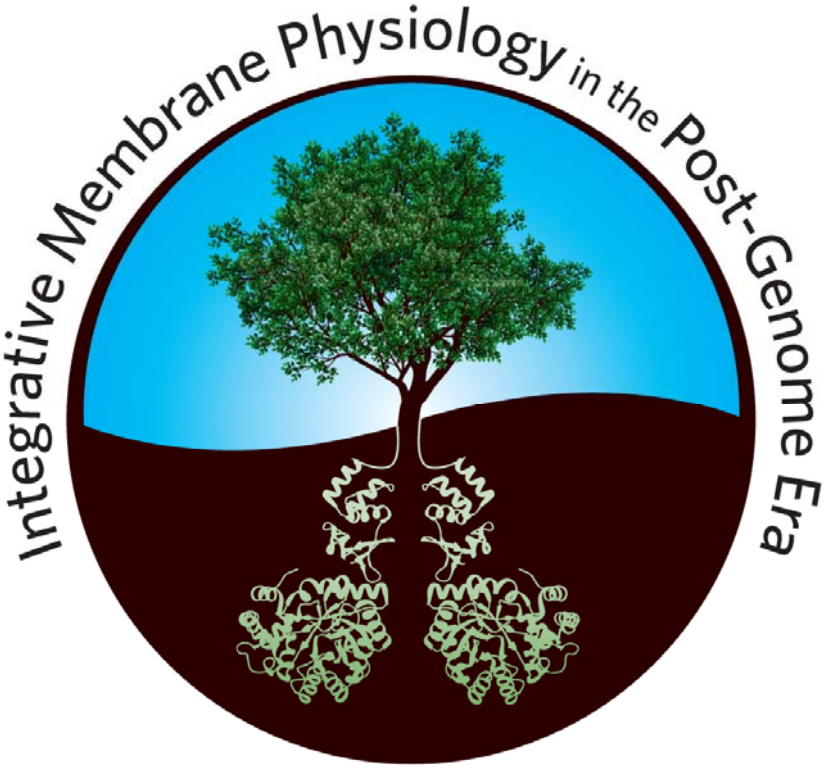


organized by Jerod S. Denton

organizing committee: Jeanne Nerbonne, Louis Ptácek, Angeles Ribera, and Alan Verkman

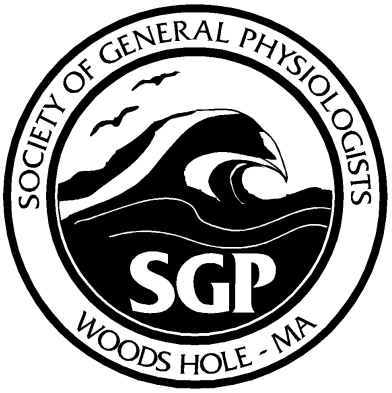
Society of General Physiologists

66th Annual Meeting and Symposium

Marine Biological Laboratory

Woods Hole, Massachusetts

Program and Abstracts



Symposium Organizer

Jerod S. Denton
Vanderbilt University

Organizing Committee

Also supported by grants from:

Meeting Site Information

Registration: Meeting registration takes place on the first floor of the Swope Center from 2:00 to 10:00 p.m. on Wednesday, September 5; and from 8:00 a.m. to noon on Thursday, September 6. Check-in is at the main desk in Swope Lobby (opposite the door) and your registration packets are at the first table on your left. If you arrive during the night, instructions are posted at Swope Center on how to contact the watchman who will have your room key.

Mail/Messages/Faxes: Private phones are available in nearly all guest rooms. Messages can be left at 508-548-3705 24 hours a day and are available for pickup at the front desk of Swope Center (phones in rooms do not have voice mail). Packages and mail should be addressed to **your name**, *Society of General Physiologists, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543-1015*. There is a fax machine in the Copy Center of the MBL Library on the second floor of the Lillie Building. It is available weekdays only, 8:00 a.m. to 5:00 p.m. The fax number is 508-540-6902 and there is a user's fee; payment must be made *in cash at the time of sending or retrieving*. Please be sure that all incoming correspondence is addressed to your name, SOCIETY OF GENERAL PHYSIOLOGISTS, care of MBL, etc.

Library Usage: The MBL Library has a world-renowned collection, and visitors are welcome at all times. The Library is staffed Monday through Friday, 8:00 a.m. to 5:00 p.m. After-hours access is provided via your MBL ID card.

Internet Access: MBL has wireless internet service throughout its campus. To use the system simply browse for wireless networks on any wireless enabled device and select "MBL-Guest". Open a web browser and you will be automatically redirected to a login page. Enter the login credentials: user name = mblguest, password = mblguest. In addition, there are 6 terminals from which you can check your email or do a web search in Swope Center. For other computer activity, the Computer Room is located in the MBL Library, Lillie 204.

Poster Instructions: Poster boards are located on the second floor of Swope Center. Posters may be up to 5 feet high and 3.5 feet wide. Thumbtacks are provided. Poster numbers and titles are listed in the abstracts section of this program.

MBL's Security System: The MBL has a new security system in all buildings. Every participant is issued an MBL ID card (proximity card) at registration. This card, to be kept with you at all times, will provide 24-hour access to the Lillie Library and Swope, the dormitory building. The main change is that many buildings, including the dormitories, have automatically locking doors. Although Swope will be open until 10 p.m., other buildings will always be locked and you will need your proximity card to gain access. If you should have any questions, both MBL and SGP personnel will be available to answer them during the course of the meeting.

Travel Information

Plane: Boston (Logan Airport) has the advantage of regular, direct bus service between Logan and Woods Hole. Boarding for the Peter Pan/Bonanza bus to Woods Hole is just outside the baggage claim area. Providence (T.F. Green Airport) also offers bus service to Woods Hole, but not directly from the airport. Be sure to check bus options online at www.peterpanbus.com/tickets/fares. If you are planning to rent a car, Green is probably the airport of choice. We suggest you don't fly into Hyannis, as local transportation between Hyannis and Woods Hole is not readily available.

Bus: Round-trip bus fare from Logan Airport to Woods Hole is $55.00; easiest is to purchase online. You can get the current bus schedule (and purchase tickets) from the Peter Pan/Bonanza website at www.peterpanbus.com/tickets/fares. The bus stop in Woods Hole, a 10-minute walk from Swope Center, is in the Steamship Authority parking lot. Walk the 100 yards or so up Luscombe Avenue to Water Street, turn left and cross the drawbridge, continue another several hundred yards and turn right on MBL Street. (Lillie Building will be on your right.) Swope is straight ahead on the Eel Pond.

Car: The meeting site is about a two-hour drive from Boston (a bit less if you're lucky), and an hour and a half from Green Airport in Providence. If you come down Route 3 and cross the Sagamore Bridge, take the first exit and follow signs for Falmouth (you will turn left at the light and follow the canal west). At the Bourne Rotary, go three-quarters of the way around and go south on Route 28. If you come down I-495, continue to the Bourne Bridge and follow Route 28 south to Falmouth. Woods Hole is about 5 miles from Falmouth, and the road is clearly marked by signs to Woods Hole and Martha's Vineyard. On arrival in Woods Hole, cross over the drawbridge and turn right onto MBL Street. Swope Center is straight ahead at the bend in the road.

Parking: If you drive to Woods Hole, remember that *parking is by permit only*. You must obtain a parking permit at the registration desk and **park ONLY at the Bar Neck parking lot**. Directions will be given to you at the registration desk. Do NOT park in any of the other MBL lots or on the street—towing is a local recreation.

66th Annual Meeting and Symposium of the Society of General Physiologists

Marine Biological Laboratory, Woods Hole, Massachusetts

September 5–9, 2012

INTEGRATIVE MEMBRANE PHYSIOLOGY IN THE POST-GENOME ERA

Organized by Jerod S. Denton

SCHEDULE OF EVENTS

Wednesday, September 5

2:00 – 10:00 **Registration and Room Assignments (Swope Center, 1st Floor)**

6:00 – 7:30 **Buffet Dinner (Swope Center, Meigs Room)**

7:30 – 7:45 **Opening Remarks (Lillie Auditorium)**
Gary Borisy, Director (Marine Biological Laboratory)
Toshi Hoshi, President (Society of General Physiologists)
Jerod Denton, Organizer

Session I KEYNOTE ADDRESS

7:45 **Michael Welsh –** HHMI, University of Iowa College of Medicine
Insights into the pathogenesis of cystic fibrosis from a new model

9:00 – 11:00 **Mixer (Swope Center, Meigs Room)**

Thursday, September 6

7:15 – 8:30 **Breakfast**

Session II: GENOMICS AND ION CHANNELOPATHIES

8:30 – 12:00 **Louis Ptácek,** UCSF, session chair

8:30 – 9:10 **Dan Roden –** Vanderbilt University School of Medicine
Translating fundamental scientific discovery to the bedside to personalize medicine: Lessons from the cardiac ion channel world

9:10 – 9:50 **Louis Ptácek –** UC San Francisco
Genetic and molecular basis of episodic disorders

9:50 – 10:10 **Short talk 1: Michael Sturek** – IUPUI
Dysfunctional smooth muscle Ca^{2+} signals underlying coronary calcification

10:10 – 10:40 **Break**

10:40 – 11:20 **Colin Nichols** – Washington University
KATP channel mutations and diabetes. Back and forth from bench to bedside

11:20 – 12:00 **William Balch** – Scripps Research Institute
Rebalancing proteostasis to manage misfolding in human disease

12:00 – 1:30 **Lunch**

2:00 – 5:00 **Poster Session**
Posters may be displayed after 8:00 a.m. on Thursday, and may stay up until Saturday evening

2:00 – 3:30 **Authors at Posters** for discussion with attendees and judging for the poster competition (for those who applied to compete)

1:45 – 2:15 **SGP Annual Business Meeting**

5:00 – 6:30 **Dinner**

Session III: DRUG DISCOVERY 1

6:30 – 9:00 **Alan Verkman,** UC San Francisco, session chair

6:30 – 7:10 **Alan Verkman** – UCSF
Aquaporin water channels as drug targets

7:10 – 7:50 **Jerod Denton** – Vanderbilt University School of Medicine
Of mosquitoes and men: Renal potassium channels as novel disease targets

7:50 – 8:10 **Short talk 2 – Matthew Loewen** – University of Saskatchewan
Influenza A-induced cytokine production correlates with altered epithelial chloride channel physiology

8:10 – 8:50 **Heike Wulff** – UC Davis
The potassium channels Kv1.3 and KCa3.1 as targets for inflammatory brain pathologies

9:00 – 11:00 **Mixer (Swope Center, Meigs Room)**

Friday, September 7

7:00 – 8:15 **Breakfast (Swope Center, Dining Room)**

Session IV: ION CHANNEL STRUCTURE AND COMPLEXES

8:15 – 12:30 **Jeanne Nerbonne**, Washington University, session chair

8:15 – 8:50 **Jeanne Nerbonne,** Washington University
Native Kv4-encoded neuronal and cardiac Kv channels function in macromolecular protein complexes

