show that ADP is a powerful inhibitor of mPTP opening and that its regulatory mechanism could be different from that of cyclophilin D.
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2487-Pos Board B473
Examining Pancreatic Islet Lipotoxicity Using Two-Photon NAD(P)H Imaging
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Pancreatic islet β-cells maintain blood glucose through the regulated secretion of insulin. A rise in blood glucose stimulates production of NAD(P)H resulting in a cascade of increased ATP/ADP ratio, closure of ATP-sensitive potassium channels, membrane depolarization, Ca2+-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 such as liver and white adipose tissue (WAT) by regulating fatty acid metabolism. To test the effects of FGF21 on 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, a substrate used in fatty acid synthesis and a regulator of fatty acid oxidation. Reducing ACC expression is critical to the normal compensation of beta-cells to lipid toxicity. We show that FGF21 decreases ACC protein levels in mouse pancreatic islets. To determine whether decreased ACC protein level acts as a protective mechanism in maintaining β-cell sensitivity to glucose, we examined changes in NAD(P)H by two photon microscopy of living islets held in a microfluidic device as a direct measure of changes in the metabolism. Our data reveals that compared to control islets, FGF21-treated islets retain the glucose-stimulated NAD(P)H response in a high fat environment. We will further examine the kinetics of the islet glucose response using time-lapse NAD(P)H imaging of islets. Overall, these studies will determine the metabolic changes that occur during the β-cell response to FGF21, and enhance understanding of how this effect can protect beta-cells from high fatty acid stress.

2488-Pos Board B474
Bcl-xL Determines the Metabolic Efficiency of Neurons, through Interaction with Mitochondrial ATP Synthase
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The anti-apoptotic BCL-2 family protein, Bcl-xL, is highly expressed in the brain. Beside its anti-apoptotic function, Bcl-xL may contribute to activity-dependent normal functioning of neurons. In this study, using a set of gain and loss of function experiments, we find that, in hippocampal neurons, Bcl-xL enhances the efficiency of neuronal energy metabolism by increasing total cellular ATP levels, while decreasing cellular oxygen use. This effect is due to the presence of Bcl-xL in the mitochondrial inner membrane, co-localized with the (F1F0) ATP synthase, shown by immunocytochemistry and immune-electron microscopy. The interaction causes a decrease in leak of H+ ions across the mitochondrial inner membrane. The decreased leak is correlated with an increase in coupling of oxidative phosphorylation. In contrast, inhibition of Bcl-xL by pharmacological or genetic means increases a leak conductance measured by patch clamping the membrane of submitochondrial particles enriched in ATP synthase complexes (SMVs). Leaky SMVs demonstrate attenuated ability to sequester H+ during ATP hydrolysis. Additionally, Bcl-xL protein directly increases ATPase activity of the purified synthase complexes, while inhibition of endogenous Bcl-xL decreases synthase activity. The exact site of the leak is being determined by cross-linking studies and by patch clamping individual protein components of the ATP synthase reconstituted into artificial lipid vesicles. Improved mitochondrial metabolic efficiency of neurons accompanying changes in leak level may result in long lasting changes in synaptic efficacy in both physiological and pathological conditions.

