effect on RyR2 SR Ca2+ release channels. This may be in part responsible for the increased arrhythmia susceptibility in Casq2-/- mice.

550-Pos

Adaptive Retuning of Small Ca2+ Fluxes in Cardiomyocyte Syncytia Predicts the Response To Pro-Arrhythmic Stimuli

Nicole C. Silvester, Philip M. Ashton, Steven R. Barberini-Jammaers, F Anthony Lai, Christopher H. George.
Cardiff University, Cardiff, United Kingdom.

Cardiac cell phenotype is driven by the interplay between large 'global' Ca^{2+} events (transients) and much smaller, spatially restricted Ca^{2+} signals. Our previous studies revealed a link between these low amplitude inter-transient Ca^{2+} fluxes, and the spatiotemporal organisation of the intra- and intercellular global Ca^{2+} transients. We hypothesised that chronic cardiac cell dysfunction may be underpinned by the incremental resetting of these Ca^{2+} fluxes that ultimately leads to perturbed Ca^{2+} homeostasis. We constructed a database comprising more than one thousand independent manipulations of the Na/K ATPase/NCX systems in spontaneously oscillating, electrically-coupled HL-1 cardiomyocytes in which inter-transient Ca^{2+} signal noise, but not mean steady state Ca^{2+} levels had been precisely modulated. Data was interrogated using our SALVO program that generates a detailed spatiotemporal profile of intra- and intercellular Ca^{2+} signals. We determined a bell-shaped relationship between incremental increases in intracellular Ca^{2+} fluxes and the propensity for intercellular dyssynchrony. Modest but sustained elevations in inter-transient Ca^{2+} fluxes protected cell syncytia from manoeuvres designed to perturb intercellular synchrony. This protective effect did not occur if inter-transient Ca^{2+} fluxes had been acutely retuned (< 20 minutes) suggesting that cellular adaptation mechanisms were involved in these phenomena. In contrast, larger elevations in inter-transient Ca^{2+} fluxes exacerbated intercellular dyssynchrony in response to pro-arrhythmic stimuli. All alterations in steady-state inter-transient Ca^{2+} fluxes were associated with altered SERCA activity and decreased cellular levels of ATP, consistent with the concept that pathological alterations in Ca^{2+} homeostasis are linked to metabolic dysfunction. Our data supports the hypothesis that small Ca^{2+} fluxes tune global Ca^{2+} events and dictate the propensity of cell syncytia to arrhythmogenic perturbation.

551-Pos

Automated Reduction of Calcium Release Site Models Via State Aggregation

Yan Hao, Peter Kemper, Gregory D. Smith.
The College of William and Mary, Williamsburg, VA, USA.

Realistic simulations of local control of cardiac EC coupling require Ca release unit (CaRU) models generated using Markov chain models of L-type Ca channels and ryanodine receptors (RyRs) as ''building blocks.'' Because compositionally defined CaRU models result in a combinatorial explosion of release site states, most whole cell simulations to date have utilized ad hoc CaRU models in an effort to maintain computational efficiency (e.g., modeling RyR clusters as a ''megachannel''). To overcome this state-space explosion, we have implemented, validated, and benchmarked several methods for automated reduction of mechanistic CaRU models that feature an automated process of state aggregation and evaluation of reduction error through comparison of the jump probability matrices of full and reduced models. When there is separation of time scales in the single channel model (e.g., fast activation and slow inactivation), we perform numerical fast/slow reduction by categorizing rate constants in the single channel model as either fast or slow, aggregating states in the expanded CaRU model that are connected by fast transitions, and calculating transition rates between lumped states using the conditional probability distribution of states within each group. For large problems where the conditional distributions can not be directly calculated from the full model, we employ iterative aggregation/disaggregation to calculate conditional distributions in a memory-efficient fashion. For problems without time scale separation, how states should be aggregated to yield good reductions can not be determined a priori. Consequently, we implemented a genetic algorithm that evolves potential schemes for state aggregation, ultimately yielding simplified CaRU models with low reduction error. We demonstrate that such automated CaRU reduction procedures can be used to accelerate multiscale models of local control of CICR in cardiac myocytes.

552-Pos

Calcium Sparks and Homeostasis in a Minimal Model of Local and Global Calcium Responses in Quiescent Ventricular Myocytes

Jana M. Hartman[1], Eric A. Sobie[2], **Gregory D. Smith**[1].
[1]The College of William and Mary, Williamsburg, VA, USA, [2]Mount Sinai School of Medicine, New York, NY, USA.

