$CaCl_2$ 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

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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

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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

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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

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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

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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.