Intracellular Calcium Channels and Calcium Sparks and Waves I

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Dyssynchronous Heart Failure is Associated with Spatially Heterogeneous Spark Density in Left Ventricular Cardiomyocytes

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Dyssynchronous heart failure (DHF) is associated with structural and functional remodeling in cardiomyocytes from subcellular to whole organ level. Several studies have demonstrated remodeling of Ca^{2+} signaling and excitation-contraction coupling. This study aimed at quantification of the subcellular spatial distribution of diastolic local Ca^{2+} release events (sparks) in cardiomyocytes and their remodeling in DHF.

Adult canines were used as control and DHF models. DHF was induced by right ventricular tachypacing at 200 beats per minute. Left ventricular cardiomyocytes were isolated via enzymatic digestion, loaded with Fluo-4 and Di-8-ANEPPS, and imaged with rapid scanning confocal microscopy (Zeiss LSM 5 Duo). Acquisition of 2D image sequences was initiated 460 ms after stimulation of the myocytes. Images were acquired at a xy resolution of 0.1 µm with a field of view of 102.4 µm (x) x 25.6 µm (y). Image sequences comprised 100 frames at 9.3 ms per frame. Image analysis involved cell segmentation and spark detection. We compared spark density in regions 0-10 µm and 10-40 µm from the longitudinal cell end.

Our analyses yielded a similar diastolic spark density in control and DHF cells (0.0119 ± 0.0023 vs 0.0155 ± 0.0027 sparks/µm2/s, respectively). In control cells spark density was homogeneously distributed. In contrast, DHF cells exhibited a decreased spark density within 0-10 µm versus 10-40 µm from the longitudinal cell end (0.0101 ± 0.0039 vs 0.0227 ± 0.0047 sparks/µm2/s, respectively).

DHF is associated with a spatially heterogeneous remodeling of spark density. This finding provides a foundation for understanding basic mechanisms of arrhythmogenesis in heart failure cells. The heterogeneity of spark density may result from heterogeneous structural remodeling, for instance, remodeling of ryanodine receptor clusters and the transverse tubular system.

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Influence of RyR2 Inhibition Kinetics on Calcium Sparks and Waves in a 3D Model of a Cardiac Cell

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Although excess Ca^{2+} release through RyR2 in diastole is a key factor in arrhythmia, not all RyR2 antagonists have an antiarrhythmic action. Flecainide, carvedilol and dantrolene are RyR2 inhibitors with an anti-arrhythmic blocking action whereas tetracaine is a RyR2 inhibitor with a pro-arrhythmic action. Block by these agents is known to have different RyR2 blocking kinetics and different effects on Ca^{2+} sparks and waves. However, it is not clear how the different RyR2 blocking kinetics produce different calcium release properties in intact cells.

We have developed a 3D model of Ca^{2+} dynamics, which is based on realistic morphological parameters and RyR2 kinetics. By including different kinetic models for block of RyR2, we find that open state block of RyR2 stabilises Ca^{2+} release. In contrast, the closed state block of RyR2 has the effect of increasing the positive feedback of Ca^{2+} on SR Ca^{2+} release; the net result is an increase in the amplitude of Ca^{2+} sparks, destabilization of SR Ca^{2+} cycling.

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Effects of Triad Geometry and RyR Gating Scheme on Simulated Skeletal Muscle Sparks

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Computer models of calcium sparks have been an important tool in their investigation, especially in understanding the way how the regenerative calcium signal is initiated and terminated.

The aim of the present work was to use an existing 3D model with distributed sarcoplasmic reticulum (SR) for cardiac sparks and demonstrate the effects of altered calcium release unit (CRU) geometry and ryanodine receptor (RyR) gating on the generation and termination of calcium release events in skeletal muscle.

First, only the geometry of CRU was modified to match the triad structure found in skeletal muscle cells. This was achieved by arranging RyRs in double rows in the terminal cisternae (TC) membrane on each side of the T-tubule and by reducing the diameter of the T-tubule. Such a model was unable to produce the regenerative phase of the calcium spark following the stochastic opening of a single RyR. Next, the gating of RyRs was modified by shifting its calcium sensitivity to the left to mimic the baseline activity of skeletal-type RyR. This model reproduced the rising phase and the peak of the spark, but was unable to model its complete termination as observed in events measured in Saponin-permeabilized skeletal muscle fibers. Refilling of the TC had to be severely constrained or more complex gating schemes had to be introduced in order to make the termination of simulated sparks similar to that seen in the experiments.

These results indicate that both the exact geometry of CRU and the gating scheme of RyR contribute to the generation and termination of localized calcium release events to a great extent and, therefore, these details have to be taken into account when their characteristics and role are examined in skeletal muscle.

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Subcellular Ca Channel Distribution and Ca Alternans in Atrial Myocytes

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Unlike ventricular myocytes, atrial myocytes have a much less well-developed T-tubular network, especially in rodents. In this study, we carried out computer simulations to investigate the consequences of a T tubular network on the development of spatially discordant Ca alternans in atrial myocytes. Our study was motivated by experimental observations in isolated mouse atrial myocytes, which express both Cav1.2 and Cav1.3 channels. Knocking out Cav1.3, but not Cav1.2, promoted spatially discordant Ca alternans in which Ca amplitude alternates out-of-phase in different regions of the myocyte. Based on observations in sinoatrial myocytes that Cav1.2 channels are mainly distributed on the surface of the cell while Cav1.3 channels are more uniformly distributed throughout the whole cell, in our computer model, we coupled both types of channels to Ca release units on the surface of the cell, but only Cav1.3 to Ca release units in the interior of the cell. The cell model was paced with a clamped voltage waveform at a pacing cycle length of 300 msec. Under control, Ca transient was normal, and no alternans were observed. When Cav1.2 channels were removed, the Ca transient amplitude decreased, but alternans did not occur. When Cav1.3 channels were removed, however, Ca alternans occurred. The magnitude of the whole-cell Ca transient alternans varied over time, exhibiting a modulated pattern. The line scan showed spatially discordant alternans. These simulations show that the subcellular distribution of Ca channels in relation to Ca release units has important effects on the proclivity for spatially discordant Ca alternans. Specifically, a high fraction of orphaned RyR clusters in the center of the myocyte facilitates spatially discordant Ca alternans.

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Complex Early and Delayed Afterdepolarization Dynamics caused by Voltage-Calcium Coupling in Cardiac Myocytes

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Early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs) are voltage oscillations known to cause cardiac arrhythmias. Complex voltage oscillations that manifest as EADs and DADs have been widely observed in experimental studies. However, the mechanisms leading to these complex EAD and DAD behaviors have not been well studied. Here we performed computer simulations using an AP model with detailed spatiotemporal calcium (Ca) cycling incorporating stochastic openings of Ca channels and ryanodine receptors to investigate the effects of voltage-Ca coupling on EAD and DAD dynamics. Simulations were complemented by experiments in mouse ventricular myocytes. We investigated the following aspects of bidirectional voltage-Ca coupling on EAD and DAD dynamics: 1) modulation of phase-2 EAD dynamics by Ca cycling properties; 2) modulation of DAD dynamics by voltage; 3) complex EAD-DAD dynamics induced by bidirectional voltage-Ca coupling; 4) the role of spontaneous Ca oscillations