like ursodeoxycholic acid (UDCA) and tauroursodeoxycholic acid (TUDCA), are cytoprotective and inhibit cell death. The mechanisms associated with these distinct effects are not entirely clear. However, the effect of hydrophilic bile acids seems to be related with the blockage of a series of processes that converge on mitochondrial damage. Bax is a pro-apoptotic protein that belongs to the superfamily of the Bcl-2 proteins and is involved in mitochondrial pore formation. Submicellar concentrations of cytoprotective bile acids have been shown to modulate Bax concentration in mitochondria, suggesting that these molecules may interact directly with the protein. In this study, our objective was to evaluate the affinity of bile acids to recombinant Bax protein, making use of fluorescence spectroscopy (FRET and fluorescence anisotropy), as well as Fluorescence Correlation Spectroscopy (FCS). Our results show that the cytoprotective bile acids UDCA and TUDCA associate with recombinant Bax protein with high affinity, while the cytotoxic bile acid DCA only seems to be able to adsorb to the protein with much lower affinity. Notably, the binding site for UDCA seems to be located in a hydrophobic pocket of the protein. This interaction could be responsible for the disruption of Bax translocation to the mitochondrial outer membrane in the presence of UDCA and/or TUDCA.
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3071-Pos Board B501

MAC Inhibitors Neutralize the Pro-Apoptotic Effects of Tbid

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Since our initial characterization of the iMACs, different di-bromocarbazole derivatives with anti-apoptotic function have been developed and tested in several mouse models of brain injury and neurodegeneration [13-21]. Owing to the increased therapeutic potential of anti-apoptotic di-bromocarbazole derivatives, we sought to expand our knowledge of the mechanism of action of these small molecule inhibitors. We investigated the kinetics of MAC inhibition in mitochondria from wild type, Bak, and Bax knockout cell lines using patch clamp electrophysiology, fluorescence microscopy, ELISA, and quantitative western blot analyses. Our results show that iMACs work through at least two mechanisms: 1) by blocking relocation of the cytoplasmic Bax protein to mitochondria and 2) by disassembling Bax oligomers in the outer membrane. A comparison of the inhibitory effects over channel conductance and cytochrome c release suggests that the iMACs interacted with both Bax and Bak with similar kinetics. Interestingly, wild type mitochondria were more susceptible to inhibition than the Bak or Bax knockouts. A quantitative western blot analysis showed that wild type mitochondria had lower steady state levels of Bak, which suggests an uneven stoichiometry of the MAC components.

3072-Pos Board B502

Tyrosine Phosphorylation of Mitochondrial Ca^{2+} Uniporter Regulates Mitochondrial Ca^{2+} Uptake

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Mitochondrial Ca^{2+} has a critical role for balancing cell survival and death. Ca^{2+} influx into mitochondrial matrix is mediated primarily by the mitochondrial calcium uniporter (MCU). However, the signaling pathways that regulate MCU channel functions via post-translational modifications of MCU are completely unknown. Here we show that adrenergic signaling induces MCU tyrosine phosphorylation and accelerates mitochondrial Ca^{2+} uptake in cardiac cells. Adrenergic signaling induces activation of proline-rich tyrosine kinase 2 (Pyk2) and translocation into the mitochondrial matrix; enhancing the interaction between Pyk2 and MCU, which subsequently accelerates mitochondrial Ca^{2+} uptake via Pyk2-dependent MCU tyrosine phosphorylation. MCU contains 15 tyrosine residues (5 in the N-terminus, 0 in the pore-forming region, 4 in transmembrane domains and 6 in the C-terminus), which are conserved across all eukaryotic species. Among them, only 3 of these tyrosine residues (Y157 at N-terminus, Y288, and Y316 at C-terminus in mouse MCU) remained as potential phosphorylation candidate sites for protein tyrosine kinases using phosphorylation prediction programs. We mutated these tyrosine residues to phenylalanine and generated non-phosphorylation mimetic MCU mutants (MCU-YFs). We confirmed that only two tyrosine sites were phosphorylated in response to adrenergic stimulation *in situ* using cell lines stably expressing MCU-YFs. In addition, overexpression of these MCU-YFs failed to increase mitochondrial Ca^{2+} uptake in response to cytosolic Ca^{2+} elevation by thapsigargin, whereas wild-type MCU transfection dramatically accelerates mitochondrial Ca^{2+} uptake compared to non-transfected cells. In summary, MCU contains Pyk2-specific phosphorylation site(s) and Pyk2-dependent tyrosine phosphorylation of MCU can modulate its channel functions and regulate mitochondrial Ca^{2+} uptake.

