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**

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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**

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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**

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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**

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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**

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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 ($11.5 \pm 0.6$ ms vs $9.5 \pm 0.6$ ms, $P<0.05$) and longer lasting (FWHM=$24.5 \pm 0.7$ ms vs $21.6 \pm 1.0$ 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

disorganized T-tubular structure in failing myocytes, which is also known to promote $Ca^{2+}$ release dyssynchrony. Specifically, we observed irregular gaps between adjacent tubules where $Ca^{2+}$ release was markedly delayed, occurring only after $Ca^{2+}$ diffusion from regions where tubules were present. Thus, slowed and dyssynchronous $Ca^{2+}$ release in failing myocytes results from a combination of altered ryanodine receptor function and T-tubule disorganization. We suggest that the sub-population of slow, small amplitude $Ca^{2+}$ sparks in CHF may represent ryanodine receptors which are functionally uncoupled from their neighbours.

**555-Pos**

**Cardiotrophin-1: Another "player" in Cardiac Calcium Handling**

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Cardiotrophin-1 (CT-1) is a cytokine member of the interleukin-6 superfamily produced by cardiomyocytes and fibroblasts in the heart, in situations of haemodynamic overload, or in the presence of humoral factors as aldosterone. CT-1 is able to induce hypertrophic growth and dysfunction of cardiomyocytes *in vitro*. Moreover, plasma levels of CT-1 are elevated in patients with cardiac hypertrophy and heart failure (HF) and correlated with the severity of the disease. On the other hand, it is well established that alterations in calcium handling are involved in cardiac dysfunction during HF. However, it is yet unknown whether CT-1 modulates $Ca^{2+}$handling in cardiomyocytes. Here we analyzed CT-1 effects on $[Ca^{2+}]_i$ handling in rat single cardiomyocytes. The L-type calcium current ($I_{CaL}$) was registered using whole-cell patch-clamp technique. Intracellular calcium $[Ca^{2+}]_i$ transients and $Ca^{2+}$ sparks were viewed by confocal miscroscopy in cardiomyocytes loaded with the fluorescence $Ca^{2+}$ indicator Fluo-3 AM. Treatment of cardiomyocytes with 1 nM CT-1 for 30 min induced a significant increase in $I_{CaL}$ density compared to control cells (at $-10$ mV: $-16.0 \pm 0.9$ vs. $11.9 \pm 0.7$ pA/pF; $P < 0.01$). The activity of ryanodine receptors (RyRs), estimated by $Ca^{2+}$ spark frequency, was significantly increased in cardiomyocytes treated with CT-1 ($Ca^{2+}$ sparks$\cdot s^{-1}\cdot 100\ \mu m^{-1}$: $2.3 \pm 0.3$ vs. $4.3 \pm 0.5$; $P < 0.01$). Moreover, we observed that the increase in the total $Ca^{2+}$ spark frequency produced by CT-1 could be attributable to the increased propensity of some clusters of RyR to release $Ca^{2+}$repetitively. Thus, we conclude that CT-1 is able to alter $Ca^{2+}$ handling in isolated cardiomyocytes, enhancing the $Ca^{2+}$ influx through L-type $Ca^{2+}$ channel and the $Ca^{2+}$release from sarcoplasmic reticulum through RyRs.

**556-Pos**

**Occurrence of Spontaneous Sparks in Ventricular Myocytes From Junctional and Non-junctional Ryr Clusters**

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In isolated rabbit ventricular myocytes, we found a significant number of ryanodine receptor (RyR) clusters that are not associated with the sarcolemma (non-junctional RyRs). The contribution of non-junctional RyR clusters to calcium transients is unclear. Here, we investigated if these non-junctional RyRs are able to produce spontaneous local calcium release events (sparks), and compared the probability of non-junctional versus junctional sparks. We imaged spontaneous sparks in cells loaded with fluo-4 and bathed in Tyrode solution with dextran (Molecular Weight: 10 kDa) linked to Texas Red dye. We evoked spontaneous sparks using field stimulation in the presence of 1 μM isoproterenol and 4 mM calcium. After 5 stimuli applied with a frequency of 0.5 Hz, we simultaneously imaged the sarcolemma and spontaneous sparks using a line scan confocal microscope (Biorad MRC-1024). Furthermore, a 3D image stack of the Texas Red associated signal was acquired to identify the sarcolemma including the transverse tubular system. We classified sparks as non-junctional if their distance to the sarcolemma is larger than 1 μm. All other sparks were assumed to be junctional. In measurements on 12 isolated cells, 38 sparks (51%) were identified as non-junctional, 36 (49%) as junctional. Our measurements clearly demonstrate that non-junctional RyR clusters are able to release calcium and produce spontaneous sparks. We expect that our approach for distinguishing between non-junctional and junctional sparks underestimates the number of non-junctional sparks. If this is true, the probabilities of the types of sparks are similar to probabilities of the two types of RyR clusters identified in related immunolabeling and microscopic studies. This would suggests that spark generation probability of RyR clusters does not depend on their type.

