intermembrane space of mitochondria. MAC is a potential therapeutic target, as modulation of its opening could induce or prevent cell death. Compounds previously found to block Bax-induced release of fluorescein from liposomes were tested for their ability to directly inhibit the channel activity of MAC. Patch clamp techniques were applied to proteoliposomes containing mitochondrial outer membranes of apoptotic FL5.12 cells to monitor MAC activity. Several antagonists irreversibly blocked MAC with the IC50's ranging from 25 to 900 nM. These Inhibitors of MAC, or iMACs, were also effective in preventing cytochrome c release and progression of apoptosis induced by IL3 deprivation or staurosporine treatment. A matrix-targeted GFP facilitated visualization of the collapse of the mitochondrial network during staurosporine-induced apoptosis; this collapse was also prevented by iMACs. The action of these inhibitors demonstrates the tight links between MAC activity, cytochrome c release and apoptosis. Future studies will evaluate the impact of MAC on mitochondrial dynamics.

2721-Pos Board B691

Voltage-gated Potassium Channel In Brain Mitochondria

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Transient cerebral ischemia is known to induce endogenous adaptive mechanisms such as the activation of mitochondrial ATP regulated potassium channels or Ca^{2+} regulated large conductance potassium channels that can prevent or delay neuronal injury. In this study a single channel activity was measured after patch-clamp of the mitoplasts isolated from gerbil hippocampus. In 70% of the all patches, a potassium selective current was recorded with mean conductance 109 $\pm$ 6 pS in symmetrical 150 mM KCl solution. The patch-clamp single channel studies showed properties of the voltage-gated potassium channel (Kv channel). We found that ATP/Mg^{2+} complex and Ca^{2+} ions had no effects on observed activity of ion channel. Observed channel was blocked by negative voltage and margatoxin (MgTx) a specific Kv1.3 channel inhibitor. The inhibition by MgTx was irreversible. We conclude that gerbil hippocampus mitochondria contain voltage-gated potassium channel (mitoKv) with properties similar to the surface membrane Kv1.3 channel which can play a role in control function of mitochondria as well as in ischemia-reperfusion phenomenon.

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Large-conductance Calcium-activated Potassium Channel In Neuronal Mitochondria

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Large-conductance calcium-activated potassium (BK) channels are expressed in the plasma membrane of various cell types. Interestingly, recent studies provide evidence for existence of this channel also in mitochondria. The goal of the present study was to find a candidate for the regulatory component of the large conductance calcium activated potassium channel in neurons. A combined approach of western blot analysis, high-resolution immunofluorescence and immunoelectron microscopy with the use of antibodies directed against four distinct beta subunits demonstrated the presence of the BK channel beta4 subunit in the inner membrane of neuronal mitochondria in rat brain and cultured neurons. Additionally, channel activity was measured with the use of patch-clamp technique.

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Biophysical Mechanism of Converting Apoptosis Regulator Bcl-2 from a Protector to a Killer in Cancer Cells By A Short Nur77-derived Peptide

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Bcl-2 can be converted into a pro-apoptotic molecule by nuclear receptor Nur77. The development of Bcl-2 converters as anti-cancer therapeutics has been explored by us. We reported recently the identification of a Nur77-derived Bcl-2 converting peptide (NuBCP) and its enantiomer, which induce apoptosis of cancer cells in vitro and in animals. The apoptotic effect of NuBCP enantiomers and their activation of Bax are not inhibited but rather potentiated by Bcl-2. Using fluorescence polarization assays, we determined that NuBCP enantiomers bind both quantitatively and stoichiometrically to the Bcl-2 loop, which shares the characteristics of structurally adaptable regions with many cancer-associated signaling proteins. NuBCP-9 enantiomers act as molecular switches to dislodge the Bcl-2 BH4 motif exposing its BH3 motif. Mechanistically we demonstrated, using fluorescence quenching based liposome assays, that NuBCP-9-induced Bcl-2 conformational change not only neutralizes Bcl-2's inhibition of Bax-mediated membrane permeabilization but also exposes the Bcl-2's BH3 motif that in turn neutralizes Bcl-X_L's inhibition of Bax like BH3 motif-derived peptides and compounds. Our results provide mechanistic insight into Bcl-2 conversion and identify a new direction for developing Bcl-2-based cancer therapeutics.

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2724-Pos Board B694

Respiratory Complex I Dysfunction Due to Mitochondrial DNA Mutations Shifts the Voltage Threshold for Opening of the Permeability Transition Pore toward Resting Levels

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We have studied mitochondrial bioenergetics in HL180 cells (a cybrid line harboring the T14484C/ND6 and G14279A/ND6 mtDNA mutations of Leber hereditary optic neuropathy, leading to an about 50% decrease of ATP synthesis) and XTC.UC1 cells (derived from a thyroid oncocytoma bearing a disruptive frameshift mutation in the MT-ND1 gene, which impairs complex I assembly). Addition of rotenone to HL180 cells and of antimycin A to XTC.UC1 cells caused fast mitochondrial membrane depolarization that was prevented by treatment with cyclosporin A, intracellular Ca2+ chelators, and antioxidant. Both cell lines also displayed an anomalous response to oligomycin, with rapid onset of depolarization that was prevented by cyclosporin A and by overexpression of Bcl-2. These findings indicate that depolarization by respiratory chain inhibitors and oligomycin was due to opening of the mitochondrial permeability transition pore (PTP). A shift of the threshold voltage for PTP opening close to the resting potential may therefore be the underlying cause facilitating cell death in diseases affecting complex I activity. This study provides a unifying reading frame for previous observations on mitochondrial dysfunction, bioenergetic defects and Ca2+ deregulation in mitochondrial diseases. Therapeutic strategies aimed at normalizing the PTP voltage threshold may be instrumental in ameliorating the course of complex I-dependent mitochondrial diseases.

2725-Pos Board B695

Bax Enhances the Permeabilization of the Mitochondrial Outer Membrane Induced by Ceramide Channels: Implications on the Regulation of the Initiation of Apoptosis

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Background: Bax is a pivotal pro-apoptotic Bcl-2 family protein that localizes to the mitochondrial outer membrane (MOM) during apoptosis and causes MOM permeabilization to proteins (MOMP). Earlier studies have demonstrated that upon an apoptotic stimulus, ceramide levels often greatly increase in cell membranes, including in the MOM. Elevation of ceramide in the MOM is sufficient to cause MOMP without requiring Bcl-2 family proteins. Moreover ceramide induced MOMP is reversed/prevented by the anti-apoptotic protein, Bcl-xL. **Methods:** Using rat liver or yeast mitochondria, the MOMP was measured with a dynamic cytochrome c accessibility assay. Ceramide's channel-forming ability was also assessed using a defined system: planar phospholipid membranes. Only C_{16}-ceramide was used. **Results:** We found that Bax induces MOMP by apparently enlarging ceramide channels. While ceramide forms