sensitive potassium channels leading to depolarization of mitochondrial membrane potential followed by mitochondrial ROS formation and respiratory dysfunction. This concept was supported by observations that ethidium bromide-induced mitochondrial damage suppressed angiotensin-II-dependent increase in Nox1 and oxidative stress. In another example hypoxia was used as a stimulator of mitochondrial ROS formation and by using pharmacological and genetic inhibitors, a role of mitochondrial ROS for the induction of NADPH oxidase *via* PKCε was demonstrated. The third model was based on cell death by serum withdrawal that promotes the production of ROS in human 293T cells by stimulating both the mitochondria and Nox1. By superior molecular biological methods the authors showed that mitochondria were responsible for the fast onset of ROS formation followed by a slower but long-lasting oxidative stress condition based on the activation of an NADPH oxidase (Nox1) in response to the fast mitochondrial ROS formation. Finally, a cross-talk between mitochondria and NADPH oxidases (Nox2) was shown in nitroglycerin-induced tolerance involving the mitochondrial permeability transition pore and ATP-sensitive potassium channels. The use of these redox signaling pathways as pharmacological targets is briefly discussed.

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5L.4 The significance of thermogenesis for the ageing process

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Impaired thermogenesis is one of the features of ageing. Activity and recruitment of mitochondria in brown adipose tissue is important for the thermogenic needs of animals. Therefore thermogenesis, both at the level of brown-fat mitochondria and at the intact animal level, was examined here in PolgA mtDNA polymerase mutant mice (mtDNA mutator), a mouse strain exhibiting numerous mutations of mtDNA and several features of premature aging. At the mitochondrial level, as compared with wild-type mitochondria, on all three substrates investigated (pyruvate, palmitoyl-l-carnitine and glycerol-3-phosphate), UCP1-dependent oxygen consumption was significantly reduced in mutant mitochondria, similarly to maximal oxidative capacity (FCCP-response), indicating impaired thermogenesis. In intact mice, at environmental temperatures below 20 °C, mtDNA mutator mice were unable to further increase their metabolism and went into torpor. Response to adrenergic stimulus (NE injection) was significantly reduced in mtDNA mutator mice. Thus, mtDNA mutation led to lower activity of brown-fat mitochondria and impaired thermogenesis; i.e. also in this respect, mtDNA. Mutator mice mimicked normal ageing. Maintaining the mice at thermoneutral temperature (30 °C) ameliorated many of the ageing symptoms. Remarkably, the life span of the mice at thermoneutrality was increased by around 100 days, emphasizing the significance of thermogenesis for the ageing process.

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5L.5 Mitochondrial fatty acid oxidation and oxidative stress: Lack of reverse electron transfer-associated production of reactive oxygen species

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Lipotoxicity results from accumulation of lipids in non-adipose tissue and is partly attributed to an impairment of mitochondrial physiology by elevated level of nonesterified fatty acids (FFA). Typical activities of FFA are uncoupling and modulation of cellular ROS generation. Here, we examined the likely ability of fatty acid oxidation to initiate ROS generation by reverse electron transfer (RET). RET from succinate to NAD^+ is known to be accompanied by high generation of reactive oxygen species (ROS). In contrast, oxidation of fatty acids by mitochondria, despite being a powerful source of $FADH_2$, does not exhibit RET-associated ROS generation. Oxidation of carnitine esters of medium- and long-chain fatty acids by rat heart mitochondria is accompanied by neither high level of $NADH/NAD^+$ nor intramitochondrial reduction of acetoacetate to β-hydroxybutyrate, comparable to those accompanying succinate oxidation, although it produces the same polarization of the inner mitochondrial membrane, evidenced by high transmembrane potential ($\Delta\psi$). Also in contrast to the oxidation of succinate, where conversion of the pH difference between the mitochondrial matrix and the medium into $\Delta\psi$ by addition of nigericin results in a decrease of ROS generation, the same treatment during oxidation of octanoylcarnitine produces a large increase of ROS. Analysis of respiratory chain complexes by Blue Native polyacrylamide gel electrophoresis revealed bands that could tentatively point to supercomplex formation between complexes II and I and complexes II and III. However, no such association could be found between complex I and the electron transferring flavoprotein that participates in fatty acid oxidation. It is speculated that structural association between respective respiratory chain components may facilitate effective RET.

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5L.6 Mitochondrial Ca^{2+} and ROS crosstalk signaling

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Mitochondria are central to cellular energy metabolism as the source of much of the cell's ATP, as well as being a hub for cellular Ca^{2+} and redox signaling pathways. Mitochondrial Ca^{2+} and morphology are effectors of ATP synthesis, yet Ca^{2+} overload and morphological deformation can lead to mitochondrial dysfunction and ultimately cell death. Moreover, Ca^{2+} uptake by mitochondria is actively involved in shaping cellular Ca^{2+} dynamics by regulating the concentrations of Ca^{2+} within microdomains between mitochondria and Ca^{2+} transporters existing in nearby sarco/endoplasmic reticulum and plasma membranes. Reactive oxygen species (ROS) are generated, at least in part, as a consequence of ATP production in the mitochondria and are important for cellular signaling, yet contribute to oxidative stress and cellular damage. ROS are important for regulating the activity of redox sensitive enzymes and ion channels within the cell, including Ca^{2+} channels. For both Ca^{2+} and ROS, a delicate balance exists between the beneficial and detrimental effects on mitochondria. In this presentation, I bring together current data on mechanisms of mitochondrial Ca^{2+}-mediated ROS generation and mitochondrial fission. I propose a model for crosstalk between Ca^{2+} and ROS signaling pathways within mitochondrial microdomains, focusing on the role of mitochondrial fission machinery and mitochondrial permeability transition pores as a modulator of Ca^{2+} dynamics and ROS generation.

