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Ca^{2+} uptake was largely inhibited in the presence of the mitochondrial phosphate carrier inhibitor N-ethylmaleimide; however, mode 1 uptake was still observed, i.e., bulk Ca^{2+} uptake through mCU mode 2 was more Pi- dependent than mode 1. These experiments demonstrate another distinction between mCU modes 1 and 2 and contribute to an understanding of their possible physiological roles in mitochondrial function as either a signal for regulating energetics or as a Ca^{2+} sink.

Oxidative Phosphorylation & Mitochondrial Metabolism

1544-Pos Board B436
Role of Mitochondrial Morphology in Bioenergetics
Hakjoo Lee[1], Bong Sook Jhun[2], **Yisang Yoon**[1].
[1]Georgia Health Sciences University, Augusta, GA, USA, [2]Thomas Jefferson University, Philadelphia, PA, USA.
Mitochondria in cells undergo constant morphological changes mainly through fission and fusion. However, functional significance of mitochondrial fission and fusion is not fully understood. To test the importance of mitochondrial morphology in maintaining mitochondrial function, first, we used glucose-stimulated insulin secretion in pancreatic β-cells as an experimental model because insulin secretion upon elevated plasma glucose concentration requires intact mitochondrial function. Increased ATP production in mitochondria from glucose metabolism induces plasma membrane depolarization and subsequent increase of cytosolic Ca^{2+} triggers insulin exocytosis. We found that glucose stimulation of the β-cell line INS-1E induces transient mitochondrial shortening and recovery. Inhibiting mitochondrial fission by expressing the dominant-negative fission mutant DLP1-K38A abolished the dynamic change of mitochondrial morphology in glucose stimulation. Importantly, we discovered that abolition of the glucose-induced mitochondrial morphology change suppresses glucose-stimulated insulin secretion. Measuring respiration under fission inhibition showed an increase of mitochondrial uncoupling, and thus significantly diminished the mitochondrial ATP production in response to glucose stimulation. Further evaluation of mitochondrial membrane potential in primary hepatocytes revealed that inhibition of mitochondrial fission induces large-scale fluctuations of the potentiometric fluorescence in mitochondria within cells. Frequencies and intervals of the fluorescence oscillation were random and insensitive to inhibitors of anion channels and mitochondrial permeability, and superoxide scavenger. This suggests that the fission inhibition-induced fluctuation of the inner membrane potential is a previously unrecognized unique phenomenon. These observations demonstrate that inhibition of mitochondrial fission induces a large-scale fluctuation of the mitochondrial inner membrane potential, which is functionally reflected in mitochondrial uncoupling. Taken together, our findings indicate that mitochondrial fission plays a role in regulating the coupling efficiency of oxidative phosphorylation.

1545-Pos Board B437
Effects of Reactive Oxygen Species on NFAT Activation and Translocation in Adult Rabbit Ventricular Myocytes
Stefanie Walther, Joshua N. Edwards, Lothar A. Blatter.
Rush University Medical Center, Chicago, IL, USA.
Nuclear factor of activated T cells (NFAT) transcription factors play a key role during cellular remodeling associated with cardiac hypertrophy and heart failure (HF). Evidence suggests that reactive oxygen species (ROS) are integral to the progression of cardiac hypertrophy and HF. Therefore, we aimed to determine the role of ROS in the activation and translocation of NFAT.
Adult rabbit ventricular myocytes were infected with recombinant adenoviruses encoding for NFAT-GFP fusion proteins (isoforms NFATc1 and NFATc3). The subcellular distribution of NFAT was quantified as the ratio of NFATnuc to NFATcyt fluorescence (RNFAT) and nuclear-cytosolic NFAT translocation was expressed as changes of RNFAT. Under basal unstimulated conditions, NFATc3 was predominantly localized in the cytoplasm, whereas NFATc1 displayed a nuclear localization.
Acute exposure to the inhibitors of oxidative phosphorylation Rotenone, Antimycin A, Oligomycin and FCCP resulted in the activation and translocation of NFATc1 (60 - 145 % increase of RNFAT) into the nucleus. These inhibitors did not induce translocation of NFATc3; however, exposure to Hydrogen Peroxide (H2O2) or Ruthenium Red (inhibitor for the mitochondrial Ca uniporter), resulted in the activation and translocation of NFATc3 (but not NFATc1). The H2O2-induced NFATc3 translocation was attenuated in the presence of the antioxidant N-acetylcysteine.
These data identify a ROS-induced activation and translocation of NFAT in adult ventricular myocytes.