2489-Pos Board B475
Decrease in a Leak Conductance Associated with Mitochondrial Complex V and Improved Bioenergetic Efficiency may Underlie Cytoprotection of at-Risk Neurons by Dexpramipexole
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Chronic neurodegenerative disorders have been linked to mitochondrial dysfunction. Pharmaceutical agents which reduce mitochondrial dysfunction may provide therapeutic benefit by protecting neurons at risk. In the current study we have investigated the effects of dexpramipexole ((6R)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine), a drug currently under clinical study in ALS patients and a putative mitochondria-targeted neuroprotectant. Mitochondria isolated from proteasome-inhibitor-injected rat brain had large or intermediate-level multiconductance channel activity not present in controls; dexpramipexole inhibited this activity. Dexpramipexole was cytoprotective over a concentration range that represented the $EC_{80\text{-}100}$ for the effects on the conductances, suggesting that full inhibition may be required for significant cytoprotection. To focus on the relationship of dexpramipexole to cell bioenergetics, recordings were made from submitochondrial vesicles enriched in complex V (SMVs). Control SMVs were leaky in response to stimulus voltage, and currents were attenuated in a concentration-dependent manner by application of ATP or dexpramipexole. Dexpramipexole enhanced the rate of ATP hydrolysis and synthesis by complex V in a concentration-dependent manner and maintained ATP production in cultured hippocampal neurons and SHSY5Y cells. Using two methods, the Seahorse respirometry system and an oxygen sensor that detects oxygen flux in single neurons, we determined that dexpramipexole decreases cellular oxygen use, consistent with the effect on inhibiting inner membrane proton leak. Removal of F1 from SMVs eliminated the effects of dexpramipexole (but not ATP) on the inhibition of conductance, and significantly attenuated binding of ^{14}C-dexpramipexole. Dexpramipexole also eliminated the switch from mitochondrial to glycolytic respiratory parameters elicited by treatment of cultured cells with PSI. These data suggest that an increase in efficiency of oxidative phosphorylation produced by dexpramipexole may result from inhibition of complex V leak conductance, enhancing the survivability of neurons.

2490-Pos Board B476
Regulation of Mitochondrial Respiration by Different Tubulin Isoforms in Vivo
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Studies of regulation of mitochondrial outer membrane selective permeability of cardiac and brain cells in vitro (isolated mitochondria) and in situ (permeabilized cells) condition has revealed remarkable differences between kinetic parameters of respiration activation. This is probably a result of the specific control of voltage dependent anion channel (VDAC) permeability by some cytoskeleton proteins, among them may be some isoforms of tubulin. Our recent results with immunofluorescence and Western blot analysis have shown that possible candidates could be β-tubulin isoforms 2c (IVb) or 4 (IVa). The aim of this study was to construct, express and purify different β-tubulin isoforms and their C-terminal tail truncated recombinants and to investigate their effect on regulation of mitochondrial respiration.
Therefore we have designed a GST-tagged β-tubulin isoform constructs to express the recombinant proteins in BL21(DE3)pLys cell line. The proteins were purified in native conditions and utilized to study the kinetics of mitochondrial respiration regulation. The reconstitution experiments with isolated brain mitochondria and GST-TUBB2c or with GST-TUBB4 were carried out. The preliminary results showed that using GST-TUBB2c there were two populations of mitochondria with different properties; the Km value for ADP of the second population of mitochondria was significantly higher, compared to the first population. Therefore, it's possible to conclude that β2c-tubulin is one possible candidate of factor X, which is able to restore the low permeability of VDAC. As the β-tubulin C-terminal tail has been shown to be responsible for selective regulation of VDAC permeability, we have also produced truncated β-tubulin isoforms which lack the C-terminal tail and investigated their effect on mitochondrial respiration. Our results show that β-tubulin 2c isoform is able to restore the diffusion restrictions for ADP which is common for permeabilized cells with oxidative metabolism.

2491-Pos Board B477
Knockdown of VDAC Isoforms Decreases Mitochondrial Membrane Potential and Modulates the Effects of Free Tubulin in HepG2 Hepatoma Cells
Eduardo N. Maldonado, David N. DeHart, Jyoti R. Patnaik, John J. Lemasters.
BACKGROUND: Mitochondrial membrane potential ($\Delta\Psi$) depends on flux of metabolites through voltage dependent anion channels (VDAC) in the mitochondrial outer membrane. *In vitro*, tubulin binding induces VDAC closure. In intact cells, nocodazole (Ncz) increases free tubulin and decreases $\Delta\Psi$, whereas paclitaxel (Ptx) promotes tubulin polymerization and increases $\Delta\Psi$. These effects are attributed to closing and opening of VDAC. Here, we hypothesize that VDAC knockdown will decrease $\Delta\Psi$ and block the inhibitory effect of free tubulin on $\Delta\Psi$. **METHODS:** HepG2 cells were transfected with individual siRNAs for VDAC1/2/3 (5 nM, Ambion) for 48 h. To assess $\Delta\Psi$, fluorescence of tetramethylrhodamine methylester (TMRM) was measured by confocal microscopy. **RESULTS**: VDAC1/2/3 relative mRNA abundance by