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

release flux was observed 3.9 ms after initiation of Ca release when all release sites became active. Ca entry flux was ten times smaller than Ca release flux. When SR Ca release was eliminated after treatment with thapsigargin and caffeine, identical Ca entry flux was observed as in control conditions. In summary, ultra-fast confocal imaging allows investigation of Ca signals with time resolution similar to patch clamp technique, however in a much less invasive fashion.

1416-Pos Board B260

Automatic Calcium Spark Detection and Analysis in Time Series of Two-Dimensional Confocal Images

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Calcium sparks are highly localized, brief calcium transients reflecting elementary calcium release from the sarcoplasmic reticulum that can occur either spontaneously or can be triggered during excitation contraction coupling in cardiac myocytes. They hold physiological as well as pathophysiolgical importance. Classically, calcium sparks were almost exclusively analyzed in line scan images because of technical limitations in the acquisition process consequently neglected their 2D spatial properties and distribution inside the cell.

During recent years the increased performance of confocal microscopes enabled high spatial and temporal resolution imaging. This allowed recording of calcium sparks from individual myocytes in time series of two-dimensional confocal images at acquisition rates exceeding 200Hz.

Here, we introduce an automatic three-dimensional approach for such analysis. Following cell border recognition we utilised locally ''derivative-like'' functions for spark-detection allowing the algorithm to analyse the temporal and spatial properties of calcium sparks. Such an approach revealed a highly robust spark-detection process, even when partial or global calcium waves occurred intermittently. 2D-gaussian fits over time were used to quantify such properties including amplitudes, decay time, frequency, spatial position and spread for further analysis. In addition, the algorithm automatically performs cluster analysis of the sparks found in order to identify common spark sites.

In an initial proof-of-concept study, we analysed spontaneous calcium sparks from various origins within the mouse heart (ventricle, left/right aria). The major result of this study was that the sparks display very distinct and characteristic properties that can be linked to physiology of the particular host cell.

Such a novel approach enables us to automatically analyze large populations of local calcium signals and thus fosters the use of such algorithms for novel high-content screening applications.

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1417-Pos Board B261

Image-Based Monte Carlo Modeling of Ca^{2+} sparks in Ventricular Myocytes

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The geometry of a dyadic calcium release unit (CRU) plays a critical role in regulating intracellular Ca^{2+} spark generation and furthermore the excitation-contraction (E-C) coupling in ventricular myocytes. A number of computational models have showed how the geometries of T-tubules and junctional sarcoplasmic reticulum (jSR) and distributions of L-type Ca^{2+} channels (LCCs) and ryanodine receptors (RyRs) could affect local Ca^{2+} signaling, but almost all of them were based on simple domain geometries such as rectangular or cylindrical shapes. For this reason, incorporating image-based realistic geometric models into mathematical simulation is timely and expected to provide more accurate simulation of many biological processes, in particular, the Ca^{2+} spark formation in ventricular myocytes.

The present study has two goals. First, we adopt a chain of image and geometric processing approaches to construct realistic 3D models (represented by high-quality surface and volumetric meshes) of both T-tubules and junctional SR from electron microscopy images of adult mouse cardiomyocytes. The distributions of LCCs and RyRs are obtained in two ways: random assignment and image-based localization (for RyRs only). The second goal of the present study is the use of Monte Carlo methods to model the randomness of release and diffusion of individual Ca^{2+} ions within a narrow dyadic cleft. To this end, the M-Cell software package, combined with the realistic geometries obtained in the first goal, is used to simulate how Ca^{2+} sparks are generated in a single dyad (or CRU) and how they change temporally and spatially with different geometries of membrane structures and distributions of channeling proteins.

