agent colchicine that is known to increase free tubulin, mitochondrial potential, as measured by tetramethylrhodamine methyester (TMRM) uptake, decreased. Inhibition of PKA activity with H89 increased mitochondrial potential and, furthermore, reversed mitochondrial depolarization induced by colchicine. To further explore a role VDAC-tubulin interaction in cell proliferation and survival, we studied the effect of erastin, the selective anti-tumour agent involved in the RAS-RAF-MEK signalling pathway, on VDAC blockage by tubulin. We found that erastin reverses tubulin blockage of VDAC isolated from HepG2 cells, promoting the unblocked state of the channel. Our findings suggest a novel functional link between serine/threonine kinase signalling pathways, mitochondrial respiration, and highly dynamic microtubule network which is characteristic of carcinogenesis and cell proliferation.

2216-Plat

The Isoform Specific N Terminus of VDAC2 is Dispensable for tBid Induced Cytochrome C Release

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VDAC proteins represent a main component of the outer mitochondrial membrane (OMM). The VDAC family is composed of 3 isoforms with more than 70 percent similarity. Although their primary role was known to be in ion and metabolite transport between mitochnodria and cytosol, it has been discovered that VDACs are involved in apoptotic pathways. We have recently found that specifically the VDAC2 isoform is needed for tBid-induced cytochrome c release, due to its role in supporting Bak insertion to the OMM (Roy et al.2009. *EMBO Rep.*10:1341-7). Sequence analysis of the mammalian VDAC isoforms shows a unique N terminal end in VDAC2, which is likely to be exposed to the cytoplasm. To test the functional significance of this N terminus, the first 12 amino acids were removed from VDAC2 or were added to VDAC1. VDAC2$^{-/-}$ MEF cells that are resistant to tBid, were rescued with these constructs and cytochrome c release was monitored in permeabilized cells by single cell imaging and immunoblotting. These studies revealed that cytochrome c release upon tBid application is effectively supported by both full length VDAC2 and the VDAC2 N-terminal deletion mutant. Furthermore, VDAC1 extended by the N terminus of VDAC2 failed to restore cytochrome c release in VDAC2$^{-/-}$ MEFs. Thus the N terminus of VDAC2 seems to be dispensible for tBid-induced OMM permeabilization. Currently, we are designing different mutations which target some addititional unique sequences in VDAC2 to find the specific domain that is essential for tBid induced cytochrome c release.

2217-Plat

Patch-Clamp Analysis of Mitochondrial Ca2+ Uniporter in Different Tissues

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The mitochondrial Ca2+ uptake has been recognized as a central player in cellular pathophysiology for decades. Pathways of Ca2+ transport across the inner mitochondrial membrane (IMM) were investigated indirectly with biochemical techniques and most recently by applying the patch clamp technique to mitoplasts isolated from mammalian systems. Patch-clamp recording of the IMM have proved to provide an unambiguous picture of mitochondrial Ca2+ uptake under strictly controlled conditions.

In this study, we evaluated and compared the biophysical properties of mitochondrial Ca2+ uptake in different mouse tissues. Freshly isolated mitoplasts from mouse heart, skeletal muscle, liver, kidney, spleen and brown adipose tissue were patch clamped in the whole-mitoplast configuration. Voltage step and ramp protocols covering the whole range of physiological potentials were applied to elicit the inwardly rectifying Ca2+current sensitive to RuR known as MCU. The distribution of MCU current densities between tissues was as follows: spleen > brown adipose tissue ≈ skeletal muscle > kidney > liver >> heart. Interestingly, MCU current density in heart was about 10 times smaller than in skeletal muscle. Our results support the view of a differential tissue activity of MCU, which can be explained by either a different distribution of MCU channel and/or by a different expression of its regulatory subunits confering various modes of physiological regulation.

