changes in spark properties occurred in absence of significant changes in SR Ca^{2+} content measured by rapid caffeine application. These data suggest that loss of triadin has a drastic effect on spark properties, possibly by altering the number of RyR2 and/or the RyR2 cluster size.

1406-Pos Board B250

Ryanodine Receptor Sensitization Alters Local And Global Sarcoplasmic Reticulum Calcium Release Termination Threshold In Rabbit Ventricular Myocytes

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Dynamic measurements of Ca within the sarcoplasmic reticulum ([Ca]SR) using low-affinity Ca indicators give critical insight into the role of [Ca]SR in Ca release termination. Here we used a low dose of caffeine to examine the effects of ryanodine receptor (RyR) sensitization on local and global SR Ca release in rabbit ventricular myocytes. In field stimulated myocytes (1 Hz), application of 250 μM caffeine caused an initial 44% increase in amplitude of action potential-induced [Ca]SR depletion. This resulted in unloading of the SR (27% decrease in steady state diastolic [Ca]SR) and a lowering of the termination level for global release (28% decrease in systolic [Ca]SR). A single stimulus protocol was used to examine the effects of caffeine on SR Ca release after varying [Ca]SR. At all [Ca]SR levels where release was observed, caffeine increased the [Ca]SR depletion amplitude by lowering the global termination level of release. We next studied the effects of caffeine on local SR Ca release events in permeabilized myocytes by simultaneously measuring cytosolic Ca sparks with associated local [Ca]SR depletions (Ca blinks). Under control conditions, Ca sparks terminated at a fixed [Ca]SR depletion threshold, irrespective of initial [Ca]SR. Application of 200 μM caffeine caused an immediate increase in Ca spark frequency (58%), amplitude (8%), duration (23%), and spatial width (13%), and decreased the Ca blink termination level below the control threshold level. Taken together, these data suggest that sensitization of the RyR produces an increase in SR Ca release by decreasing the [Ca]SR termination level for release at individual release junctions.

1407-Pos Board B251

Altered Ryanodine Receptor Sensitivity after ß-Adrenergic Stimulation of Guinea-pig Ventricular Myocytes

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In cardiac muscle, chronic ß-adrenergic stimulation has been proposed to induce arrhythmogenic Ca^{2+} leak from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyRs). However, the contribution of altered RyR Ca^{2+} sensitivity to the physiological response to sympathetic activation has proven difficult to study in intact cardiomyocytes, mainly due to accompanying alterations in global SR Ca^{2+} content, diastolic cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), and inward Ca^{2+} current (I_{Ca}). Here, we studied whole-cell Ca^{2+} release and spontaneous Ca^{2+} leak (Ca^{2+} sparks) under identical experimental conditions before and after ß-adrenergic stimulation by isoproterenol (Iso), with confocal Ca^{2+} imaging of the fluorescent Ca^{2+} indicator fluo-3. Under whole-cell voltage-clamp conditions, we controlled the extent of SR Ca^{2+} loading by trains of I_{Ca}. UV flash-induced uncaging of Ca^{2+} from DM-nitrophen was employed as an invariant trigger for whole-cell Ca^{2+} release. At matched SR Ca^{2+} content, whole-cell Ca^{2+} release was increased by ~20% in Iso. This enhancement could be attributed to increased spatiotemporal synchronization of Ca^{2+} release, evidenced by more homogenous Ca^{2+} release throughout the cell and higher maximal rate of Ca^{2+} release. When studying spontaneous SR Ca^{2+} leak, very rare Ca^{2+} sparks were seen in control conditions. However, at similar SR Ca^{2+} content and $[Ca^{2+}]_i$, we observed a ~4 fold increase in the number of Ca^{2+} sparks in Iso. Furthermore, a ~4 fold increase in Ca^{2+} spark frequency also became apparent within 2′ in quiescent cells without increased SR Ca^{2+} content. These results support the notion of a sensitized RyR after ß-adrenergic stimulation, both in response to rapid elevations of $[Ca^{2+}]_i$ and at diastolic $[Ca^{2+}]_i$, and by consequence an increased propensity for arrhythmogenic Ca^{2+} leak and Ca^{2+} wave propagation. *Support: SNF.*

