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Modeling the Effects of Genetic Manipulations of Calsequestrin on Local Calcium Release and Depletion in Cardiac Myocytes

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situ (an SCR) occurs in a synchronized fashion; however, the mechanism responsible for synchronizing SCR activity in coupled myocardium is unknown. Since others have reported that Ca can diffuse through gap junctions (GJ), we hypothesize that uncoupling cells by blocking GJ will desynchronize, and attenuate SCR activity. Methods: To test this hypothesis high resolution optical mapping of Ca (Indo-1AM) from the anterior surface of the Langendorff perfused guinea pig heart (n=5) was performed in hearts under high Ca conditions ([Ca2+]e=5.5mM), with and without carbonoxolone (CBX, 50μM) to reduce GJ coupling. Endocardial cryoablation were performed to eliminate Purkinje fibers and cytochalasin-D (7μM) was administered to remove motion artifact. Fifteen seconds of rapid pacing (350-160 ms cycle length) followed by a pause was used to induce SCR activity. Results: In all preparations, SCR activity was observed across the entire mapping field before and after CBX. With CBX, the amplitude of SCR activity increased (+14.8%, $p < 0.05$) and its time to peak occurred earlier (−11.2%, $p < 0.01$) compared to no CBX. CBX also decreased the range of local SCR time to peaks across the mapping field (−17.2%, $p < 0.05$), suggesting that uncoupling myocytes synchronizes spontaneous calcium release across cells. There was no statistical difference in the occurrence of triggered activity before and during CBX. Conclusions: These results demonstrate that the occurrence of spontaneous calcium release in tissue (an SCR) does not require Ca diffusion though GJs. In fact, spontaneous calcium release in tissue is paradoxically enhanced during GJ inhibition.

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Synchronization of Spontaneous Calcium Release Waves Among Myocytes in Intact Heart Determines the Magnitude of Delayed Afterdepolarizations and Triggered Activity

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Intracellular Ca waves occur as the result of spontaneous Ca release (SCR) during Ca overload. Ca waves activate Na-Ca exchange, causing delayed afterdepolarizations (DAD) which can achieve threshold and produce triggered extrasystoles. It is not known how these single cell events depolarize enough myocytes in intact heart to produce a triggered beat. We combined experimental observations with computer simulations to explain how SCR synchronization among myocytes brings a critical tissue mass to threshold. Confocal microscopy was used to measure SCR waves in groups of myocytes in the LV epicardium of rat hearts loaded with fluo-4AM. Contraction was abolished with cytochalasin-D and blebbistatin. Raising extracellular [Ca] and rapid pacing protocols were used to increase sarcoplasmic reticulum (SR) Ca load and induce Ca waves. As Ca load increased, the number of myocytes giving waves increased. Both the wave latency and the variability (SD) of wave latency decreased with increasing Ca load. Similar results were obtained in isolated rat ventricular myocytes, indicating that the reduction in latency interval and variability represent intrinsic properties of SR release in Ca overload. Computer simulations demonstrate that decreasing wave latency and variability determine the rate and magnitude of increased cytoplasmic [Ca] and therefore determine the timing and magnitude of the DAD. The synchrony of SCR waves among myocytes therefore determines the likelihood of achieving threshold and producing a triggered beat. These results demonstrate that intrinsic properties of SR Ca release are responsible for Ca wave synchronization during Ca overload, causing DADs to reach threshold and produce triggered arrhythmias.

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Looking at the Trigger for CICR During Rat Cardiac Action Potentials

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It is generally accepted that cardiac CICR is triggered by L-type Ca2+ channel activation during the action potential. At the microscopic level, CICR is revealed by calcium sparks whose activation by single L-type Ca2+ channels has been demonstrated. In this study, we have tried to visualize the Ca2+ influx (that triggers CICR) during normal action potentials and when the L-type Ca2+ channel is partially blocked and/or during the application of an L-type Ca2+ channel gating modifier FPL64176. Using Fluo-4 and high speed confocal line scanning we have detected the rise in Ca2+ that precedes SR Ca2+ release. When ~90% of L-type Ca2+ channels are blocked with 10 uM nifedipine, L-type Ca2+ influx is seen as an increase in fluorescence of ~2 %/ms, which is 8% of the rate of rise of Ca2+ associated with Ca2+ sparks. This is associated with a latency for Ca2+ spark activation of typically 9 ms; assuming a Kd for fluo-4 of 800 and a resting Ca2+ of 65nM the trigger in these conditions equates to a current of ~1 nA for a 30 pL cell. An unexpected finding was that FPL64176 did not dramatically decrease the latency for Ca2+ spark activation, as might be expected if many short L-type Ca2+ channel openings are required to activate CICR. In addition, no 'sparklets' were observed as might be expected if L-type Ca2+ channels are located almost exclusively in the junctional space and Ca2+ sparks activate with minimal delay.

