Platform: Cardiac, Smooth, and Skeletal Muscle Electrophysiology

977-Plat
Ionic Mechanisms that Underlie Ventricular Action Potential Prolongation following Loss of Caveolin-3 in Adult Transgenic Mice

Vignesh Ramchandran[1], Thomas O'Hara[1], Yogananda S. Markandeya[2], Ravi C. Balijepalli[2], Timothy J. Kamp[2], Natalia A. Trayanova[1].
[1]Johns Hopkins University, Baltimore, MD, USA, [2]University of Wisconsin, Madison, WI, USA.

Caveolin proteins are involved in establishing membrane microstructure, lipid raft organization, and cell signaling. In the heart, caveolin-3 (Cav3) predominates. Inherited or disease-induced Cav3 loss increases risk of sudden cardiac death (SCD). We aimed to explore connections between Cav3 loss and arrhythmogenic changes in the ventricular action potential (AP) by investigating the Cav3 dependence of ionic currents. Drugs commonly used to disrupt or remove Cav3 in cultured cells exclude any compensatory process likely to occur in vivo. This motivated us to engineer a novel conditional Cav3 knockout (Cav3-/-) mouse that survives to adulthood. We isolated ventricular cells for electrophysiological experimentation.

AP duration (APD90) was prolonged from 24 ± 4 ms in WT to 96 ± 9 ms in Cav3-/-, and several currents were affected. Reduced peak: L-type Ca^{2+} current (ICaL), 21%; slow K^+ current, 81%; transient outward K^+ current, 57%; steady state outward K^+ current (Iss), 43%. Late Na^+ current was enhanced ~10-fold. These changes were partially offsetting - preventing a simple account for the APD90 increase. To relate changes in currents to changes in the AP, we developed a computational representation of Cav3-/- based on the Morotti et al. mouse ventricular cell model and defined by fractional change in currents.

Unexpectedly, the relatively small change in relatively small Iss caused 33% of total simulated AP prolongation. Though Iss conductance was reduced, peak Iss actually increased in the dynamic setting of the simulated AP. Early in the AP, lower Iss indirectly enhanced inward currents (importantly late ICaL) by extending the plateau phase, which in turn allowed Iss to more fully activate. This Iss/ICaL process largely accounted for the pro-arrhythmic APD90 increase following Cav3 loss and is therefore a candidate target for normalizing SCD risk.

978-Plat
Diabetes Slows Heart Rate via Electrical Remodeling of K^+ Currents in Sinoatrial Node Myocytes

Joshua R. St. Clair, Emily J. Sharpe, Andrew Hagar, Clayton Garthe, Julie Juchno, **Cathy Proenza**.
Physiology & Biophysics, University of Colorado, Aurora, CO, USA.

Diabetes mellitus is associated with sinoatrial node dysfunction, as evidenced by an increased risk of atrial fibrillation, pacemaker implantation due to bradycardia and cardiac death in diabetic patients. While sinoatrial node myocytes (SAMs) generate the spontaneous action potentials (APs) that initiate each heartbeat, little is known about how diabetes affects SAMs directly. In this study, we used streptozoticin (STZ) -treated mice as a model of diabetic hyperglycemia. Four weeks after STZ injections, we found that both intrinsic heart rate (measured during autonomic blockade) and maximum heart rate (measured during restraint stress) were reduced in diabetic animals compared to pretreatment values. Current-clamp recordings from acutely isolated SAMs from diabetic animals revealed corresponding reductions in spontaneous AP firing rates. AP waveform analysis showed that the reduced firing rates in diabetic cells resulted from a prolongation of the AP duration and a slowing of the rate of repolarization. Accordingly, we observed significant decreases in steady state and transient outward K^+ current densities in whole-cell voltage-clamp recordings from SAMs from diabetic animals. Diabetes caused little or no change in voltage-gated calcium currents in diabetic SAMs. The effects of diabetes on the AP waveform and firing rate were mimicked by application of 10 μM 4-aminopyridine in current clamp experiments and when the transient outward K^+ current was reduced in a mathematical model of the sinoatrial AP. These results suggest that diabetic sinoatrial node dysfunction results in part from electrical remodeling of K^+ currents in sinoatrial node myocytes, which paradoxically slows the spontaneous AP firing rate and thus heart rate.

979-Plat
Cardiac Specific Leucine-Rich Repeat Containing 10 (LRRC10) Protein Interacts with and Regulates the $Ca_v1.2$ L-Type Ca^{2+} Channels

Marites T. Woon[1], Adrian C. Grimes[2], Courtney R. Reynolds[2], Matthew J. Brody[3], Youngsook Lee[4], Ravi C. Balijepalli[2].
[1]Medicine, University of Wisconsin, Madison, WI, USA, [2]Medicine, University of Wisconsin, Madison, Madison, WI, USA, [3]University of Cincinnati, Cincinnati, OH, USA, [4]University of Wisconsin, Madison, Madison, WI, USA.

