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Genetic and Animal Models for Ion Channel Function in Physiology and Disease

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ANDREA MEREDITH and MARK NELSON

1. Role on TRPM4 in Human Cardiac Disorders.
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The TRPM4 channel is one of the 28 transient reception potential channels expressed in the human body. It is expressed in a wide range of cells and tissues, but its function in physiology and pathophysiology is scarcely understood. Recently, its roles in human disorders such as cardiac electrical disturbances and multiple sclerosis have been demonstrated. In this talk, new data on genetic variants found in human *TRPM4* linked to cardiac conduction defect, congenital atrioventricular block and Brugada syndrome will be presented. Our group is also currently developing more specific and potent TRPM4 small molecule inhibitors. Recent data on the characterization of these inhibitors used as either chemical chaperones or tools to understand the physiology of TRPM4 in vivo will be shown. Finally, we will present our new findings on the characterization of the cardiac electrical properties of a cardiac-specific TRPM4 knockout mouse line that we generated.

2. The Late L-type Ca Current as a Target for a New Class of Antiarrhythmics.
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There is an unmet need for an effective pharmacological therapy for cardiac arrhythmias to circumvent the limitations of class IV antiarrhythmics (e.g., diltiazem and verapamil) that block the peak L-type Ca current ($I_{Ca,L}$), compromising EC coupling. We recently discovered that early afterdepolarizations (EADs) of the cardiac action potential (AP), well-recognized triggers of fatal arrhythmias, can be potently suppressed by reduction of the late $I_{Ca,L}$ component, without perturbing peak $I_{Ca,L}$ (Madhvani et al. 2015. *J. Gen. Physiol.* 145:395–400). Motivated by these findings, we are studying pilot compounds that could constitute a new, safer class of antiarrhythmics that do not compromise inotropy. One of the promising drugs is roscovitine, a purine analogue. We observed a selective reduction of late versus peak $I_{Ca,L}$ by roscovitine in both native $I_{Ca,L}$ from isolated rabbit ventricular myocytes under AP clamp and human $Ca_V1.2$ channels expressed in *Xenopus* oocytes. The EAD-suppressing ability of this drug was tested in isolated rabbit ventricular myocytes after induction of a stable EAD regimen with 600 μM H_2O_2. The oxidative stress caused EADs in 81% (95% CI: 66–95%) of the APs, prolonging AP duration (APD_{90}) from 276 ms (CI: 97–433 ms) to 840 ms (CI: 285–1,967 ms; $n = 4$). Addition of roscovitine (20 μM) completely abolished EADs and restored the APD_{90} to 189 ms (CI: 155–222 ms). Moreover, roscovitine did not significantly perturb the Ca transient, implying the preservation of normal excitation–contraction coupling. We also tested roscovitine on isolated perfused aged rat hearts exposed to 0.1 mM H_2O_2 to induce EAD-mediated ventricular tachycardia/fibrillation (VT/VF). Roscovitine addition suppressed these VT/VF in five out of six hearts within 10 min of perfusion. These findings set the basis for the development of a conceptually new class of antiarrhythmics (L-type Ca channel gating modifiers) that selectively reduce late $I_{Ca,L}$, with minimal or no effect on contractility.

3. HIV-tat Increases Connexin43 Expression and Alters Trafficking in Human Astrocytes: Role in NeuroAIDS.
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HIV-associated neurocognitive disorders (HAND) are a major complication in at least half of the infected population despite effective antiretroviral treatment and immune reconstitution. HIV-associated CNS damage is not correlated with viral replication, but instead is associated with mechanisms that regulate inflammation and neuronal compromise. Our data indicate that one of these mechanisms is mediated by gap junction channels. Normally, gap junction channels shut down under inflammatory conditions. However, HIV infection up-regulates Cx43 expression and maintains gap junctional communication. Here, we demonstrate that HIV-tat, the transactivator of the virus, and no other HIV proteins tested, increases Cx43 expression and maintains functional gap junctional communication in human astrocytes. Cx43 up-regulation is mediated by binding of the HIV-tat protein to the Cx43 promoter, resulting in increased Cx43 mRNA and protein, as well as increased gap junctional communication. Therefore, we propose that HIV-tat DNA persistent in the host genome is responsible for continued expression of the HIV-tat protein and that the resulting increased Cx43 expression allows spread of intracellular toxic signals generated in a few HIV-infected cells into surrounding uninfected cells. HIV-tat, which is membrane permeable, gets out of the infected cells and into adjacent cells where it increases Cx43 expression. Formation of gap junctions requires Cx43 in both apposed cells, and apoptosis is observed across astrocyte–astrocyte, astrocyte–neuron, and astrocyte–endothelial cell contacts. In the current antiretroviral era, where HIV replication is often completely suppressed, viral proteins such as HIV-tat are still produced and released from infected cells. Thus, blocking the effects of HIV-tat could be a new strategy to

reduce the damaging consequences of HIV infection of the CNS (see Berman et al. 2016. *J. Neuroinflammation.* 13:54. doi:10.1186/s12974-016-0510-1).

4. Cross Species Function and Pharmacology of CFTR: Implications for Animal Models of Cystic Fibrosis.
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Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). Animal models of CF exhibit phenotypic variation, for example mice with the most common CF-causing mutation F508del do not develop the lung pathology observed in CF patients. Models with closer physiology to humans, including pig and ferret, have therefore been developed, and a sheep model has been proposed. To understand better the function of CFTR orthologues from model species, we used single-channel recordings to compare human, mouse, and sheep wild-type (WT) and F508del-CFTR and determine their response to the potentiator ivacaftor (VX-770; Vertex Pharmaceuticals).

The gating behavior of sheep CFTR closely resembled that of human CFTR, whereas mouse CFTR showed prolonged openings to a subconductance state (O_1) with brief transitions to the fully open state (O_2). Sheep CFTR single-channel current (*i*) and open probability (P_o) were increased compared with those of human CFTR. However, the *i* and P_o of mouse O_2 were reduced compared with human CFTR. The F508del mutation reduced the P_o of sheep CFTR less severely than its effect on human CFTR, whereas the mutation was without effect on mouse O_2. For human and sheep CFTR, ivacaftor potentiated WT and F508del-CFTR; the drug was without effect on mouse WT and F508del-CFTR. Both human and sheep F508del-CFTR showed thermal instability at 37°C, whereas mouse F508del-CFTR remained stable at this temperature.

Our data indicate that CFTR from human, sheep, and mice show variation in function and pharmacology with relevance for animal models of CF. They also demonstrate that the F508del mutation does not have the same impact in CFTR orthologues from diverse species. These observations have implications for understanding the structure, function, and pharmacology of CFTR.

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5. Physiological Foundations of Susceptibility to Periodic Paralysis Revealed by Knock-In Mouse Models.
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Hypokalemic periodic paralysis (HypoKPP) is a dominantly inherited disorder of skeletal muscle with recurrent episodes of severe weakness lasting hours to days, associated with low serum K^+ (<3.5 mM). Weakness is caused by reduced fiber excitability resulting from sustained depolarization of the V_{rest}. Missense mutations, almost always at arginine residues of S4 voltage-sensor domains (VSDs), in $Ca_V1.1$ (60% of families) or $Na_V1.4$ (20%) are established causes of HypoKPP. Expression studies of HypoKPP mutant $Na_V1.4$ channels in oocytes revealed a common anomaly, the gating pore current conducted by a leaky VSD that is permissive to cation flux at hyperpolarized potentials and nonconducting when depolarized ($V_{1/2}$ = −52 mV). Based on oocyte data, the gating pore conductance in HypoKPP muscle at a resting potential of −90 mV is estimated to be 10 μS/cm^2 or 1% of the total resting conductance.

Muscle from knock-in mutant mice ($Ca_V1.1$-R528H or $Na_V1.4$-R669H) has a HypoKPP phenotype with severe loss of force in low K^+ challenge, and voltage-clamp studies confirmed gating pore currents for mutant $Ca_V1.1$ as well as $Na_V1.4$ channels. Simulations of fiber excitability reveal a mechanism by which a small gating pore conductance may produce susceptibility to anomalous depolarization in low K^+. When the K^+ current that sets V_{rest} is conducted by Kir channels, then this outward current amplitude is compromised and V_{rest} depolarizes if external K^+ is low enough (even though E_K is more negative). In normal muscle, this paradoxical depolarization occurs for K^+ <2 mM. The HypoKPP gating pore current shifts this critical K^+ to the 3–4-mM range, creating V_{rest} bistability of −90 mV and −60 mV. The pathogenic depolarized V_{rest} is favored by raised internal Cl^-, and reducing Cl^- influx via inhibition of the NKCC transporter with bumetanide prevents low-K^+–induced loss of force in HypoKPP mice.

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6. Selective Activation of Muscarinic Receptors in Dentate Gyrus-CA3 Promotes Hyperexcitability and Seizure Susceptibility.
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Epileptogenesis describes the complex, plastic changes that alter a normal brain into a hyperexcitable network debilitated by recurrent seizures. The dentate gyrus (DG) and granule cell neurons control excitatory inputs into the hippocampus. Voltage-gated KCNQ2/3 K^+ channel (M-channel) current maintains homeostatic control over neuronal resting membrane potential and

firing frequency. M-current is sensitive to suppression by muscarinic acetylcholine receptor activation. Cholinergic input to M1 muscarinic receptors and the dominant function of M-current in neuronal excitability suggest involvement in epileptogenesis pathways. We hypothesized that in DG granule cells, muscarinic receptor activity induces maladaptive changes in which M-current is more susceptible to muscarinic depression, conferring hyperexcitability and recurrent seizures.

We induced epileptogenesis by transgenic expression of muscarinic "Designer Receptors Exclusively Activated By Designer Drug" (DREADD) exclusive to the DG-CA3 circuit using Cre-loxP mice. Mice were administered the designer drug clozapine-N-oxide (CNO) to elicit remote activation of the muscarinic DREADDs. EEG electrodes were implanted in mice to record electrographic seizure activity. Stimulation of the muscarinic DREADD by CNO delivery was repeated once daily, and seizure behavior was scored. Active and passive discharge properties and M-channel currents of DG granule cells were characterized with patch-clamp electrophysiology in the brain slice.

Muscarinic stimulation of DG granule cells was sufficient to induce epileptogenic activity in vivo, as mice displayed focal seizure activity after administration of CNO. Repeated kindling of seizures resulted in generalized clonic seizures that persisted for 30–60 s. DG granule cells exhibited robust M-current suppression and greater hyperexcitability upon DREADD receptor stimulation.

Muscarinic receptor stimulation in the DG-CA3 circuit is sufficient to induce epileptiform excitation that progressed into network seizures. Chemogenetic spatial and temporal control represents a promising advancement in understanding epileptogenesis over traditional chemoconvulsant models. The interaction of muscarinic receptors and M-channels may strongly contribute to maladaptive, epileptogenic changes in the DG to promote seizure.

7. Dissecting Phenotypes by Gene Deletion in a Mouse Model of Dravet Syndrome. **WILLIAM CATTERALL**, *Department of Pharmacology, University of Washington, Seattle, WA 98195*

Dravet syndrome is a complex genetically dominant neuropsychiatric disease involving intractable epilepsy, profound cognitive deficit, autistic-like phenotypes, and a high frequency of sudden unexplained death in epilepsy (SUDEP). It is caused by loss-of-function mutations in the gene *SCN1A* encoding brain sodium channel Nav1.1, which specifically impair action potential firing by GABAergic inhibitory interneurons and disinhibit neural circuits in the brain. Deletion of Nav1.1 channels specifically in forebrain GABAergic interneurons using the Cre-Lox method recapitulates all of the manifestations of this disease, including epilepsy, SUDEP, cognitive deficit, and autistic-like behaviors. These results demonstrate that disinhibition of neural circuits by mutation of Nav1.1 channels causes both epilepsy and comorbidities in Dravet syndrome. Dissection of the cellular basis for Dravet syndrome further with the Cre-Lox method shows that mutation in parvalbumin-expressing fast-spiking interneurons causes pro-epileptic effects and autistic-like behaviors, deletion in somatostatin-expressing interneurons causes pro-epileptic effects and hyperactivity, and deletion in both types of interneurons gives synergistic effects on epilepsy and SUDEP and a partial phenotype for cognitive deficit. Sudden death is caused by excess parasympathetic outflow from the central nervous system during and following seizures, which causes intense bradycardia and frequently leads to sudden cardiac death by ventricular arrhythmia within 1–2 min. Further genetic dissection of pathophysiology and novel approaches to therapeutics for Dravet syndrome will be discussed.

8. The MAGUK CASK Regulates the Cardiac Sodium Channel Nav1.5, Which is Mediated by Calcineurin. MORGAN CHEVALIER, SARAH VERMIJ, SABINE NAFZGER, JEAN-SEBASTIEN ROUGIER, and HUGUES ABRIEL, *Department of Clinical Research, University of Bern, Bern, Switzerland*

Background: The voltage-gated Na^+ channel $Na_v1.5$ is responsible for the rapid depolarization phase of the cardiac action potential (AP). $Na_v1.5$ is present in different membrane domains in cardiomyocytes and interacts with specific partners such as MAGUKs (membrane-associated guanylate kinase), which are key regulators of ion channels. The MAGUK family member CASK (calcium/calmodulin-dependent serine protein kinase) regulates ion channels in the brain. Here, we investigate the role of CASK in the regulation of $Na_v1.5$ in cardiomyocytes.

Methods and results: First, immunostainings in isolated mouse cardiomyocytes show that CASK is exclusively expressed at the lateral membrane. We also showed the SAP97-independent interaction of CASK with Nav1.5 by pulldown experiments with the Nav1.5 C-terminal domain in SAP97 KO and WT cardiomyocytes. To assess the functional consequences of the interaction between CASK and $Na_v1.5$ channels, patch-clamp experiments in cardiac-specific CASK KO cardiomyocytes revealed that I_{Na} is increased by 40% without any significant modifications of the steady-state activation and inactivation. Additionally, AP recordings revealed that the AP threshold is significantly lower in CASK KO cardiomyocytes, while dV/dt and resting membrane potentials are not modified. Concomitantly, Western blots in CASK KO or WT cardiomyocytes revealed an increase in the expression of $Na_v1.5$. All together, these data show that CASK is a negative regulator of $Na_v1.5$ channels.

CASK also interacts with the calcium-dependent serine-threonine phosphatase calcineurin in cardiomyocytes, which inhibits its activity. To assess the role of calcineurin in the CASK-dependent regulation of $Na_v1.5$, I_{Na} was recorded in CASK-silenced TSA-201 cells. Interestingly, treatment with the calcineurin inhibitor cyclosporin A (10 μM) abolished the effect of CASK silencing on I_{Na}.

Conclusion: According to these results, CASK appears to be as negative regulator of Nav1.5, which activity is mediated by calcineurin.

9. Optogenetic Induction of Cortical Spreading Depressions. DAVID Y. CHUNG,[1,2,*] HOMA SADEGHIAN,[1] FUMIAKI OKA,[1,3] TAO QIN,[1] CENK AYATA,[1,2] *[1]Neurovascular Research Unit, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114; [2]Stroke Service and Neuroscience Intensive Care Unit, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114; [3]Department of Neurosurgery, Yamaguchi University School of Medicine, Ube, Japan; *presenting author*

Background: Cortical spreading depression (CSD) has a critical role in the pathogenesis of secondary damage after traumatic brain injury, ischemic stroke, intracerebral hemorrhage, and aneurysmal subarachnoid hemorrhage. Experimental studies targeting CSDs continue to be important in the development of new therapeutic approaches; however, current CSD induction methods are cumbersome, cause primary brain injury, and obscure events at the nidus of the CSD.

Methods: We developed a minimally invasive method for the study of CSDs. Transgenic mouse lines expressing channelrhodopsin (ChR2) in cortical neurons were used to determine regional thresholds for optogenetic precipitation of CSDs through intact skull. In vivo laser speckle and Doppler flowmetry, bright field imaging, and electrocorticography were used to detect CSDs. Additionally, we measured local field potential shifts (LFP) and extracellular potassium using an ion-selective glass microelectrode inserted within the area of light stimulation.

Results: We were able to induce CSDs in multiple regions without causing primary brain injury. We observed regional differences in thresholds for optogenetically-induced CSDs (from lowest to highest threshold): (1) whisker barrel, (2) motor, (3) sensory, and (4) visual cortex. CSDs were reliably induced in whisker and motor cortices for all tested stimulation paradigms; however, it was not always possible to predict whether a CSD could be induced in sensory or visual cortex. Dynamic changes in LFP and increased extracellular potassium concentrations at the site of light stimulation preceded precipitation of a CSD.

Conclusions: Minimally invasive optogenetic stimulation through intact skull can reliably induce CSDs and enables observation of changes in extracellular potassium at the origin of a CSD. Optogenetic induction of CSDs in ChR2 transgenic mice is a potentially useful method for the study of secondary brain injury and can serve as an important tool for the future study of fundamental mechanisms of the phenomenon.

10. Bringing Bioelectricity to Light. **ADAM COHEN**, *HHMI, Harvard University, Cambridge, MA 02138*

We are developing tools for all-optical electrophysiology: simultaneous optical perturbation and optical measurement of membrane voltage in electrically active cells. We developed a protein-based fluorescent voltage indicator, QuasAr3, derived from a gene found in a Dead Sea microorganism. When expressed in neurons or cardiac myocytes, this gene reports firing via flashes of near-infrared fluorescence. We paired the reporter with a blue light–activated ion channel, called CheRiff. Using patterned optical stimulation, we have probed excitability, synaptic transmission, and network dynamics in primary neurons in culture, in human iPSC-derived neurons, in acute brain slice, and in vivo. We have also explored electrical activity in cardiomyocytes, vascular endothelial cells, and even bacteria. I will also describe applications to human iPSC-derived models of ALS and epilepsy and to modeling arrhythmia in human iPSC-derived cardiomyocytes.

11. Physiological Levels of Extracellular Histones Increase TRPV4 Activity While Trauma Levels Increase P2X-Mediated Ca^{2+} Influx. DANIEL COLLIER,[1] NURIA VILLALBA,[1] ADRIAN SACKHEIM,[2] KALEV FREEMAN,[1,2] and MARK NELSON,[1,2] *[1]Department of Pharmacology and [2]Department of Surgery, University of Vermont, Burlington, VT 05405*

Elevated levels of histone proteins are found in the circulation of patients following traumatic injury (healthy: 2.3 μg/ml, 4 h post trauma: 10–230 μg/ml; Abrams et al. 2013. *Am. J. Respir. Crit. Care. Med.* 187 2:160–169) and are associated with vascular dysfunction, coagulopathy, sepsis, and poor patient outcome. However, the mechanism and specific ion channels involved have not been identified. We used resistance-sized mouse mesenteric arteries (MAs) to study the effects of extracellular histones on the vasculature. To study the spatial and temporal characteristics of histone-induced endothelial cell (EC) Ca^{2+} signals, we used spinning disk confocal microscopy of *en face* MAs from EC-specific genetically encoded Ca^{2+} indicator mice (GECI, cx40-GCaMP5-mCherry). Physiological histone levels (1 μg/ml, unfractionated histone protein mixture of H1, H2a, H2b, H3, and H4) increased local Ca^{2+} events that were inhibited by 10 nM GSK219, a TRPV4-specific antagonist. Trauma levels of histones (10 μg/ml), within seconds of application, triggered large Ca^{2+} events that propagate within and between

ECs. 10 μg/ml histones increased intracellular Ca^{2+} within minutes, and exposure for 30 min induced EC death and decreased EC-dependent dilation in ex vivo pressurized MAs. Propagating events were due to Ca^{2+} influx rather than IP_3R-mediated release from intracellular stores, yet events were not sensitive to inhibition or genetic ablation of TRPV4. Propagating events were suppressed by selective and nonselective purinergic antagonists (100 μM amiloride, 1 μM TNPATP, and 50 μM Suramin), suggesting a role for ionotropic purinergic receptor (P2X)–mediated Ca^{2+} influx in ECs. The data demonstrate that circulating histones are robust activators of Ca^{2+} signaling in the vascular endothelium from physiology to disease.

12. The Wistar Kyoto Rat Strain as a Model for Irritable Bowel Syndrome. JULIE E. DALZIEL,[1] WAYNE YOUNG,[1] KARL FRASER,[1] SHALOME BASSETT,[1] and NICOLE C. ROY,[1,2] *[1]Food Nutrition and Health Team, Food and Bio-based Products Group, AgResearch, Palmerston North, New Zealand; [2]Riddet Institute, Massey University, Palmerston North, New Zealand*

The Wistar Kyoto (WKY) rat strain is hyper-responsive to stress and considered a model for stress-associated functional gastrointestinal (GI) disorders such as irritable bowel syndrome (IBS). Although GI transit and microbiota composition are altered in IBS, these aspects have not been thoroughly investigated in WKY rats. We therefore compared GI transit in WKY with Sprague Dawley (SD) rats and characterized differences in cecal microbiome composition and plasma metabolites associated with stress. Transit of metallic beads was tracked along different regions of the GI tract over 12 h by high resolution x-ray imaging. Stomach emptying (4 h) and transit to the small (9 h) and large intestine (12 h) were assessed using a rating scale to classify bead location (Dalziel et al. 2016. *Neurogastroenterol. Motil.* in press). Plasma extracts were analyzed with both positive and negative ionization modes of lipid and HILIC (polar compounds) liquid chromatography mass spectrometry (LC-MS) streams. Cecal microbial composition was determined by Illumina MiSeq 16S rRNA amplicon sequencing and analysis using the QIIME pipeline. In WKYs, 77% of beads were retained in the stomach compared with 35% retention for SDs. Subsequent transit was decreased by 36% at 9 h and by 21% at 12 h in WKY compared with SDs. Interestingly, excluding those retained in the stomach at 9 h, transiting beads had moved 39% further through the small intestine over 4–9 h for WKY compared with SDs. Phylogenetic differences between rat strain cecal microbiota communities were clearly distinguished and plasma metabolite differences detected. The results demonstrate impaired stomach emptying, yet rapid small intestine transit, in WKY rats compared with SD animals. This was unexpected and reveals that the slower GI transit in WKY can be largely attributed to delayed stomach emptying. These observations suggest that WKY rats may also be considered an appropriate model for functional gastric disorders.

13. Developing a New Approach for the Treatment of Timothy Syndrome. IVY E. DICK,[1,2] WORAWAN B. LIMPITIKUL,[1] JENNIFER BABICH,[1] WANJUN YANG,[1] MASAYUKI YAZAWA,[3] and DAVID T. YUE,[1] *[1]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21205; [2]Department of Physiology, University of Maryland, Baltimore, MD 21201; [3]Columbia University Stem Cell Initiative, New York, NY 10032*

Timothy syndrome (TS) is a multisystem disorder, including neurological impairment, autism, and cardiac action potential prolongation (long QT) with life-threatening arrhythmias. The underlying basis of TS is typically a single point mutation in either exon 8a (TS type 1) or exon 8 (TS type 2) of the $Ca_V1.2$ Ca^{2+} channel, such that the expression pattern of these mutually exclusive exons significantly contributes to the overall severity of the disease. The effect of these mutations on the channel is a disruption of both voltage- and Ca^{2+}-dependent regulation. However, despite the known effects on Ca^{2+} channel function, blockers of these channels such as verapamil have only partially counteracted the severe symptoms of TS patients. Here, we show that this is likely due to a differential effect of verapamil on TS versus wild-type channels. In particular, the use-dependent block of verapamil is significantly attenuated in the context of the TS channel, thus decreasing the efficacy of verapamil in TS patients. We therefore propose an alternate therapeutic strategy in which inclusion of the TS-containing exon is decreased through manipulation of the splice expression pattern via an antisense oligonucleotide. Remarkably, treatment of induced pluripotent stem cells (iPSCs) derived from a TS type 1 patient resulted in not only a decrease of the deleterious exon, but a corresponding increase in the non-affected exon. Further, treatment of the cells normalized the action potential duration, validating the functional efficacy of the treatment strategy. Overall, the potential benefits for TS patients are significant, and the approach may serve as a model for developing new therapeutic strategies for any channelopathy in which the mutation occurs within a mutually exclusive exon.

