

1583-Pos Board B493**Alterations in Mitochondrial State 4→3 Transition Underlie Stress-Induced Energetic-Redox Imbalance and Myocyte Dysfunction in Diabetic Mice**

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Diabetes is a syndrome in which mitochondrial alterations may participate in the dysfunction, and ultimately failure of several organs. The diabetic heart in particular can be subjected to both glycemic level oscillations and increased workload (via β -stimulation), events that may further deteriorate its mechanical properties. Here we show that when diabetic heart (*db/db*) cells are challenged with high levels of extracellular glucose (30 mM) and concomitant β -adrenergic stimulation via isoproterenol (ISO), intracellular GSH is greatly diminished while superoxide and H_2O_2 generation substantially increased. The typical enhancement in sarcomere shortening and whole Ca^{2+} transient driven by β -adrenergic stimulation is blunted in *db/db* cells, along with hampered relaxation and Ca^{2+} reuptake. Mitochondria isolated from *db/db* hearts subjected to high-glucose/ISO regimen exhibit markedly decreased coupling ratios from respiratory complexes I, II, and IV while ROS emission is greatly increased, under both forward and reverse electron transport. These changes reflect a profound alteration in the crucial (energy supplying) state 4→3 transition that ultimately results in lowered ATP synthesis and in a “non-stop” reactive oxygen species (ROS) emission in the presence of ADP. Incubating high-glucose/ISO treated *db/db* myocytes with cell-permeable GSH restores intracellular GSH, blunts ROS production and fully rescues contractility/relaxation in these myocytes. These results show the direct role played by an oxidized redox environment in triggering the negative synergy among mitochondrial ROS and energetics leading to the E-C coupling impairment. Our study maps mitochondrial sites, i.e. state 4→3 transition, that in diabetic heart cells account, at least in part, for excess ROS emission and loss in energy supply when metabolically and energetically challenged.

1584-Pos Board B494**The Role of Reactive Oxygen Species in Contractile Dysfunction Following Ischemia/Reperfusion**

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Myocardial stunning occurs following ischemia-reperfusion, and is characterized by depressed contractility and elevated resting tension that is distinct from dysfunction associated with tissue damage or death. A wide variety of factors have been shown to contribute to post-ischemic myocardial dysfunction including cytosolic Ca^{2+} overload, acidosis, and the damaging effects of degradative enzymes and free radicals such as reactive oxygen species (ROS). These mechanisms act over a wide range of timescales. Intracellular Ca^{2+} , Na^+ , and pH return to near pre-ischemic levels within a matter of minutes, while ROS production persists for up to three hours. The symptoms of myocardial stunning can persist even longer, indicative of a modified redox environment in the myocyte. Studies have revealed a number of sources of ROS production during reperfusion, but the mitochondria's role in regulating the balance of electron-carrying redox couples implicates cellular respiration as a critical source of ROS. In the modeling work presented here, we link a mathematical model of mitochondrial respiration and ROS production with a model of excitation-contraction coupling in the guinea pig myocyte. Experimentally determined ROS-dependent modulation of ryanodine receptors, sarcoplasmic reticulum (SR) Ca^{2+} ATPase, Na^+ - K^+ ATPase and myofilaments is incorporated into our model. Using this integrated model, we show that contractile dysfunction associated with myocardial stunning persists after ROS-mediated alterations in protein function, ROS-related changes in Na^+ - K^+ ATPase flux lead to altered intracellular Na^+ , which modulates diastolic intracellular Ca^{2+} via the Na^+ - Ca^{2+} exchanger. Reduced SR Ca^{2+} ATPase activity and increased ryanodine receptor leak cause decreased SR Ca^{2+} load. Finally, ROS-related alteration of myofilament proteins reduces their Ca^{2+} sensitivity, which also contributes to decreased contractile force. Taken together, all of these mechanisms lead to contractile dysfunction caused by mitochondria-derived ROS.

