mitochondrial redox response to increased work. Isolated cardiomyocytes were field stimulated and fractional shortening simultaneously recorded with epi-fluorescence measurements of NAD(P)H and FAD. Cells were paced at 0.5Hz and the stimulation frequency step increased to 1Hz, 2Hz and 3Hz in order to increase work intensity. NAD(P)H was excited at 340nm (fluorescence collected 455-480nm) and FAD was excited at 430nm (fluorescence collected 505-600nm).
Increasing the stimulation frequency from 0.5Hz to 2Hz and 3Hz, but not 1Hz, resulted in a decrease in NAD(P)H fluorescence and an increase in FAD fluorescence, indicating oxidation of the cell environment. Reducing work intensity back to 0.5Hz pacing led to immediate recovery of metabolite fluorescence. Addition of 2mM NaCN established a completely reduced mitochondrial environment, leading to NAD(P)H fluorescence increasing to a maximum and FAD fluorescence decreasing to a minimum. Subsequent step increase in stimulation to 3Hz caused no change in NAD(P)H or FAD fluorescence. Treatment with 2μM FCCP established a completely oxidised state, resulting in NAD(P)H fluorescence falling to a minimum and FAD fluorescence increasing to a maximum. Pacing at 3Hz in this state again led to no change in metabolite fluorescence, confirming the response to increased work was mitochondrial in origin. Increasing stimulation frequency to 3Hz in the presence of the movement uncoupler cytochalasin D, minimising cell contraction, also led to no change in NAD(P)H or FAD fluorescence, thus confirming that contractile work was the cause of the change in mitochondrial redox state.
In conclusion, the response to increased work intensity in cardiomyocytes is oxidation of the cell, suggesting that the mitochondria are initially unable to maintain NAD(P)H/$FADH_2$ supply in order to cope with increased metabolic demand.

1246-Pos Board B90
Trimetazidine Effects On The Mitochondrial Metabolism During Rabbit Heart Failure
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Background: We have previously shown that the anti-ischemic agent trimetazidine (TMZ) rescued $[Ca^{2+}]_i$ transient and mechanical alternans in ventricular myocytes from rabbits with non-ischemic heart failure (HF), induced by combined aortic insufficiency and stenosis. The cardioprotective action of TMZ has been linked to the inhibition of free fatty acid (FFA) oxidation, however the underlying mechanism remains poorly defined. The aim of this study was to determine whether the plasma levels of FFA (total ($[FFA]_{tot}$) and unbound to albumin ($[FFA]_u$)) are elevated in rabbit HF and whether TMZ affects mitochondrial metabolism. **Methods and Results:** We found that both $[FFA]_{tot}$ and $[FFA]_u$ were significantly elevated in HF rabbits. $[FFA]_u$ increased 4-times during HF (from 13 ± 4 to 53 ± 7 nM) while the $[FFA]_{tot}$ increased only two-fold (from 58 ± 16 to 121 ± 29 μM), demonstrating that $[FFA]_u$ is a reliable biomarker of HF. Furthermore, using TMRM fluorescence confocal microscopy and a Strathkelvin micro volume precision respirometry system, we determined that mitochondrial complex II activity was significantly elevated (+72%) during HF, while complex I activity was decreased (-90%). Cell treatment with TMZ had no effects on the complex I activity in control (+6%), while it increased (+26%) the activity of complex I under HF conditions. Moreover, TMZ reversed complex II activity in HF myocytes (-55%), while it had no effect on complex II activity in control cells (-10%). The oxidation of palmitoylcarnitine, the upstream substrate for FFA oxidation, was decreased 32% by TMZ, while TMZ had no effect on complex IV activity. Furthermore, FADH-mediated auto-fluorescence levels were significantly elevated in HF myocytes treated with TMZ. **Conclusion:** TMZ suppresses the elevated activity of mitochondrial complex II while it increases the depressed activity of complex I in rabbit HF, and therefore it preserves metabolic reserve of the cell.