1408-Pos Board B252

Beta-Adrenergic Stimulation Does Not Affect Calcium Sparks Refractoriness in Ventricular Myocytes

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Cardiac Ca^{2+} sparks are intracellular Ca^{2+} release events from clusters of ryanodine receptors (RyR2) in the junctional sarcoplasmic reticulum (jSR). L-type Ca^{2+} channels (LCC) are located in the nearby apposing sarcolemma (SL) mainly at the transverse tubules. Cellular depolarization permits local Ca^{2+} influx through LCC that activates RyR2 clusters by Ca^{2+}-induced Ca^{2+}-release (CICR). Ca^{2+} sparks also occur during diastole due to the finite opening rate of the RyR2s that are sensitive to both cytosolic Ca^{2+} ($[Ca^{2+}]_i$) and to SR Ca^{2+} ($[Ca^{2+}]_{SR}$). There is a significant (at least 50%) depletion of jSR Ca^{2+} during each Ca^{2+} spark and this depletion (measured as Ca^{2+} blinks, Brochet et al. 2005) suggests that refractoriness of Ca^{2+} sparks is due to the reduction of $[Ca^{2+}]_{SR}$ (Sobie et al. 2006). Additional factors (beyond $[Ca^{2+}]_i$ and $[Ca^{2+}]_{SR}$) have been reported to affect the opening and closing rates of RyR2. Here we examine RyR2 modulation by protein kinase A (PKA) during Beta-adrenergic stimulation. Studying RyR2 refractoriness is complicated because it overlaps with I_{Ca} restitution and with the slower SR Ca^{2+} uptake by SERCA. We assessed RyR2 refractoriness in permeabilized ventricular myocytes from phospholamban-KO mice by studying repeated spontaneous Ca^{2+} sparks at the same Ca^{2+} release location in the absence and presence of cAMP (10 μM). We observed under control conditions that Ca^{2+} spark amplitude restoration (time constant ~70 ms) was ~2 fold slower than the reported jSR Ca^{2+} refilling. RyR2 phosphorylation did not affect Ca^{2+} sparks amplitude restoration, and the Ca^{2+} spark frequency distribution peak was slightly diminished, with small increases at longer delays. We conclude that under conditions when neither LCC nor $[Ca^{2+}]_{SR}$ can change to influence Ca^{2+} sparks rate or Ca^{2+} sparks refractoriness, RyR2 phosphorylation by PKA activation does not alter RyR2 refractoriness.

1409-Pos Board B253

Phosphorylation of Ryanodine Receptor At Serine-2809 Modulates Sarcoplasmic Reticulum Ca Release in Rabbit Ventricular Myocytes

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The role of protein kinase A (PKA)-dependent phosphorylation of cardiac ryanodine receptor (RyR) is highly controversial. Here we studied a functional link between RyR phosphorylation at serine-2809 (PKA-specific site) and sarcoplasmic reticulum (SR) Ca release and leak in cardiomyocytes. We simultaneously measured intra-SR free Ca ($[Ca]_{SR}$) with Fluo-5N and cytosolic Ca with Rhod-2 in permeabilized rabbit ventricular myocytes. RyR phosphorylation at site serine-2809 was measured with a phospho-specific antibody (Badrilla, UK). We found that cAMP (10 μM) increased Ca spark frequency by ~2.6 times. This effect was associated with an increase in SR Ca load from 0.84 to 1.24 mM. PKA inhibitory peptide (10 μM) abolished cAMP-mediated increase of SR Ca load and spark frequency. When SERCA was completely blocked by thapsigargin, cAMP did not affect RyR-mediated Ca leak. The lack of cAMP effect on RyR function can be explained by almost maximal phosphorylation of RyR at serine-2809 after membrane permeabilization and also argues against the functional importance of another PKA-specific site (serine-2031) for SR Ca release. This high phosphorylation level of RyR could be due to a shift of the balance between protein kinase and phosphatase activity after permeabilization. Preventing this increase in phosphorylation with staurosporine (1 μM) decreased RyR-mediated SR Ca leak. Surprisingly, further dephosphorylation of RyR at serine-2809 with protein phosphatase 1 (PP1; 2 U/ml) markedly increased Ca leak. However, it is important to note that PP1 and staurosporine possibly affected other phosphorylation sites of RyR as well. In conclusion, our results provide direct evidence that RyR phosphorylation at serine-2809 modulates channel function and SR Ca release in rabbit ventricular myocytes.