14. Structural Elements of Peripheral Coupling Sites Regulating Cerebral Artery Smooth Muscle Contractility. SCOTT EARLEY, *Department of Pharmacology, Center for Cardiovascular Research, University of Nevada School of Medicine, Reno, NV 89557*

Junctional membrane complexes (JMCs) are static structures that regulate close contacts (~20 nm) between the plasma membrane (PM) and sarcoplasmic reticulum (SR) and are critically important for the excitability and contractility of cardiac and skeletal muscle

cells. In smooth muscle cells (SMCs), JMCs occur as sites of peripheral coupling between the PM and the SR. These sites may be important for the activity of Ca^{2+}-sensitive ion channels on the PM by Ca^{2+} released from the SR, such as the activation of large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) by Ca^{2+} sparks generated by release of Ca^{2+} from the SR through type 2 ryanodine receptors (RyR2). Here we investigate the structural basis and functional significance of peripheral coupling in native contractile cerebral artery SMC. High-resolution live-cell confocal imaging experiments using PM-, SR-, and tubulin-specific fluorescent dyes revealed distinct arching microtubule structures beneath the peripheral SR proximal to the PM. These structures were also apparent when ground state depletion with individual molecule return (GSDIM) super-resolution nanoscopy was used to image single microtubule filaments. Surprisingly, GSDIM images also showed that immunolabeled RyR2 clusters selectively colocalized with microtubules in freshly isolated SMCs. To investigate the influence of microtubule arches on the formation of peripheral coupling sites at the molecular level, a combination of high-resolution live cell confocal microscopy and GSDIM super-resolution nanoscopy was used. SMCs labeled with PM- and SR-specific dye showed areas of peripheral coupling that were disrupted when nocodazole (10 μM) was used to depolymerize microtubules, but not when the actin cytoskeleton was disrupted with a combination of latrunculin B (1 μM) and swinholide A (0.1 μM). Peripheral coupling was also maintained during maximal agonist-induced contraction (uridine trisphosphate, 30 μM). Freshly isolated SMCs were immunolabeled with anti–BK_{Ca}-α subunit (BKα) and anti-RyR2 and imaged using GSDIM super-resolution nanoscopy. Using object-based analysis, we found that 1.9 ± 0.4% of BK_{Ca} puncta colocalized with RyR2, far greater than the colocalization of these two proteins in randomized control images (0.2 ± 0.1%, $n = 12$, $p < 0.001$). In addition, nocodazole treatment (10 μM, 1 h), significantly reduced colocalization of BKα and RyR2 ($n = 12$, $P < 0.05$), suggesting that intact microtubule networks are required for the formation of this signaling complex. Additional experiments were performed to establish functional consequences of disrupting peripheral coupling sites. Ca^{2+} sparks were recorded using high-speed, high-resolution confocal Ca^{2+} imaging in smooth muscle cells loaded the Ca^{2+} indicator Fluo-4AM. Cells treated with nocodazole showed no change in Ca^{2+} spark amplitude and rise time and a slight decrease in frequency. Ca^{2+} spark duration, decay time, and spatial spread were significantly increased. In perforated-patch clamp electrophysiology experiments ($V_H = -30$ mV), nocodazole treatment abolished spontaneous Ca^{2+}-dependent BK_{Ca} activity, recorded as spontaneous transient outward currents (STOCs), suggesting that microtubule depolymerization uncouples Ca^{2+} spark-dependent activation of BK_{Ca} channels. Pressure myography was used to determine the effects of this response on arterial contractility. Myogenic vasoconstriction of cerebral resistance arteries (∼100–200 μm) treated with nocodazole was significantly great than controls, likely due to loss of BK_{Ca} activity, which has a negative feedback on the membrane potential during pressure-induced vasoconstriction. Taken together, these findings demonstrate that microtubule-dependent formation of peripheral coupling sites is functionally significantly for the regulation of membrane excitability and contractility of arterial SMC.

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15. Recombinant ApoL1 Confers pH-Dependent Anion Permeability to Phospholipid Vesicles.

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Background: Variants in ApoL1 confer increased risk of certain chronic kidney diseases in people of African ancestry. ApoL1 has been reported to function as an ion channel, but reports vary on the nature of this activity. We sought to characterize ApoL1 ion transport activity more fully.

Methods: Recombinant N-terminal His-tagged ApoL1 was expressed in bacteria and purified using methods of Thompson and Finkelstein (2015. *Proc. Natl. Acad. Sci.* 112:2894–2899). Ion permeability was assessed using vesicle-based, voltage-dependent Cl and K efflux assays using ion-selective electrodes. Single channel properties were investigated using the tip-dip lipid bilayer approach. Protein structure was probed using intrinsic tryptophan fluorescence.

Results: The preparation yields large amounts of very highly purified His-tagged ApoL1, which is active in a trypanosome killing assay. Direct addition of ApoL1 to phospholipid vesicles yields robust Cl-selective permeability that supports voltage-driven Cl transport. The activity shows a strong dependence on pH, with a sharp drop in activity as pH is raised above 6.5. Activity is linearly dependent on mass of protein and shows strong dependence on lipid composition of the vesicles, requiring the presence of negatively charged phospholipids. We do not find cation-selective permeability when assayed at either pH 5.0 or 7.5. Biophysical studies of ApoL1 in solution reveal a significant structural transition in the same pH range in which the channel is activated (pH 7–6.5). In tip-dip bilayers, ApoL1 inserts at low pH, generating transitions with a range of conductances between 2 and 10 pS and with a nonrectifying current-voltage relationship. We do not find enhanced channel activity if the bath solution is changed to pH 7.5.

Conclusions: Purified recombinant ApoL1 can insert directly into phospholipid membranes at low pH and function as an anion-selective channel. Whether the disease-associated variants show altered channel properties remains to be determined.

16. Trafficking of the Cardiac Sodium Channel $Na_V1.5$ is Regulated by the Lateral Membrane-Specific Protein CASK Through Its GUK and L27B Domains.
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Introduction: Targeting and clustering of transmembrane proteins to specialized membrane domains of cardiac myocytes (CMs) are critical for proper cardiac function. MAGUK proteins have emerged as key partners in this organization. We have previously reported that the MAGUK protein CASK localizes at lateral membranes (LMs) of CMs, in association with the dystrophin–glycoprotein complex (DGC). We have also shown that CASK interacts with the main cardiac sodium channel, $Na_V1.5$, and negatively regulates the sodium current (I_{Na}).

Methods: Using adenoviral transfer technology to manipulate adult CMs, high resolution 3-D deconvolution and total internal reflection fluorescence (TIRF) microscopy, GST pull-down, biotinylation, RT-qPCR, and patch clamp electrophysiology, we investigated the mechanism of CASK-dependent regulation of I_{Na} in CMs and examined the role of CASK in the organization of the $Na_V1.5$ macromolecular complex at LMs.

Results: Using a purified protein CASK, we showed that CASK directly binds to $Na_V1.5$ C terminus via the PDZ-binding motif SIV, regardless of syntrophin presence. Overexpression of WT CASK decreased I_{Na} in CMs, whereas overexpression of CASK constructs deleted for either the L27B or GUK domain restored I_{Na}. Neither the expression level of *scn5a* nor the total amount of $Na_V1.5$ proteins was modified upon CASK silencing or overexpression. Furthermore, the protein transport inhibitor brefeldin-A prevented I_{Na} and $Na_V1.5$ surface expression increase in CASK-silenced CMs. Finally, quantification of $Na_V1.5$ expression at the LM and ID revealed that the LM pool was only increased upon CASK silencing.

Conclusion: CASK directly interacts with $Na_V1.5$ and controls its surface expression by regulating anterograde trafficking and/or stabilization at the LM. Both L27B and GUK domains of CASK are likely involved in this regulation. These results strengthen the concept of differentially regulated pools of $Na_V1.5$ and suggest that CASK could participate in maintaining low level of $Na_V1.5$ at the LM.

17. Excitation-Secretion Coupling in Zebrafish Pancreatic Islets.
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Zebrafish are becoming more frequently used to model development and metabolic diseases as well as to screen for genes modulating many pathways of interest. However, many questions regarding basic physiology in the zebrafish remain unanswered. In mammalian pancreata, ATP-sensitive potassium channels (K_{ATP} channels) and voltage-dependent calcium channels (VDCCs) link metabolism and membrane excitability to insulin secretion and are critical in controlling blood glucose. Whether this process occurs in zebrafish and to what extent it is essential for overall glucose homeostasis are not well understood. While zebrafish possess orthologous genes for many mammalian proteins, whether key proteins are expressed or functional in the pancreas has not previously been determined. We characterized K_{ATP} channels and VDCCs in islets and individual β cells isolated from zebrafish and show that the fish pancreatic cells express functional K_{ATP} channels with similar subunit composition, pharmacology, and function as their mammalian counterparts. We also show that these channels are essential for metabolic control of insulin secretion ex vivo using isolated islets and in vivo via intraperitoneal glucose tolerance tests. Further, we demonstrate that fish β cells possess functional calcium channels that are active when islets are exposed to high glucose, sulfonylureas, or membrane depolarization, indicating that calcium fluxes in these cells are downstream of K_{ATP}-dependent membrane depolarization. Strikingly, in contrast to mammals, zebrafish β cells are not electrically coupled, perhaps explaining differences in glucose tolerance between fish and mammals. Further, we have developed essential components for screening zebrafish for modulators of whole body responses to β-cell membrane inexcitability. Given that some but not all of the mammalian components for metabolism-secretion coupling are present in zebrafish, the fish provide an opportunity to understand the evolutionary conservation of many essential components of insulin secretion as well as to identify the pathways modulating whole-body responses to islet electrical inexcitability.

18. Underlying Mechanisms of Remission in a Mouse Model of Neonatal Diabetes.
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Gain-of-function (GOF) mutations in the K_{ATP} channel cause the channels to remain open despite rising glucose levels preventing insulin secretion, triggering neonatal diabetes mellitus (NDM). Individuals with identical mutations can have different levels of symptom severity and treatment efficacy, ranging from permanent

to transient NDM, suggesting other factors modulate responses to β-cell inexcitability. Mice expressing tamoxifen-inducible K_{ATP}–GOF mutations in pancreatic β-cells develop severe diabetes following transgene induction, which persists if untreated. However, if mice are treated with glibenclamide (GB) for two weeks (starting at disease induction) they show two distinctive outcomes at the end of treatment: (a) one group of mice enters into remission (TND), with normalization of glucose which persists long after treatment ended, and (b) another group develops severe and permanent diabetes (PND). Notably, remission in TND mice is not an off-target effect of GB, as some mice also remit with insulin treatment. To explore causes of these outcomes, we subjected K_{ATP}–GOF mice to hyperinsulinemic-euglycemic clamps. These reveal that insulin sensitivity is greatly reduced in PND mice but improved in TND mice. Initial tracer studies show no differences between groups in glucose uptake rates to explain the divergent baseline blood glucose and clamp glucose infusion rates. To explore the temporal link between changes in insulin sensitivity and treatment response, insulin tolerance tests before disease induction and at the end of GB treatment were done. These reveal that blood glucose diverges before changes in insulin sensitivity. In addition, plasma lipids are elevated in PND mice and not in TND mice, but only after differences in glucose appear. Together, the results suggest that insulin sensitivity changes are a result of rather than a cause of diverging blood glucose in TND and PND and suggest that other signaling pathways may determine which individuals enter remission in NDM.

19. Ionic Bases of Conditioned Inhibition-Produced Changes in *Hermissenda* Type B Photoreceptors. JOSEPH FARLEY, *Neuroscience, Indiana University, Bloomington, IN 47405*

While the neural mechanisms involved in simple forms of associative learning have been extensively studied, most research has focused upon paradigms in which stimuli are repeatedly paired. In contrast, little is known about the mechanisms underlying conditioned inhibition (CI), where an organism learns that one stimulus signals the absence of a second. In aversive conditioning paradigms, CI learning is often referred to as safety-signal learning. Our previous research has shown that CI in *Hermissenda*, established by repeated explicitly-unpaired (EU) presentations of light and rotation, separated by a fixed, lengthy temporal gap, results in increased phototactic behavior and decreased photoresponses and spike activity of ocular type B photoreceptors. Here I describe the ionic bases of these changes. Intracellular recordings confirmed our prior reports that B cells from EU-animals showed selective decreases in light-evoked generator potentials and spike frequencies. Voltage clamp analyses of type B cells revealed that two somatic K^+ currents (I_A and $I_{K\text{-}Ca}$) were increased for days following CI training. Elimination of $I_{K\text{-}Ca}$ through chelation of intracellular Ca^{2+} abolished the CI-produced difference in light response, while 4-aminopyridine block of I_A abolished CI-produced difference in spiking. Peristimulus time histogram analyses of spiking in B cells from untrained and random control animals revealed bimodal distributions, with the second longer mode being blocked by 4-AP and reflective of I_A. In B cells from EU-trained animals, the second mode was more prominent than in control cells, indicative of greater I_A. Hodgkin-Huxley style computational models constructed for B cells that incorporated the changes in I_A and $I_{K\text{-}Ca}$ produced by paired- or EU-conditioning produced changes in simulated light responses and spiking that mimicked those observed in physiological studies. These data are the first to correlate biophysical changes in ionic conductance systems with CI-produced alterations in excitability of identified neurons that are mediators of CI memory.

20. Mechanisms of Suppression of Kv1.1 Channel Activity by Aβ(1–42). JOSEPH FARLEY, KRISTI DEBOEUF, and MOHAMMAD FARIDUL ISLAM, *Neuroscience, Indiana University, Bloomington, IN 47405*

Many studies have found that Aβ-peptides participate in the pathogenesis of Alzheimer's disease (AD), leading to disruption of calcium (Ca^{2+}) homeostasis and eventual cell death. The mechanisms underlying these effects remain unclear. Our work suggests that Aβ inhibition of voltage-dependent K^+ channel (e.g., Kv1.1) activity is among the earliest steps. Our previous work, using murine Kv1.1 expressed in *Xenopus* oocytes, elucidated a pathway in which Ca^{2+}-dependent activation of protein phosphatase 2B (PP2B/calcineurin), protein kinase C (PKC), protein tyrosine kinases (PTKs), and RhoA all participate to produce rapid and strong suppression of Kv1.1 activity. This pathway is recruited by a variety of stimuli that increase $[Ca^{2+}]_i$, including GPCRs that couple to $G_{q/11}$–PLC, calcium ionophore (A23187), and LGICs that flux Ca^{2+}. Because Kv1.1 and related channels are activated during an action potential and regulate Ca^{2+} influx during depolarization and inhibition of Kv1 channels is often neurotoxic, we speculate that Aβ suppression of Kv1 channels could lead to hyperexcitability, altered synaptic transmission, disruption of Ca^{2+} homeostasis, and neurotoxicity. We assessed the effects of Aβ(1–42) peptide (AnaSpec, monomers and low-*n* oligomers) on Kv1.1 channels in oocytes. Aβ(1–42) (10 nM to 1 μM) produced a dose-dependent inhibition of macroscopic Kv1.1 current: ∼50% reductions within 30 m for 1 μM. Reverse sequence (40–1) peptide and other controls failed to suppress Kv1.1. Aβ suppression of Kv1.1 was partially Ca^{2+} and PP2B dependent, being reduced by ∼50% when cells were either loaded with BAPTA-AM or exposed to the PP2B-inhibitor, cyclosporine A. The Aβ-stimulated

Ca^{2+} increase did not occur via Ca^{2+} influx. Complete removal of extracellular Ca^{2+} failed to attenuate Aβ suppression of Kv1.1. Instead, Aβ appears to release Ca^{2+} from internal stores. Single channel recording results suggest the possibility that Aβ suppression of Kv1.1 also involves direct protein–protein interaction of Aβ with Kv1.1 channel subunits.

21. Looking for the Line Between Agonists and Inhibitors. Prokaryotic Sodium Channels and Allosteric Modulation of Voltage-Sensitive Gating. ROCIO K. FINOL-URDANETA,[1,2] JEFF R. MCARTHUR,[2] RACHELLE GAUDET,[2] DENIS TIKHONOV,[3] BORIS ZHOROV,[3,4] and ROBERT J. FRENCH,[1] *[1]Department of Physiology and Pharmacology, University of Calgary, Calgary, Alberta, Canada T2N 4N1; [2]Harvard University, Department of Molecular and Cellular Biology, Cambridge, MA 02138; [3]Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg 194223, Russia; [4]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada L8S 4L8*

Both agonistic biological toxins, such as batrachotoxin (BTX), and peptide inhibitors, such as μ-conotoxins (μCTXs), show use-dependent, apparently allosteric actions on voltage-gated sodium (Nav) channels. At this time, available high-resolution structural data, and the relatively simple, homo-tetrameric structures, of prokaryotic Nav channels offer an excellent opportunity to better understand these complex ligand–channel interactions. We have recently found that both BTX (Finol-Urdaneta et al. 2016. *Biophys. J.* 110:109a.) and μCTXs show potent interactions with NavBac channels. For BTX concentrations in the range of 1–10 μM, NaChBac channel modulation was facilitated, under voltage clamp, by trains of conditioning depolarizing pulses. Tail currents, on repolarization, increased in amplitude and slowed throughout the train, coincident with a negative shift in activation voltage. μCTX PIIIA inhibited NaChBac in the picomolar range (IC50 ∼0.5 pM; Finol-Urdaneta et al. 2015. *Biophys. J.* 108:583a.), even more potent than striking earlier predictions (Chen and Chung. 2012. *Biophys. J.* 102:483–488.). Inhibition/block approached a quasi–steady-state, while being monitored with repeated depolarizing test pulses to −10 mV (Vh = −120 mV). Furthermore, at concentrations well below the IC50, single-pulse inactivation decay rates increased. Thus, actions of both of these structurally disparate toxins were facilitated by voltage protocols that normally drive transitions of the channels from resting to activated states. Given that the binding sites for BTX and the μCTXs appear to be on opposite sides of the selectivity filter, these data seem to be consistent with considerable flexibility and coupling along the pore lining.

We explored BTX interactions with a NaChBac model, based on a cryo-EM structure of NavCt (Tsai et al. 2013. *J. Mol. Biol.* 425:4074–4088.). Modeling and mutagenesis studies suggest that the BTX-binding site is separate from the μCTX receptor but overlaps residues homologous to those important for both local anesthetic and BTX binding in eukaryotic channels.

22. Cardiac Dysfunction in Duchenne Muscular Dystrophy: Role of Connexin 43. DIEGO FRAIDENRACH,[1] PATRICK GONZALEZ,[1] NATALIA SHIROKOVA,[2] JAYALAKSHMI RAMACHANDRAN,[2] and **JORGE E. CONTRERAS**,[2] *[1]Department of Cell Biology and Molecular Medicine and [2]Department of Pharmacology, Physiology, and Neuroscience, Rutgers University, New Jersey Medical School, Newark, NJ 07103*

Duchenne muscular dystrophy (DMD) is characterized by the lack of dystrophin in cardiac and skeletal muscle cells. This leads to progressive loss of functional myocardium, to heart failure, and to damage of skeletal muscle. In the working myocardium, gap junctions located at intercalated disks mediate proper intercellular propagation of electrical signals and contractility. The gap junction channels are composed of connexin protein. Recently, we found that connexin 43 (Cx43), the dominant connexin in the ventricular myocardium, is up-regulated and mislocalized from the intercalated disks to lateral regions in cardiomyocytes of DMD mice and humans. These lateralized Cx43 proteins seem to be in the form of active unopposed plasma membrane channels ("hemichannels") rather than junctional channels. Strikingly, we found that specific pharmacological inhibition of Cx43 hemichannels prevents isoproterenol-induced arrhythmias and death in DMD mice. Furthermore, we found that the lateralized Cx43 hemichannels in DMD cardiomyocytes cause increased membrane permeability, which may cause damage to cardiac myocytes via enhanced free radical production and cytosolic Ca^{2+} increase. Together, these results suggest that the DMD-induced mislocalization of Cx43 has pathological consequences regarding both cardiac function and myocyte health/survival. This is a novel and potent mechanism by which DMD causes cardiac pathology. Because Cx43 lateralization occurs in many cardiac pathologies (e.g., hypertension, ischemic reperfusion, myocardial infarction, and hypertrophy), we propose that opening of these hemichannels can have a key role in many cardiovascular diseases.

23. Genomic Insights from Comparative Analyses of Teleost Fish: Implications for Myopathies Related to Excitation-Contraction Coupling. JENS FRANCK, *Department of Biology, University of Winnipeg, Winnipeg, MB, Canada R3B 2E9*

Excitation-contraction (EC) coupling describes the relationship between the depolarization of the muscle membrane and the subsequent contraction of the muscle cell. In skeletal muscle cells, the depolarization of the muscle membrane triggers a conformational change

in the L-type calcium channel ($Ca_V1.1$). In skeletal muscle, the $Ca_V1.1$ directly interacts and mechanically gates the intracellular ryanodine receptor (RyR) channel to release calcium from the intracellular stores of the sarcoplasmic reticulum, a mechanism termed depolarization-induced calcium release (DICR). In contrast, the L-type channel in cardiac muscle ($Ca_V1.2$) opens in response to the depolarization signal, and the extracellular calcium subsequently acts as a ligand to gate open the intracellular RyR channels, a mechanism termed calcium-induced calcium release (CICR). The DICR mode of calcium release is believed to be a vertebrate innovation. We previously described fiber type–specific expression of RyR1 paralogues in slow twitch (ryr1a) and fast twitch (ryr1b) muscle fibers in fish (Franck et al. 1998. *Amer. J. Physiol.* 275: C401–C415; Darbandi and Franck. 2009. *Comp. Biochem. Physiol. B.* 154:443–448). More recently, it has been reported that the α_{1S} subunits (α_{1S}) of the multimeric $Ca_V1.1$ channel are also duplicated and expressed discretely in the slow twitch (α_{1S}-a) and fast twitch (α_{1S}-b) muscles of zebrafish (Schredelseker et al. 2010. *Proc. Natl. Acad. Sci.* 107:5658–5663). Interestingly, the $Ca_V1.1$ channels in teleosts do not conduct any extracellular calcium and rely solely on the DICR mode of intracellular calcium release (Schredelseker et al. 2010. *Proc. Natl. Acad. Sci.* 107:5658–5663). Diseases related to abnormalities in the EC coupling process include malignant hyperthermia (MH), hypokalemic periodic paralysis (HypoKPP), and central core disease (CCD). Mutations in both the RyR1 and α_{1S} genes have been linked to MH. In the α_{1S} gene, five mutations, R174W, R1086H, R1086C, R1086S, and T1354S have been identified as causative (Bannister and Beam. 2013. *Biochim. Biophys Acta.* 1828:1587–1597). The T1354S mutation is proposed to act by increasing the rate of inward calcium current through the $Ca_V1.1$ channel and an increase in caffeine sensitivity of the intracellular RyR1 channel (Pirone et al. *Am. J. Physiol. Cell Physiol.* 299:C1345–C1354). In light of the fact that teleost fish do not rely on extracellular calcium, it is interesting to observe that the α_{1S} genes in teleosts all encode S at position 1354. This observation therefore provides potential insight into the relationship between $Ca_V1.1$ and RyR1 coupling in disease states such as MH.

