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 (D). We aim 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 Cav3 knockout (Cav3-/-) mouse that survives to adulthood. We isolated ventricular cells for electrophysiological experimentation.

AP duration of PD90 was prolonged from 24 ± 4 ms in WT to 96 ± 9 ms in Cav3-/-, and several currents were affected. Reduced peak: Ltype Ca2⁺ 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

Diabetic 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 elevated rates of reentrant arrhythmias. While sinoatrial node myocytes (SAMs) from diabetic animals reveal corresponding reductions in spontaneous AP firing rate, the mechanisms that underlie this phenomenon are untested. Here we have taken advantage of genetically modified mice harboring various PLN mutantions (PLN-/-, N27A, S16A, T17A) and RYR2 mutations (S2808A and S2814A) results from genetically modified mice harboring various PLN mutantions (PLN-/-, N27A, S16A, T17A) and RYR2 mutations (S2808A and S2814A) to investigate the role of CaMKII and PKA in diabetic heart rate (HR) control. We show that 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 ‘Ca2⁺ clock’ homeostatic proteins. The Ca2⁺ clock mechanism for cardiac pacing relies on SR proteins governing SR Ca2⁺ uptake and release. PKA and CaMKII can phosphorylate membrane voltage-dependent (V1/2, -21.7mV) or the H150A mutation (V1/2, -16.4mV). Finally, co-immunoprecipitation and western blots showed that LRRC10 associates with Cav1.2 subunit but not the Ca2⁺ subunit. Moreover, co-expression of the LRRC10 H150A mutation disrupted the association of Cav1.2 with LRRC10. We conclude that LRRC10 may directly associate with Cav1.2 subunit and regulate the LTCC function by enhancing the surface expression, density and biophysical properties of the Cav1.2.

980-Plat

The Ca2⁺ 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 ‘Ca2⁺ clock’ homeostatic proteins. The Ca2⁺ clock mechanism for cardiac pacing relies on SR proteins governing SR Ca2⁺ uptake and release. Inhibition of SR Ca2⁺ release by ryanodine slows HR but we are unaware of any studies testing the potential for slowing SR Ca2⁺ uptake to reduce HR. Phosphoholamban (PLN) is a negative regulator of SERCA that acts to slow SR Ca2⁺ uptake. PLN phosphorylation is catalyzed by PKA, at serine 16, or CaMKII, at threonine 17. PKA and CaMKII can also promote SR Ca2⁺ release by catalyzing phosphorylation of the ryanodine receptor (RYR2). CaMKII phosphorylation at Ser 2814 and PKA phosphorylation at Ser 2808 can increase RyR2 Ca2⁺ leak that drives cell membrane depolarization inward current through the Na/Ca2⁺ 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 mutations (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