3073-Pos Board B503

Cardioprotective Roles of Neuronal Ca^{2+} Sensor-1 during Stress

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Dysregulation of Ca^{2+} homeostasis in cardiomyocytes often results in heart failure. Identifying molecular targets that regulate cardiomyocyte survival is of therapeutic importance. Neuronal Ca^{2+}-sensor-1 (NCS-1) is an EF-hand Ca^{2+}-binding protein, which is important for excitable cell functions. We previously found that NCS-1-deficient *($Ncs1^{-/-}$)* mice had excess neonatal mortality (*Circ. Res. 2011*). The aim of the present study is to examine whether NCS-1 plays beneficial roles in cardiac survival during stress and the possible mechanisms underlying these effects. Neonatal mouse ventricular myocytes or whole hearts from wild-type (WT) and *$Ncs1^{-/-}$* mice were subjected to stressors, and the resistance to stress was evaluated. *$Ncs1^{-/-}$* mouse hearts were more susceptible to stress induced by oxidative impairment and ischemia-reperfusion injury. Stress-induced activation of phosphatidylinositol 3-kinase (PI3K)/Akt signaling, a major survival pathway, was substantially reduced in the *$Ncs1^{-/-}$* group, and overexpression of NCS-1 or the constitutive active form of Akt increased the survivalrate of *$Ncs1^{-/-}$* myocytes. Cellular ATP levels, as well as mitochondrial respiration rates (both basal and maximal O_2 consumption) were significantly depressed in *$Ncs1^{-/-}$* myocytes; especially with oxidative stress. Furthermore, intracellular Ca^{2+} handling was more easily dysregulated in stressed *$Ncs1^{-/-}$* myocytes than WT myocytes. Since NCS-1 levels were increased by stress, the data suggest that NCS-1 is a survival-promoting factor, which is upregulated by stress stimuli. Interestingly, however,supra-physiological NCS-1 expression was toxic to cells. Taken together, our data suggest that moderate NCS-1 expression during stress promotes cardiomyocyte survival by maintaining proper Ca^{2+} handling, which is required for activation of Akt survival pathways and mitochondrial function.

3074-Pos Board B504

Initiation of Electron Transport Activity and a Decrease of Oxidative Stress Occur Simultaneously during Embryonic Heart Development

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Mitochondria in early embryonic hearts are not thought to produce ATP, yet they do produce reactive oxygen species (ROS) that regulate myocyte differentiation. To assess changes in ATP and ROS generation in the developing heart, we measured mitochondrial oxygen consumption, the activity of the complexes (Cx) 1 and 2 of the electron transport chain (ETC), ETC supercomplex assembly, and ROS in embryonic mouse hearts. At embryonic day (E) 9.5, mitochondrial ETC activity and oxidative phosphorylation (OXPHOS) are not coupled, even though the ETC complexes are present. We show that Cx-1 is able to accept electrons from the Krebs cycle, but enzyme assays that specifically measure electron flow to ubiquinone or Cx-3 show no activity at this early embryonic stage. At E11.5, mitochondria appear functionally more mature; ETC activity and OXPHOS are coupled and respond to ETC inhibitors. In addition, the assembly of highly efficient respiratory supercomplexes containing Cx −1, −3, and −4, ubiquinone, and cytochrome *c* begins at E11.5, the exact time when Cx-1 becomes functional activated. At E13.5, ETC activity and OXPHOS of embryonic heart mitochondria are indistinguishable from adult mitochondria. In contrast, generation of reactive oxygen species (ROS), as measured with Amplex Red, is high at E9.5 and drops significantly by E11.5, coinciding with activation of the ETC. In summary, our data suggest that between E9.5 and E11.5 dramatic changes occur in the mitochondria of the embryonic heart, which result in a decrease of ROS generation and an increase in OXPHOS due to the activation of Cx-1 and the formation of supercomplexes.

3075-Pos Board B505

The Stoichiometry between MICU1 and MCU Determines the Different Mitochondrial Ca^{2+} Uptake Phenotypes in Heart and Liver

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Mitochondrial Ca^{2+} uptake is central to oxidative metabolism and cell death signaling. The first clues to its molecular mechanism have emerged from the recent identification of the mitochondrial Ca^{2+} uniporter's pore forming protein (MCU) as well as its regulators. Among the regulators, MICU1 shows striking