1410-Pos Board B254

Properties Of Sarcoplasmic Reticulum Ca Leak In Rabbit Ventricular And Atrial Myocytes

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To investigate properties of sarcoplasmic reticulum (SR) Ca leak in ventricular and atrial myocytes, we simultaneously measured Ca sparks and intra-SR free Ca ($[Ca]_{SR}$) after sarcolemma permeabilization. SR Ca leak ($\Delta[Ca]_{SR\text{-}total}/s$) was measured over a wide range of SR Ca loads after complete SERCA inhibition with thapsigargin. We found that in both tissues the ryanodine receptor (RyR) was the main contributor to SR Ca leak. RyR-mediated leak occurred in part as Ca sparks, but also as non-spark-mediated leak. Additionally, there was a component of SR Ca leak that was insensitive to RyR inhibitors. In contrast to ventricular cells, atrial SR had a slower total leak rate mainly due to a smaller contribution from RyR non-spark-mediated leak. As result of this, atrial myocytes had a higher SR Ca load under control conditions (1.4 mM) than ventricular (0.8 mM). RyR type-2 expression levels were similar in both types of cells suggesting that observed differences in SR leak are due to difference in RyR regulation. Activation of IP_3 receptors (IP_3R) increased total SR Ca leak rate more than 2-fold in atrial myocytes, but only slightly affected leak in ventricular myocytes. This finding agrees with higher (more than 3 times) IP_3R type-2 and -3 expression levels in atrial than in ventricular myocytes. In conclusion, ventricular myocytes have a more ''leaky'' SR than atrial cells due to higher

RyR activity at resting condition. However, SR Ca leak in atrial myocytes can be facilitated significantly during activation of IP_3-dependent signaling pathways.

1411-Pos Board B255

Changes In Cytosolic Ca^{2+} Have Greater Effects On SR Ca^{2+} Leak Than Changes In SR Ca^{2+}

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Attention has recently focused on preventing arrhythmias by controlling sarcoplasmic reticulum (SR) Ca^{2+} ''leak''. Increased leak in ventricular myocytes is associated with regenerative Ca^{2+} waves and delayed afterdepolarizations, leading to arrhythmias. Studies that have measured SR Ca^{2+} leak have not examined changes in $[Ca^{2+}]_{SR}$ independent of changes in $[Ca^{2+}]_i$, causing a degree of uncertainty as to which factor plays a greater role. Our current work explores the possibility that changes in $[Ca^{2+}]_i$ have a greater effect on leak than changes in $[Ca^{2+}]_{SR}$.

In quiescent rat ventricular myocytes, we recorded steady-state Ca^{2+} levels, then blocked the ryanodine receptors (RyRs) with a saturating concentration of tetracaine. Using the calcium indicator fluo-3, we recorded changes in $[Ca^{2+}]_i$ using a confocal microscope and analyzed the data using leak calculations that took into account underlying assumptions about cytosolic and SR buffers.

