linescanning after 488nm excitation and recording emission at 505-530nm in intact Fluo-3-loaded cardiomyocytes (2uM) at 37°C and at [Ca2+] 1.2mM and 5.0mM. These studies showed that spontaneous wave frequency was higher at 5.0mM than 1.2mM Ca2+. Post-MI HF cardiomyocytes had ~twice the wave frequency compared to sham-operated controls. Regular ExTr post-MI improved exercise capacity and induced reverse remodeling. ExTr also reduced the frequency of spontaneous waves at both Ca2+ 1.2mM and 5.0mM, although it did not completely normalize spontaneous Ca2+ waves. ExTr also increased the ratio between aborted and complete waves at Ca2+ 1.2mM, but not Ca2+ 5.0mM. No effects were found on spontaneous wave velocity. This suggests that ExTr partly improved the control of diastolic Ca2+ by reducing the frequency of spontaneous Ca2+ waves and by improving the ability of the cardiomyocyte to eliminate a spontaneous wave after its generation, but before its propagation. Finally, we repeated these studies in the presence of the nitric oxide synthase inhibitor L-NAME, to study the contribution of nitric oxide. This did not have any effects.

1402-Pos Board B246

Upregulation Of Cam Kinase IIδ Modulates Spontaneous Ca^{2+} Wave Properties In A Rabbit Model Of Heart Failure

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In ventricular cardiomyocytes, CaMKIIδ (calcium/calmodulin-dependent protein kinase IIδ) is known to modulate Ca^{2+}-handling proteins of the sarcoplasmic reticulum (SR). Dysregulation of Ca^{2+}-handling is evident in cardiomyopathy and may be mediated by changes in CaMKIIδ expression and/or activity. The present study assesses CaMKIIδ expression and activity in rabbit left ventricular(LV) whole homogenates one week after coronary artery ligation(CAL). Changes in CaMKIIδ are translated to changes in SR function in isolated LV cardiomyocytes.

Quantitative immunoblotting of CaMKIIδ protein revealed expression was increased by ~2-fold in CAL (0.096 ± 0.01(sham)vs.0.214 ± 0.042(CAL) normalised mean ratio, (n=7). Similarly, CaMKIIδ activity was increased by ~1.5-fold in CAL (0.168 ± 0.022 (sham) vs. 0.247 ± 0.028 (CAL) pmolPO_4^-inc/min/μg protein, n=7). In isolated permeabilised cardiomyocytes, spontaneous Ca^{2+} waves were studied to assess changes in function associated with upregulation of CaMKIIδ. This was accomplished by measuring changes in Ca^{2+} wave properties in the presence and absence of AIP (autocamtide-2-related inhibitory peptide). Cells were incubated with a modest concentration (300nM) of AIP for >30 min before use; these were then loaded with fluo-3 and fluorescence was monitored by confocal linescan microscopy with subsequent conversion to Ca^{2+}. In cells from sham animals, no changes in Ca^{2+} waves were observed in the presence of AIP. In cells from LVD animals AIP caused Ca^{2+} waves to be reduced in frequency ($-20.8 \pm 3.4\%$), increased minimum Ca^{2+} ($+17.4 \pm 5.0\%$) as well as increased rate of decline ($+15.8 \pm 2.0\%$). These changes are consistent with CaMKIIδ playing an increased role in SR Ca^{2+} handling following CAL. Since CaMKIIδ expression and activity are both significantly increased in this model, increased SR Ca^{2+} handling could occur via CaMKII-mediated effects on SERCA activity producing increased SR accumulation of Ca^{2+}. This would result in an elevated sensitivity to β-adrenergic stimulation that could be arrhythmogenic.

1403-Pos Board B247

Ca^{2+} Wave Development in Ventricular Cardiomyocytes from Mice with Inducible Knockout of SERCA2

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Ca^{2+} waves develop when Ca^{2+} is spontaneously released from the sarcoplasmic reticulum (SR). Ca^{2+} then diffuses through the cytosol and triggers further release from neighboring ryanodine receptors. SERCA2 has been proposed to affect Ca^{2+} wave development in ventricular cardiomyocytes in two ways: 1) By its role as regulator of SR Ca^{2+} content. 2) By its influence on cytosolic Ca^{2+} in a propagating Ca^{2+} wave. We have studied the effect of an isolated reduction of SERCA2 abundance on Ca^{2+} wave development. Knockout of the *Serca2* gene in cardiomyocytes was induced by a single i.p. injection of tamoxifen in *Serca2*$^{flox/flox}$ Tg(αMHC-MerCreMer) mice. Serca2$^{flox/flox}$ mice served as controls. Experiments were performed on ventricular cardiomyocytes with a 53% reduction in SERCA2 protein expression without any changes in expression of the L-type Ca^{2+} channel, Na^+-Ca^{2+}-exchanger or plasma membrane Ca^{2+} ATPase. In field stimulated cells SERCA2 mediated rate of Ca^{2+} reuptake was reduced by 42%. Basic characteristics of excitation-contraction-coupling were as expected with a 16% reduction in Ca^{2+} transient amplitude, SR Ca^{2+} content reduced by 16% and peak Ca^{2+} current increased by 40%. When SR Ca^{2+} content was increased by 10mM external Ca^{2+}, only 27% of voltage clamped cardiomyocytes from knockout mice developed Ca^{2+} waves compared to 50% of control cells. Confocal imaging showed that Ca^{2+} waves in knockout mice propagated at 16% lower velocity, possibly due to a 12% reduction in Ca^{2+} wave amplitude. We conclude that decreased SERCA2 abundance reduces the overall propensity for Ca^{2+} wave development, and decreases Ca^{2+} wave velocity.

