activates a novel, calcium-independent, PKCα-dependent signaling pathway, which results in mitochondrial depolarization. As a result, mitochondrial dysfunction is likely to be a key contributor to the pathophysiology of gas embolism injury. Further, this connection between the endothelial surface layer and endothelial mitochondria may also play an important role in vascular homeostasis and disease.

1105-Plat

Calcium-Induced ROS Generation during Ischemia Triggers mPTP-Dependent Cell Death during Reperfusion

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Impairment of mitochondrial function is a central event of ischemia-reperfusion (I/R) injury leading to tissue damage and cell death. We studied the relationship between mitochondrial Ca^{2+} overload, reactive oxygen species (ROS) generation, opening of the mitochondrial permeability transition pore (mPTP) and cell death in rabbit ventricular myocytes exposed to simulated I/R. Changes in mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) were measured with X-Rhod-1, ROS generation with Mito-Sox Red, mPTP opening as mitochondrial calcein red release, and cell death as lactate dehydrogenase release. I/R was induced by exposing cells to glucose-free Tyrode solution containing 20 mM 2-deoxyglucose and 2 mM NaCN, pH 6.4, followed by superfusion with standard Tyrode solution. No cell death was observed after 20 min of ischemia despite a significant increase in $[Ca^{2+}]_m$, ROS and mPTP opening, however cell death increased significantly after 15 min of reperfusion. The Ca^{2+} uniporter blocker Ru360 partially prevented $[Ca^{2+}]_m$ increase and completely abolished ROS generation and cell death when applied during I/R, however application of Ru360 only during reperfusion did not protect from cell death. Scavenging ROS with superoxide dismutase mimetic MnTBAP or antioxidant Trolox prevented reperfusion-induced cell death. Blocking mPTP during ischemia by cyclosporine A or depletion of mitochondrial inorganic polyphosphate did not provide protection against cell death during reperfusion, but instead led to increased mitochondrial ROS accumulation. However, inhibiting mPTP opening during reperfusion with cyclosporine A attenuated cell death. We conclude that mPTP-dependent cell death during reperfusion is mediated by Ca^{2+}-dependent ROS generation during ischemia. Moreover, our data suggest that mPTP opening during ischemia could serve as ROS escape pathway from mitochondria and thereby attenuate mitochondrial ROS accumulation and ROS-mediated cell damage during subsequent reperfusion.

1106-Plat

Dynamic Measurement of Ca^{2+}-Induced Changes in Organelle-Specific Redox Microdomains

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Multiple interactions between Ca^{2+}-signalling and reactive oxygen species (ROS) production are thought to exist and be of both physiological and pathophysiological relevance. However, until recently, the study of ROS in the context of Ca^{2+}-signalling has been hampered by ROS probes limited in both specificity and targeting. To measure ROS in discrete subcellular domains, we compared the genetically-encoded ratiometric H_2O_2 sensor HyPer with the redox de-sensitized derivative SypHer to control for non-specific changes. These probes, targeted to the ER lumen, ER membrane (cytosolic face), cytosol, mitochondrial matrix and outer membrane (OMM) and the IP_3-receptor, are positioned to assess redox changes at a local level. In resting conditions, the ER lumen showed >3-fold increased HyPer ratio compared to the outside of the ER membrane, bulk cytosol and mitochondrial matrix and OMM. In the ER lumen, IP_3-linked Ca^{2+}-mobilization induced a prounounced, downward shift in HyPer ratio, whereas little or no change was detected in other compartments. In permeabilized cells, mitochondrial Ca^{2+}-overload was accompanied by substantial increase in H_2O_2 detected in the matrix and at the OMM. These data demonstrate that redox environments within individual cellular compartments constitute a complex and dynamic interrelationship with the concentration of free Ca^{2+}. The ER lumen is highly oxidized and exhibits profound decreases in H_2O_2 concommitant with Ca^{2+}-release. Interestingly, these changes are not transmitted to the outer leaflet of the ER membrane or to other compartments. Mitochondria resist redox changes under moderate stimulation, however, during mitochondrial Ca^{2+}-overload and collapsed membrane potential, strong H_2O_2 generation is detectible in the matrix and at the mitochondrial surface. The ROS generated and detected at locations immediately apposed to Ca^{2+}-transport proteins, such as the IP_3-receptor, may modify their function at a local level and contribute to the feed-forward cycle of Ca^{2+}-dysregulation and subsequent cell death.