When extracellular Ca^{2+} ($[Ca^{2+}]_e$) was increased from 0.5 mM to 1.0 mM at rest, leak increased 37% (9 ± 1.019 vs. 12.3 ± 1.108 μM/s), $[Ca^{2+}]_i$ increased 6.6% (98.9 ± 0.09 vs. 105.4 ± 3.2 nM, and $[Ca^{2+}]_{SR}$ decreased 5.2% (489 ± 21 vs. 464 ± 23 μM). We also compared leak in resting cells versus leak in the same cells immediately after pacing for 10 s at 1 Hz. At 1 mM $[Ca^{2+}]_e$, pacing increased leak by 17.9% (12.3 ± 1.108 vs. 14.5 ± 8.8 μM/s), increased $[Ca^{2+}]_i$ by 9.4% (105.4 ± 3.2 vs. 115.3 ± 5.7 nM), but increased $[Ca^{2+}]_{SR}$ by only 1.0% (463.7 ± 22.7 vs. 468.2 ± 26.8 μM). Qualitatively similar results were obtained after pacing in 0.5 mmol $[Ca^{2+}]_e$. These results suggest that $[Ca]_i$ plays a larger role in determining diastolic SR Ca^{2+} leak than $[Ca]_{SR}$.

1412-Pos Board B256

EPAC Does Not Affect Diastolic Sarcoplasmic Reticulum Ca^{2+} Release in Rabbit Ventricular Myocytes

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Recent evidence gathered in ventricular myocytes from rodents points out that the EPAC pathway is a strong promoter of the release of Ca^{2+} from the sarcoplasmic reticulum (SR; Pereira et al., 2007, *J. Physiol*. 583:685-94). Encouraged by the above observations, we studied the effects of 2 μM 8-CPT (a specific EPAC activator) on the diastolic SR Ca^{2+} release in rabbit ventricular myocytes. Our initial studies used epifluorescence and focused on the relationship between the SR load and the diastolic SR Ca^{2+} release (i.e., the so called SR Ca^{2+} leak-load relationship). Contrary to the observations in rodents, our rabbit ventricular myocytes displayed no alterations of the leak-load relationship upon 8-CPT application. Since the leak-load relationship requires a steady state to be reached prior to the measurements, we also tested for non steady-state effects of EPAC stimulation using confocal microscopy. We studied the frequency and properties of Ca^{2+} sparks during the first 30 seconds of rest decay following 2 minutes of 8-CPT application and field stimulation at 1 Hz. Our results showed no effects of EPAC stimulation on the spark frequency or the spatio-temporal properties of the sparks. In summary, our results suggest that EPAC does not affect diastolic SR Ca^{2+} release in rabbit ventricular myocytes. Future studies will target the species dependence of the effect of EPAC and the effect of SR $[Ca^{2+}]$ upon the release properties under these conditions.

Calcium Fluxes, Sparks, and Waves II

1413-Pos Board B257

Ca Alternans in Cardiac Myocytes: Relating Macroscopic Behavior to Microscopic Ca Release Properties

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Beat-to-beat alternation in the intracellular Ca (Ca_i) transient (Ca_i alternans) causes pulsus alternans and electrocardiographic T-wave alternans, conditions associated with cardiac arrhythmias and sudden death. The whole cell Ca_i transient represents the summed activity of thousands of individual Ca sparks, i.e. Ca released locally by Ca release units (CRU). However, the extent to which the global behavior of the whole cell Ca_i transient mirrors the microscopic behavior of individual CRU units is unclear. We derived a one-dimensional iterated map of CRU behavior in which we could independently adjust the probabilities of random triggering of Ca sparks, recruitment of Ca sparks from adjacent CRUs, and CRU refractoriness following a Ca spark. After verifying that these three local properties (randomness, recruitment and refractoriness) could sustain an ensemble (global) alternans in a two-dimensional cellular automata network, we developed a physiologically-detailed subcellular Ca cycling model containing a network of coupled stochastic CRU which replicated the iterated map predictions. We find that a number of experimentally-reported phenomena, including whole cell Ca_i alternans, Ca waves in the presence of high spatial cooperativity, graded whole-cell Ca release, and a steep dependence of fractional SR Ca release on SR Ca load, emerge naturally from the collective behavior of individual CRUs depending on the balance of these three properties. A striking prediction is that microscopic CRU behavior does not always mirror collective CRU behavior, e.g. during whole cell Ca_i alternans, Ca sparks from individual CRU do not consistently alternate. In addition, whole cell Ca_i alternans is not generally dependent on alternans of diastolic SR Ca content. The findings provide novel multiscale insights into how global Ca signaling properties emerge from simple microscopic CRU properties.