We present a minimal whole cell model that accounts for both local and global aspects of Ca signaling in quiescent ventricular myocytes. This includes random spontaneous Ca sparks, changes in myoplasmic and SR [Ca] mediated by the balance of stochastic release and reuptake by the SR, and the feedback of myoplasmic and SR [Ca] on spark frequency. We correlate our modeling results with recent experiments showing that tetracaine, an inhibitor of RyRs, causes a transient suppression of Ca sparks followed by an increase in SR [Ca], partial recovery of spark frequency, and an increase in Ca spark duration [Zima et al. Biophys. J. 94(5):1867, 2008]. Using release sites composed of clusters of two-state RyRs with Ca activation (but no Ca inactivation or luminal regulation), we find that mean spark duration is a biphasic function of the RyR Ca-activation rate constant (closed dwell time). In spite of the fact that spark duration is biphasic, the aggregate release flux and bulk SR [Ca] overload are monotone functions of RyR closed dwell time. The same degree of SR overload and balance of stochastic release and reuptake can be achieved by high-frequency short-duration or low-frequency long-duration Ca sparks, depending on the mechanism of RyR inhibition (i.e., whether RyR open probability is reduced by increasing the closed dwell time or decreasing the open dwell time). Our calculations suggest that the hidden flux mediated by stochastic Ca release events below detection threshold are suppressed more strongly by tetracaine than observable release events.

553-Pos

Mechanisms of Spontaneous Calcium Wave Generation During Beta-Adrenergic Stimulation in Rabbit Ventricular Myocytes

Timothy L. Domeier, Lothar A. Blatter.
Rush University, Chicago, IL, USA.

The beta-adrenergic signaling pathway represents the principal positive inotropic mechanism of the heart. While the effects of beta-adrenergic stimulation on L-type Ca channel Ca influx and SERCA-mediated sarcoplasmic reticulum (SR) Ca uptake are well established, the effects on SR Ca release through ryanodine receptor (RyR) release clusters remains highly controversial. Here, we examine SR Ca release in rabbit ventricular myocytes in the form of spontaneous Ca waves during beta-adrenergic stimulation with isoproterenol under controlled cytosolic and SR [Ca]. Cytosolic Ca was monitored using high-affinity Ca indicators indo-1 or rhod-2, while SR Ca was measured directly using the low-affinity Ca indicator fluo-5N or indirectly using the amplitude of the cytosolic Ca transient in response to 10 mM caffeine. Under control conditions, Ca waves were not observed following rest from 0.75 Hz pacing. In the presence of isoproterenol (500 nM), SR Ca content increased by 34% and spontaneous Ca waves were observed in 67% of cells during rest after pacing. However, when post-rest cytosolic Ca and SR Ca content were experimentally matched to control conditions using low extracellular Ca (100 uM versus 2 mM) and SERCA inhibition (7.5 uM cyclopiazonic acid), spontaneous Ca waves were never observed in the presence of isoproterenol. In contrast, pharmacological sensitization of the RyR with 250 uM caffeine induced Ca waves under control conditions (8/12 cells) and in the presence of isoproterenol at matched cytosolic Ca and SR Ca content (7/12). Together, these data suggest that spontaneous Ca release during beta-adrenergic stimulation is a result of increased RyR sensitivity in response to increased SR Ca content, and is not due to direct alterations in RyR function by the beta-adrenergic signaling cascade.

554-Pos

Alterations in Ca^{2+} Sparks and T-Tubules Promote Slowed, Dyssynchronous Ca^{2+} Release in Failing Cardiomyocytes

William E. Louch, Halvor K. Mørk, Karina Hougen, Ivar Sjaastad, Ole M. Sejersted.
University of Oslo, Oslo, Norway.

In heart failure, cardiomyocytes exhibit slowing of the rising phase of the Ca^{2+} transient which contributes to the impaired contractility in this condition. We investigated the underlying mechanisms in a murine model of congestive heart failure (CHF). Myocardial infarction (MI) was induced by left coronary artery ligation, and at 10 weeks post-MI, mice exhibited symptoms of CHF including reduced cardiac function and increased lung weight. Cardiomyocytes were isolated from viable regions of the septum, and septal myocytes from SHAM-operated mice served as controls. Confocal line-scan imaging revealed a slowed rate of rise of Ca^{2+} transients (fluo-4 AM, 1 Hz) in CHF cells, which largely resulted from spatially non-uniform Ca^{2+} release. Ca^{2+} sparks recorded in resting myocytes were also slower to peak in CHF than SHAM (11.5 ± 0.6 ms vs 9.5 ± 0.6 ms, $P<0.05$) and longer lasting (FWHM=24.5 ± 0.7 ms vs 21.6 ± 1.0 ms, $P<0.05$). The mean increase in these measurements resulted from a sub-population of sparks in CHF cells with very long rise times but small amplitudes. Local Ca^{2+} transients (width=2 μm) measured at the same coordinates as these sparks were also slow to rise, indicating that altered Ca^{2+} spark kinetics contributed to the dyssynchronous Ca^{2+} release pattern in CHF. As well, di-8-ANEPPS staining revealed