APD_{90} beat-to-beat variability (BVR) was larger in KO and, consistently, the incidence of early afterdepolarizations was higher in KO than WT (respectively, 66% and 18%). In contrast, KD cells showed a slight prolongation of APD_{90} (96 ± 7.9 ms), but BVR and EADs incidence were comparable to WT. While voltage-gated K^+ currents (I_{to}, I_{Kur} and I_{ss}) were strongly downregulated in KO, KD myocytes showed only a small reduction of I_{to}. I_{CaL} density was significantly lower in both KO and KD mice at negative potentials only. Consistently, both KD and KO mice showed a positive shift of both steady-state activation ($\Delta V_{1/2}$ vs WT: KO 4.2 mV, KD 5.5 mV) and inactivation curves ($\Delta V_{1/2}$ vs WT: KO 3.2 mV, KD 3.4 mV). Addition of isoproterenol (100 nM) resulted in I_{CaL} enhancement, whose magnitude was larger in KO compared to KD and WT myocytes (% increase,WT: 58 ± 6%,KD:45 ± 7%, KO:95.5 ± 12%). These data suggest distinct action related to either kinase and scaffold functions: while the former has an impact on I_{CaL} biophysical properties, the latter prevents arrhythmias in murine ventricular myocytes by preserving K^+ currents.

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Mass Spectrometry-Based Analysis of the Phospho-Proteome for Cardiac Dyssynchrony and Resynchronization Therapy

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In patients suffering from heart failure, conduction abnormalities that create cardiac dyssynchrony worsen morbidity and mortality. However, a pacemaker-based treatment termed Cardiac Resynchronization Therapy (CRT) can significantly restore function and mortality. Our previous work implicated post-translational signaling cascades in modulating this recovery. To further understand the mechanism of recovery, we aimed to catalogue, as completely as possible, the phospho-proteome in canine models of normal cardiac function (Con), cardiac dyssynchrony (Dys), and CRT. The models were generated by implanting pacemakers and pacing 6-weeks dyssynchronously (Dys), 3-weeks dyssynchronously and 3-weeks CRT (CRT), or not at all (Con). Each cardiac tissue sample (n=4 per group) was split into three parts: myofilament-enriched, myofilament-depleted, and phospho-tyrosine enriched. Each part was phospho-enriched and run on an Orbitrap Elite. By one-way ANOVA and Holm-Sidak post-hoc test, there were 104 sites Ser/Thr sites and 85 Tyr sites that were changed in at least one group. These sites were assigned one of three categories based on the pattern of changes: ''Dyssynchrony, Not Fixed by CRT'', ''Fixed by CRT'', or ''CRT Only/Exacerbated by CRT''. Next we predicted the kinase for each phosphorylation site (Group-based Prediction System 2.1). Many tyrosine kinases were identified, although no kinase showed specificity to any category. However, the Ser/Thr kinase CK2 was predicted to target a majority of the ''Fixed By CRT'' category, and was highly specific for this group, as CK2 was not predicted to target sites in any of the other categories. Thus, CRT appears to act as a broad CK2 activator. In the future, these sites need to be validated as CK2 targets. Moreover, the effect of CK2 activation on cardiomyocyte function, sarcomere shortening and calcium transient, will be explored, considering these are already implicated in recovery with CRT.

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Modulation of Action Potential Alternans by IKs in Myocardial Infarction

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Background: Patients with post myocardial infarction (MI) have increased incidence of ventricular arrhythmias. The slowly activating K^+ channels (IKs) has been reported to be downregulated consistently but in different degrees in the post-MI hearts. The role of downregulation of IKs in arrhythmogenesis in post MI remains to be defined.
Hypothesis: We hypothesize that significant suppression of IKs in post MI impairs the rate-dependent and Ca^{2+}-dependent augmentation of repolarization reserve resulting in increased alternans and thereby arrhythmias.
Methods: We evaluated the effect of IKs on alternans in post MI using transgenic rabbit model of Long QT Type 1 (LQT1) lacking IKs. LQT1 and littermate control (LMC) rabbits underwent MI procedures and optical mapping was performed to record Vm and Ca^{2+} simultaneously from the 3 week post-MI hearts (n=9 LQT1-MI, 4 LQT-MI Sham, 10 LMC-MI, 9 LMC-Sham hearts).
Results: The dispersion of APD was enhanced in LQT1-MI compared to LQT1-Sham and LMC-MI (αAPDmax-min = 28 ± 4 ms vs. 20 ± 3 ms and 27 ± 6 ms). APD alternans was more pronounced in LQT1-MI, accompanied with large amplitude Ca^{2+} alternans, and often spatially discordant (see traces and restitution curves in panel A&B). Spatially discordant alternans in LQT1-MI was highly dynamic, preceded by sudden appearance of Ca^{2+} nodal lines near the MI border zone (panel C, dark lines), associated with high VF induction (n=9/9 LQT1-MI vs. 1/4 LQT1-sham and 7/10 LMC-MI).
Conclusion: Our results demonstrate the importance of IKs as a repolarization reserve in MI and that significant downregulation of IKs may promote spatially discordant Ca^{2+} and APD alternans and VF induction.

