Mitochondria are the major source of reactive oxygen species (ROS) during the ischemia/reperfusion (I/R) and the same time one of the most susceptible compartment to the ROS damage. Oxidative stress and mitochondria dysfunction are believed to be main reasons of acute kidney injury after I/R. Inhibition of ROS production inside the mitochondria could protect these organelles from dysfunction during I/R and thus prevent cell death. For this purpose a new type of antioxidant molecules was designed. Due to the delocalized positive charge the antioxidant accumulates in mitochondrial matrix in concentrations highly exceeding its concentration in the cytosol or in the intracellular space. The aim of the work was to investigate the ability of mitochondria-targeted antioxidant 10-(plastoquinonyl)-decylrhodamine (SkQR1) to prevent oxidative stress and protect kidney from acute kidney injury on the model of 40-min ischemia of rat kidney. For the evaluation of ROS production and mitochondria membrane potential after I/R, DCF- and TMRE-loaded renal cortex tissue slices were analyzed by confocal microscopy. Malonic dialdehide (MDA)-products in kidney, blood urea nitrogen (BUN) and creatinine level were investigated 48 h after I/R. Histological study of renal tissue was also held. It was revealed that 40-min I/R led to the burst of ROS production, decrease of mitochondria membrane potential and fragmentation of mitochondrial reticulum. creatinine and BUN level increased 48 h after I/R about 4 and 6 times, respectively; MDA-products increased more than twice. Tubular lesions were observed by histological examination. Intraperitoneal injection of SkQR1 before I/R partly normalized ROS production and prevented mitochondria damage. The MDA-products in kidney were diminished. Administration of SkQR1 had a beneficial effect on kidney function: creatinine and BUN level decreased and there were minimal pathological changes in the kidney. We conclude that mitochondria-targeted antioxidant SkQR1 was able to normalize mitochondria functioning during I/R, prevent oxidative stress and having beneficial effect on acute kidney injury.

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5P.9 Effect of flavonolignans derived from silybin on mitochondrial production of reactive oxygen species

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Flavonolignans exert cytoprotective and anticancer effects generally ascribed to their antioxidant properties. We have tested seventeen chemically modified derivatives of the naturally occurring silybin and two derivatives of the flavonol quercetin to assess their alleviating effect on mitochondrial reactive oxygen species (ROS) production. We have used isolated intact rat heart mitochondria (RHM) and detected the mitochondrial production of reactive oxygen species using the Amplex Red assay for fluorometric monitoring of H_2O_2. Silybin titration (0.01–20 μM) resulted in only a mild decrease in the mitochondrial ROS production. The most prominent decrease in the detected ROS production was found in the case of 2,3-dehydrosilybinic acid, 2,3-dehydrosilybin (DHS) and its 3-*O*-methyl, 7-*O*-galloyl and 23-*O*-galloyl derivatives. *O*-Methylation at a position seven reverted the anti-oxidant effect of 3-*O*-methyl-2,3-dehydrosilybin. The half-maximum inhibitory concentration of DHS was found to be 0.15 mM. Moreover, the detection of mitochondrial respiration and membrane potential indicated that DHS and other tested compounds which decrease the mitochondrial ROS production also uncouple oxidative phosphorylation, an effect analogous to that of the synthetic uncoupler FCCP. In addition, we found DHS and its derivatives to be more effective uncouplers than quercetin. The similarity of the behavior between FCCP, DHS and selected derivatives suggests a direct protonophoretic mechanism. In summary, our data support previous studies indicating the ability of several bioflavonoids to uncouple respiration. These data further extend our previous results showing that DHS and several of its derivatives are more potent scavengers of ROS than silybin and we attribute these effects to their innate uncoupling properties.

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5P.10 Proton transfer and reactive oxygen species in the cytochrome bc_1 complex

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The mitochondrial cytochrome bc_1 complex links electron transfer from ubiquinol to cytochrome *c* by a protonmotive Q cycle mechanism in which ubiquinol is oxidized at center P and ubiquinone is reduced at center N [1,2]. E272 of the conserved PEWY loop of most cytochrome *b* has been suggested as ligand in the enzyme–substrate complex and as proton acceptor in parallel proton-electron transfer towards heme b_L [3]. E272D and E272Q mutations support the importance of the residue for correct ubiquinol oxidation, showing effects such as lowered ubiquinol cytochrome *c* reductase activity, elevated bypass reactions, and altered K_M for ubiquinol oxidation [4]. However, these effects may also be indirect and the role of E272 as direct ligand of ubiquinol is debated. Furthermore, E272 is not fully conserved across all species. We suggested that in *Beta-* and *Gamma-proteobacteria* of which the PEWY glutamate is substituted by valine or leucine, a glutamate equivalent to yeast H253 is conserved, which could take over the proton transfer function. To challenge this hypothesis, single and double substitutions of H253 and E272 have been constructed in *Saccharomyces cerevisiae*. Eight variants were produced and the detergent-solubilized and purified complexes were characterized. The mutations affect cytochrome *c* reductase activity and provoke reactive oxygen species production. Mechanistic implications for ubiquinol oxidase and the control of deleterious bypass reactions will be discussed.

