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.

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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+}].

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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 $CaCl_2$.

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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.

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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.

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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