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

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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

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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 pre-treatment 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

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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

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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

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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

secondary to potentiated sarcoplasmic reticulum Ca release and enhanced Na/Ca exchange (NCX), and can be abolished by the I_{Na} blocker tetrodotoxin, but not by the selective (in ventricles) late I_{Na} blocker ranolazine.
Aim. Since repolarization in human atrial myocytes is relatively rapid and potently modulated by Ca (as in mouse ventricle), we investigate whether the same EAD mechanism may occur in human atria. Indeed, phase-3 EADs have been suggested to underlie re-initiation of atrial fibrillation (AF) after termination upon autonomic stimuli - well recognized AF triggers.
Methods: We integrated a Markov model of I_{Na} (also describing the channel interaction with ranolazine, similar to work by Clancy) in our human atrial myocyte model. To recapitulate experimental results, we simulated rapid cell pacing (10 Hz) in the presence of Acetylcholine (0.1 μM) and Isoproterenol (1 μM), and assessed EAD occurrence upon return to sinus rhythm (1 Hz).
Results: Cellular Ca overload during fast pacing results in a transient period of hyper-contractility after return to sinus rhythm. Here, fast repolarization and enhanced NCX facilitate I_{Na} reactivation via the canonical gating mode (i.e., not late I_{Na} burst mode), which drives EAD initiation. Notably, in action potential clamp experiments, an EAD waveform elicits a lidocaine-sensitive inward current during the EAD upstroke in pig atrial cells. Simulating ranolazine administration reduces peak I_{Na} and leads to a faster repolarization, during which I_{Na} fails to reactivate.
Conclusions: Our simulations suggest that non-equilibrium I_{Na} reactivation critically contributes to arrhythmias in human atrial myocytes. Ranolazine might be beneficial in this context by blocking peak (not late) atrial I_{Na}.

982-Plat
Probing the Trafficking Routes of KCNQ1 and KCNE1 After Their ER Exit
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Background: KCNQ1 (Q1, pore-forming channel subunit) and KCNE1 (E1, regulatory subunit) associate to form the slow delayed rectifier (I_{Ks}) channel, important for cardiac action potential repolarization. Although Q1/E1 are obligatory partners in cardiomyocytes, in adult ventricular myocytes the two are not well colocalized: E1 is on the lateral cell surface while Q1 is mainly in the intracellular junctional SR. This appears to contradict data from heterologous expression or non-cardiac cell types, which suggest that Q1/E1 assemble into I_{Ks} when they are still in ER or soon thereafter. The current study is designed to distinguish between 2 scenarios. First, Q1 and E1 traffic from ER to the plasma membrane together, followed by Q1 internalization to the ER/SR compartment. Alternatively, Q1 and E1 traffic on different routes from ER to their separate destinations.
Methods: COS-7 cells transfected with Q-GFP/E1-dsR are cultured in presence of brefeldin A (BFA) for 12 – 16 hr, to allow protein translation without ER exit. Cells are imaged in absence of BFA but presence of cycloheximide+dynasore (blocking protein translation and endocytosis). Time-lapse images (Zeiss 710, 37°C) of 4-um optic slice (to maximize capture of trafficking events in thin cytosol of COS-7 cells) are recorded till the Q1-GFP and E1-dsR distribution reaches quasi steady-state (> 2 hr).
Results: BFA removal allows ER exit and Golgi reconstruction. E1-dsR exits ER in distinct vesicles, more frequently at the cell periphery. These vesicles gradually cluster to the peri-nuclear region, entering the post-BFA Golgi. Q1-QFP stays in the ER compartment for > 2 hr. Its ER location is confirmed by FRAP experiments.
Conclusion: Our data support the second scenario. Experiments are in progress to test whether E1-dsR and Q1-GFP behave the same when expressed in a cardiac myocyte environment.

