENaC expression in all four types of arteries from hypertensive animals and improved ACh-mediated vasodilation in mesenteric arteries. Conclusion: The study identifies increased ENaC

expression as a contributing factor for impaired vaso-reactivity in this rat model of hypertension. In addition, spironolactone treatment of hypertensive rats normalized ENaC expression and ACh-mediated responses were similar to the responses observed in arteries from normotensive animals. This indicates that vascular ENaC is as a promising target for the treatment of hypertension.

6.10

High-salt diet and aldosterone: focus on CaSR-RAS on plasma and urinary outcomes in salt-sensitive rats

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The CaSR regulates Ca2+-mediated release of parathyroid hormone (PTH) from parathyroid cells by sensing small changes in serum Ca2+concentration. The receptor is also involved in the regulation of cellular Ca2+ levels independent of PTH secretion. The renin-angiotensin-aldosterone system (RAAS) plays vital roles in the development of hypertension, which is associated with vascular and renal dysfunction, and treatment is difficult because the mechanisms are not fully understood. Studies show that adequate Ca2+ intake (1.0 to 1.5 g/day) is critical for optimal BP regulation, and randomized controlled trials have revealed significant reductions in hypertension risk and BP levels in humans. In the kidney, the CaSR is expressed along the nephron and plays an important role, along with the Na+ /Ca2+ exchanger (NCX), in the regulation of tubular Ca2+, Na+, and water reabsorption. In the present study, we analyzed the effects of a high salt diet on plasma aldosterone, sex hormones, expression of the CaSR and its downstream signaling complements as well as RAAS proteins in male and female Dahl salt-sensitive (SS) rats. Our data show statistically significant decreases in both plasma and urinary aldosterone, renin, and estradiol in SS females than males fed high salt diets. Furthermore, the high salt diet increased the expression of NCX1 and CaSR and decreased its downstream signaling proteins (PKCa, GRK2) as well as potassium in SS females compared to males. Thus, CaSR signaling is linked to RAAS to control hypertension.

6.11

Aldosterone-independent regulation of ENaC and Na and K excretion

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We investigated the regulation of ENaC under conditions of dietary Na depletion and K loading in mice lacking the gene for aldosterone synthase (AS) and the ability to produce aldosterone. We used amiloride-sensitive whole-cell currents (INa) in principal cells of the cortical collecting duct and proteolytic cleavage of the γENaC subunit in whole kidneys to assess the activation of ENaC. Under control conditions no INa could be detected and a minimal amount of cleaved γENaC was observed in both wild-type (AS+/+) and knockout (AS-/-) animals. After 24 hours on a low-Na diet, the amount of cleaved γENaC increased in AS+/+ (1.7 ± 0.2 fold) but not AS-/- animals (1.0 ± 0.2 fold). Under these conditions urinary Na excretion was higher in AS-/- (62 ± 12 nmoles/min) compared with AS+/+ (11 ± 2 nmoles/min) mice. After 7 days of Na depletion INa was significantly higher in AS+/+ (270 ± 70 pA) than in AS-/- (54 ± 18 pA) animals, and the amount of cleaved γENaC was also significantly higher only in WT mice (2.3 ± 0.5 fold). Both genotypes had similar reductions in Na excretion at this time. However in the AS-/- mice this was accompanied by a large reduction in GFR (AS+/+: 177 ± 5 ml/min; AS-/-: 51 ± 5 ml/min). These animals were able to completely conserve Na but at the expense of reduced glomerular filtration. Animals fed a high-K diet (3% K) for 1 week lost weight and had only a moderately increased rate of K excretion. We therefore assessed responses to an acute dietary K load. Animals were habituated to a the high-K diet for 48 hours, fasted overnight and then re-fed with either a high-K or a low-K diet for 5 hours. AS-/- and AS+/+ mice ate comparable amounts of high-K food and excreted comparable amounts of K during the refeeding period. However plasma K was

substantially higher in AS-/- (6.5 ± 0.7 mM) compared with AS+/+ (3.5 ± 0.5 mM). The amount of cleaved γENaC was elevated in both genotypes, although the increase was smaller in the knockouts (AS+/+: 2.9 ± 0.3 fold; AS-/- : 2.1± 0.2 fold). These results suggest that elevated aldosterone secretion facilitates excretion of a K load in WT mice, at least in part through processing and activation of ENaC. In the absence of aldosterone, ENaC can still be upregulated by through a mechanism that requires much higher levels of extracellular K.

6.12

Mineralocorticoid receptor antagonism in acute hyperkalemia: is aldosterone a must-have?

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Introduction: Hyperkalemia is one of the most potent ways to increase aldosterone levels in the blood. A large number of scientific evidence has shown that aldosterone increases K+ excretion by the kidneys and therefore is essential in potassium homeostasis. The increase in extracellular K+ also promotes intense urinary flow that is associated with high K+ clearance. However, aldosterone is also usually associated with Na+ retention and reduced urinary flow. Hence, the real role of aldosterone in the severe hyperkalemia context is yet to be determined: is high aldosterone a mere consequence of hyperkalemia or is this hormone actively fighting against potassium overload? Objective: To analyze the time-dependent role of aldosterone in the acute hyperkalemia induced by intragastric KCl injection. Methods: Male Holtzman rats weighing 300-320g received a pretreatment of 80mg.kg-1 s.c. injection of spironolactone (SL) dissolved in sunflower seed oil or only oil (VEH). Three hours after pretreatment, all animals received 3,5 mL of 2.3M KCl solution through gavage. For the next 12 hours, three random animals (n=3 in all results) from both groups were placed inside metabolic cages for urine analysis from hours 0-3; 3-6; 6-9; and 9-12 respectively after KCl administration. After the urine collection, the animals were anesthetized for blood analysis. Glomerular filtration rate was measured from creatinine clearance and fractional sodium and potassium excretion (FKE) were estimated from the sodium and potassium measures from urine and serum. Results: The animals of both groups developed an intense hyperkalemia 3 hours after KCl administration (KCl + VEH = 8,1mM [K+]; KCl + SL = 8,4mM [K+]; ns). These K+ levels reduced equally in both groups at the 6th hour period (KCl + VEH = 5,3mM [K+]; KCl + SL = 5,5mM [K+]; ns). However, in the 9th and 12th hour, K+ remained higher in SL treated group (KCl + VEH = 4,5mM [K+]; KCl + SL = 5,4mM [K+]; $p<0,05$, at 9th hour and KCl + VEH = 4,0mM [K+]; KCl + SL = 4,8mM [K+]; $p<0,05$, at 12th hour). Aldosterone followed a similar pattern, increasing in the SL treated rats at 9th and 12th hour after KCl administration (KCl + VEH = 126mg/dL [Aldo]; KCl + SL = 366mg/dL [Aldo]; $p<0,05$, at 9th hour and KCl + VEH = 42mg/dL [Aldo]; KCl + SL = 423mg/dL [Aldo]; $p<0,05$, at 12th hour). FKE showed strong positive correlation with urine output (Pearson correlation = 0,99, $p<0,001$, during all collection periods). There was no difference in the natremia between groups; however, both groups increased Na+ content in serum after 6th hour of KCl administration (KCl + VEH or SL = 134,2mM [Na+] at 3rd hour; KCl + VEH or SL = 142mM [Na+] at 9th hour, $p<0,05$). Conclusion: The 2,3M KCl gavage promotes rapid hyperkalemia independent of SL treatment. However, previous treatment with SL reduced the homeostatic drive for extracellular K+, maintaining higher levels in the serum after 6h. This can explain why aldosterone levels were higher and increased at the later periods of the protocol. Interestingly, even with SL, K+ was eliminated in the urine with a strong correlation with urine output, questioning the role of aldosterone in intense hyperkalemia as serum K+ above 5,5mM promoted no differences among the groups. Finally, the high urinary output possibly increased free water clearance, concentrating Na+ in the serum. Further work is needed in order to elucidate the role of aldosterone hyperkalemia. Financial aid: FAPESP (2018/02194-0), CNPq

6.13

Probiotics effectively restore the function and structure of damaged kidney in gout

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Introduction Gout is extremely spread pathology involving kidney, liver and joints, is met more often in men. Gout has deep interplay with metabolic syndrome (MetS), including obesity and leads to chronic renal failure in every fourth patient. Sonography can clearly determine symptoms of gouty nephropathy also at early stages, can be used as main method for screening and monitoriung treatment. Modulating gut microbiome has great potential to improve metabolic health in gout. The Aim: was to study the efficacy of individualized probiotic intervention on signs of gout and MetS by monitoring sonographic diagnostic markers of gouty nephropathy. Material and methods We included twelve patients (age 32-67 years) with BMI>30, waist circumference (WC)>110, who met criteria of MetS with hyperuricemia (the levelsof uric acid over 400 µmol/L) and increased blood preassure. All patients underwent extensive general clinical, lab tests; multiparameter ultrasound (US) of liver, kidneys, joints, measuring visceral fat (FV). The typical symptoms of gouty nephropathy in sonographic display were documented. Patients were given probiotics (B. animalis VKB / B. animalis VKL strains at a dose 108 CFU daily during 10 days); in cases of liver fibrosis - L. delbrueckii subsp. bulgaricus IMV B-7281, B. ?nimalis VKB, L. casei IMV B-7280 (considered individually according to the knowledge on treatment mechanics obtained from in vivo in vitro studies and existing evidence). Results. We registered ultrasound signs of symptoms of gouty nephropathy in all patients, namely detection of small hyperechoic inclusions in parenchyma (chalk-stone), increase in resistive index (RI) in segmental vessels over 0.7, thinning of parenchyma (less than 13 mm), fibrotic changes in parenchyma, hilly kidney margins, anaechoic strips under the capsule. Weight, BMI, WC and VF decreased, liver structure, blood pressure improved, joints tophacae decreased in size, normalized creatinine levels (under 90 µmol/L) and uric acid levels (under 250 µmol/L) in all patient after focused probiotic administation. Most signs of gouty nephropathy improved, namely increasing in kidneys size and parenchyma, decreased IR under 0.7, decreased petrifications, small cystic lesions; fibrotic changes retained in parenchyma after short-term treatment. In 6 patients microsplenia was detected, size improved to norm after treatment. Conclusion Short-term individualized probiotic therapy is effective to treat the signs of MetS and hyperuricemia and can successfully restore the function and structure of damaged kidney in gout.

6.14

Sex-dependent protection from high fat diet-induced metabolic disease in mice lacking normal levels of TrpC6 and/or ASIC2 and β ENaC proteins

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Degenerin proteins (β ENaC and ASIC2) and TrpC6 have been implicated in cardiovascular function. However, their roles and potential interaction in metabolic disease has not been studied. To begin to assess this interaction, we evaluated the impact of a high fat diet (HFD) in mice lacking normal levels of TrpC6 (TrpC6-/-) and/or ASIC2 (ASIC2-/-) and β ENaC (β ENaCm/m) on a mixed genetic background. Twenty week old male and female mice were placed on a 60% HFD for 12 weeks. Body weight was measured weekly and body composition (non-invasive ECHO MRI) and fasting blood glucose were measured at 0, 4, 8 and 12 weeks. Data are presented as mean ± SEM; order of data presentation is ASIC2-/-/β ENaCm/m, ASIC2-/-/β ENaCm/m/TrpC6-/- vs wildtype (WT). At 20 weeks of age, female ASIC2-/-/β ENaCm/m (n=9), but not ASIC2-/-/β ENaCm/m/TrpC6-/- (n=12) mice, weighed less than WT (n=9)(22.3± 0.5, 23.7± 0.8 vs 25.5± 0.6 g). Both ASIC2-/-/β ENaCm/m and ASIC2-/-/β ENaCm/m/TrpC6-/-gained less weight than WT

after 12 weeks HFD (13.4± 0.8, 12.8± 1.5 vs. 20.8± 0.8 g). Total body fat (16.2± 0.5, 17.8± 1.2 vs 23.5± 0.7 g) and lean body masses (20.2± .04, 19.5± 1.3 vs 24.1± 0.6 g) were reduced in female ASIC2-/-/β ENaCm/mand ASIC2-/-/β ENaCm/m/TrpC6-/-mice. In contrast, male ASIC2-/-/β ENaCm/m(n=10), but not ASIC2-/-/β ENaCm/m/TrpC6-/-(n=6), mice had lower body weight compared to WT (n=11) at 20 weeks (30.5± 0.4, 34.1± 0.8 vs. 34.4± 1.1 g). Body weight gain in male ASIC2/β ENaCm/m and ASIC2-/-/β ENaCm/m/TrpC6-/- mice were not quite different than WT (11.7± 1.6, 12.4± 1.5, vs. 16.1± 1.5 g). Male total body fat (14.6± 1.1, 16.8± 1.0 vs. 18.6± 0.8 g) and lean body masses (28.5± 0.7, 29.7± 0.6 vs. 32.5± 0.5 g) were less in ASIC2-/-/β ENaCm/mand ASIC2-/-/β ENaCm/m/TrpC6-/- compared to WT. Fasting blood glucoses were also lower in female (154± 4, 172± 6 vs. 201± 6 mg/dL) and male (147± 25, 181± 3 vs. 228± 11 mg/dL) ASIC2-/-/β ENaCm/mand ASIC2-/-/β ENaCm/m/TrpC6-/-mice compared to WT. In females, liver (0.94± 0.04, 0.85± 0.04 vs. 2.18± 0.37 g) and liver fat masses (0.035± 0.009, 0.016± 0.007 vs.0.341± .109 g), as well as percent liver fat (3.5± 0.7, 1.5± 0.9 vs. 13.8± 2.5%), were reduced in ASIC2-/-/β ENaCm/m and ASIC2-/-/β ENaCm/m/TrpC6-/-mice after HFD. In males, liver mass was lower in ASIC2-/-/β ENaCm/m and ASIC2-/-/β ENaCm/m/TrpC6-/-mice (1.67± 0.14, 1.77± 0.13 vs. 2.48± 0.17 g). However, liver fat mass (0.23± 0.08, 0.17± .06 vs. 0.46± .06 g) and percent liver fat were only lower in ASIC2-/-/β ENaCm/m/TrpC6-/-mice (11.1± 3.4, 8.8± 2.6 vs. 17.6± 1.6%). These highly novel findings suggest that ASIC2, β ENaC and/or their interaction with TrpC6, robustly protects against HFD induced-metabolic disease in female mice. The mechanisms underlying this response will be examined in future studies. This work was supported by NIH P01HL051971, P20GM104357, P20GM121334 and R01HL136684 sources.

6.15

Effects of dietary potassium restriction and K+ loading on blood pressure and renal tubular Na+ transport

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It is well documented that dietary potassium intake inversely correlates with blood pressure. Yet, it is unclear how potassium restriction leads to hypertension, or how potassium excess causes a natriuresis despite elevated aldosterone levels. Our goal was to study the effects of dietary potassium on blood pressure, acid/base balance, and ion transport in wild-type SV129 mice. These mice were fed either 1) K+ deficient, 2) control, 3) high K+ basic, or 4) high KCl diets for 10 days. We monitored BP using radiotelemetry probes, urine electrolyte excretion via metabolic cages, and transporter expression via immunofluorescence, western blots, and diuretic challenges. Interestingly, despite the induction of hypokalemia, extreme K+ depletion had no effect on blood pressure. In contrast, K+ loading augmented blood pressure by ~10mmHg. To determine whether these unexpected effects were dependent on NaCl intake, we challenged mice with 1% saline. The K+ deficient mice developed an increase in blood pressure (~8 mmHg), whereas K+ replete mice exhibited no significant change in blood pressure with saline challenge. Notably, just 10d of K+ restriction was associated with diabetes insipidus, evidenced by polyuria and a decrease in AQP2 expression. This was associated with an increase in sodium transporters in the upstream tubule, likely the cause of salt sensitivity. The elevated blood pressure on the K+ loaded diet correlated with elevated aldosterone levels and increased ENaC activation. During K+ loading, the type of anion (basic vs. chloride-rich) had a considerable effect on key transporters along the tubule, despite no differences on blood pressure. In our model, the effect of dietary K+ on blood pressure was coupled to NaCl intake, and the

inverse relationship between dietary K+ intake and blood pressure was only observed in the setting of NaCl loading. Our data strongly suggest that AQP2 expression should be closely monitored to accurately interpret such results. Further, the accompanying anionic content should be taken into consideration when modeling the physiologic effect of K+ intake on tubular salt transport and blood pressure.