24. Development of a Novel, In Vivo Assay To Screen Pharmacological Agents During Axolotl Larval Tail Regeneration and Its Use To Identify Ion Channels Involved in Critical Cellular Processes. BRANDON M. FRANKLIN, S. RANDAL VOSS, and JEFFREY L. OSBORN, *Department of Biology, University of Kentucky and Biomedical Science Research Group, LLC, Lexington, KY 40506*

Soft tissue repair is a critical function of living organisms required to restore anatomical structure and physiological function following tissue insult. A better understanding of molecular mechanisms that control cellular dynamics is crucial for the development of clinical therapies in the treatment of trauma, degenerative diseases and cancer. The current study used a novel, in vivo assay of larval tail regeneration coupled with a pharmacological screen of ion channel antagonists to identify molecular targets underlying ion channel regulation of critical cell processes. Individual channels identified by the assay were qualitatively classified as having delayed or completely inhibited regenerative growth. Channels that delayed or slowed regeneration were inferred to have influenced some critical cellular dynamic such as proliferation, migration, survival, and/or differentiation. These channels were anoctamin1/2 (Ano1/2), $K_V2.1/2.2$, L-type CaV channels, and H/K ATPases. Inhibiting channels were concluded to be involved in some other cellular process essential for initiating regeneration. These channels were GlyR, $GABA_AR$, $K_V1.5$, and SERCA pumps. Chloride channel inhibition with DIDS, DNDS, or T16a(inh)-A01 effectively slows tail regeneration. Cellular proliferation was assessed via EdU incorporation assay, which revealed that mesenchymal-like cell proliferation was inhibited (control: 33.5 ± 3.2%; DIDS: 18.13 ± 2.25%; DNDS: 19.64 ± 3.79%; T16a(inh)-A01: 19.65 ± 1.85% EdU+ cells) but did not affect proliferation in either neural or epidermal tissues. Analysis of mRNA expression with qPCR showed down-regulation of p44/42 MAPK signaling components in response to Ano1 blockade (T16a(inh)-A01). HEK 293 cells were used to further elucidate the mechanisms involved in Cl^- channel–mediated cell proliferation and Ano1-amplified Ca^{2+} signaling. These results indicate that upon amputation, Ano1 is activated and, through amplification of Ca^{2+} flux, regulates proliferation via p44/42 MAPK signaling. We propose that Ano1 amplifies Ca^{2+} signaling by acting as a countercurrent ion channel to offset localized fluctuations in membrane potentials. Current research is pursuing this hypothesis using fluorescent ion imaging.

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25. Regulation of Cerebral Artery Endothelial TRPV4 Channel Function by cGMP-Dependent Protein Kinase. KALEV FREEMAN,[1] ADRIAN BONEV,[1] DANIEL COLLIER,[1] NURIA VILLALBA,[1] OSAMA HARRAZ,[1] SWAPNIL SONKUSARE,[2] and MARK NELSON,[1] *[1]Department Surgery, University of Vermont, Burlington, VT 05405; [2]University of Virginia, Charlottesville, VA 22908*

Cerebral arteries (CAs) have uniquely different vasodilatory mechanisms than mesenteric arteries (MAs); yet, both arteries have functional endothelial TRPV4 channels. In MAs, TRPV4 Ca^{2+} events in myoendothelial projections (MEPs) control vasodilation. We measured vasodilation in response to the TRPV4 agonist GSK1016790A (GSK101) in similarly sized mouse CA

and MAs. The concentration of GSK101 necessary to induce CA vasodilation was 10× higher than for MAs (EC50, 322 nM vs. 32 nM). We next measured elementary TRPV4 Ca^{2+} influx events (sparklets) in CAs from mice expressing a genetically encoded endothelial Ca^{2+} biosensor (GCaMP2). The amplitude and quantal levels of TRPV4 sparklets were the same in CAs as MAs. Because the coupling of elementary Ca^{2+} influx events depends on A-kinase anchoring protein (AKAP150), we analyzed the relationship of sparklets to AKAP and MEPs in CAs. CAs had the same TRPV4 coupling strength between sparklet events and similar proximity to AKAP150 at MEPs, as MAs. However, the baseline TRPV4 open channel probability in CAs was substantially lower. In conventional whole cell patch clamp of CA endothelial cells, introduction of nonhydrolyzable ATP increased TRPV4 currents. In perforated patch clamp experiments, inhibition of cGMP-dependent protein kinase (PKG) with Rp-8-Br-PET-cGMP caused a 227 (±15) % increase in TRPV4 currents in response to 300 nM GSK101; yet, this did not completely replicate the 10× difference between tissue beds. Ca^{2+} imaging experiments in CAs in the presence of Rp-8-Br-PET-cGMP or NO inhibitor L-NNA showed that both interventions increase Ca^{2+} sparklets in CAs. These data suggest phosphorylation of the TRVP4 channel by PKG, accounts for approximately twofold regulation in TRPV4 function in CAs. However, our data also suggest that other endogenous factors, possibly other kinases, must also be involved in strong negative regulation of TRPV4 channels in the brain, possibly to protect CA endothelial cells from excessive Ca^{2+} influx.

26. Decrypting Variants of Unknown Significance in Cardiac and Brain Channelopathies. **ALFRED GEORGE**, *Northwestern University Feinberg School of Medicine, Chicago, IL 60611*

Genetic testing has become standard-of-care for many diseases including channelopathies affecting cardiac rhythm such as the congenital long-QT syndrome (LQTS) and those associated with severe childhood epilepsy. However, interpreting genetic test results is often confounded by the discovery of "variants of unknown significance" for which there is insufficient data to establish whether or not a particular variant predisposes to a disease. This problem is particularly vexing for genetic disorders with strong allelic heterogeneity and a preponderance of "private" mutations. For channelopathies, in vitro functional assessments have been valuable for determining the potential pathogenicity of variants discovered in the research setting, but the value of this approach for variant classification in the clinical setting has not been evaluated. Functional annotation experiments (e.g., patch-clamp recording) have become the gold standard in assessing the likely pathogenicity of ion channel variants, but the extreme time and labor intensity of this approach are insufficient to tackle the thousands of known variants.

We have implemented a high-throughput (dual 384-well), automated patch-clamp system to enable functional annotation and pharmacological profiling of human ion channel variants at an unprecedented scale. Data from studies of cardiac potassium channels in LQTS as well as brain sodium and potassium channels associated with early onset epileptic encephalopathy will be presented to illustrate the power of this approach.

27. Pericyte-Mediated Alterations of Blood Flow Distribution at Capillary Bifurcations in a Genetic Model of Cerebral Ischemic Small Vessel Disease. ALBERT L. GONZALES,[1] THOMAS A. LONGDEN,[1] FABRICE DABERTRAND,[1] BO SHUI,[2] MICHAEL I. KOTLIKOFF,[2] and MARK T. NELSON,[1] *[1]Department of Pharmacology, University of Vermont, Burlington, VT 05404; [2]Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853*

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common heritable cause of stroke and vascular dementia and is caused by dominant mutations of NOTCH3, which is expressed only in smooth muscle cells and pericytes in the brain. Pericytes cover a significant portion of the vast capillary network within the brain. However, only pericytes closest to the terminal arterioles are contractile. The goal of the current study is to elucidate the functional role of contractile pericytes at capillary bifurcations and to examine the consequence of the NOTCH3 mutation on pericyte-controlled capillary blood flow. We tested the hypothesis that junctional pericytes asymmetrically control the luminal diameter of branches at capillary bifurcations independently of one another and, in doing so, are capable of controlling the distribution of red blood cells (RBCs) within the capillary system. Using a novel transgenic mouse expressing a genetically encoded Ca^{2+} indicator in contractile pericytes (acta2-GCaMP5-mCherry), we characterize the ion channels contributing to local Ca^{2+} signals regulating the asymmetry constriction by the pericyte processes of capillary branches. Using in vivo multiphoton microscopy, we observed distribution of RBCs at capillary junctions in correlation with the angle of bifurcation and diameter of daughter branches. Using a transgenic mouse model of CADASIL, we detected a disruption in RBC flow at capillary bifurcations, suggesting that pericytes play an integral role in controlling the directional flow of blood within the capillary bed.

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28. BK Channel α and γ1 Subunits Assemble with a Stoichiometry of Up To 4:4, But One γ1 Is Sufficient To Produce the Full γ_1-Induced Gating Shift. VIVIAN GONZALEZ-PEREZ,[1] MANU BEN-JOHNY,[2] and CHRISTOPHER J. LINGLE,[1] *[1]Department of Anesthesiology, Washington University School of Medicine, St Louis, MO,63110; [2]Department of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, 21205*

Ca^{2+} and voltage-gated K^+ channels of large conductance (BK) are symmetrical complexes whose minimal functional unit is a tetramer composed of four identical α-subunits. BK α-subunits can coassemble with auxiliary subunits belonging to two different families, β and γ, which drastically influence channel gating. The presence of β2 subunit produces a complete inactivation of BK currents, and the inactivation rate at single channel level is a functional reporter of the number of β2 subunits/channel. This methodology has established that up to four β-subunits can assemble with the α-subunits in a BK channel complex, where each one contributes an identical additive increment to the total β-induced gating shift. However, the stoichiometry of the assembly of α and γ subunits is still unknown. To solve it, we designed an "inactivating" γ1 subunit (chimera β2/γ1) and measured the inactivation rates at the single channel level. Our results show that, similar to β subunits, up to four β2/γ1 subunits can assemble in BK channels expressed in *Xenopus* oocytes. Unlike β subunits, the presence of one $\beta 2/\gamma_1$ subunit in a single BK channel is sufficient to produce the full γ_1-induced gating shift. Our results confirm the "all-or-none" type of functional regulation produced by γ1 on BK channels.

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29. Munc13 Control of Presynaptic Voltage-Gated Ca^{2+} Channels. GERALDINE GOUZER, MINGYU XUE, and TIMOTHY RYAN, *Department of Biochemistry, Weill Cornell Medicine, New York, NY 10065*

Synaptic transmission relies on precise control of presynaptic voltage-gated Ca^{2+} channels to drive exocytosis of neurotransmitters. Given the steep relationship between Ca^{2+} influx and release probability, it is critical to understand the machinery responsible for tuning Ca^{2+} influx at nerve terminals and how this is coordinated with tuning of the release machinery itself. We previously showed that the critical active zone SNARE assembly molecule Munc13-1, which is essential for all known forms of fast neurotransmitter release, also controls the gating of voltage-gated Ca^{2+} channels in nerve terminals (Calloway et al. 2015. *eLife*. 4:e07728). Munc13-1 contains a SNARE-binding Mun domain and an adjacent tandem C1-C2B domain that mediates both plasma membrane and Ca^{2+} channel interactions. The C1 domain of Munc13 was previously shown to be critical for mediating the strong modulatory impact of the diacylglycerol (DAG) mimetic phorbol-ester on synaptic transmission, presumably by tuning SNARE assembly such as to lower the energy barrier for fusion. We show here that activation of the C1 domain in Munc13 likely disrupts the C2 domain interaction with Ca^{2+} channels since application of phorbol ester rapidly decreases action potential–driven presynaptic Ca^{2+} influx by ∼25%. Genetic ablation of Munc13 reduces Ca^{2+} influx by a similar amount, and the remaining AP-driven Ca^{2+} influx is no longer sensitive to phorbol ester. Furthermore phorbol-ester modulation of AP-driven presynaptic Ca^{2+} influx is eliminated when Munc13-1 is replaced with a variant harboring a point mutation in the C1 domain that renders it insensitive to DAG. Our data are thus consistent with a model by which interactions of the C1 domain with the plasma membrane changes the positioning of the C2B domain with respect to the Ca^{2+} channel, breaking the interaction, while in turn positioning the Mun domain to drive more favorable SNARE assembly and potentiation of neurotransmitter release at the expense of lowered Ca^{2+} influx.

30. Physiological and Behavioral Comorbidities in a Zebrafish Model for Dravet Syndrome. BRIAN P. GRONE and SCOTT C. BARABAN, *Department of Neurological Surgery, University of California, San Francisco, San Francisco, CA 94143*

Mutations in *SCN1A*, which encodes Nav1.1, cause Dravet syndrome (DS), a catastrophic childhood epilepsy. In addition to early-life seizures, DS patients experience comorbid conditions such as movement disorders, sleep disturbances, anxiety, early fatality, and cognitive decline. To study the functional consequences of *SCN1A* mutations, we use zebrafish with a loss-of-function mutation in *scn1lab*, one of two zebrafish homologues of *SCN1A*. Homozygous *scn1lab*$^{s552/s552}$ mutant larvae exhibit early-life seizures, metabolic deficits, and early death. To establish zebrafish as a model for quantifying comorbid conditions in DS, we performed a battery of in vivo assays with *scn1lab*^{s552} mutants between 3 and 6 d postfertilization (dpf). To study motor activity during a seizure, we used high-speed video imaging with simultaneous EEG recording in head-fixed zebrafish. In mutants ($n = 8$), long-duration ictal events were associated with high-velocity, complex sinusoidal tail deflections >50 degrees and lasting 600–1200 ms; interictal events were associated with briefer tail movement and smaller angle deflections. To study anxiety, we used locomotion tracking to monitor exploratory behavior in an open field arena. Mutants exhibited impaired exploratory behavior, with significantly increased time spent freezing and more time spent in the periphery. To evaluate nighttime arousal disturbances, we tracked larvae in 96-well plate format for 24 h. Locomotor activity during night (sleep phase) was higher in mutants compared with controls. To assess cardiac function, we measured

heart rate using video recordings. No significant differences in heart rate compared with controls were observed. We also quantified the distribution of onset of fatality, which occurs in the first 14 dpf. Our results demonstrate conserved features of movement disorders, anxiety, sleep disturbances, and early fatality in *scn1lab* mutant zebrafish. These studies establish the zebrafish as a model for studying comorbid disease states, in addition to seizures, in a zebrafish DS model.

31. Endothelial PIP_2 Bidirectionally Modulates TRPV4 and Kir2.1 Signaling in the Brain Capillary Endothelium. OSAMA F. HARRAZ[1] and MARK T. NELSON,[1,2] *[1]Department of Pharmacology, College of Medicine, University of Vermont, Burlington, VT 05405; [2]Institute of Cardiovascular Sciences, University of Manchester, Manchester, England, UK*

Brain capillaries form a sensory web that can detect neuronal activity. We recently showed that brain capillary endothelial cells (cECs) express ideal candidates for the transduction of Ca^{2+} and electrical signals, namely TRPV4 and Kir2.1 channels, respectively. Here, we tested the hypothesis that the same regulatory pathway can simultaneously exert opposite effects on TRPV4 and Kir2.1 channels in freshly isolated cECs from C57BL/6 mouse brain. TRPV4 currents were inhibited by the inclusion of hydrolysable ATP in the patch pipette. Scavenging phosphatidylinositol 4,5-bisphosphate (PIP_2) or inhibition of its synthesis reversed ATP effects. In contrast, Kir2.1 currents were unaffected by exogenous PIP_2 or ATP. Kir2.1 current, however, declined over time, and this rundown did not occur when PIP_2 was included in the pipette. Activation of G protein–coupled receptors (G_qPCRs) hydrolyzes PIP_2 to produce IP_3 and diacylglycerol (DAG). We tested the hypothesis that G_qPCR activation through hydrolysis of PIP_2 has divergent consequences on TRPV4 and Kir2.1 currents. Prostaglandin E2 (PGE2; an agonist of the G_qPCR EP1), which has been implicated in neurovascular coupling, relieved the inhibition of TRPV4 channels by intracellular ATP. The latter effect was attributed to PIP_2 hydrolysis rather than enhanced IP_3/Ca^{2+} or DAG/PKC signaling. On the other hand, Kir2.1 current rundown was significantly augmented by PGE2. In conclusion, our studies support the concept that PIP_2 tonically inhibits TRPV4 channels and activates Kir2.1 channels in brain capillary endothelial cells. GqPCR activation acts as a molecular switch to alter the balance between electrical (Kir2.1) and Ca^{2+} (TRPV4) signaling, which would change the signaling modality to upstream penetrating arterioles and likely would have profound effects on the control of blood flow into the brain.

32. Whole Exome Sequencing in 50,000 Clinic Patients Leads to Discovery of a Novel Kir3 Mutation Implicated in Arrhythmia. CASSANDRA M. HARTLE, BRYN S. MOORE, and TOORAJ MIRSHAHI, *Weis Center for Research, Geisinger Clinic, Danville, PA 17837*

Through collaboration with the Regeneron Genetics Center, we have performed whole exome sequencing on more than 50,000 individuals for whom we have comprehensive clinical data from the electronic health records including ECG records. We have discovered a large number of variants in ion channels that we are assessing for association with various disease states. As a first example, we identified a patient who carried a novel truncation mutation in *KCNJ3* gene (Kir3.1 or GIRK1 channel). The patient had a strong personal and family history of heart disease and has since died of a suspected arrhythmia. Kir3.1 and Kir3.4 (GIRK4) form a hetero-tetramer, traffic to the cell surface, and comprise I_{KACh} in atria. The stop-gain mutation, R286stop identified in this patient, removes the vast majority of the channel C terminus. To test the functional relevance of this novel mutation to the patient's arrhythmia, we Flag tagged Kir3.1 on the N terminus and mutated Arginine 286 to a stop codon (R286stop). In HEK293 cells, R286stop degraded more rapidly compared with wild-type Kir3.1. That degradation was prevented by proteasome inhibitors. Kir3.4 and WT Kir3.1 interact with R286stop but also appear to degrade when coexpressed with R286stop, suggesting a dominant negative effect on the interacting proteins caused by the truncated channel. Kir3.1 alone resides in the ER and is targeted to the plasma membrane when coexpressed with Kir3.4. R286stop, on the other hand, localizes to the ER and vesicle type puncta in the cell and does not traffic to the plasma membrane when Kir3.4 is coexpressed. Interestingly, R286stop traps some of the Kir3.4 inside the cell, reducing its cell surface expression. Our data suggest the R286stop mutation could have resulted in diminished I_{KACh}, leading to the arrhythmia in the patient.

33. Discovery of a Persistent Inward Current in Skeletal Muscle with Characteristics Suggesting It Plays a Central Role in Triggering Myotonia in Myotonia Congenita. AHMED HAWASH, ANDREW VOSS, and MARK RICH, *Wright State University, Dayton, OH 45435*

Myotonia congenita is caused by loss-of-function mutations of the skeletal muscle chloride channel (ClC-1) and is characterized by involuntary repetitive firing of action potentials. Patients with myotonia congenita experience muscle dysfunction, mainly due to muscle stiffness. For over 40 yr, it has been accepted that a buildup of K^+ in the transverse tubular system during trains of evoked action potentials is responsible for the sustained firing of spontaneous action potentials. If K^+ buildup is the cause, opening K^+ channels should worsen myotonia. However, our preliminary studies show that opening K^+ channels using retigabine lessened myotonic firing. This strongly suggests that K^+ buildup cannot be the sole cause of myotonia. We thus hypothesized that an additional depolarizing event, which remains active for seconds (the length of a myotonic run),

drives the repetitive action potentials in myotonia. A possible mechanism involves persistent inward currents (PICs), which are resistant to fast inactivation and enable repetitive firing in motor neurons. To look for a PIC in skeletal muscle, we used slow ramp protocols under voltage clamp in dissociated flexor digitorum brevis fibers of ClC-1–null (myotonic) mice. We identified a PIC in myotonic skeletal muscle with characteristics needed to drive the repetitive involuntary firing seen in myotonia congenita. We also determined that a similar PIC is present in wild-type muscle, suggesting the current has a hitherto overlooked physiological role. We suggest that a physiological role of skeletal muscle PIC may be to sustain repetitive firing during prolonged activation of muscle.

34. Kv12-Encoded Channels Selectively Regulate Nighttime Firing Rates in the Suprachiasmatic Nucleus. TRACEY O. HERMANSTYNE,[1] DANIEL GRANADOS-FUENTES,[2] ERIK D. HERZOG,[2] and JEANNE M. NERBONNE,[1] *[1]Departments of Medicine and Developmental Biology, Washington University School of Medicine and [2]Department of Biology, Washington University, St. Louis, MO 63130*

Kv12.1 and Kv12.2 K^+ channels, which generate outwardly rectifying currents in the subthreshold voltage range, are strong candidates for regulating daily rhythms in suprachiasmatic nucleus (SCN) membrane excitability. We explored the hypothesis that Kv12.1 and/or Kv12.2 contribute to the daily variations in the resting and active properties of neurons in the SCN that are critical for circadian rhythms in physiology and behavior. Short hairpin RNAs (shRNAs) selectively targeting Kv12.1 or Kv12.2 were used to acutely "knockdown" Kv12.1 or Kv12.2 expression in the adult mouse SCN. Current-clamp recordings in acute SCN slices revealed that the mean input resistances of SCN neurons at night were significantly ($P < 0.05$) higher in Kv12.1 (1.2 ± 0.3 GΩ)- and Kv12.2 (1.8 ± 0.3 GΩ)-targeted shRNA-expressing SCN neurons, compared with WT SCN neurons (0.9 ± 0.1 GΩ). In addition, mean ± SEM nighttime action potential thresholds were significantly ($P < 0.05$) more hyperpolarized in Kv12.1-targeted shRNA-expressing (-32.6 ± 2.1 mV) and Kv12.2-targeted shRNA-expressing (-30.1 ± 2.2 mV) than in WT (-24.1 ± 1.8 mV) SCN neurons. Furthermore, compared with WT SCN neurons (-46.2 ± 1.4 mV), the mean ± SEM nighttime resting membrane potential was significantly ($P < 0.05$) more depolarized in Kv12.1-targeted shRNA-expressing SCN neurons (-37.3 ± 3.3 mV). Mean ± SEM repetitive firing rates measured at night were also significantly ($P < 0.01$) higher in Kv12.1 (4.9 ± 1.1 Hz)- and Kv12.2 (3.6 ± 0.8 Hz)-targeted shRNA-expressing SCN neurons when compared with WT SCN neurons (0.7 ± 0.2 Hz). Parallel daytime experiments revealed no significant differences in passive membrane properties and repetitive firing rates in WT and Kv12.1- and Kv12.2-targeted shRNA-expressing SCN neurons. Taken together, these results reveal that the targeted "knockdown" of Kv12.1 or Kv12.2 selectively regulates nighttime firing rates in the SCN with very little effect on daytime firing activity. These observations suggest a critical role for Kv12.1- and Kv12.2-encoded K^+ channels in regulating the "down-state" nighttime electrical activity in the SCN.

35. Impaired Calcium Release in Skeletal Muscle Fibers with Cav1.1 Channels Carrying the Hypokalemic Periodic Paralysis-Causing Mutation R528H. ERICK O. HERNÁNDEZ-OCHOA,[1] FEN FEN WU,[2] STEPHEN C. CANNON,[2] and MARTIN F. SCHNEIDER,[1] *[1]Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD 21201; [2]Department of Physiology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095*

Hypokalemic periodic paralysis (HypoPP) is a genetic disease that presents with severe and recurrent episodes of weakness triggered by reduced serum potassium. Mutations at arginine residues in the S4 segments of the voltage sensor domains (VSD) in the voltage-gated calcium channel (Cav1.1) are associated with HypoPP. We developed a knock-in Cav1.1 mutant with one arginine-to-histidine substitution (R528H) located at the S4 of the second VSD to investigate abnormal muscle excitability in HypoPP. R528H mice recapitulated the disease in a gene dosage–dependent manner; muscle force was reduced and became exacerbated after low K^+ challenge, muscle fibers displayed abnormal excitability and aberrant "gating pore" currents–ionic fluxes conducted via a crevice of the channel through which the S4 moves–that increase the susceptibility to paradoxical depolarization in low extracellular [K^+] distinctive of HypoPP. Interestingly, we also reported that depolarization-induced Ca^{2+} transients were impaired in fibers from homozygous R528H mice. Here, we further investigate the impact of the R528H mutation on voltage-dependent Ca^{2+} release using Ca^{2+} imaging, membrane dyes, voltage-clamp, and confocal microscopy techniques. Depolarization-induced rhod-2 Ca^{2+} transients were significantly decreased in muscle fibers from homozygous R528H mice when compared with heterozygous or wild-type counterparts, as were the Ca^{2+} transients and Ca^{2+} release calculated from F/F_0 signals. The altered Ca^{2+} release was not due to differences in internal releasable Ca^{2+} or alterations in fiber and T-tubule morphology, suggesting Ca^{2+} release defects and further dysfunction of the Cav1.1. The impact of R528H mutation on Ca^{2+} release suggests the hypothesis that S4 from second VSD contributes to Cav1.1/RyR1 electromechanical coupling and Ca^{2+} release in skeletal muscle. Further mutations in the VSDs of Cav1.1 are needed to explore the mechanistic underpinnings of Cav1.1 voltage sensing and excitation-contraction coupling.