1247-Pos Board B91
Mitochondrial Dynamics In Heart Cells: Very Low Amplitude High Frequency Fluctuations In Adult Cardiomyocytes And Flow Motion In Non-beating Hl-1 Cells
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The arrangement and movements of mitochondria were quantitatively studied in adult rat cardiomyocytes and in the cultured continuously dividing non beating NB HL-1 cells with differentiated cardiac phenotype.
Mitochondria were stained using fluorescent dye MitoTrackerGreen, a dye associated with inner membrane of mitochondria, and studied by fluorescent confocal microscopy. Imaging during different time intervals made it possible to visualize the 2-dimensional movements and dynamics of cardiac mitochondria.
In adult cardiac cells mitochondria were always arranged very regularly in a crystal-like manner and did not show any changes in their position during 30 min of low speed scanning. However, high speed scanning (pixel dwell time 3 ms, time interval between images 400 ms) revealed very rapid fluctuations of the positions of fluorescence centers which followed the pattern of a random walk movement within the limits of the internal space of mitochondria, most probably due to transitions between condensed and orthodox configurational states of matrix and inner membrane as a result of functioning of transmembrane metabolite carriers. No evidence for mitochondrial fusion or fission was found in adult cardiomyocytes.
In contrast, in NB HL-1 cells, mitochondria were arranged as a dense tubular network, in permanent fusion, fission and displacement with high velocity around 90 nm/s.
The differences observed are related to specific structural organization of the cells, and most probably due to differences in mitochondria-cytoskeleton organization. Intracellular local restrictions of diffusion of adenine nucleotides and metabolic feedback regulation of respiration via phosphotransfer networks are also different in these cells.

1248-Pos Board B92
Cgp-37157 Abrogates The Adverse Effect Of Ouabain On Mitochondrial Energetics
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Cardiac glycosides have been used to treat heart failure for more than 200 years and their major effect is to inhibit the Na^+/K^+ pump. Inhibition of the pump on the sarcolemma of cardiac myocytes elevates intracellular Na^+ ($[Na^+]_i$), resulting in a positive inotropic effect by increasing Ca^{2+} load. However, our previous work demonstrated that elevated $[Na^+]_i$ impairs mitochondrial energetics by blunting mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) accumulation. Moreover, we showed that CGP-37157, an inhibitor of $[Ca^{2+}]_m$ efflux, restored $[Ca^{2+}]_m$ accumulation and improved mitochondrial energetics. Here, we investigated the effects of ouabain with or without CGP-37157 on $[Na^+]_i$ and NADH production in isolated cardiomyocytes and examined the effects on hemodynamics and Oxygen consumption (mVO_2) in whole hearts. Application of ouabain to isolated myocytes elevated $[Na^+]_i$ in a dose-dependent way. During 1 Hz stimulation, the NADH/NAD+ redox potential in ouabain treated myocytes was decreased significantly, whereas NADH levels were well maintained in the presence of CGP-37157. In whole-heart studies, ouabain increased LVDP, +dP/dt, and -dP/dt, and addition of CGP-37157 further increased +dP/dt and -dP/dt. When isoproterenol was employed to increase cardiac work, LVDP was not increased, but +dP/dt and -dP/dt were increased by 57% and 52%, respectively, in hearts without concomitant CGP-37157 treatment. In isoproterenol-treated hearts also exposed to CGP-37157, LVDP increased by 30%, and +dP/dt and -dP/dt were increased by 75% and 53%, respectively. Whole heart mVO_2 increased by 18% after ouabain treatment and by 25% after isoproterenol administration compared to baseline. With concomitant CGP-37157 treatment, ouabain increased mVO_2 by 32% and isoproterenol increased mVO_2 by 53%. Our findings revealed an adverse effect of the glycoside on mitochondrial energetics and indicate that CGP-37157 can prevent this impairment. In addition, inotropic responses to both ouabain and isoproterenol were enhanced in the presence of CGP-37157.

1249-Pos Board B93
Mitochondrial Energetics During Transients Following Substrate And Ca2+ Additions. Modeling And Experimental Studies
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Ionic equilibria are known to be dramatically altered in failing hearts, as well as during and after ischemic injury. Ion transport across the mitochondrial inner membrane has been shown to modulate the energetic performance of mitochondria. Consequently, it is critical to thoroughly understand the interrelationship between ion fluxes and energetics. With this aim in mind, here we continue to develop our computational model of mitochondrial energetics to account for pH regulation, Na+/H+ cotransport, and the Pi carrier, and study their effects on mitochondrial energy production and Ca2+ handling mediated by the Ca2+

uniporter and Na+/Ca+ exchanger. Since time-dependent behavior represents the most stringent criterion of model validation, we perform direct comparisons of simulation results with experimental data obtained after challenging isolated mitochondria from guinea pig hearts with substrate and Ca2+ additions in the presence of different Na+ concentrations. Experimentally, we measured NADH and mitochondrial membrane potential (ratiometrically determined with TMRM) to monitor mitochondrial energetics. The model is able to reproduce the time course of NADH and membrane potential upon addition of 5mM glutamate plus malate followed by 2mM Pi and 1mM ADP. Moreover, the model recapitulates the NADH recovery profile after Ca2+ addition (0.1 to 0.5uM) during state 3 or state 4 respiration in the presence of either 5 or 15mM Na+. The results indicate that the computational model employed is able to account for the response of mitochondrial energetics to all experimental conditions tested. This work is supported by NIH grant R33HL87345.