6.16

PIP_2 regulates ENaC through multiple electrostatic interactions

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Phosphatidylinositol 4,5-bisphosphate (PIP2) regulates the activity and gating of many different ion channels to include the epithelial Na+ channel (ENaC). To date, understanding of the biophysical interaction between ENaC and PIP2 has been limited to indirect observation using functional assays. The cryoEM structure of trimeric ENaC containing α, β and γ subunits was recently elucidated. However, the cytoplasmic amino and carboxy termini, which are believed to bind PIP2, were not resolved in earlier structural analyses. Thus, structural insight about how intracellular ENaC termini might interact with PIP2 remains obscure. In this study, we used steady state intrinsic fluorescence and microscale thermophoresis to compare the binding affinities of PIP2 and its analogues to synthetic peptides corresponding to putative PIP2 binding sites within the amino and carboxy termini of ENaC. We found that the extreme amino terminus of the β ENaC subunit has the strongest electrostatic interaction with the PIP2 headgroup (Kd ~4-10 uM). We also identified two other much weaker PIP2 binding sites within the cytoplasmic termini of γ and β ENaC subunits. Using the CIBN-CRY2 optogenetic dimerization system to modify cellular levels of PIP2, we showed that removal of the 5' phosphoryl group from PIP2 reduces Na+ entry into cells transfected with ENaC; and that mutation to the PIP2 binding sites in γ and β ENaC likewise reduce PIP2-dependent Na+ entry into cells transfected with ENaC. These results are consistent with PIP2 binding to ENaC playing a structural role in stabilizing the open conformation of channel via moderate and weak electrostatic interactions within the cytoplasmic termini. This work is supported by NIH/HHLBI T32 HL07446 and NIH/NIDDK R01 DK113816 to JDS.

6.17

Effects of dietary habits on hypertension among medical students

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Hypertension is one of the leading public health problems in most countries. This study was conducted to find out how dietary patterns can affect or cause hypertension and its prevalence among the population on medical school campus. The cross-sectional study involved a survey of 90 respondents aged 16 to 57 years using the random sampling technique. Google forms were used to collect the data and data analysis was done using Microsoft excel sheets. The results were based majorly on two factors i.e. diet and lifestyle. Considering the analysis on these factors, only 4.5% participants had high cholesterol intake, whereas 16.6% participants take heavy amounts of sugars and only 3.3% participants had high caffeine intake. Prevalence of smoking was 11.1% and heavy alcohol consumption was 4.07%. Contradictory to the expected results, only 6.7% population was found to be diagnosed by hypertension while 40-45% of people among the selected population were at risk of developing hypertension. 30.7% population did not have any exercise habits which is a risk factor leading to hypertension.

6.18

A mechanism to reduce cysts in autosomal dominant polycystic kidney disease by inducing fluid absorption via CFTR, NHE3 and ENaC.

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Autosomal dominant polycystic kidney disease (ADPKD), caused by malfunction of either PC1 or 2, is associated with progressive enlargement of cysts, leading to a decline in function and renal failure. We demonstrated previously that VX-809, a CFTR corrector, conventionally used to manage cystic fibrosis, reduces cyst growth in mouse models. To address the mechanism of how this occurs, we used proximal tubule-derived, cultured Pkd1-knockout cells and the Pkd1fl/fl; Pax8rtTA; TetO-cre mouse model which, when treated with doxycycline, allows the ablation of PC1 in renal tubular epithelial cells. These mice, when injected with doxycycline develop multiple large cysts. We found that cysts are reduced when the mice are treated with VX-809 and renal function improved. VX-809 treatment of cultured Pkd1-knockout cells increased by two-fold the activity of the sodium proton exchanger, NHE3, (as measured by the rate of change in intracellular pH induced by changes in extracellular Na+). To study this further we assessed the location of NHE3 in the cystic kidneys using confocal microscopy. In mice treated with doxycycline, NHE3 was present in large cysts but was primarily colocalized with Rab11, a marker of recycling endosomes. In the mice treated with both doxycycline and VX-809, NHE3 now colocalized with the plasma membrane marker E-cadherin consistent with the increase in NHE3 activity. Similarly, VX-809 also increased the apical localization of ENaC. In mice treated with both doxycycline and VX-809, CFTR protein levels increased. When mice were treated with doxycycline and large cysts developed CFTR was colocalized with calnexin, the ER marker, and with E-cadherin but when mice were treated with doxycycline and VX-809, surprisingly CFTR colocalization with the Na+/K+ ATPase), a marker for the basolateral membrane, increased 2 fold. Interestingly, basolateral localization of CFTR occurs in the sweat duct, a normally Cl-absorbing epithelium. Conclusion: The data suggest that VX-809 reduces cyst size in the PC1-null mice by promoting an absorptive phenotype. Given that administration of VX-809 is safe, this drug potentially offers a new way to treat patients with ADPKD.

7: SYMPOSIUM 3A: SIGNALING AND REGULATION OF ENAC AND OTHER EPITHELIAL CHANNELS/ TRANSPORTERS

7.1

ENaC regulation by the mTORC2-SGK1 signaling module

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Aldosterone and ENaC play central roles in potassium (K+) homeostasis, and maintaining normal blood K+ concentration by controlling the driving force for K+ secretion in principal cells of the distal nephron. SGK1, a key intermediary between aldosterone and ENaC, is under dual regulation: the aldosterone-regulated mineralocorticoid receptor stimulates SGK1 gene transcription, while the master kinase, mTORC2 (the type 2 mTOR complex), phosphorylates SGK1 to stimulate its activity. The physiologically relevant regulators of mTORC2 remain poorly characterized. We have found that in principal cells, both K+ itself and angiotensin II act directly in distal nephron principal cells to activate the mTORC2-SGK1 signaling module and thereby stimulate ENaC. The K+ effect requires basolateral Kir4.1 channels (possibly in the form of Kir4.1/5.1 heteromultimers) but does not depend on changes in aldosterone, or on enhanced distal delivery of Na+ from upstream nephron segments. The angiotensin II effect is mediated by AT1R and requires PKC activity, which induces a change in subcellular localization of mTORC2. Mechanisms of specificity and differences between angiotensin II and K+ will be discussed.

7.2

Role of microRNAs in aldosterone signaling and ENaC regulation

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MicroRNAs (miRs) are non-coding RNAs between 18-23 nucleotides long and function primarily to degrade target mRNA and prevent protein synthesis. The role of miRs is increasingly being realized in many aspects of kidney physiology, from development, ion homeostasis and in disease. Aldosterone regulates miR expression in the aldosterone-sensitive distal nephron where they act in concert with long established protein participants to fine-tune aldosterone signaling. Aldosterone regulated miRs serve two primary functions. First, miRs can alter the expression of channels, transporters and associated proteins to regulate Na+ reabsorption across the distal nephron epithelial cells. Our previous studies identified 2 regulatory proteins that serve this purpose. This first involved upregulation of the scaffold protein Ankyrin-G (ANK3). ANK3 facilitated the delivery of ENaC to the apical surface of CCD cells to increase Na+ transport. Follow-up studies investigated the miRs that were induced by aldosterone and we identified a cluster of miRs that were increased in response to aldosterone stimulation, miRs-23,34,27. These miRs targeted the endocytic accessory protein intersectin 2 (Itsn2) to inhibit ENaC endocytosis. A reduction in Itsn2 expression reduced the formation of clathrin coated pits that endocytose ENaC resulting in increased ENaC residency at the apical surface of mouse cortical collecting duct cells (mCCDs). Both pathways increase ENaC expression at the apical surface of CCD cells resulting in increased Na+ transport in line with the characterized role of aldosterone. The second function of the miRs is to act as local negative feedback regulators of aldosterone signaling. Several miR groups are upregulated by long-term aldosterone stimulation to feedback and inhibit elements of the renin-angiotensin-aldosterone signaling cascade (RAAS). In mice this includes members of the miR-466 family, and in mouse and humans it involves the miR-17~92 cluster (specifically miR-19). Upregulation of these miRs after extended aldosterone stimulation in mCCD cells and in mouse kidney (24-96hrs) results in reduced expression of the mineralocorticoid receptor (MR) and the serum and glucocorticoid kinase (SGK1). By chronically inhibiting proteins that are needed for aldosterone signal transduction in the CCD, the miRs act as a rheostat to prevent overactivity that would be associated with extended aldosterone stimulation. By preventing the upregulation of these miRs we can demonstrate that MR and SGK1 expression is increased and that the negative feedback by aldosterone-induced miRs is blocked. A significant increase in both ENaC-mediated transport and aldosterone sensitivity of the mCCD cells results. The action of the miRs is reminiscent of other physiological pathways and constitutes a form of aldosterone escape that prevents excessive sodium reabsorption. The miR pathways involved in altering sodium transport directly or acting as negative feedback regulators will be presented. Funding source: NIH-NIDDK R01: DK102843

7.3

AMPK regulates ENaC via a tripartite inhibitory complex

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The metabolic sensor AMP-activated protein kinase (AMPK) inhibits ENaC, a key regulator of salt reabsorption by the kidney and thus total body volume and blood pressure (1). Earlier work demonstrated that AMPK regulates ENaC by promoting the association of the E3 ubiquitin ligase Nedd4-2 with ENaC with subsequent ENaC ubiquitination and degradation in various epithelial tissues (2). Recently, we found that AMPK promotes the association of the PAK-interacting exchange factor β 1Pix, 14-3-3 proteins, and the ubiquitin ligase Nedd4-2 into a complex that inhibits ENaC by enhancing Nedd4-2 binding to ENaC and ENaC degradation. Functional β 1Pix is required for

ENaC inhibition by AMPK and promotes the AMPK-dependent phosphorylation of Nedd4-2 at a site critical for Nedd4-2 stability in mouse kidney cortical collecting duct (CCD) cells (3). New findings reveal that AMPK also directly phosphorylates β 1Pix in vitro. Among several AMPK phosphorylation sites on β 1Pix detected by tandem mass spectrometry, one site was validated as functionally significant. Compared to wild-type β 1Pix, overexpression of a phosphorylation-deficient β 1Pix mutant significantly attenuated ENaC inhibition and the AMPK-activated interaction of both β 1Pix and Nedd4-2 to 14-3-3 proteins in CCD cells. Overexpression of a β 1Pix deletion-tract mutant unable to bind 14-3-3 proteins decreased the interaction between Nedd4-2 and 14-3-3 proteins, suggesting that 14-3-3 binding to β 1Pix is critical for the formation of a β 1Pix/Nedd4-2/14-3-3 complex. With expression of a general peptide inhibitor of 14-3-3-target protein interactions (R18), binding of both β 1Pix and Nedd4-2 to 14-3-3 proteins were reduced, and AMPK-dependent ENaC inhibition was also attenuated. Altogether, our results demonstrate the importance of AMPK-mediated phosphorylation of Nedd4-2 and β 1Pix, which promotes 14-3-3 interactions with β 1Pix and Nedd4-2 to form a tripartite ENaC inhibitory complex, in the mechanism of ENaC regulation by AMPK.

7.4

Elevated sodium activates the NLRP3 inflammasome in antigen presenting cells through an ENaC-dependent mechanism

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Hypertension is a major public health issue due to the profound risk of developing cardiovascular (CVD) diseases, including stroke, heart failure, and kidney disease. Approximately 50% of hypertensive patients are salt-sensitive, and reducing dietary sodium (Na+) decreases both blood pressure and CVD risk. The precise mechanisms of how elevated Na+ leads to hypertension are still not well defined. The role of the renal amiloride-sensitive epithelial Na+channel (ENaC) in blood pressure regulation has been well studied, however, we recently found that dendritic cells (DCs) in response to increases in extracellular [Na+] exhibit an ENaC-dependent activation of NADPH-oxidase, superoxide production, isolevuglandin (IsoLG)-protein adduct formation, and cytokine secretion which promote hypertension. In this study, we hypothesized that elevated Na+activates the NLRP3 inflammasome in antigen presenting cells (APCs) through an ENaC-dependent mechanism. To test this hypothesis, we isolated human monocytes from 11 volunteers and cultured them in either normal salt (150mM NaCl) or high-salt (190 mM NaCl) for 72 hours. Using high-throughput sequencing of cDNA (RNAseq), we found that high-salt treatment markedly increased inflammasome component NLRP3 (1759 ± 260.8 vs 2994 ± 487.6 relative expression, $p<0.05$), pyroptotic and apoptotic caspases, and pro-inflammatory cytokines, including IL-1β (1144 ± 350.2 vs 4160 ± 785.0 relative expression, $p<0.01$) transcription in human-derived monocytes. In additional experiments, we cultured mouse splenocytes in normal-salt or high-salt media with or without co-treatment with the ENaC inhibitor, amiloride (20 mM). Using flow cytometry, we found that high-salt increased both monocyte and DC production of IL-1β, which was confirmed through an ELISA assay detecting release of IL-1β (2.131± 0.733 vs 12.75 ± 1.108 pg/mL, $p<0.0001$) into the culture media. Treatment with amiloride prevented production of IL-1β (12.75 ± 1.108 vs 1.905 ± 0.3495 pg/mL, $p<0.0001$) in both monocytes and DCs. Our findings suggest a role for ENaC-dependent NLRP3 inflammasome activation in APCs in response to high-salt diet, which may represent a promising approach to treatment of salt-induced hypertension.

7.5

Postprandial effects on ENaC-mediated sodium absorption

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Recent studies have validated that postprandial increases in insulin directly influence renal Na+ transport to reduce urinary Na+ excretion following a meal. While there are a number of renal transporters that respond to insulin stimulation, which of these transporters are involved in this Na+ sparing mechanism under normal physiological conditions is not fully established. The epithelial sodium channel (ENaC) is the rate limiting step for sodium reabsorption in the distal nephron and its regulation by insulin is well established; however, the regulatory effect of acute hyperinsulinemia (as would result from a meal) on ENaC under normal conditions was previously unknown. This study hypothesized that increased ENaC activity in the postprandial time frame contributes to Na+ reclamation to prevent Na+ wasting from the osmotic load of a meal. To test this, we gave fasted Sprague Dawley a high carbohydrate supplement to simulate a meal and analyzed changes to single-channel ENaC activity and protein expression. Additionally, we assessed protein expression of other renal transporters involved in sodium homeostasis known to be regulated by insulin signaling. As ENaC is classically regulated by the renin-angiotensin-aldosterone system (RAAS) hormones, we also interrogated circulating levels of RAAS metabolites to exclude them as contributing to changes in ENaC activity. We found that 4 hours post carbohydrate stimulus, ENaC open probability increased relative to controls in split-open isolated collecting duct tubules (0.34 ± 0.06 vs. 0.70 ± 0.07, $n \geq 5$, $p < 0.01$), while the number of channels at the surface and total ENaC α- and β -subunit levels remained unchanged. We also examined expression levels of the Na+-Cl- cotransporter (NCC), Na+-K+-2Cl- cotransporter (NKCC2), and Na+-K+-ATPase (NKA); however, there were no significant differences in expression of NCC, NKCC2, or NKA. Additionally, using mass spectrometry-based analysis, we interrogated different metabolites of RAAS signaling between the stimulated and fasted rats and found no significant changes in aldosterone or angiotensin metabolite levels. These results demonstrate that acute hyperinsulinemia following a meal increases ENaC activity independent of the RAAS signaling cascade. Our study demonstrates that ENaC regulation via insulin is a likely contributor to an evolutionary mechanism for preserving sodium and volume loss after a meal, and that this regulation is distinct from classical ENaC regulation by RAAS.