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36. Monoaminergic Synaptic Transmission at the Merkel Cell–Neurite Complex. BENJAMIN U. HOFFMAN, YOSHICHIKA BABA, and ELLEN A. LUMPKIN, *Department of Dermatology, Columbia University, New York, NY, 10032*

Of the five cardinal senses, touch is the least understood. Due to the complexity of the peripheral nervous system, mechanisms of how we encode touch stimuli have remained elusive. In the skin, gentle touch is mediated in part by Merkel cells—epidermal cells that cluster in highly touch-sensitive skin regions, including fingertips, whisker follicles, and touch domes. In complex with myelinated (Aβ) somatosensory afferents, they form high acuity receptors that mediate slowly adapting type I (SAI) responses. The Merkel cell–neurite complex detects pressure, represents shapes and edges, and guides dexterous hand movements. We hypothesized that Merkel cells release monoamine-containing vesicles at SNARE-dependent synapses to excite firing in tactile afferents. To test this hypothesis, we selectively expressed tetanus toxin light chain (TeNT) in epidermal cells in vivo in order to disrupt SNARE-mediated synaptic vesicle release. Using ex vivo skin-nerve recordings, we showed that Merkel cells require functional SNARE proteins to mediate canonical SAI responses. Indeed, in response to mid-range stimuli, TeNT-expressing Merkel cells were unable to mediate sustained firing in tactile afferents. Moreover, to show that Merkel cells package and release monoamines as neurotransmitters, we employed a fluorescent neurotransmitter analogue to label Merkel cells in semi-intact epidermal preparations. Merkel cells loaded neurotransmitter analogues into acidic vesicles in a VMAT2-dependent manner. Finally, neurotransmitters were released from Merkel cells in response to depolarizing stimulation. For the first time, these experiments reveal that Merkel cells employ SNARE-dependent, regulated release of neurotransmitters to signal to tactile afferents and set the stage to study the receptor pathways through which this information is transduced.

37. A Novel Pain Assay Using a Transgenic Mouse Expressing $hNa_V1.7$ with an Inherited Erythromelalgia Mutation: Comparison with Traditional Models for Inflammatory and Neuropathic Pain. S. HOWARD,[1] G. BANKAR,[1] K. NELKENBRECHER,[1] M. WALDBROOK,[1] Z. XIE,[1] K. KHAKH,[1] E. CHANG,[1] C. YOUNG,[1] S. LIN,[1] J.P. JOHNSON,[1] L.E. SOJO,[1] A. LINDGREN,[1] N. CHAHAL,[1] S. CHOWDHURY,[1] S. DECKER,[1] I. HEMEON,[1] C.M. DEHNHARDT,[1] J. CHANG,[2] B. SAFINA,[2] D.P. SUTHERLIN,[2] D. HACKOS,[2] C.L. ROBINETTE,[1] and C.J. COHEN,[1] *[1]Xenon Pharmaceuticals, Burnaby, BC, Canada; [2]Genentech, South San Francisco, CA 94080*

Loss-of-function mutations in $Na_V1.7$ result in congenital indifference to pain with little or no effect on motor or cognitive function. Moreover, gain-of-function mutations result in inherited erythromelalgia (IEM) and other painful conditions. A humanized transgenic mouse was developed for in vivo testing of target engagement of $hNa_V1.7$. BAC transgenic FVB mice incorporated $hNa_V1.7$ with the IEM mutation I848T. Transgenic mice express high levels of $hNa_V1.7$ mRNA in DRGs and olfactory bulbs, areas of high expression of the endogenous channel. A subcutaneous hind paw injection of the sodium channel agonist aconitine elicits a reproducible and quantifiable flinching/licking response for up to 60 min. The aconitine effects are dependent on the presence of the transgene since a comparable injection of aconitine into wild-type mice induces minimal nocifensive behaviors. The model provides a well-defined PK-PD relationship and facilitates the rapid evaluation of compounds. The analgesic activity of sodium channel blockers was characterized both in the transgenic IEM assay and in traditional pain assays using littermate wild-type mice. We have used two acylsulfonamide s that are potent inhibitors of [^{3}H]GX-545 binding to $Na_V1.7$. This ligand targets a binding site on VSD4 that has a high degree of molecular selectivity among Na_Vs (Ahuja et al. 2015. *Science.* 350:aac5464). One compound has limited selectivity against $Na_V1.1$, $Na_V1.2$, and NaV1.6, while the second is >10-fold selective against these isoforms. However, with allowance for modest differences in IC_{50} for mouse versus human $Na_V1.7$, both compounds are equipotent in the IEM target engagement assay and in assays using littermate wild-type mice. The assays include inflammatory pain induced by complete Freund's adjuvant or formalin or neuropathic pain caused by treatment with streptozotocin. These comparative studies with compounds that differ in selectivity among Na_Vs indicate that block of $Na_V1.7$ alone is sufficient for broad analgesic activity against inflammatory and neuropathic pain.

38. Pyramidal Neuron Subpopulation Excitability Increases with Reduced Function and Expression of Voltage-Gated Na^+ Channels in the *Scn1b*-Null Mouse Model of Dravet Syndrome. JACOB HULL, LARISA KRUGER, HEATHER O'MALLEY, and LORI ISOM, *Department of Neuroscience, University of Michigan, Ann Arbor, MI 48105*

Mutations in *SCN1B*, encoding voltage-gated Na^+ channel (VGSC) β1 subunits, result in pediatric epilepsies including Dravet syndrome (DS), an epileptic encephalopathy. While the majority of DS patients have de novo mutations in *SCN1A*, patients with two loss-of-function *SCN1B* alleles also have DS. Brain VGSCs contain one pore forming α and two modulatory β subunits. The effects of *Scn1b* on excitability are cell type and brain region specific, and thus studying *Scn1b*-null mice may help identify dysfunctional DS microcircuits. We investigated the effects of *Scn1b* deletion at the level of individual pyramidal neuron populations in p14-21–null

and WT mice. We found no changes in excitability of pyramidal neurons in acute slices of visual cortex layer 2/3 ($n = 19$, $n = 9$) versus WT ($n = 16$, $n = 10$) or layer 5 ($n = 16$, $n = 8$) versus WT ($n = 12$, $n = 7$). However, null layer 6 (L6) ($n = 22$, $n = 19$) versus WT ($n = 19$, $n = 11$) and subicular pyramidal neurons ($n = 9$, $n = 4$) versus WT ($n = 13$, $n = 6$) had increased firing in 1s current injections and increased input resistance (R_{In}). To investigate the role of somatic Na^+ current density (I_{Na}), we pulled nucleated patches from L6-null ($n = 9$, $n = 8$) and WT ($n = 8$, $n = 8$) and subicular-null ($n = 4$, $n = 4$) and WT ($n = 8$, $n = 8$) pyramidal neurons, finding 43% and 39% decreases in I_{Na} density, respectively ($P < 0.05$). Action potential (AP) phase plots in 1s trains showed no change during the first AP and attenuation of both the axon initial segment and somatic components in later APs in null L6 neurons versus WT ($P < 0.05$). ^{3}H-STX binding to cortical membranes showed a 35% reduction in VGSCs in null ($n = 5$) versus WT ($n = 3$) ($P < 0.05$). Reduced I_{Na} suggests K^+ channels regulating R_{In} may underlie hyperexcitability in *Scn1b*-null neurons. Future work will test this hypothesis. Identification of affected neuron populations and the contributing ion channels may guide development of treatments for DS.

39. Discovering Epilepsy Mechanisms in Dravet Syndrome. **LORI L. ISOM**, *University of Michigan Medical School, Ann Arbor, MI 48105*

Neuronal channelopathies cause various brain disorders including epilepsy, migraine, and ataxia. Despite the development of mouse models, pathophysiological mechanisms for these disorders are poorly understood. One particularly devastating channelopathy is Dravet syndrome (DS), a severe childhood epileptic encephalopathy (EE) with a high risk of sudden unexplained death in epilepsy (SUDEP). DS is typically caused by de novo dominant mutations in *SCN1A*, encoding the voltage-gated Na^+ channel $Na_v1.1$. Although SUDEP is the most devastating consequence of epilepsy and the leading cause of epilepsy mortality, astonishingly little is understood about its causes, and no biomarkers exist to identify at-risk epilepsy patients. Heterologous expression of mutant $Na_v1.1$ channels suggests haploinsufficiency, raising the question of how loss of Na^+ channels underlying action potentials produces hyperexcitability. Data from DS mouse models indicate both decreased Na^+ current (I_{Na}) in interneurons, implicating disinhibition, and increased I_{Na} in pyramidal cells, implicating hyperexcitability, depending on genetic background, brain area, and animal age. To understand the effects of *SCN1A* DS mutations in human neurons, we derived forebrain-like neurons from two DS subjects by induced pluripotent stem cell (iPSC) reprogramming of patient fibroblasts and compared them with iPSC-derived neurons from human controls. We found that DS patient-derived neurons have increased I_{Na} density and spontaneous bursting in both bipolar- and pyramidal-shaped neurons. Because $Na_V1.1$ is also expressed in heart, a compelling idea is that altered I_{Na} in DS cardiac myocytes, in addition to central neurons, may lead to arrhythmias and contribute to SUDEP. To test this hypothesis, we used the iPSC method to derive cardiac myocytes from fibroblasts of DS subjects. Our data suggest that a subset of DS subjects shows increased cardiac myocyte I_{Na} and excitability, which may be predictive of SUDEP risk. In parallel studies of a DS human mutant *SCN1A* knock-in mouse model, we observed spontaneous seizures and SUDEP in the mice, increased ventricular cardiac myocyte I_{Na} density, and ventricular arrhythmias at the time of SUDEP. To discover potential common mechanisms of SUDEP, we studied changes in cardiac excitability in two additional EE models, a *SCN8A* knock-in model of EIEE13 and a *Scn1b*-null model of DS. Both models showed cardiac arrhythmias with changes in I_{Na} and calcium handling in ventricular myocytes. Parallel studies of human patient–derived *SCN8A*- and *SCN1B-EE* linked iPSC neurons and cardiac myocytes are in progress. The ultimate goal of our work is to provide a greater understanding of the mechanisms of DS and related EEs that may lead to novel therapeutic agents for epilepsy and SUDEP prevention.

40. Regional Differences in Transient Outward Potassium (I_{to}) Currents and the Role of I_{to} in Human Ventricular Repolarization. ERIC K. JOHNSON,[1] STEVEN SPRINGER,[1] WEI WANG,[1] ANNA ZHANG,[1] EVELYN M. KANTER,[1,2] KATHRYN A. YAMADA,[1] and JEANNE M. NERBONNE,[1,3] *[1]Department of Medicine, Division of Cardiology, [2]Department of Surgery, and [3]Department of Developmental Biology, Washington University in St. Louis, St. Louis, MO 63130*

The rapidly activating and inactivating Ca^+-independent transient outward potassium current, I_{to}, has been described in the hearts of many mammals, where it contributes to the early phase of action potential repolarization and to the generation of normal cardiac rhythms. Dysregulation of I_{to} has been implicated in Brugada syndrome and in hypertrophied and failing hearts and is associated with increased risk of potentially life-threatening arrhythmias. The biophysical properties and functional role(s) of I_{to} in the human heart, however, are poorly understood. To fill this knowledge gap, voltage-clamp studies were performed in myocytes isolated from the sub-epicardial (LV Sub-Epi), epicardial (LV Epi), endocardial (LV Endo), and sub-endocardial (LV Sub-Endo) regions of the left ventricle. A gradient of I_{to} densities was identified with higher densities in LV Sub-Epi and LV Epi than in LV Sub-Endo and LV Endo myocytes. The voltage dependences and kinetics of I_{to} activation and inactivation were similar in all LV cell types. Analysis of the kinetics of I_{to} recovery from inactivation revealed two distinct

I_{to} components: in LV Sub-Epi myocytes, the mean ± SEM time constant of recovery was 19.3 ± 0.3 ms (n = 21), reflecting expression of $I_{to,f}$, whereas the time constant of recovery in LV Sub-Endo myocytes was 1,067 ± 45 ms (n = 37), reflecting expression of $I_{to,s}$. In LV Epi (n = 42) and LV Endo (n = 39) myocytes, recovery was bi-exponential, consistent with the presence of both $I_{to,f}$ and $I_{to,s}$.

To explore the role(s) of I_{to} in shaping human ventricular action potential (AP) waveforms, a mathematical model of I_{to} was generated and used in dynamic-clamp experiments, and the effects of changing I_{to} density and inactivation kinetics on AP properties were determined. Manipulation of I_{to} density had pronounced effects on notch potentials with minimal effects on other AP properties. Manipulation of I_{to} inactivation kinetics had dramatic effects on both the shapes and durations of action potentials.

41. Patient-Specific Induced Pluripotent Stems as a Platform for Precision Therapeutics in the Treatment of an Inherited Cardiac Arrhythmia. **ROBERT KASS**, *Columbia University, New York, NY 10027*

Understanding the genetic basis for differential responses to drug therapies is a key goal of precision medicine. Induced pluripotent stem cells (iPSCs) offer a unique system to investigate the pharmacology of disease processes in therapeutically and genetically relevant primary cell types in vitro. In this presentation, we report the use of patient-derived iPSCs to understand limitations of clinical regimen that had failed to control cardiac arrhythmias in a long QT syndrome patient who was found to have a de novo *SCN5A* LQT-3 mutation and a polymorphism in *KCNH2*, the gene for LQT2. Analysis of the molecular pharmacology of ion channels expressed in cardiomyocytes differentiated from these iPSCs (iPCS-CMs) revealed drug interactions that included off target inhibition of the *KCHN2* (hERG) channel that could be minimized by altering heart rate. The improved in vitro pharmacologic approach correlated with improved management of arrhythmias in the patient and provides support for this approach in developing precise patient-specific clinical regimens.

42. Elucidating the Mechanisms Underlying the Regulation of Voltage-Gated Ca^{2+} Channels Using Transgenic Mice. ALEXANDER KATCHMAN,[1] LIN YANG,[1] and **STEVEN MARX**,[1,2] *[1]Division of Cardiology, Department of Medicine and [2]Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, NY 10027*

Cav1.2, the sarcolemmal L-type Ca^{2+} channel, plays a key role in regulation of signaling in the brain and the heart. In the heart and brain, abnormalities in Cav1.2 function are associated with diseases, such as autism, heart failure, and hypertrophy. A detailed molecular understanding of Cav1.2 regulation in myocytes and brain has been hampered, however, by the inability to recapitulate and then dissect in heterologous expression systems key aspects of Cav1.2 function. We have developed novel tools to surmount major obstacles that have limited progress in the field and allow us to probe molecular aspects of Cav1.2 regulation, using biochemical and electrophysiological techniques, within the context of cardiomyocytes and neurons, but with the power of a heterologous expression system. Using a transgenic (TG) approach that enables selective and reliable expression of FLAG-epitope–tagged, dihydropyridine-resistant Cav1.2 channel subunits, harboring mutations at key regulatory sites or covalently linked to regulatory components, in adult cardiomyocytes and at all stages of development, we have explored the mechanisms responsible for adrenergic regulation of Cav1.2. We found that Ser1700 and Thr1704 are not required for β-adrenergic stimulation of Cav1.2 in adult cardiomyocytes. Naturally, in a protein as large as α1C, there are numerous intracellular consensus PKA phosphorylation sites, although many may be not surface exposed. Rather than continue the search for single phosphorylated residues using in vitro techniques (which has not been fruitful), we created a transgenic mouse with Ala substitutions of 17 and 22 potential PKA sites and found that Cav1.2 channels harboring these 17 and 22 Ala substitutions were functional, trafficked to the dyad, and were appropriately regulated by adrenergic stimulation, implying that these residues are not required for adrenergic regulation. We have concluded that direct phosphorylation of the α1C subunit by PKA may not be required for adrenergic regulation of Cav1.2. We have also sought to determine whether proteolytic cleavage of a1C is required for the adrenergic stimulation of Cav1.2. Since proteolytic cleavage cannot be reconstituted in heterologous expression, there is no effective way to study the process, other than in native tissues. Although deletion of the 1798NNAN motif did not alter the proteolytic cleavage of α1C, cleavage likely occurs in this general region, however, based upon the molecular weight of the truncated α1C. Within the region, there are other similar motifs including 1794NANI1797. Although the protease responsible for cleavage of α1C is not known, it has been speculated to be calpain-like. A conserved PEST sequence, a motif rich in Pro, Glu, Ser, and Thr that has been proposed to serve as substrate recognition sites for calpains, is just N-terminal to Ala1800. We have created a transgenic mouse line with deletion of both the PEST sequence (1769DTESP) and 1794NANINNANN1802. Data will be presented concerning these mice. In summary, using novel transgenic approaches, we have clarified key mechanisms responsible for adrenergic regulation of Ca^{2+} channels.

43. TRPV1-Mediated Ca^{2+} Influx in Arterial Smooth Muscle. MASAYO KOIDE, ELIZABETH HUGHES, ARSALAN U. SYED, SWAPNIL SONKUSARE, INESSA MANUELYAN, MARK T. NELSON, and GEORGE C. WELLMAN, *Department of Pharmacology, University of Vermont College of Medicine, Burlington, VT 05405*

Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels (VDCCs) has traditionally been viewed as the major Ca^{2+} entry pathway in arterial smooth muscle (ASM) to control contraction as well as modulate excitability and transcription. Here, we provide direct evidence for another major Ca^{2+} entry pathway—the TRPV1 channel—in ASM of specific vascular beds. Using mice with a genetically-encoded Ca^{2+} indicator (GCaMP5-tdTomato) linked to endogenous TRPV1 expression, we have found functional expression of TRPV1 channels in ASM throughout the external carotid artery (ECA) territory. Using "optical" patch clamping techniques (Sonkusare et al. 2012. *Science.* 336:597–601), we provide the first measurements of Ca^{2+} influx through single TRPV1 channels ("TRPV1 sparklets") and show that this unitary input of Ca^{2+} dwarfs (fivefold) the flux through a single VDCC. In TRPV1-postive arteries (e.g., facial artery), physiological stimuli such as adrenergic agonists and endocannabinoids cause robust increases in ASM Ca^{2+} and vasoconstriction through TRPV1 activation. Our data indicate that when ASM TRPV1 are activated and become the dominant contributor to membrane conductance and Ca^{2+} entry, VDCC-dependent regulation of arterial diameter is lost. We propose that TRPV1-mediated vasoconstriction causes increased resistance in the ECA territory, leading to a redistribution of blood flow from ECA to the internal carotid and cerebral arteries, which do not possess functional TRPV1 channels. Further, under conditions of ASM TRPV1 activation, endogenous hyperpolarizing vasodilator mechanisms would remain intact in cerebral arteries but would be short-circuited in the external carotid territory, amplifying increases in cerebral blood flow. In summary, our data suggest that Ca^{2+} influx mediated via ASM TRPV1 channels can profoundly impact the regulation of arterial diameter and blood flow.

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44. Enhanced Astrocyte Ca^{2+} Signaling Contributes to Pathological Neurovascular Coupling After Subarachnoid Hemorrhage. MASAYO KOIDE, ANTHONY C. PAPPAS, and GEORGE C. WELLMAN, *Department of Pharmacology, University of Vermont, Burlington, VT 05405*

Neurovascular coupling (NVC) is a unique function within the brain to increase local blood flow in regions of active neurons. We have previously demonstrated an inversion of NVC, or neuronal activation-induced vasoconstriction rather than vasodilation, in brain slices from subarachnoid hemorrhage (SAH) model rats (Koide et al. 2012. *Proc. Natl. Acad. Sci.* 109:E1387–E1395). This pathological neuronally-evoked vasoconstriction coincided with the emergence of astrocyte endfeet high-amplitude Ca^{2+} signals (eHACS). In the present study, we examined the mechanism underlying SAH-induced eHACS. Using two-photon fluorescent and infrared-differential interference contrast microscopy, astrocyte endfeet Ca^{2+} and arteriolar diameter were recorded in brain slices from SAH model animals. We observed both high amplitude (eHACS, peak Ca^{2+} >500 nM) and control-like astrocyte Ca^{2+} signals in the absence of electrical or chemical stimulation that were abolished by cyclopiazonic acid (a sarco/endoplasmic reticulum Ca^{2+} ATPase inhibitor) or U73122 (a phospholipase C inhibitor). These data indicate that these spontaneous endfoot Ca^{2+} signals are IP_3-mediated intracellular Ca^{2+} release events. Interestingly, either the broad-spectrum purinergic receptor inhibitor, suramin, or a cocktail of P2Y receptor inhibitors blocked eHACS, but not control-like Ca^{2+} events. Further, suramin treatment restored vasodilatory NVC in brain slices from SAH model animals. These results indicate that P2Y purinergic signaling contributes to SAH-induced eHACS and inversion of NVC. Moreover, these data demonstrate that the amplitude of spontaneous astrocyte Ca^{2+} signaling can profoundly impact NVC. Abnormal astrocyte Ca^{2+} oscillations are observed in various disease states such as Alzheimer diseases, epilepsy, and small vessel diseases. Thus, investigating and targeting molecular mechanisms of abnormal astrocyte Ca^{2+} signaling may improve cerebral blood flow and provide benefit to individuals with a range of brain pathologies.

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45. Ethanol-Induced Gating Alterations in Homomeric slo1 Versus β1-Containing BK Channels. GURUPRASAD KUNTAMALLAPPANAVAR and ALEX M. DOPICO, *Department of Pharmacology, College of Medicine, The University of Tennessee Health Science Center, Memphis, TN 38163*

Calcium and voltage-gated, large conductance potassium (BK) channels result from tetrameric association of slo1 proteins (BK α subunits). In most mammalian tissues, BK channels consist of α and β subunits. The latter do not form channels themselves but affect the BK phenotype (Torres et al. 2014. *Front. Physiol.* 5:383). Four β subtypes have been identified, with β1 being predominantly expressed in smooth muscle (Brenner et al. 2000. *J. Bio. Chem.* 275:6453–6461). Intoxicating levels of ethanol (10–100 mM) usually increase the open probability (Po) of homomeric slo1 channels while decreasing Po of β1-containing BK complexes (Dopico et al. 2014. *Front. Physiol.* 5:466). The gating modifications leading to these differential ethanol actions on BK Po have remained

unresolved. We tested ethanol action on slo1 (cbv1) and β1 subunits cloned from rat cerebral artery myocytes and expressed in *Xenopus* oocytes. Single channel and macroscopic currents were obtained from inside-out patches before and after 1–2 min exposure to 50 mM ethanol. We used the Horrigan Aldrich (HA) allosteric model of BK channel gating to address which allosteric parameters and gating processes were altered by ethanol. Data show that ethanol-induced change in cbv1-mediated ionic current is associated with a ×9 time increase in the channel's apparent calcium binding affinity (K_d = 9.02 ± 1.4 vs. 1 ± 0.07), whereas there is no significant change in voltage-dependent ($V_h(J)$ = 155 vs. 155.1 ± 2; z_J = 0.6 ± 0.04 vs. 0.57 ± 0.02; D = 19.7 ± 1.4 vs. 19.7 ± 0.61) or intrinsic gating parameters ($L_0 = 2.3 \times 10^{-6} \pm 6.3 \times 10^{-7}$ vs. $2.1 \times 10^{-6} \pm 2.8 \times 10^{-7}$; z_L = 0.34 ± 0.05 vs. 0.35 ± 0.04). On the other hand, ethanol modification of cbv1+β1-mediated current is due to reduced allosteric interactions between calcium binding-channel opening (i.e., decreased allosteric factor C by ∼50%; 27.2 ± 2.3 vs. 13.54) and calcium binding-voltage sensor activation (i.e., decreased allosteric factor E by ∼50%; 9.5 ± 1.6 vs. 4.06 ± 0.5). Thus, while ethanol increases the apparent calcium affinity of both slo1 and slo1+β1 channels, ethanol-induced disruption of allosteric coupling in slo1+β1 override drug-induced decrease in K_d, leading to overall decrease in Po.