1546-Pos Board B438
Unpolymerized βII Tubulin in Regulation of Mitocondrial Function in Muscle Cells
Minna Karu[1,2], Rafaela Bagun[3], Madis Metsis[1], Alexei Grichine[3], Kersti Tepp[2], Tuuli Käämbre[2], Valdur Saks[2,3], Rita Guzun[3].
[1]Tallinn University of Technology, Tallinn, Estonia, [2]National Institute of Chemical Physics and Biophysics, Tallinn, Estonia, [3]Joseph Fourier University, Grenoble, France.
The importance of microtubular system in shaping the organization of intracellular energy metabolism and regulating mitochondrial functioning is becoming increasingly more evident. Our previous studies with cardiac cells have shown that regulation of mitochondrial outer membrane (MOM) permeability for ADP by dimeric αβ-tubulin is important for efficient cross-talk between mitochondria and contraction apparatus through phoshocreatine energy transfer pathway. This regulation was specifically related with tubulin isoform βII after showing its mitochondrial localization in cardiac cells and verifying its concomitant expression with mitochondrial creatine kinase (MtCK). However the exact mechanism of this regulation is still rather elusive and studied mainly in cardiac cells. To determine if βII tubulin expression is specific only to oxidative muscle cells with high MtCK activity and to gain further insight to the role of βII tubulin in energy metabolism, we have analyzed the relationship between βII tubulin expression, mitochondrial respiration regulation and their intracellular positioning in striated muscles with different metabolic phenotype. In this study we provide further proof for the functional importance of βII tubulin in regulation of mitochondrial respiration in striated muscles. We show that both oxidative and glycolytic muscles express βII tubulin, but the presence of unpolymerized βII tubulin is significantly lower in glycolytic muscle cells concomitant with higher MOM permeability for ADP. Analysis of mitochondria and βII tubulin localization reveals that in oxidative muscle cells mitochondria are positioned in close vicinity to βII tubulin with high degree of colocalization which is much less prevalent in glycolytic muscles. Together our results show that βII tubulin displays both structural and regulatory role in striated muscle cells and its distribution and polymerization level has direct impact on regulation of mitochondrial ADP sensitivity and efficiency of mitochondria coupling with contraction apparatus.

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Substrate Oxidation Control of Respiratory Rates in Primary Hepatocytes
Anil Noronha Antony, Cynthia Moffat, Aditi Swarup, Erin Seifert, Jan B. Hoek.
Thomas Jefferson Universiy, Philadelphia, PA, USA.
The goal of our studies is a bioenergetics profiling of primary rat hepatocytes using the Seahorse-XF analyzer in order to assess adaptation in response to metabolic stress or disease. Cellular oxygen consumption rates (JO2) were compared in enriched medium (DMEM) vs a balanced salt solution (HBSS) without added substrates. Hepatocytes exhibited higher basal JO2 in DMEM compared to HBSS and showed a proportional increase in oligomycin-insensitive JO2. The fractional increase in JO2 by uncoupler was higher in DMEM than in HBSS, presumably due to substrate supply by amino acids present in DMEM. These data suggests that substrate oxidative pathways exert significant control over basal and uncoupled respiration rates in primary rat hepatocytes. To further test this hypothesis, we assessed JO2 under different substrate conditions, in DMEM or HBSS medium. Addition of mono-methylsuccinate (MMS), a mitochondrial Complex III substrate, resulted in a large concentration- dependent stimulation of basal JO2 of hepatocytes in HBSS but a more limited stimulation in DMEM, likely reflecting availability of alternate substrates. In DMEM, physiological glucose concentrations (11mM) had little stimulatory effect, while higher concentrations (25mM) inhibited O2 uptake, thus exhibiting a ''Crabtree-like'' effect, which was not overcome by uncoupler treatment. This inhibitory effect of high glucose was not evident in HBSS, where basal JO2 increased with higher concentrations of glucose. Oligomycin-insensitive JO2, as a fraction of basal O2 uptake remained similar under all substrate conditions in DMEM and HBSS, apart from a small decrease at the highest MMS concentration. These results suggest a significant control exerted by substrate oxidative pathways over basal and uncoupler-stimulated respiration rates in primary rat hepatocytes. Electron supply may limit the rate of uncoupled respiration in hepatocytes, underestimating the reserve capacity in the electron transport chain. Supported by NIH grants AA018873 and AA017261.