$CaCl_2$ after 37°C IRI and 0% after 17°C IRI. This study shows that hypothermia prevents IRI damage through pathways restricting mitochondrial Ca^{2+} loading and preserves mitochondrial redox state and respiration. Moreover, mitochondria protected during ischemia with hypothermia were more resistant to Ca^{2+}-induced mPTP opening and oxidative phosphorylation was better preserved. Hypothermia might prevent conformational changes in the F_1F_0-AT-Psynthase and the ADP/ATP carrier, leading to better mitochondrial function and a resistance to mPTP opening as the ADP/ATP carrier is associated with mPTP opening.

3827-Pos

Buffer Magnesium Limits Mitochondrial Calcium Uptake but not Matrix Calcium Buffering in Response to ADP

Age D. Boelens, Ranjan K. Dash, Johan Haumann, Amadou K.S. Camara, Mohammed Aldakkak, Ashish K. Gadicherla, David F. Stowe.
Medical College of Wisconsin, Milwaukee, WI, USA.

Mg^{2+} is known to limit Ca^{2+} uptake by mitochondria through the Ca^{2+} uniporter. Changes in matrix Ca^{2+} concentration are an important signaling pathway in mitochondrial function as well as in apoptosis. In a previous study we showed an increase in matrix free Ca^{2+} in response to added ADP in $MgCl_2$ free buffer. Because of the presumed role of Mg^{2+} in mitochondrial regulation of Ca^{2+} we explored the effects of buffer Mg^{2+} on matrix Ca^{2+} uptake and buffering in isolated mitochondria. Guinea pig heart mitochondria were isolated by differential centrifugation, loaded with the fluorescent dye Indo 1 AM and then suspended in respiration media, containing 1 mM of EGTA, with or without added 1 mM $MgCl_2$. To the mitochondrial suspension was added 0.5 mM pyruvic acid, either 0.25, 0.5 or 0.75 mM $CaCl_2$, and 250 μM ADP. Adding 0.25, 0.5 and 0.75 mM Ca^{2+} caused a dose-dependent increase in matrix Ca^{2+} of 14, 35 and 45%, respectively, in the group without Mg^{2+} in the buffer, and 6, 18 and 42%, respectively, in the group with Mg^{2+} in the buffer. The differences in uptake between Mg^{2+} and no Mg^{2+} groups were significant in the 0.25 and 0.5 mM groups, but not in the 0.75 mM group. The additional increase in matrix free Ca^{2+} in response to ADP without Mg^{2+} was 9, 11 and 9% for the 0.25, 0.5 and 0.75 mM Ca^{2+} groups, respectively. These additional increases in matrix free Ca^{2+} with ADP were not significantly altered by Mg^{2+}. We conclude that external Mg^{2+} alters the uptake of Ca^{2+} into the mitochondrial matrix, but does not alter the increase in matrix ionized Ca^{2+} after addition of ADP.

3828-Pos

Complex I and F_0F_1-ATP Synthase Mediate Membrane Depolarization and Matrix Acidification by Isoflurane in Mitochondria

Danijel Pravdic, Naoyuki Hirata, David F. Stowe, Zeljko J. Bosnjak, **Martin Bienengraeber**.
Medical College of Wisconsin, Milwaukee, WI, USA.

Introduction: Short application of volatile anesthetic isoflurane at reperfusion after ischemia exerts strong protection of heart and cardiac mitochondria against injury. Mild depolarization and acidification of mitochondrial matrix are involved in the protective mechanism, but the molecular basis for these changes is not known. In this study we investigated the electron transport chain, F_0F_1-ATP synthase and mitochondrial ion channels as potential targets of isoflurane in mitochondria.

Methods: We have measured mitochondrial respiration, membrane potential, matrix pH, matrix swelling, and H_2O_2 release in isolated mitochondria in the presence and absence of isoflurane (0.5 mM). Pyruvate/malate, succinate/rotenone, or ascorbate/TMPD, were used as substrates for complex I, II and IV, respectively. Guanosine-diphosphate (GDP), oligomycin, paxilline and 5-hydroxydecanoic acid (5-HD) were used to probe involvement of uncoupling proteins, F_0F_1-ATP synthase, mitochondrial ATP- and Ca^{2+}-sensitive K^+ channel. Nigericin, a K^+/H^+ exchanger, was used to manipulate the matrix pH.

Results: With pyruvate/malate as substrates, isoflurane inhibited mitochondrial respiration by $23 \pm 4\%$, depolarized membrane potential by $2.7 \pm 0.7\%$ and decreased matrix pH by $11 \pm 3\%$. With complex II and complex IV-linked substrates, respiration was not changed, but isoflurane still decreased matrix pH and depolarized $\Delta\Psi_m$. Depolarization and matrix acidification were only attenuated by oligomycin, but not GDP, paxilline, or 5-HD. Isoflurane did not induce matrix swelling, but decreased H_2O_2 release in the presence of succinate in an oligomycin and matrix pH sensitive manner.