8:50 – 9:30 **Daniel L. Minor Jr.** – UC San Francisco
Structural insights into ion channel function and modulation

9:30 – 10:10 **Bernhard Bettler** – University of Basel
Molecular insights into the regulation of $GABA_B$ receptor responses

10:10 – 10:30 **Break**

10:30 – 10:50 **Short talk 3 – Janice Robertson** – Brandeis
Measuring the dimerization free energy of a CLC Cl^-/H^+ antiporter in lipid bilayers by single molecule fluorescence analysis

10:50 – 11:30 **James S. Trimmer** – UC Davis
Ion channel proteomics and dynamic regulation of cell physiology

11:30 – 11:50 **Short talk 4 – Celine Marionneau** – INSERM
Mass spectrometry-based identification of native cardiac Nav1.5 channel phosphorylation sites

11:50 – 12:30 **Eric S. Bennett** – University of South Florida College of Medicine
Regulated and aberrant sialylation modulate cardiac electrical signaling

12:30 – 2:00 **Lunch**

Afternoon free: Harbor cruises in local waters

5:15 – 6:45 **Dinner (Swope Center, Dining Room)**

Session V: GENETIC MODEL ORGANISMS

6:45 – 9:00 **Angela Ribera,** University of Colorado, session chair

6:45 – 7:20 **Angeles B. Ribera –** University of Colorado
Novel regulation of sensory neuron sodium current

7:20 – 7:55 **Miriam Goodman** – Stanford
***Deconstructing sensory transduction with* C. elegans**

7:55 – 8:30 **Paul Garrity** – Brandeis
Overheated and highly irritated: Thermal and chemical sensing from the Cambrian to the sushi bar

8:30 – 9:05 **Kristin Scott** – HHMI/UC Berkeley
Molecular basis for water taste in Drosophila

9:05 – 11:00 **Mixer (Swope Center, Meigs Room)**

Saturday, September 8

7:00 – 8:15 **Breakfast (Swope Center, Dining Room)**

Session VI: DRUG DISCOVERY 2

8:15 – 12:30 **Jerod Denton,** Vanderbilt University, session chair

8:15 – 8:50 **Baldomero Olivera** – University of Utah
Conotoxins as therapeutics for pain

8:50 – 9:10 **Short talk 5 – Jorge Contreras** – UMDNJ
Calcium regulation in wild type and mutants D50N/Y human connexin26 (hCx26) hemichannels

9:10 – 9:45 **David Weaver** – Vanderbilt University School of Medicine
Discovery and characterization of potent and selective small molecule GIRK activators

9:45 – 10:05 **Short talk 6 – Daniel Basilio** – Weill Cornell Medical College (Cranefield awardee)
A kinetic analysis of protein transport through the anthrax toxin channel

10:05 – 10:30 **Break**

10:30 – 11:05 **Sven-Eric Jordt** – Yale University
Sensory TRP channels in airway chemosensation and inflammation

11:05 – 11:25 **Short Talk 7 – Leigh Plant** – University of Chicago (Cranefield awardee)
SUMO modification of cell surface Kv2.1 potassium channels regulates the activity of rat hippocampal neurons

11:25 – 12:00 **Pierre-Jean Corringer** – Institut Pasteur
X-ray structure of general anesthetics bound to their principal target, pentameric channel receptors

12:00 – 12:30 **Round table discussion:** ***The future of membrane transport***
Jerod Denton, Robert Kass, Jeanne Nerbonne, Louis Ptácek, Angela Ribera

12:30 – 2:00 **Lunch**

2:00 – 5:00 **Poster Session II**

Session VII KEYNOTE ADDRESS 2
(N.B.: This will be held in the Meigs Room, pre-banquet mixer to follow)

5:00 – 6:00 **Robert S. Kass** – Columbia University
Molecular pharmacology of ion channels expressed in induced pluripotent stem cells guides clinical therapy of a long QT variant 3 patient

7:00 – 8:30 **Clambake & Awards Ceremony (Swope Center, Dining Room)**

8:30 **–** 10:30 **Mixer (Swope Center, Meigs Room)**

Sunday, September 9

7:00 – 8:30 **Breakfast (Swope Center, Dining Room)**

10:00 **Checkout deadline**. Participants may pick up a box lunch after checkout.

SPEAKER SUMMARIES AND ABSTRACTS OF PAPERS AT THE SIXTY-SIXTH ANNUAL MEETING OF THE SOCIETY OF GENERAL PHYSIOLOGISTS

Integrative Membrane Physiology in the Post-Genome Era

Marine Biological Laboratory

Woods Hole, Massachusetts

5–9 September 2012

Organized by

JEROD S. DENTON

1. Rebalancing Proteostasis to Manage Misfolding in Human Disease. **WILLIAM E. BALCH**, *Scripps Research Institute*

The cell exploits the emergent properties of proteostasis, a biological system of folding chaperones, trafficking pathways and degradation systems managed by folding stress responsive signaling pathways of high clinical relevance, to promote human healthspan (2008. *Science*. 319:916; 2011. *Curr. Opin. Cell Biol.* 23:126). The proteostasis network (PN) generates and maintains proteome balance inside and outside diverse cell, tissue, and organ environments in the germline, during development and in response to disease and aging (2010. *Science*. 367:766; 2011. *Nature*. 471:42). Physical, pathological, and inherited challenges to the basic biophysics (the energetics) of the biological fold can compromise proteome balance. By use of systems level proteomic, genomic, and bioinformatic tools, we are building a dynamic, multi-layered view of the healthy biological protein fold and the changes that occur in response to energetically compromised folding stress such as is observed in neurodegenerative disease, diabetes, COPD/emphysema, cancer, and cystic fibrosis. We are finding that chemical biology management of the PN can alter the composition of the local proteostasis program to restore function across a broad spectrum of human disease. The discovery of tools that redirect the activity of biological folding systems highlights the potential value of the dynamic emergent properties of the PN to therapeutically rebalance function of the proteome to benefit human healthspan.

2. Regulated and Aberrant Sialylation Modulate Cardiac Electrical Signaling. **ERIC S. BENNETT**, *University of South Florida, College of Medicine*

Cardiac electrical signaling is dependent on the orchestrated activity of various types of voltage-gated ion channels (VGICs). VGICs are extensively modified by protein glycosylation, a sequential process that involves hundreds of glycogenes and often results in the addition of a negatively charged sialic acid residue(s) to glycan termini. Our lab and others demonstrated that VGIC sialylation modulated channel gating through isoform-specific, saturating electrostatic mechanisms. Using genomic and proteomic analyses, we described that the cardiac glycome, defined as the full set of glycan structures produced in the heart, varies between atria and ventricles and is altered differentially during development of each cardiac chamber. The regulated expression of a single sialyltransferase was sufficient to modulate AP waveforms and gating of less sialylated Na_v consistently, indicating that regulated sialylation modulates cardiomyocyte activity. Recently, we turned to question whether and how the aberrant (reduced) sialylation associated with the >40 distinct forms of congenital disorders of glycosylation (CDG) contribute to the altered electrical signaling that is prevalent in CDG. To begin our analysis, we compared changes in cardiac excitability and conduction using a knockout strain of a second sialyltransferase, ST3Gal4, which is uniformly expressed throughout the developing heart. ECG recordings indicated aberrant conduction with increased susceptibility to arrhythmias. This was confirmed using optical mapping techniques, with conduction anomalies presenting in 90% of the $ST3Gal4^{-/-}$ hearts but in only 10% of control hearts. $ST3Gal4^{-/-}$ myocytes isolated from the left ventricular apex (LVA) demonstrated increased action potential duration, with consistent changes observed in both voltage-gated Na^+ and K^+ channel gating (but not in current densities). The data suggest that aberrant protein sialylation directly alters cardiomyocyte excitability, likely through changes in voltage-gated Na^+ and K^+ channel gating, and this results in altered conduction and increased susceptibility to arrhythmias. We will discuss how regulated and aberrant glycosylation, particularly sialylation, are important modulatory mechanisms that impact physiological and pathological cardiac electrical signaling.

3. Molecular Insights into the Regulation of $GABA_B$ Receptor Responses. **BERNHARD BETTLER**, *University of Basel, Switzerland*

Molecular cloning revealed that functional $GABA_B$ receptors are formed by the heteromeric assembly of $GABA_{B1}$ with $GABA_{B2}$ subunits. However, cloned $GABA_{B(1,2)}$ receptors failed to reproduce the functional diversity observed with native $GABA_B$ receptors. Using functional proteomics, we recently demonstrated that native $GABA_B$ receptors are high molecular weight complexes of $GABA_{B1}$, $GABA_{B2}$, and members of a subfamily of the "potassium channel tetramerization domain-containing" (KCTD) proteins (Schwenk et al. 2010. *Nature*. 465:231–235). Coassembly with KCTD proteins changes kinetic and pharmacological properties of the $GABA_{B(1,2)}$ core receptor in a KCTD subtype-specific manner. I will provide an update of the effects of the KCTD proteins on recombinant and native $GABA_B$ responses.

4. Ossabaw Miniature Swine with a Loss-of-Function AMP Kinase γ3 Mutation Have Augmented Electrocardiographic ST Segment Elevation during Myocardial Ischemia. AARTI CHAWLA, STEPHEN SPENCER, MOUHAMAD ALLOOSH, JAMES BYRD, KIEREN MATHER, and MICHAEL STUREK, *Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN 46202*

Myocardial ischemia activates AMP kinase (AMPK), which modulates electrophysiology. A spontaneous point mutation from Val199→Ile in the AMPK γ3

subunit of Ossabaw swine impairs AMPK function. We hypothesized that ischemia would elicit greater electrocardiographic ST segment elevation (STE) in AMPK mutant compared to nonmutant swine. Ischemia induced by balloon occlusion of the circumflex artery was verified by angiography. AMPK mutants showed significant STE within 2 min of ischemia, whereas nonmutants showed an STE of <2 mm during 15 min of ischemia. The percentage of pigs reaching STE criterion of 5 mm was greater in mutants versus nonmutants ($P < 0.05$). Intracoronary infusion of the AMPK activator AICAR (0.5 mM) for 10 min before coronary occlusion reduced profound STE in AMPK mutants, but completely prevented STE in nonmutants. The percent age of pigs reaching STE criterion again was greater in mutants versus nonmutants during ischemia. Compound C, an inhibitor of AMPK, tended to decrease time to reach STE criterion compared to AICAR. Metabolic syndrome induced by hypercaloric atherogenic diet was similar in mutants and nonmutants. Mutants reached the STE criterion in 4 min, but the nonmutants did not in the 15-min occlusion, thus showing a striking cardiac phenotype. Furthermore, pharmacological activation of ATP-sensitive K^+ (K_{ATP}) channels by intracoronary infusion of pinacidil elicited STE and coronary vasodilation, whereas blockade of K_{ATP} with glibenclamide blocked STE. In conclusion, functional cardiac AMPK and pharmacological AMPK activation decrease ischemia-induced ST segment elevation, probably by decreasing K_{ATP} channel current. Support by NIH HL062552.