1418-Pos Board B262

Markov Chain Models Of Coupled Calcium Channels: Kronecker Representations And Iterative Solution Methods

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Mathematical models of calcium release sites derived from Markov chain models of intracellular calcium channels exhibit collective gating reminiscent of the experimentally observed phenomenon of stochastic calcium excitability (i.e., calcium puffs and sparks). Calcium release site models are stochastic automata networks that involve many functional transitions, that is, the transition probabilities of each channel depend on the local calcium concentration and thus the state of the other channels. We present a Kronecker structured representation for calcium release site models and perform benchmark stationary distribution calculations using both exact and approximate iterative numerical solution techniques that leverage this structure. When it is possible to obtain an exact solution, response measures such as the number of channels in a particular state converge more quickly using the iterative numerical methods than occupation measures calculated via Monte Carlo simulation. In particular, multi-level methods provide excellent convergence with modest additional memory requirements for the Kronecker representation of calcium release site models. When an exact solution is not feasible, iterative approximate methods based on the power method may be used, with performance similar to Monte Carlo estimates. This suggests approximate methods with multi level iterative engines as a promising avenue of future research for large-scale calcium release site models.

1419-Pos Board B263

Ca^{2+} Sparks Generate Depolarizing STICs Causing Contraction And Asthmatic Hyperresponsiveness In Airway Smooth Muscle Cells

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Ca^{2+} sparks are well known to be essential for controlling the relaxation of cerebral artery SMCs; however, the functional importance of this local Ca^{2+} signaling in other types of SMCs remains to be determined. Thus, the aim of this study was to investigate the role of Ca^{2+} sparks in airway SMCs. Our data reveal that spontaneous Ca^{2+} sparks could activate spontaneous transient inward currents (STICs) at the resting membrane potential and spontaneous transient outward currents (STOCs) at more positive membrane potentials in mouse airway SMCs. Application of ryanodine to block ryanodine receptors (RyRs) abolished spontaneous Ca^{2+} sparks without altering the whole-cell cytosolic Ca^{2+} levels in single airway myocytes and decreased the resting muscle tension in isolated airway rings, whereas activation of RyRs with a low concentration of caffeine had opposite effects. Iberiotoxin, a selective blocker of big-conductance Ca^{2+}-activated K^+ channels, eliminated STOCs, but did not affect either spontaneous Ca^{2+} spark activity or resting muscle tension. In contrast, NPPB, an inhibitor of Cl^- channels, reduced resting muscle tension. The effect of NPPB was prevented in the presence of the selective voltage-dependent Ca^{2+} channel blocker nifedipine. We have also found that the activity of Ca^{2+} sparks in single asthmatic mouse airway SMCs and in-vivo airway resistance in asthmatic mice were significantly increased. Interestingly, ryanodine caused a stronger relaxation in asthmatic airway smooth muscle. Taken together, these findings suggest that spontaneous Ca^{2+} sparks can activate Ca^{2+}-activated Cl^- channels and then generate STICs, causing membrane depolarization, opening of voltage-dependent Ca^{2+} channels, extracellular Ca^{2+} influx and contraction in airway SMCs. Moreover, Ca^{2+} sparks and attendant STICs are both increased in asthmatic airway SMCs, which may contribute to asthmatic airway hyperresponsiveness.

1420-Pos Board B264

Decreased Ca^{2+} Spark Frequency and RyR2 Expression in Cerebral Arteries Following Subarachnoid Hemorrhage

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Ca^{2+} sparks represent local and transient increases in intracellular Ca^{2+} caused by the coordinated opening of ryanodine receptors (RyRs) located in the sarcoplasmic reticulum of muscle. Contrary to the contraction caused by global Ca^{2+} increases, Ca^{2+} sparks promote smooth muscle relaxation via activation of plasmalemmal large-conductance Ca^{2+}-activated potassium (BK) channels leading to membrane potential hyperpolarization and decreased activity of voltage-dependent Ca^{2+} channels. Here, we examined whether impairment of this vasodilator pathway contributes to enhanced cerebral artery constriction associated with subarachnoid hemorrhage (SAH). Using a rabbit SAH model, Ca^{2+}