1585-Pos Board B495**Ec Coupling is Preserved in Nex Knockout Mice Exposed to Metabolic Inhibitors**

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To determine whether ablating the cardiac sodium-calcium exchanger (NCX) prevents couplon loss during metabolic inhibition (MI), we recorded Ca transients or resting Ca spark frequency (CaSpF) in ventricular myocytes isolated from WT and ventricular-specific NCX knockout (KO) mice. In field stimulated myocytes loaded with 10 μ M Fluo-3 AM, application of the mitochondrial and glycolytic inhibitors FCCP (50 nM) and 2-DG (10 mM) reduced Ca transients by $54.2 \pm 3.3\%$ in WT but only by $19.0 \pm 6.0\%$ in KO ($p < 0.05$). Similarly, MI suppressed action potential evoked Ca sparks in patch clamped WT myocytes loaded with 1 mM fluo-3 salt by $60.0 \pm 8.2\%$ ($p < 0.05$), but in KO by only

$33.0 \pm 3.4\%$. The reduction in I Ca during MI was similar for both WT and KO and SR Ca stores remained at control levels in both cell types during MI. In patch clamped myocytes loaded with 1 mM K5Fluo-3 and held at -75 mV, MI reduced resting CaSpF in WT by $92.4 \pm 6.7\%$, ($p=0.008$) but had no effect on CaSpF in KO. Abolishing Na and Ca gradients by using the same “internal” solution for both the bath and pipette (current clamp mode, $V_{rest} = 0$ mV), eliminated the KO's resistance to MI, causing CaSpF to decline to a similar extent in both WT and KO ($59.0 \pm 6.2\%$ and $60.7 \pm 10.9\%$, respectively). This finding suggests that elevated diadic cleft Ca, a major characteristic of KO myocytes (Pott et al, Biophys J, 92: 1431, 2007; Neco et al. Biophys J, 99: 755, 2010), maintains effective triggering of SR Ca release during MI despite reduced I Ca .

1586-Pos Board B496**Mechanisms Regulating the Organellar Ca^{2+} Dynamics in Intact Hearts During Ischemia-Reperfusion**

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The accumulation of Ca^{2+} during ischemia, in both cytosol and sarcoplasmic reticulum (SR) of ventricular cardiomyocytes, contributes to the condition known as myocardial stunning. This not only disrupts normal Ca^{2+} dynamics in the contractile cells but also makes them more prone to apoptosis and/or necrosis. Therefore, the identification of the mechanisms underlying this phenomenon is important for understanding functional changes that occur during cardiac infarction. We examined the role of acidosis, inhibition of Na^+ / Ca^{2+} exchanger (NCX) and altered restitution of Ca^{2+} release as factors that can potentially be involved in the intracellular Ca^{2+} accumulation. Pulsed Local Field Fluorescence experiments on Langendorff-perfused mouse hearts reveal that a decrease in Tyrode solution's pH of from 7.4 to 6.2 diminished the amplitude of Ca^{2+} transients ($52 \pm 3\%$ of control) without elevation of diastolic Ca^{2+} level (measured with rhod-2 for cytosol and mag-fluo-4 for intra-SR). This suggests that acidosis alone cannot be responsible for accumulation of Ca^{2+} in myocytes. The combination of low pH (6.2) and partial (25%) replacement of Na^+ with Li^+ (to inhibit NCX) resulted in 1.4-fold increase in resting diastolic [Ca^{2+}] in cytosol suggesting that the NCX activity might contribute to ischemia-induced changes in intracellular Ca^{2+} content. The restitution of Ca^{2+} release, measured with double-pulse protocols, slowed down during the ischemic period. Under these conditions, the decrease of Ca^{2+} release from SR might promote an accumulation of Ca^{2+} in this cellular compartment. Simultaneous measurements of intracellular action potentials (AP) and Ca^{2+} transients revealed that the restitution of AP is not the limiting step for Ca^{2+} release restitution during ischemia. Interestingly, the slow restitution persisted during reperfusion leading to an amplification of Ca^{2+} alternans. The study was supported by NIH R01-HL-084487 grant.

1587-Pos Board B497**Optical Measurement of Action Potential in Adult Ventricular Myocytes**

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Due to their shape and fragility primary cells such as neurons or cardiomyocytes cannot enter automated membrane potential measurements on patch-clamp robots. Instead contact-free optical approaches would be advantageous. So far procedures based on potentiometric dyes are lacking one or several of the following properties: (i) ability for quantification, (ii) sufficiently fast response time to follow action potentials (APs), (iii) sufficiently high signal-to-noise ratio to avoid signal averaging, (iv) sensor-induced alterations of AP properties. We show that the small molecule dye di-8-ANEPPS and the novel genetically encoded sensor “Mermaid” provide quantitative potential information. Based on an improved optical design, individual AP could be recorded with an exceptional signal-to-noise-ratio. The sensors were validated using the patch-clamp technique, confocal microscopy and fluorescence lifetime imaging in combination with global unmixing procedures. When applying such approaches we identified distinctly different pharmacological profiles of APs between adult and neonatal cardiomyocytes. We concluded that optical recordings of individual APs can be performed using small molecule dyes and novel genetically encoded biosensors without disturbing the properties of APs. Additionally we have demonstrated the superiority of adult over neonatal cardiomyocytes for pharmacological investigations such as QT-screens.