5. X-ray Structure of General Anesthetics Bound to Their Principal Target, Pentameric Channel Receptors. **PIERRE-JEAN CORRINGER**, *Institut Pasteur, France*

Pentameric channel receptors, including nicotinic acetylcholine and GABAA receptors, play a key role in fast excitatory and inhibitory transmission in the nervous system and are the target of numerous therapeutic and addictive drugs. They carry several neurotransmitter-binding sites, which govern the opening of a transmembrane ion channel. Extensively expressed in animals, they were recently found in several bacteria, especially the homolog from Gloeobacter violaceus (GLIC), which functions as a proton-gated ion channel, and the homolog from Erwinia chrysanthemi (ELIC). The simplified architecture of these archaic homologues, as well as their prokaryotic origin, allowed solving the first x-ray structures of integral membrane ELIC and GLIC in a closed and apparently open conformation, respectively. Comparative analysis of ELIC and GLIC suggests that receptor activation occurs through a symmetrical quaternary twist and tertiary deformation, according to a global transition that couples channel opening with reorganization of the binding pockets for neurotransmitters and allosteric effectors. In addition, recent co-crystallization of GLIC with allosteric inhibitors that are clinically used as general anesthetics reveals the mechanism of action at the membrane of these amphipathic molecules and will help in the designing of new drugs targeted to pentameric channel receptors.

6. Of Mosquitoes and Men: Renal Potassium Channels as Novel Disease Targets. **JEROD DENTON**, *Vanderbilt University School of Medicine*

A growing body of physiological and genetic evidence suggests that certain members of the inward rectifier potassium (Kir) channel family expressed in renal epithelial cells may represent targets for novel classes of diuretics to lower blood volume and pressure. We will discuss our ongoing work using high-throughput screening, medicinal chemistry, conventional and automated patch-clamp electrophysiology, and x-ray structure-guided mutagenesis to develop potent and highly selective small-molecule probes to assess the integrative physiology and therapeutic potential of Kir1.1, Kir4.1, and Kir7.1 channels. Kir channels are phylogenetically ancient and have evolved to play important physiological roles in lower organisms as well. We will also discuss our work aimed at developing Kir channel inhibitors to induce "renal" failure in the malarial vector *Anopheles gambiae* as novel disease–vector control insecticides.

7. High-Resolution Proteomics Unravel Full Molecular Diversity of Native AMPA Receptor Complexes in the Mammalian Brain. **BERND FAKLER**, *University of Freiburg, Germany*

AMPA-type glutamate receptors (AMPARs) are responsible for a wide variety of processes in the mammalian brain including fast excitatory neurotransmission, postsynaptic plasticity, or synapse development. We show by using comprehensive and quantitative proteomic analyses that native AMPARs are macromolecular complexes with an unappreciated large molecular diversity. This diversity results from coassembly of the known AMPAR subunits, pore-forming GluA and three types of auxiliary proteins, with 21 newly identified constituents, mostly secreted proteins or transmembrane proteins of different classes. Their integration at distinct abundance and stability establishes the heteromultimeric architecture of native AMPAR complexes: a defined core with a variable periphery resulting in an apparent molecular mass between 0.6 and 1 MDa. Coassembly of the newly identified constituents changes the gating properties of AMPARs and provides novel molecular links to the "protein dynamics" fundamental for the complex role of AMPARs in formation and operation of glutamatergic synapses.

8. Overheated and Highly Irritated: Thermal and Chemical Sensing from the Cambrian to the Sushi Bar. **PAUL GARRITY**, *Brandeis University*

Thermal and chemical detection in animals often involves cation channels of the transient receptor potential (TRP) family. My lab is studying the function and evolution of TRP-mediated sensory detection, focusing on TRPA1, an ion channel well known for its role in sensing wasabi, tear gas, and other pungent agents. Our recent work investigating the cellular and molecular basis of *Drosophila* TRPA1's ability to respond to thermal as well as chemical stimuli will be presented, with a particular emphasis on how TRPA1's properties have been modulated over evolution to endow different cells and different species with distinct behaviors.

9. Deconstructing Sensory Transduction with *C. elegans*. **MIRIAM GOODMAN**, *Stanford University School of Medicine*

The ability to detect touch is conserved from echinoderms to humans but remains poorly understood. My research group studies the nematode *C. elegans*, a simple animal that has only 30 mechanoreceptor neurons, to understand how our sense of touch works. We focus on two classes of mechanoreceptor neurons: the six touch receptor neurons (TRNs) that detect touch and the paired ciliated ASH neurons that detect noxious mechanical cues. I will discuss work from my lab that combines genetic dissection and classical genetic analysis with in vivo electrophysiology to understand the molecular events that give rise to the sense of touch. Additionally, I will also discuss recent work using *C. elegans* to understand the extraordinary sensitivity and robustness of thermosensation.

10. Sensory TRP Channels in Airway Chemosensation and Inflammation. **SVEN-ERIC JORDT**, *Yale University*

The human respiratory system is highly sensitive to damage by hazardous chemicals and pathogens. Reflex responses such as cough, sneezing, and glandular secretions are thought to protect the airways from toxic exposures, promoting the inactivation and removal of chemical threats and pathogens from the respiratory tract. Respiratory reflexes are triggered by chemosensory trigeminal and vagal sensory nerve fibers innervating the airways. These nerve fibers are activated by a wide range of reactive electrophiles, oxidants, acids, bases, organic solvents, and particulates. Only little is known about the molecular mechanisms enabling chemosensory nerves to detect such a wide variety of stimuli. Using a combination of physiological, molecular, and genetic approaches, we identified TRPA1, a TRP ion channel, as a receptor for multiple reactive stimuli in airway sensory neurons. Transient receptor potential (TRP) ion channels are membrane receptor proteins that integrate chemical and physical stimuli to induce cation flux and neuronal excitation. We found that TRPA1 mediates neuronal responses to a multitude of chemicals, including acrolein, the major irritant in cigarette smoke, noxious terpenes, tear gas agents, asthma-inducing isocyanates, and chlorine. Using barometric plethysmography, we observed that TRPA1-deficient mice lacked respiratory irritant responses to chlorine and other oxidants, supporting a major role for TRPA1 in the initiation of respiratory reflexes. TRPA1-activating stimuli such as cigarette smoke, chlorine, and aldehydes are frequent triggers of asthma attacks. Endogenous TRPA1 agonists, including reactive oxygen species, hypochlorite, and lipid peroxidation products, are generated by lung-infiltrating immune cells and have been recognized as potent drivers of allergen-induced airway inflammation in asthma. Using the murine ovalbumine model, we examined the role of TRPA1 in inflammatory responses in murine asthma. Genetic ablation of TRPA1 diminished allergen-induced leukocyte infiltration in the airways, reduced cytokine and mucus production, and almost completely abolished airway hyperreactivity to cholinergic stimuli. This phenotype was recapitulated by treatment of wild-type mice with TRPA1 antagonists. $Trpa1^{-/-}$ mice displayed deficiencies in chemically and allergen-induced release of proinflammatory neuropeptides in the airways. In summary, our data suggest that TRP channels are a key integrator of interactions between the chemical environment and the immune and nervous systems in the airways, triggering acute reflex responses and modulating airway inflammation after inhaled allergen challenge. TRP channels may represent promising pharmacological targets for the treatment of cough, asthma, and other inflammatory conditions.

11.* Molecular Pharmacology of Ion Channels Expressed in Induced Pluripotent Stem Cells Guides Clinical Therapy of a Long QT Variant 3 Patient. **ROBERT S. KASS**, *Columbia University*

With increasingly powerful, efficient, and accessible sequencing technology, the promise of personalized medicine seems within reach, and yet the complexities of human disease remain daunting. Understanding the basis for individual and differential responses to drug therapies remains challenging despite advances from genome-wide analysis. An emerging view is that an alternative approach is to focus on diseases caused by single gene mutations and to investigate the impact of these mutations within the context of the background genetic makeup of the mutation carrier. Here, I report on the use of inducible pluripotent stem cells (iPSCs) derived from a patient harboring a spontaneous mutation in the heart Na^+ channel as well as a common polymorphism in the hERG potassium channel to understand the mechanistic basis for resistance to gene-

targeted therapy based on heterologous expression and to test for potentially more effective therapies. We find previously undetected off-target drug effects that explain the individual's poor therapeutic response and identification of a simpler therapeutic regimen that, to date, has been effective in controlling arrhythmias in the patient.

12. Mass Spectrometry-based Identification of Native Cardiac Nav1.5 Channel Phosphorylation Sites. CELINE MARIONNEAU,[1] CHERYL F. LICHTI,[2] PIERRE LINDENBAUM,[1] FLAVIEN CHARPENTIER,[1] JEANNE M. NERBONNE,[4] R. REID TOWNSEND,[2,3] and JEAN MEROT,[1] [1]*Institut du Thorax, INSERM UMR1087, CNRS UMR6291, Nantes, France;* [2]*Department of Medicine,* [3]*Department of Cell Biology and Physiology, and* [4]*Department of Developmental Biology, Washington University Medical School, Saint Louis, MO 63110*

Cardiac voltage-gated Na^+ (Nav) channels are key determinants of action potential waveforms, refractoriness, and propagation, and direct phosphorylation of Nav1.5 pore-forming (α) subunits has been suggested to be critical in regulating various aspects of channel function. Although previous studies have suggested roles for specific kinases and amino-acid residues in mediating these effects, the basal, in situ phosphorylation sites on the Nav1.5 protein have not been identified, and the impact of basal phosphorylation on the expression and the functioning of native cardiac Nav1.5 channels is unknown. A mass spectrometry (MS)-based proteomic approach was developed and exploited here to characterize in situ cardiac Nav channel complexes and to identify native Nav1.5 phosphorylation sites. Using anti-NavPAN–specific antibodies (directed against all Nav α subunits), Nav channel complexes were immunoprecipitated from adult wild-type mouse heart, and MS analyses led to the reliable identification of the Nav1.5 protein. These analyses also resulted in the identification of three other Nav α subunits, the Nav1.4, Nav1.3, and Nav1.9, as well as of some of the previously identified Nav channel associated/regulatory proteins, such as the Calmodulin, the CamKII δ subunit, and the fibroblast growth factor 13. In addition to interacting proteins, phosphoproteomic analyses of purified cardiac Nav1.5 protein identified 11 serine/threonine phosphorylation sites, of which nine are novel. With the exception of one residue located in the cytoplasmic N terminus, all the phosphorylation sites identified are in the first intracellular linker loop, suggesting a critical role for this region in phosphorylation-dependent regulation of Nav1.5 channel expression and functioning. Interestingly, commonly used phosphorylation site prediction algorithms did not accurately predict these newly identified in situ Nav1.5 phosphorylation sites. The results of this analysis, therefore, provide the first unbiased in situ phosphorylation map of cardiac Nav1.5 channels and demonstrate that native cardiac Nav1.5 channels are highly phosphorylated.