8: SYMPOSIUM 3B: SIGNALING AND REGULATION OF ENAC AND OTHER EPITHELIAL CHANNELS/ TRANSPORTERS II

8.1

WNK kinases and cell volume regulation

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When challenged by hypertonicity, dehydrated cells recover their volume through regulatory volume increase (RVI). In most cells, this process is mediated by the bumetanide-sensitive cotransporter NKCC1, which is phosphorylated and activated by the WNK-SPAK/OSR1 pathway. How cells sense a reduction in volume and transduce the RVI signal despite a strengthened ionic milieu that favors low WNK activity remains unclear. Here, I will describe our efforts to unravel this mechanism using a combination of high-speed live cell imaging, optogenetic, and biochemical approaches in gene-edited cells. We have found that during cell shrinkage and macromolecular crowding caused by reduced cell water, WNK1 dynamically condenses into punctate liquid droplets, leading to pathway autoactivation, cytosolic exchange, and NKCC1 phosphorylation within the RVI time course. The formation of WNK1 condensates was driven by its large intrinsically disordered C-terminus, whose strong regional disorder tendency was highly conserved despite poor sequence

homology across evolution. This disorder-encoded phase behavior allows WNK kinases to react to a crowded cytosol, bypass the ionic strength problem caused by cellular dehydration, and rescue cell volume via mass action. Thus, our findings indicate that in addition to their well-appreciated role as chloride sensors, WNK kinases are physiological crowding sensors that phase separate to coordinate a conserved hyperosmotic stress response. I will wrap up by discussing how crowding-induced phase transitions in the WNK signaling pathway might play a role in DCT physiology, where the WNK-SPAK/OSR1 pathway forms WNK bodies – membraneless electron-hypodense condensates that amplify the potassium stress response via KS-WNK1, a functional disordered scaffold for WNK kinases.
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8.2

From SPLUNC1 to SPX-101, novel peptidomimetics to treat sodium hyperabsorption in the CF lung

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CF airways are characterized by abnormal anion/cation transport that promote airway surface liquid (ASL) dehydration, causing a detrimental reduction in mucus clearance. Impaired mucociliary clearance promotes mucus plugging, bacterial colonization and chronic inflammation. Restoration of ASL volume to physiological levels can be achieved by reducing Na+ absorption via inhibition of the epithelial Na+ channel (ENaC). We have previously shown that the secreted airway protein, short palate lung and nasal epithelial clone 1 (SPLUNC1), can inhibit ENaC via direct binding of SPLUNC1's N-terminal S18 region to ENaC subunits. SPLUNC1 is an allosteric inhibitor of ENaC. Indeed, SPLUNC1's S18 region binds extracellularly to ENaC leading to rapid, NEDD4-2-dependent ubiquitination and internalization of ENaC. SPLUNC1-mediated ENaC inhibition is diminished in CF patients, both due to (i) pH-sensitive inhibition of SPLUNC1-ENaC interactions in the CF environment and (ii) neutrophil-elastase-mediated degradation of SPLUNC1 in CF ASL. However, therapeutic inhibition of ENaC may serve to restore ASL hydration and mucociliary clearance, potentially reversing and preventing progression of airway obstructive disease in CF. We have developed a SPLUNC1 peptidomimetic, called SPX-101, that is resistant to degradation by neutrophil elastase. Inhaled SPX-101 does not cross the epithelial boundary and hence, has little effect on ENaC in the kidney. Further, this peptide increases ASL height in CF airway cultures, causes an increase in mucus clearance rates in sheep treated with CFTR inhibitors and prolongs survival in bENaC overexpressing mice. In this talk, we shall discuss the progression from SPLUNC1 to S18 to SPX-101 , as well as preclinical data in CF patients with Class 1 CFTR mutations, that are not responsive to Vertex compounds will also be discussed. SPX-101 is currently being evaluated in CF patients in ongoing clinical trials.
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8.3

Direct effect of potassium on ENaC regulation and potassium secretion in collecting duct cells: role of mTORC2/SGK1 signaling

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The balance between potassium (K+) intake and excretion is crucial in maintaining electrolyte homeostasis in mammals. In the principal cells of the distal nephron, electrogenic Na+ transport by the epithelial sodium channel (ENaC) generates the electrical driving force for K+ secretion across the apical membrane. The role of aldosterone in the control of ENaC regulation has been well studied. Aldosterone stimulates the gene transcription of the ENaC-regulatory kinase, SGK1 which subsequently undergoes phosphorylation and activation by mTOR

complex 2 (mTORC2). However, the possibilities that ENaC can also be regulated in an aldosterone independent mechanism have been demonstrated by several studies. In this study, we identified a direct effect of K+ in cortical collecting duct principal cells to stimulate ENaC and enhance K+ secretion, and investigated the underlying mechanism. In mice an acute K+ load enhances benzamil-inducible Na+ excretion (a reflection of ENaC activity) in parallel with benzamil suppressible kaliuresis indicating increased K+ excretion prior to significant change in plasma aldosterone level; moreover, this early effect on ENaC is mineralocorticoid receptor (MR) independent. Whole cell patch clamp studies in intact, isolated mouse collecting duct, and electrophysiological studies in cultured collecting duct cells reveal a rapid alteration in ENaC activity in response to changes in extracellular [K+] on the blood side of the cells, involving basolateral membrane K+-channel activity. The stimulation of ENaC activity by increased extracellular K+ is mediated by mTORC2/SGK1 signaling pathway. The K+-induced increase in SGK1 phosphorylation by mTORC2 is selective and highly dependent on WNK1, but interestingly, without the requirement of WNK1 kinase activity, which is distinct from the regulation of NCC by WNK1. Taken together, our data strongly support the idea that K+ is sensed locally by principal cells to activate WNK1-mTORC2-SGK1 signaling cascade to activate ENaC and to stimulate its own secretion. We propose that this local effect acts in concert with aldosterone and increased Na+ delivery from upstream nephron segments to sustain K+ homeostasis. Acknowledgements
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8.4

High mobility group box-1 protein regulates lung epithelial sodium channel activity via the receptor for advanced glycation end products

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Background and Significance: The epithelial sodium channel (ENaC) plays an important role in maintaining proper airway hydration and has been shown to be hyperactive in the cystic fibrosis (CF) lung. In CF, ENaC dysfunction leads to dehydrated airways, chronic inflammation, and a progressive decline in lung function. High mobility group box-1 (HMGB-1) is a nuclear and cytoplasmic protein known to play an important role in activating inflammatory responses and has also been shown to be elevated in the CF lung. Our aim was determine if a causal connection exists between elevated HMGB-1 and hyperactive ENaC in the CF lung. Successful completion of our studies can lead to new therapeutic strategies for CF. Hypothesis: We hypothesize that HMGB-1 signals via the receptor for advanced glycation end products (RAGE) to increase ENaC activity and inflammation in the airways. Methods and Results: We measured equivalent short circuit current (Isc) and single channel ENaC open probability (Po) in human small airway epithelial cells (SAEC) in the presence or absence of human HMGB-1 peptide (40 nM). HMGB-1 significantly increased amiloride-sensitive ENaC Isc by -6.5± 0.93 μA/cm2;$p<0.01$ after 5 minutes, while pretreatment with 1 μM FPS-ZM1 (a RAGE inhibitor) attenuated HMGB-1 effects. HMGB-1 increased ENaC Po from 0.15± 0.03 to 0.28± 0.04;$p<0.01$. Pretreatment with FPS-ZM1 for 1 hour inhibited the HMGB-1 mediated increase in Po. Mean absolute total immune cell counts were significantly higher in bronchoalveolar lavage fluid (BALF) from mice intraperitoneally (IP) injected with HMBG-1 (27.4×103± 8.8×103 cells) vs vehicle injected mice (5.2×103± 1.3×103 cells);$p<0.05$. Masson’s Trichrome labeling showed that IP injected HMGB-1 significantly increased pulmonary fibrosis. IP injection of HMBG-1 and FPS-ZM1 did not lead to an increase in pulmonary fibrosis. Flow cytometric

analysis of HMGB-1 and vehicle injected mice showed significant increases in IL-1β, IL-10, IL-6, IL-27, IL-17A, IFN-β, and GM-CSF;$p<0.05$. Confocal microscopic imaging and analysis of SAECs indicate that CF cells have more cytoplasmic HMGB-1, along with a larger nucleus (277± 8.7 μm2) than normal SAEC (187± 5.7 μm2);$p<0.01$. Treating CF SAEC with 1 μM amiloride for 24 hours significantly decreased cytoplasmic HMBG-1 and nucleus size to 198± 6.8 μm2;$p<0.01$. Conclusions: HMGB-1 signals via RAGE to increase ENaC Po and inflammation in the CF lung. HMGB-1 activation of ENaC may play an important role in osmotic stress, nuclear swelling, and increases translocation of nuclear proteins. Outcomes from this study proposes that HMGB-1 signaling to RAGE plays a critically important role in perpetuating lung ENaC dysfunction and inflammation in CF patients. Funding This work was supported by NIH 1 R01 HL137033-01A1 awarded to MH.

9: SYMPOSIUM 4A: ENAC BIOGENESIS AND TRAFFICKING

9.1

Novel mechanisms of diuretic resistance revealed by single cell analysis

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Furosemide, a widely prescribed diuretic for edema-forming states, inhibits sodium reabsorption in the thick ascending limb (TAL) of the nephron. Tubular adaptation to diuretics has been observed but the range of mechanisms along the nephron has not been fully explored. Using morphometry, we found that furosemide induces renal tubular epithelial hyperplasia selectively in distal nephron segments. In contrast, we found progressive cellular hypertrophy in both proximal and distal nephron segments. Using single cell RNA sequencing we detected furosemide-induced apoptosis and ~50% fewer TAL cells in mice and significant down-regulation in human sections. We also detected an increase in DCT cells. By differential expression in specific cell types, changes in hypertrophic and ion transport genes with furosemide treatment were enriched in distal nephron segments. For example Pkd1, Akt, Foxo3 were significantly and differentially expressed in hypertrophied distal nephron segments. We also mapped a gradient of transcriptional changes congruent with enhanced distal sodium transport specific to each of four cell clusters. Collectively, we demonstrate cell type-specific forms of tubular remodeling including selective hyperplasia with growth of DCT cells, progressive hypertrophy and expression of gene signatures related to cell growth in the distal nephron, death of TAL cells, and heterogeneity of differentially expressed genes across cell clusters. These findings highlight novel mechanisms of diuretic resistance, provide a repository of transcriptional responses to a common drug, and expand the implications of long-term loop diuretic use for human disease.

9.2

Regulation of renal ion transport by ubiquitylation and phosphorylation networks

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The kidney plays a major role in ion homeostasis, and is able to maintain constant plasma concentration of ions such as sodium, potassium and chloride. Thereby, signaling pathways involving ubiquitylation and phosphorylation have been shown to play a major role in the control of ion balance. Ubiquitin-protein ligases such as NEDD4-2 or KLHL3/CUL3 have been shown to have major impact on the activity and the trafficking of ion channels such as ENaC or transporters such as the Na,Cl-cotransporter NCC. These ubiquitylation events can be controlled by protein kinases, as is the case for SGK1 that controls the action of NEDD4-2; on the other hand the WNK kinases WNK1 and WNK4 are negatively controlled by KLHL3/CUL3. I will discuss these mecanisms in my presentation.

9.3

Regulation of ENaC by the ER lumenal, molecular chaperone, GRP170

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The epithelial sodium channel (ENaC) is responsible for sodium reabsorption in a variety of epithelia. In the kidney ENaC is expressed in the distal nephron where it regulates salt and water homeostasis and, therefore, blood pressure. ENaC gain of function mutations result in Liddle syndrome and high blood pressure, whereas loss of function mutations results in Pseudohypoaldosteronism Type 1 (PHA1), salt-wasting and low blood pressure. In the lung ENaC function is linked to the pathology of cystic fibrosis and ENaC is a drug target for cystic fibrosis patients. ENaC is a member of the degenerin family of ion channels and is a heterotrimeric channel composed of an α, β and γENaC subunit. Each subunit is composed of two transmembrane domains, a large extraceullar loop and short N- and C- termini. While the ENaC subunits are ~30-40% identical they are subject to differential post-translational modifications and cellular regulatory pathways [1]. ENaC subunits are synthesized and assemble in the endoplasmic reticulum (ER). However, ENaC subunits that fail to assemble are targeted for degradation by the ER associated degradation (ERAD) pathway. ERAD substrates are recognized by molecular chaperones, ubiquitinated, retrotranslocated to the cytosol and degraded by the cytosolic 26S proteasome. To characterize the ENaC degradation pathway, we developed a yeast ENaC expression system, which we used to identify a unique subset of molecular chaperones that are required for ENaC degradation [2]. For example, the ER lumenal Hsp70-like chaperone, Lhs1, is required for αENaC degradation, but is dispensable for the ERAD of the β or γENaC subunit [3]. In contrast, assembly of the ENaC heterotrimer blocks Lhs1 targeted degradation of the α-subunit. Specifically, we found that intersubunit interactions between ENaC transmembrane domains are required to block Lhs1-dependent degradation [4]. We confirmed that the mammalian homolog of Lhs1, GRP170, also targets αENaC for ERAD. Conversely, when all three ENaC subunits are present GRP170 promotes forward trafficking and the surface expression of ENaC. Next to study the role of GRP170 in regulating ENaC in the mammalian kidney, we generated an inducible, kidney-tubule specific, GRP170 knock-out (KO) mouse. We hypothesized that in the absence of GRP170 ENaC surface expression would be reduced. Preliminary data suggest that GRP170 is required for efficient salt-reabsorption and water retention. In addition, the GRP170 KO mice display elevated aldosterone levels, reduced plasma Na+ levels and increased plasma K+ levels, all of which are consistent with ENaC dysregulation. The role of GRP170 in the quality control, trafficking and regulation of other ion channels and transporters is unknown and is the subject of both ongoing but future studies by our group. Supported by Pittsburgh Center for Kidney Research (DK79307) and R01DK117126 (PI: Buck). 1. Buck and Brodsky, Curr Opin Nephrol Hypertens. 2018 2. Buck et al., Mol Biol Cell. 2010 3. Buck et al. J Biol Chem. 2013 4. Buck et al., Biochem J. 2017

9.4

A conserved region in the N-terminus of α-ENaC regulates proteolytic processing during anterograde transport

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Expression, function and regulation of epithelial sodium channels (ENaC) is required for salt homeostasis. The α-, β - and γ-ENaC subunits assemble into heteromers in the endoplasmic reticulum (ER), which are further processed and proteolyzed in the Golgi/TGN en route to the plasma membrane. Surprisingly, the molecular determinants involved in anterograde transport of ENaC from the ER to the plasma membrane remain largely uncharacterized. We have recently demonstrated that a splice variant of

the rat α-ENaC (deletion of aa 34-82) reaches the plasma membrane in a form that is not processed by furin in Xenopus oocytes. Here, we show that a similar sequence in the human α-ENaC, when excised, reaches the mammalian cell surface in a predominantly uncleaved form. Using deletion mutagenesis, we have identified a 13-amino acid stretch in the N-terminus of human α-ENaC that regulates processing by furin-like convertases. Using cell surface biotinylation and whole-cell INa measurements, we have determined that ENaC lacking the 13 amino acid stretch reach the cell surface as efficiently as WT ENaC and could be activated by exogenous protease treatment. Hughey and colleagues have identified a C-terminal region in α-ENaC that regulates exit from the ER. Our results suggest a role for the N-terminus of α-ENaC in a distal compartment of the anterograde transport pathway. Identification of trafficking branch points that mediate impairment of furin-mediated processing will help illuminate the anterograde transit routes for surface expression of ENaC.

11: POSTER SESSION 2

11.1

Myeloid mineralocorticoid receptor contributes to lung inflammation and vascular remodeling in experimental pulmonary hypertension

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Objective: Previous studies have indicated that mineralocorticoid receptor (MR) regulates systemic vascular function and contributes to cardiovascular disease by promoting cell proliferation and fibrosis, but the underlying mechanism remains unclear. We hypothesize that myeloid-MR plays a pivotal role in the cross-talk between macrophages (MØs) and pulmonary fibroblasts, promoting pulmonary inflammation-mediated vascular remodeling of pulmonary hypertension (PH). Methods: Male myeloid-specific MR knockout (MyMR-KO) mice and their littermate controls (MyMR-intact) were exposed to Sugen5416(Su)/hypoxia to induce PH or normoxia for 4 weeks. We measured right ventricular (RV) hemodynamics, cardiomyocyte size, pulmonary vascular muscularization and collagen deposition, and perivascular lung inflammation. In vitro, human pulmonary adventitial fibroblasts (hFibs) were treated with conditioned medium from MyMR-KO MØs (vs. MyMR-intact) and expression of profibrotic genes was measured. Results: SU/hypoxia produced similar increases in RV systolic pressure and RV mass in both MyMR-KO and MyMR-intact mice. However, MyMR-KOs showed attenuated pulmonary vascular remodeling compared with MyMR-intact mice with decreased muscularization and arterial wall collagen deposition. RV cardiomyocyte area was also reduced in MyMR-KO mice. Moreover, lung inflammation was attenuated in MyMR-KO mice with down-regulation of TNFα expression and reduced infiltration of Galectin-3 (Gal-3) positive cells in the lungs compared to MyMR-intact mice. When exposed to conditioned medium from MyMR-KO MØs, cultured hFibs significantly decreased mRNA expression of profibrotic genes: collagen Iα1 (Col IA1), transglutaminase 2 (TG2) and α-smooth muscle actin (α-SMA), but not Ki67, a proliferative gene. Conclusions: Our results suggest that MR in MØs contributes to experimental PH via increased accumulation of Gal-3 positive inflammatory cells in the perivascular area, followed by production of paracrine factors that activate adventitial fibroblasts towards a profibrotic phenotype.

