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Mitochondrial ROS Production Contributes to Generation of Ca Waves During Beta-Adrenergic Receptor Stimulation in Rabbit Ventricular Myocytes

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Beta-adrenergic receptor (beta-AR) activation leads to positive inotropic effect but can also increase diastolic sarcoplasmic reticulum (SR) Ca release in the form of pro-arrhythmic Ca waves. Beta-AR activation increases cellular energy demand and mitochondrial reactive oxygen species (ROS) production. We investigated the role of mitochondrial ROS in the generation of Ca waves during beta-AR stimulation. In electrically stimulated myocytes, application of ISO (0.1 microM) led to the occurrence Ca waves during diastole. The frequency of Ca waves increased over time during ISO application, with a particular steep increase after 6 minutes. Frequency of ISO-mediated Ca waves positively correlated with mitochondrial ROS production measured with Mito-SOX (a dye specifically targeted to mitochondria). The mitochondria specific antioxidant Mito-Tempo (5 microM) effectively prevented the ISO-mediated ROS production, as well as ISO-mediated Ca waves. Inhibition of the mitochondrial respiration with rotenone (0.3 microM) produced similar preventive effects on mitochondrial ROS production and Ca wave generation. Measurements of intra-SR free Ca ([Ca]SR) showed an initial increase of [Ca]SR (SR Ca load) followed by a gradual decline over time during ISO application. This decline of [Ca]SR was the result of increased SR Ca leak particularly in the form of Ca waves. Mito-Tempo did not affect the initial increase in SR Ca load, however, the ROS scavenger significantly prevented the [Ca]SR decline in the presence of ISO. Furthermore, Mito-Tempo significantly reduced ISO-mediated augmentation of SR Ca leak. These results suggest that mitochondrial ROS production play an important role in the generation of Ca waves during beta-AR stimulation.

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Calcium-Mediated Arrhythmia Substrates Associated with Oxidative Stress during Myocardial Infarction

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Following myocardial infarction (MI), ventricular arrhythmias commonly originate from the MI border zone (BZ) but the cause for this is not clear. Increased oxidative stress is a hallmark of MI and an important mechanism of intracellular calcium dysregulation. We hypothesize that following MI, increased oxidative stress in the infarct BZ creates a substrate for calcium-mediated arrhythmias. Methods: Male Lewis rats (n=12) underwent ligation of the left-anterior descending artery. At 4 weeks, hearts were isolated and high-resolution optical mapping of intracellular calcium (Indo-1AM) and reactive oxygen species (ROS, dichlorofluorescein diacetate) were performed. Blebbistatin (7μM) was used to eliminate motion artifact. Calcium transient alternans (CaT_Alt) and multicellular-spontaneous calcium release (mSCR) were induced by rapid pacing (200–670 bpm) before and after the CaMKII blocker KN-93. Sites within 2mm of the anatomical scar border where designated as BZ. All other sites in the mapping field outside the BZ and scar were considered remote. Results: CaT_Alt and mSCR activity were significantly greater in the MI BZ ($15 \pm 3\%$, $20 \pm 2\%$) as compared to remote sites ($3.3 \pm 2\%$, $p<0.01$; $9.0 \pm 2\%$, $p<0.001$), respectively. Additionally, ROS density was increased by $283 \pm 53\%$ ($p<0.001$) in the BZ compared to remote regions, which was confirmed offline by tissue sample analysis. Interestingly, treatment with KN-93 significantly decreased CaT_Alt in the BZ by $50 \pm 10\%$ ($p<0.05$) but did not decrease mSCR activity. Conclusions: These results demonstrate that increased ROS in the infarct BZ is associated with a significant increase in calcium-mediated arrhythmia substrates (CaT_Alt and mSCR). In addition, CaMKII activation may be a mechanism of CaT_Alt but not spontaneous calcium release in the BZ, suggesting multiple calcium regulatory targets of oxidative stress associated with MI.