Conclusion: Our results indicate that isoflurane inhibited the electron transport chain at the site of complex I and also modified F_0F_1-ATP synthase. Both effects lead to an acidification of the mitochondrial matrix which is beneficial at the time of reperfusion. K^+ channels and uncoupling proteins are likely not involved in these direct effects of isoflurane on isolated mitochondria.

3829-Pos

Ca^{2+} Enhances ROS Generation from Inhibited Complex I but not from Inhibited Complex III with Nadh-Linked Substrate

Mohammed Aldakkak, David F. Stowe, Age D. Boelens, Johan Haumann, Ranjan K. Dash, Matthias L. Riess, Amadou K.S. Camara.
Medical College of Wisconsin, Milwaukee, WI, USA.

Mitochondrial electron transport chain complexes can be major sources of ROS. Several mechanisms are responsible for modulating ROS production, possibly including mitochondrial Ca^{2+} uptake. Here we tested effects of added buffer $CaCl_2$ on ROS generation from complex I in the presence of rotenone, and from complex III in the presence of antimycin A. Guinea pig heart mitochondria (n=6) were isolated by differential centrifugation and suspended in respiration media containing amplex red and horseradish peroxidase to measure the rate of H_2O_2 generation. Increasing concentrations of buffered $CaCl_2$ were added to the mitochondrial suspension. Complex I substrate pyruvate (10 mM) or complex II substrate succinate (10 mM) was added followed by either rotenone (10 μM) or antimycin A (5 μM) to block complex I or III, respectively. Compared to no added $CaCl_2$ in the respiratory buffer, the slope of the H_2O_2 signal in the presence of pyruvate + rotenone increased respectively by 1.3 ± 0.1, 2.1 ± 0.2, 3.4 ± 0.4, 4.5 ± 0.3 times with 10, 25, 50, and 100 μM added external $CaCl_2$. In contrast, H_2O_2 generation from complex III in the presence of antimycin A did not change with increasing $CaCl_2$, whereas H_2O_2 generation from complex I in the presence of succinate (due to reversed electron flow) decreased with increasing buffer $CaCl_2$. Moreover, H_2O_2 generation from complex III in the presence of antimycin A and rotenone in mitochondria supported with succinate did not change with increased buffer $CaCl_2$. We conclude that adding $CaCl_2$ to the buffer enhances H_2O_2 generation from complex I only during blocked downstream electron transport. This emphasizes the impact of matrix Ca^{2+} loading on electron leak leading to free radical formation only under conditions of inhibited electron flow at complex I.

3830-Pos

Identification of the Mitochondrial Carrier that Provides *yarrowia Lipolytica* with a Fatty Acid- Induced and Nucleotides- Sensitive Uncoupling Protein- Like Activity

Luis Alberto Luévano[1], Eva Moyano[2], Mario García de Lacoba[2], Eduardo Rial[2], Salvador Uribe- Carvajal[1].
[1]Instituto de Fisiología celular, UNAM, Mexico City, Mexico, [2]Centro de investigaciones biológicas, Madrid, Spain.

Uncoupling proteins (UCPs) are mitochondrial carriers distributed throughout the eukaryotic kingdoms. While genes coding for UCPs have been identified in plants and animals, evidences for the presence of UCPs in fungi and protozoa are only functional. Here, it is reported that in the yeast Yarrowia lipolytica there is a fatty acid-promoted and GDP-sensitive uncoupling activity indicating the presence of a UCP. The in silico search on the Y. lipolytica genome led to the selection of two genes with the highest homology to the UCP family, XM_503525 and XM_500457. By phylogenetic analysis, XP_503525 was predicted to be an oxaloacetate carrier while XP_500457 would be a dicarboxylate carrier. Each of these two genes was cloned and heterologously expressed in Saccharomyces cerevisiae and the resulting phenotype was analyzed. The transport activity of the two gene products confirmed the phylogenetic predictions. In addition, only mitochondria isolated from yeasts expressing XP_503525 showed bioenergetic properties characteristic of a UCP: the proton conductance was increased by linoleic acid and inhibited by GDP. It is concluded that the XM_503525 gene from Y. lipolytica encodes for an oxaloacetate carrier although, remarkably, it also displays an uncoupling activity stimulated by fatty acids and inhibited by nucleotides.