11.2

Endothelial mineralocorticoid receptor deletion protects female mice from obesity-induced endothelial dysfunction and modulates vascular TRPV4 expression and function

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Background: The incidence of obesity is increasing, particularly among women. One of the earliest vascular consequences of obesity is

endothelial cell (EC) damage, resulting in impaired arterial dilation. Extracellular calcium entry via transient receptor potential vanilloid 4 (TRPV4) channels can activate endothelial nitric oxide (NO) synthase (eNOS) and/or calcium-activated potassium (K) channels to mediate microvessel dilation. Levels of the mineralocorticoid hormone aldosterone are elevated in obesity and our lab recently demonstrated that deletion of the mineralocorticoid receptor (MR) from EC in mice rescues endothelial dysfunction specifically in obese females by inducing a switch from K channel to NO-mediated dilation. The combined role of EC-MR and TRPV4 in regulating this process has not been explored in females or during obesity. Methods: Female EC-MR wildtype or knockout (EC-MR-KO) littermates were fed control or high fat diet for 12 weeks to produce a model of diet-induced obesity with elevated fasting glucose. Mesenteric vessels were isolated to quantify mRNA by QRT-PCR or protein by immunoblotting. Mesenteric microvessel function was measured by wire myography. Results: Obesity alone did not affect expression of TRPV4, however mesenteric vessel TRPV4 mRNA increased significantly in obese EC-MR-KO mice compared to obese MR-intact mice. Conversely, TRPV4 protein expression was significantly decreased in vessels from obese EC-MR-KO females. TRPV4 function in mesenteric microvessels was measured by direct TRPV4 activation with GSK101 (30nM) or inhibition with GSK219 (100nM) during wire myography. In MR-intact females, the peak GSK101-induced vasodilation was significantly increased by obesity (69± 5.2% in obesity vs 44± 5.2% on normal chow, p=0.006) and this change was lost in EC-MR-KO mice. TRPV4 inhibition significantly increased endothelial-dependent vasodilation to acetylcholine in vessels from obese MR-intact females, but again, had no effect in obese EC-MR-KO littermates. Downstream of TRPV4-mediated calcium entry, K channel activation results in vasodilation. However, the degree of vasodiliation to the IK/SK channel activator NS309 (1μM) was unaffected by EC-MR-KO or obesity. TRPV4, MR, and eNOS can also colocalized in caveolin-rich domains of the plasma membrane, where eNOS is unable to be activated by calcium. eNOS mRNA expression was increased in vessels from EC-MR-KO females on control diet and this effect was abrogated by obesity. Total caveolin protein expression was not changed by exposure to obesity, but is significantly decreased in obese EC-MR-KO mice. Summary: Taken together, these results indicate that EC-MR may directly modulate the expression, activity and/or composition of plasma membrane signaling domains of vascular TRPV4 channels specifically in obese females. This research gives novel insights to the pathophysiology of vascular dysfunction in obesity and the potential importance of sex and obesity-specific treatments, including MR antagonists, for microvascular dysfunction. Grant support: NIH T32HL007609(LAB), R25GM066567(BVC), R01HL095590(IZJ)

11.3

Long extracellular beta strand in ENaC gating

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Epithelial Na+ channel (ENaC) activity is modulated by Na+ self-inhibition which reflects an open probability reduction initiated specifically by extracellular Na+. While many extracellular factors affect ENaC activity through hampering or boosting Na+ self-inhibition, the allosteric mechanisms of this inhibition remain unresolved. Previous studies have implicated extracellular finger, knuckle, thumb and palm domains as well as the junctions between extracellular domains and transmembrane domains in the Na+ self-inhibition. In this study, we investigated the role of the palm domain b10 strand traversing nearly the entire extracellular domain at the trimeric symmetry axis. All twelve b10 residues of mouse aENaC were individually mutated to a Cys residue. Through comparing WT and mutant mouse ENaCs expressed in Xenopus oocytes, we identified two mutations (aV508C and aY517C) that reduced and three mutations (aS507C, aT509C and aN510C) that increased the magnitude of Na+ self-inhibition.

Other seven Cys mutants show a similar Na+ self-inhibition response to WT channels. Both aV508C and aY517C mutants showed an approximately 50% greater amiloride-sensitive current than WT, however, the difference did not reach statistical significance ($p>0.05$), which might be attributed to a limited number of examined cells (n=12-13 in each group). Interestingly, aV508 of mouse ENaC is homologous to aV481 of human ENaC. aV481M was previously reported as a gain-of function variant (PMID: 27582106). We also examined accessibility of the introduced sulfhydryl groups to positively changed MTSET in the first seven mutants (S507 through K512). Only the aS507C and aK512C mutants were modified by externally applied MTSET, evidenced by an irreversible decrease in currents. Our results suggest that the beginning and ending b10 residues in a subunit may contribute to the mechanism of Na+ self-inhibition whereas the majority residues in the middle of the longest b strand are not involved in the Na+ inhibition. Instead, the middle segment of b10 may indeed function as a scaffold as previously suggested for ASIC (PMID: 22842900), to maintain the core structure of the extracellular domain facilitating other peripheral and mobile regions to change conformations in response to various signals. (Supported by DK079307, DK051391 and HL147818)

11.4

ENaC-α agonism can induce endothelial dysfunction in control female mice only independently of endothelial mineralocorticoid receptors

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Emerging clinical evidence indicates that women have a more pronounced cardiovascular benefit to mineralocorticoid receptor (MR) antagonism compared to men. Published data from our group demonstrates that female mice and humans endogenously express higher levels of endothelial MR compared to males, which predisposes females to endothelial mineralocorticoid receptor-induced endothelial dysfunction. In addition, we have shown that leptin-induced aldosterone-mediated endothelial dysfunction in female mice is ablated by amiloride, an inhibitor of the epithelial sodium channel (ENaC). Recent reports indicate that endothelial MR activation induces dysfunction in endothelial cells and that amiloride-sensitive aldosterone-induced Na+ influx can promote endothelial stiffness in female mice. However, whether control female mice are more sensitive to ENaC-like channel-mediated endothelial dysfunction compared to males has not been directly tested. Therefore, we hypothesized that agonism of the α subunit of ENaC, induced by the TIP peptide, could induce moderate endothelial dysfunction in control female mice. We infused male and female Balb/C wild-type mice with TIP peptide (50 mg/dose, 3x) over a period of 7 days. Endothelial-dependent acetylcholine-induced vasorelaxation responses in aorta of female mice were moderately impaired following TIP treatment (2-way ANOVA with repeated measures, $^*P<0.05$), however, no impairment was induced in male mice. No significant differences were observed with TIP treatment in endothelial-independent (sodium nitroprusside) relaxation or phenylephrine-induced constriction responses with TIP peptide treatment in either male or female mice. To test whether ENaC-α agonism is downstream of the effects of endothelial MR activation, we infused TIP peptide into female mice with selective deletion of endothelial MR. We observed that endothelial MR deletion did not protect female mice from ENaC-α agonist-induced endothelial dysfunction ($^*P<0.05$). Neither sodium nitroprusside or phenylephrine responses were affected by TIP peptide in female mice with deletion of endothelial MR. Collectively, these data indicate that ENaC-α agonism induces moderate endothelial dysfunction, independently of endothelial MR expression, in control female mice exclusively. This indicates that endothelial MR-induced increases in ENaC activity may be a pathway for endothelial impairment in females

under these specific conditions, as other reports indicate positive effects of endothelial ENaC-a activation during bacterial infection.

11.5

Effect of amiloride on epithelial sodium channel among adult Nigerian subjects

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Polymorphisms of the β -ENaC subunit has been recorded among Nigerians. Amiloride has been shown to reduce blood pressure especially among Blacks though with varied results. This study was designed to determine the effect of amiloride on ENaC activity after salt ingestion among adult Nigerians. Age-matched normotensive (NT, n=38) and hypertensive (HT, n=52) subjects participated in the study. Ethical clearance (CM/COM/8/Vol. XXI) was obtained from the College of Medicine, Univ. of Lagos. All subjects gave informed written consent and tests were carried out in accordance with Helsinki Declaration. After baseline measurements, subjects were salt-loaded with 200mmol Na+/day for 5 days. After one week wash-out period, subjects ingested a combination of the salt-load and 5mg amiloride tablets daily for 5 days in subjects with blood pressure >110 mmHg (systolic) and >60 mmHg (diastolic) only. Repeat BP measurements and blood tests were carried out after each intervention. Data were compared with ANOVA and Neuman-Keuls post-hoc tests using GraphPad Prism version 5. Significance was accepted at $p<0.05$. Significant pressor responses to salt-loading were recorded in NT and HT subjects ($p<0.01$). Salt+amiloride resulted in significant decreases in systolic and diastolic blood pressures compared with baseline and salt-loading in NT ($p<0.05$; $p<0.01$ respectively) and HT ($p<0.001$; $p<0.001$ respectively) subjects. At baseline and after salt-loading, plasma Na+ was higher ($p<0.05$) while urine Na+ excretion was significantly lower ($p<0.05$) in HT subjects compared with NT. Following ingestion of salt+amiloride, plasma Na+ was reduced to below baseline levels in both groups. Plasma K+ decreased after salt-loading in both groups and increased significantly ($p<0.05$) after salt+amiloride in HT. Plasma Renin Activity (PRA) was lower among NT at baseline but increased significantly ($p<0.05$) following salt-loading; there was a reduction following salt+amiloride ($p<0.05$). Aldosterone was lower ($p<0.001$) among NT and reduced below baseline in both NT ($p<0.001$) and HT with salt+amiloride. We conclude that amiloride reversed the pressor and other effects of enhanced ENaC activity in adult Nigerians. We acknowledge the Tertiary Education Trust Fund (TETFUND) for funding this work. References: Elias et al., (2014). Clin & Exp Hypertension 41(2):144-151 Pratt JH (2005). J Am Soc Nephrol 16:3154-3159 Baker EH et al., (2002). Hypertension 40: 13-17

11.6

Epithelial sodium channel modulation by acid and anions in salt taste

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The epithelial sodium channel (ENaC) functions in the maintenance of extracellular volume balance. In the tongue, ENaC functions as a salt taste receptor, where ENaC is exposed to wide fluctuations in ionic content. For example, many foods and beverages that we ingest are highly acidic. To test the effects of acid on ENaC, we measured ENaC current. Exposure of ENaC to acid (pH 2.5) increased ENaC current more than two-fold. We found that this increase in current resulted from an increase in open-state probability. In human subjects, we found that exposure of the tongue to acid enhanced salt taste. Foods and beverages can also vary in their anion composition, and in previous work, we identified anion binding sites in the extracellular domain of ENaC. In the presence of Cl-, we found that acid enhanced ENaC current. Likewise, acid stimulated ENaC when Cl- was replace with Br-, phosphate, gluconate, or a number of other anions. In contrast, when Cl- was replaced with sulfate, acid failed to increase ENaC current. To determine a dose-response

relationship for sulfate, we tested mixtures of Cl- and sulfate. Half-maximal inhibition occurred at ~5 mM sulfate, a concentration which is present in some foods and beverages. Through site-directed mutagenesis, we identified a potential anion binding site at the interface between the β and γ subunits that was necessary for the response to sulfate. In summary, we found that ENaC activity is modulated by acid and by sulfate. This modulation may alter our perception of salt in foods and beverages.

11.7

The stimulation of the basolateral Kir4.1/Kir5.1 in distal convoluted tubule is involved in augmenting NCC activity of Nedd4-2-deficient mice.

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Background: We have previously demonstrated that Nedd4-2 inhibits Kir4.1/Kir5.1 by increasing Kir4.1 ubiquitination. The aim of the present study is to test hypothesis that deletion of Nedd4-2-induced stimulation of NCC is in part achieved by increasing Kir4.1/Kir5.1 activity in the DCT. Methods: We have used electrophysiology, immunoblotting, immunostaining and renal clearance to study the basolateral Kir4.1/Kir5.1 channel activity in the DCT and to examine the activity of thiazide-sensitive Na-Cl cotransporter (NCC) in WT, Ks-Nedd4-2 knockout (KO) and Ks-Kir4.1/Nedd4-2 double KO mice. Results: Deletion of Nedd4-2 increased the activity and expression of basolateral Kir4.1 in the DCT and hyperpolarized DCT membrane. Also, the expression of phosphorylated NCC (pNCC) / total NCC (tNCC) and thiazide-induced natriuresis was significantly increased in Ks-Nedd4-2 KO mice. To test the possibility that Nedd4-2 deletion-induced stimulation of DCT hyperpolarization may be involved in stimulating NCC activity in Ks-Nedd4-2 KO mice, we generated Ks-Kir4.1/Nedd4-2 double KO mice. The double deletion of Nedd4-2 and Kir4.1 in the kidney largely abolished the basolateral K+ conductance and depolarized DCT membrane. Moreover, the expression of pNCC/tNCC and thiazide-induced natriuresis were significantly inhibited in the double KO mice. However, deletion of Nedd4-2 or double deletion of Kir4.1/ Nedd4-2 in the kidney increases the expression of full-length and cleaved ENaCα. Finally, Ks-Kir4.1/Nedd4-2 double KO mice have significant renal K+ wasting and were hypokalemic while Ks-Nedd4-2 KO mice were normokalemic. Thus, hypokalemia and renal K+ wasting in the double KO mice were reminiscent to the Ks-Kir4.1 KO mice. Conclusion We conclude that Nedd4-2 regulates the activity of basolateral Kir4.1/Kir5.1 in the DCT and that the deletion of Nedd4-2-induced stimulation of the basolateral K channels is at least in part responsible for increasing NCC activity.

11.8

MLP regulates ENaC activity in renal distal convoluted tubule cells

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The epithelial sodium channel (ENaC) is regulates blood pressure by fine-tuning distal nephron sodium reabsorption. Our previous work has shown that ENaC gating is regulated by anionic phospholipid phosphates including phosphatidylinositol 4,5-bisphosphate (PIP2). The PIP2-dependent regulation of ENaC is mediated by the myristoylated alanine-rich protein kinase C substrate-like protein-1 (MLP-1). MLP-1 is a reversible source of PIP2 at the plasma membrane. Both MARCKS and ENaC are regulated by proteolysis. Our previous work has shown that ENaC proteins are present in distal convoluted tubule (DCT) cells. We examined MLP-1 regulation of ENaC in DCT-15 cells. We wanted to show if ENaC is regulated by MLP-1 cleavage. The properties of DCT-15 cells are similar to those of in situ cells of the DCT-2 nephron segment. We used monolayers of DCT-15 cells on permeable polyester supports to measure transepithelial voltage, resistance, and current. Our results show that DCT-15 cells had

relatively low resistances (206 ± 5.71 Ω-cm2, n=36) and small, but measurable amiloride-sensitive currents (2.87 ± 0.237 μA, n=36), showing that these cells contain functional ENaC. 100 nM aldosterone significantly increased mean DCT-15 cell current (from 2.87 ± 0.237, n=36, to 5.33 ± 0.395μA, n=8). Wild-type MLP-1 ran as a doublet at apparent MW of 40 and 33 KDa despite having 21 KDa predicted MW. To further examine MLP in DCT-15 cells, we constructed several MLP mutants. WT: a full length wild-type protein; S3A: 3 substitutions in the effector domain to prevent phosphorylation; S3D: 3 substitutions in the effector domain to mimic constitutive phosphorylation; GA: one substitution to prevent myristoylated. Each mutant was tagged with either N-terminal 3XFLAG or C-terminal m-Cherry. Transfection with MLP mutants modified current in DCT-15 cells: current was highest in S3A and lowest in S3D; the current after transfection with either construct was significantly different from WT. In Western blots, when transfected with 3XFLAG tagged MLP mutants, the expression of the full length of MLP at 52 KDa) increased in mutant S3A-MLP transfected DCT-15 cells and decreased in S3D-MLP transfected DCT-15 cells. Whereas the expression of the full length MLP was not significantly different fromS3A-MLP and S3D transfected DCT-15 cells when tranfected with cherry-tagged MLP mutants. A small molecular weight protein about 25 kDa was detected in 3X-tagged-MLP-mutant-tranfected DCT cells, and the expression of this possible fragment of MLP in S3D tranfected cells was significantly higher than in S3A transfected cells. In cell transfected with mCherry mutants, full length protein was detected with products corresponding to the WT bands at 40 and 33 KDa (when corrected for mCherry's size) and a third band at lower MW (about 28 KDa). The sum of the small Flag-tagged (N-terminal fragment) and the small mCherry-tagged (C-terminal Fragment) is almost equal to the the size of the full-length protein suggesting that the two bands represent two cleaved pieces ogf MLP-1. Our results suggest a complicated role for proteolytic processing in MLP-1 regulation.
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11.9

The differential effects of aldosterone and high salt on kidney injury

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The steroid hormone aldosterone (aldo) is responsible for mediating the restoration of fluid and electrolyte homeostasis. While it plays this important physiological function, inappropriate chronic elevation of aldo has been shown to increase renal injury and inflammation. However, many of the studies characterizing aldo's role in kidney injury have been conducted in mice provided high sodium via either food or water, with full factorial experiments not being performed to separately analyze the independent effects of aldo and sodium. To further our understanding of how aldo and sodium act alone and in synergy to cause renal damage, we examined four groups of mice: a control group receiving standard 0.4% NaCl chow, a high salt diet (HSD) group receiving 8% NaCl chow for 4 weeks, an aldo group given a subcutaneous minipump implant delivering aldo at 240mg/kg/day for two weeks, and a combination group receiving both HSD for 4 weeks and aldo administration during the final two weeks. Urinary analysis revealed that both aldo alone and the combination treatment caused an increase in urinary protein excretion. However, qPCR showed that while transcript levels of the kidney injury markers KIM1 and NGAL were increased approximately 10-fold with aldo alone, these levels were increased 100-fold with combination treatment. Furthermore, histology revealed that while aldo alone caused some tubule injury, combination treatment led to tubular dilation and indications of fibrosis exceeding those of aldo treatment alone. At a transcript level, genes associated with inflammation, including those producing IL-1b, MCP-1, TNFa, and IL-6, were most elevated with combination treatment as compared to either HSD or aldo alone. Together, these data

emphasize that while aldo alone does lead to renal injury and inflammation, there is a synergistic effect when it is administered with HSD, therefore experiments should be careful to separate the effects of these stressors acting alone versus in combination.