1107-Plat

ER-Mitochondrial Ultrastructure in the Liver of Normal and Ethnaol-Fed Rats

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Chronic alcohol consumption causes severe pathology in liver, associated with altered metabolism and reduced ATP production. Liver mitochondria isolated from alcohol-fed animals show decreased capacity for electron transport and ATP synthesis, impaired calcium handling, and increased ROS. In parallel, the endoplasmic reticulum (ER) also shows increased ROS and a stress response. The close correlation between defects in mitochondria and ER is matched by recent evidence of the functional integration of cellular responses (including calcium signaling) involving these organelles. To evaluate the ultrastructural basis of alcohol-induced changes, we performed transmission electron microscopy and electron tomography studies of rat liver. Hepatocytes showed a large number of mostly round mitochondrial cross sections, which occupied ~20% of the cytoplasmic area. The cell cytoplasm in chronic ethanol-fed (9 months) condition exhibited reduced particle density, indicative of cell swelling, and contained large lipid vesicles. The mitochondria were generally intact but showed narrower intermembrane spacings within cristae and at the organelle periphery, consistent with low-scale matrix swelling. In both control and ethanol-fed conditions, mitochondria were typically surrounded by extensive ER, with cisternae sometimes sandwiched between neighboring mitochondria. In one case, ER was prominent at a site of mitochondrial fusion/fission. As previously reported, regions of close ER-mitochondrial association (20-60 nm) contained numerous ''tethers'' between outer mitochondrial membranes (OMM) and adjacent ER. While individual tethers were discernible, dense granular material (including ribosomes) within OMM-ER interfaces interfered with accurate quantitation of tethers. However, membrane spacings could be readily measured. It was found that the mitochondrial surface in close association with ER was significantly reduced in the chronic ethanol-fed condition as compared to control (17.5 ± 7% vs. 39 ± 2%). This might be expected to cause reduced calcium signaling between ER and mitochondria in liver after chronic alcohol ingestion.

1108-Plat

Mitochondrial Nm23-H4/NDPK-D is Multifunctional: Intermembrane Cardiolipin Transfer Linked to Apoptosis

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Nm23-H4/NDPK-D forms symmetrical homohexameric complexes in the mitochondrial intermembrane space. The well established function of the enzyme is phosphotransfer activity as a nucleoside diphosphate kinase (NDPK), using mitochondrial ATP to regenerate nucleoside triphosphates. Nm23-H4 is further known to strongly bind *in vitro* to anionic phospholipids, mainly cardiolipin, and *in vivo* to inner mitochondrial membranes. We show here that such protein/lipid complexes inhibit NDPK activity but are necessary for Nm23-H4 to function in selective intermembrane lipid transfer. Nm23-H4-deficient HeLa cells expressing either wild-type Nm23-H4 or a membrane-binding deficient mutant were analyzed by membrane fractionation and LC-ESI-MS. Data revealed that wild-type Nm24-H4 increased cardiolipin content in the outer mitochondrial membrane as compared to mutant enzyme. This correlated with higher susceptibility of wild-type enzyme expressing cells to rotenone-induced apoptosis as seen by increased annexin V binding, elevated caspase 3/7 activity and stimulated release of cytochrome c into the cytosol. Molecular modeling of Nm23-H4 binding with cardiolipin reveals potential intermembrane transfer mechanisms. We propose that Nm23-H4 acts as a lipid-dependent mitochondrial switch with dual function, allowing either for phosphotransfer or for cardiolipin transfer, with a role in apoptotic signaling.