3831-Pos

Silybin Derivatives Modulate Thyroid Hormone-Mediated UcP2 Expression in Neonatal Rat Cardiomyocytes

Martin Modriansky[1], Eva Gabrielova[1], Radek Gazak[2], Martin Jaburek[3], Pavel Kosina[1], Jan Vacek[1], Vladimir Kren[2].
[1]Palacky University, Olomouc, Czech Republic, [2]Institute of Microbiology, Prague, Czech Republic, [3]Institute of Physiology, Prague, Czech Republic.

Thyroid hormones (TH) govern cardiac phenotype including myocardial bioenergetics, a finely tuned process, possibly by affecting expression of a number of proteins. Chronic hyperthyroidism is associated with cardiac hypertrophy, which may lead to serious heart problems perhaps through higher expression of uncoupling protein 2 (UcP2), which is present in the failing heart. We were investigating effects of silybin (SB) and dehydrosilybin (DHSB) on TH-regulated cardiomyocyte bioenergetics, including UcP2 expression levels.

Both substances displayed concentration dependent down-regulation of UcP2 expression induced by THs in neonatal rat cardiomyocytes. Because DHSB uncoupled the respiration of isolated rat heart mitochondria while limiting reactive oxygen species (ROS) formation, it may be affecting UcP2 expression in a feedback control fashion. However, SB does neither. Therefore we explored the possibility of both substances limiting TH uptake into cardiomyocytes. TH presence in cardiomyocytes was evaluated by mass-spectrometry and we did not observe any limitation to the uptake of hormones. Because TH actions are primarily mediated by nuclear thyroid receptors and their transcriptional activation, we used reporter plasmid system to assess the SB and DHSB effect on thyroid hormone receptor transcriptional activity in cardiomyocytes. Our data suggest that SB and DHSB modulation of TH-mediated UcP2 expression is not related to their antioxidant ability (DHSB) or lack thereof (SB). Rather both substances influence TH-related processes by affecting the TH-dependent signaling pathway with possible beneficial effects in hyperthyroid patients.
(Supported by GACR 303/08/0658 and MSM 6198959216)

3832-Pos

Uncoupling and Inward Migration of Subsarcolemmal Mitochondria in Rat Heart during Early Diabetes

Chun-Hong Shao, Ming Li, Aydin Tay, Matthew C. Zimmerman, Lyudmila S. Shlyakhtenko, William G. Mayhan, Gisela Beutner, Shey-Shing Sheu, Keshore Bidasee.
University of Nebraska Medical Ctr, Omaha, NE, USA.

Myocyte loss is an established feature in hearts of both individuals with diabetes mellitus and in several animal models of the disease. Studies attribute this to an increase in apoptosis resulting from elevation in cytoplasmic cytochrome *c* and activation of caspase-3. To date, spatial, structural and functional changes subsarcolemmal mitochondria (SSM), which protect myocytes from circulating insults, undergo during early diabetes remains poorly characterized. Using the streptozotocin-induced diabetic rat model we show that after 5-6 weeks of diabetes, SSM disaggregate and migrate inwards. Diabetic SSM (dSSM) also exhibited increased biogenesis, were smaller with more compact cristae, possessed higher citrate synthase activity, produced more reactive oxygen species (ROS), increased interaction with sarcoplasmic reticulum (SR), and took up more Ca^{2+}. Atomic force microscopy also revealed that forty percent of dSSM also possessed a circumferential ''ribbon-like'' structure and 12% of these were leaky. dSSM also contained 65% less superoxide dismutase-I and 66% less connexin 43, a protein that regulates the activity of mitochondria K_{ATP} channels and opening of the mitochondrial permeability transition pore. Insulin-treatment blunted these changes. The inward migration of SSM during diabetes is likely to leave myocytes vulnerable to plasmalemmal Ca^{2+} spikes resulting from the barrage of circulating agonists. Persistent increases in ROS production and lower connexin 43 content are also likely to trigger leaking of dSSM and elevate cytoplasmic levels of cytochrome c and apoptosis-inducing factors. Thus, we propose that compensatory changes to ensure adequate ATP production and maintenance of ionic homeostasis during diabetes switches SSM from protecting myocytes to inducing their demise. (This work was funded in part by grants from NIH to WGM and KRB and AHA to MCZ).

3833-Pos

FGF21 and Pancreatic Islet Fatty Acid Metabolism

Mark Y. Sun[1], Dawn M. Kilkenny[1], Jonathan V. Rocheleau[1,2].
[1]University of Toronto, Toronto, ON, Canada, [2]University Health Network, Toronto, ON, Canada.