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46. Pharmacological and Genetic Modifiers of a $Ca_v2.1$ Channelopathy. **AMY LEE**, *University of Iowa, Cedar Falls, IA 50614*

Voltage-gated ion channels exhibit complex properties, which can be targeted in pharmacological therapies for disease. We have found that the pro-oxidant, tert-butyl dihydroquinone (BHQ), modulates $Ca_v2.1$ Ca^{2+} channels in ways that oppose defects in channel gating and synaptic transmission, resulting from a familial hemiplegic migraine mutation (S218L). BHQ slows deactivation, inhibits voltage-dependent activation, and potentiates Ca^{2+}-dependent facilitation of $Ca_v2.1$ channels in transfected HEK293T cells. These actions of BHQ help offset the gain of function and reduced Ca^{2+}-dependent facilitation of $Ca_v2.1$ channels with the S218L mutation. Transgenic expression of the mutant channels at the *Drosophila* neuromuscular junction causes abnormally elevated evoked postsynaptic potentials and impaired synaptic plasticity, which are largely restored to the wild-type phenotypes by BHQ. Our results reveal a mechanism by which a $Ca_v2.1$ gating modifier can ameliorate defects associated with a disease-causing mutation in $Ca_v2.1$. We are currently screening for genetic modifiers of "flygraine" phenotypes in S218L-expressing flies in an effort to identify new signaling pathways that may be targeted in novel therapies.

47. Using Knockout Approaches To Tease Apart Physiological Roles of Slo Family Ion Channels and the Contributions of Regulatory Subunits. **CHRISTOPHER LINGLE**, *Washington University School Medicine, St. Louis, MO 63130*

The Slo family of four mammalian genes encode large conductance K^+ channels that are regulated by cytosolic soluble ions, Slo1 by Ca^{2+}, Slo2.1 and Slo2.2 by Na^+, and Slo3 by alkalization. In some cases, additional regulatory b and g subunits help define key tissue-specific physiological properties. In previous work, important advances in understanding the physiological roles of Slo1 BK-type channels have been gained from development of KO animals for the Kcnma1 (BK α subunit) gene, the Kcnmb1 (BK β1) gene, and the Kcnmb4 (BK β4) gene. Such models have provided insight into potential roles of the BK channel in hypertension, epilepsy, and motor coordination with additional roles likely to be identified. Now, new mouse models of other Slo family subunits have become available, including Kcnu1 (Slo3), Lrrc52 (Slo3 γ2 subunit), Kcnmb2 (BK β2), Slo2.1, and Slo2.2. Together, the Slo3 and Lrrc52 KOs reveal a Slo3/Lrrc52 partnership is critical to mouse fertility. The BK β2 KO reveals a critical role of β2 containing BK channels in shaping repetitive firing and, unexpectedly, that the absence of β2 subunits may favor spontaneous slow-wave bursting in some cells. Slo2.2 KO results in acute sensory phenotypes and, most obviously, an enhanced initial response to itch stimuli that arises from a reduced threshold for action potential generation in DRG neurons. Although the different physiological systems influenced by these subunits are only beginning to be examined, in each case this general approach has so far revealed novel aspects of physiological roles of these ion channels which had not been previously anticipated.

48. Translational Control of Ion Channel Composition. FANG LIU,[1] DAVID K. JONES,[1] WILLEM DE LANGE,[2] and **GAIL A. ROBERTSON**,[1,3] *[1]Department of Neuroscience, [2]Department of Pediatrics, and [3]Cardiovascular Research Center, Wisconsin Institutes of Medical Research, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705*

Ion channel function depends on subunit composition and stoichiometry. How the subunits find each other and assemble into complexes with the appropriate composition is poorly understood. We observed that alternate transcripts encoding hERG1a and 1b subunits, which assemble to produce ion channels mediating cardiac repolarization, are physically associated during translation. This association was first suggested by the observation that shRNA specifically targeting either hERG1a or 1b transcripts reduced levels of both transcripts when they were co-expressed heterologously. Native hERG1a and 1b transcripts in cardiomyocytes

derived from human-induced pluripotent stem cells were similarly knocked down by either shRNA, whereas KCNE1 or RYR2 levels were unaffected. The shRNA targeted transcripts undergoing translation, as opposed to aggregates destined for degradation, as confirmed by reductions in corresponding protein levels assayed by Western blot, quantitative immunocytochemistry, and membrane current recordings. To further test the hypothesis that co-knockdown of transcripts reflects a physical association, we immunoprecipitated nascent hERG1a protein from polysomal preparations and found that both the hERG1a and 1b transcripts co-purified. This association occurred even when translation of 1b protein was prevented by mutating the translation start site, indicating that the association could not be explained by cotranslational association of the nascent proteins and is likely attributable to the action of an RNA-binding protein. The physical association of transcripts encoding different subunits provides the spatial proximity required for nascent proteins to interact during biogenesis and may represent a general mechanism facilitating assembly of heteromeric protein complexes involved in a range of biological processes.

49. $cPLA_2\alpha^{-/-}$ Sympathetic Neurons Exhibit Increased Membrane Excitability and Loss of N-Type Ca^{2+} Current Inhibition by M_1 Muscarinic Receptor Signaling.
LIWANG LIU,[1] JOSEPH V. BONVENTRE,[2] and ANN R. RITTENHOUSE,[1] *[1]University of Massachusetts Medical School, Worcester, MA 01655; [2]Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115*

Group IVa cytosolic phospholipase A_2 ($cPLA_2\alpha$) mediates GPCR-stimulated arachidonic acid (AA) release from phosphatidylinositol 4,5-bisphosphate (PIP_2), located in plasma membranes. We previously found in superior cervical ganglion (SCG) neurons that PLA_2 activity is required for voltage-independent N-type Ca^{2+} (N-) current inhibition by M_1 muscarinic receptors (M_1Rs). These findings are at odds with an alternative model, previously observed for M-type K^+ (M-) current inhibition, where PIP_2 dissociation from channels and subsequent metabolism by phospholipase C suffices for M-current inhibition. To resolve $cPLA_2\alpha$'s importance, we investigated its role in mediating voltage-independent N-current inhibition of ~40% that follows application of the muscarinic agonist oxotremorine-M (Oxo-M). Using a multidisciplinary approach that combined lipid pharmacology, G_q signaling, gene knockout technology, and ion channel biophysics, we found that $cPLA_2\alpha$ is required for M_1R-mediated, voltage-independent N-current inhibition. $cPLA_2\alpha$'s role appears specific for voltage-independent N-current inhibition since voltage-dependent inhibition by M_2/M_4 R signaling remained intact in $cPLA_2\alpha^{-/-}$ SCG neurons. Moreover, M-current inhibition occurred independently of $cPLA_2\alpha$, documenting divergent M_1R signaling mediating M-current and N-current inhibition. These findings support an emerging idea that multiple phospholipases act in highly specific ways to decrease PIP_2 levels at or near N-channels following M_1R stimulation. To determine $cPLA_2\alpha$'s functional importance at the neuronal level, we compared action potential firing of $cPLA_2\alpha^{+/+}$ and $cPLA_2\alpha^{-/-}$ SCG neurons. Decreases in latency to first firing and in interspike interval resulted in a doubling of firing frequency in $cPLA_2\alpha^{-/-}$ neurons. These unanticipated findings identify $cPLA_2\alpha$ as a tonic regulator of neuronal membrane excitability.

50. Modeling Epileptic Encephalopathies With Patient-Derived Neurons.
YU LIU, ANDREW TIDBALL, LUIS LOPEZ-SANTIAGO, YUKUN YUAN, JACY WAGNON, MIRIAM MEISLER, LORI ISOM, and **JACK PARENT**, *University of Michigan Medical School, Ann Arbor, MI 48109*

Reprogramming somatic cells to a pluripotent state via the induced pluripotent stem cell (iPSC) method offers an unparalleled approach for neurological disease modeling using patient-derived neurons. My laboratory has applied the iPSC approach to model severe childhood genetic epilepsies with patient-derived cells. I will provide some background on disease modeling with iPSCs and then discuss our work modeling epileptic encephalopathies caused by sodium channel mutations. We initially generated patient-derived neurons to study epilepsy mechanisms in Dravet syndrome (DS), a catastrophic childhood epilepsy caused by de novo dominant mutations and haploinsufficiency of the *SCN1A* gene that encodes the voltage-gated sodium channel $Na_v1.1$. The talk will describe our findings of altered sodium currents and excitability in DS patient neurons. Then I will describe recent work examining potential epilepsy mechanisms in another severe childhood epilepsy caused by putative gain-of-function mutations in the *SCN8A* gene that encodes $Na_v1.6$. Compared with control iPSC neurons, mutant SCN8A patient-derived neurons show increased persistent sodium current and hyperexcitability. Using a multi-well multi-electrode array for drug screening, we are validating the model with drugs known to work or to be ineffective in patients with SCN8A-associated epilepsy. I will also describe related work in mouse models of these disorders. Taken together, our work suggests that the iPSC approach offers great promise for modeling childhood epileptic encephalopathies and should provide a useful platform to identify novel therapies.

51. Critical Role of Nitric Oxide in Capillary-to-Arteriole Electrical Signaling in the Brain.
THOMAS A. LONGDEN[1] and MARK T. NELSON,[1,2] *[1]Department of Pharmacology, College of Medicine, University of Vermont, Burlington, VT 05405; [2]Institute of Cardiovascular Sciences, University of Manchester, Manchester, England, UK*

Brain capillary ECs are ideally positioned to detect and respond to local neuronal activation by signaling for increases in blood flow, but the role of capillaries in this context has so far received little attention. We have shown that capillary ECs are capable of detecting potassium (K^+) ions released during neuronal activity, which leads to inward rectifier K^+ (K_{IR}) channel-mediated retrograde electrical signaling to dilate upstream PAs and increase CBF (Longden et al. 2015. *J. Gen. Physiol.* 146:10A). Nitric oxide (NO) is required for the proper function of multiple neurovascular coupling pathways in the brain, including K^+-evoked cerebral blood flow responses in vivo (Dreier et al. 1995. *J. Cereb. Blood Flow Metab.* 15:914–919). Here, we aimed to explore whether NO contributes to capillary-to-arteriole electrical signaling in the brain.

Using the perforated configuration of the patch clamp technique, we observed capillary EC K_{IR} currents sensitive to 100 µM Ba^{2+}. Interestingly, incubation of cells with the NO synthase inhibitor L-NNA (100 µM) markedly reduced K_{IR} channel current density. Using an in vivo cranial window model to enable visualization of microcirculatory hemodynamics in the brain, we observed that selective delivery of K^+ to capillaries produced robust hyperemia within seconds, due to activation of capillary K_{IR} channels. This response was almost abolished upon superfusion of the cortical surface with 100 µM L-NNA.

Brain capillaries constitute an active sensory web, converting changes in external K^+ into rapid upstream electrical signaling to regulate blood flow into the brain. These results suggest that NO has a critical role in maintaining capillary K_{IR} channel activity to permit ongoing electrical communication with upstream arterioles.

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52. Alteration of Gating Properties in $Ca_V3.1$ Channel Induced by Aspartic Residues in Its Pore. IGNACIO LÓPEZ-GONZÁLEZ, EDGAR GARZA-LÓPEZ, and TAKUYA NISHIGAKI, *Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, UNAM Av. Universidad #2001, Col. Chamilpa, Cuernavaca, Morelos, Mexico CP 62210*

The simple and classical view of voltage-gated ion channels proposes that amino acid residues of the pore selectivity filter do not have a strong influence on the gating properties of the channel. However, previous reports have questioned this classical point of view. Regarding low voltage–activated (LVA) Ca^{2+} channels, it has been demonstrated that substitutions of pore aspartate residues (D) by glutamate residues (E) in domains III and IV alters the channel gating properties with the same tendency (Talavera et al. 2003. *J. Gen. Physiol.* 121:529–540), suggesting certain pore symmetry. In the present report, we evaluated the role of pore (E) residues of domains I and II on the $Ca_V3.1$ channel gating properties. Our results indicate that substitution of (E) residues in the pore loops of domains I and II by (D) residues differentially modify the gating properties of $Ca_V3.1$ channel. The single mutant channel (DEDD, domain I) presented slight uncoupling between the activation and inactivation processes, with a more stable inactivation state and a slower recovery from inactivation without change in the deactivation kinetics. In contrast, the single mutant channel (EDDD, domain II) presented a less stable close state, allowing an easier channel transition to the open state, with inactivation kinetics similar to WT $Ca_V3.1$ channels and slower deactivation kinetics. At last, the double mutant channel (DDDD, domains I and II) presented completely uncoupled activation and inactivation processes; faster activation, inactivation, and deactivation kinetics; and slower deactivation process than the WT $Ca_V3.1$ channel. Taking into account our results, we can conclude that the selectivity filter residues of LVA Ca^{2+} channels have a differential influence on the gating properties of this Ca^{2+} channel subfamily, suggesting a pore pseudosymmetry. In a speculative way, we propose these new mutations in the selectivity filter of the $Ca_V3.1$ channel could be important in some types of epilepsies (Chen et al. 2003. *Ann. Neurol.* 54:239–243; Mckeown et al. 2006. *Acta Pharmacol. Sin.* 27:799–812; Heron et al. 2007. *Ann. Neurol.* 62:560–568).

53. FLARE Biosensors for Multiparametric Imaging. MICHELE L. MARKWARDT,[1] BRIAN ROSS,[2] JENNIFER MCFARLAND,[1] JIN ZHANG,[2] and **MARK A. RIZZO**,[1] *[1]Departiment of Physiology, University of Maryland School of Medicine, Baltimore MD, 21201; [2]Department of Pharmacology, University of California, San Diego, La Jolla, CA 92093*

Optical tools designed to track activation of key cell signaling pathways have revealed the spatial and temporal dynamics of intracellular communication with unprecedented resolution. Even so, many of these studies are practically limited to tracking a single endpoint because of limitations set by the intrinsic optical properties of the sensors. This is problematic because intracellular communication pathways interact in complex ways in time and space. For example, calcium and cAMP signals frequently display oscillatory temporal dynamics and tight spatial compartmentalization that can influence one another. Thus, better tools are needed to permit quantitative measurement of multiple signaling pathways in living cells and tissues and enable studies of complex cellular processes, including how cells integrate information from competing signals and how progressive diseases affect a cell's ability to process information. Here, we describe a novel FRET-biosensor strategy that is optimized for multiparameter measurements in living

cells, both cultured and in vivo, using homotransfer fluorescence anisotropy reporters (FLAREs). The FLARE sensors are generally constructed from existing, validated heterotransfer sensors, but include FRET pairs of the same color that permit FRET detection using fluorescence polarization microscopy. Single-color FLARE biosensors for calcium, cAMP, voltage, myosin kinase, and myosin phosphorylation have been constructed in multiple colors, enabling measurement of up to three intracellular signals in a single preparation. The general principle of the method will be discussed, as well as evidence supporting the utility of this technique for in vivo imaging in living organisms. In addition, we present evidence that inducing progressive endoplasmic reticulum (ER) stress in excitable cells leads to ER calcium retention and reduced cytoplasmic calcium. This cripples cell–cell communication by decoupling GPCR signaling from activation of ER calcium channels through calcium-induced calcium release.

54. Progressive Aortic Valve Stenosis in the Mouse with a Gain-of-Function Mutation in $Ca_V1.2$ (*CACNA1C*). MAIKO MATSUI,[1] KUSHAL KADAKIA,[2] ERIC WEI,[2] DANIEL SINDEN,[1] and GEOFFREY PITT,[1] *[1]Weill Cornell Medical College, Cornell University, New York, NY 10065; [2]Duke University Medical Center, Durham, NC 27710*

Calcific aortic valve stenosis (CAVS) is a life-threatening disorder affecting ~2% of people >65 years. Previously thought to be due to "wear and tear," studies show that osteoblast-like and osteoclast-like cells are found in human CAVS and osteogenic pathways such as BMP and Wnt signaling pathways are activated in calcific valves. Importantly, genome-wide association studies recently identified *CACNA1C*, encoding the α subunit of voltage-dependent L-type calcium channel $Ca_V1.2$, as a new CAVS susceptibility gene, and expression quantitative trait loci (eQTL) mapping suggested that increased Ca^{2+} influx through the channel drove the phenotype. Beyond this genetic association, little is known about roles for $Ca_V1.2$ in the aortic valve and how increased Ca^{2+} influx through $Ca_V1.2$ contributes to CAVS.

In this study, we exploited a *Cacna1c* reporter line to define expression patterns in valves and found *Cacna1c* is expressed primarily in the annulus of adult aortic valves.

We generated two mouse lines. For both mouse models, we evaluated valves by histology to assess CAVS and examined transcriptional responses by qPCR.

The first mouse line, A G406R knock-in mutation in *Cacna1c*, which decreases channel inactivation and leads to Ca^{2+} influx, displayed chondrocyte-like transformation of cells in the attachment of the cusps to the annulus. These areas were markedly thickened compared with wild-type littermate controls and showed evidence of accelerated calcification. In the second mouse line, we activated a G406R mutant *Cacna1c* transgene specifically in valve interstitial cells (VICs) with a Cre recombinase driven by the transcription factor *Scleraxis* (*Scx*). Ectopic activation of the G406R mutant transgene in VICs by *Scx-Cre* resulted in marked thickening of the valve cusps. Our results indicate that increased Ca^{2+} influx through $Ca_V1.2$ in the aortic valve leads to thickened valves and calcification. Ectopic expression of the mutant $Ca_V1.2$ in valve cells demonstrates that the role of $Ca_V1.2$ is cell autonomous for the development of CAVs.

55. Molecular Mechanisms of K_{ATP} Channel Mutations in Cantu Syndrome. CONOR MCCLENAGHAN,[1,2] MONICA SALA-RABINAL,[1,2] PAIGE COOPER,[1,2] RISHA SHAH,[1,2] HAIXIA ZHANG,[1,2] THERESA HARTER,[1,2] CHRIS EMFINGER,[1,2] BLANCHE SCHWAPPACH,[3] MARIA REMEDI,[2,4] and COLIN NICHOLS,[1,2] *[1]Department of Cell Biology and Physiology, and [2]The Centre for the Investigation of Membrane Excitability and Disease, Washington University School of Medicine, St. Louis, MO 63130*; *[3]Department of Molecular Biology, University Medicine Göttingen, Germany*; *[4]Division of Endocrinology, Metabolism and Lipid Research, Washington University School of Medicine, St. Louis, MO 63130*

Cantu syndrome (CS) is a rare condition characterized by craniofacial dysmorphology, hypertrichosis, osteochondrodysplasia, and various cardiovascular symptoms including cardiomegaly (Cantú et al. 1982. *Hum. Genet.* 60:36–41). ATP-sensitive potassium channels (K_{ATP} channels) are formed as hetero-octameric complexes of Kir6.x and SUR subunits. The recent identification of gain of function (GoF) mutations in *ABCC9* (SUR2) and *KCNJ8* (Kir6.1) in numerous CS patients implicates K_{ATP} channels in the syndrome (Harakalova et al. 2012. *Nat. Genet.* 44:793–796; Cooper et al. 2014. *Hum. Mutat.* 35:809–813). How these mutations affect channel function and how this relates to the pathophysiology of CS is the subject of current investigation. Here, we demonstrate that two hitherto uncharacterized mutations, R1150W (R1154W in hSUR2) and S1050Y (S1054Y in hSUR2), result in increased currents in the presence of Mg-nucleotides yet have no effect on ATP inhibition in the absence of magnesium, demonstrating that these mutations augment Mg-nucleotide activation. Interestingly, the effect of the R1150Q mutation on nucleotide regulation appears more subtle. This mutation occurs close to an exon/intron boundary, and characterization of the recently developed CS mouse SUR2[R1150Q] indicates that the mutation results in alternative splicing of SUR2A and subsequent expression of a transcript encoding for a nonfunctional subunit (R1150Q-Δexon 30). Further investigation suggests that decreased SUR2 protein expression may be compensated by increased SUR1 in ventricular myocytes, which would contribute to K_{ATP} GoF due to the increased Mg-nucleotide sensitivity of the SUR1 subunit. These preliminary results point toward diverse molecular

mechanisms of exonic SUR2 mutations in CS, including modulation of splicing in addition to direct alteration in nucleotide sensitivity. The strategy to further delineate the effects of K_{ATP} channel mutations in CS, including biophysical characterization and transgenic mouse studies, is discussed.

56. Mechanistic Analysis of SNP effects on human BK Channel Properties. BETH A. MCNALLY, AMBER E. PLANTE, and ANDREA L. MEREDITH, *University of Maryland School of Medicine, Baltimore, MD 21201*

The human large conductance calcium-activated potassium channel (BK) is encoded by a single gene (KCNMA1), and we recently found six single nucleotide polymorphisms (SNPs) in the KCNMA1 coding region that alter BK channel properties under standard testing conditions (0, 1, 10, and 100 μM Ca). Here, we selected three of these SNPs (A138V, C495G, and R800W) and further probed potential mechanisms of their altered BK channel properties. Since an autism-linked SNP (A138V) is in close proximity to the BK channel Mg^{2+} coordination site, specifically residue D164 (D99 in mbr5), we examined Mg^{2+} gating. We found that physiological Mg^{2+} concentrations (1 and 3 mM) resulted in a leftward G-V shift for both A138V and WT. A138V was significantly right shifted at 1 mM Mg^{2+}/10 μM Ca^{2+} ($P < 0.05$) when compared with WT. Second, we examined whether the C495G SNP alters the redox/oxidation of the channel, since this residue (C430 in hSlo1) was previously shown to contribute to the rightward shifting effects of cysteine oxidation on BK channel properties. Next, we examined whether the rightward shifting effect of the R800W SNP was due to size or charge by testing R800A, R800E, and R800Q mutations, respectively. Lastly, we used the human gain-of-function mutation, D434G, which increases BK currents by altering the coupling of Ca^{2+} binding and channel opening to assess the distinct mechanistic effect of C495G and R800W on channel properties. The D434G leftward shift was not altered in the presence of C495G, whereas the R800W rightward shift persisted in the presence of D434G, suggesting that R800W and D434G can exert distinct and opposite effects on channel properties. Together, these results begin to reveal the gating mechanisms acted upon by human SNP variation and suggest a disease-linked mutation could be mitigated by SNP variation at distal residues.