Cardiac L-type Ca^{2+} channels (LTCC) play essential role in multiple cellular processes including excitation-contraction coupling, signaling and gene regulation. Diverse families of regulatory and scaffolding proteins regulate the LTCCs in the cardiomyocytes. Leucine-rich repeat containing 10 (LRRC10) is a cardiac-specific scaffolding protein that plays a critical role in heart development and function. Recently we have demonstrated that the *Lrrc10*-null (*Lrrc10*$^{-/-}$) mice develop dilated cardiomyopathy and *Lrrc10*$^{-/-}$ cardiomyocytes exhibit reduced LTCC current ($I_{Ca,L}$). However, it is unclear how LRRC10 regulates LTCC function in the heart. To investigate the role of LRRC10 in the regulation of LTCCs, we co-expressed the WT LRRC10 with LTCC channel complex comprising of the $Ca_v1.2$, β_{2CN2}, $\alpha_2\delta$ subunits in HEK293 cells and performed whole-cell patch clamp experiments. Co-expression of LRRC10 significantly enhanced (225%) the peak LTCC current ($I_{Ba,L}$ -75 ± 7 pA/pF) density compared to LTCC alone (-33 ± 3 pA/pF). We then introduced a single point mutation (substituted alanine for histidine) in the putative functional interaction site at amino acid position 150 (H150A) of LRRC10. Co-expression of LRRC10H150A caused significant reduction (27%) in $I_{Ba,L}$ (-57 ± 4 pA/pF) compared to WT LRRC10 co-expression. Furthermore, G/G_{max} analysis revealed a significant shift in the voltage dependence of activation to more negative potentials with the co-expression of either the WT LRRC10 ($V_{1/2}$,-21.7mV) or the H150A mutation ($V_{1/2}$, −25.1mV) compared to LTCC alone ($V_{1/2}$, −16.4mV). Finally, co-immunoprecipitation and western blot analysis in HEK293 cells demonstrated that LRRC10 associates with $Ca_v1.2$ subunit but not the Ca_{v2} subunit. Moreover, co-expression of the LRRC10 H150A mutation disrupted the association of $Ca_v1.2$ with LRRC10. We conclude that LRRC10 may directly associate with $Ca_v1.2$ subunit and regulate the LTCC function by enhancing the surface expression, density and biophysical properties of the $I_{Ca,L}$.

980-Plat
The Ca^{2+} Clock is Not Governed by a Single CaMKII or PKA Phosphorylation Site for Fight or Flight Responses

Yuejin Wu[1,2], William J. Kutschke[2], Hector H. Valdivia[3], Xander H.T. Wehrens[4], Mark E. Anderson[1,2].
[1]Medicine, Johns Hopkins Medical Institutions, Baltimore, MD, USA, [2]Internal Medicine, University of Iowa, Iowa City, IA, USA, [3]Internal Medicine, University of Michigan, Ann Arbor, MI, USA, [4]Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, USA.

Both CaMKII and PKA contribute to fight or flight heart rate (HR) increases in response to isoproterenol or activity. CaMKII and PKA promote HR increases, at least in part, by actions on 'Ca^{2+} clock' homeostatic proteins. The Ca^{2+} clock mechanism for cardiac pacing relies on SR proteins governing SR Ca^{2+} uptake and release. Inhibition of SR Ca^{2+} release by ryanodine slows HR but we are unaware of any studies testing the potential for slowing SR Ca^{2+} uptake to reduce HR. Phospholamban (PLN) is a negative regulator of SERCA that acts to slow SR Ca^{2+} uptake. PLN phosphorylation is catalyzed by PKA, at serine 16, or CaMKII, at threonine 17. PKA and CaMKII can also promote SR Ca^{2+} release by catalyzing phosphorylation of the ryanodine receptor (RyR2). CaMKII phosphorylation at Ser 2814 and PKA phosphorylation at Ser 2808 can increase RyR2 Ca^{2+} leak that drives cell membrane depolarizing inward current through the Na^+/Ca^{2+} exchanger. Despite the mounting evidence that CaMKII and PKA sites on PLN and RyR2 are important for cardiac pacing, the relative importance of these sites is unknown and the potential for any particular site to exert a controlling influence over fight or flight physiology is untested. Here we have taken advantage of genetically modified mouse models where CaMKII and PKA sites are specifically ablated to interrogate the role of each site and determine if any of these SR protein sites exercises a decisive influence on HR responses to isoproterenol or activity. The results from genetically modified mice harboring various PLN mutantions (PLN-/-, N27A, S16A, T17A) and RYR2 mutations (S2808A and S2814A) suggest that established CaMKII and PKA sites do not, by themselves, control fight or flight HR responses.

981-Plat
Ranolazine Prevents Phase-3 Early Afterdepolarizations in Human Atrial Myocytes by Inhibiting Na Current Non-Equilibrium Reactivation

Stefano Morotti[1], Andrew D. McCulloch[2], Donald M. Bers[1], Andrew G. Edwards[3,4], **Eleonora Grandi**[1].
[1]Department of Pharmacology, UC Davis, Davis, CA, USA, [2]Department of Bioengineering, UC San Diego, San Diego, CA, USA, [3]Institute for Experimental Medicine, Oslo University Hospital Ullevål, Oslo, Norway, [4]Simula Research Laboratory, Lysaker, Norway.

Background: We have previously shown that non-equilibrium reactivation of the Na current (I_{Na}) drives Isoproterenol-induced phase-3 early afterdepolarizations (EADs) in failing mouse ventricular myocytes. EAD initiation is