oscillatory frequencies >0.864Hz. Ouabain treatment (100nM, 45mins) ablated both intercellular synchrony and the precise temporal ordering of Ca^{2+} transients and under these conditions small, localised Ca^{2+} fluxes were intimately linked to the shapes and intercellular synchronisation of global Ca^{2+} transients. Our data suggests that during normal Ca^{2+} homeostasis, manoeuvres that alter Ca^{2+} transient 'shape' do not modulate the extent of intercellular synchrony. However, under conditions of imposed Ca^{2+} cycling dysfunction, modulation of small dynamic Ca^{2+} fluxes may tune Ca^{2+} transients and modify the extent of intercellular synchronisation.

1397-Pos Board B241

Dynamic Changes Of Local Ca Sensed By Ca-dependent Currents In Cardiac Myocytes

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In cardiac cells, Ca release from the sarcoplasmic reticulum (SR) is a local event that occurs in the subsarcolemmal space. In this study, we investigated dynamic changes of subsarcolemmal Ca sensed by Ca current (ICa) and Na/Ca exchanger (NCX) during SR Ca release.

In pig ventricular myocytes, membrane currents were recorded using whole-cell voltage-clamp with Fluo-3 as indicator for global Ca. SR Ca release was triggered through activation of ICa during steps from -70 to -35 mV. At this potential, ICa showed release-dependent inactivation and recovery. The step at -35 mV was interrupted at different time intervals by a step to -70 mV or 0 mV to measure the time course of NCX and availability of ICa respectively. NCX tail currents were converted to subsarcolemmal Ca using steady-state dependence of NCX on global Ca during caffeine application. Release-dependent inactivation of ICa at +10 mV was assessed by subtraction analysis of two pulses with different amplitudes of Ca release during repetitive stimulation in Na-free conditions after caffeine.

Subsarcolemmal Ca reached its peak value immediately after the trigger pulse, where global Ca increased more slowly and to a lesser extent. Maximal inactivation and recovery of ICa occurred 20-30 ms after the step to -35 mV, with a faster time course than changes in global Ca, but slower than maximal NCX activation. At a more positive potential of +10 mV, inactivation of ICa was maximal at 12.7 ± 0.78 ms (n=10).

In conclusion, local Ca sensed by NCX and ICa during triggered release considerably differs from changes in global Ca. The discrepancy between time courses of local Ca effects on NCX and ICa is currently unexplained and may be related to the longer latency for Ca channels at more negative potentials.

1398-Pos Board B242

Pathways of Abnormal Stress-Induced Calcium Influx into Dystrophic mdx Cardiomyocytes

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In Duchenne muscular dystrophy, deficiency of the cytoskeletal protein dystrophin leads to well-described defects in skeletal muscle, but also to dilated cardiomyopathy, which accounts for about 20% of the mortality. However, the precise mechanisms leading to cardiomyocyte cell death and dilated cardiomyopathy are not well understood. One hypothesis to explain the dystrophic muscle phenotype suggests that the lack of dystrophin leads to membrane instability during mechanical stress and to the activation of not yet identified calcium (Ca^{2+}) influx pathways. In the present study, potential Ca^{2+} entry pathways initiating damaging intracellular signals were explored with confocal imaging and pharmacological tools. Modest osmotic shocks were applied to isolated *mdx* cardiac myocytes, which are an established model for dystrophy. Osmotic shocks mimic some characteristics of stress encountered by the cells *in vivo*. Our results confirm that stretch-activated channels (SACs) and sarcolemmal microruptures play an important role in the initial Ca^{2+} entry, with the latter pathway also permeable for the dye FM1-43. Interestingly, our findings also suggest that Ca^{2+} influx pathways which are more prominent in cardiac than in skeletal muscle synergistically contribute to the observed Ca^{2+} responses (e.g. the L-type Ca^{2+} channels or the Na^{+}-Ca^{2+} exchange (NCX) importing Ca^{2+} subsequent to some Na^{+} entry via the aforementioned primary pathways). This additional complexity needs to be considered when targeting abnormal Ca^{2+} influx as a treatment option for dystrophy. Supported by SNF, MDA & SSEM.

1399-Pos Board B243

Origin And Propagation Velocity Of Ca^{2+} Waves Determine The Kinetics Of Transient Inward Currents (I_{ti}) In Cardiomyocytes

