transmembrane flux/exchange of the metabolites. These transporters may possess novel and as yet unidentified characteristics that are in proportion to their critical role in cell metabolism. Evidence has been presented that they function as homodimers *(1)* even though each subunit appears to posses its own metabolite transport path *(2)*. We have expressed some of these transporter subunits as bacterial inclusion bodies. Homodimers but not complexes with more than two subunits can be formed and such dimers are able to catalyze metabolite transport or exchange when incorporated into liposomes. We find now that dimers can also be formed between subunits of different transporters (carriers), i.e. between phosphate and oxaloacetate or between phosphate and dicarboxylate. These experiments suggest that the subunit interface of different transporters must be very much alike. Heterodimers have never been purified from or identified in mitochondria. The homodimers are formed during insertion into the mitochondrial membrane *(3)*. These membrane insertion steps are very important since a heterodimer of different transporter subunits can lead to an inappropriate exchange of metabolites that can be deleterious to oxidative phosphorylation and to the link between metabolic reactions of the matrix and the cytosol.

(1) J. Biol. Chem. (1998) 273 14269.
(2) Nature (2003) 426 39.
(3) Science (2003) 299 1747.

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Role of Zinc Transporter ZnT5 In PKC Signaling And Cardiac Cell Survival

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Zinc transporter ZnT5 was determined to protect from intracellular zinc overload. We asked whether ZnT5 participates in intracellular signaling and regulation of cell function. Using adult rat cardiomyocytes and cultured atrial cells, HL-1, we investigated interaction of ZnT proteins with protein kinase C (PKC). ZnT5 was found to co-precipitate with PKC isoforms δ and ϵ. Complex formation was zinc-dependent: zinc-depletion with the specific chelator TPEN promoted interaction. PKC activators increased ZnT5 association with PKC ϵ and dissociated PKC δ from the complex. To determine functional significance we assessed translocation of PKC isoforms in HL-1 cells. The cells were transfected with ZnT5cDNA or the mutated dominant negative (DN) form. When ZnT5 expression was modified, localization of both PKC isoforms was altered, particularly pronounced with DN ZnT expression that resulted in disappearance of PKCδ from the Golgi complex. ZnT5 was found to localize in the Golgi in proliferating cultured HL-1 cells but not in adult cardiomyocytes where sarcomeric pattern was observed. Overexpression of ZnT5 enhanced proliferation of HL-1 cells. High demand for ZnT5 in the cells with high rate of proliferation was confirmed in the developing embryos and embryonic bodies. ZnT5 was prevalent in the areas of highly proliferating cells belonging to inner cell mass and not the differentiated ones surrounding basement membrane stained for laminin. Biochemical experiments confirmed that highly proliferating cells in embryonic bodies at stage D2 have higher expression of ZnT5 but not α-actin as compared to D7 stage of development. The expression of α-fetal protein (α-FP) at day 7 indicates differentiation. The data suggest that in addition of controlling zinc homeostasis, ZnT5 zinc transporter plays an important role in signaling. Both functions are likely required for cell survival, proliferation and therefore cardioprotection and embryonic development.

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Mrp4 Is A Transmembrane Export Pump Acting As An Endogenous Regulator Of Cyclic- Nucleotides Dependent Pathways

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Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are second messengers which regulate many biological processes. They can be eliminated by active efflux transporters, namely the multidrug resistance proteins MRP4 and MRP5. To delineate the role of MRP4/5, we studied arterial smooth muscle cells in which the role of cyclic nucleotide levels on proliferation has been well-established.

Methods: Human SMCs were isolated from the coronary artery media from patients. Small interfering RNAs (siRNA) specific for MRP4 mRNA were designed and validated. Adenovirus encoding MRP4 short hairpin RNA (Ad-shMRP4) were used for *in vivo* studies.