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Modeling the Effects of Genetic Manipulations of Calsequestrin on Local Calcium Release and Depletion in Cardiac Myocytes

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Cardiac calsequestrin (CASQ2), a Ca buffer localized to the junctional SR (jSR) of cardiac myocytes, is known to bind to the RyR-triadin-junctin complex, participate in the luminal regulation of RyRs, and modulate Ca spark activity. To investigate the functional role of CASQ2 during spontaneous Ca sparks, we constructed a hybrid CTMC-ODE stochastic simulation of a Ca release site model composed of 100 Lee-Keener RyRs [J. Theor. Biol. 253:668-679, 2008] that includes Ca activation, Ca inactivation, CASQ2-RyR binding, and the dynamics of myoplasmic and luminal domain Ca and buffer concentrations. Myoplasmic and network SR [Ca] were determined by balancing the simulated average release flux and reuptake mediated by SERCA. The model reproduces average properties of spontaneous sparks in normal myocytes including spark amplitude, blink nadir, and junctional SR recovery time. Parameter studies were performed to interpret the effects of known arrhythmogenic CASQ2 mutants [Terentyev et al., Biophys. J. 95(4):2037-2048, 2008] on average spark properties. Increases in the total amount of CASQ2 resulted in increased spark amplitudes and increased jSR recovery times (observed in myocytes overexpressing wildtype CASQ2); shallower nadirs were observed in simulations but not experiment. Increasing the Kd of CASQ2 and Ca binding decreases spark amplitude and jSR recovery time (observed in myocytes expressing the dominant negative mutation CASQ2-DEL that suppresses CASQ2-Ca binding); in simulations (but not experiment) release-reuptake balance leads to increased network SR [Ca]. Increasing the Kd of CASQ2 and RyR binding is associated with decreased network SR [Ca], as in myocytes expressing CASQ2-R33Q, a variant with dominant negative effects on interactions of CASQ with RyRs. The implications of these results to mechanism of release regulation by CASQ2 will be discussed.

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Loss of Calsequestrin (Casq2) in the Heart Increases Spark Frequency and Alters Spark Properties

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Lack of Casq2 causes spontaneous Ca2+ releases from the sarcoplasmic reticulum (SR) and catecholaminergic-polymorphic ventricular tachycardia. We tested the hypothesis that lack of Casq2 alters elementary Ca2+ release events (Ca2+ sparks) by comparing spark properties of ventricular myocytes isolated from wild-type (WT) and Casq2 null (Casq2-/-) mice. Sparks were recorded in line-scan mode and analyzed with SparkMaster. Spark mass was calculated as amplitude×1.206×FWHM3. SR Ca2+ load was measured by rapid application of caffeine. In intact Casq2-/- myocytes stimulated with 100nM isoproterenol, spark amplitude and spark width (FWHM) increased compared to WT (0.77 ± 0.019 vs. 0.39 ± 0.02 Δ(F/Fo), and 2.4 ± 0.03 vs. 1.3 ± 0.05μm, 1095 and 105 sparks respectively), resulting in larger spark mass (20 ± 1.4 vs. 2.2 ± 0.4 Δ(F/Fo).μm3). Time-to-peak and spark duration (FDHM) were 2.5-fold longer and spark frequency was 4-fold higher in Casq2-/- myocytes (2.4 ± 0.2 vs. 0.5 ± 0.08 sparks×100μm-1×s-1). Spark-mediated leak (spark mass×spark frequency) was much larger (47.6 vs. 1.2 Δ(F/Fo) μm3). In saponin-permeabilized myocytes, spark-mediated leak and spark frequency were also higher in Casq2-/- myocytes (249.2 vs. 171.3 Δ(F/Fo)μm3, and 9.5 ± 1 vs. 4.2 ± 1 sparks×100μm-1×s-1, 587 and 333 sparks for Casq-/- and WT respectively), but the differences between Casq-/- and WT were less pronounced compared to intact myocytes. This may be a consequence of the increased spark-mediated SR Ca2+ leak resulting in significantly decreased SR Ca2+ load in permeabilized Casq2-/- cells (−25% at baseline and −31% with 50μM cAMP, n= 10-16 myocytes per group).
Conclusions: Lack of Casq2 in cardiac myocytes increases the spark frequency and the spark-mediated leak. This is still observed in permeabilized cells despite decreased SR Ca2+ load, suggesting that this is due to a primary Casq2