Pancreatic islet β-cells maintain blood glucose through the regulated secretion of insulin. A rise in blood glucose stimulates β-cell production of NAD(P)H and increases the ATP/ADP ratio resulting in a cascade of events including closure of ATP-sensitive potassium channels, membrane depolarization, Ca^{2+}-influx, and insulin secretion. During the course of Type II diabetes, the glucose stimulated insulin response is dampened by glucose and lipid toxicity. It has recently been shown that the novel endocrine factor, FGF21, protects metabolically active tissues by regulating fatty acid metabolism. To test this effect in islet β-cells, we measured the levels of Acetyl-CoA carboxylase (ACC) in response to FGF21. ACC is an enzyme involved in the synthesis of malonyl-CoA, the substrate used in fatty acid synthesis and a regulator of fatty acid oxidation. We show that FGF21 causes an increase in ACC levels in βTC3 cells, a pancreatic islet β-cell line. We propose that this increase in ACC acts as a protective mechanism for maintaining β-cell sensitivity to glucose by lowering β-oxidation of fats for energy. To measure fatty acid metabolism in the islet, we will extend our biochemical studies of ACC to mouse islet tissue. Furthermore, we will examine mitochondrial metabolism of β-cells in the presence of fatty acid using two photon microscopy of NAD(P)H. More specifically, we will examine glucose-stimulated mitochondrial NAD(P)H response of β-cells under normal and high fat environments. Overall, these studies will determine whether metabolic changes in the β-cells occur under varying nutritional states and understand the effects of FGF21 regulation of ACC levels in controlling β-cell metabolism.

Computational Methods III

3834-Pos

A New Semi-Explicit Solvation Model: Fast Physics for Better Results

Charles Kehoe, Christopher Fennell, Ken Dill.
UCSF, San Francisco, CA, USA.

Computational physicists, chemists, and biologists have a critical need for better models of water and aqueous solutions. We present an exciting new solvation model called Semi-Explicit Assembly, which combines the speed of the fastest continuum models available with the strong physical basis and discrete water treatment afforded by explicit solvent simulations. We base our model on several simple physical properties of water as a solvent, collected directly from explicit solvent simulations for individual atomic solutes. As a first test and application of our method, we compute solvation free energies based on dispersion and electrostatics. Our approach, which is purely physical and involves no fitting of parameters to data sets, executes as fast as the popular Generalized Born solvation model, but with substantially improved accuracy in prediction of experimental solvation free energies. Also, the structure of our model means that improvements in simulation forcefields will improve our results as well. All of this comes without any artificial parameter adjustments; our model's properties are the same as those used directly in molecular dynamics. Our model's energetic accuracy and detailed structural information have wide-ranging implications for molecular modeling research.

3835-Pos

Non-Linear Analysis of Voltage Clamp Data in the Investigation of Mechanisms of Inherited Arrhythmias

Ashley Raba, Jacques Beaumont.
State University of New York at Binghamton, Binghamton, NY, USA.

Several mutations in genes encoding cardiac ion channels render the heart at high risk of incidence of life-threatening arrhythmias. Although the genes, loci, and phenotypes have been identified, the mechanism by which these mutations lead to fatal arrhythmias is still poorly understood. Progress on this problem requires a thorough quantification of the phenomena involved over multiple scales. An aspect of this is the precise quantification of membrane current kinetics. Here, we present a methodology that addresses this problem and apply it to the testing of a hypothesis on the initiation of abnormal beats in LQT2 and LQT3 syndromes.

We show that the traditional estimation of the functions of voltage composing the Hodgkin-Huxley model through non-linear least square fitting (NLLSF), has numerous limitations and present a novel non-linear method that overcomes these limitations.

An important result is the demonstration that we can determine a-priori whether the voltage clamp data fully constrains the model in a given voltage range. Then, based on voltage clamp data gathered in two complementary protocols, we can evaluate the voltage dependence of the steady state through a sequence of non-linear transformations, i.e. an inversion. The voltage dependence of the time constants is obtained by inverting the model at each data point and applying constraints as well as continuity criteria on the inverted solution. Importantly, we show how the methodology allows us to derive experimental protocols constraining the model; thus allowing us to thoroughly test our hypothesis.

In conclusion, we have presented a theory to perform a high quality non-linear analysis of voltage clamp data and applied it to provide credence to a plausible mechanism for the initiation of arrhythmias in LQT2 and LQT3 syndromes.

3836-Pos

Misty Mountain Clustering: Application to Fast Unsupervised Flow Cytometry Gating

Istvan P. Sugar, Stuart C. Sealfon.
Mount Sinai School of Medicine, New York, NY, USA.

Flow cytometry experiments record, in individual cells, the fluorescent intensity of different fluorophores that correspond to features such as the levels of specific proteins. An assay typically generates a large number (order 10^6) of data points in a two or