13. Dysfunction of Coronary Smooth Muscle Ca^{2+} Regulation in the Progression of Metabolic Syndrome and Coronary Artery Disease in Ossabaw Miniature Swine. MIKAELA L. MCKENNEY,[1] MEREDITH C. KOHR,[1] MOUHAMAD ALLOOSH,[1] KYLE A. SCHULTZ,[1] L. NICOLE BELL,[2] JOHNATHAN D. TUNE,[1] and MICHAEL STUREK,[1] [1]*Department of Cellular and Integrative Physiology and* [2]*Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202*

Coronary smooth muscle (CSM) cell Ca^{2+} regulation was studied in the Ossabaw swine model of metabolic syndrome (MetS) and coronary artery disease (CAD). MetS was induced by excess calorie atherogenic diet for 9 or 12 months and compared to lean controls on normal diet. Isometric tension studies on isolated coronary arteries revealed that 9 months of MetS increased tension development to K depolarization versus lean controls, whereas 12 months reduced tension versus control. MetS increases CAD and CSM Ca^{2+} dysregulation. CSM were isolated enzymatically and digitally imaged with the fluorescent Ca^{2+} indicator fura-2. There was no difference in basal Ca^{2+} levels between groups. We released sarcoplasmic reticulum (SR) Ca^{2+} stores with caffeine. The peak Ca^{2+} release was increased in 9-month and decreased in 12-month MetS versus lean. This Ca^{2+} response largely represents the caffeine-sensitive SR Ca^{2+} store capacity. After the SR store depletion, a sustained Ca^{2+} signal above basal levels remained in the 9-month MetS group, reflecting store-operated Ca^{2+} entry and decreased Ca^{2+} extrusion ability. Intravascular ultrasound and micro-computed tomography imaging showed greater atherosclerosis and extracellular calcification in 12- versus 9-month MetS. Collectively, these data suggest that CSM undergo dedifferentiation from a contractile to a synthetic phenotype defined by CSM proliferation and migration and increased intracellular Ca^{2+} signaling. As CAD further progresses, CSM dedifferentiate to an osteogenic phenotype with decreased intracellular Ca^{2+} signaling and increased extracellular calcification.

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14. Effects of GLP-1 Receptor Agonist on Ca^{2+} Handling of Coronary Smooth Muscle Cells from Metabolic Syndrome Ossabaw Swine with Coronary Artery Disease. MIKAELA L. MCKENNEY, DANIEL SUH, MOUHAMAD ALLOOSH, KYLE A. SCHULTZ, L. NICOLE BELL, NAGA CHALASANI, and MICHAEL STUREK, *Department of Cellular and Integrative Physiology and Division of Gastroenterology/Hepatology, Indiana University School of Medicine, Indianapolis, IN 46202*

Chronic effects of the glucagon-like peptide (GLP-1) receptor agonist AC3174 were studied in Ossabaw swine with metabolic syndrome (MetS). MetS was induced by hypercaloric atherogenic diet for 6 months. Subcutaneous injections of placebo or AC3174 were given twice daily for 6 months after induction of MetS. Consistent with clinical effects, AC3174 attenuated food consumption from 96 ± 1% to 82 ± 2% of the allotted amount ($P < 0.05$), and weight gain decreased from 116.2 ± 2.4 kg to 99.5 ± 5.8 kg, respectively.

MetS increases coronary artery disease (CAD) and coronary smooth muscle (CSM) dysfunction of intracellular Ca^{2+} handling. CSM cells were isolated enzymatically from the coronary arteries, and Ca^{2+} was digitally imaged with fluorescent fura-2. There was no difference in basal Ca^{2+} levels. In normal physiological salt solution, we released sarcoplasmic reticulum (SR) stores with caffeine, which binds to ryanodine receptors in the SR membrane. AC3174-treated cells showed a larger peak increase in Ca^{2+} in response to caffeine, $\Delta F360/380 = 0.26 \pm 0.02$, compared to placebo cells, $\Delta F360/380 = 0.16 \pm 0.01$ ($P < 0.001$). In CSM from healthy lean pigs, acute exposure to AC3174 markedly increased activity of the sarcoplasmic–endoplasmic reticulum Ca^{2+} ATPase (SERCA). The increased SERCA may explain increased SR Ca^{2+} store in CSM of MetS pigs. Recovery time to basal levels after the SR store depletion was not different between groups, but a sustained Ca^{2+} signal was present in the treated cells, implying there is greater Ca^{2+} influx after chronic AC3174 treatment. The sustained fluorescence ratio from treated cells was 0.07 ± 0.01 compared to a lesser signal of 0.04 ± 0.003 from placebo cells ($P < 0.05$). It appears chronic AC3174 treatment elicits greater Ca^{2+} release and influx in MetS. Our interpretation is that AC3174 attenuates the decline in CSM Ca^{2+} handling associated with more severe CAD and dedifferentiation of CSM from a contractile to an osteogenic phenotype.

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15. Computational Ligand- and Structure-based Drug Design Methodologies Applied to Ion Channels and Other Membrane Proteins. **JENS MEILER**, *Vanderbilt University*

We present a framework that generates quantitative structure activity relationship (QSAR) models from high-throughput screening (HTS) data using a consensus of machine-learning approaches and custom-optimized descriptor sets to optimize prediction accuracy. The framework offers a series of new algorithms to apply the resulting QSAR models for virtual screening, scaffold hopping, pharmacophore mapping, and design of focused libraries in hit-to-lead optimization. We combine this ligand-based approach to drug discovery with structure-based comparative modeling and ligand-docking approaches using the Rosetta program. Application examples include potassium channels, G protein–coupled receptors, and serotonin transporter.

16. Structural Insights into Ion Channel Function and Modulation. **DANIEL L. MINOR JR.**, *University of California, San Francisco, San Francisco, CA*

Ion channels are fundamental components of biological electrical signaling networks. These proteins control the passage of ions across the cell membrane and generate the bioelectricity that is essential for life. Much of the future of neuroscience, cardiovascular research, and design of biomimetic membrane systems lies in understanding the molecular details of how these protein machines function, how they are regulated, and how they are integrated into large macromolecular complexes within the cell. To address these issues, my laboratory seeks to uncover the basic mechanisms by which ion channels act through the use of a multidisciplinary approach that includes genetic selections, x-ray crystallography, solution biophysical methods, and electrophysiology. We are interested in understanding the high-resolution structures of channel proteins, their regulatory factors, and the conformational changes that accompany channel action as well as in the development novel means to manipulate channel action in vivo. Recent advances from these efforts will be presented.

17. Native Kv4-encoded Neuronal and Cardiac Kv Channels Function in Macromolecular Protein Complexes. **JEANNE M. NERBONNE**, *Washington University*

Voltage-gated K^+ (Kv) channels are key determinants of membrane excitability in the nervous and cardiovascular systems, functioning to control resting membrane potentials, to shape the waveforms of action potentials and repetitive firing patterns, and to regulate the responses to neurotransmitters and neurohormones. Consistent with this functional diversity, multiple types of Kv currents, with distinct time- and voltage-dependent properties and cellular/subcellular expression patterns, have been identified. Rapidly activating, inactivating, and recovering Kv currents, typically referred to as I_A (A-type) in neurons, for example, regulate repetitive firing rates and action potential back-propagation into dendrites and modulate the responses to synaptic inputs, influencing synaptic plasticity. Currents with similar biophysical properties, referred to as $I_{to,f}$ (fast transient outward), expressed in cardiomyocytes, control the early phase of myocardial action potential repolarization. Several recent studies have demonstrated critical roles for pore-forming (α) subunits of the Kv4 subfamily in the generation of native neuronal I_A and cardiac $I_{to,f}$ channels. Studies in heterologous cells have

also suggested roles for a number of Kv channel accessory and regulatory proteins in the generation of I_A and $I_{to,f}$ channels. The application of quantitative mass spectrometry–based proteomic analysis is being exploited increasingly as a rapid and, importantly, unbiased approach to identify the components of native macromolecular protein complexes. Recent studies exploiting these approaches to identify the components of native neuronal (and cardiac) Kv4-encoded channel complexes and the experimental approaches being exploited to define the physiological roles of newly identified putative accessory and regulatory channel subunits will be discussed.

18. KATP Channel Mutations and Diabetes: Back and Forth from Bench to Bedside. **COLIN NICHOLS**, *Washington University*

Cloning of the molecular components of the ATP-insensitive K_{ATP} channel led to structure–function analyses by site-directed mutations that provide a coherent molecular model that is now well supported by homologous Kir channel crystal structures. These molecular analyses led us to develop transgenic mice models of gain-of-function, ATP-insensitive channels, which in turn demonstrated the critical role of K_{ATP} channel closure in insulin secretion. These transgenic mice expressing these mutant channels in pancreatic β cells typically died as neonates because of profound diabetes with ketoacidosis. This led us to predict the subsequent discovery that similar mutations cause human neonatal diabetes mellitus (NDM). This realization has in turn led to most neonatal diabetics receiving a dramatic lifestyle change in switching from injected insulin to the channel-blocking sulfonylurea therapy. Novel transgenic mice carrying an ATP-insensitive mutant K_{ATP} channel subunit under control of Cre recombinase have permitted in-depth exploration of the mechanistic progression of the disease and treatment options. Constitutive expression in pancreatic β cells (by RIP-driven expression) causes neonatal diabetes that becomes progressively more severe, with growth retardation and loss of both islet insulin content and β cell architecture. When expression is induced in adult β cells (under tamoxifen-inducible PDX promoter control), diabetes ensues within 2 weeks, with similar secondary consequences. Chronic sulfonylurea treatment avoids diabetes and maintains insulin content if initiated before disease onset but is ineffective after diabetes has developed, and in human NDM, sulfonylureas can replace insulin therapy, but dosing and efficacy are highly variable. To gain insight to drug dosing and efficacy in NDM, we treated xNDM mice with high-dose glibenclamide for 6 days only at the beginning of disease induction. Approximately 70% of mice develop severe diabetes after treatment cessation, but ~30% maintain near-normal blood glucose indefinitely, because of a compensatory increased insulin sensitivity. The results imply that there is a critical window early in disease onset, during which compensatory mechanisms can develop, switching the disease from permanent to an essentially transient form. We propose that such a mechanism underlies the fact that the same mutation can cause both transient or permanent NDM in humans, and that a worsened prognosis for sulfonylurea therapy in older patients might be the result of chronically poor glycemic control during years of insulin therapy, leading to gradual loss of β cells.