11.10

Characterization of mice with a hypomorphic ENaC γ subunit

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ENaC is expressed in numerous epithelia. In kidney, complete channels include α, β , and γ subunits and participate in Na retention during periods of extracellular fluid volume depletion. Reports have suggested the α and γ subunits are expressed in bone periosteum, where the channel has been postulated to contribute to osteoblast activity. Additionally, renal tubular ENaC activity could influence bone architecture and density through alteration of divalent cation handling. Perinatal mortality of γ subunit knock-out mice has hindered examination of the subunit's physiologic roles. We have developed a viable mouse γ subunit global hypomorph to assist in exploring the requirement for this subunit in various tissues. In these mice, plasma aldosterone levels are elevated, and blood pressures are lower, relative to controls. We observe increased blood K on a high K diet. Bone parameters, including bone mineral density, cortical thickness, trabecular thickness, inter-trabecular spacing, bone tissue volume/total volume, surface/volume, and connectivity density did not differ as compared to litter-mate controls. These results provide no evidence for a role of this subunit in bone physiology. On-going studies are exploring the role of ENaC's γ subunit in other extra-renal tissues.

11.11

Epithelial sodium channel (ENaC) in endothelium modulates vascular reactivity with a high-salt diet

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The epithelial sodium channel (ENaC) is best known for its role in fluid and electrolyte balance, however, it has more recently been shown to be involved in pressure- and flow-induced responses in endothelial cells. In cell culture, endothelial ENaC is upregulated in response to high aldosterone and extracellular [Na+]. While in vivo experiments have been performed examining the role of endothelial ENaC under physiological conditions and in animals given a Western diet, the in vivo effects of endothelial ENaC in the setting of high salt diet (HSD) and aldosterone are unclear. To test this, we divided mice into four treatments: the first group received control chow (0.4% NaCl), the second group received four weeks of 8% NaCl diet, the third group received two weeks of aldosterone administration via a subcutaneous minipump (240mg/kg/day), and the fourth group received both high salt diet and aldosterone. Using pressure myography, we found that none of the treatments significantly reduced acetylcholine (Ach) responsiveness in the thoracodorsal artery (TDA) of mice, but when amiloride was added to the lumen of the vessels to understand the role ENaC in vasodilation, responses were varied among the different treatments. The greatest difference in Ach responsiveness between control and amiloride treated vessels was noted in the high salt diet treated animals. In the majority of the vessels taken from mice treated for four weeks with high salt diet, amiloride treatment shifted the Ach dose response curve to the right, suggesting that ENaC was promoting vasodilation in these vessels. However, when this high salt regimen was extended to eight weeks, amiloride addition to the lumen of vessels shifted the Ach-dose response to the left, suggesting that ENaC was limiting vasodilation at this time point. At both time points, serum aldosterone was decreased as compared to control treated animals as measured by ELISA. Together these data

suggest that aldosterone may not be required for ENaC's expression and activity in endothelial cells. They additionally demonstrate that endothelial ENaC has a role in promoting the vascular dysfunction seen with chronic high salt intake, but that this role may stem from a compensatory mechanism as ENaC seems to promote vasodilation in animals given a high salt diet for shorter periods of time. Ongoing studies are examining the role of nitric oxide and eNOS phosphorylation status in the endothelial response to a HSD.

11.12

Epithelial Na^+ channel activation by cleavage coevolved with the terrestrial migration of vertebrates

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The epithelial Na+ channel (ENaC) is critical to regulating extracellular fluids through its activity in several epithelia, including the aldosterone-sensitive distal nephron and colon, and the airway and alveoli. Human ENaC subunits are subject to activating proteolysis. During processing in the trans-Golgi network, furin cleaves the α subunit twice. This liberates an imbedded 26-mer inhibitory tract, moderately activates the channel, and leaves 30 kDa and 65 kDa fragments as parts of the channel complex. Furin cleaves the γ subunit once. This event has no effect on channel activity, but leaves 18 kDa and 75 kDa fragments as parts of the channel complex. Once at the cell surface, one of a number of proteases may cleave the γ subunit in a region ~35-50 residues distal to the furin cleavage site. This second cleavage event of the γ subunit also releases an imbedded inhibitory tract, 43-residues when prostasin is the second protease, and greatly activates the channel. To examine the evolution of ENaC cleavage sites, we generated a phylogenetic tree of ENaC and ENaC-like subunits to identify key branch points in the evolution of ENaC subunits. The four ENaC subunits arose from three gene duplication events. First, a single gene diverged to an α/Δ precursor and a β /γ precursor. Shortly after, the β /γ precursor diverged to β and γ subunits. These two events occurred in time for the emergence of jawless fishes, as lampreys have α, β, and γ subunits. Later, but prior to the terrestrial migration, α and Δ diverged from the α/Δ precursor; the lobe-finned coelacanth is the first known species to have all four subunits. We observed that the activating cleavage sites in the α and γ subunits appeared around the transition to terrestrial life. Among the fishes, only the Australian lungfish γ subunit had two cleavage sites, and all tetrapod α and γ subunits had two apparent cleavage sites. We ran nested likelihood models in BayesTraits and found a significant relationship between terrestrial status and having two cleavage sites in the α and γ subunits. Both sites showed a statistically significant coevolutionary pattern with the terrestrial state (proximal site, $p = 0.004$; distal site, $p = 0.006$). The sites also showed a coevolutionary pattern with each other ($p = 1x10-7$). In contrast, the C-terminal PY motif, which is important for aldosterone regulation through Sgk1/Nedd4-2, did not show a dependence on terrestrial status ($p = 0.58$). Furthermore, reconstruction of ancestral ENaC subunits predict that neither site was present at key gene duplication events (proximal site, $p = 0.01$; distal site, $p = 0.003$), suggesting that each of the cleavage sites in the α and γ subunits arose independently. The vertebrate terrestrial migration is associated with many changes, including accessibility to dietary Na+, differences in osmotic stress, and the development of lungs. One or a combination of these factors may have provided the selection pressure to develop this form of channel regulation.

11.13

Wnt4(+) cell mapping and changes in ENaC regulatory genes in furosemide-treated mice

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Furosemide, a commonly prescribed loop diuretic to treat hypertension and edema-forming states, increases sodium excretion by inhibiting reabsorption via the sodium-potassium 2 chloride cotransporter (NKCC2) in the thick ascending limb. Chronic use of loop diuretics can lead to resistance and the need for higher doses and/or additional classes of diuretics with adverse effects. Using tubular morphometry and single cell RNA sequencing of vehicle- and furosemide-treated mice, we found that furosemide induces significant nephron segment-specific heterogeneity in tubular remodeling (hyperplasia, hypertrophy, apoptosis) and in gene expression. On examination of differential expression between vehicle and furosemide treated mice, we demonstrate that a small cluster of Wnt4-enriched cells harbor large relative gene expression changes. These cells were markedly enriched for several transcripts from the Wnt signaling system: Wnt4, Wnt7b, and Wnt 9b, compared to CCD principal cells. In pseudotime plots, Wnt4(+) cells preceded CCD principal cells and multiple other cell types, supporting prior observations that Wnt4(+) cells are a development precursor of tubular epithelia. With furosemide treatment, these cells decreased expression of their identifying markers (Wnt4, Wnt7b) and increased markers of principal cells (Scnn1b, Scnn1g, Hsd11b2, and Nr3c1). Ongoing studies seek to understand the functional consequences of these changes toward a principal cell gene programme. In conclusion, furosemide-induced changes in Wnt4-enriched cells may represent transdifferentiation, a novel mechanism of tubular remodeling and diuretic resistance.

11.14

Angiotensin and aldosterone receptor antagonism attenuates angiotensin II- induced hypertension in sprague dawley rats

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The inappropriate activation of the renin-angiotensin-aldosterone system (RAAS) contributes to hypertension and cardiovascular damage. The blockade of the Ang II receptor (via ARB) robustly ameliorates hypertension, more so than the antagonism of the mineralocorticoid receptor (via eplerenone) alone. Moreover, a consequence of chronic MR blockade (MRb) is exacerbation of plasma aldosterone levels, which may contribute to the failure of MRb to completely ameliorate hypertension. However, the combined effects of ARB and MRb simultaneously on arterial pressure and renal Na+ regulation are not well-defined during Ang II-induced hypertension. While we expected ARB alone and in combination would completely ameliorate the Ang II-induced hypertension, we hypothesized that the addition of MRb would have the added benefit of increased UNaV. To test our hypothesis, SBP and urinary Na+ were measured in five groups of Sprague- Dawley male rats: (1) untreated controls, (2) Ang II infused (80 ng/min x 28d), (3) Ang II + AT1 receptor blocker (ARB; 10 mg losartan/kg/d x 21 d), (4) Ang II + MRb (Epl; 100 mg eplerenone/d x 21d), and (5) Ang II + ARB + Epl (combo; 21 d). Both the ARB and combo group attenuated Ang II- induced hypertension, while the Epl treatment only prolonged the onset of AngII- induced hypertension. The Epl treatment exacerbated urinary aldosterone excretion over the 21 days (Epl: 275 ± 119; Control: 64 ± 18 pmol/d), which was confirmed by plasma aldo on day 28. However, the exacerbation of aldo was abolished with the co-treatment with ARB. Despite some group effects in UNaV early in the

study, by day 28, there were no detectable group effects. This lack of a change in UNaV at the end of the study was consistent with a lack of a change in g-ENaC content; however, the combination of both receptor blockers decreased the expression of a- ENaC by 42% when compared to control. These results suggest that during Ang II-dependent hypertension, the contribution of aldosterone to the volume-dependent effects of the hypertension appears to be minimal especially as it pertains the ENaC-mediated component of renal Na+ regulation.

11.15

Serum and glucocorticoid-regulated kinase 1 (SGK1) up-regulates ENaC in the uterus for pro-inflammatory shift in term and preterm labor

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We have recently demonstrated in humans and mouse models that uterine ENaC together with its downstream inflammatory signaling plays an essential role in term and preterm labor (EMBO Mol Med 2018). The surge in cortisol levels, a major glucocorticoid in humans, is observed in pregnant women as approaching toward the end of pregnancy. SGK1 is phosphorylated and activated by glucocorticoids and promotes ENaC expression in many other systems. We therefore investigated possible involvement of SGK1 in regulation of ENaC in the uterus at term and preterm labor. Our results showed that uterine SGK1 is activated at labor in mice. Placental SGK1 expression is upregulated in women with spontaneous preterm labor as compared to those with term labor. Analysis of the human transcriptome database reveals significant and positive correlation of SGK1 with ENaC alpha subunit (ENaCα) as well as labor-associated pro-inflammatory factors in labored birth groups (both term and preterm), but not the non-labored group. In human endometrial cells in vitro, treatment with cortisol enhances ENaCα and COX-2 expression, which is significantly attenuated by SGK1 knockdown. Therefore, these results have suggested a role of SGK1 in up-regulating ENaC in the uterus for pro-inflammatory shift in term and preterm labor.

11.16

Primary aldosteronism decreases insulin secretion and increases insulin clearance in humans

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Primary aldosteronism is associated with an increased risk of diabetes, but the mechanism is uncertain. We enrolled 9 patients with primary aldosteronism and assessed insulin secretion and insulin sensitivity beforte and after 3-12 months after either adrenalectomy (n-6) or medical treatment (n=3). Plasma 8AM Aldosterone (p=0.02) and 11-deoxycorticosterone (p=0.02) decreased after treatment, whereas cortisol was unchanged (p=0.57). Systolic blood pressure (131.1± 3.6 vs 124.3± 7.7; p=0.43), serum potassium (3.7± 0.1 vs 4.1± 0.1 mEq/L; p=0.063), and creatinine (1.1± 0.1 vs 1.2± 0.1 mg/dL; p=0.35) were similar after treatment. During hyperglycemic clamps, we infused glucose to increase plasma glucose to a target of 200mg/dL. The insulin response (218.6± 39.8 vs 403.1± 42.0 pM insulin mean at 90-120 min, p=0.004) and L-arginine stimulated insulin response increased compared to Pre-Treatment assessment. C-peptide results were similar to insulin alterations. Comparison to control subjects matched for age, race, sex, and BMI indicated that insulin secretion after treatment was comparable to healthy individuals. During hyperinsulinemic-euglycemic clamps, insulin clearance (steady state insulin divided by insulin infusion rate) increased after treatment (855± 68 vs 615± 57 mL/min, p=0.031). Insulin sensitivity decreased after treatment (30.7± 2.1 vs 18.5± 1.6 M/I; p=0.016), but disposition index was unchanged (acute insulin response x M/I; p=0.81). Insulin secretion increases after treatment for primary aldosteronism. This effect was explained in part

by decreased insulin clearance. These changes are accompanied by a decrease in insulin sensitivity and no change in the disposition index.

11.17

Differential effects of acute vs. chronic dietary potassium intake on plasma potassium concentration and NCC phosphorylation and expression

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Diets high in potassium are associated with short-term blood pressure reductions and long-term cardiovascular benefits in humans. Dietary potassium may regulate blood pressure via its effects on NCC-mediated sodium excretion. Over the short-term in humans, and over days to weeks in mice, potassium intake has been reported to affect plasma potassium concentration which regulates NCC phosphorylation and protein expression. We investigated the differential effect of acute vs. chronic dietary potassium intake on plasma levels and NCC phosphorylation and expression in which dietary potassium intake was varied over weeks and again over 4 hours in a 2x2 comparison. Mice were fed either a 5% KCl (high, HK) or 0.7% (normal, NK) diet for 18-35 days, fasted 4 hours before sacrifice, and gavaged with 5% of their daily K intake (gavage) or water (vehicle) 30 minutes before sacrifice. Plasma potassium was increased by acute dietary potassium (gavage) in the HK compared to NK group (5.94 ± 0.43 vs. 4.08 ± 0.12 mmol/L respectively, $p < 0.01$). However, it was no different in HK compared to NK mice that did not receive the KCl gavage (vehicle) (3.81 ± 0.07 vs. 3.93 ± 0.03 mmol/L respectively, $p = 0.11$). Concordant with plasma potassium levels, dietary potassium decreased phospho-Thr53 NCC only when gavaged (1.00 ± 0.18 vs. 0.16 ± 0.06, NK vs. HK respectively, $p < 0.05$) and was no different in acute vehicle-gavaged, chronic HK-fed compared to NK-fed mice (1.00 ± 0.10 vs. 0.93 ± 0.27, NK vs. HK respectively, $p = 0.82$). Total NCC abundance was decreased in chronically HK-fed compared to NK-fed mice (1.00 ± 0.09 vs. 0.80 ± 0.22, NK vs. HK respectively, $p = 0.04$). Chronic HK-fed mice that were not fasted had a higher plasma potassium (4.22 ± 0.16 vs. 3.79 ± 0.13, $p < 0.05$) and lower pNCC abundance compared to NK-fed mice (1.00 ± 0.85 vs. 0.24 ± 0.08, NK vs. HK respectively, $p = 0.03$). Chronic HK-fed mice had a trend toward a lower sodium excretion in response to NCC inhibition than mice on a NK diet (0.23 ± 0.02 vs. 0.16 ± 0.01 umol/min, NK vs. HK respectively, $p = 0.10$). In conclusion, chronic high potassium diets reduce total NCC expression which correlates with decreased natriuretic sensitivity to NCC inhibition. In contrast, acute dietary potassium can increase plasma potassium levels and decrease NCC phosphorylation, but does not correlate with NCC activity, as measured by natriuretic sensitivity.

