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.

546-Pos

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

J. Andrew Wasserstrom[1], Yohannes Shiferaw[2], Satvik Ramakrishna[1], Heetabh Patel[1], Matthew J. O'Toole[1], Amanda Pappas[1], Asrar Khan[1], Rishi Arora[1], Gary L. Aistrup[1].
[1]Northwestern University Feinberg School of Medicine, Chicago, IL, USA, [2]California State University - Northridge, Northridge, CA, USA.

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.

547-Pos

Looking at the Trigger for CICR During Rat Cardiac Action Potentials

Cherrie H.T. Kong, Mark B. Cannell.
University of Auckland, Auckland, New Zealand.

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.

548-Pos

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

Ryan P. Carpenter[1], Sandor Gyorke[2], Gregory D. Smith[1].
[1]The College of William and Mary, Williamsburg, VA, USA, [2]The Ohio State University, Columbus, OH, USA.

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.

549-Pos

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

Eleonora Savio Galimberti[1], Sabine Huke[1], SylvainJ. Le Marchand[2], David W. Piston[2], Bjorn C. Knollmann[1].
[1]Vanderbilt University, Department of Medicine, Division of Clinical Pharmacology, Nashville, TN, USA, [2]Vanderbilt University, Department of Molecular Physiology & Biophysics, Nashville, TN, USA.

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

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