19. Store-operated cAMP Signaling in the Regulation of Colonic Secretory Function. JONATHAN M. NICHOLS, ISABELLA MAIELLARO, JOANNE ABI-JAOUDE, SILVANA CURCI, and ALDEBARAN M. HOFER, *VA Boston Healthcare System, Brigham and Women's Hospital, Harvard Medical School, Boston, MA*

Apical cAMP-dependent CFTR Cl^- channels are essential for efficient vectorial movement of fluid into the lumen of the colon. In colonic cell lines (e.g., T84), it has been postulated that Ca^{2+}-dependent Cl^- channels also participate in this process. In contrast, evidence for an apical Ca^{2+}-dependent Cl^- current has not been reported in the native colonic epithelium. In fact, it has long been held that Ca^{2+}-mediated transepithelial anion secretion depends absolutely on the presence of cAMP-dependent CFTR, even though CFTR is apparently not activated directly by Ca^{2+}. We recently described a new mode for eliciting elevations in intracellular cAMP that is triggered by decreases in Ca^{2+} content within the ER lumen. This process required the luminal ER Ca^{2+} sensor known as STIM1 and was particularly prominent in certain colon- and airway-derived cultured cells. We termed this process store-operated cAMP signaling, or SOcAMPS. Here, we assessed the extent to which SOcAMPS participates in epithelial Cl^- transport as measured by transepithelial short-circuit current (I_{sc}) in polarized T84 monolayers. In agreement with previous findings, we found that in Ca^{2+}-free conditions, the Ca^{2+} ionophore ionomycin and the Ca^{2+}-mediated agonist carbachol stimulate Cl^- secretion. The effect was enhanced in the presence of the phosphodiesterase inhibitor IBMX and inhibited by the CFTR inhibitor $CFTR_{inh}$-172. The response persisted after BAPTA-AM treatment and was unaffected by Ba^{2+} (inhibits Ca^{2+}-activated K^+ channel) or flufenamic acid (inhibits Ca^{2+}-activated Cl^- channels). Parallel imaging measurements of FRET-based reporters for cAMP (EpacH30 and EpacH90) and of PKA activity (AKAR4) showed that depletion of Ca^{2+} stores resulted in increased cAMP levels and PKA activation. We propose that a discrete component of the Ca^{2+}-activated secretory activity in the colon derives from SOcAMPS. This alternative mode of cAMP production could contribute to the actions of

diverse xenobiotic agents that disrupt ER Ca^{2+} homeostasis, leading to diarrhea.

20. Conotoxins: Developing Combination Neuropharmacology Targeted to Ion Channels and Receptors. **BALDOMERO OLIVERA**, *University of Utah*

The 700 different species of cone snails have extremely complex venoms, each with many hundreds of different peptidic toxins. The vast majority of these are targeted to ion channels and receptors. Prior work done on the venoms of fish-hunting cone snails has shown that in effect, cone snails use a combination drug therapy strategy: multiple toxins, each targeted to a specific receptor/ion channel subtype, act together towards a common physiological end point. We call such combinations of conopeptides toxin "cabals." The consequence of this combination strategy is an extremely diverse set of peptides in every venom. Enough *Conus* venom peptides have been characterized so that we can adopt the combinatorial strategy of the snails for biomedically useful purposes. The utility of combinations of peptides ("diagnostic cabals") for functionally profiling the neuronal subtypes present at a particular anatomical locus in the mammalian nervous system will be discussed. One *Conus* venom peptide has become an approved drug for intractable pain, and five others have undergone human clinical trials. Ultimately, by having a large array of highly specific peptides for a set of desirable molecular targets, the use of multiple conopeptides or conopeptide-like pharmacological agents for combination drug therapy should become an increasingly feasible approach to address some of the more intractable neurological pathologies.

21. Genetic and Molecular Basis of Episodic Disorders. **LOUIS J. PTÁČEK**, *University of California, San Francisco, San Francisco, CA*

Episodic phenomena are common in humans. These include (but are not limited to) seizures, headaches, cardiac arrhythmias, episodic movement disorders, and periodic paralyses. These disorders have strong genetic determinants and often affect people who are completely normal between attacks. Although episodic disorders of the brain, heart, and muscle seem quite different on the surface, they share many similarities. They often come on in childhood or adolescence and frequently improve with aging. In addition to being episodic, attacks in all of these disorders can often be precipitated by stress, fatigue, and some dietary factors. The medications used to treat these disorders overlap significantly. Thus, insights gained by study of any of these disorders can impact our understanding of the others. Beginning in the early 1990s, we set out to clone genes responsible for a group of disorders called the familial periodic paralyses. We now know that mutations in four different ion channel genes expressed in muscle are responsible for a large majority of patients with these diseases. Three of these genes (*SCN4A, CACNA1S*, and *CLCN1*) are muscle specific, but the fourth (*KCNJ2*) is widely expressed. *KCNJ2* mutations cause one form of periodic paralysis called Andersen-Tawil Syndrome (ATS). Not surprisingly, ATS also has other features including cardiac arrhythmias, developmental features, and a neurocognitive phenotype. Thyrotoxic periodic paralysis (TPP) usually appears as a sporadic disorder that resolves with treatments of the underlying thyrotoxicosis. Based on our knowledge of the molecular basis of familial periodic paralyses, we were able to clone a novel gene (*KCNJ18*) encoding an inwardly rectifying potassium channel and showed that it is mutated in some TPP patients. Knowledge of the genetic and molecular basis of these disorders provided the foundation for the entire channelopathy field, which now includes many channel genes that are mutated in cardiac arrhythmias, migraine headache variants, and epilepsies. Such insights lead to better classification systems for patients and will ultimately lead to better therapies. More recently, we've cloned genes for a number of disorders including epilepsy that encode proteins of unknown function. We know that these are not ion channels and are exploring the normal role of these proteins in neuron, circuit, and brain function. We are also interested in understanding how the mutations lead to the clinical phenotypes. Understanding of these new pathways may lead to novel therapeutic targets for episodic disorders such as epilepsy, migraine, and movement disorders like Parkinson's disease.

22. Melanopsin Expression Confers Light Sensitivity to Neurons of the Outer Nuclear Layer in the avian retina. JORGE RAMÍREZ,[1,3] ANDRÉS HERNÁNDEZ,[1,3] ATTICUS PINZÓN,[1,3] ENRICO NASI,[2,3,4] and MARÍA DEL PILAR GOMEZ,[1,3,4] *[1]Departamento de Biología and [2]Instituto de Genética, Universidad Nacional de Colombia, Bogotá; [3]Centro Internacional de Física, Bogotá; [4]Marine Biological Laboratory, Woods Hole, MA*

In mammals, the discovery of melanopsin and intrinsic light responsiveness in a small population of retinal ganglion cells (ipRGCs) paved the way for understanding light regulation of nonvisual functions, like the pupillary reflex or the entrainment of circadian rhythms. The scarcity of ipRGCs, however, is a hurdle for investigating melanopsin signaling mechanisms. In the avian retina, melanopsin expresses abundantly in the outer nuclear layer too, but its functionality is unknown. We used the chicken embryo retina as a model system to investigate possible physiological roles of melanopsin in non-ganglion cells. A polyclonal antibody targeting both chicken melanopsin isoforms was validated by Western blot and used to corroborate the distribution pattern in fixed retina sections; strong

immunoreactivity occurred in areas that likely include horizontal, bipolar, and some amacrine cells. Retinas were enzymatically dissociated to yield morphologically well-preserved isolated neurons; their physiological viability was tested with whole cell recording. Although voltage-gated currents were found in the different cell types, initial recordings failed to reveal direct changes in membrane current by photostimulation. However, light could reversibly modulate voltage-gated currents. Fluorescence imaging in cells loaded with calcium indicators demonstrated a Ca fluorescence increase in selected bipolar cells and small neurons, likely to comprise a subtype of amacrine cells. Higher sensitivity measurements, using a photomultiplier and pulsed light to extend the recording period, showed that in a minority of bipolar cells, light evoked Ca responses with a long latency and a slow time course, spanning minutes. A reassessment of electrical responses occurring on such a long time scale revealed a small inward current (tens of pA) with a similar time course. These observations indicate that intrinsic photosensitivity is not confined to rods, cones, and some ganglion cells, but extends to additional retinal cell types. Its physiological role remains to be investigated.

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23. Inward Rectifier Potassium Channels as Novel Insecticide Targets in Disease-carrying Mosquitoes. RENE RAPHEMOT,[1,2] PETER M. PIERMARINI,[3] KLAUS W. BEYENBACH,[4] COREY HOPKINS,[2] CRAIG W. LINDSLEY,[2] and JEROD S. DENTON,[1,2] *[1]Department of Anesthesiology and [2]Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232; [3]Department of Entomology, The Ohio State University, Wooster, OH 44691; [4]Department of Biomedical Sciences, Cornell University, Ithaca, NY 14853*

Diseases such as dengue fever, yellow fever, and malaria are transmitted to humans through the bite of an infected mosquito. Thus, mosquitoes represent one of the largest threats to global health. Efforts to reduce mosquito populations are being challenged by the emergence of resistance to commonly used control agents. Thus, the development of insecticides with new modes of action is essential for controlling the transmission of mosquito-borne diseases. We therefore initiated an insecticide-development campaign targeting inward rectifier potassium (Kir) channels, which play essential physiological roles in diverse species and have yet to be targeted for insecticide development in any insect. We report that a barium-sensitive Kir channel cloned from the Malpighian (renal) tubules of the yellow fever mosquito *Aedes aegypti* (AeKir1) was functionally expressed in HEK293 cells. A fluorescence-based, thallium-flux assay was developed to support a high-throughput screening (HTS) campaign for small-molecule modulators of AeKir1. By screening a focused library of mammalian Kir channel antagonists, a moderately potent inhibitor of AeKir1, termed VUXXX, was discovered and found to inhibit urine production in isolated Malpighian tubule assays. Medicinal chemistry is being used in an effort to improve the potency and selectivity of VUXXX for AeKir1 over mammalian Kir channels. From a screen of ~30,000 compounds, >100 novel AeKir1 inhibitors have been confirmed. HTS, medicinal chemistry, and electrophysiology are being used to develop a panel of drug-like inhibitors of mosquito Kir channels to test for the first time if chemical induction of "renal failure" represents a viable method for controlling mosquito populations.

