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.

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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.

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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.

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Triadin Deletion Alters Calcium Sparks in Murine Cardiomyocytes

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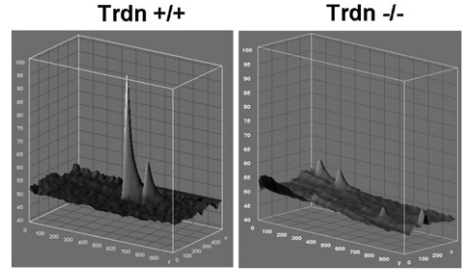
Gene-targeted deletion of the sarcoplasmic reticulum (SR) protein triadin (Trdn-/-) causes 50% reduction in ryanodine receptor (RyR2) Ca^{2+} release channels and cardiac calsequestrin, and a 50% decrease in the size of t-tubule SR junctions in mouse heart muscle. Here we report on the Ca^{2+} spark 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.