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

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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

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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

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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.

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

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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.