11.18

Age and sex-specific reference ranges are needed for the aldosterone/renin ratio

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Background: The aldosterone/renin ratio (ARR) is the standard screening test for primary aldosteronism (PA). Current guidelines define ARR>70 (pmol/L)/(mIU/L) as abnormal regardless of age or sex. However, fluctuations in female hormones over the menstrual cycle influence the ARR. Objective: To characterise variations in the ARR according to age and sex. Methods: A retrospective analysis of 466 clinically indicated ARRs at Monash Health from December 2016 – June 2018 was conducted. Patients who took spironolactone, oral contraceptive pill, were pregnant or had a known adrenal condition (including untreated PA) were excluded. Results: Among patients aged 20-39 years (N=74), females had significantly higher median aldosterone (373.5 vs 231 pmol/L), lower median renin (16.5 vs 23.5 mIU/L), and higher median ARR (20.75 vs 10.49) than males. However,

females had lower median systolic (135 vs 145 mmHg, p=0.021) and diastolic (89 vs 96.5 mmHg, p=0.007) blood pressure (BP). These sex differences were not observed in the 40 – 59 years (n=161) or 60 – 79 years (n=157) age groups. When females were divided into pre- and post-menopausal groups (at an arbitrary age cut-off of 45 years), those ≤45 had significantly higher median aldosterone levels (364 vs 273 pmol/L, p=0.047), lower systolic BP (139 vs 148 mmHg, p=0.002), but higher diastolic BP (89.5 vs 80 mmHg, p=0.001).

Conclusion: Our findings highlight the need for age- and sex-specific ARR reference ranges, particularly for younger women who have a significantly higher ARR that is not associated with higher BP.

12: SYMPOSIUM 5A: MR IN THE VASCULATURE

12.1

Sex-specific mechanisms of resistance vessel endothelial dysfunction in obesity

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The epidemic of obesity that affects more women than men worldwide is one of the major risk factors for cardiovascular disease. The mechanisms underlying obesity-induced endothelial dysfunction and blood pressure increase have been suggested to be sex-specific. In this talk, I will present data on the pathophysiological role of endothelial mineralocorticoid receptor (MR) activation for the endothelial dysfunction in obese females. I will also show how the perivascular adipose tissue might contribute for the obesity-induced endothelial dysfunction in males. The differences in the molecular mechanisms triggering endothelial dysfunction in male and female obesity support the need for a sex-specific therapeutic approach for patients at cardiometabolic risk.

12.2

Sex differences in the role of the smooth muscle cell mineralocorticoid receptor in cardiovascular aging

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Aging is universal and is the most significant risk factor for cardiovascular disease. Clinical data supports that the time course for developing aging-associated cardiovascular disease occurs differentially in males vs. females. We previously demonstrated that male mice with the mineralocorticoid receptor deleted from smooth muscle cells (SMC-MR-KO) are protected from cardiovascular aging. The purpose of this study was to determine whether there are sex differences in the role of SMC-MR in cardiovascular aging. Vascular MR mRNA expression increases at 12 months of age in males but not until 18 months of age in females. We therefore compared microvascular contractile function and large vessel structure in male and female mice aged 3 months (young adult), 12 months (middle aged) and 18 months (old) with MR-intact to SMC-MR-KO littermates. We previously identified miR-155 as a regulator of the angiotensin II type 1 receptor (AT1R) that is negatively regulated by MR in the aging male vasculature. We now confirm that the rise in MR in aging vessels is accompanied by a concomitant down-regulation of vascular microRNA (miR)-155 at 12 months in males and at 18 months in females, however, this is prevented by SMC-MR-KO in males only. Functionally, we find that AT1R-mediated vasoconstriction of mesenteric resistance vessels is increased at 12 months in males and at 18 months in females and was prevented in SMC-MR-KO mice of both sexes. We next explored aging-related changes in vascular structure by measuring aortic pulse wave velocity to quantify vascular stiffness, a parameter that rises with age in humans and correlates with risk of cardiovascular disease and death. Vascular stiffness increases significantly by 12 months in males but not until 18 months in females and that is prevented by SMC-MR-KO. Carotid fibrosis increased at 12

months in males vs. 18 months in females, and is attenuated by SMC-MR-KO in males only. Cardiac function declines at 18 months in both males and females, but is attenuated by SMC-MR-KO in females only. In summary, the time course and mechanisms of cardiovascular aging are distinct between males and females with SMC-MR playing a differential role in males versus females. Furthermore, these studies suggest that sex-specific therapies may be essential to improve CVD outcomes in the aging population.

12.3

Role of the myeloid mineralocorticoid receptor in vascular inflammation in atherosclerosis

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Background: Elevated plasma aldosterone is associated with increased risk of heart attack and stroke, and mineralocorticoid receptor (MR) antagonists reduce cardiovascular-related mortality. Heart attack and stroke are caused by rupture of atherosclerotic plaques with abundant pro-inflammatory leukocytes. In mouse atherosclerosis models, MR antagonists reduce plaque size and inflammation, particularly the proportion of pro-inflammatory Ly-6Chi myeloid cells. Deficiency of MR in endothelial but not smooth muscle cells attenuates plaque inflammation, but neither reproduced the decrease in pro-inflammatory myeloid cells observed with systemic MR blockade. Here we test the hypothesis that myeloid MR contributes to atherosclerotic plaque inflammation by modulating the myeloid cell phenotype in vivo. Methods and Results: LysM-Cre/MRfl/fl mice were crossed with ApoE-/- mice to generate atheroprone mice with MR specifically deleted from myeloid cells (MyMR-KO). PCR amplification of the floxed MR locus confirmed that the MR was specifically deleted in macrophages by at least 50% and only in MyMR-KO mice. Two month old male and female MyMR-KO and MR-intact littermates were fed high-fat diet for 8 weeks. Aortic root plaque area was significantly decreased in MyMR-KO vs. MR-intact mice (p=0.028), but there was no difference in the percent of the plaque composed of neutral lipid, necrotic core, or collagen content by Oil Red O and Masson's trichrome staining. Immune cell populations within aortic arch plaques were then quantified by flow cytometry. The numbers of macrophages and T cells in the aortic arch were significantly decreased in MyMR-KO vs. MR-intact mice (p=0.007, p=0.041 respectively), while the other populations (granulocytes, monocytes, B cells) were not different by genotype. Although females had larger plaques, they were less inflamed with significantly fewer plaque myeloid and T cells compared to male mice (p=0.013, p=0.008 respectively). Interestingly, the ratio of Ly-6Chi:Ly-6Clo monocytes was significantly increased in MyMR-KO vs. MR-intact mice (p<0.0001), but this was driven by a reduction in Ly-6Clo monocytes in MyMR-KO mice (p=0.020). Myeloid MR knockout did not affect hematopoiesis in the bone marrow or spleen, suggesting that differences in plaque inflammation observed between MyMR-KO and MR-intact mice are likely due to changes in recruitment, proliferation, and/or survival of immune cells. Intravital microscopy data studies will quantify leukocyte rolling along the endothelium in vivo in response to TNFα to assess the role of MyMR in leukocyte recruitment. Summary and Conclusions: Deficiency of myeloid MR attenuates atherosclerotic plaque size and inflammation and reduces plaque macrophages and T cells. Female mice have larger plaques but significantly fewer plaque myeloid and T cells compared to male mice. This study suggests that a decrease in plaque inflammation from blocking myeloid MR may contribute to the improved patient outcomes observed in clinical trials with MR antagonists. Funding Sources: This work was supported by grants from the National Institutes of Health HL095390 (to IZJ) and the American Heart Association EIA 18290005 (to IZJ).

12.4

Aldosterone and angiotensin II increase aortic stiffness and endothelial dysfunction via an action of oxidative stress on the endothelial sodium channel

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Renin-angiotensin-aldosterone system activation promotes accelerated arterial stiffening in obese men and women. Importantly, arterial stiffening increases the risk of cardiovascular and chronic kidney disease. There is emerging evidence that activation of the epithelial sodium channel in endothelial cells (ECs) (EnNaC) promotes endothelial stiffness and impaired nitric oxide (NO) production, which, in turn, contributes to arterial remodeling, inflammation and stiffening. However, the role that mineralocorticoid receptor and angiotensin II (Ang II) receptor type 1 signaling leads to EnNaC activation in arterial stiffening is not fully understood. Thereby, we hypothesized that deletion of the alphsubunit of EnNaC would decrease both aldosterone (Aldo) and Ang II- mediated EnNaC activation with consequent improvement in EC stiffening and NO mediated relaxation. Aldo (250 ng/kg/day)and Ang II (500 ng/kg/day) were administered by continuous infusion (Alzet osmotic pump) to 9 month old wild type (WT) and EnNaC alpha subunit KO mice for four weeks. The antioxidant tempol (2 mM/L) was added to the drinking water in a cohort of Ang II treated mice. Both Aldo and Ang II increased whole cell sodium current in ECs, on patch clamp that was temporally- related to aortic EC stiffness on atomic force microscopy and impaired aortic relaxation. These abnormalities were associated with increased oxidative stress. Interestingly EnNaC alpha subunit deletion prevented Aldo induced aortic stiffness and impaired NO dependent aortic relaxation both of which were associated with a decrease in oxidative stress suggesting role of oxidative stress in ECMR/ EnNaC mediated aortic stiffening and endothelial dysfunction. Moreover, antioxidant mediated improvement in endothelial/arterial stiffness and endothelial dysfunction induced by Ang II were similar in EnNaC alpha KO treated with tempol and Ang II compared to EnNaC KO mice infused with Ang II without tempol. These results suggest a role for oxidative stress as both an upstream regulator and a downstream mediator of EnNaC signaling in Ang II mediated aortic stiffening and endothelial dysfunction. In summary, these data demonstrate a critical role for oxidative stress in Aldo and Ang II mediated EnNaC activation, endothelial cell stiffness, impaired endothelium-dependent vascular relaxation and heightened arterial stiffness.

12.5

The novel non-steroidal MR antagonist finerenone improves metabolic parameters via ATGL-mediated lipolysis of brown adipose tissue in high-fat diet fed mice

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Herein, we evaluated metabolic effects of the novel non-steroidal MR antagonist Finerenone (FIN) on adipose tissues in a mouse model of diet-induced obesity (HFD, with 60% kcal from fat). After 3 months of normal diet (ND) or HFD+/-FIN (0.1 g/kg of diet), C57BL6 male mice fed HFD showed increased body weight and impaired glucose tolerance. HFD+FIN group did not show changes in body weight gain, compared with HFD group. FIN treatment improved glucose tolerance, as shown by a significant reduction of the area under curve of intraperitoneal glucose tolerance tests at 20 min after glucose injection. Histological analysis of brown adipose tissue (BAT) showed increased multilocularity in the HFD+FIN group, as compared to the HFD group. Differently, FIN did not affect adipocyte size in white adipose depots, suggesting a BAT-specific effect. We then analyzed mRNA and protein expression of UCP-1 and PGC1-alpha, showing a significant increase of both markers in BAT of the HFD+FIN group. Interestingly, we observed a significant increased phosphorylation of AMPK at T172 in BAT of the HFD+FIN group. Such increase was associated with activation of adipose triglyceride lipase (ATGL) phosphorylation at S404 and a concomitant inhibitory phosphorylation of hormone sensitive lipase (HSL) at S565. In order to better understand FIN effects in BAT, we wondered if FIN was able to affect adipose differentiation of T37i brown adipocytes. In this cell line, after 7 days of differentiation along with treatment with FIN, we confirmed increased expression of UCP-1 and PGC1-alpha in FIN-treated adipocytes, as observed in vivo. Furthermore, we analyzed mitochondrial dynamics (fission/fusion), a process potentially affecting mitochondrial abundance and efficiency. In particular, we observed a reduction of mitochondrial fission with concomitant reduction of mitophagy in FIN-treated T37i cells, suggesting that FIN is able to preserve the integrity of mitochondria. We hypothesize that free fatty acids, derived from increased ATGL-mediated lipolysis, could increase fatty acids oxidation and energy expenditure, determining favorable metabolic effects in high-fat diet fed mice.

13: SYMPOSIUM 5B: MR STRUCTURE AND ROLE IN THE HEART AND LUNGS

13.1

Enhanced endothelium epithelial sodium channel signaling prompts left ventricular diastolic dysfunction in obese female mice

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Increased activation of membrane epithelial sodium channels in endothelial cells (ECs) (EnNaC) occurs in states of cardiovascular oxidative stress, increased vascular stiffness and impaired nitric oxide mediated cardiovascular relaxation. Our previous studies in female mice fed a Western diet (WD) have shown that activation of EC mineralocorticoid receptor signaling promotes expression and translocation of EnNaC to the surface of ECs contributing to arterial pro-fibrotic and inflammatory responses as well as macrophage infiltration and M1 polarization. Emerging information also supports

EnNaC involvement in high salt/DOCA-induced cardiac fibrosis in non-hypertensive rodents. However, a specific role for EnNaC signaling in the development of cardiac stiffness and diastolic dysfunction has not been examined in the setting of diet-induced obesity (DIO). Accordingly, we hypothesized that inhibition of EnNaC activation with amiloride or endothelium specific deletion of the α subunit of EnNaC (αEnNaC-/-) would prevent cardiac stiffening and diastolic dysfunction with DIO in female mice. Female αEnNaC KO (EnNaC-/-), wild-type littermate and C57BL6/J females were fed a WD containing high fat (46%), sucrose (17.5%), and high fructose corn syrup (17.5%) for 12-16 weeks. C57BL6/J females were treated with or without a low dose of amiloride (1mg/kg/day). Low dose amiloride administration attenuated WD-induced impairment of left ventricular initial filling rate, relaxation time, and cardiac relaxation as measured by high resolution magnetic resonance imaging (MRI). Further, this DIO induced cardiac diastolic dysfunction was associated with increases in cardiac oxidative stress, activation of pro-fibrotic signaling TGF-β 1, and interstitial fibrosis. Amiloride administration prevented WD-induced increases in endothelium permeability and this effect of amiloride was associated with decreased expression of claudin-5 and occluding and a reduction of total MΦ recruitment (CD11b) and M1 polarization (CD11c). Furthermore, αEnNaC-/- prevented DIO-induced increases in inward sodium currents in isolated pulmonary ECs. The DIO induced abnormalities occurred in conjunction with in vitro cardiomyocyte stiffening (Atomic force microscopy) as well as increased left ventricular filling pressure and myocardial performance index examined by in vivo Doppler in αEnNaC wild-type littermates on a WD. Collectively, these findings support the notion that DIO related EnNaC activation mediates coronary artery endothelium dysfunction which, in turn, promotes cardiac oxidative stress and proinflammatory responses, resulting in cardiomyocyte stiffness, followed by cardiac fibrosis and diastolic dysfunction in females.