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5L.7 What are the sources of hydrogen peroxide production by heart mitochondria?

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Several mitochondrial enzymes, the respiratory complexes I and II, oxoglutarate dehydrogenase, free dihydrolipoamide dehydrogenase, and monoamine oxidase are potential contributors to overall intra- and extramitochondrial production of hydrogen peroxide generated either directly or via intermediate formation of superoxide anion. At least three intramitochondrial enzymes, Mn-superoxide dismutase, glutathione peroxidase, and catalase are involved in further reduction of superoxide and H_2O_2. The intramitochondrial steady-state level of hydrogen peroxide and its external production are thus resulted from an interplay between these enzyme activities. We measured the rates of H_2O_2 and superoxide generation by heart mitochondrial preparations of different degree of resolution: (i) intact coupled mitochondria, (ii) inside-out submitochondrial particles (SMP), (iii) alamethicin-treated mitochondria (A-mito, uncoupled mitochondria, permeable for low mol. mass components), and (iv) soluble matrix proteins and purified fractions derived there from. The NADH- and succinate-supported superoxide generation by SMP are strongly suppressed at high physiologically relevant concentrations of either NADH or NAD^+. Hydrogen peroxide formation by A-mito assayed under optimal conditions for complex I-mediated reaction (low NADH in the presence of rotenone) is only partially sensitive to complex I-specific active site-directed inhibitor, NADH-OH. The residual inhibitor-insensitive activity is strongly and specifically stimulated by NH_4^+. A soluble matrix located protein fraction (mol. mass of about 50 kDa) responsible for the ammonia-dependent NAD(P)H-supported hydrogen peroxide formation was purified. It catalyzes NADH:lipoamide and NADPH:glutathione oxidoreductase reactions and shows significant homology with dihydrolipoamide dehydrogenase. The data suggest that in heart mitochondria the soluble matrix located protein(s), not the respiratory chain components, are the major contributor(s) to hydrogen peroxide formation. Whether relative contributions of the respiratory chain components and matrix located flavoproteins to the extra- and intramitochondrial hydrogen peroxide production is the same in other than heart tissues remain to be established.

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5L.8 Oxidative stress-dependent p66Shc phosphorylation in skin fibroblasts of children with mitochondrial disorders

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p66Shc, the growth factor adaptor protein, can have a substantial impact on mitochondrial metabolism through regulation of cellular response to oxidative stress. We investigated relationships between the extent of p66Shc phosphorylation at Ser36, mitochondrial dysfunctions and an antioxidant defence reactions in fibroblasts derived from five patients with various mitochondrial disorders (two with mitochondrial DNA mutations and three with methylglutaconic aciduria and genetic defects localized, most probably, in nuclear genes). We found that in all these fibroblasts the extent of p66Shc phosphorylation at Ser36 was significantly increased. This correlated with a substantially decreased level of mitochondrial superoxide dismutase (SOD2) in these cells. This suggest that SOD2 is under control of the Ser36 phosphorylation status of p66 protein. As a consequence, an intracellular oxidative stress and accumulation of damages caused by oxygen free radicals are observed in the cells.

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Posters

5P.1 Inhibition of the α-ketoglutarate dehydrogenase-mediated reactive oxygen species generation by lipoic acid

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Dihydrolipoamide dehydrogenase (LADH) is a flavo-enzyme that serves as a subunit of α-ketoglutarate dehydrogenase complex (α-KGDHC). Reactive oxygen species (ROS) generation by α-KGDHC has been assigned to LADH (E3-subunit) and explained by the diaphorase activity of E3. Dysfunctions of α-KGDHC and concurrent ROS-production have been implicated in neurodegeneration, ischemia–reperfusion and other pathological conditions. In this work we investigated the intimate details of ROS-generation by isolated LADH and α-KGDHC. We found a parallel generation of superoxide and hydrogen peroxide by the E3-subunit of α-KGDHC which could be blocked by lipoic acid (LA) acting on a site upstream of the E3-subunit. The pathologically relevant ROS-generation (at high NADH/NAD + ratio and low pH) in the reverse mode of α-KGDHC could also be inhibited by LA. Our results contradict the previously proposed mechanism for pH-dependent ROS-generation by LADH, showing no disassembling of the E3 functional homodimer at acidic pH using a physiologically relevant method for the examination. It is also suggested that LA could be beneficial in reducing the cell damage related to excessive ROS-generation under pathological conditions.

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5P.2 Measuring mitochondria-derived reactive oxygen species in cell culture: Challenges and limitations

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