983-Plat
Trafficking and Gating Mechanisms of HERG1A C-Terminus (LQTS-2) Truncation Mutations on HERG1A-HERG1B Hetero-Multimeric Channel
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In heart, HERG1A-HERG1B subunits generate the rapid component of delayed rectifier potassium current, IKr, critical for normal repolarization. Congenital mutations in HERG1A lead to long QT syndrome-2 (LQTS-2), a condition that increases susceptibility to fatal arrhythmias. Many LQTS-2 mutations that are localized to HERG1A C-terminus generate truncated variants and their behavior when co-expressed with HERG1B and their (patho) physiological effects in heart are largely unknown. Therefore, we compared the physiological impact and mechanistic bases of two HERG1A C-terminus truncation mutations (G965X, R1014X). Bungarotoxin-binding optical assays for channel cell-surface expression and electrophysiological recordings were carried out in Human Embryonic Kidney cells. R1014X displayed normal surface density whereas, the G965X mutant, displayed a significant decrease in channel surface density, which was fully rescued by wild-type HERG1A-HERG1B subunits. Homo-multimeric (mutant+HERG1B), G965X and R1014X channel subunits yielded currents with severely reduced current amplitude. When co-expressed with wild-type HERG1A, both mutants exerted a dominant negative effect but to different extents: G965X current amplitude was partially rescued while R1014X current remained unchanged. Homo-multimeric mutant channels displayed a significant rightward shift in the activation curve which was partially (G965X), or not (R1014X) rescued with wild-type HERG1A. The data reveal these mutants exert a purely biophysical effect on hetero-multimeric (mutant HERG1A + wild-type HERG1A + HERG1B) channels. These mechanistic insights may enhance therapies for LQTS-2 mutations. Furthermore, we show for the first time that the R1014X shows normal surface expression, but is non-functional and exerts a strong dominant-negative effect on wild-type HERG1A channels which is in contrast to existing data that demonstrates that R1014X causes HERG1A channel dysfunction by defective trafficking. Finally, studies that explore the functional impact of these mutations in heart will provide novel information that will be more predictive of disease penetrance.

984-Plat
Dominant Negative Consequences of a HERG 1B Mutation Associated with Intrauterine Fetal Death
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The human ether-a-go-go related gene (hERG) encodes the voltage-gated potassium channel responsible for conducting the rapid delayed rectifier potassium current (IKr). Reduced IKr slows cardiac action potential repolarization and is an underlying cause of cardiac arrhythmias associated with both inherited and acquired long QT syndrome (LQTS). Two subunits comprise the hERG channel, hERG 1a and hERG 1b, both of which represent critical components of cardiac repolarization. R25W is a hERG 1b mutation associated with a case of intrauterine fetal death and previously shown to reduce heteromeric hERG 1a/1b current density in Chinese hamster ovarian cells1. The mechanism of current reduction and a direct link to cardiac dysfunction has not been described for R25W. We expressed R25W in HEK cells and human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) to define the molecular, biophysical, and physiological consequences of R25W. R25W significantly reduced 1a and 1b protein levels to 76.4 ± 6.0% and 65.2 ± 7.8% of control, respectively, in HEK cells coexpressing hERG 1a and 1b. In iPSC-CMs, R25W transfection significantly reduced peak tail IKr (0.8 ± 0.1 pA/pF, n = 10, $p < 0.05$) compared to either native IKr (1.7 ± 0.2 pA/pF, n = 5) or wild type 1b transfected iPSC-CMs (1.5 ± 0.3 pA/pF, n = 7). Interestingly, wild-type 1b did not affect peak tail IKr whereas transfection of either 1a alone (35.9 ± 11.0 pA/pF, n = 5) or 1a/1b together (24.8 ± 11.2 pA/pF, n = 5) dramatically increased current density. These data identify R25W as a dominant negative mutation that reduces native IKr and provide new evidence that hERG 1b fails to traffic as a homomeric channel in human cardiomyocytes. Supported by NIH/R01HL081780 and the UW Training Program in Translational Cardiovascular Science (T32HL007936).
1. Crotti et al., 2013, JAMA.

Platform: Membrane Pumps, Transporters, and Exchangers II

985-Plat
On the Na^+/H^+ Selectivity of Membrane Transporters and Enzymes: Experimental and Theoretical Studies of an ATP-Synthase Rotor Ring
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Electrochemical gradients of sodium and protons are the primary driving forces for a wide array of cellular processes mediated by membrane proteins, such as energy conversion, solute uptake and multi-drug extrusion. The factors that confer ion specificity to these systems are poorly understood. Sodium and proton-driven systems of different functionality are often found in the same organism, and membrane proteins within the same functional family frequently feature distinct specificities despite a high similarity in their structures. Therefore, it appears that the specificity of H^+/Na^+-coupled systems is largely