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24. Novel Regulation of Sensory Neuron Sodium Current. **ANGELES B. RIBERA**, *University of Colorado*

Neurological symptoms of hyperthyroidism include seizures and tremor, while those of hypothyroidism comprise cognitive impairment, decreased nerve conduction velocity, peripheral neuropathy, and cerebellar ataxia. These neurological phenotypes implicate voltage-gated currents, especially sodium channels, as likely targets of thyroid hormone regulation. Our research has shown that thyroid hormone regulates sensory neuron sodium current. Our work takes advantage of the genetic, molecular, physiological, and imaging approaches possible in the zebrafish embryo model. The thyroid hormone T4 rapidly up-regulates the density of voltage-gated sodium current in zebrafish sensory neurons. This rapid effect involves T4 signaling via the plasma membrane integrin, αVβ3, rather than nuclear receptors. Pharmacological studies implicate p38 MAPK and protein phosphatases as downstream mediators of T4's action. Further, T4-αVβ3 signaling targets sodium channels containing the Nav1.6a α subunit, but not another sensory neuron sodium channel isotype, Nav1.1la. These findings raise the possibility that defects in T4-αVβ3 signaling contribute to the neurological symptoms of thyroid disorders.

25. Translating Fundamental Scientific Discovery to the Bedside to Personalize Medicine: Lessons from the Cardiac Ion Channel World. **DAN RODEN**, *Vanderbilt University School of Medicine*

The genomic era is producing a rapidly increasing dataset of common and rare DNA variants that are implicated as modulators of protein function. In the cardiac ion channel domain, the genome-wide association study paradigm has linked common DNA variants with electrophysiologic traits ranging from variability in normal ECG indices of conduction or repolarization to arrhythmia susceptibility. At the other end of the frequency spectrum, rare variants are well

recognized as causes of monogenic diseases like long QT syndrome or catecholaminergic polymorphic ventricular tachycardia. It is also becoming increasingly clear that interactions among rare and common genetic variants, and between genetic variants and environmental influences, determine clinical phenotypes. One important environmental variable is drug exposure: the field now recognizes individuals with arrhythmia susceptibility alleles, who may not display clinical phenotypes until exposure to inciting drugs.

26. Bax/Bak Orchestrates Ion Transport, Apoptosis, and Inflammatory Response of Host Cells to *Pseudomonas aeruginosa* Quorum-sensing Molecule Homoserine Lactone. CHRISTIAN SCHWARZER, ZHU FU, MARK GRABINER, STACEY SHUAI, JASON KIM, and TERRY E. MACHEN, *Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720*

P. aeruginosa, which accumulates in the lungs of cystic fibrosis (CF) patients, uses *N*-(3-oxododecanoyl)-homoserine lactone (C12) as a quorum-sensing molecule. C12 also affects host cells: activating the IP_3 receptor, stimulating cAMP and CFTR, and triggering the ER unfolded protein response and apoptosis, and both pro- and antiinflammatory responses. The present work tested whether bax and bak were involved in the effects of C12 on fibroblasts. In wild-type mouse embryo fibroblasts (wt MEFs), 50 μM C12 elicited apoptosis-associated blebbing of plasma membranes, condensation of nuclei, release of cytochrome C from mitochondria, and activation of caspase 3/7. C12 also caused the ER to release Ca^{2+} and mitochondria to depolarize. C12 caused none of these effects in $bax^{-/-}/bak^{-/-}$ (dko) MEFs. C12 caused NF-κB p65 to enter the nucleus from the cytosol, but also inhibited NF-κB–regulated luciferase expression in wt but not in dko MEFs. C12 stimulated expression but inhibited secretion of proinflammatory cytokines IL-6 and KC in untreated and in TNFα-treated wt MEFs. In contrast, C12 did not affect NF-κB or expression or secretion of cytokines in either untreated or in TNFα-treated dko MEFs. Thus, C12-triggered events all required bax/bak, indicating that the responses were mediated through a common mechanism that shares many characteristics with pattern-associated molecular pattern receptors (e.g., TLRs and NODs). It is proposed that C12 regulates bax/bak, which causes: (a) depolarization of mitochondria; (b) release of cytochrome C (leading to activation of caspase 3/7 and apoptosis); (c) activation of IP_3R (and release of Ca^{2+} from the ER into the cytosol, activation of STIM1 and adenylate cyclase, and cAMP/PKA and CFTR); and (d) activation of NF-κB and expression of cytokine genes but inhibition of cytokine production and secretion resulting from effects of C12 to trigger ER stress and inhibit protein synthesis.

Supported by NIH, CFRI.

27. Molecular Basis for Water Taste in *Drosophila*. **KRISTIN SCOTT**, *HHMI/University of California, Berkeley, Berkeley, CA*

The detection of water and the regulation of water intake are essential for animals to maintain proper osmotic homeostasis. Drosophila and other insects have gustatory sensory neurons that mediate the recognition of external water sources, but little is known about the underlying molecular mechanism for water taste detection. Here, we identify a member of the degenerin/epithelial sodium channel family, ppk28, as an osmosensitive ion channel that mediates the cellular and behavioral response to water. We use molecular, cellular, calcium imaging, and electrophysiological approaches to show that *ppk28* is expressed in water-sensing neurons and that loss of *ppk28* abolishes water sensitivity. Moreover, ectopic expression of *ppk28* confers water sensitivity to bitter-sensing gustatory neurons in the fly and sensitivity to hypo-osmotic solutions when expressed in heterologous cells. Ongoing studies are testing the model that ppk28 directly responds to membrane stretch induced by low osmolarity. These studies link an osmosensitive ion channel to water taste detection and drinking behavior, providing the framework for examining the molecular basis for water detection in other animals.

28. Ion Channel Proteomics and Dynamic Regulation of Cell Physiology. **JAMES TRIMMER**, *University of California, Davis, Davis, CA*

As physiology enters the post-genomic era, a major goal is the translation of genomic sequence information into a molecular understanding of the mechanisms of neuronal information processing and transfer. My laboratory's research focuses on the biochemical pathways and networks of protein–protein interactions and posttranslational modifications that dynamically regulate intra- and intercellular signaling in mammalian neurons. In particular, we are interested in dynamic regulation of voltage-sensitive ion channel abundance, localization, and function through dynamic protein–protein interactions mediated by reversible protein phosphorylation. Modern proteomic techniques have allowed for insights into protein networks, posttranslational modifications, which allow for the design of experiments to directly test their role in regulating ion channels, and the subsequent impact of such regulation on cell physiology. Our approach in general involves monoclonal antibody–based immunopurification of native ion channels from mammalian brain and other tissues, and the subsequent analysis of the components of the purified channel complexes, and posttranslational modification, by tandem mass spectrometry. The obtained data is then used in experiments to determine the role of these

protein–protein interactions and modifications. Although our studies are primarily aimed at a molecular understanding of how neuronal ion channels generate and maintain the fidelity of neuronal signaling, and how these processes can be dynamically regulated to generate neuronal plasticity, the insights gained from these studies has broad implications across all of physiology. Such information is necessary for an increased understanding not only of the normal functional plasticity of cells but also of disease states where cellular function is altered, and the response of cells to acute external insults such as ischemia and drugs of abuse.

29. Aquaporin Water Channels as Drug Targets. **ALAN VERKMAN**, *University of California, San Francisco, San Francisco, CA*

The aquaporins are a family of membrane water channels, some of which also transport glycerol. They are involved in a wide range of physiological functions and human diseases, including water/salt homeostasis, exocrine fluid secretion, glaucoma, cancer, epilepsy, obesity, and epidermal hydration. At the cellular level, aquaporin-mediated osmotic water transport across cell plasma membranes facilitates transepithelial fluid transport, cell migration, and neuroexcitation; aquaporin-mediated glycerol transport regulates cell proliferation, adipocyte metabolism, and epidermal water retention. Genetic diseases caused by loss-of-function mutations in aquaporins include nephrogenic diabetes insipidus and congenital cataracts. The neuroinflammatory demyelinating disease neuromyelitis optica is marked by pathogenic autoantibodies against astrocyte water channel aquaporin-4 (AQP4). There remain broad opportunities in aquaporin-based diagnostics and therapeutics. There is great promise in the development of small-molecule aquaporin modulators for therapy of some types of refractory edema, brain swelling, neuroinflammation, glaucoma, epilepsy, cancer, pain, and obesity. We recently introduced anti-AQP4 monoclonal antibody blocker therapy as a novel approach to treat neuromyelitis optica, in which a high-affinity nonpathogenic antibody blocks binding of pathogenic autoantibodies to AQP4.

30. Influenza A–induced Cytokine Production Correlates with Altered Epithelial Chloride Channel Physiology. TARYN A. WAUGH, JOHN C.H. CHING, TARA D. GUINN, EMILY E. JOHN, RILEY A. GLEW, and MATTHEW E. LOEWEN, *Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada, S7N 5B4*

Upon influenza infection, respiratory epithelium lining the airways produces cytokines that aid in fighting the infection. These same cytokines by themselves have been shown to increase chloride channel function. Chloride movement through these channels creates the osmotic drive to hydrate the airways. However, the excessive increase in cytokine production during an influenza infection could contribute to excessive chloride channel activity and airway overhydration. This could explain, in part, the disruption of the fluid balance in the lungs and the resulting pulmonary edema that occurs during severe influenza infections. In this study, using polarized Calu-3 respiratory epithelial cells, we measured cytokine production by real-time quantitative RT-PCR and Bio-Plex suspension array. We simultaneously measured chloride channel function by means of short-circuit current produced by a Calu-3 monolayer in a Ussing chamber in response to agonists and chloride channel blockers. Viral protein production was shown along with an initial increase in cytokines at 24 hours after infection, including a large increase in antiinflammatory cytokines such as IL-4. Interestingly, no change in short-circuit current response was found at 24 hours after infection. However, further increases in proinflammatory cytokines at 48 hours after infection, such as IL-8 and IL-6, did correlate with a change in the agonist-induced short-circuit current response. However, the observed decrease in cAMP-induced short-circuit current, presumably mediated by CFTR in Calu-3 cells, did not correlate with the significant increases in CFTR mRNA induced by the infection. In conclusion, influenza infection either directly or in concert with cytokine production can induce transcriptional expression of chloride channels but simultaneously inhibit their function. This study is the first step in understanding the pathophysiology that drives fluid into the lungs during an influenza A infection.

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31. Discovery and Characterization of Potent and Selective Small Molecule GIRK activators. **C. DAVID WEAVER**, *Vanderbilt University School of Medicine*

G protein–coupled receptor-activated inward rectifying potassium (K^+) channels (GIRK, $K_{ir}3$, *KCNJ*) are a family of G protein–coupled receptor-modulated potassium channels. GIRK channels are comprised of homo- and heterotetrameric subunit combinations with broad distribution in the central nervous system and in the periphery. In the heart, for instance, the GIRK 1/4 subunit combination forms the conductance known as IK_{Ach}, a conductance important in cardiac function. GIRK 1/2, 1/3, and 2/3 combinations are expressed in different brain regions and thought to play varying roles with implications in epilepsy, pain, and addiction. IRK pharmacology, particularly with respect to activation, remains very poorly understood, with the only reported

small molecule activators being alcohols (including ethanol), some volatile anesthetics, and the natural product naringin. The most potent of these, naringin, shows potency of ~100 μM and little selectivity among GIRK subunit combinations. We have used a combination of thallium flux-based high-throughput screening, whole cell voltage-clamp electrophysiology, and technology-enabled medicinal chemistry to discover and characterize the first reported highly potent, effective, and selective small molecule activators of GIRK ($K_{ir}3$, *KCNJ*) potassium channels. These molecules are poised to enable an extension of our understanding of the role of GIRK channels in physiological and pathophysiological processes as well as a beginning to explore the therapeutic potential of GIRK activation.

