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