parenchyma. To assess the impact of SAH on global cytosolic Ca^{2+} and arteriolar diameter, intact freshly-isolated parenchymal arterioles (PAs; 30-60 μm diameter) were loaded with the ratiometric Ca^{2+} indicator fura-2. At physiological intravascular pressure (40 mmHg), PAs from SAH animals exhibited significantly elevated cytosolic Ca^{2+} (349 ± 10 nM vs. 248 ± 14 nM) and developed significantly greater (~2-fold) myogenic tone compared with PAs from sham-operated animals. The L-type voltage-dependent Ca^{2+} channel (Ca_V) blocker nimodipine caused ~90% reduction in Ca^{2+} and tone in PAs from both groups suggesting elevated PA Ca^{2+} following SAH results from enhanced L-type Ca_V activity. Increased Ca_V activity may reflect Ca_V upregulation or membrane potential (V_M) depolarization of arteriolar smooth muscle. When V_M was clamped at K^+ equilibrium potential using elevated extracellular K^+ (60 mM; −22 mV), Ca^{2+} and tone were similar between groups, suggesting surface Ca_V density is unchanged by SAH. To examine whether suppression of voltage-dependent K^+ channel (K_V) activity contributes to V_M depolarization, outward K^+ currents were measured in isolated PA myocytes using conventional whole cell patch clamp electrophysiology. Currents sensitive to 4-aminopyridine, a K_V channel blocker, were reduced by ~70% (at +40 mV) in myocytes from SAH animals compared with controls. Taken together, our results suggest decreased K_V channel activity causes V_M depolarization, increased Ca_V activity, elevated cytosolic Ca^{2+} and enhanced constriction of PAs following SAH. Impaired regulation of PA diameter may contribute to local ischemia and neurological deterioration following SAH.

Calcium Fluxes, Sparks & Waves I

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Nitric Oxide Can Mediate Beta-Adrenergic- and CaMKII-Dependent Spontaneous Ca^{2+} Waves in Cardiac Myocytes, Independent of PKA Activation

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Increased diastolic SR Ca leak can initiate spontaneous Ca waves (SCaWs). SCaWs activate inward Na/Ca exchanger current causing an arrhythmogenic delayed afterdepolarization. Here we examine SCaWs in ventricular myocytes isolated from rabbit hearts. Myocytes did not exhibit SCaWs at baseline conditions, but 43% did when exposed to isoproterenol (ISO). This ISO-induced increase in activity was reversed by inhibition of CaMKII by KN93, but not with PKA inhibition by H89. At similar $[Ca]_{SRT}$ (121 μM) myocytes treated with ISO plus KN93 had significantly fewer SCaWs versus those treated with ISO or ISO plus H89 (0.2 ± 0.28 vs. 1.1 ± 0.28 & 1.29 ± 0.39 SCaWs $cell^{-1}$, respectively). We attribute this increase in activity to the previously characterized CaMKII-dependent increase in RyR-dependent leak. We also find that SR Ca leak is increased by the nitric oxide (NO) donor, SNAP; and this NO-dependent effect is also completely reversed by KN-93. We also show the increase in leak to be dependent on nitric oxide synthase 1 (NOS1) activity. At comparable SR Ca load (132 μM) ISO treated myocytes have significantly higher leak vs. control (8.4 vs. 3.8 μM). The ISO-induced leak (at constant SR Ca load) was attenuated by the NOS1 inhibitor, SMLT, but not the NOS3 inhibitor, L-NIO (3.5 vs. 6.8 μM). Moreover, ISO causes an upward trend in myocyte [NO] (sensed by the NO-dependent dye, DAF-2 A), and the NOS inhibitor, L-NAME significantly attenuated the development of SCaWs. Together this data suggests a novel pathway in which β1-adrenergic receptor activation stimulates NO production via NOS1, which in turn activates CaMKII to increase RyR gating, SR Ca leak, SCaWs and delayed afterdepolarizations.

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On the "spark Frequency Vs. Leak Rate" Relationship in Ventricular Myocytes: A Study in the Rabbit

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Recent studies have linked heart disease to mutated or hyperactive ryanodine receptors (RyRs) in ventricular myocytes, renewing the interest on how the sarcoplasmic reticulum (SR) Ca release process works during diastole. We addressed two questions of potential clinical relevance: (1) whether the SR Ca leak rate (J_{leak}) can be entirely explained by Ca sparks, and (2) whether the spark-dependent fraction of J_{leak} can be varied upon RyR phosphorylation. We used confocal microscopy to simultaneously measure J_{leak} and Ca sparks in Fluo 4-loaded rabbit ventricular myocytes. Control cells (C; n = 47) were paced at 0.5 Hz, while isoproterenol-treated cells (I; n = 14, [Isoproterenol] = 125 nM) were paced at 0.25 Hz to match the SR loads (C = 136.4 ± 5.6 μmoles/l cytosol; I = 126 ± 9.2 ; P = 0.47 in *t*-test with Welch correction). J_{leak} was quantified as in Shannon et al. (2002; *Circ Res* 91:594-600), but using a lower K_m for the forward rate of uptake in the I group. Although J_{leak} did not significantly differ among the groups (C = 10.87 ± 0.93 μM/s; I = 13.12 ± 2.53 ; P = 0.42), the spark frequency was more than doubled in the isoproterenol-treated cells (C = 1.21 ± 0.15 sparks * $(100\ \mu m)^{-1}$ * s^{-1}); I = 2.82 ± 0.51; P = 0.0082). These findings point to an increase in the spark-dependent fraction of J_{leak} upon RyR phosphorylation (for a given SR load), while suggesting an enhancement of Ca-induced RyR coupling relative to the influence of stabilizing RyR couplers.