57. Rhythmic Regulation of BK Splice Variants in the Circadian Clock. **ANDREA MEREDITH**, *University of Maryland School of Medicine, Baltimore, MD 21201*

BK Ca^{2+}-activated K^+ currents exhibit diverse properties across tissues. The functional variation in voltage- and Ca^{2+}-dependent gating underlying this diversity arises from multiple mechanisms, including alternate splicing of *Kcnma1*, the gene encoding the pore-forming (α) subunit of the BK channel, phosphorylation of α subunits, and inclusion of β subunits in channel complexes. One physiologically-integrated system for studying the molecular basis of BK current diversity is found in the brain's central clock, the suprachiasmatic nucleus (SCN) of the hypothalamus. We identified a novel role for BK channels in the daily patterning of neuronal activity in the SCN, correlated with a day versus night difference in BK macroscopic current levels, but the channel-based mechanisms that produce rhythmic action potential activity are not fully understood. In the SCN, alternative splicing is under circadian control, and the abundance of two *Kcnma1* splice variants (BK_{SRKR} and BK_0) differs between day and night. Currents recorded from "day" BK_{SRKR} channels in HEK293 cells had a significantly right-shifted current-voltage relationship across a range of Ca^{2+} concentrations, slower activation, and faster deactivation, compared with "night" BK_0 channels. These effects were dependent upon phosphorylation of S642, a constitutive serine immediately preceding the SRKR insert, and were abolished by alkaline phosphatase or mutation of S642. To test this mechanism in daytime SCN neurons, alkaline phosphatase was applied intracellularly, resulting in increased BK current in response to both step- and action-potential commands. The change in BK current was correlated with a decrease in native spontaneous action potential firing rate. This work identifies alternative splicing of BK channels as a genetic mechanism that may contribute to the dynamic regulation of excitability in the circadian pacemaker.

58. Defining New Mechanisms Underlying Cardiovascular Disease. **PETER J. MOHLER**, *Davis Heart and Lung Research Institute, The Ohio State University Medical Center, Columbus, OH 43210*

Our research is focused on the mechanisms underlying the targeting and regulation of membrane-associated (ion channels, transporters, and receptors) and signaling proteins in cardiac and other excitable cells. In particular, we are interested in the role of membrane-associated ankyrin and spectrin family of polypeptides in the targeting and function of ion channels and transporters as well as kinases and phosphatases. A primary focus of the laboratory is the role of the ankyrin-G–based pathway for targeting voltage-gated sodium channels to the intercalated disc of cardiomyocytes. We have discovered a direct requirement of ankyrin-G for Na channel targeting and have linked human Na channel arrhythmia mutations with loss of ankyrin-G binding, and Na channel targeting resulting in defects in Na channel function and myocyte excitability. A second line of work in the laboratory establishes that loss-of-function mutation in ankyrin-B is the basis for a human cardiac arrhythmia syndrome associated with sinus node dysfunction, repolarization defects, and polymorphic

tachyarrhythmia in response to stress and/or exercise ("ankyrin-B syndrome"). Additionally, our work revealed that reduction of ankyrin-B in mice results in reduced levels and abnormal localization of Na/Ca exchanger, Na/K ATPase, and InsP3 receptor at T-tubule/SR sites in cardiomyocytes and leads to altered Ca^{2+} signaling and extrasystoles that provide a rationale for the arrhythmia. These studies establish a physiological requirement for ankyrins and spectrins in localization of a variety of ion channels in excitable membranes in the heart and demonstrate a new class of functional "channelopathies" due to abnormal cellular localization of functionally related ion channels and transporters. More recently, we have developed a third line of research in the laboratory focused on the molecular mechanisms underlying kinase and phosphatase targeting in excitable cardiomyocytes. Specifically, work from our laboratory has shown the importance of CaMKII and PP2A targeting for myocyte and cardiac function.

59. Potentiation of Excitatory Synaptic Transmission in the Superficial Dorsal Horn by Low Concentrations of Kv Channel Inhibitors. TANZIYAH MUQEEM,[1] VITOR PINTO,[2] and MANUEL COVARRUBIAS,[1] *[1]Department of Neuroscience and Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, PA 19107; [2]ICVS/3Bs, University of Minho, Braga, Portugal*

Kv3.4 channels underlie a majority of the high-voltage activating K^+ current in dorsal root ganglion (DRG) nociceptors and are expressed in all functional compartments of these neurons, including synaptic terminals. Kv3.4 channels in DRG neurons regulate the rate of action potential repolarization in a manner that depends on phosphorylation of the channels' inactivation domain. Additionally, Kv3.4 channel down-regulation is implicated in the pathophysiology of chronic pain induced by spinal cord injury. We hypothesize that, through their ability to facilitate action potential repolarization, Kv3.4 channels help keep nociceptive synaptic transmission in check. Kv3.4 channel down-regulation would, therefore, promote pain transduction. To test this hypothesis, we have investigated excitatory synaptic transmission in the superficial dorsal horn under conditions that inhibit the Kv3.4 current in DRG nerve terminals. We used an ex vivo rat cervical spinal cord preparation suitable for whole-cell patch clamping of secondary neurons in the dorsal horn. Since Kv3.4 channels are hypersensitive to submillimolar concentrations of 4-aminopyridine (4-AP) and tetraethylammonium (TEA), we tested their effects on excitatory post-synaptic currents (EPSCs) in laminae I and II of the dorsal horn. We found that 500 µM TEA potentiates the EPSCs by 53% ($P < 0.001$, paired t test, $n = 3$). Similarly, 50 µM 4-AP potentiates the EPSCs by 59% ($P < 0.001$, paired t test, $n = 3$). We conclude that the Kv3.4 channel expressed in nociceptors is a plausible regulator of nociceptive synaptic transmission in the spinal cord. Currently, we are testing additional inhibitors to evaluate possible contributions of other K^+ channels and exploring more specific knockdown strategies to assess the role of the Kv3.4 channel as a regulator of nociception in vivo.

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60. Potassium Channelopathy-Like Defect Underlies Early-Stage Cerebrovascular Dysfunction in a Genetic Model of Small Vessel Disease. **MARK NELSON**, *University of Vermont, Burlington, VT 05405*

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), caused by dominant mutations in the Notch3 receptor in vascular smooth muscle, is a genetic paradigm of small vessel disease (SVD) of the brain. Recent studies using transgenic (Tg)Notch3R169C mice, a genetic model of CADASIL, revealed functional defects in cerebral (pial) arteries on the surface of the brain at an early stage of disease progression. Here, using parenchymal arterioles (PAs) from within the brain, we determined the molecular mechanism underlying the early functional deficits associated with this Notch3 mutation. At physiological pressure (40 mmHg), smooth muscle membrane potential depolarization, and constriction to pressure (myogenic tone) were blunted in PAs from TgNotch3R169C mice. This effect was associated with an ~60% increase in the number of voltage-gated potassium ($K_V1.5$) channels, which oppose pressure-induced depolarization. Inhibition of K_V1 channels with 4-aminopyridine or treatment with the epidermal growth factor receptor agonist heparin-binding EGF (HB-EGF), which promotes K_V1 channel endocytosis, reduced K_V current density and restored myogenic responses in PAs from TgNotch3R169C mice, whereas pharmacological inhibition of other major vasodilatory influences had no effect. K_V1 currents and myogenic responses were similarly altered in pial arteries from TgNotch3R169C mice, but not in mesenteric arteries. Interestingly, HB-EGF had no effect on mesenteric arteries, suggesting a possible mechanistic basis for the exclusive cerebrovascular manifestation of CADASIL. The metalloproteinase inhibitor, TIMP3, accumulates in the extracellular matrix of PAs from the CADASIL mouse model and in human CADASIL patients. Genetic overexpression of TIMP3 mimicked the effects of CADASIL on K_V current density and the loss of myogenic tone. Collectively, our results indicate that increasing the number of K_V1 channels in cerebral smooth muscle produces a mutant vascular phenotype akin to a channelopathy in a genetic model of SVD.

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61. Cantu Syndrome: Multi-Organ Complexities from KATP Gain-of-Function. **COLIN G. NICHOLS**,[1] HAIXIA ZHANG,[1] PAIGE E. COOPER,[1] JIN-MOO LEE,[1] ELAINE L. SHELTON,[2] MICHAEL J. DAVIS,[3] MARK A. LEVIN,[1] MARIA S. REMEDI,[1] GAUTAM K. SINGH,[1] and DOROTHY K. GRANGE,[1] *[1]Washington University School of Medicine, St. Louis, MO 63110; [2]Vanderbilt University School of Medicine, Nashville, TN 37232; [3]University of Missouri School of medicine, Columbia, MO 65212*

Cantu syndrome (CS, MIM 239850) is a complex congenital syndrome with multiple cardiovascular features. Hypertrichosis is a constant feature, with coarse facial features, generalized macrosomia, and macrocephaly typically presenting at birth. Cardiovascular features include enlarged and hypercontractile hearts, hypertension, pericardial effusion, persistent patent ductus arteriosus (PDA) and fetal brain circulation, and lymphedema. In the last three years, we have demonstrated that GOF mutations in the genes encoding both the regulatory (*ABCC9*) and pore-forming (*KCNJ8*) subunits of K_{ATP} channels underlie the disease in the majority of patients, but such findings have not elucidated the mechanisms underlying the myriad syndrome features. Our development of relevant transgenic animal models and a unique research CS clinic have permitted extensive characterization of disease phenotype and have led us to unique insights to the underlying pathology of cardiac, vascular, and lymphatic features.

62.* Precision Physiology and Rescue of Ion Channel Disorders: The Gate is Now Wide Open. **JEFFREY NOEBELS**, *Baylor College of Medicine, Houston, TX 77030*

A steady drumroll of genetic discoveries linking ion channel mutations to human disease highlights the urgent need for a deeper understanding of how altered biophysics and cell biology of channel function can be therapeutically reversed. Ion channel subunits now comprise the single largest gene family underlying disorders of heart, muscle, and brain, and the most frequently tested for precision clinical diagnosis of a broad phenotypic spectrum of cognitive disability, neuropsychiatry, epilepsy, sensorimotor dysfunction, and neurodegenerative disease. These disorders collectively constitute an enormous public health burden, with a greater number of life years diminished or lost than cancer. The significance of each variant, which in some cases spells the difference between lifelong disability or sudden death, requires accurate functional interpretation in order to stimulate drug discovery and guide the use of mutation-specific therapies.

What is missing from this brave new agenda? There is a profound lack of functional information regarding the majority of roughly 400 genes and their myriad splice forms, as well as the unexpected complexity of their coordinate regulation and interaction within cells. This deficit poses a major obstacle to successful therapeutic translation of precision diagnostic testing. The results of clinical exomes point to recurring mysteries that are impossible to ignore: seemingly slight or no changes in gating kinetics may lead to devastating disease, implicating the involvement of unknown nonpore functions. Shared subunit expression in heart and brain may lead to either unpredictable patterns of compensatory functional sparing or devastating patterns of selective vulnerability.

The arrival of genetic testing has permanently altered the ion channel basic research mandate. Focused studies of the functional biology of human channel mutations are essential to the success of precision medicine. New basic/clinical collaborative approaches are required to uncover, validate, and model meaning, either singly or in complex combinations. Fortunately, rather than distracting from the fundamental goal of defining canonical protein function, a focus on the cell and clinical biology of mutant channels accelerates discovery of unsuspected aspects of channel gating, interaction domains, location, remodeling, and epistasis. At higher levels of network organization, mutations provide insight into activity-driven wiring of developing brain circuitry. Finally, genetic editing strategies are already beginning to repair point mutations of ion channels in model systems to confirm and explore the disease mechanism. The rich biology and powerful approaches explain why ion channel mutations represent some of the most intriguing and medically essential molecular lesions to understand and treat.

63. Targeting Sodium Channel Slow Inactivation as a Novel Therapeutic Approach in Myotonia Congenita. KEVIN NOVAK,[1] WILLIAM ARNOLD,[2] ALAN SANDERSON,[2] JOHN KISSEL,[2] and MARK RICH,[1] *[1]Wright State University, Dayton, OH 45435; [2]The Ohio State University, Columbus, OH 43210*

Patients with myotonia congenita have muscle hyperexcitability due to loss-of-function mutations in the chloride channel in skeletal muscle, which causes spontaneous firing of muscle action potentials (myotonia), producing muscle stiffness. In patients, muscle stiffness lessens with exercise, a change known as the warmup phenomenon. Our goal was to identify the mechanism underlying warmup and to use this information to guide development of novel therapy. Exercise was mimicked in vitro in individual muscle fibers by stimulating 5,000 muscle action potentials at a rate of 20 Hz. Action potential morphology was assessed before and after the repeated stimulation. Significant decreases in action potential height and rate of rise (dV/dt) suggested increased sodium channel slow inactivation contributes to warm up. These data suggested that enhancing slow inactivation of sodium channels might offer effective therapy for myotonia. Lacosamide and ranolazine enhance

slow inactivation of sodium channels and are approved by the US Food and Drug Administration for other uses in patients. We compared the efficacy of both drugs with mexiletine, a sodium channel blocker currently used to treat myotonia. In vitro studies suggested that both lacosamide and ranolazine were superior to mexiletine. However, in vivo studies in a mouse model of myotonia congenita suggested that side effects could limit the efficacy of lacosamide. Ranolazine produced fewer side effects and was as effective as mexiletine at a dose that produced none of mexiletine's hypoexcitability side effects. Based on our preclinical studies, we performed an open label, proof of concept trial in patients with myotonia congenita to assess the tolerability and efficacy of ranolazine. Participants demonstrated less self-reported stiffness, improved motor performance, and had reduced electromyographic myotonia. No participant discontinued ranolazine due to side effects. These results suggest ranolazine is well tolerated at doses that significantly improve motor symptoms and lessen myotonia and support a future controlled trial.

64. $Na_v1.1$ in Aδ Fibers Mediates Mechanical Pain Signaling: Relevance for Migraine Pathogenesis. JEREMIAH D. OSTEEN,[1] JOHN GILCHRIST,[2] FRANK BOSMANS,[2] and DAVID JULIUS,[1] *[1]Department of Physiology, University of California, San Francisco, San Francisco, CA 94143; [2]Department of Physiology and Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205*

Migraine headache is a common, debilitating condition characterized by recurrent attacks of headache pain, sensory sensitivity and nausea. These attacks are sometimes preceded by aura, a sensory disturbance resulting from cortical spreading depression in the brain. Subsequent migraine pain and photophobia arise from sensitization of trigeminal primary afferents, including release of pro-inflammatory peptides. A third hallmark is vasodilation of the meningeal vasculature, which increases pressure on trigeminal afferents. The etiology of migraine has been a subject of debate, since the separation of cause and effect between brain, trigeminal, and vascular changes is difficult to distinguish. Recently, the $Na_v1.1$ subtype of voltage-gated sodium channel has been associated with an inherited form of migraine (familial hemiplegic migraine type 3—FHM3). $Na_v1.1$ is widely expressed in the brain, and migraine mutations are currently thought to act through a CNS-initiated mechanism. Here, we describe a spider toxin, Hm1a, which selectively activates $Na_v1.1$. Following injection of Hm1a into the hind paw of a mouse, we see acute pain behaviors as well as sensitization to mechanical, but not thermal stimuli, including evidence of robust central sensitization. We find that $Na_v1.1$ resides in primary afferent sensory neurons, including myelinated Aδ fibers, but not within the most well-studied nociceptor class, the unmyelinated c fibers. Interestingly, we find that the main effects of Hm1a on $Na_v1.1$ mirror the effects of several studied FHM3 mutations: Hm1a shifts steady-state inactivation to more depolarized potentials and inhibits slow inactivation. In addition, Hm1a targets the $Na_v1.1$ domain IV voltage sensor, the same location around which many FHM3 mutations cluster. Taken together, our results demonstrate a peripheral role for $Na_v1.1$ in the development of mechanical pain and implicate hyperexcitability of $Na_v1.1$-expressing Aδ fibers in FHM3 pathogenesis. Based on these data, Hm1a can now be used to further probe migraine both centrally and peripherally, thereby helping to untangle migraine etiology.

65. Cysteine Mutagenesis Reveals TMC1 Residues that Contribute to Mechanotransduction. BIFENG PAN,[1] XIAO-PING LIU,[1] YUKAKO ASAI,[1] KIYOTO KURIMA,[2] ANDREW J. GRIFFITH,[2] and JEFFREY R. HOLT,[1] *[1]Department of Otolaryngology, F.M. Kirby Neurobiology Center, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115; [2]Molecular Biology and Genetics Section, National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, MD 20892*

TMC1 and TMC2 have emerged as strong candidates for the hair cell transduction channel. A growing body of evidence supports a direct role for these molecules as necessary components of the channel. *Tmc1/Tmc2* mRNAs are expressed in auditory and vestibular hair cells at the right time during development. TMC1 and TMC2 proteins are localized to the tips of shorter row stereocilia. The proteins interact with other known components of the hair cell transduction complex including PCDH15 and LHFPL5. Deletion of *Tmc1* and *Tmc2* results in complete loss of conventional hair cell transduction, and reintroduction of either *Tmc1* or *Tmc2* cDNA leads to restoration of mechanotransduction. Hair cells expressing the *Beethoven* point mutation in *Tmc1* have lower single-channel conductance, lower calcium permeability, and lower sensitivity to block by dihydrostreptomycin than cells expressing wild-type *Tmc1*. These data are consistent with the hypothesis that TMC1 and TMC2 can function as pore-forming subunits of the transduction channel. However, the pore region within the TMC1 topology has not been definitively identified.

To investigate structure–function relationships in TMC1 we generated AAV vectors that encoded mutant TMC1 sequences, introduced at several strategic sites within the TMC1 amino acid sequence. The vectors were injected via the round window membrane into the cochleas and vestibular organs of *Tmc1/Tmc2* double knockout mice. We recorded conventional mechanotransduction from AAV-transduced auditory and vestibular hair cells and assayed for changes in hair cell transduction currents and selectivity. The data provide further evidence supporting a direct role for TMC1 in hair cell transduction and suggest a revised topology.

66. G Protein–Coupled Receptor (GPCR) Signaling Underlies the Nicotine-Induced Up-Regulation of alpha 7 (α7) Nicotinic Acetylcholine Receptors (nAChRs) Expressed in *Xenopus* Oocytes. JAYHARSH PANCHAL, KRISTI DEBOEUF, MOHAMMAD FARIDUL ISLAM, and JOSEPH FARLEY, *Neuroscience, Indiana University, Bloomington, IN 47405*

α7 nAChRs are widely distributed throughout the nervous system, playing important roles in learning, memory, several disease and neurodegenerative processes, and nicotine addiction. A variety of agonists and antagonists produce functional and/or numerical up-regulation of α7 Rs in different cells, implicating multiple signaling pathways/mechanisms. Prolonged nicotine exposure can also up-regulate α7 nAChRs, which may contribute to nicotine addiction. We found approximately twofold up-regulation of murine α7 nAChRs in *Xenopus* oocytes following 12 h of 100 µM nicotine and extensive washout. Nicotine up-regulation was dependent upon intracellular Ca^{2+}, being abolished by BAPTA-AM, and involved several Ca^{2+}-dependent enzymes (e.g., PP2B and PKC). However, up-regulation was independent of Ca^{2+} influx, being unaffected by removal of extracellular Ca^{2+}. The M3-M4 loop of α7 contains a conserved G protein–binding cluster (GPBC). G protein signaling by α7 Rs has previously been shown in neurons and PC12 cells (Kabbani et al. 2013. *BioEssays.* 25:1025–1034). Here, we show that GPCR signaling mediates nicotine up-regulation of α7 nAChR. We observed that a substance P-analogue peptide (a putative specific inhibitor of G-α-q/11 binding to GPCRs) prevented nicotine-induced up-regulation of α7 Rs. However, the substance P-analogue also significantly reduced control α7 R currents, suggesting that its effects were nonspecific. Therefore, we mutated the α7 nAChR GPBC (RMKR to AAAA; denoted as α7 344-347A) to block interaction of G-α-q with the GPBC. Receptor expression levels, peak current amplitude, and kinetics were equivalent for mutant and wild-type (wt) α7 Rs. But nicotine-up-regulation of α7 344-347A R was completely inhibited. In contrast, prolonged exposure to the cell-permeable, competitive antagonist methyllycaconitine (MLA), produced approximately twofold up-regulation of both wt and mutant α7 Rs, which were unaffected by BAPTA-AM. MLA up-regulation of mutant and wt α7 Rs may result from a chaperone-like mechanism (Lester et al. 2009. *APPS J.* 11:167–177). GPCR signaling of α7 Rs is critical for their nicotine-up-regulation.

67. TRPC Channel Remodeling in a Mouse Model of Essential Hypertension: The TRPC3-TRPC6 Game of Thrones. M. TERESA PEREZ-GARCIA, INES ALVAREZ-MIGUEL, PILAR CIDAD, and JOSE R. LOPEZ-LOPEZ, *Department of Biochemistry and Physiology and Institute of Biology and Molecular Genetics (IBGM), School of Medicine, Universidad de Valladolid, Spain*

Essential hypertension involves a gradual, sustained rise in total peripheral resistance caused by an increased vascular tone. A model has been proposed in which the combination of membrane depolarization and higher L-type Ca^{2+} channel activity conspires to generate augmented Ca^{2+} influx into vascular smooth muscle cells (VSMCs), contraction and vasoconstriction. The search for culprit ion channels that can drive membrane depolarization has provided several candidates, including some members of the TRPC family. TRPC3 and TRPC6 are DAG-activated, nonselective cationic channels contributing to stretch- or agonist-induced depolarization. Conflicting information regarding changes in TRPC3 or TRPC6 functional expression associated to hypertension has been reported. However, although TRPC3-TRPC6 channels can form homo- or heteromultimers, the possibility that differences in their association pattern may change their functional contribution to vascular tone is largely unexplored.

We probe this hypothesis using a model of essential hypertension (BPH mice) and its normotensive control (BPN mice). We characterize homo- and heterotetramers with electrophysiological recordings in TRPC3-, TRPC6-, or TRPC3/6-transfected CHO cells. Nonselective basal or hypotonic stimulus-activated cationic currents were elicited with all the studied combinations. However, TRPC currents were sensitive to the selective antagonist Pyr10 only when TRPC6 was present. Meanwhile, intracellular anti-TRPC3 antibody selectively blocked TRPC3-mediated currents.

In isolated mesenteric VSMCs, basal and agonist-induced currents were more sensitive to Pyr3 and Pyr10 in BPN cells. Consistently, myography studies in pressurized mesenteric arteries showed a larger Pyr3/10-induced vasodilation in BPN arteries. mRNA and protein expression data (explored with qPCR and proximity ligation assay) support changes in TRPC3 and TRPC6 proportion and assembly, with a higher TRPC3 channel contribution in BPH VSMCs which could favor cell depolarization in the hypertensive phenotype. These differences in functional and pharmacological properties of TRPC3 and TRPC6 channels depending on their assembly as homo- or heterotetramers could represent novel therapeutical opportunities.

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68. The KLHL1 KO as a Model of Altered T-Type Ca Channel Function. PAULA P. PERISSINOTTI,[1] YUNGUI HE,[2] ELIZABETH MARTÍNEZ-HERNÁNDEZ,[1] ELIZABETH A. ETHINGTON,[1] ERIC ALMAZÁN,[1] ALISSA ZEGLIN,[1,2] MICHAEL D. KOOB,[3] and **ERIKA S. PIEDRAS-RENTERÍA**,[1,4] *[1]Cell and Molecular Physiology Department and [2]Stritch School of Medicine, Loyola University Chicago, Chicago, IL 60660; [3]Institute for*

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The physiological consequences of the global deletion of the Kelch-like 1 protein (KLHL1) in mice will be discussed. KLHL1 is a neuronal actin-binding protein that modulates $Ca_V2.1$ P/Q-type and $Ca_V3.2$ T-type channel activity. Decreased expression and function of these two channel types is seen when KLHL1 is acutely down-regulated using specific shRNA; however, the KLHL1 KO mouse displays tissue-specific Ca current homeostasis that can result in normal, decreased, or increased Ca currents. Altered Ca channel function and Ca current levels, neuronal excitability changes, and synaptic function are detected in this model.

