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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 H₂O₂. 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 H₂O₂ 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 NH₄⁺. 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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