**557-Pos**

**Alteration of Ryanodine Receptor-Mediated Calcium Release in Heart Failure**

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The decrease in contractility in heart failure associates with impaired cellular $Ca^{2+}$ homeostasis that is in part due to altered ryanodine receptor (RyR) function. We studied properties of sarcoplasmic reticulum (SR) $Ca^{2+}$ release in normal and failing rabbit ventricular myocytes using simultaneous measurements of cytosolic ($[Ca^{2+}]_i$) and intra-SR free $Ca^{2+}$ ($[Ca^{2+}]_{SR}$). At a given SR $Ca^{2+}$ content, fractional SR $Ca^{2+}$ release during action potential stimulation was higher in failing than nonfailing myocytes, suggesting increased sensitivity of RyRs in heart failure. In permeabilized myocytes, SR $Ca^{2+}$ content and $Ca^{2+}$ spark frequency were decreased in heart failure, while $Ca^{2+}$ spark amplitude was similar between failing and nonfailing myocytes. To compare these two groups further, SR $Ca^{2+}$ content was experimentally decreased in nonfailing myocytes to the level observed in failing myocytes using SERCA inhibition. When SR $Ca^{2+}$ content was matched, both $Ca^{2+}$ spark frequency and amplitude were markedly increased in failing myocytes, showing that RyRs are more sensitive to release activation. By monitoring $[Ca^{2+}]_{SR}$ during $Ca^{2+}$ sparks, we also observed that the $[Ca^{2+}]_{SR}$ level for spark termination was significantly lower in myocytes from failing hearts. Because $Ca^{2+}$ sparks are a major contributing factor to diastolic SR $Ca^{2+}$ leak, we compared the properties of SR $Ca^{2+}$ leak in normal and failing myocytes. In failing myocytes SR $Ca^{2+}$ leak was significantly faster, particularly at high $[Ca^{2+}]_{SR}$ where $Ca^{2+}$ sparks are the predominant pathway for SR $Ca^{2+}$ leak. These data show that during the progression of heart failure, modifications to RyRs alter both activation and termination of local SR $Ca^{2+}$ release events. At a given SR $Ca^{2+}$ content these effects may increase fractional SR $Ca^{2+}$ release and preserve contractility during systole, however at the cost of increased diastolic SR $Ca^{2+}$ leak and SR depletion.

**558-Pos**

**Abnormal Intra-Store Calcium Handling and Arrhythmogenesis in Heart Failure**

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Heart failure (HF) patients are known to have increased susceptibility to ventricular arrhythmias. Although abnormal intracellular calcium (Ca) cycling is recognized as an important contributor to the pathogenesis of ventricular arrhythmias, the specific cellular and molecular mechanisms of these arrhythmias remain to be defined. The objective of present study was to investigate the sub-cellular mechanisms of Ca-dependent arrhythmia using time-resolved Ca imaging in the cytosolic and sarcoplasmic reticulum (SR) luminal compartments and the patch-clamp technique in a canine model of tachypacing-induced HF. When rhythmically paced in the presence of the β-adrenergic agonist, isoproterenol, HF myocytes displayed a higher frequency of diastolic Ca waves than control myocytes. In both HF and control myocytes, diastolic Ca waves occurred when [Ca]SR rose above a certain threshold level, which was significantly lower in HF than in control myocytes. Ca signaling refractoriness determined as the time delay between systolic SR Ca depletion and Ca wave initiation was significantly reduced in HF myocytes. Electrical and Ca signaling activities exhibited several distinct potentially arrhythmogenic patterns, including: 1) delayed afterdepolarizations and extrasystolic action potentials (APs) linked to diastolic spontaneous Ca waves; 2) intermittent prolongations of AP duration associated with pre-systolic spontaneous Ca waves and post-systolic triggered Ca waves; and 3) disorganized release uncoupled from myocyte electrical activity. The level of [Ca]SR threshold for spontaneous Ca waves and the time to attain the threshold during the pacing cycle were critical in determining the type of arrhythmogenic abnormality. These experiments suggest a common mechanistic framework for apparently different arrhythmic phenotypes and provide new insights into the relationship between abnormal Ca release and arrhythmogenesis in HF.

**559-Pos**

**Impaired Function of Cardiac Ryanodine Receptors in An Experimental Model of Metabolic Syndrome**

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Metabolic syndrome (MS) has become a global epidemic. In Mexico, the prevalence of MS has increased in the last 10 years together with obesity and type-2