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A Guinea Pig Model for Heart Failure-Associated Sudden Cardiac Death

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Sudden cardiac death (SCD) is the leading cause of mortality in patients with heart failure (HF); However, the mechanisms underlying SCD are incompletely understood. In the present study, we developed a guinea pig model for HF-associated SCD. Male adult guinea pigs underwent ascending aortic constriction (AC) in addition to isoproterenol (iso) challenge by daily *i.p.* injection of iso at 1 mg/kg in week1 and 2 mg/kg after week 1. Iso treatment at the selected doses for 4 weeks had no significant effects on cardiac function and geometry in sham control, however, iso treatment worsened cardiac function in AC animals. Animal with AC and iso challenge (ACi) displayed earlier decompensation and higher incidence of SCD compared to AC group. ~60% of ACi animals died without overt symptom of HF within 4 weeks. Electrocardiography (ECG) indicated QT prolongation and high incidence of premature ventricular beat (PVB) and ventricular fibrillation (VF) in ACi animals. Electrophysiological study, performed in isolated ventricular myocytes from ACi and sham hearts in week4, revealed prolonged action potential (AP) (APD_{90}: 279ms ± 15 in ACi vs 203ms ± 17 in sham) and reduced potassium currents (Ik1 at −150mV was −28.4pA/pF ± 0.8 in ACi vs −47.6pA/pF ± 2.3 in sham; Tail current of I_{kr} and I_{ks} at +50mV were 0.28pA/pF ± 0.03 and 0.30 ± 0.03 in ACi vs 0.55pA/pF ± 0.02 and 0.54pA/pF ± 0.03 in sham, respectively) in ACi animals. Our results demonstrated that the combination of AC and iso challenge produces high incidence of arrhythmia and SCD in guinea pig with features of prolonged QT and reduced repolarization reserve, thus providing a novel model for HF-associated arrhythmia and SCD.

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Iron Overload Promotes Arrhythmias via ROS Production and Mitochondrial Membrane Potential Depolarization

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Genetic disorders or frequent blood transfusions often cause iron overload which can lead to arrhythmias. However, the underlying mechanism is not well known. In the present study, we assess the hypothesis that iron-overload promotes arrhythmias via reactive oxygen species (ROS) production and mitochondrial membrane potential ($\Delta\Psi_m$) depolarization, opening the mitochondrial permeability transition pore (mPTP) and promoting Ca waves. ROS levels were measured by DCF fluorescence. Perfusion with Fe^{2+} (1 – 10 mM) significantly increased relative ROS levels. For example, 1 mM Fe^{2+} raised ROS levels 465 ± 14% compared to the control ($p < 0.01$). TMRM fluorescence decrease was used as an index of $\Delta\Psi_m$ depolarization. Fe^{2+} significantly decreased TMRM fluorescence (1 mM: 8.1 ± 2.3%; 10 mM: 94.8 ± 2.9 %), compared to the baseline in myocytes, indicating $\Delta\Psi_m$ depolarization by Fe^{2+}. Intracellular Ca^{2+} was imaged with Fluo-4-AM. Spontaneous Ca waves were induced in high external Ca^{2+} (4 mM). Furthermore, Fe^{2+} significantly increased the rate of Ca waves: 1 mM Fe^{2+} significantly increased the rate from 23.9 ± 1.7 to 39.8 ± 7.6 min^{-1} ($p < 0.05$). Fe^{2+}-promoted Ca waves were significantly reduced by the mPTP inhibitor cyclosporine A (CsA; 1 μM). ECGs were recorded from Langedorff-perfused hearts. Arrhythmia induction testing was conducted through programmed S_1-S_2 stimulation. Hearts treated with Fe^{2+} (1 mM) alone significantly increased arrhythmia scores compared to hearts pretreated with CsA prior to Fe^{2+} ($p < 0.05$), indicating mPTP inhibition can ameliorate the proarrhythmic effects of iron-overload. These observation were confirmed in the cyclophilin D knockout (CypD KO) mice, in which mPTP opening is impaired. In conclusion, our results suggest that exogenous Fe^{2+} treatment increases ROS production, depolarizes the $\Delta\Psi_m$, opens the mPTP, and promotes Ca waves and arrhythmogenesis.