1414-Pos Board B258

Analysis of Calcium Alternans in a Cardiac Myocyte Model that Uses Moment Equations to Represent Heterogenous Junctional SR Calcium

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The recently introduced ''probability density approach'' to modeling local control of CICR in cardiac myocytes [Williams et al. Biophys. J. 92(7):2311-28, 2007] and associated moment closure technique [95(4):1689-703, 2008] can reproduce whole cell voltage-clamp protocols high-gain Ca release that is graded with changes in membrane potential. This modeling formalism represents heterogeneous local Ca signals in a population of diadic subspaces and junctional sarcoplasmic reticulum (jSR) depletion domains using a system of differential-algebraic equations for the time-evolution of the zeroth, first, and second moments of probability density functions for jSR [Ca] jointly distributed with calcium release unit (CaRU) state. Here we show that a cardiac myocyte model that uses moment equations to represent heterogenous jSR Ca can exhibit Ca alternans when periodically stimulated by depolarizing voltage pulses, and makes predictions regarding the distribution of jSR [Ca] across a large population CaRUs as a function of stimulation frequency and cellular parameters such as the rate of diffusive transfer between network and junctional SR. Factors promoting alternating Ca responses in the moment closure model are analyzed and compared to analogous mechanism in a minimal ''common pool'' model with comparatively simple SR and PM fluxes. We derive load-release and release-reuptake curves for both models, and investigate how model parameters influence these relations and the existence and stability of steady-state periodic Ca responses during repetitive depolarizing voltage pulses. Specifically, we find that increasing SR Ca leak, RyR sensitivity, and maximum release flux decreases the steepness of load-release curves, shifts load-release curves to smaller SR loads, and increases the critical simulation frequency resulting in Ca alternans.

1415-Pos Board B259

New Insight Into Cardiomyocyte Ca Signaling Obtained By Fast Confocal Imaging

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With ultra-fast 1D-(x-t) and 2D-(x-y-t) confocal microscopy (Zeiss LSM 5 Live) we studied the spatio-temporal properties of Ca sparks and Ca transients. Ca sparks which originated from subsarcolemmal sarcoplasmic reticulum (SR) release sites in atrial myocytes were elongated in the longitudinal direction of the cell. Ca sparks corresponding to Ca release from non-junctional SR in atrial myocytes and junctional SR in ventricular myocytes were variable spatially with some events being symmetrical and others asymmetrical. Anisotropic sparks occurred in transverse as well as longitudinal direction. Ca sparks originating from non-junctional SR and recorded in line-scan (x-t) mode at 40,000 lines/s revealed a step-like appearance in space (time-dependent step-like increase of width from the point of origin) and amplitude during the activation phase of the spark. These steps in space and amplitude may represent the sequential opening of individual ryanodine receptor (RyR) channels in a release cluster and support the notion that sparks represent Ca release from a group of RyRs. Mathematical analysis of global Ca transients recorded from field-stimulated ventricular myocytes at high temporal resolution allowed separation of Ca entry from Ca release flux. After the electrical stimulus a latency period of 2.5 ms was required to activate sarcolemmal Ca channels. SR Ca release was initiated with an additional delay of 3.0 ms. Maximal Ca