13.2

Structural determinants of activation of the mineralocorticoid receptor: an evolutionary perspective

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The mineralocorticoid receptor (MR) has been highly conserved across vertebrate evolution. the zebra fish (Danio rerio) (z)MR for instance shares ~50% identity with the human (h)MR. In terrestrial vertebrates the MR mediates the regulation of sodium homeostasis by aldosterone (aldo) and also acts as a receptor for cortisol (or corticosterone in rodents). The MR also binds progesterone (prog) which acts as an antagonist of the hMR; the commonly used MR antagonist, spironolactone (spiro), is based on the prog molecule. A curious feature of corticosteroid receptor evolution is that although the MR is present in teleosts (ray-finned fish), these lack aldo synthesis. Despite this, teleost MR respond to aldo and cortisol but in contrast to the hMR, the teleost MR is activated by both prog and spiro. We sought to define the molecular basis of these divergent responses of the zMR and hMR to spiro and prog using MR chimeras and molecular modeling. A series of zMR:hMR chimerias coupled with reciprocal site-directed mutagenesis of putative critical amino acids in the two MR was screened using a conventional transactivation assay in CV-1 cells and then confirmed in a zebra fish cell line (ZF-4 cells incubated at 28C). Each MR chimera/mutant was examined using aldo or spiro/prog alone, or both together. The structural consequences of the critical differences were assessed using molecular dynamic simulation (MD) based on the crystal structures of the MR ligand-binding domain. Substitution of a leucine (amino acid 856 in the zMR) by threonine (amino acid 870 in the hMR) in helix 8 of the ligand-binding domain of the zMR confers the antagonist response to prog/spiro. This leucine is conserved across fish species whereas threonine (serine in rodents) is conserved in terrestrial vertebrate MR. This difference is not a product of the heterologous system in that an agonist response to prog with

the zMR is also seen in a homologous system, the ZF-4 cells. MD identified an interaction of the leucine in helix 8 with a highly conserved leucine in helix 1 that stabilises the agonist conformation including the interaction between helices 3 and 5, an interaction which has previously been characterised. This switch in the MR coincides with the evolution of terrestrial vertebrates and of aldosterone synthesis. It was perhaps mandatory if the appearance of aldosterone as a specific mediator of the homeostatic salt retention was to be tolerated. The conformational changes also provide novel insights into the structural basis of agonism versus antagonism in steroid receptors, with potential implications for drug design in this important therapeutic target. In addition we have confirmed that this agonist response to prog in the zMR is observed in a homologous system which argues that prog is indeed a physiological agonist for the teleost MR with a corollary that studies in fish using spiro as an MR antagonist should be interpreted with caution.

13.3

The quaternary structure of the mineralocorticoid receptor depends on ligand and DNA binding

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The mineralocorticoid and glucocorticoid receptors (MR and GR) are evolutionary related nuclear receptors with high sequence conservation in the ligand- and DNA-binding domains (LBD and DBD). Both receptors are activated by glucocorticoids, but MR can be selectively activated by aldosterone. In addition, both receptors bind a shared hormone response element (HRE) with the same DNA consensus sequence. MR and GR physically and functionally interact, potentially contributing to cell type-specific corticosteroid signaling. It has recently been proposed that DNA binding promotes liganded GR self-association as tetramers. Here we investigated whether MR adopts a similar quaternary arrangement after receptor activation. To that end we used a fluorescence imaging technique, Number & Brightness (N&B), in a system where receptor-DNA interaction can be studied in live cells in real time. Our results show that MR adopts a tetrameric organization in the nucleoplasm upon agonist binding (aldosterone or corticosterone), suggesting that unlike GR, MR does not need DNA binding to form tetramers. MR interaction with a gene target promotes quaternary complexes with an average of 7 receptor units. Interestingly, an MR antagonist, spironolactone, induced dimerization, but not tetramerization in the nucleoplasm. With corticosterone, GR can incorporate to MR complexes partially displacing MR monomers. The structural determinants of MR oligomerization were studied using different mutants. Truncation of the LBD only partially reverts higher order oligomerization, consistent with previously reported data showing that similar mutants retain residual activity. Mutant P654R, analogous to mutant P481R mimicking DNA binding in GR, increases the number of MR monomers forming a complex in the nucleoplasm after aldosterone stimulation, but not at HREs. Our results indicate that the nature of the ligand (agonist or antagonist) and specific binding to HRE determines the final quaternary structure of active MR.

13.4

Vascular cell-specific roles of mineralocorticoid receptors in pulmonary hypertension

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Abnormalities that characterize pulmonary arterial hypertension (PAH) include an impairment in the structure and function of pulmonary vascular endothelial (EC) and smooth muscle cells (SMC). Aldosterone levels are elevated in human PAH and in experimental pulmonary hypertension (PH), while inhibition of

the aldosterone-binding mineralocorticoid receptor (MR) attenuates PH in multiple animal models. This study explored the specific role of MR in ECs and SMCs in PH using cell specific MR knockout mice exposed to sugen/hypoxia-induced PH. MR inhibition with spironolactone, at a dose that does not impact systemic blood pressure, significantly reduces right ventricular (RV) systolic pressure. However, this is not reproduced by MR deletion in either SMCs or ECs. Similarly, MR inhibition attenuates the increase in RV cardiomyocyte area independent of vascular MR with no effect on RV weight or interstitial fibrosis. RV perivascular fibrosis is significantly decreased by spironolactone and is reproduced by specific deletion of MR from ECs. MR inhibition significantly reduces pulmonary arteriolar muscularization, independent of EC-MR or SMCMR. Finally, the degree of perivascular inflammation in pulmonary vessels is attenuated by MR antagonism and is fully reproduced by SMC-specific MR deletion. These studies demonstrate that, in the sugen/hypoxia PH model, systemic-MR blockade significantly attenuates the disease and vascular MR has cell- and tissue-specific effects, with EC-MR contributing to RV perivascular fibrosis and SMC-MR stimulating pulmonary perivascular inflammation. The role of MR in pulmonary vascular muscularization and RV cardiomyocyte hypertrophy may be mediated by MR in other cell types such as cardiomyocytes and/or inflammatory cells.

14: CLINICAL PLENARY LECTURE

14.1

The role of non- steroidal MR antagonists and new potassium binders for the treatment of cardiovascular disease

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The steroidal mineralocorticoid receptor antagonists (s- MRAs) spironolactone and eplerenone have been shown to be effective in reducing mortality and hospitalizations for heart failure in patients with heart failure and a reduced ejection fraction (HFrEF), are likely effective in patients with heart failure and a preserved ejection fraction (HFpEF) and spironolactone is the drug of choice in patients with resistant hypertension . The use of these s-MRAs has however been limited by the occurrence of hyperkalemia (HK). Despite a class 1 indication in international guidelines for patients with HFrEF the s-MRAs are often not initiated due to the fear of inducing HK and when initiated are often discontinued within the first several due to HK. New nonsteroidal MRAs (ns-MRAS) such as Finerenone are under investigation and appear to be at least as effective as the s-MRAs in patients with HFrEF as well as those with diabetes mellitus (DM) and or chronic kidney disease (CKD) but with a lower incidence of HK. These ns-MRAs if shown to be effective in patients with HF, DM and or CKD could further reduce cardiovascular mortality. Alternatively new potassium binding agents such as patiromer or lokalma may be used in conjunction with the s-MRAs to treat and or prevent HK. The potassium binder sodium polystyrene sulfate (SPS) has been available for many years but its use has been limited by the occurrence of bowel necrosis and the occurrence of edema and loss of blood pressure control in patients with hypertension due to the fact that it exchanges potassium for sodium. The new potassium binding agents have recently become available for clinical use and are not associated with bowel necrosis. There are however important differences between the new potassium binders –patiromer and lokalma – in that patiromer exchanges potassium for calcium and lokalma exchanges potassium for sodium , although less than SPS. The availability of the new potassium binders along with the likely availability of new ns-MRAs holds the promise of more effective blood pressure control in patients with resistant hypertension with DM and or CKD, prevention of the progression of CKD, as well as a further reduction in mortaity and hospitalizations for heart failure in patients with HFrEF and HFpEF.

15: SYMPOSIUM 6A: NORMAL AND PATHOGENIC REGULATION OF ALDOSTERONE BIOSYNTHESES

15.1

Development of an inducible mouse model of aldosteronism

William Rainey[1], Mathey Taylor[1]
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Adrenal steroid production relies on regulated expression of five cytochrome P450s that direct steroid production toward mineralocorticoids or glucocorticoids. Differential expression of the steroid biosynthetic enzymes, including the P450s, determines the zone-specific production of steroid hormones. The zona glomerulosa produces aldosterone due to its sole expression of aldosterone synthase (CYP11B2), while the zona fasciculata produces cortisol due to expression of the closely related 11β - hydroxylase (CYP11B1). Normally CYP11B2 and aldosterone production are under tight control of the renin-angiotensin II (AngII) system. Dysregulated expression of adrenal CYP11B2 and aldosterone production leads to the most common adrenal disease, primary aldosteronism. Primary aldosteronism results from renin-independent expression of CYP11B2. Because of its clinical significance, we have focused on defining the normal and pathologic mechanisms controlling CYP11B2 expression. The predominant hormonal regulator of CYP11B2 is AngII, which acts through glomerulosa-specific Gq signaling pathways. Our working hypothesis has been that Gq signaling is the dominant regulator of glomerulosa differentiation and its capacity to express CYP11B2. Recently, we generated transgenic mice with adrenal cortex expression of Gq-coupled designer receptors exclusively activated by designer drugs (DREADDs). Adrenal-wide ligand activation of Gq DREADD triggered a disorganization of adrenal functional zonation with induction of CYP11B2 in CYP11B1-expressing fasciculata cells. This resulted in renin-independent aldosterone production and hypertension. All parameters were reversible following termination of DREADD Gq activation. These findings demonstrate that activation of fasciculata Gq signaling is sufficient to disorganize normal adrenocortical CYP11B2 expression and suggest that the Gq pathway plays an important role in differentiation of glomerulosa to fasciculata. This transgenic mouse also provides an inducible and reversible in vivo model of hyperaldosteronism for the testing of primary aldosteronism therapeutics (including inhibitors of CYP11B2).

15.2

A gain of function mutation in CLCN2 chloride channel gene causes primary aldosteronism

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Primary aldosteronism is the most common and curable form of secondary arterial hypertension. Germline mutations in genes coding for ionic channels were associated to familial and early-onset forms of PA, but the pathophysiology of a large proportion of cases is still unknown. To identify new genes involved in primary aldosteronism we performed whole exome sequencing in germline DNA from 12 patients with young onset hypertension and PA diagnosed before age 25 ys. Two index cases were investigated together with their parents and unaffected sibling to search for de novo variants. A de novo heterozygous c.71G>A/p.Gly24Asp mutation was identified in the CLCN2 gene, coding for the voltage-gated ClC-2 chloride channel, in a patient with hypertension and hypokalemia due to primary aldosteronism diagnosed at age 9 ys. CLCN2 is expressed in the human and mouse adrenal cortex and patch-clamp analysis of glomerulosa cells of mouse adrenal gland slices revealed hyperpolarization-activated Cl- currents that were abolished in Clcn2-/- mice. The ClC-2 p.Gly24Asp mutation, located in a well conserved inactivation domain of the chloride channel, abolished the voltage- and time-dependent gating of ClC-2. The resulting almost ohmic currents strongly increased the Cl- conductance at resting potentials. Expression of ClC-224Asp in adrenocortical H295R-S2 cells led to increased aldosterone production, which is paralleled by increased expression of

CYP11B2, coding for aldosterone synthase. This involves plasma membrane depolarization, opening of voltage-gated calcium channels and increased calcium influx through voltage-gated calcium channels. Conversely, knock-down of endogenous ClC-2 by shRNA diminished aldosterone production and reduced CYP11B2 expression. In conclusion, a gain-of-function mutation in the ClC-2 chloride channel underlies a genetic form of secondary arterial hypertension. Increased Cl- currents induced by the ClC-2 p.Gly24Asp mutation depolarize the zona glomerulosa cell membrane, thereby opening voltage-gated calcium channels which trigger autonomous aldosterone production by increasing intracellular Ca2+ concentrations. These results highlight for the first time the important role of chloride in aldosterone biosynthesis and identify ClC-2 as the foremost chloride conductor of resting glomerulosa cells.

15.3

Circulating MicroRNAs as diagnostic biomarkers of primary aldosteronism

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Aldosterone is a key cardiovascular hormone which, when present in excess, accounts for substantial cardiovascular morbidity. Its overproduction in primary aldosteronism (PA) is detected in ~10% of hypertensives although this figure may increase significantly if simpler, less expensive, non-invasive diagnostic techniques were available. Timely and accurate detection of aldosterone excess, combined with a comprehensive understanding of the mechanisms underlying aldosterone biosynthesis are therefore key to preventing the adverse cardiovascular effects. MicroRNAs (miRNAs) have emerged as key regulators of human gene expression, affecting numerous biological processes including aldosterone biosynthesis. miRNAs are a class of non-coding small RNAs, 21–23 nucleotides in length, that bind the 3' untranslated region of target messenger RNAs, resulting in down-regulation of gene expression through inhibition of translation or promotion of messenger RNA degradation. In higher eukaryotes, miRNA impact on gene expression may be comparable to that of transcription factors, and they have been proposed to act as 'genetic hormones', acting on tissues far from their site of production. Finally, their release into the circulation, has led to their emergence as biomarkers for certain conditions. We propose that circulating miRNAs represent a minimally-invasive biomarker that will enable accurate diagnosis of PA. The ENS@T-HT project is designed to define specific 'omics' signatures for endocrine hypertension. Our focus has been the profiling of circulating miRNAs in plasma, in order to identify those of diagnostic value and also highlight novel biological pathways relevant to PA. Initial miRNA profiling is now complete and preliminary analysis confirms differential miRNA expression in PA, essential hypertensives and normotensive subjects. Confirmation of the diagnostic miRNA signature is now being tested in a prospective study population. Our findings indicate the potential for circulating miRNAs as diagnostic biomarkers for PA. As we identify those miRNAs that show the most marked and consistent dysregulation in PA we will turn our attention to their biological roles in order to gain insight into its pathology. This will complement our previous studies of the adrenal cortex, which have shown that components of the aldosterone biosynthetic pathway are directly targeted by miRNAs. By combining our studies of tissue and circulating miRNAs, we hope to gain fresh insight into the development of PA and identify potential targets for therapeutic intervention.

15.4

The retinoic acid receptor a contributes to the development of primary aldosteronism by regulating adrenal cortex structure through interactions with Wnt and Vegfa signaling

Sheerazed Boulkroun[1,2], Rami M El Zein[1,2], Audrey H Soria[1,2], Jose Felipe Golib Dzib[3], Amanda J. Rickard[1,2], Fabio L Fernandes-Rosa[1,2], Benoit Samson-Couterie[1,2], Isabelle Giscos-Douriez[1,2], Angelique Rocha[1,2], Marko Poglitsch[4], Celso E Gomez-Sanchez[5], Laurence Amar[1,2,6], Norbert B Ghyselinck[7], Arndt Benecke[3], Maria-Christina Zennaro[1,8]

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Rationale: Primary aldosteronism (PA) is the most frequent form of secondary arterial hypertension. While somatic mutations in aldosterone producing adenomas (APA) are responsible for increased aldosterone production, adenoma formation may involve additional mechanisms. The identification of new determinants of APA development may offer new clues for the care of patients with secondary hypertension. Objective: To identify mechanisms involved in the development of PA and, in particular, specific signaling pathways responsible for abnormal cell proliferation and APA formation. Method and Results: We have performed a transcriptome analysis on 48 APA and investigated correlations of gene expression with their genetic, morphological and functional characteristics. We identified retinoic acid receptor alpha (RARα) signaling as a central molecular network involved in APA formation. To understand how RARα modulates adrenal structure and function, we have explored the adrenal phenotype of 12 and 52 weeks old male and female Rarα knockout mice. Inactivation of Rarα in mice led to major structural and functional disorganization of the adrenal cortex in both sexes, with modifications of the vessel architecture and extracellular matrix and reduced expression of steroidogenic genes. These abnormalities were due to increased proliferation, decreased Vegfa expression and modifications in extracellular matrix components. Rarα inactivation reduces non-canonical Wnt signaling, without affecting the canonical Wnt pathway nor PKA signaling. Adrenal cortex disorganization persisted with aging, while molecular abnormalities affecting Wnt signaling and Vegfa or steroidogenic gene expression regressed. Conclusions: Our study suggests that Rarα contributes to normal adrenal cortex development, by modulating Wnt and Vegfa signaling. We propose a model in which interaction between retinoic acid, Wnt and Vegf signaling pathways is required for normal development of the adrenal cortex. Dysregulation of this interaction may contribute to abnormal cell proliferation and APA development in human, creating a propitious environment for the emergence of specific driver mutations in APA.

16: SYMPOSIUM 6B: INTEGRATED REGULATION OF RENAL ION TRANSPORT

16.1

The role of ENaC in hyperoxia-induced preterm lung injury.

