can be, at least partially, due to their interactions with the endogenous pool of fatty acids.

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5P.21 The effect of Ca^{2+} on reactive oxygen species generation in brain mitochondria in the absence of permeability transition

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Glutamate excitotoxicity is a key element in the pathomechanism of acute (ischemia–reperfusion) and chronic (Alzheimer's disease, Parkinson's disease, Huntington's disease) neurological disorders. Stimulation of glutamate receptors results in the elevation of intracellular [Ca^{2+}] which can activate reactive oxygen species (ROS) generation. The aim of our experiments was to study the effects of high micromolar Ca^{2+} concentrations on the H_2O_2 generation in isolated guinea pig brain mitochondria, supported by NADH-generating substrates; glutamate plus malate. H_2O_2 formation was detected extramitochondrially by Amplex red assay. In parallel with ROS formation NAD(P)H autofluorescence was detected. Mitochondrial membrane potential ($\Delta\psi_m$) was measured by safranine O and TMRM fluorescence respectively. Swelling of mitochondria was detected by light scattering. Permeability transition pore (PTP) opening was measured by calcium induced calcium release and by quenching of calcein fluorescence. PTP was prevented by ADP, a very efficient inhibitor of mitochondrial permeability transition. In the presence of ADP 50 μM Ca^{2+} (500 nmol/mg protein) did not induce PTP opening but enhanced mitochondrial H_2O_2 release by 81 ± 18%. Mitochondria were able to take up calcium; after a transient depolarization $\Delta\psi_m$ was restored, even hyperpolarization was detected and parallel with these NAD(P)H fluorescence was increased. With 300 μM Ca^{2+} membrane potential collapsed without recovery and H_2O_2 release was unchanged. At 300 μM [Ca^{2+}] in the presence of ADP, mitochondria were unable to complete Ca^{2+}-uptake but no signs of PTP were detected in the time-frame of the experiments. In highly polarized mitochondria, in the presence of ATP or oligomycin ROS production was elevated, Ca^{2+} failed to stimulate mitochondrial ROS generation and hyperpolarization did not follow the Ca^{2+}-induced depolarization. It is suggested that in the presence of nucleotides the effect of Ca^{2+} on mitochondrial ROS release is related to changes in $\Delta\psi_m$. The increased ROS release evoked by Ca^{2+} in the presence of ADP in isolated mitochondria is unrelated to PTP and would not explain the extensive cellular ROS production observed during glutamate excitotoxicity.

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5P.22 Cardiolipin: Altered content and fatty acid composition in mitochondria from mtDNA mutator mice

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We have recently demonstrated that the assembly and turnover of the mitochondrial respiratory chain complexes I, III and IV (but not complexes II and V) are altered in mitochondria from mtDNA mutator mice [1]. Since it is known that cardiolipin is essential for assembly and stability of respiratory chain complexes, we have elected to study this phospholipid in mitochondria from mtDNA mutator mice. The content of mitochondrial phospholipids was analysed by two-dimensional high performance thin layer chromatography (2D-HPTLC). The content of cardiolipin was significantly lower in liver and skeletal muscle mitochondria from mtDNA mutator mice than in wild-type mitochondria. To analyse the fatty acid composition of cardiolipin, gas chromatography/flame ionization detection or electron ionization-mass spectrometry (GC/FID or EI MS) was applied. The content of the polyunsaturated *n-6* fatty acids was remarkable lowered in the cardiolipin fraction from skeletal muscle and liver mitochondria of mtDNA mutator mice, as compared with wild-type mice. Mitochondrial phospholipids were also studied in mice chronically treated with mitochondria-targeted antioxidant (plastoquinone derivative, SkQ1), added to drinking water. The content of cardiolipin and its fatty acid composition were normalised in mtDNA mutator mitochondria after treatment with SkQ1. We conclude that cardiolipin content is decreased and its fatty acid composition markedly altered in mitochondria from mtDNA mutator mice. The cause and significance of these alterations are of interest, considering the special role of cardiolipin in mitochondrial bioenergetics.

Reference

[1] Edgar D *et al.* (2009) *Cell. Metab.* **10:** 131–138.

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5P.23 Antioxidant defence systems and generation of reactive oxygen species in osteosarcoma cells with defective mitochondria: Effect of selenium

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Mitochondrial diseases originate from mutations in mitochondrial or nuclear genes encoding for mitochondrial proteome. Neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP) syndrome is associated with the T8993G transversion in *ATP6* gene which results in substitution at the very conservative site in the subunit 6 of mitochondrial ATP synthase. Defects in the mitochondrial respiratory chain and the ATPase are considered to be accompanied by changes in the generation of reactive oxygen species (ROS). This study was aimed to elucidate effects of selenium on ROS and antioxidant system of NARP cybrid cells with 98% of T8993G mutation load. We found that selenium decreased ROS generation and increased the level and activity of antioxidant enzymes such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR). Therefore, we propose selenium to be a promising therapeutic agent not only in the case of NARP syndrome but also other diseases associated with mitochondrial dysfunctions and oxidative stress.

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5P.24 Response of *Acanthamoeba castellanii* mitochondria to hydrogen peroxide stress

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The purpose of this study was establishing the response of energetic parameters of amoeba *Acanthamoeba castellanii* mitochondria respiring with different respiratory substrates to oxidative stress caused by hydrogen peroxide in mitochondria isolated from H_2O_2-treated and control cells. Applied concentration of H_2O_2 (0.7–1.4 mM) slowed down growth of *A. castellanii* batch culture and increased amount of death cells. However, mitochondria from H_2O_2-treated cells did not display any substantial impairment. In the presence of an inhibitor of alternative oxidase, a slight increase in cytochrome pathway activity was found and no marked changes in coupling parameters were observed. Moreover, our results show no change in membrane potential and mitochondrial outer membrane integrity. A significant increase in activity of energy-dissipating systems, cyanide-resistant alternative oxidase and uncoupling protein, was observed, which may indicate their role as endogenous antioxidant systems under stress conditions in unicellular organisms.

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