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

Johan Haumann, Cory Hartman, Ranjan K. Dash, Mohammed Aldakkak, Amadou K.S. Camara, David F. Stowe.
Medical College of Wisonsin, Milwaukee, WI, USA.

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+

Johan Haumann, David F. Stowe, Ranjan K. Dash, Mohammed Aldakkak, Runa Patel, Matthew R. Vernon, Daniel A. Beard, Amadou K.S. Camara.
Medical College of Wisonsin, Milwaukee, WI, USA.

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

Natalja Timohhina[1], Kersti Tepp[1], Rita Guzun[2], Tuuli Käämbre[1], Peeter Sikk[1], Ülo Puurand[3], Valdur Saks[1,2].
[1]Laboratory of Bioenergetics, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia, [2]Laboratory of Bioenergetics, INSERM U884, Joseph Fourier University, Grenoble, France, [3]Tallinn University of Technology, Tallinn, Estonia.

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

Helena M. Viola[1], Evan Ingley[2], Peter G. Arthur[1], Livia C. Hool[1].
[1]The University of Western Australia, Crawley, Australia, [2]Western Australian Institute of Medical Research, Perth, Australia.

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

Huang Li-Ju, Kuo Li-Wei, Yang Rei-Cheng.
Kaohsiung Medical University, Kaohsiung, Taiwan.

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

is a major reason of bioenergetic failure in sepsis, little attention has been given on ATP conservation. We recently identified one of the differentially expressed genes, mitochondrial ATPase inhibitor protein (IF1), which is down-regulated in late septic liver. Hence, the purpose of this study was to evaluate the expression of IF1 and mitochondrial F_0F_1-ATPase activity using a rat model of sepsis induced by cecal ligation and puncture (CLP). We also further analyzed whether the IF1 protein expression could be modulated by HIF-1, which is one of the dominant transcriptional factors under hypoxic condition. The results showed that the elevated mitochondrial F_0F_1-ATPase activity is concomitant with the decline of intramitochondrial ATP concentration in late septic liver. In addition, mRNA and the mitochondrial content of IF1 were decreased in late sepsis. Additionally, decreased nuclear HIF-1α protein was followed by reduced IF1 mRNA expression in late sepsis. Furthermore, increased level of HIF-1α protein was concomitant with IF1 protein augmentation under hypoxic condition or $CoCl_2$ (HIF-1α activator) treatment. Antisense oligonucleotide against HIF-1α greatly decreased the IF1 protein level in clone 9 epithelia cell line. Down-regulation of HIF-1α expression with RNA interference also led to decrease expression of IF1 and elevate the mitochondrial F_0F_1-ATPase activity in the presence of Bis-Tris buffer (pH6.5). In conclusion, these results are the first time to suggest that suppression of IF1 expression and subsequent elevated mitochondrial F_0F_1-ATPase activity might contribute to the bioenergetic failure in septic liver and HIF-1α might play a crucial role in regulating the IF1 protein expression.

1255-Pos Board B99

Calcium-Mediated Translocation of Fission Protein DLP1 to Mitochondria and Augmentation of Reactive Oxygen Species (ROS) Levels in Heart

Jennifer Hom, Yisang Yoon, Sergei Nadtochiy, Paul Brookes, Shey-Shing Sheu.
University of Rochester, Rochester, NY, USA.

Background: Mitochondrial fission/fusion/movement plays a critical role in bioenergetics, Ca^{2+} homeostasis, redox signaling, and apoptosis. We have recently shown that Ca^{2+} regulates mitochondrial fission, ROS production, and mitochondrial permeability transition (MPT) in several cell types. The adult heart mitochondria *in vivo* are continuously exposed to cytosolic Ca^{2+} transients. They situate orderly in the sarcomeres so that their movement is minimal. Here we test the hypothesis that cardiac muscle cells contain DLP1 and its translocation to mitochondria is regulated by Ca^{2+}. The translocation of DLP1 is a critical step for Ca^{2+}-mediated ROS increases.

Results: Western blots showed fission proteins DLP1 and hFis were present in the mitochondria of adult and neonatal ventricular myocytes. To raise cytosolic Ca^{2+}, we used: 1) 50 mM KCl to open L-type Ca^{2+} channels, 2) 1 μM thapsigargin to inhibit Ca^{2+} uptake into sarcoplasmic reticulum, and 3) combination of electrical and β-adrenergic receptor stimulation to increase the frequency and magnitude of Ca^{2+} transients. All three interventions increase translocation of DLP1 to mitochondria ($p<0.01$) and elevation of ROS levels (3 fold). Overexpression of dominant-negative mutant DLP1-K38A led to web-like interconnected long mitochondria in cultured neonatal rat ventricular myocytes that no longer respond to the Ca^{2+}-mediated ROS increases. The translocation of DLP1 was also observed in mitochondria isolated from Langendorff hearts perfused with 10 nM isoproterenol ($p<0.05$) suggesting the Ca^{2+}-mediated mitochondrial fission could occur *in vivo*. Current experiments are addressing whether the DLP1 translocation could promote MPT openings.

Conclusion: Adult cardiac myocytes possess the key proteins involved in mitochondrial fission. Ca^{2+} increases DLP1 translocation to mitochondria and thus augments ROS generation. Therefore, the mitochondrial fission machinery could play a critical role in physiological regulation of cardiac energy metabolism and ROS homeostasis.

1256-Pos Board B100

The Dual Control Of Insulin Secretion By Increased Calcium Influx And A Factor Related To Increased Metabolism

Seung-Ryoung Jung, Benjamin J. Reed, Ian R. Sweet.
University of Washington, Seattle, WA, USA.