Further on, in order to investigate the putative direct contribution of Uncoupling Protein Isoform 2 and 3 (UCP2/3) to the uniporter, we investigated MCU properties in wild type (WT) mouse tissues where these two proteins have been found to have a major role and compared its properties in the correspondent tissues of UCP2 and UCP3 KO mice. We found no significant differences in calcium currents of any of the WT tissues studied compared to their UCP2/3 KO counterparts.

2218-Plat

SR-Mitochondrial Ultrastructure in the Heart of Normal and Ethanol-Fed Rats

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Chronic alcoholism causes various forms of stress in the heart, and this is often accompanied by mitochondrial dysfunction. A putative source of mitochondrial dysfunction is SR-mitochondrial stress and the ensuing mitochondrial Ca^{2+} overload. To test this possibility, first we isolated mitochondria from chronic ethanol-fed (6 months) and paired control rats, and documented ethanol-dependent sensitization to Ca^{2+} and ROS-induced permeabilization. To evaluate the ultrastructural basis of the mitochondrial impairments, we performed transmission electron microscopy (TEM) and electron tomography studies of left ventricular muscle. The overall ultrastructure of the myocytes became less organized in the ethanol-fed condition. Also, a considerable fraction of the mitochondria lost the highly ordered structure of the cristae and showed an increase in the cross-sectional area. However, the size of the SR-mitochondrial associations and the gap-width at the interface was unchanged. Evaluation of the SR-mitochondrial tethers by electron tomography indicates that they occur in clusters and display heterogeneity in length, as previously reported for ER-mitochondrial tethers in normal rat liver (Csordas et al, 2006, J Cell Biol, 174:915). Overall tether length distribution appears to be unaltered by alcohol ingestion. Analyses of effects of alcohol ingestion on tether membrane-surface density and structural classes (linear, V, Y and more complex shapes) is in progress, as is 3D analysis of SER and mitochondrial inner- and outer-membrane topologies. (Supported by NIH grant 1RC2AA019416.)

2219-Plat

Bcl-xL Increases Bax Mitochondrial Localization and Activation in Non-Apoptotic Cells

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We report that, in non-apoptotic pro-lymphocyte cells, Bcl-xL induced an increase of the mitochondrial localization of Bax up to the level found in apoptotic cells. Furthermore Bcl-xL stimulated both the active conformation of Bax and increased the total amount of Bax inserted in the outer mitochondrial membrane. In order to further investigate the molecular mechanisms involved in this effect, different Bax and Bcl-xL mutants were co-expressed in yeast. We found that wild-type Bcl-xL increased the mitochondrial localization of both wild-type Bax and of an active mutant Bax-S184D, while mutant Bcl-xL-G138A did not have this effect. Further, wild-type Bcl-xL protected Bax-S184D against proteolytic degradation. Finally, we found that a C-terminally truncated mutant of Bcl-xL, that lost its capacity to inhibit Bax-induced release of cytochrome c, still increased the mitochondrial localization of wild-type Bax, thus promoting the release of cytochrome c. These data suggest that Bcl-xL, by acting as a modulator of mitochondrial Bax localization, may play an active role along the pathway leading to Bax activation.

2220-Plat

The Physiological Response of Intact *Ex Vivo* Mitochondria upon Apoptotic Stress: Insight into the Regulation of Apoptosis at a Mitochondrial Level

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Apoptosis - programmed cell death - is vital for multicellular organisms to dispose of redundant, damaged or infected cells. This mechanism becomes dysfunctional in cancer where tumor cells survive drug-induced death signals. Reversely, upregulated apoptosis can induce premature neuronal cell death in amyloidogenic diseases such as Alzheimer's disease (AD) or Amyotrophic Lateral Sclerosis (ALS). Mitochondrion, the power plant of the cell, contains an arsenal of proteins which regulate programmed cell death. There, the pro- and anti-apoptotic Bcl-2 proteins meet at the mitochondrial membrane to decide the fate of a cell. How the molecular activities of the anti-apoptotic