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Significance: Oxygen supplementation is an important component of intensive care for preterm infants and many patients with water in the lungs. Preterm lungs have 25% more water in the lungs than term infants. ENaC serves as the rate limiting step for net salt and water transport across an epithelium, and therefore,

plays an important role in resolving water in the lungs. Our lab has shown that during high O2 exposure, the level of glutathione disulfide (GSSG) is significantly increased, and that GSSG significantly decreases ENaC activity. Herein, we investigate whether chronic O2 treatment contributes to lung injury via S-glutathionylation dependent down-regulation of ENaC using a mouse model of preterm lung injury. Results from our studies could lead to new treatment options for O2-induced lung injury, and addresses the unmet clinical needs of preterm infants. Hypothesis: We hypothesize that S-glutathionylation dependent down-regulation of ENaC activity plays an important role in hyperoxia-induced lung injury. Methods: We employed the term mouse model of hyperoxia-induced lung injury, and a gain-of-ENaC function C57Bl6 mouse model which overexpresses the Scnn1b gene (referred to as Scnn1b hereafter). Scnn1b and WT litter mates were maintained on 85% or 21% O2 from birth up to postnatal days (PN) 11-15. A subset of normoxic WT C57Bl6 mice were nasally insufflated with 400 µM GSSG from PN 1-11. Lung injury and development were assessed by morphometric analysis (radial alveolar counts) of Masson's trichrome stained tissue; hydroxyproline assays quantified fibrosis. The Evan's blue assay was used to measure rate of alveolar fluid clearance. Quantitative redox proteomic characterization of S-glutathionylation in lung tissue was conducted using mass spectrometry. Results: Our studies show that newborn mice maintained on 85% O2 from birth up to PN 11-15 can be used as a biologically relevant model for O2-induced lung injury since chronic hyperoxia correlated with increased fibrosis and attenuated lung development. Scnn1b mice were protected from O2 injury; Masson's Trichrome staining showed less fibrotic tissue in hyperoxic Scnn1b mice compared to hyperoxic WT littermates. Radial alveolar counts (a reliable measure of lung maturation) increased from 8.1± 0.29 in (hyperoxia) WT pups to 9.9± 0.35 in (hyperoxia) Scnn1b pups. Overexpression of lung ENaC, under both normoxic and hyperoxic conditions, correlated positively with increased alveolar fluid clearance. Nasal insufflation of GSSG significantly decreased alveolar fluid clearance and increased lung injury. A more pronounced decrease in the levels of S-glutathionylation in response to hyperoxic conditions were observed in Scnn1b mice than those from WT. Conclusion: Scnn1b over-expression in mouse lung increases ENaC activity, which translates into improved fluid clearance rates and protection from hyperoxia-induced injury. S-glutathionylation plays a potential significant role in such protection. Future studies will evaluate the role of S-glutathionylated proteins in the regulation of lung fluid clearance, and evaluate the therapeutic value of reversing lung GSH/GSSG redox potentials in hyperoxia-induced lung injury. Funding: R01HL137033-01

16.2

Regulation of renal ion transport and blood pressure by the CRL3-WNK-SPAK pathway

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The rare disease Familial Hyperkalemic Hypertension (FHHt) primarily involves dysregulation of the thiazide-sensitive Na+-Cl- Cotransporter (NCC), expressed along the distal convoluted tubule (DCT) in the kidney. Identification of the genes mutated that cause FHHt has revealed a complex NCC regulatory pathway. NCC is activated by phosphorylation through the With-No-Lysine [K] (WNK) kinases and Ste20-related Proline Alanine Rich kinase (SPAK) pathway. WNK-SPAK activity is in turn regulated by the Cullin3 Ring Ligase (CRL3) ubiquitin protein-ligase complex, which modulates WNK abundance. Our studies using genetically modified mice have provided several insights into CRL3-WNK-SPAK function in vivo. We showed that disruption of SPAK, which directly phosphorylates NCC, mimics the human disease Gitelman syndrome caused by inactivating NCC mutations (Cell Metab 2011). We identified a kidney-enriched truncated form of SPAK (KS-SPAK) which lacks the kinase domain and competitively inhibits activity of SPAK and the closely related kinase Oxidative Stress Response 1 (OSR1) (Cell Metab 2011 and Am J Physiol Renal Physiol 2013). In vivo we

found that KS-SPAK is expressed along the renal thick ascending limb where it may inhibit OSR1-stimulated activity of the Na+-K+-2Cl- Cotransporter 2 (NKCC2). We also demonstrated that SPAK and OSR1 play critical roles in potassium homeostasis (J Physiol 2016) as components of the DCT plasma [K+] we collaboratively identified (Cell Metab 2015 and J Am Soc Nephrol 2017). Our recent work has focused on the mechanisms by which FHHt-causing disrupt CRL3 function. We showed that the FHHt-causing Cullin3 mutant inappropriately degrades the WNK-binding CRL3 substrate adaptor KLHL3 (J Clin Invest 2014) and does not simply cause FHHt by inducing its own degradation (JCI Insight 2017). Funding: NIH (NIDDK) R01DK098141 References Cell Metab. 2011 Sep 7;14(3):352-64 Am J Physiol Renal Physiol. 2013 Dec 15;305(12):F1687-96 J Clin Invest. 2014 Nov;124(11):4723-36 Cell Metab. 2015 Jan 6;21(1):39-50 J Physiol. 2016 Sep 1;594(17):4945-66 J Am Soc Nephrol. 2017 Jun;28(6):1814-1825 JCI Insight. 2017 Dec 21;2(24) J Physiol. 2016 Sep 1;594(17):4945-66

16.3

WNK regulation of ion transport in the malpighian tubule

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Background and Objective Mutations in WNK (With No Lysine (K)) kinases in mice and humans result in abnormal serum potassium concentration and blood pressure. WNKs phosphorylate and activate SPAK and OSR1 kinases, which phosphorylate SLC12 sodium chloride and sodium-potassium-2-chloride cotransporters. Our lab has shown that this cascade is conserved in the Drosophila Malpighian (renal) tubule, and that chloride and the scaffold protein Mo25 regulate pathway activity 1,2. Here, we examine roles for potassium and Mo25 in regulating Drosophila and mammalian WNKs. Methods We used differential scanning fluorimetry and mass spectrometry to measure WNK kinase domain thermal stability and autophosphorylation in vitro in the presence of varying concentrations of potassium. We examined the activity of DmWNK (Drosophila WNK) and mammalian WNKs in the Drosophila Malpighian tubule, using phosphorylation of transgenically expressed kinase-dead rat SPAK as a readout. We developed baths with varying extracellular potassium and fixed intracellular chloride concentrations (measured using the transgenic sensor ClopHensor). We measured intracellular potassium in the tubule using inductively coupled plasma mass spectrometry. We measured transepithelial ion flux in Malpighian tubules in which DmWNK was knocked down and replaced by mammalian WNK3 or WNK4, with or without Mo25. Results Potassium directly binds to the kinase domain of DmWNK (Drosophila WNK) and human WNK1 in vitro, as assayed by differential scanning fluorimetry, and inhibits autophosphorylation and activation of DmWNK and HsWNK3 (human WNK3) kinase domains. In the tubule, high potassium bath, which increases intracellular potassium concentration, inhibits DmWNK and mammalian WNK activity in both 16 mM and 30 mM intracellular chloride conditions, and also inhibits chloride-insensitive mammalian WNK activity. HsWNK3 rescues ion transport in DmWNK knockdown tubules in both basal and stimulated conditions. MmWNK4 (mouse WNK4) is not able to rescue unless co-expressed with Mo25 in basal conditions, and chloride-insensitive MmWNK4 co-expressed with Mo25 rescues DmWNK knockdown in stimulated conditions. Rescue was achieved with RnWNK1 (rat WNK1) in basal conditions, but not in stimulated conditions, regardless of Mo25 co-expression or mutating the chloride-binding site. Conclusion Our studies uncover novel regulatory mechanisms for WNK pathway signaling, including direct effects of potassium and differential effects of Mo25 on mammalian WNK paralogs, which may further increase the dynamic range of WNK signaling in different tissues.

16.4

Interleukin 6 activation of the epithelial sodium channel in the distal convoluted tubule and cortical collecting duct via Rac1

Oishi Paul[1], Auriel Moseley[1], Chang Song[1], Otor Al-Khalili[1], Celso Gomez-Sanchez[2], Douglas Eaton[1], Brandi Wynne[1]

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Hypertension (HTN) is a primary global health concern. HTN is an inflammatory disease that is characterized by excessive sodium (Na+) reabsorption and increased pro-inflammatory cytokines, such as interleukin 6 (IL-6). Aldosterone (Aldo) is the primary ligand for the mineralocorticoid receptor (MR), which is expressed along the distal nephron referred to as the aldosterone-sensitive distal nephron (ASDN). Although studies demonstrate a beneficial effect of MR inhibition during HTN, Aldo levels are not always increased, suggesting an alternate MR activation pathway. The epithelial sodium channel (ENaC) is located in both the late distal convoluted tubule (DCT2) and cortical collecting duct (CCD), and is major Na+ transport protein. Data from our laboratory have shown that IL-6 can activate the MR in vitro, and that pharmacological inhibition of Rac reduces mineralocorticoid response element (MRE) activation. We then hypothesized that IL-6 activates the MR via the small GTPase Rac1 in the distal nephron, increasing ENaC activity. To investigate this, we measured transepithelial voltage and resistance using a voltohmmeter (EVOM), and calculated current in both murine distal convoluted tubule (mDCT15) and cortical collecting duct (mpkCCD) cells. Cells were transfected with a vector containing wild-type (WT), Rac1 knock-down (KD) or constitutively active Rac1 (overexpression, OE) and then treated with vehicle, Aldo [100nM] or IL-6 [100ng/mL]. Amiloride was applied to confirm current was ENaC-dependent. Data are shown as a fold change of current before and after treatments (1hr). We observed a significant increase in amiloride-sensitive current in both mDCT2 and mpkCCD cells following both Aldo and IL-6 treatments. Interestingly, the magnitude of current was considerably higher in the DCT2 (10.04 vs.1.98 fold change/baseline), as compared to the CCD. Further, we found that with Rac1 KD, there was a complete inhibition of IL-6 induced Na+current (-1.16 fold change/baseline) and that IL-6 did not further increase current in cells with Rac1 OE. Our data are the first to investigate ENaC activity in the DCT2. Additionally, we demonstrate that IL-6 is a strong stimulus for ENaC activity in both the DCT2 and CCD. Our data suggest that Rac1 may play an important role in IL-6 mediated activation of the MR in the DCT2. Our data reveals a potential mechanism of MR activation during HTN, without increased Aldo levels.

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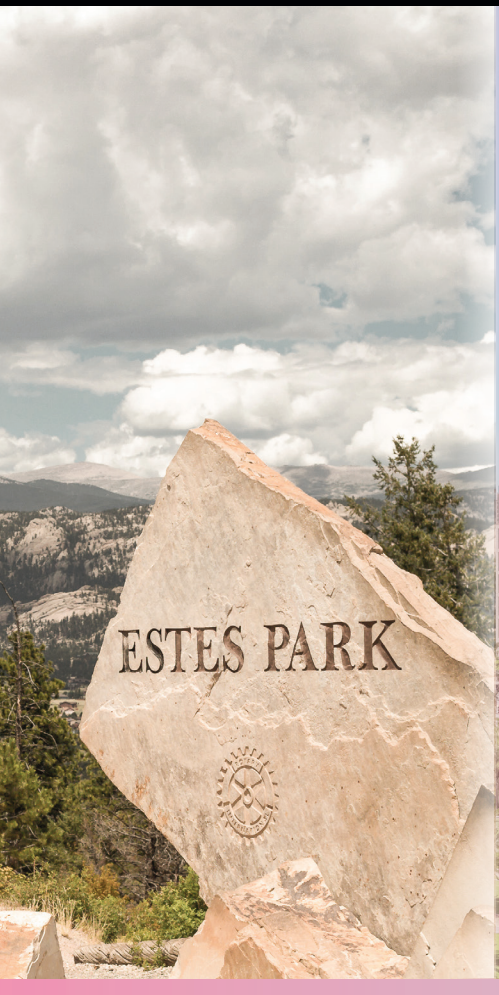

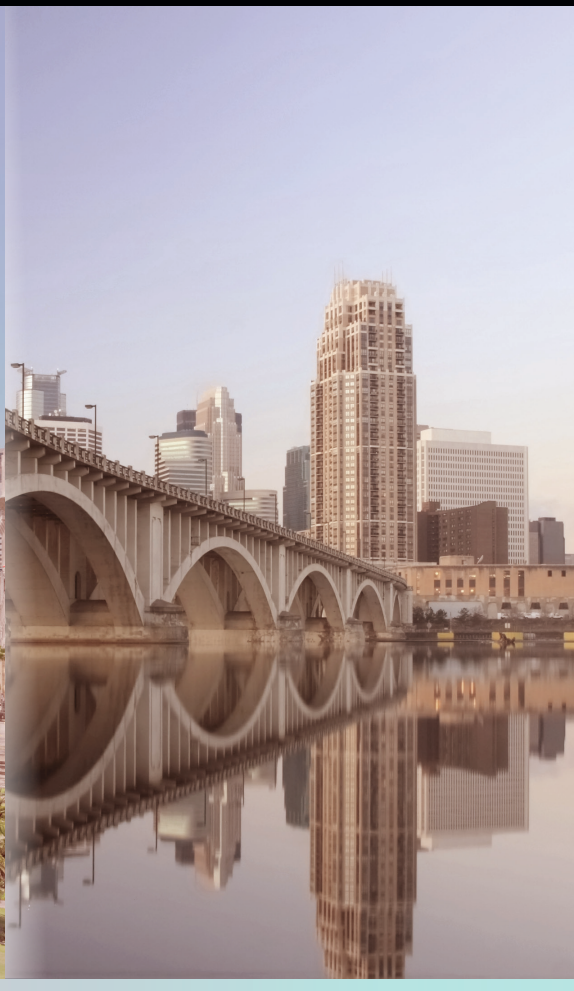

INQUIRY COLLABORATION DISCOVERY

2019

APS Aldosterone and ENaC in Health and Disease: The Kidney and Beyond
October 2–6, 2019
Estes Park, Colo.
the-aps.org/enac

2020

APS Annual Meeting at Experimental Biology 2020
April 4–7, 2020
San Diego
the-aps.org/EB

Institute on Teaching and Learning
June 21–26, 2020
Minneapolis

Eleventh International Conference on Heme Oxygenase and Related Enzymes: From Physiology to Therapeutics
June 28–July 1, 2020
Los Angeles

Integrative Physiology of Exercise
October 2020
Location TBD

APS members enjoy discounted registration.

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ADDENDUM TO THE PROGRAM BOOK

Program Updates:

Thursday, October 3, 2019

8:00 a.m. – 8:30 a.m. **2.1** Aldosterone-independent activation of mineralocorticoid receptor in salt-sensitive hypertension and glomerular diseases
Toshiro Fujita, *The Univ. of Tokyo*
New Title: Aldosterone-independent MR activation in salt-sensitive hypertension

Saturday, October 5, 2019

8:00 a.m. – 10:00 a.m. **12.0** **Symposium 5A: MR in the vasculature**
Pavilion Theater, 1st Floor
Chairs: **Frederic Jaisser**, *INSERM*
***Jun Yang**, *Monash Univ*.**
(Replacing **Iris Jaffe**, *Tufts Medical Center)*

9:30 a.m. – 9:45 a.m. **12.5** The novel non-steroidal MR antagonist finerenone improves metabolic parameters via ATGL-mediated lipolysis of brown adipose tissue in high-fat diet fed mice
Massimiliano Caprio, *San Raffaele Roma Univ.*
Replacing **Vincenzo Marzolla**, *IRCCS San Raffaele Pisana*

Sunday, October 6, 2019

8:00 a.m. – 10:00 a.m. **15.0** **Symposium 6A: Normal and pathogenic regulation of aldosterone biosynthesis**
Pavilion Theater, 1st Floor
Chairs: **Eleanor Davies**, *Univ. of Glasgow*
***Iris Jaffe**, *Tufts Medical Center*
(*Replacing **Jun Yang**, *Monash Univ.)*

10:15 a.m. – 12:00 p.m. **16.0** **Symposium 6B: Integrated regulation of renal ion transport**
Pavilion Theater, 1st Floor
Chairs: **Daniela Rotin**, *Hospital for Sick Children and Univ. of Toronto*
(Replacing **David Ellison**, *Oregon Health Science Univ.)*
Peter Snyder, *Univ. of Iowa*
(Replacing **Johannes Loffing**, *Univ. of Zurich)*

11:45 a.m. – 12:00 p.m. **16.4** Interleukin 6 activation of the epithelial sodium channel in the distal convoluted tubule and cortical collecting duct via Rac1
Oishi Paul, *Emory Univ.*
Talk has moved to **Session 2A, Thursday, October 3, 2:45 – 3:00 p.m.**