1250-Pos Board B94

Characterizing The Calcium Uniporter: Effect Of Partial Depolarization On Calcium Flux

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Introduction: Mitochondrial (m) Ca^{2+} uptake occurs mainly via the Ca^{2+} uniporter (CU) and is dependent on the electrical and chemical gradient. We measured Ca^{2+} uptake at deceasing membrane potentials ($\Delta\Psi_m$) in isolated mitochondria. **Methods:** m[Ca^{2+}], $\Delta\Psi_m$, pH, and NADH fluorescence were measured using indo-1, rhodamine 123, BCECF and autofluorescence, respectively, in isolated guinea pig heart mitochondria. After energizing with pyruvic acid, 0, 10, 20, 30 or 100 μM of the protonophore dinitrophenol (DNP) was added to reduce $\Delta\Psi_m$ to 0, 3, 5, 9 and 80% of the maximal depolarization elicited by the protonophore CCCP, after which 10 and 25 mM [$CaCl_2$] ([Ca^{2+}] = 80 and 130 nM) were added. **Results:** Partial depolarization resulted in decreased Ca^{2+} uptake. Adding 25 μM Ca^{2+} without DNP gave a Ca^{2+} uptake of 44 nM/s. Partial depolarization decreased Ca^{2+} uptake in a dose dependent fashion (30, 28, 18, 10 nM/s with 10, 20, 30 or 100 nM DNP). Adding 10 μM $CaCl_2$ gave an uptake of 4.1, 3.6, 2.5, 2.8, 0.9 nM/s with 0, 10, 20, 30, 100 μM DNP, respectively. After 10, 20 and 30 nM DNP, m[Ca^{2+}] did not attain a steady state after the initial Ca^{2+} uptake. DNP alone decreased matrix pH, and addition of $CaCl_2$ caused additional decreases in pH. **Conclusion:** We demonstrate the importance of the electrical and chemical gradients for Ca^{2+} uptake. We show that mild depolarization reduced the rate of Ca^{2+} influx, but it did not decrease total steady-state m[Ca^{2+}] after 10 min. Only full depolarization of $\Delta\Psi_m$, as observed with 100 μM DNP, resulted in a lower total m[Ca^{2+}]. These results provide additional insight in understanding the dynamic vs steady-state transport of Ca^{2+} via the CU and its mutual dependence on $\Delta\Psi_m$ and extra-matrix [Ca^{2+}].

1251-Pos Board B95

ADP/ATP Antiport and ADP Phosphorylation Increase Mitochondrial Free Ca2+

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Introduction: Matrix free [Ca^{2+}] (m[Ca^{2+}]) is believed to be a key regulator of mitochondrial function. The effect of differential buffering of calcium by ADP, ATP and P_i on m[Ca^{2+}] levels has not been examined. We tested how m[Ca^{2+}] is increased by ADP/ATP transport and phosphorylation, and if increased m[Ca^{2+}] alters the bioenergetic state. **Materials and Methods:** Guinea pig heart mitochondria were isolated by differential centrifugation. Respiration and m[Ca^{2+}], using indo-1 fluorescence, and corrected for NADH autofluorescence, were measured. After energizing mitochondria with 0.5 mM pyruvic acid, 0, 10, 25 μM $CaCl_2$ (16, 88, 130 nM [Ca^{2+}]) was added to the suspension before adding 250 μM ADP, in the presence or absence of ADP/ATP carrier blocker carboxyatractyloside (CATR) or F_1F_0ATPase blocker oligomycin (OMN). **Results:** m[Ca^{2+}] increased proportionately with addition of $CaCl_2$. ADP caused an additional increase to 100 ± 6% in m[Ca^{2+}] after 25 μM $CaCl_2$. This was due to lesser binding of ADP vs. ATP to Ca^{2+}. The rise in m[Ca^{2+}] after ADP was reversed after all ADP was converted to ATP. With OMN the increase after ADP was lower (18 ± 6%), but remained elevated as ADP was not phosphorylated to ATP. CATR completely blocked the ADP -induced increases in m[Ca^{2+}] because matrix ADP transport was blocked. State 2 and 4 respiration, but not state 3, increased 14% and 18% with 25 μM $CaCl_2$. NADH decreased with ADP alone, but NADH was not altered by adding $CaCl_2$. **Discussion:** These results show that ADP transport into mitochondria and ADP conversion to ATP have significant effects on m[Ca^{2+}]. Acutely changing buffer [$CaCl_2$] has limited effects on redox state, although m[Ca^{2+}] is believed to stimulate several dehydrogenases. However the $k_{0.5}$ (1 μM) for this effect is only reached by adding ADP after 25 μM $CaCl_2$.