Results: MRP4 was over-expressed in serum-induced proliferating SMC as well as in atherosclerotic plaques in human coronary arteries and in neo-intima of injured rat carotid arteries. Inhibition of MRP4 by siRNA blocked VSMC proliferation *in vitro*. In balloon-injured rat carotid arteries, intima/media ratios were significantly lower in Ad-shMRP4-infected arteries than in Ad-shLuciferase-infected arteries (0.65 ± 0.1 *vs* 1.05 ± 0.2; $p<0.03$). *In vitro*, MRP4 inhibition significantly increased intracellular cAMP and cGMP levels. A PKA inhibitor (PKI) but not the PKG inhibitor (KT5823) completely reversed the anti-proliferative effect of MRP4 inhibition. The level of pCREB increased by $329 \pm 18.8\%$ ($p=0.003$) on MRP4 inhibition. **Conclusion**: We provide first evidences that MRP4 acts as an independent endogenous regulator of cyclic nucleotides intra-cellular levels in vascular smooth muscle cells

Calcium Fluxes, Sparks, and Waves I

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Synchronized Spontaneous Calcium Release Events Throughout The Intact Heart

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Intracellular calcium (Ca) dysregulation associated with cardiac disease (e.g. heart failure) has been linked to mechanisms of ventricular arrhythmias. Such arrhythmias can arise at the sub-cellular level from delayed afterdepolarizations that are due to spontaneous calcium release from the sarcoplasmic reticulum. The mechanisms that govern an aggregate of sub-cellular spontaneous calcium release events at the tissue level (i.e. an SCR) are not well understood. We hypothesize that in tissue, an SCR can be significantly influenced by ryanodine receptor (RyR) function. **Methods:** High resolution optical mapping of Ca (Indo-1AM) from the anterior surface of the Langendorff perfused guinea pig heart (n=4) was performed in hearts under high Ca conditions ($[Ca^{2+}]_e$=5.5mM), with and without caffeine (CAFF, 1mM) to enhance RyR open probability. Endocardial cryoablation was performed to eliminate Purkinje fibers and cytochalasin-D (7μM) was administered to remove motion artifact. Fifteen seconds of rapid pacing (400-160 ms cycle length) followed by a pause was used to induce SCR activity. **Results:** In all preparations**,** synchronized SCR activity was observed across the entire anterior surface of the heart with and without CAFF. SCR activity increased in magnitude and occurred earlier with decreasing pacing cycle length. With CAFF, the amplitude of SCR activity increased (+10.9%, $p < 0.05$) and occurred earlier (+15.6%, $p < 0.05$). CAFF also decreased the spatial heterogeneity of SCR onset across the mapping field (-37.4%, $p < 0.05$), suggesting that increased RyR open probability enhances the synchronization of SCR activity. **Conclusions:** These results demonstrate that sub-cellular spontaneous calcium release events (an SCR in tissue) occur over a broad region of the intact heart and are enhanced when RyR open probability is increased. SCR activity may be an important mechanism of arrhythmogenesis in heart disease associated with calcium dysregulation.

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Investigating the Ca^{2+}-Cycling Basis of Rhythmicity and Synchronicity in Coupled Cardiomyocyte Monolayers

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Arrhythmia arises from the breakdown of synchronous ion handling. Abnormal intracellular Ca^{2+} cycling has emerged as a primary driver for subsequent arrhythmogenic perturbations in sarcolemmal Na^+ and K^+ fluxes. Consequently, therapeutic manipulation of intracellular Ca^{2+} handling represents a key target for new anti-arrhythmic strategies. In order to better understand the potential modes of modulating Ca^{2+} cycling that may underpin feasible anti-arrhythmic approaches, we focussed on dissecting the relationships between the spatiotemporal aspects of Ca^{2+} release and intercellular (dys)synchrony. Ca^{2+}-dependent fluo-4 signals recorded in spontaneously oscillating, electrically-coupled cardiomyocytes were decoded using the **S**ynchronicity-**A**mplitude-**L**ength and **V**ariability of **O**scillation (SALVO) program that describe 'contractile' and 'non-contractile' aspects of Ca^{2+} handling. In 40 separate experiments (n> 500 cells), intercellular synchronisation of Ca^{2+} release and sequestration was relatively constant ($42.7 \pm 2.5\%$) over wide ranges of Ca^{2+} transients profiles (transient areas of 7 - 117 units) and oscillatory frequencies (0.026 - 2.17Hz). Surprisingly, the relative areas of Ca^{2+} transients (reflecting the amplitude and kinetics of Ca^{2+} release and sequestration) were not linked to the oscillatory frequency. Although high levels of intercellular synchrony persisted despite a remarkable plasticity in both the frequencies and shapes of Ca^{2+} transients, we found that the transients became more uniformly ordered at

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.

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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.

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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.

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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.

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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.

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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