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5P.11 ATP concentration change in *Caenorhabditis elegans*

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Several lines of evidence have suggested that energy metabolism regulation deeply related to longevity of organisms. For example, most age-related genes were correlated with energy metabolism. Also, dysfunction of mitochondria increases longevity of several organisms. However, relationship between aging and production/consumption of ATP in organisms has been rarely known. In this study, we have attempted to determine the change of ATP concentration during aging in nematodes by both bulk phase and molecular imaging analysis. We report the change of ATP concentration during aging in nematode.

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5P.12 Comparison of superoxide production of rat brain mitochondria analyzed with hydroethidine and MitoSOX

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The production of superoxide generation is implicated for several types of neurodegenerative diseases and aging. Despite the progress in characterising the ROS effects on cell function, the mechanisms of cellular superoxide formation are less well understood. Considerable difficulties and artefacts are observed with different methods for detection of ROS, and in particular superoxide. In this work we compared MitoSOX and Hydroethidine, the well known dyes for superoxide detection in living cells and tissue slices, for the suitability to detect superoxide in isolated rat brain mitochondria. Hydroethidine (HET) is used to visualize superoxide localized in cytoplasm. For more targeted detection of ROS production in the mitochondria, hydroethidine is modified by conjugating this dye to triphenylphosphonium (MitoSOX). We observed that, unlike hydroethidine, MitoSOX allows to detect superoxide generation in isolated rat brain mitochondria respiring on the complex II substrate succinate. This superoxide generation, detected by MitoSOX, was sensitive to uncoupler and rotenone. This indicates that it is due to reversed electron flow caused ROS generation by Complex I. Similarly, rotenone increased the superoxide generation detected by MitoSOX but not HET in the presence of the complex I substrates glutamate + malate, α-ketoglutarate and pyruvate + malate. Since the MitoSOX is assumed to be accumulated at the inner side of mitochondrial inner membrane, this indicates that Hydroethidine and MitoSOX probably detect mitochondrial superoxide production in different local compartments.

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5P.13 Tissue specific effects of MnSOD knockout in mice

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A critical role of mitochondrial dysfunction and oxidative damage has been hypothesized in both aging and neurodegenerative diseases. Mitochondria are the main source of reactive oxygen species in cells because 2–4% of the oxygen consumed by mitochondria is converted to superoxide anions by the electron transport chain and moreover mitochondria have restricted protection against oxidative stress. To determine the importance of mitochondrial oxygen species toxicity, we analyzed heart muscle tissue and fibroblasts from mutant mice, with deficiencies in the mitochondrial antioxidant enzyme manganese superoxide dismutase (MnSOD) generated in three different ways. The knockout mouse models have been produced by disruption of different regions of Sod2 gene (Li Y *et al.* (1995) *Nat. Genet.* **11**: 376–381; Lebovitz RM *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**: 9782–9787). In agreement with literature in heart muscle we observed aconitase deficiency (Melov S *et al.* (1999) *Proc. Natl. Acad. Sci. USA* **96**: 846–851). In comparison with wild type animals the knockout mice showed only one third of aconitase activity. In contrast, the fibroblast cultures from these mice did not show any alteration of aconitase activity. In the digitonin treated fibroblast the resting state of respiration was elevated, while active state respiration was not affected. Our findings demonstrate strong tissue specificity effect of MnSOD knockout.

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5P.14 Clear-up of Redox state under hypoxia

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Hypoxic adaptations participate in numerous physiological (i.e. strenuous exercise) and pathological situations (i.e. ischemia and tumor development). The mitochondria, as the oxygen sensor, serve as a primary regulatory element within this process. By-products of mitochondrial metabolism, free radicals or reactive oxygen and nitrogen species (ROS/RNS), participate, in balance with antioxidant shield, in redox status of the cell. Redox signaling was shown to be a principal regulator of metabolic responses to low oxygen, mainly through HIF1-mediated reprogramming of gene expression. We have attempted here to clarify the reported controversies in ROS/RNS production during various stages of hypoxic adaptations. We have found that the amount of ROS/RNS production differs in the course of hypoxic adaptation and reflects involvement of mitochondrial metabolism. Moreover, we have located the production sites of ROS/RNS and characterized also selected scavenging system. Finally, we have correlated the redox status with mitochondrial metabolism and morphology.

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5P.15 Do semiquinones formed by mitochondrial complex I contribute to reactive oxygen species production?

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Mitochondria have been identified as a major source of the oxidative and nitrosative stresses that can compromise cellular homeostasis. Mutations in several mitochondrial enzymes are now recognised as the cause of disease states, and severe mitochondrial dysfunction and elevated radical production are implicated in neurodegenerative pathologies including Alzheimer's and Parkinson's. Therefore, it is important to characterise the sites and