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ROS Dependent Modulation of Calcium Sparks in Cardiomyocytes

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Mitochondrial regulation of cytosolic calcium ($[Ca^{2+}]_i$) is thought to depend on the mitochondrial inner membrane potential ($\Delta\Psi_m$). $\Delta\Psi_m$ arises from activity of the electron transport chain and is thought to play a critical role in all ion movements across the inner membrane. With $\Delta\Psi_{mito} \approx -150$ mV to -200 mV, there is clearly a strong electrochemical potential for the movement of Ca^{2+} from the cytosol (about 100 nM) into the matrix (around 100 nM). If significant rapid Ca^{2+} influx occurs, then $\Delta\Psi_{mito}$ per se should influence the time-course, frequency, magnitude and other characteristics of Ca^{2+} sparks and the $[Ca^{2+}]_i$ transients. Tetramethylrhodamine methyl ester (TMRM) was used to monitor $\Delta\Psi_{mito}$ in freshly isolated rat cardiomyocytes and photon stress was used to depolarize the mitochondria. In addition to the changes in electrochemical potential for Ca^{2+} entry, depolarization of mitochondria was associated with an increase in cellular reactive oxygen species (ROS) as measured by DCF (as has been previously reported by several laboratories). Quantitative analysis of these findings permits us to separate the influence of each on the changes in Ca^{2+} signaling observed. Consistent with findings by Zhou et al., 2011, we report a role for local [ROS] in altering Ca^{2+} signaling.

References
Zhou, L., Aon., M.A., Lui, T., O'Rourke, B. Dynamic modulation of Ca^{2+} sparks by mitochondrial oscillations in isolated guinea pig cardiomyocytes under oxidative stress. *JMCC*. 2011. 51(5):632–9.

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Ryanodine Receptor Use-Dependent Block Suppresses Ca^{2+} Waves in Permeabilized Casq2-/- and RyR2-R4496C Myocytes

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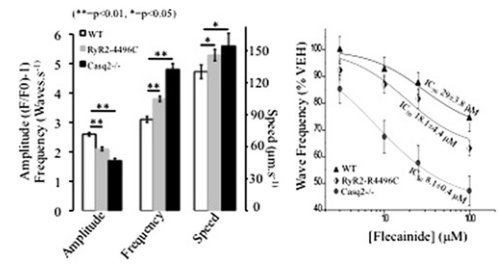
Background-Casq2 and RyR2 mutations can both cause catecholaminergic-polymorphic ventricular tachycardia (CPVT). Leaky RyR2s (increased open probability) generate Ca^{2+} waves, delayed afterdepolarizations and ventricular arrhythmias. We hypothesize that the RyR2 open-state inhibitors flecainide (FLEC) and R-propafenone (RPROP) abolish this sequence of events in both CPVT genotypes.

Methods and Results-Using confocal microscopy, we recorded Ca^{2+} waves in permeabilized myocytes from casq2-/-, RyR2-R4496C and wild-type (WT) mice. Casq2-/- myocytes showed the highest wave frequency and speed and the lowest amplitude, RyR2-R4496C exhibited intermediate values and WT had the lowest values (Figure). We next obtained FLEC and RPROP concentration-response curves and calculated IC_{50} and efficacy of wave inhibition. Both FLEC and RPROP reduced all wave parameters with higher potency (lower IC_{50}) and efficacy in casq2-/- and RyR4496C compared to WT (FLEC: IC_{50} 12μM vs 22μM vs 49μM; efficacy 43% vs 29% vs 21% for casq2-/-, RyR2-R4496C and WT respectively. RPROP: IC_{50} 18μM vs 28μM; efficacy 36% vs 17% for RyR2-R4496C and WT respectively; n=10-60 cells per group).

Conclusion-RyR2 activity determines the potency and efficacy of open-state blockers for suppressing arrhythmogenic Ca^{2+} waves in permeabilized myocytes from casq2-/- and RyR2-R4496C CPVT models.



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Ca^{2+} Wave Velocity in Cardiomyocytes is Regulated by Ryanodine Receptor Ca^{2+} Sensitivity and SR Ca^{2+} Content

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Arrhythmias can be elicited by sudden release of Ca^{2+} from the sarcoplasmic reticulum (SR) via the SR Ca^{2+} release channel (RyR) in cardiomyocytes. Such release may initiate a self-propagating process called a Ca^{2+} wave, which may trigger a spontaneous action potential. We hypothesized that likelihood of arrhythmia is augmented when waves propagate more rapidly, and investigated the role of RyR Ca^{2+} sensitivity and SR Ca^{2+} content. Ca^{2+} waves were studied in isolated ventricular cardiomyocytes from mice using confocal microscopy and Ca^{2+} fluorescence. Waves preceding spontaneous action potentials propagated more rapidly than those that did not generate action potentials ($p<0.05$). Thus, mechanisms controlling wave speed may determine arrythmogenic potential. We investigated effects of increased RyR Ca^{2+} sensitivity by rapidly exposing cells to 1 mM caffeine. The first wave