Calcium influx is required for sustained insulin secretion, but its mechanism of action is not understood. As activation of calcium channels (by BayK 8644) at sub-threshold (3 mM) levels of glucose increased cytosolic calcium without affecting insulin secretion (ISR) or oxygen consumption (OCR), there is evidence that a secondary factor related to increased metabolism is also essential for insulin secretion. To further characterize this metabolic factor, the relationship between calcium influx, OCR and ISR were measured using a perifusion system in response to fuels entering metabolic pathways at different entry points, and an inhibitor of L-type calcium channels (nimodipine). After acquiring baseline measurements at 3mM glucose, the substrate was changed to either 20 mM glucose, or 3 mM glucose plus either 10 mM alpha-ketoisocaproate (KIC), 2 mM glutamine/10 mM leucine or 10 mM glyceraldehyde, followed by the addition of 5 microM nimodipine. The addition of each of the first three substrates lead to a similar increase in all measured parameters, and the subsequent exposure of islets to nimodipine elicited a near-complete suppression of ISR, a 30-40% decrease in glucose stimulated OCR, and a 40-50% decrease in glucose stimulated calcium levels. In contrast, glyceraldehyde stimulated ISR similarly to glucose, but only increased OCR by about 30%. Nimodipine completely reversed the effect of glyceraldehyde on OCR, so the absolute decrements in OCR in response to nimodipine in the presence of all four of the substrates were similar. Taken together, this data supports a new conceptual model of insulin secretion where calcium influx activates a highly energetic and essential process that is linked to the regulation of insulin secretion and requires a factor generated downstream of the TCA cycle.

1257-Pos Board B101

Effects of Glucose on Mitochondrial Function of Insulin-Producing INS-1 Insulinoma Cells

Eduardo N. Maldonado.
Medical University of South Carolina, Charleston, SC, USA.

BACKGROUND: Highly differentiated INS-1 832/13 cells are widely used as a model for glucose-stimulated insulin secretion (GSIS) similar to pancreatic beta cells. In the current view of GSIS, glucose metabolism leads to pyruvate formation, which is oxidized by mitochondria generating ATP. Mitochondrial ATP transported to the cytosol in exchange for cytosolic ADP via adenine nucleotide translocators (ANT) closes K_{ATP} channels. K_{ATP} channel closing causes plasma membrane depolarization which in turn opens voltage-dependent Ca^{2+} channels, triggering exocytosis of insulin granules. Our **AIM** was to evaluate mitochondrial function in INS-1 cells in relation to glucose stimulation. **METHODS:** Respiration of INS-1 cells incubated with 0, 3 or 15 mM glucose was determined in a Seahorse XF24. Mitochondrial and plasma membrane polarization was assessed by confocal microscopy of TMRM and Di-BAC_4, respectively. **RESULTS:** Glucose maximally increased respiration and insulin secretion at 15 mM. GSIS was blocked by rotenone, a mitochondrial respiratory chain inhibitor. Mitochondrial polarization was maintained in the absence of glucose and remained constant with increasing glucose to 15 mM. Blocking of mitochondrial ATP synthase by oligomicin inhibited respiration, depolarized mitochondria and hyperpolarized the plasma membrane. Mitochondrial depolarization after oligomycin was prevented by NIM811, an inhibitor of the mitochondrial permeability transition (MPT). Tolbutamide, a K_{ATP} channel blocker, reversed hyperpolarization of the plasma membrane. **CONCLUSIONS**: These findings indicate that mitochondrial function in INS-1 cells is preserved in the absence of glucose and that an increase of mitochondrial membrane potential is not required for GSIS.

1258-Pos Board B102

Assessment of Respiration-Dependent Intra- and Extramitochondrial ATP Turnover: HepG2 Cancer Cells do not Utilize ATP from Oxidative Phosphorylation in the Cytosol

Eduardo N. Maldonado.
Medical University of South Carolina, Charleston, SC, USA.

BACKGROUND: Mitochondrial ATP is transported to the cytosol in exchange for ADP through the adenine nucleotide translocator (ANT). Carboxyatractyloside (CAT) and bongkrekic acid (BA) specifically inhibit ATP delivery to the cytosol via ANT, whereas oligomycin (OL) inhibits all ATP synthesis by oxidative phosphorylation. Our **AIM** was to assess respiration-dependent intra and extramitochondrial ATP turnover in HepG2 human hepatocarcinoma cells and cultured rat hepatocytes stimulated by ureagenic substrates. **METHODS**: Overnight cultured rat hepatocytes were stimulated with ureagenic substrates (in mM: 5 Na-lactate, 5 L-ornithine, 3 NH_4Cl). HepG2 cells were incubated in Hank's solution or permeabilized with digitonin in intracellular buffer plus 0.5 mM ADP and 5 mM succinate. Respiration was measured using a Seahorse XF24. **RESULTS**: Ureagenic substrates increased respiration by hepatocytes progressively 2 to 3-fold over an hour. Subsequent addition of OL inhibited respiration to basal levels, whereas BA and CAT inhibited ureagenic respiration by ~65%. Partial inhibition by ANT blockers was consistent with utilization of both intra- and extramitochondrial ATP in the urea cycle. In non-permeabilized HepG2 cells, OL inhibited respiration by ~60% but BA and CAT had no effect, whereas in permeabilized HepG2 cells, OL, BA and CAT each inhibited respiration equally. In **CONCLUSION,** respiration inhibited by OL reflects total ATP turnover linked to oxidative phosphorylation, whereas respiration inhibited by BA or CAT reflects extramitochondrial turnover of ATP formed by oxidative phosphorylation. The difference is intramitochondrial ATP turnover. In intact HepG2 cells, ATP generated by oxidative phosphorylation was not utilized in the cytosol, although HepG2 cells contain functional ANT.