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Ca^{2+} waves are propagating increases in intracellular [Ca^{2+}] caused by chain-reaction Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum (SR). In ventricular myocytes, Ca^{2+} waves provoke transient inward currents that are the consequence of electrogenic extrusion of a fraction of the Ca^{2+} wave into the extracellular space via mainly the Na^{+}/Ca^{2+} exchanger. Although Ca^{2+} waves cause arrhythmogenic delayed afterdepolarizations (DADs), they also provide an anti-arrhythmic mechanism to deplete a fraction of SR Ca^{2+} load. Therefore, it is important to understand Ca^{2+} waves and their associated currents. In this study we used BiNiX, a photosensitive compound that releases paraxantine (a caffeine analog) upon UV illumination to activate cardiac SR Ca^{2+} release channels and induce Ca^{2+} waves; concurrently ionic currents were monitored. Focal photolysis (~10 μm) of BiNiX usually caused a local [Ca^{2+}] rise that initiated a Ca^{2+} wave, which propagated throughout the entire myocyte. Altering the site of photolysis (i.e. origin of the Ca^{2+} wave) dramatically modified the kinetics of the resulting I_{ti}. Increasing the turnover rate of the SR Ca^{2+}-ATPase by various mechanisms accelerated Ca^{2+} wave propagation and the kinetics of the ensuing I_{ti}. We developed a minimal model of the Ca^{2+} wave-activated I_{ti} that takes into account propagation velocity and origin. Simulated and experimental data showed remarkable agreement. For each cell, when I_{ti}s predicted by the model were injected in current-clamp mode, the role of Ca^{2+} wave origin and propagation velocity in the development of DAD could be measured. These results suggest that the rate of Ca^{2+} release from the SR during a Ca^{2+} wave and the activation kinetics of the consequent I_{ti} determine the magnitude of the DAD and, in turn, the likelihood of reaching threshold to trigger an arrhythmogenic action potential.

1400-Pos Board B244

The Inter-Relationship Between Calcium Transient And Spontaneous Calcium Wave Frequency In Adult Rabbit Ventricular Cardiomyocytes

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The inter-relationship between the electrically stimulated calcium transient frequency (STF) and the spontaneous calcium wave frequency (SWF) at the same mean intracellular [Ca^{2+}] was quantified in isolated rabbit cardiomyocytes. Field stimulation (37°C, 1.8mM [Ca^{2+}]) of Fura 2/4FAM loaded cells over a range of frequencies (0.5-4.0Hz) raised the mean intracellular [Ca^{2+}] from 62.0 ± 7.46 to 315 ± 64.7nM respectively. In a separate set of experiments (without field stimulation) SWF was determined at a range of mean intracellular [Ca^{2+}] in voltage clamped cells. Mean intracellular [Ca^{2+}] was dictated by altering holding voltage for 2min periods from -80 to +80mV at an extracellular [Ca^{2+}] ranging from 1.8-5.4mM. Spontaneous Ca^{2+} waves increased from 0.3 to 0.8 waves.s^{-1} when intracellular [Ca^{2+}] increased from 340-760nM respectively. Field stimulation of cells in the presence of 150nM isoproterenol (ISO) over a STF of 0.5-4.0Hz raised the mean intracellular [Ca^{2+}] from 170 ± 4.93 to 1030 ± 102nM. The relationship between mean intracellular [Ca^{2+}] and SWF under voltage clamp conditions in the presence of ISO was shifted to the left compared to control. The net effect of ISO is to increase the SWF/STF ratio at each mean intracellular [Ca^{2+}] value. Spontaneous Ca^{2+} waves were observed between stimulated Ca^{2+} transients in ISO at a STF of 0.5-2.0Hz where the SWF/STF had the highest values. But spontaneous waves were not evident at mean intracellular [Ca^{2+}] values reached at 3.0-4.0Hz corresponding to lower SWF/STF values. This quantitative analysis suggests that sarcoplasmic reticulum (SR) Ca^{2+} release that depends entirely on SR Ca^{2+} load will be intrinsically slow compared to normal heart rates and therefore unlikely to occur during diastole. The data suggests that other factors are required to increase the intrinsic rate of SR Ca^{2+} release sufficiently to precipitate release during the diastolic interval.

1401-Pos Board B245

Exercise Training Reduces Spontaneous Ca2+ Waves In Cardiomyocytes From Post-myocardial Infarction Heart Failure Rats

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Arrhythmias cause ~50% of deaths in heart failure (HF), but no satisfactory treatment exists. An underlying scenario is the impaired control of cardiomyocyte intracellular diastolic Ca2+. Exercise training (ExTr) has the potency to correct abnormal Ca2+ handling in experimental models of HF, but several aspects remain unstudied. We induced myocardial infarctions (MI) by coronary artery ligation in Sprague-Dawley rats, which subsequently resulted in HF. MI was evidenced by echocardiography, indicating that $40 \pm 5\%$ infarction of the left ventricle (LV), whereas HF was evidenced by increased LV end-diastolic pressures and decreased contraction-relaxation rates and exercise work capacity. Pathological remodeling was evidenced by increased LV cardiomyocyte lengths and widths. Spontaneous Ca2+ waves were measured by confocal

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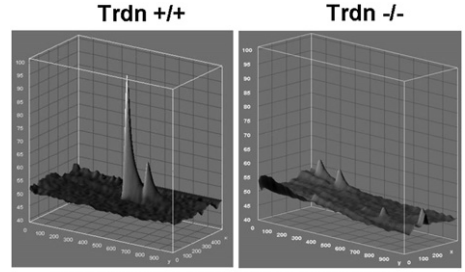
Gene-targeted deletion of the sarcoplasmic reticulum (SR) protein triadin (Trdn-/-) causes 50% reduction in ryanodine receptor (RyR2) Ca^{2+} release channels and cardiac calsequestrin, and a 50% decrease in the size of t-tubule SR junctions in mouse heart muscle. Here we report on the Ca^{2+} spark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