Here, we will discuss our most recent findings on the KLHL1 KO mouse as a model to assess altered T-type channel expression. The hypothalamus from KO mice exhibits slightly decreased $Ca_V3.2$ expression as expected for this model, however $Ca_V3.1$ levels are highly up-regulated, resulting in increased LVA channel activity, increased excitability of pro-opiomelanocortin–positive neurons, and T type channel-mediated resistance to the satiety hormone leptin, as well as abnormal responses after a 20-h period of food deprivation. In contrast, KO dorsal root ganglia display decreased LVA $Ca_V3.2$ expression without additional compensations, resulting in decreased LVA currents, decreased DRG excitability, and increased threshold to mechanical pain. In summary, the KLHL1 KO mouse model is an excellent system to explore how moderate Ca channel function changes affect neuronal tissues. Our results also establish T-type channels as possible targets for obesity treatment.

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69. The Role of $Ca_V1.2$ L-Type Ca^{2+} Channels in Physiology and Disease. **GEOFFREY PITT**, *Weill Cornell Medicine, New York, NY 10065*

Calcium channels modulate the final signal transduction step in excitable cells such as neurons, hormone-secreting cells, and cardiac myocytes and thus are central for their function. The $Ca_V1.2$ L-type Ca^{2+} channel encoded by *Cacna1c* is subject to temporally regulated and tissue-specific alternative splicing. We have generated several new models including gain-of-function and loss-of-function $Ca_V1.2$ mouse models, some of which affect only one of two alternatively spliced and mutually exclusive exons. These various models display tissue-specific phenotypes that reflect the alternative splicing. The phenotypes include various behavioral abnormalities that mimic certain neuropsychiatric diseases, cardiac arrhythmias, developmental abnormalities, and disorders of hormone regulation. Not only do they reveal novel and unexpected roles for $Ca_V1.2$ in physiology and disease, but they also uncover exon-specific functions that demonstrate the complex regulation of channel splicing and the exon-specific contributions to disease.

70. Single Nucleotide Polymorphisms (SNPs) Alter Current from Human BK Channels. AMBER E. PLANTE, BETH A. MCNALLY, MICHAEL H. LAI, and ANDREA L. MEREDITH, *University of Maryland School of Medicine, Baltimore, MD 21201*

One mechanism with the potential to alter channel properties is natural genetic variation via nonsynonymous single nucleotide polymorphisms (SNPs). We identified six SNPs (A138V, C495G, N599D, R800W, R640Q, and R645Q) in the large-conductance, Ca^{2+}-activated potassium (BK) channel gene (*Kcnma1*) predicted to alter channel structure and function. To determine whether these SNPs alter BK currents, we introduced each SNP into a human muscle-type (BK_{QEERL}) and a brain-type (BK_{VYR}) BK splice variant. Functional properties were investigated in HEK293T cells using standard voltage-clamp protocols. C495G and R800W affected the conductance-voltage relationship across multiple Ca^{2+} conditions in both BK_{QEERL} and BK_{VYR}. C495G and R800W consistently produced leftward and rightward shifts in the $V_{1/2}$ values, respectively, that were similar across both splice variant backgrounds. Expressing C495G/R800W in parallel produced a right-shifted effect similar to R800W, but unlike R800W alone, this rightward shift was reduced at 10 µM Ca^{2+}. In contrast to C495G and R800W, two additional SNPs, A138V (an autism-linked SNP) and N599D, had variable effects on current properties. A138V and N599D produced rightward shifts in the $V_{1/2}$ in 0 Ca^{2+}, but N599D also produced a leftward shift at 10 µM Ca^{2+}. Next, to test whether SNP effects persist with posttranslational modifications, we treated patches with alkaline phosphatase, which resulted in increased BK channel activity attributed to dephosphorylation of Serine 642 in these conditions. We found the leftward shift due to C495G and rightward shift due to R800W persisted in dephosphorylating conditions. A combination of two SNPs (R640Q/R645Q) predicted to disrupt phosphorylation of Serine 642 produced partial effects on $V_{1/2}$ values at intermediate Ca^{2+} concentrations. These results implicate naturally occurring human genetic variation as a potential modulator of BK channel properties across splice variant backgrounds. Furthermore, SNPs exert effects on the conductance-voltage relationship of the channel that are additive with each other and with posttranslational modifications.

71. Role of Chloride Intracellular Channels (CLICs) Proteins in Maintaining Cardiac Mitochondrial Physiology. DEVASENA PONNALAGU, SHUBHA GURURAJA RAO, AHMED TAFSIRUL HUSSAIN, and HARPREET SINGH, *Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA 19102*

Chloride (Cl) intracellular channels (CLICs) are a unique class of ion channel proteins existing in both soluble and integral membrane form. CLICs are widely conserved in prokaryotes as well as eukaryotes. Six paralogues of CLICs (CLIC1-CLIC6) are known in mammals. They are multifunctional proteins maintaining renal physiology, apoptosis, and tumorigenesis. In addition, using pharmacological inhibitor (IAA-94), CLICs have been implicated in ablation of myocardial infarction (MI) due to ischemia-reperfusion (IR) injury, but the molecular identity and mechanism of cardioprotection was not elucidated. This intrigued us toward understanding the significance of each CLIC in maintaining cardiac physiology and their respective role in cardioprotection from IR injury. In this study, we show that CLIC1, CLIC4, and CLIC5 are the predominant paralogues present in the cardiac tissue. CLIC4 and CLIC5 localize to the mitochondria of adult cardiomyocytes as well as Percoll-purified mitochondria, but not CLIC1, indicating CLIC4 and CLIC5 as mitochondrial channel proteins. We also observe unique distribution of CLIC4 and CLIC5 in mitochondria as CLIC4 is enriched in outer mitochondrial membrane and CLIC5 in inner mitochondrial membrane (IMM). These results for the first time established the molecular component of IMM Cl channel. Functionally, we have observed that CLIC5 knockout (KO) mice show significant increase in ROS production ($P \leq 0.05$, $n = 3$) as compared with wild-type mice, whereas CLIC1 and CLIC4 mice did not show any significant difference in the ROS production. Interestingly, only CLIC4 KO mice showed a difference ($\sim$10%, $n = 5$) in the cardiac mitochondrial calcium retention capacity as compared with wild-type mice. These results strongly establish CLIC4 and CLIC5 as mitochondrial ion channels and also their significance in maintaining cardiac mitochondrial function. As CLICs are implied to play a role in cardio protection from IR injury, role of CLIC4 and CLIC5 in cardioprotection needs to be further investigated.

72. Regulation of Neurogenesis and Astrocyte Function by Store-Operated Calcium Channels. **MURALI PRAKRIYA**, *Northwestern University, Chicago, IL 60611*

Store-operated calcium channels (SOCs) are a major pathway for calcium signaling in virtually all animal cells and serve a wide variety of functions ranging from gene expression, motility and secretion to tissue and organ development and the immune response. SOCs are activated by the depletion of Ca^{2+} from the endoplasmic reticulum (ER), triggered physiologically through stimulation of a diverse set of metabotropic surface receptors. The identification of the STIM proteins as ER Ca^{2+} sensors and the Orai proteins as store-operated channels 10 yr ago has enabled rapid progress in understanding the mechanisms of SOC function and their physiological roles in various tissues. In this talk, I will discuss our recent work in using genetically modified mice for probing the physiological roles of SOCs in regulating neurogenesis and gliotransmitter release from astrocytes.

73. HIV-Associated Cardiovascular Disease: Role of Connexin43. LISA PREVEDEL,[1,2] CAMILLA MOROCHO,[1] MICHAEL V. BENNETT,[3,*] SUSAN MORGELLO,[4,5] and ELISEO A. EUGENIN,[1,2] *[1]Public Health Research Institute (PHRI), Newark, NJ 07103; [2]Department of Microbiology, Biochemistry, and Molecular Genetics, Rutgers New Jersey Medical School, Rutgers the State University of NJ, Newark, NJ 07103; [3]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461; and [4]Department of Neurology and [5]Departments of Pathology and Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY 10029; *presenting person*

Currently, HIV-infected individuals are living longer due to effective antiretroviral treatment (ART). Despite block of viral replication, several non-AIDS comorbidities, including heart disease, have become a major cause of death in the HIV-infected population. However, the mechanisms involved are poorly described. Here we report changes in the expression and distribution of Connexin43 (Cx43) in vast areas of the heart in correlation with loss of cardiac function using postmortem human heart tissue obtained from HIV-infected and uninfected individuals. Cx43 is the main component of gap junctions in cardiomyocytes at the intercalated disks and mediates propagation of the cardiac action potential. We found that Cx43 expression is increased in HIV infected individuals and its localization is altered, which may contribute to the increased heart disease. Cx43 expression was examined by qRT-PCR and Western blot, and protein distribution was evaluated by immunolabeling and confocal microscopy. Van Kossa staining for calcium showed increased calcium deposition in areas with compromised Cx43 expression and localization in association with altered structure evaluated by electron microscopy. Cx43 expression was up-regulated at both mRNA and protein levels and Cx43 was mislocalized to the lateral membranes of the cardiomyocytes in addition to its normal presence at the intercalated disks. Regions of the tissue with mislocalized Cx43 also exhibited calcium overload, mitochondrial degeneration/proliferation, and compromise of myofibrils. All of these changes were independent of viral replication, CD4 counts, inflammation, and previous or ongoing ART. Our results demonstrate up-regulation of Cx43 in the hearts of HIV individuals. Furthermore, these areas also

have calcium and mitochondrial dysregulation as well as myofibril compromise, despite successful block of HIV replication. We propose that persistent viral DNA expresses HIV-tat protein, which alters Cx43 expression and distribution, leading to chronic heart damage and increased sensitivity to additional stress.

74. Electrical Remodeling in the Sinoatrial Node Associated with Diabetes and Aging. **CATHERINE PROENZA**, *University of Colorado School of Medicine, Aurora, CO 80045*

Cardiac pacemaking is driven by the generation of spontaneous action potentials (APs) by specialized pacemaker myocytes in the sinoatrial node of the heart. Sinoatrial node dysfunction limits cardiac output, $VO_{2\text{-}max}$ and functional independence for older people and for people suffering from diabetes. Development of new treatments for sinoatrial node dysfunction requires understanding of the underlying molecular mechanisms that are altered by aging or disease. To this end, mice are an established model system for both aging and diabetes, the ion channels that control pacemaking are highly conserved in mice and humans, and we have found that aging and diabetes slow maximum and intrinsic heart rates in mice as they do in humans. Slower heart rates in both aged and diabetic mice resulted from slower spontaneous AP firing rates in isolated sinoatrial node myocytes (SAMs). However we have found that aging and diabetes are associated with different changes in AP waveform parameters and membrane currents. Aging slows sinoatrial myocyte AP firing rate mainly via hyperpolarization of the maximum diastolic potential and slowing of the spontaneous diastolic depolarization rate. In contrast, diabetes slows AP firing rate mainly by increasing AP duration. In accordance, we have found that aging is associated with a hyperpolarizing shift in the voltage dependence of the funny current (I_f), whereas diabetes is associated with a reduction in the transient outward current (I_{to}) in sinoatrial myocytes. Thus, I_f and I_{to} represent distinct potential pharmacological targets for treatment of sinoatrial node dysfunction in aging or diabetes, respectively.

75. Intracellular Fibroblast Growth Factor 14 Has Differential Effects on the Intrinsic Excitability of Hippocampal Pyramidal Neurons and Cerebellar Purkinje Neurons. JOSEPH L. RANSDELL,[1] YARIMAR CARRASQUILLO,[2,*] MARIE K. BOSCH,[2] DAVID M. ORNITZ,[2] and JEANNE M. NERBONNE,[1,2] *[1]Department of Medicine and [2]Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO 63110; *Present address: Division of Intramural Research, National Center for Complementary and Integrative Health (NCCIH), Bethesda, MD 20892*

Intracellular fibroblast growth factors (iFGF11–14) are a unique subgroup of FGF proteins that are functionally distinct from canonical FGFs. iFGFs function intracellularly and are known to bind to the pore-forming α (α) subunits of voltage-gated sodium (Nav) channels, although recent reports suggest that iFGFs may interact with additional types of voltage-gated ion channels. iFGF14 is expressed throughout the CNS and is the locus of mutations responsible for spinal cerebellar ataxia 27 (SCA27), an autosomal-dominant disorder characterized by ataxia and cognitive decline. Mice harboring a targeted disruption in the *Fgf14* locus (*Fgf14*$^{-/-}$) recapitulate many of the phenotypes seen in SCA27. Whole-cell recordings from *Fgf14*$^{-/-}$ hippocampal CA1 pyramidal neurons in acute slices revealed that the loss of iFGF14 does not affect evoked firing frequency. Analysis of phase plots, however, revealed a hyperpolarizing shift in the voltage dependence (dV/dt) of the rising phase of the action potential. Voltage-clamp experiments demonstrated that the voltage dependence of activation of the Nav conductance was significantly ($P < 0.01$) hyperpolarized in *Fgf14*$^{-/-}$ pyramidal neurons ($V_{1/2} = -39$ mV), compared with WT ($V_{1/2} = -30$ mV) pyramidal neurons. These results contrast with the effects of iFGF14 in cerebellar Purkinje neurons, in which loss of iFGF14 caused a dramatic reduction in spontaneous firing, and the voltage dependence of steady-state inactivation of the Nav currents was significantly ($P < 0.001$) hyperpolarized ($V_{1/2} = -73$ mV), compared with WT ($V_{1/2} = -55$ mV) Purkinje neurons. Importantly, immunohistochemical experiments revealed that the loss of iFGF14 does not affect Nav α subunit localization at the axon initial segments in either hippocampal CA1 pyramidal or cerebellar Purkinje neurons. Taken together, these results demonstrate that expression of iFGF14 has cell type–specific effects: iFGF14 regulates the voltage dependence of activation of the Nav currents in hippocampal CA1 pyramidal neurons and regulates the voltage dependence of steady-state inactivation of the Nav currents in cerebellar Purkinje neurons.

76. Fast Voltage-Gated Sodium Channel Activity in Huntington's Disease. ERIC J. REED,[1] MARK M. RICH,[2] and ANDREW A. VOSS,[1] *[1]Department of Biological Sciences and [2]Department of Neuroscience, Cell Biology, and Physiology, Wright State University, Dayton, OH 45435*

Huntington's disease is caused by expanded CAG repeats in the *huntingtin* gene and results in cognitive problems as well as muscle weakness, chorea, rigidity, and dystonia. Most of the research on Huntington's disease has focused on neurodegeneration. Recent studies have begun to find peripheral defects that may help explain the debilitating motor symptoms of the disease. For example, we have shown that skeletal muscle from transgenic R6/2 Huntington's disease mice is hyperexcitable because of decreases in the resting chloride and potassium currents (Waters et al. 2013. *Proc. Natl. Acad.*

Sci. 110:9160–9165). Other groups have speculated that the fast sodium currents may also be disrupted in the R6/2 mice. To fully examine membrane excitability, we measured the fast voltage-gated sodium currents in voltage clamped flexor digitorum brevis and interosseous muscle fibers from late-stage R6/2 mice and age-matched siblings. Because of their speed and size, the fast voltage-gated sodium currents are notoriously difficult to measure in mature nerve or muscle. We were able to record the sodium currents by reducing the extracellular [Na^+] to reduce the size of the current and recording from small fibers to minimize space clamp issues. We found little to no change in R6/2 sodium current peak amplitude or voltage-dependent activation and inactivation compared with control. These measurements give us a more complete understanding of the hyperexcitability in Huntington's disease skeletal muscle. Because we did not find any defects R6/2 sodium currents, therapeutics that decrease hyperexcitability by targeting the fast voltage-gated sodium channels may be effective in treating the motor defects of Huntington's disease.

77. Temperature, pH, and Calcium: Arrhythmogenic Triggers in Mixed Long QT3 and Brugada Syndrome.
PETER C. RUBEN, COLIN H. PETERS, and MENA ABDELSAYED, *Department of Biomedical Physiology, Simon Fraser University, Burnaby, BC V5A 1S6, Canada*

Sudden cardiac death (SCD) is a major cause of mortality, afflicting more than a quarter million people annually in the United States. Although there are a number of causes for SCD, inherited genetic mutations account for a substantial proportion of deaths in victims under the age of 40 yr. Rare forms of inheritable cardiac disease underlying SCD include Long-QT3 (LQT3) and Brugada syndromes (BrS1), both of which arise as a consequence of channelopathies caused by mutations in the SCN5a gene that encodes the cardiac voltage-gated sodium channel, NaV1.5. One NaV1.5 mutant, E1784K, is among several that cause a mixed channelopathy, having the characteristics of both LQT3 and BrS1. We studied E1784K channels expressed in *Xenopus* oocytes and mammalian cells and found that several physiological factors, all of which are normal by-products of intense exercise, exacerbate the biophysical defects caused by the mutation itself. Voltage clamp recordings of ionic and gating currents show changes in a range of biophysical properties in E1784K, compared with wild-type NaV1.5 channels, when temperature is raised, extracellular pH is reduced, or cytosolic Ca^{2+} is elevated. These biophysical changes are predicted to be arrhythmogenic. We incorporated our biophysical results into a ventricular action potential model and found that, at high heart rates, the effects of temperature, pH, or calcium are individually arrhythmogenic. These results lead us to the conclusion that catastrophic arrhythmias may be triggered by intense exercise in individuals carrying the SCN5a mutation underlying the E1784K form of mixed syndrome.

78. CRISPR/Cas-Mediated Genome Engineering.
ROB TAFT, *The Jackson Laboratory, Bar Harbor, ME*

CRISPR (clustered regularly interspaced short palindromic repeats) is a powerful genetic tool, giving scientists unprecedented precision to alter genomic DNA—from generating transgenics to correcting mutations. The main CRISPR system includes an enzyme, Cas9, that cuts DNA. This system is programmable and can be directed to precise sequences in the genome via an RNA-based guide molecule. This genome-editing technology has developed rapidly and has already been applied to a range of biological systems and disease areas since the first work in mouse and human cells less than three years ago. The power of CRISPR techniques stem not only from their precision, but also from their ease of use, most notably by significantly reducing the time to produce transgenic alleles. The promises of precise, combinatorial, efficient methods for genomic engineering are exciting, and applying CRISPR technology to a variety of model organisms is expected to quickly advance scientific understanding of disease mechanisms by allowing researchers to ask complex questions and find answers much faster than with traditional gene targeting approaches. This talk will cover how CRISPR/Cas has been used successfully in mice to generate endogenous knock-in alleles, conditional ("floxed") mutations, as well as multiple mutations in a single generation. The discussion will focus on how this technology compares with similar technologies and technical challenges and practical considerations.

79. Selective Antagonists of $Na_V1.6$ Prevent Electrically Induced Seizures in a Mouse Model of EIEE13.
PARISA TARI, CELINE DUBE, KULDIP KHAKH, ELAINE CHANG, NOAH SHUART, CLINT YOUNG, SOPHIA LIN, ZHIWEI XIE, RICHARD DEAN, ANDREA LINDGREEN, LUIS SOJO, ABID HASAN, WEI GONG, MICHAEL GRIMWOOD, THILO FOCKEN, CHARLES J. COHEN, and J.P. JOHNSON JR., *Xenon Pharmaceuticals, Burnaby, BC V5G 4W8, Canada*

CNS neurons express three voltage-gated sodium channels, $Na_V1.1$, $Na_V1.2$, and $Na_V1.6$. $Na_V1.1$ is the dominant isoform in inhibitory interneurons, and block of $Na_V1.1$ is believed to be proconvulsant since patients with heterozygous null mutations in the SCN1A gene encoding $Na_V1.1$ have early infantile onset epileptic encephalopathy 6 (EIEE6, a.k.a. Dravet syndrome). $Na_V1.2$ and $Na_V1.6$ are expressed in excitatory neurons, and mutations in the SCN8A gene encoding $Na_V1.6$ cause another catastrophic epilepsy, EIEE13. EIEE13 patients seize as early as the first day postpartum. Patients exhibit

cognitive and physical delay including ataxia and muscular hypotonia. Many patients never gain the ability to speak or walk. Most EIEE13 mutations identified are gain of function, so selective inhibition of $Na_V1.6$ is a promising interventional approach. Consistent with this idea, some EIEE13 patients respond to nonselective Na_V inhibitors, such as phenytoin. Inhibiting $Na_V1.6$, while sparing $Na_V1.1$, and thus inhibitory interneuron function, should be more effective and better tolerated. Recombinant mice with a patient identified mutation (N1768D) display symptoms of epilepsy (Wagnon et al. 2015. *H. Mol. Genet.* 24:506–515). Mice have reduced seizure thresholds, spontaneous seizures, and sudden death. We evaluated the ability of $Na_V1.6$ inhibitors to prevent seizures in a 6-Hz psychomotor seizure assay in N1768D mice and found them effective. Drugs with little selectivity for $Na_V1.6$, novel compounds that block $Na_V1.6$ and $Na_V1.2$, but spare $Na_V1.1$, and novel $Na_V1.6$ selective compounds all prevented seizures in N1768D mice. In vitro inhibition of $Na_V1.6$ combined with brain exposure was predictive of in vivo efficacy. Block of additional sodium channels by less selective compounds did not impact efficacy, suggesting the assay is a good surrogate for evaluating $Na_V1.6$ inhibition and occupancy. Thus, directly addressing the underlying mechanism of disease by selective block of $Na_V1.6$ can improve seizure resistance in this EIEE13 model.

Sponsor: Charles Cohen

80. The Cardiac Sodium Channel Nav1.5 Occurs at Three Distinct Pools in Cardiomyocytes. SARAH VERMIJ, DIANA SHY, JEAN-SÉBASTIEN ROUGIER, and HUGUES ABRIEL, *Department of Clinical Research, University of Bern, Bern, Switzerland*

The voltage-gated cardiac sodium channel Nav1.5 is responsible for the rapid upstroke of the cardiac action potential. Mutations in its gene *SCN5A* are associated with many severe cardiac disorders, including Brugada and congenital long-QT syndrome. The phenotypic diversity may be explained by the suspected three pools of Nav1.5: at the intercalated disc (ID), in the lateral membrane (LM), and in the T-tubular membrane (TTM). These pools seem to be differentially regulated: mice that lack the three last amino acids of Nav1.5 (ÄSIV) loose Nav1.5 only at the LM, leading to a reduction in sodium current.

This project aims to correlate Nav1.5 to markers of the three pools in isolated cardiomyocytes by immunofluorescent stainings and the proximity ligation assay (PLA) DuoLink.

In isolated cardiomyocytes, we showed that Nav1.5 colocalized with the ID makers connexin43 (Cx43) and plakophilin-2 (PKP2) at the ID and with syntrophin and dystrophin at the LM. PLA experiments confirm that Nav1.5 is within close proximity (<40 nm) with Cx43, PKP2, and syntrophin. Stainings with the TTM marker Bin1 and Nav1.5 showed a clear overlap in fluorescence profiles. The distance between fluorescence peaks is ~2 µm, specific for T-tubules. Interestingly, preliminary data suggest that the overlap between Bin1 and Nav1.5 is stronger in ÄSIV than in wild-type mice.

To conclude, our data strongly suggest that Nav1.5 is present in three pools in cardiac cells: at the ID, the LM, and the TTM. Moreover, we showed that each pool is differentially regulated by specific interacting proteins: PKP2 and Cx43 at the ID and syntrophin and dystrophin at the LM. Protein interactions at the TTM remain unknown. Next, we will use super-resolution microscopy to investigate Nav1.5 clusters at the LM and the TTM and localization of interacting proteins.