1588-Pos Board B498**The Differential Action of Cytocalasin D in T-tubular Remodelling of Ventricular Myocytes**

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Cytocalasin D (cytoD) is a fungal metabolite that inhibits cytokinesis by blocking formation of contractile microfilament structures resulting in multinucleated cells, reversible inhibition of cell movement, and the induction of

cellular extrusion. However, in muscle cells reports are controversial and vary from actin stabilization to actin disruption at concentrations of 40 μM . The aim of this study was to investigate the putative cytoD effects on adult cardiomyocytes in culture and how it can be used as a pharmacological tool in single cell models. This investigation is based on our optimized serum-free culture method for adult rat ventricular myocytes.

Initially, for global calcium transients we performed repetitive dose response relationships over the time course of 6 days to identify the optimal cytoD concentration. At this identified concentration we analyzed action potentials, calcium handling (incl. post rest behavior and caffeine induced calcium release) and cellular contractility. In addition, we investigated cytoD-mediated changes in the T-tubular system and actin cytoskeleton by confocal and STED microscopy, respectively.

In contrast to previous reports we were able to identify a cytoD concentration that resulted in a high degree of morphological and functional conservation of properties found in freshly isolated cells over a time course of one week.

In conclusion our findings represent a further significant improvement for single cell models of cardiomyocytes, enabling chronic stimulation and an extended time period for adenoviral protein expression. The identified relationship between actin filament modulation and prevention of T-tubular loss might shed new light on the loss of T-tubules in cardiac diseases.

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Modelling the Structure and Function of Cardiac Cell Transverse-Axial-Tubules

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Modelling the Structure and Function of Transverse-Axial-Tubules

The past decades have seen significant strides being made in understanding the structure and function of the heart using experimental and computational approaches. Mathematical models of the heart can now represent the anatomical structure of the whole-heart and simulate the heart beat using biophysically based methods on the computer. At the cellular-level, however, mathematical models typically simulate the function using black-box mass kinetics approaches and make several simplifying assumptions regarding the spatial scale and organization of the key cellular components in excitation-contraction coupling. While these studies have been pivotal in gaining quantitative insights on cardiac cell activity, a deeper understanding of structure function relationships at the cellular scale can only be gained by incorporating more realistic models of the geometry. With this aim, we present an anatomically realistic finite element model of the cardiac cell sarcolemma, and its transverse-axial tubule system. Confocal microscopy images of the sarcolemma and t-tubules of an isolated wistar rat myocyte were acquired, segmented and skeletonized. A two-dimensional surface mesh was fitted to the sarcolemma data, and one-dimensional finite elements were used to represent the t-tubules from the skeletonized image in three-dimensional space. This representation will form the basis to simulate calcium diffusion into the cell cytosol during the excitation phase of excitation-contraction coupling. We present preliminary results from these simulations, and provide first quantitative insights into cardiac cell excitation in 3D.

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Enhanced Propagation of Calcium-Induced Calcium-Release (CICR) from the Cell Periphery to the Core Increases Contractility of Detubulated Myocardium

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Mechanics and Excitation-Contraction-Coupling were studied in rat ventricular trabeculae and myocytes before and after formamide-induced detubulation. Detubulation was checked using FM4-64 membrane staining and dual-photon microscopy. Electrical uncoupling of t-tubules from sarcolemma was confirmed by simultaneous action potential recordings at surface and t-tubular membranes (Sacconi et al., this meeting). Global and regional (periphery/core) Ca^{2+} -transients were measured either under confocal microscopy or in wide-field with a high-speed CCD-camera from Fluo4-loaded myocytes. At low $[\text{Ca}^{2+}]_o$ (1mM, 2Hz, 30°C) twitch tension of detubulated trabeculae was reduced to ~40% of controls and the time course of contraction was significantly slower. Under the same conditions, the global Ca^{2+} -transients were depressed and slowed down in detubulated cells vs. controls. Regional Ca^{2+} -transients were synchronous and homogeneous in control myocytes while in detubulated cells they were mostly restricted to a subsarcolemmal ring with the cell core displaying lower and delayed Ca^{2+} -signals. The results are consistent with detubulation inducing a shift from synchronous CICR to a propagated form of CICR that is slower and

may not recruit deep myofibril layers. At high $[\text{Ca}^{2+}]_o$ (>8mM), or following post-rest potentiation, twitch tension of detubulated trabeculae was unchanged compared to controls, though contractions were prolonged. At high $[\text{Ca}^{2+}]_o$, or at resting stimulation frequency, the Ca^{2+} gradient between cell periphery and core was markedly reduced in detubulated myocytes while the time-lag between the two Ca^{2+} -transients was still present. The results suggest that CICR propagation from cell periphery to the core can be enhanced and contractility improved in detubulated myocardium by increasing the Ca^{2+} -trigger and the SR Ca^{2+} -load. Interventions that increase RyR2 open probability may also enhance CICR propagation in detubulated myocytes. This has been confirmed by comparing the effects of caffeine (200 μM) on control and detubulated myocytes.