32.* Insights into the Pathogenesis of Cystic Fibrosis from a New Model. **MICHAEL WELSH**, *HHMI/ University of Iowa School of Medicine*

Lung disease causes most of the morbidity and mortality in cystic fibrosis (CF). To better understand CF pathogenesis, we generated pigs with *null* and *ΔF508* alleles of the *CFTR* gene. Within months of birth, CF pigs spontaneously develop hallmark features of CF lung disease, including airway inflammation, airway remodeling, mucus accumulation, airway obstruction, and infection. Their lungs contained multiple bacterial species, suggesting an equal opportunity host defense defect. At birth, lungs of CF pigs showed no inflammation but failed to eradicate bacteria as effectively as wild-type pigs. These results suggest that impaired bacterial elimination is the pathogenic event that initiates a cascade of inflammation and pathology in CF lungs. These newborn pigs provide an unprecedented opportunity to investigate the mechanisms that impair host defense and initiate disease because they allow CF–non-CF comparisons without confounding secondary consequences of the disease. They also give us the chance to study mechanisms in vivo, in samples obtained from airways, and in primary cultures of airway epithelia.

33. The Potassium Channels Kv1.3 and KCa3.1 as Targets for Inflammatory Brain Pathologies. **HEIKE WULFF**, *University of California, Davis, Davis, CA*

The voltage-gated Kv1.3 and the calcium-activated potassium channel KCa3.1 are involved in the activation of T cells, macrophages, and microglia by regulating membrane potential and calcium signaling and have been proposed as potential antiinflammatory drug targets. We previously designed potent and selective small molecule inhibitors for both channels, PAP-1 for Kv1.3 and TRAM-34 for KCa3.1, and demonstrated that these compounds can prevent or treat various autoimmune diseases and inflammatory conditions in rodents, such as delayed-type hypersensitivity, contact dermatitis, type 1 diabetes, inflammatory bowel disease, atherosclerosis, and transplant vasculopathy. More recently we observed strong KCa3.1 immunoreactivity on activated microglia in infarcted rat brain and therefore tested TRAM-34 in middle cerebral artery occlusion (MCAO) with 7 days of reperfusion. Compound administration starting 12 h after reperfusion reduced infarct area by 50%, $ED1^+$ activated microglia and TUNEL-positive neurons in the infarcted hemisphere, and improved neurological deficit. When examining the effects of KCa3.1 and Kv1.3 blockers on microglia in vitro, we found that both TRAM-34 and PAP-1 inhibit amyloid-beta oligomer-induced microglia activation and microglia-mediated neuronal toxicity without affecting phagocytosis of fibrillar amyloid-beta. This observation raises the exciting possibility that KCa3.1 and Kv1.3 blockers might preferentially target microglia activities involved in neuronal killing without affecting beneficial functions such as scavenging of debris. Taken together with previous work implicating Kv1.3 and KCa3.1 in the pathogenesis of multiple sclerosis, these results suggest Kv1.3 and KCa3.1 as novel targets for CNS pathologies involving inflammation.

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Late Abstracts

34. Impact of blood pressure, body weight, age, and blood lipids on Pulse wave velocity in HIV positive participants in a semi urban African setting. [1]AWOTEDU K.O, [1]NAMUGOWA A. [1]IPUTO J.E. *[1]Dept of Physiology, Walter Sisulu University, Mthatha, South Africa*

There is increased risk of cardiovascular events in HIV positive patients. This includes dementia, stroke and death. Arterial stiffness plays an important part in these cardiovascular events. Measurement of (aortic pulse wave velocity; PWV) provides some of the strongest evidence concerning the prognostic significance of large artery stiffening. In this study we aimed to investigate the relationship between blood pressure, anthropometry, age, blood lipid profile and pulse wave velocity. This was a cross-sectional study comprising of 169 participants (62 males and 107 females). 63 of these were HIV negative (group A), 54 HIV positive participants not on treatment (group B), and 52 were HIV positive on treatment (group C). PWV was assessed using the Sphygmocor Vx. Statistically, ANOVA was used for variables with normal distribution and Non parametric tests were used for variables with skewed distribution. Notable significant differences were seen in the means of some variables across all the 3 groups. These included PWV, High Density Lipoprotein Cholesterol, Total Cholesterol, Heart rate, and Age. The mean PWV value for group B (7.212±2.1671) was greater than that for group C (6.843± 1.173) which in turn was more than group A (6.381±1.673); P=0.037. Blood pressure and Age were independent predictors of pulse wave velocity in all groups of participants using multivariate analysis. Our findings showed that HIV infected patients with or without antiretroviral therapy have increase arterial stiffness which is associated with an increased cardiovascular risk. Arterial stiffness was more pronounced on participants who are antiretroviral naïve. Measurement of arterial stiffness in these patients might help in early diagnosis of patients who are at risk of cardiovascular diseases.

35. In *vivo* Profile of SKA-19, a Mixed $K_{Ca}2$ Activator and $Na_v1.2$ Blocker, Working in Synergy to Reduce Neuronal Activity. NICHOLE COLEMAN, DAVID PAUL JENKINS, BRANDON BROWN, HEIKE WULFF. *Department of Pharmacology, University of California, Davis, CA*

Activators of neuronal potassium channels and blockers of neuronal sodium channels decrease neuronal excitability and may provide benefit for treating disorders characterized by neuronal hyperexcitability such as epilepsy and ataxia. Of particular interest are the small-conductance calcium-activated channels $K_{Ca}2.1$-2.3, which underlie the apamin sensitive medium afterhyperpolarization, and the voltage-gated $Na_v1.2$ channel. Both channels play important roles in neuronal excitability either through action potential generation ($Na_v1.2$) or regulation of firing frequency ($K_{Ca}2$). In the present study, we evaluated the effects of SKA-19 (6-((trifluoromethyl)thio)benzo[*d*]thiazol-2-amine) an orally bioavailable, mixed $K_{Ca}2.3$ opener (EC_{50} = 14.4 mM) and $Na_v1.2$ blocker (IC_{50} = 5 mM), in a broad range of rodent seizure models. SKA-19 was effective against maximal electroshock (MES) in both rats (MES, ED_{50} = 1.6 mg/kg i.p.) and mice (MES, ED_{50} = 4.9 mg/kg i.p.), in the amygdala kindling model of tonic seizures (full protection from seizure at 15 mg/kg) in rats and in the 6-Hz model of psychomotor seizures in mice (ED_{50} = 12.2 mg/kg). Antiseizure efficacy in all models was observed at doses significantly lower than the toxic dose (TD_{50} = 14.3 mg/kg, rat; 29.7 mg/kg, mice). SKA-19 further reduced the acute pain response in the formalin pain model during the first 20 minutes significantly, but had no effect in the inflammatory pain phase. From these findings we propose that $K_{Ca}2$ channel activating activity combined with blocking of the neuronal sodium channel $Na_v1.2$ exerts broad-spectrum antiepileptic activity in rodents in a synergistic manner.

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36. Correlating Over-active K_{ATP} Channels in β-Cells with Diabetes Progression. PAIGE COOPER and COLIN NICHOLS. *Center for the Investigation of Membrane Excitability Diseases*

and Department of Cell Biology and Physiology, Washington University School of Medicine, 660 South Euclid Avenue, Saint Louis, MO 63110

The pancreatic beta (β) cell is responsible for insulin production and release, and insulin release is tightly linked to cell metabolism and membrane excitability. The ATP sensitive potassium (K_{ATP}) channel is a key link between β-cell metabolism and membrane potential: metabolism of glucose normally leads to closing of K_{ATP} channels as the ATP/ADP ratio increases. K_{ATP} closure then results in membrane depolarization followed by Ca influx and insulin release. However, ATP-insensitive K_{ATP} channels remain open in response to increased metabolism, leading to reduced membrane depolarization and downstream reduction in insulin release, a mechanism that underlies neonatal diabetes and a contributory factor in type 2 diabetes (Remedi and Nichols. 2009. Cell Metab. 10:442-53). By combining a neonatal diabetes-causing point mutation (K185Q) and amino-terminal deletion (del N30) in the Kir6.2 subunit of the K_{ATP} channel, we have developed a mouse model of this disorder. The transgene is controlled by a tamoxifen-induced cre-recombinase system permitting temporal control of transgene induction. By removing islets from transgenic animals at specific times after transgene induction, we can assess the dependence of whole animal glucose levels on beta-cell K_{ATP} channel activity. We measured on-cell K_{ATP} channel activity and ATP-sensitivity of excised patches, and correlated this with blood glucose level at the time of isolation. In non-diabetic normoglycemic islets (blood glucose 121 $\pm$ 6 mg/dl), on-cell activity was 6% maximum off-cell activity (8.5 $\pm$ 1.6 pA per patch at -50 mV, n=4), and $K_{1/2,ATP}$ ([ATP] causing half-maximal inhibition) was 5.5 $\pm$ 1.2 μM, whereas in acutely diabetic islets (blood glucose 359 $\pm$ 34 mg/dl, 8-9 days following disease-induction), on-cell activity (2.95 $\pm$ 0.84 pA) was 20 % of maximum on-**C**ell activity (14.3 $\pm$ 3.1 pA per patch, n=13), and $K_{1/2,ATP}$ was 46.8 $\pm$ 2.1 μM. The results confirm a significant shift of ATP-sensitivity as causal in the onset of diabetes, without marked change in beta-cell K_{ATP} channel density.

37. Quartz Crystal Microbalance: A sensitive, label free and biochemically modifiable tool to provide insights into thin biofilms and membranes. CARSTEN HABER, *3T Analytik, Gartenstrasse 100, 78532 Tuttlingen, Germany*

The quartz crystal microbalance (QCM) is based on the piezoelectric effect which, in reverse mode, causes a quartz crystal sensor substrate operated at an AC frequency to resonate. In 1959, Sauerbrey established an equation which describes a linear relationship between mass adsorbed to the surface and the resonant frequency of the crystal in air. This relationship can be exploited to quantitatively assess energy dissipating properties of the bound surface mass. In parallel, a weaker interaction with the solution adjacent to the QCM sensor surface provides real-time insights into liquid viscosity-density changes. This phenomenon is referred as damping or dissipation. QCM type sensors have in the past decade been extensively employed to the study of a wide range of (bio)molecular interactions at the solution-surface interface, in biopolymers and biochemical systems. Surface derivatizations and attachment strategies used by investigators to reveal multi-layer interactions of thin films and membranes will be presented.