81. Gain-of-Function Mutations of BK Potassium Channels and Pro-Excitatory Effects. BIN WANG, VLADISLAV BUGAY, LING LING, HUI-HSUI CHUANG, ADELINE ORTS-DEL IMMAGINE, JASON PUGH, DAVID B. JAFFE, and **ROBERT BRENNER**, *University of Texas Health Sciences Center, San Antonio, TX 78229*

While ion channel drugs have long been the mainstay of epilepsy therapies, genetics sometimes reveal paradoxical effects of ion channels that caution simplistic interpretation and drug targeting. Large conductance calcium- and voltage-activated potassium (BK) channels present a key example where a potassium channel gain-of-function mutation can be pro-excitatory, and blockers of BK channels protect against seizures. Using a number of BK channel gain-of-function mouse models, we have attempted to understand the mechanisms underlying pro-excitatory effects of BK channels in dentate gyrus neurons of the hippocampus. We indeed observed electrographic seizures in a mouse model of the human D434G (D369G in mouse), BK gain-of-function epilepsy mutation, and also in genetic knockout of the inhibitory β4 subunit. Pharmacological studies in the β4 knockout suggest that pro-excitatory effects of BK channels are dependent on spike-evoked ryanodine receptor calcium release. Ryanodine receptors were found to feed calcium to fast-gated BK channels, which in-turn increase the fast-afterhyperpolarization (fAHP) and shorten the interspike interval. The pro-excitatory ryanodine receptor, BK channel functional coupling was also revealed in the epileptic gain-of-function R2474S ryanodine receptor mutation mice. R2474S mice were found to have a larger BK-dependent fAHP and increased spike frequency. These findings indicate that fast-gated BK channels promote excitability in excitatory neurons by increasing the fAHP amplitude, which in turn shortens the time to the subsequent spike. Using dynamic clamp to systematically alter the fAHP amplitude during spiking, we confirmed the cause and effect between fAHP amplitude and interspike interval. In summary, selective functional coupling of fast-gated BK channels with ryanodine receptors suggest that BK channels may serve as effectors of endoplasmic reticulum calcium dysregulation in epilepsy and other diseases.

82. Limb-Girdle Muscular Dystrophy 2L Is Caused by a Defect in Phospholipid Scrambling Mediated by ANO5.
JARRED M. WHITLOCK, KUAI YU, YUANYUAN CUI, and H. CRISS HARTZELL, *Department of Cell Biology, Emory University School of Medicine, Atlanta, GA 30322*

Limb girdle muscular dystrophies (LGMDs) are the fourth most common inherited muscle disorder. Most LGMDs are caused by recessive mutations in one of 23 different genes. LGMD2L, one of the most common LGMDs, usually presents after the third decade and is characterized by asymmetric lower limb weakness/atrophy, elevated serum creative kinase levels, and exercise intolerance. LGMD2L is linked to mutations in *ANO5/TMEM16E,* a member of the Anoctamin/TMEM16 superfamily of ion channels and regulators of Ca^{2+}-dependent phospholipid scrambling (Ca^{2+}-PLS). We propose that ANO5 regulates Ca^{2+}-PLS that plays a role in muscle repair. PLS is a process where phosphatidylserine, a major signaling molecule normally sequestered in the inner leaflet of the plasma membrane, is translocated to the external leaflet and exposed to the extracellular space. The fusion of myoblasts with one another and with muscle fibers plays a major role in repair of muscle fibers that have been damaged by mechanical stress during activity. Phosphatidylserine exposure occurs coincidently with myoblast fusion and participates in this process. Here we demonstrate that ANO5 expression elicits Ca^{2+}-PLS and a nonselective ionic current associated with lipid translocation. An *Ano5*$^{-/-}$ knockout mouse recapitulates many features of human LGMD2L, in particular defective muscle repair. Myoblasts isolated from this mouse exhibit diminished myoblast fusion in vitro and do not exhibit Ca^{2+}-PLS or Ano5 ionic currents, while wild-type myoblasts exhibit both activities. To determine whether Ca^{2+}-PLS is disrupted in human LGMD2L, skin fibroblasts were obtained from an LGMD2L patient and unaffected controls. Sequencing demonstrated *ANO5* mutations in both *ANO5* alleles of the patient. Western blot showed greatly diminished ANO5 expression. The LGMD2L patient fibroblasts exhibited markedly reduced Ca^{2+}-PLS, with >75% reduction in the number of cells exposing phosphatidylserine. Our findings demonstrate that ANO5 is essential for proper Ca^{2+}-PLS signaling and that this process is perturbed in patients with LGMD2L. We propose that defective Ca^{2+}-PLS reduces the ability of myoblasts to fuse during the process of muscle repair.

83. BK Channels Are Activated by Distinct Calcium Sources During Day and Night in SCN Neurons.
JOSHUA P. WHITT and ANDREA L. MEREDITH, *University of Maryland School of Medicine, Baltimore, MD 21201*

BK K^+ channels are regulated by membrane depolarization and increases in local intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). BK channels can couple to the voltage-gated Ca^{2+} channels L-, P-, Q-, and N-type, as well as to release from intracellular Ca^{2+} stores. In the suprachiasmatic nucleus (SCN), both $[Ca^{2+}]_i$ and voltage-gated Ca^{2+} channel (VGCC) currents are greater during the day. To determine whether diurnal regulation of $[Ca^{2+}]_i$ was relevant for BK channel activation in SCN, we used a pharmacological approach to identify the Ca^{2+} sources for BK current activation during the day and night in acute SCN slices. We found a 78% reduction in BK current with 10 μM nimodipine during the day, with less effect at night (10% reduction), suggesting that L-type VGCCs are the primary Ca^{2+} source for BK activation during the day. Furthermore, 71% of neurons in the SCN express inactivating BK currents (BK_i) during the day. Nimodipine abolished all the BK_i currents in the SCN, while 5 μM (S-) Bay-K 8644, an L-type VGCC current activator, produced 100% BK_i currents during the day. In contrast, at night, when nimodipine has little effect, we found a significant decrease in BK current using 10 μM dantrolene, blocking Ca^{2+} release from RyRs (63%), as well as 5 μM thapsigargin, depleting Ca^{2+} from intracellular stores (64%), suggesting that nighttime BK activation is driven by RyR-mediated store release. Lastly, 3 μM ω-conotoxin MVIIC reduced currents to a similar extent between day and night (24% and 22%, respectively), suggesting that a fraction of BK channels maintain stable Ca^{2+} channel coupling. These data demonstrate diurnal regulation of the functional coupling between BK channels and LTCCs and suggest diurnal changes in Ca^{2+} coupling could contribute to BK current inactivation in the SCN and the role of BK channel inactivation in circadian rhythmicity.

84. High-Fat Diet Improves Blood Glucose Control in a Mouse Model of Human Neonatal Diabetes: Protection by Fat?
ZIHAN YAN,[1] ALECIA WELSCHER,[1] and MARIA S. REMEDI,[1,2] [1]*Department of Medicine, Division of Endocrinology, Metabolism, and Lipid Research and* [2]*Department of Cell Biology and Physiology, Washington University in St. Louis, St. Louis, MO 63130*

Chronic hyperglycemia has been proposed to cause pancreatic β-cell dysfunction and reduction of β-cell mass (glucotoxicity) in diabetes. Gain-of-function (GOF) mutations in the K_{ATP} channel have been identified as causal of human neonatal diabetes (NDM). Mice expressing K_{ATP}-GOF channels in β-cells demonstrate severe diabetes and low circulating plasma insulin levels due to lack of insulin secretion. As disease progresses, insulin content and β-cell mass dramatically decrease, all secondary consequences of glucotoxicity, also seen in other forms of diabetes. However, comorbidity of hyperglycemia and elevated lipids (glucolipotoxicity) is also critical in diabetes. To test the contribution of elevated lipids in NDM, K_{ATP}-GOF mice were exposed to a high-fat diet (HFD) at disease onset. Surprisingly, HFD fed K_{ATP}-GOF mice demonstrate resistance to diet-induced obesity accompanied by a markedly divergent

phenotype: (1) remission of diabetes with normalization of fast and fed blood glucose (remitting) and (2) development of severe diabetes (nonremitting). Strikingly, the dramatic secondary loss of insulin content and β-cell mass observed in chow diet–fed K_{ATP}-GOF is avoided in HFD-fed mice. Unexpectedly, K_{ATP}-GOF remitting mice demonstrated a marked increase in peripheral insulin sensitivity, which precedes the normalization of blood glucose and may explain the lower circulating glucose levels. Importantly, remitting mice show reduced plasma lipids, decreased food intake, and increased energy expenditure and oxygen consumption. These results suggest that restriction of dietary carbohydrates and caloric replacement by fat may induce metabolic changes that are beneficial in terms of decreasing glucotoxicity in K_{ATP}-induced diabetes. Dietary recommendations for blood glucose control and their influences in development and progression of diabetes remain controversial. A recent report demonstrated that high-fat dairy products reduce the risk of development of type-2 diabetes. Together, our results suggest that HFD might also be protective in certain forms of diabetes, especially if this is not accompanied by weight gain and obesity.

85. Quantitative Imaging of Genetically-Encoded Metabolic Sensors in Mouse Brain. **GARY YELLEN**, *Harvard Medical School, Boston, MA 02115*

Cellular metabolism is no mere "housekeeping" function: it responds dynamically to energy demand and to changes in fuel supply, with important consequences for cellular function. To understand the dynamics of metabolism in living cells, we have developed several genetically encoded fluorescent biosensors for key metabolites—ATP and NADH—and are imaging these in neurons and astrocytes of mice, both in acute brain slices and in vivo. We have observed marked metabolic changes in response to neuronal stimulation, both at naturalistic levels and at pathophysiological levels such as those observed in epilepsy models. We are also investigating a mouse model of metabolic resistance to epileptic seizures, in which blunted glucose metabolism produces substantial resistance to induced seizures.

86. Cardiovascular Phenotypes of a Mouse Model of Cantu Syndrome. HAIXIA ZHANG,[1,2] PAIGE COOPER,[1,2] CHRISTOPHER EMFINGER,[1,2] THERESA HARTER,[1,2] MONICA SALA-RABANAL,[1,2] SCOT MATKOVICH,[3] BLANCHE SCHWAPPACH,[5] CONOR MCCLENAGHAN,[1,2] ZIHAN YAN,[4] ROBERT MECHAM,[1] MARIA REMEDI,[4] and COLIN G. NICHOLS,[1,2] *[1]Department of Cell Biology and Physiology, [2]Center for the Investigation of Membrane Excitability Diseases, [3]Department of Internal Medicine, and [4]Division of Endocrinology, Washington University School of Medicine, St. Louis, MO 63110; and [5]Department of Molecular Biology, University Medicine Gottingen, Gottingen, Germany*

Cantu syndrome (CS) is a rare disorder characterized by congenital hypertrichosis, distinctive facial features, and cardiovascular defects; the genetic causes of CS are gain-of-function mutations (GOF) of K_{ATP} channels. To understand the underlying mechanisms for CS, we used CRISPR/Cas9 genome engineering to generate CS mice carrying the SUR2[R1150Q] mutation (equivalent to human R1154Q—the most frequent mutation in CS patients). CS mice show lower blood pressure (BP), enlarged hearts, and enhanced heart output, which recapitulate cardiovascular phenotypes of CS patients. As expected for a GOF mutation, K_{ATP} channels of CS mouse ventricular myocytes show decreased ATP inhibition and increased MgADP stimulation. Interestingly, pinacidil (a SUR2 specific K_{ATP} opener) fails to lower BP in CS mice, while it significantly lowers BP in WT mice. Consistently, pinacidil activated current is much smaller in CS cardiomyocytes than in WT cardiomyocytes. However, pinacidil activates SUR2[R1150Q]/Kir6.2 recombinant channels as well as SUR2/Kir6.2 channels in Cosm6 cells, suggesting decreased sensitivity to pinacidil in CS mice is not directly derived from the SUR2[R1150Q] mutation itself. Surprisingly, cDNA sequencing from the hearts of CS mice reveals a deletion of 93 bases following R1150Q in one sequence of SUR2 cDNA, which may imply an alternative splicing event following R1150Q. In addition, SUR2A is down-regulated in CS mouse ventricles with a compensatory up-regulation in SUR1 (a more active subunit in K_{ATP} channels), which explains the decreased sensitivity to pinacidil in CS mouse. In conclusion, the SUR2[R1150Q] CS mouse recapitulates the cardiovascular phenotypes of CS patients, and provides a promising mouse model to study the mechanisms and treatment of CS. SUR2[R1150Q] GOF and SUR1 up-regulation may both contribute to the phenotypes in CS mouse. Future studies are required to investigate the phenotypes other than cardiovascular abnormalities in CS mice, as well as the splicing events caused by SUR2[R1150Q].

87. SWELL1 Is a Regulator of Adipocyte Insulin Signaling and Glucose Homeostasis. YANHUI ZHANG,[1] LITAO XIE,[1] SUSHEEL GUNASEKAR,[1] DAN TONG,[1] ANIL MISHRA,[1] WILLIAM J. GIBSON,[2] CHUANSONG WANG,[3] TREVOR FIDLER,[4] BRODIE MARTHALER,[1] ALOYSIUS KLINGELHUTZ,[5] E. DALE ABEL,[4] JESSICA SMITH,[6] ISAAC SAMUEL,[6] LEI CAO,[3] and RAJAN SAH,[1,4] *[1]Department of Internal Medicine, Division of Cardiovascular Medicine, University of Iowa, Carver College of Medicine, Iowa City, IA, 52242; [2]Harvard Medical School, Boston, MA, 02115; [3]Department of Molecular Virology, Immunology and Medical Genetics, The Ohio State University, Columbus, OH 43210; [4]Fraternal Order of the Eagles Diabetes Research Center, Iowa City, IA 52242; and [5]Department of Microbiology and [6]Department of Surgery, University of Iowa, Carver College of Medicine, Iowa City, IA 52242*

Obesity is characterized by a tremendous increase in adipose tissue that is in large part due to massive volumetric expansion of the constituent adipocytes (Farnier et al. 2003. *Int. J. Obes. Relat. Metab. Disord.* 27:1178–1186). There is a longstanding concept that metabolic disease in obesity is associated more with adipocyte size than numbers (Farnier et al. 2003. *Int. J. Obes. Relat. Metab. Disord.* 27:1178–1186; Heinonen et al. 2014. *Int. J. Obes. (Lond.).* 38:1423–1431; Lonn et al. 2010. *FASEB J.* 24;326–331; Salans et al. 1968. *J. Clin. Invest.* 47:153–165; Weyer et al. 2000. *Diabetologia.* 43:1498–1506). In support of this concept, recent studies have highlighted a connection between adipocyte size and membrane tension with adipocyte signaling and adipogenesis (Ben-Or Frank et al. 2014. *Biomech. Model Mechanobiol.* 14:15–28; Briand et al. 2014. *Diabetes.* 63:4032–4044; Pellegrinelli et al. 2014. *J. Pathol.* 233:183–195; Shoham et al. 2012. *Am. J. Physiol. Cell Physiol.* 302:C429–C441; Shoham et al. 2014. *Biophys. J.* 106:1421–1431), suggesting adipocyte-autonomous mechanisms of lipid homeostasis. To date, no molecular candidate for this adipocyte volume sensor has been proposed. Here, we combine adipocyte patch-clamp with shRNA- and CRISPR/cas9-mediated gene silencing to show that *SWELL1* (LRRC8a) encodes a volume-sensitive current in adipocytes. *SWELL1*, is a member of the leucine-rich repeat containing protein family recently discovered to be important for cytoplasmic volume-regulation (Qiu et al. 2014. *Cell.* 157:447–458; Voss et al. 2014. *Science.* 344:634–638) and phosphoinositide 3-kinase (PI3K)/Akt signaling (Kumar et al. 2014. *J. Exp. Med.* 211:929–942). We find that *SWELL1* is induced and activated in hypertrophic adipocytes in the setting of obesity and is required for adipocyte hypertrophy and glucose uptake. Moreover, *SWELL1* modulates adipocyte insulin signaling via molecular interactions with GRB2/Cav1 and PI3K-AKT pathway. In vivo, both shRNA-mediated *SWELL1* knockdown and adipose-targeted *SWELL1* deletion reduce adipocyte size and fat mass in obese mice. These studies identify the volume-sensitive molecule *SWELL1* as a cell-autonomous sensor of adipocyte size that regulates adipocyte growth, insulin sensitivity, and glucose tolerance in the setting of obesity. As *SWELL1* is broadly expressed, and PI3K-AKT pathway ubiquitous, and fundamentally intertwined with numerous signaling pathways, we anticipate that *SWELL1* signaling will be physiologically and pathophysiologically important in a multitude of different tissues and disease states.

88. Regulation of Cardiac Sodium Channel in Heart Failure by RNA-Binding Protein HuR. ANYU ZHOU,[1,2] HONG LIU,[1,2] GUANGBIN SHI,[1] and SAMUEL C. DUDLEY, JR.,[1,2] *[1]The Cardiovascular Institute, Rhode Island Hospital, Providence, RI 02903; [2]The Warren Alpert Medical School, Brown University, Providence, RI 02903*

Introduction: In patients, deletions or loss-of-function mutations of cardiac sodium channel α subunit gene SCN5A have been associated with a wide range of arrhythmias. The expression of SCN5A has been shown to decrease in failing hearts and the reduced expression of SCN5A is associated with the arrhythmia risk in heart failure. mRNA levels are determined by the balance between transcription and mRNA degradation. While transcriptional regulation of SCN5A expression has been extensively studied, little is known regarding the regulation of SCN5A mRNA degradation. We have shown that RNA-binding protein, HuR, positively regulates SCN5A mRNA expression by protecting SCN5A mRNA from degradation in vitro. To examine whether HuR modulates cardiac sodium channel expression in heart failure, we conducted this study.

Methods: A mouse model of myocardial infarction was created by the permanent ligation of the left anterior descending coronary artery. Mouse HuR coding sequences were cloned into an associate adenoviral (AAV) vector driven by cardiac troponin (cTNT) promoter. Recombinant AAV were packaged into infectious serotype 9 AAV particles. AAV-9-HuR AAV particles were injected into the MI mouse via the right jugular vein 2 wk after left anterior descending coronary artery occlusion. 2 wk after injection, mouse hearts were collected to determine the expression of cardiac sodium channel, and optical mapping studies were performed to determine arrhythmia inducibility in situ and to map conduction velocity and action potential duration.

Results: Overexpression of HuR by AAV injection increased cardiac sodium channel α subunit expression by 119.2% by Western blot. In addition, ectopic HuR also increased endogenous HuR protein level. Our optical mapping study revealed that HuR overexpression reduced arrhythmic inducibility, prevented reentry, increased conduction velocity, and decreased action potential rise time.

Conclusions: Our study results indicate that RNA-binding protein HuR can positively regulate cardiac sodium channel SCN5A expression in vivo and can reduce arrhythmia risk in heart failure. Therefore, manipulation of HuR expression may be a useful strategy to treat HF-associated arrhythmias.

89. Beta Subunits and Inherited Mutations Alter $Na_V1.5$ Pharmacology via Regulation of the Voltage-Sensing Domains. WANDI ZHU, ANGELA SCHUBERT, and JONATHAN SILVA, *Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO 63130*

Background: The pharmacology of the cardiac Na^+ channel, $Na_V1.5$, can be chamber specific and varies among patients. We probed whether the small, single transmembrane-spanning β-subunit components of $Na_V1.5$ could significantly affect channel pharmacology by regulating the voltage-sensing domains (VSDs) of $Na_V1.5$.

Methods: The NaV1.5 α subunit contains four domains (DI–DIV), each with six membrane-spanning segments (S1–S6). S1–S4 form the VSDs that convert voltage into channel gating. Previously, we created four DNA constructs, each with a cysteine engineered into the extracellular S4 of a single channel domain. RNA from these constructs was injected into *Xenopus* oocytes and expressed channels were labeled at this cysteine with TAMRA-MTS fluorophores. Ionic current and fluorescence emission that reflected changes in VSD conformation were simultaneously recorded using the cut-open oocyte configuration.

Results: In comparison with α alone, β1 and β3 cause a depolarizing shift in channel inactivation, and correlating shift in DIV-VSD activation, while β3 also affects DIII-VSD activation. DI and DII were not significantly affected. Class Ib antiarrhythmics, such as lidocaine and ranolazine, modulate DIII-VSD activation of $Na_V1.5$. Therefore, we hypothesized that this interaction would be affected by the β subunits. We observed that β3 abolished lidocaine-induced stabilization of the DIII-VSD and enhanced the ranolazine effect. Oppositely, β1 abolished ranolazine-induced stabilization of the DIII-VSD and enhanced the lidocaine effect. At the cell level, this modulation significantly affected tonic and use-dependent drug block by both lidocaine and ranolazine. Finally, the atrial fibrillation–linked β3 R6K mutation prevented DIII-VSD regulation by both molecules.

Conclusions: These results suggest that class I drug responses will be significantly altered by the β-subunits, providing a potential mechanism for chamber specificity that depends on β-subunit expression. Inherited mutations carried by the β-subunits are likely to strongly influence the patient response to therapy by these molecules.

90. The Molecular and Ionic Mechanism of Two Stable Levels of Resting Potentials of Cardiomyocytes in Hypokalemia. DONGCHUAN ZUO,[1,*] KUIHAO CHEN,[1,*] MIN ZHOU,[2] ZHENG LIU,[3] and HAIJUN CHEN,[1] *[1]Department of Biological Sciences, University at Albany, State University of New York, Albany, NY 12222; [2]Department of Neuroscience, The Ohio State University Wexner Medical Center, Columbus, OH 43210; [3]Department of Cardiology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China; *These authors equally contributed to this work*

Multiple types of cells including human cardiomyocytes exhibit two stable levels of resting potentials in physiologically or pathologically identical ionic conditions. In subphysiological extracellular K^+ concentrations ($[K^+]_o$), which occur in pathological hypokalemia, human cardiomyocytes can show both hyperpolarized and depolarized resting potentials, associating with cardiac arrhythmia. Resting potentials of human cardiomyocytes and cardiac Purkinje fibers can either spontaneously shift from hyperpolarization to depolarization in a subphysiological $[K^+]_o$ or fluctuate between two levels in a nongraded manner. Injection of small current pulses can switch resting potentials between the two levels. However, the mechanism underlying this well-known phenomenon is not well understood. We recently demonstrate that in subphysiological $[K^+]_o$ K2P1 two-pore domain K^+ channels dynamically change ion selectivity, become nonselective cation channels, and conduct inward leak cation (mainly Na^+) currents. Here, we show that under hypokalemia K2P1 channels contribute to two stable levels of resting potentials of cardiomyocytes. We employ two models of cardiomyocytes: human cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CMs) that highly express K2P1 channels and mouse HL-1 cardiomyocytes that do not express K2P1 channels nor exhibit two levels of resting potentials in subphysiological $[K^+]_o$. In subphysiological $[K^+]_o$ "matured" hiPSC-CMs and mouse HL-1 cardiomyocytes with ectopic expression of K2P1, channels can exhibit two stable levels of resting potentials and N-shaped current-voltage relationships that cross the voltage axis three times, and the first and third zero-current potentials determine the two levels of resting potentials. Removal of K2P1-like inward Na^+ currents or knockdown of K2P1 expression eliminates two levels of resting potentials in hiPSC-CMs. These results elucidate the mechanism that results in two levels of resting potentials of human cardiomyocytes in hypokalemia and demonstrate a new mechanism regulating excitability.

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