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Distribution and Clustering of Membrane Ca^{2+} Transporters Regulate Ca^{2+} Signals in Rat Ventricular Myocytes

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In ventricular cardiac cells, the t-tubule microanatomy and the distribution and clustering of membrane Ca^{2+} transporters (L-type Ca^{2+} channel, $\text{Na}^+/\text{Ca}^{2+}$ exchanger, sarcolemmal Ca^{2+} ATPase) are considered important in Ca^{2+} regulation [1]. Their variations over time may hold clues to the progression of heart diseases [1,2]. To examine how these sub-cellular mechanisms regulate the Ca^{2+} signals in rat myocytes with inhibited sarcoplasmic reticulum we used modeling approach [3]. The t-tubule geometry was derived from light microscopy images and Ca^{2+} diffusion and buffering by stationary and mobile Ca^{2+} buffers (troponin C, ATP, calmodulin, Fluo-3) were taken into account [3,4]. In agreement with experiment: (1) the L-type Ca^{2+} current density was higher along the t-tubule, the spacing between adjacent L-type Ca^{2+} clusters was assumed ~0.68 μm , and the average L-type Ca^{2+} cluster size was ~50 nm in radius [2,5]; (2) the $\text{Na}^+/\text{Ca}^{2+}$ current density was higher and uniform along the t-tubule and $\text{Na}^+/\text{Ca}^{2+}$ exchanger was located within (or close to) L-type Ca^{2+} clusters [1]; (3) the sarcolemmal Ca^{2+} pump was uniformly distributed along the sarcolemma co-localizing or not with L-type Ca^{2+} clusters. The results indicate that spatial Ca^{2+} signals critically depend on the local diffusion properties and the location and clustering of Ca^{2+} transporters along the cell membrane.

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Remodeling of the Sarcoplasmic Reticulum and T-Tubules in Cardiomyocytes Following Conditional *SERCA2* Knockout

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Heart failure is associated with reduced sarco(endo)plasmic reticulum (SR) calcium ATPase (SERCA) function. Mice with conditional cardiomyocyte specific knockout of the *Serca2* gene (KO) revealed that major reductions in SERCA and SR function are compensated by enhanced Ca^{2+} cycling across the cell membrane. Thus, cardiac function was maintained at near-normal values, at least in the short term. We hypothesize that this shift from SR to sarcolemmal Ca^{2+} fluxes is reflected in alterations in SR and sarcolemmal morphology. Seven weeks following gene disruption, some hearts were fixed for electron microscopy by perfusion with 3.5% glutaraldehyde and others for enzymatic isolation of cells for confocal microscopy and patch clamping. *Serca2* flox-flox (FF) mice served as controls. Electron micrographs revealed drastically altered SR architecture and density. SR volume was reduced from 1.74 ± 0.16 % of total cell volume in controls to 0.84 ± 0.13 % in KO. The SR area to cell volume ratio was reduced from 0.68 ± 0.06 to 0.32 ± 0.05 $\mu\text{m}^2/\mu\text{m}^3$, and 55% of the free SR appeared collapsed, with a virtually occluded lumen. Although overall cardiomyocyte dimensions were unaltered in KO compared to FF, total surface area was increased (cell capacitance = 182 pF vs 147 pF, $P < 0.05$). This resulted from increased t-tubule density, as revealed by confocal images of Di-8-Anepps stained myocytes. Specifically, Fourier analysis indicated a maintained organization of t-tubules which transversely spanned the cell, but an increased presence of tubules in the longitudinal direction. Immunocytochemical data showed that the newly grown longitudinal t-tubules contained $\text{Na}^+/\text{Ca}^{2+}$ -exchanger which co-localized with ryanodine receptors in the SR, but did not contain $\text{Ca}_v1.2$ channels. Our observations indicate that the SR is greatly reduced and collapses following *Serca2* KO. Further, proliferation of longitudinal t-tubules facilitates trans-sarcolemmal Ca^{2+} flux to compensate for declining SR function.