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Regulation of Sarcoplasmic Reticulum Calcium Leak by Cytosolic Calcium in Rabbit Ventricular Myocytes

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Diastolic sarcoplasmic reticulum (SR) Ca leak determines Ca content and, therefore, the amplitude of action potential-induced global Ca transients in ventricular myocytes. However, the pathways and properties of SR Ca leak have been poorly described. Here, we studied the effects of cytosolic [Ca] ($[Ca]_i$) on SR Ca leak in permeabilized rabbit ventricular myocytes. Using confocal microscopy we simultaneously measured intra-SR free Ca ($[Ca]_{SR}$) with fluo-5N and cytosolic Ca sparks with rhod-2, and monitored SR Ca leak as the change in $[Ca]_{SR}$ over time after complete SERCA inhibition with thapsigargin (10 μM). Increasing $[Ca]_i$ from 150 to 250 nM significantly increased SR Ca leak (by ~30%) over the entire range of $[Ca]_{SR}$. This increase in SR Ca leak associated with an increase in Ca spark frequency. Further increasing $[Ca]_i$ to 350 nM led to rapid $[Ca]_{SR}$ depletion due to the occurrence of spontaneous Ca waves. In contrast, lowering $[Ca]_i$ to 50 nM markedly decreased SR Ca leak rate (by ~60%) and nearly abolished Ca spark activity. When the ryanodine receptor (RyR) was completely inhibited with ruthenium red (50 μM), changes in $[Ca]_i$ between 50 and 350 nM did not produce any significant effect on SR Ca leak, showing that changes of $[Ca]_i$ over a physiological range alter SR Ca leak solely by regulating RyR activity. However, decreasing $[Ca]_i$ to a lower, non-physiologically level (5 nM) activated additional SR Ca leak pathway(s) that were insensitive to RyR or SR Ca ATPase inhibition. In summary, $[Ca]_i$ plays an important role in regulating SR Ca leak by activating RyR and preventing Ca leak through unspecified pathways.

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Dynamic Changes of Calcium in Sarcoplasmic Reticulum of Rabbit Atrial Myocytes

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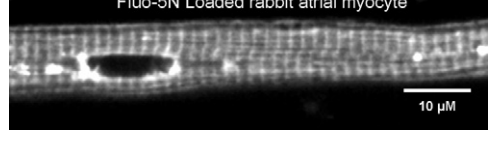
With each contraction in atrial muscle, Ca^{2+} is released from the sarcoplasmic reticulum (SR) through ryanodine receptors and then reaccumulated by the activity of the SR Ca^{2+} ATPase (SERCA). Recent experiments by us and others have examined SR Ca^{2+} concentration ($[Ca^{2+}]_{SR}$) using low affinity fluorescent Ca^{2+} sensors (e.g. fluo-5N) in ventricular myocytes (Brochet et al. PNAS 2005 102(8) 3099-104) however, similar characterization in atrial cells is missing. Here we examine $[Ca^{2+}]_{SR}$ dynamics in healthy adult rabbit atrial myocytes using fluo-5N. Fractional SR Ca^{2+}release at room temperature was 30% (n=12), where caffeine was used to determine 100% release. Restoration of $[Ca^{2+}]_{SR}$ by SERCA was consistent with the decline of $[Ca^{2+}]_i$ with each beat (1 Hz). The interconnectivity of the cell-wide SR and nuclear envelope and endoplasmic reticulum was characterized using fluorescence recovery after photobleaching (FRAP). Additional diverse modulations of atrial cell $[Ca^{2+}]_{SR}$ will be discussed.



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Spatiotemporal Profiles of Sarcoplasmic Reticulum Ca^{2+} Release in Mouse Atrial Cardiomyoctes *in situ*

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Previous studies in atrial cardiomyocytes isolated from various mammalian species have demonstrated poorly developed transverse(t)-tubules and inhomogeneities of sarcoplasmic reticulum (SR) Ca^{2+} release, but little is known of mice. Here, we examined the t-tubular organization and action potential-evoked Ca^{2+} release in *in situ* myocytes, using confocal microscopy of Langendorff-perfused hearts. Imaging of ANNINE-6plus-stained sarcolemmal membranes revealed paucity of t-tubules in atrial, but densely and regularly spaced t-tubules in ventricular myocytes. However, both myocyte types exhibited regular, striated appearance of type 2 ryanodine receptor distribution. Line-scans