1252-Pos Board B96

Selective Regulation of Mitochondrial Outer Membrane VDAC Permeability *in situ* in Permeabilized Cardiomyocytes

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The aim of this study was to investigate localized restrictions of diffusion of phosphocreatine (PCr) and adenine nucleotides across mitochondrial outer membrane (MOM). Complete kinetic analysis of mitochondrial creatine kinase (MtCK) - activated respiration *in situ* and *in vitro* showed that apparent dissociation constants of MgATP from complexes with MtCK were increased by several orders of magnitude in *situ* system in comparison with values *in vitro*. No difference of apparent dissociation constant for PCr was observed. To study the selective permeability of the VDAC in MOM *in situ*, we measured the rates of PCr synthesis and channelling from mitochondria into medium in permeabilized cardiomyocytes. An external PEP-PK system was used to trap extramitochondrial ADP and prevent PCr utilization after activation of the MtCK system by Cr. The concentrations of the ATP stayed constant as the concentration of the PCr showed linear increase in time. The rate of mitochondrial synthesis of PCr and its diffusion into medium at 5 mM ATP was equal to 0,55 μmol/min/mg giving PCr/O ratio equal to 5,5. Thus, in permeabilized CM the permeability of VDAC for PCr was high; at the same time apparent Km for ADP and for ATP rise tenfold and 100-fold respectively from *in vitro* to *in situ*. These results show that diffusion restriction through the VDAC is selective due to the interaction of the anion channels with some of the components of cytoskeletal network. We found that beta-tubulin cDNA (beta-tubulin gene M-beta-4) is present in mouse myocardium and oxidative *m. soleus* (high apparent Km(ADP)) but is absent in *m. extensor digitorum longus (*low apparent Km(ADP)). This tubulin isoform may participate in the organization of the intracellular energetic units as the regulator of VDAC permeability in oxidative muscle cells.

1253-Pos Board B97

Identifying The Site Of The Source Of Reactive Oxygen Species Within The Mitochondria After Transient Exposure Of Cardiac Myocytes To Hydrogen Peroxide

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Oxidative stress is a feature of cardiovascular disease. Hydrogen peroxide (H_2O_2) can act as a signaling molecule to mediate cardiovascular pathology. We have previously shown that transient exposure of adult guinea pig ventricular myocytes to H_2O_2 leads to further production of reactive oxygen species (ROS) from the mitochondria. We have demonstrated that exposure of myocytes to 30μM H_2O_2 for 5 min then 10U/ml catalase for 5 min to degrade the H_2O_2 caused a 65.4 ± 8.4% further increase in superoxide by the mitochondria (n=47). We tested whether transient exposure to H_2O_2 altered protein synthesis in the myocytes. Exposure of myocytes to 30μM H_2O_2 for 5 min followed by 10U/ml catalase for 5 min caused a 2-fold increase in protein synthesis measured as ^{3}H-Leucine incorporation (n=10). This suggests that a transient exposure to H_2O_2 may be sufficient to induce cardiac hypertrophy. We now wish to identify the site of ROS production in the mitochondria. Superoxide was assessed with the fluorescent indicator dihydroethidium (DHE). Exposing myocytes to 1μM DPI, which binds prior to the ROS generation site of complex I, followed by transient exposure to H_2O_2 resulted in complete attenuation of the increase in DHE signal after exposure to H_2O_2. Exposing myocytes to 1μM rotenone, which binds after the ROS generation site of complex I, followed by transient exposure to H_2O_2 resulted in a 45% reduction in the increase in DHE signal after exposure to H_2O_2. These data suggest the source of ROS production is distal to complex I. Identifying the site of production of ROS may represent a possible therapeutic target to prevent the development of cardiac hypertrophy associated with a transient exposure to H_2O_2.

1254-Pos Board B98

HIF-1α Contributes in Hepatic Bioenergetic Failure of Late Sepsis by Regulating Mitochondrial ATPase Inhibitor Protein (IF1) Expression

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Tissue hypoxia caused by inadequate tissue perfusion damages the mitochondrial function and subsequently contributes the energy deficiency in late septic liver. Although several lines of evidence revealed the decreased ATP synthesis