1404-Pos Board B248

Ca-dependency Of Cardiac SR Ca Release Reveals No Sign Of Ca-dependent Inactivation And Points To Luminal Ca As A Principal Regulator Of Release

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Calcium-induced calcium release (CICR) from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyRs) mediates periodic beating of cardiac myocytes and can occur spontaneously contributing to arrhythmia. Following Ca release, SR Ca signaling becomes refractory due to a transitory functional inactivation of the RyR channels. Although the precise causes continue to be debated, cytosolic Ca-dependent inactivation and luminal Ca-dependent deactivation are viewed as the most likely mechanisms responsible for this phenomenon. In order to examine the role of these mechanisms in controlling CICR, we investigated SR Ca release in a wide range of cytosolic Ca concentrations ([Ca]c; 1-100uM) in permeabilized canine ventricular myocytes by monitoring Ca concentration inside the SR ([Ca]SR) using the low affinity Ca indicator Fluo5N. Elevating Ca from 100nM to 1-50uM caused spontaneous oscillations of [Ca]SR manifested as periodic depletions followed by periods of reloading synchronized across the cell. While the duration of depletion intervals increased, the periods when the SR was reloaded shortened resulting in an overall increase in the frequency of [Ca]SR oscillations with increasing [Ca]c. At [Ca]c>50uM, [Ca]SR oscillations disappeared and the SR stayed continuously empty. Preloading the SR with low-affinity Ca chelators decreased the frequency of [Ca]SR oscillations in a concentration-dependent manner. Our results suggest that under conditions of continuous activation by cytosolic Ca, RyRs can periodically cycle between open and deactivated states due to effects of luminal Ca. Deactivation appears to involve desensitization to cytosolic Ca because it is overcome at high [Ca]c, which renders the channels continuously open. Inactivation by cytosolic Ca plays no detectable role in controlling SR Ca release.

1405-Pos Board B249

Triadin Deletion Alters Calcium Sparks in Murine Cardiomyocytes

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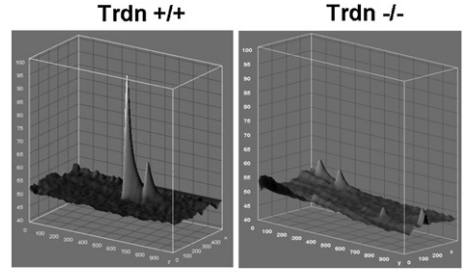
Gene-targeted deletion of the sarcoplasmic reticulum (SR) protein triadin (Trdn-/-) causes 50% reduction in ryanodine receptor (RyR2) Ca^{2+} release channels and cardiac calsequestrin, and a 50% decrease in the size of t-tubule SR junctions in mouse heart muscle. Here we report on the Ca^{2+} spark properties of Trdn-/- cardiomyocytes. Isolated ventricular myocytes from Trdn-/- mice (N=5) and wild-type littermates (Trdn+/+, N=8) were loaded with the Ca-sensitive fluorescent indicator Fluo4-AM and Ca^{2+} sparks were measured in 2mM Ca^{2+} by confocal microscopy in line scan mode. As illustrated in the figure, triadin deletion caused a dramatic reduction in spark amplitude (ΔF/Fo: Trdn-/- 0.43 ± 0.01, n=893; Trdn+/+ 0.61 ± 0.02, n=745, p<7.27E-22), spark width (FWHM (μm): Trdn-/- 2.64 ± 0.03 n=893, Trdn+/+ 2.90 ± 0.03, n=745, p<1.77E-09) and spark upstroke velocity ($\Delta(F/Fo)/\Delta t_{max}$(Δ(F/Fo)/s): Trdn-/- 31.67 ± 0.90, n=891, Trdn+/+ 58.35 ± 1.62, n=741, p<3.49E-48), whereas spark frequency was modestly increased (sparks/100μm/s: Trdn-/- 0.92 ± 0.08, n=255 myocytes, Trdn+/+ 0.71 ± 0.06, n=321 myocytes, p<0.03). The



3D surface plots of line scan images from Trdn +/+ and Trdn -/- myocytes.

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