38. Sensory TRPA1 Channels Control Inflammation and Behavioral Responses in Murine Contact Dermatitis. BOYI LIU,[1]* JASMINE ESCALERA,[1]* SHRILATHA BALAKRISHNA,[1] LU FAN,[1] ANA. I. CACERES, [1] AIWEI SUI,[1] EVE ROBINSON,[2] CHRISTINE J. KO,[2] CHRISTINA A. HERRICK,[2] and SVEN-ERIC JORDT[1]. [1]*Department of Pharmacology and [2] Department of Dermatology, Yale School of Medicine, 333 Cedar St., New Haven, CT 06520.*

Contact dermatitis is a common skin condition characterized by inflammation and pruritus. In the majority of patients, contact dermatitis is caused by sensitization and subsequent epicutaneous challenge with environmental, occupational or nutritional allergens. TRPA1 is an irritant-sensing ion channel expressed in chemosensory nerves innervating the skin. Here we characterized the role of sensory TRPA1 channels in oxa-

zolone induced acute and chronic murine contact dermatitis model. Genetic ablation of TRPA1 inhibited skin edema and inflammation in acute dermatitis. This phenotype was recapitulated by treatment of wild-type mice with HC-030031, a TRPA1 antagonist. In the chronic oxazolone model, TRPA1 deficient mice also showed reduced skin edema and inflammation. This effect was mimicked by Substance P (SP) NK1 receptor antagonist aprepitant. Furthermore, TRPA1 but not TRPV1-deficient mice showed reduced dermatitis induced scratching behavior. TRPA1 antagonists HC-030031 and A-968079 also reduced scratching behavior. Levels of 4-HNE, an endogenous TRPA1 agonist and SP were highly increased in skin samples from chronic dermatitis mice. Pharmacological blockage of NK1 but not NK2 receptor significantly reduced mice scratching behaviors. Moreover, SP per se caused scratching behaviors and excitation of dorsal root ganglion neurons in wild-type mice but these effects were both reduced in TRPA1-deficient mice. Our data suggest that activation of TRPA1 and the release of SP play crucial roles in contact dermatitis. TRPA1 may represent a promising pharmacological target for the treatment of contact dermatitis and other allergic inflammatory conditions.

39. Calcium regulation in wild type and mutants D50N/Y human connexin26 (hCx26) hemichannels. LOPEZ W, GONZALEZ J, LIU J, HARRIS AL, CONTRERAS JE. *Department of Pharmacology and Physiology, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, NJ 07103*

Due to the large size and modest selectivity of the connexin hemichannel aqueous pore, highly regulated hemichannel opening is crucial. This regulation is achieved by physiological extracellular Ca^{2+}, which drastically reduces hemichannel activity. Here we characterize the Ca^{2+} response of the hCx26 wild type and human mutations D50N/Y, which cause gain in hemichannel function and result in deafness and skin disorders. We found that in hCx26 wild-type channels deactivation kinetics are accelerated as a function of Ca^{2+} concentration, indicating that Ca^{2+} facilitates and stabilizes the closed state of the hemichannels. Mutants D50N/Y do not display changes in deactivation kinetics in response to changes in extracellular Ca^{2+} and show lower apparent affinities for Ca^{2+}-induced closing than wild-type channels. These results indicate that position D50 plays a role in (1) stabilizing the open state in the absence of Ca^{2+}, and (2) facilitating closing and stabilization of the closed state in the presence of Ca^{2+}. To explore the structural role of a negatively charged residue at position D50 at low and high Ca^{2+} concentrations, we substituted this position with a cysteine residue and performed chemical modification with a negatively charged methanethiosulfonate reagent (MTSES). D50C mutant hemichannels display properties similar to those of D50N/Y mutants. Recovery of the negative charge with chemical modification by MTSES favors open hemichannels and slow deactivation kinetics in the absence of (or low) Ca^{2+}. Conversely, in the presence of (or high) Ca^{2+}, MTSES modification favors closing of the hemichannels. These results confirm the essential role of a negative charge at position 50 for regulation by Ca^{2+}. We also provide evidence that a salt bridge between D50 and K61 in the adjacent connexin subunit stabilizes open hemichannels. Our data indicate that human mutations causing diseases at position D50 can be linked to dysfunction of hemichannel gating by Ca^{2+}. Support: R01GM099490

40. Arterial stiffness in Black Africans using EndoPAT Atamar Medical and the SphygmoCor Atcor Medical machines. *N. NDUNA, *K. AWOTEDU, *A. NAMUGOWA, *B. LONGOMBENZA. **Department of physiology, Walter Sisulu University, South Africa*

Augmentation index (AIx) is a parameter measured by pulse wave analysis and is used as a surrogate marker of arterial stiffness. The aim of this study was to describe arterial stiffness in Black Africans using the EndoPAT 2000 and SphygmoCor Vx version 7.01 software devices. A relationship between peripheral augmentation index (pAIx) measured by SphygmoCor and that

measured by EndoPAT was investigated. This was a cross-sectional observational study. pAIx was measured in 89 participants using both the SphygmoCor and EndoPAT devices. The criteria for statistical significance was P-value <0.05. All analyses were conducted using SPSS software for Windows (version 19.0, SPSS Inc., Chicago, IL, USA).Results The results showed a lower r coefficient estimate of 0.673(P<0,0001). It was concluded that even though the absolute values derived by each technique were different, there was a highly significant and positive correlation between pAIx measured by SphygmoCor and pAIx measured by EndoPAT.

41. Measuring the dimerization free energy of a CLC Cl^-/H^+ antiporter in lipid bilayers by single molecule fluorescence analysis. ROBERTSON, JANICE L.[1,2], L. FRIEDMAN[2], L. KOLMAKOVA-PARTENSKY[1], J. GELLES[2] & C. MILLER[1,2]. [1]*Department of Biochemistry and Biophysics, Brandeis University, Waltham MA, USA 02454.* [2]*Howard Hughes Medical Institute, Brandeis University, Waltham MA, USA 02454.*

Folding of alpha-helical membrane proteins involves the spontaneous assembly of non-polar helices within the lipid bilayer. How do these greasy protein surfaces choose their greasy protein partners over the similarly greasy lipid bilayer to fold faithfully into a native structure? Answering this question requires quantitative measurements of folding free energies, but these efforts have been limited by a lack of well-behaved membrane proteins that fold reversibly in the lipid bilayer. Recently, we engineered a structurally stable and functional monomeric form of the normally homodimeric Cl^-/H^+ antiporter CLC-ec1 by introducing tryptophan mutations at the dimer interface. This is a large protein, formed by 18 transmembrane helices that dimerizes via a 1200 $Å^2$ surface lined by greasy, non-polar sidechains. Studies show that the oligomeric state of this protein can be shifted between monomer and dimer with additional surface mutations or in certain lipid conditions. The reversible dimerization of CLC-ec1 provides a new, simplified model for studying the physical driving forces involved in membrane protein folding. To measure the free energy of dimerization, we quantify the equilibrium monomer and dimer populations as a function of protein to lipid density. Cysteine mutations are introduced into each subunit of CLC-ec1, which come within 35 Å when the protein is in its dimer state. The cysteines are covalently labeled with cyanine-3- or cyanine-5-maleimide dyes, and then the fluorescent protein is reconstituted into membranes. Small, unilamellar vesicles are formed to trap the protein, either monomer or dimer, into each liposome. Vesicles are adhered to a glass slide, via biotinylation or direct surface fusion, and then the fluorescence behavior is examined using total internal reflection microscopy (TIRF). The presence of a monomer or dimer is measured by counting photobleaching steps or by observing Förster resonance energy transfer (FRET) that only occurs in the dimeric state. The work described here will allow us to measure the energies of protein-protein interactions in bilayer membranes and pave the way towards identifying the key physical driving forces that nature exploits to regulate membrane protein folding.

42. *Trpm7* is required for cardiac automaticity and atrioventricular conduction. RAJAN SAH[1,2], PIETRO MESIRCA[3], XENOS MASON[1], MARJOLEIN VAN DEN BOOGERT[1], CHRISTOPHER BATES-WITHERS[1], MATTEO E. MANGONI[3], DAVID E. CLAPHAM[1,4]. [1]*Howard Hughes Medical Institute, Department of Cardiology, Manton Center for Orphan Disease, Children's Hospital Boston, 320 Longwood Ave., Enders 1309, Boston, MA.* [2]*Cardiovascular Division, Brigham and Women's Hospital, 75 Francis St, Boston, MA.* [3]*CNRS, UMR-5203, INSERM U661, Universités de Montpellier 1 & 2, Institut de Génomique Fonctionnelle, Département de Physiologie, LabEx ICST.* [4]*Department of Neurobiology, Harvard Medical School, Boston, MA.*

*T*ransient *R*eceptor *P*otential *M*elastatin *7* (*TRPM7)* is an intriguing divalent (Ca^{2+}/Mg^{2+}) permeant channel-kinase that is ubiquitously expressed and concentrated in embryonic myocardium. *TRPM7*-like currents have been described

in adult ventricular myocytes, but the functional significance of *TRPM7* in myocardium remains unexplored. Here we show that *TRPM7* is required for normal cardiac automaticity, sinoatrial node function and atrioventricular conduction. *TRPM7* disruption in cultured embryonic ventricular cardiomyocytes (EVM) significantly reduces Ca^{2+} transient firing rates, impairing automaticity *in vitro*. Likewise, morpholino mediated *TRPM7* knock-down in zebrafish embryo slows heart rate *in vivo*. Cardiac-targeted *TRPM7* deletion in mouse *(KO*) induces episodes of sinus pauses, atrioventricular node (AVN) block and cardiomyopathy. Patch-clamp of murine sinoatrial nodal cells (SAN), the specialized myocardial cell responsible for cardiac automaticity, reveals a robust *TRPM7* current, absent in *KO* SAN. Confocal Ca^{2+} imaging of isolated SAN reveal diminished Ca^{2+} transient firing rates and a blunted diastolic Ca^{2+} rise in *KO* SAN cells compared to wild-type (WT), similar to cultured EVM following *TRPM7* disruption. Moreover, action potential firing rates are diminished in *KO* SAN due to a slower diastolic depolarization rate. *Hcn4* mRNA and the pacemaker current, I_f (encoded by *Hcn4*) are diminished in both SAN and AVN from *KO* mice, contributing further to impaired diastolic depolarization and both sinus node and atrioventricular node dysfunction. We conclude that TRPM7 provides a diastolic Ca^{2+} "leak" current that contribute to diastolic membrane depolarization and myocardial automaticity. Impairments in cardiac automaticity following genetic ablation of *Trpm7* in myocardium arise in part from the elimination of TRPM7-mediated inward Ca^{2+} "leak" at hyperpolarized membrane potentials, in addition to down-regulation of *Hcn4* expression and I_f, resulting in SAN dysfunction and AVN block *in vivo*.