have shown that superoxide formation at the ubiquinol oxidation center of membrane-bound or purified cytochrome bc_1 complex is stimulated by the presence of oxidized ubiquinone when the ubiquinol reduction center (Q_i site) is blocked [1]. This indicated that the electron is transferred onto oxygen from reduced cytochrome b_L *via* ubiquinone in a reverse reaction rather than during the forward Q-cycle reaction. In intact rat heart mitochondria respiring on succinate, inhibitors (malonate, diazoxide, TTFA, and atpenin A5) of the succinate:ubiquinone oxidoreductase (complex II) stimulated mitochondrial ROS production at the Q_0 site of complex III under conditions of oxidant-induced reduction; this stimulation was greatly enhanced by uncoupling [2]. We conclude that cytochrome bc_1 complex linked ROS production is promoted by a partially oxidized rather than by a fully reduced ubiquinone pool. This mechanism of ROS production by complex III offers a straightforward rationale of how the redox state of the ubiquinone pool could play a central role in mitochondrial redox signaling.

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5P.6 Role of conformational changes in mitochondrial complex I in the hypoxic response

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Mitochondrial complex I plays a critical role in regulating cellular energy generation and the production of reactive oxygen species (ROS). Two catalytically and structurally distinct forms of mitochondrial complex I have been characterised in enzyme preparations *in vitro*: one is a fully catalytically competent, active (A)-form and the other is a dormant, silent or de-activated (D)-form. When deprived of substrate at physiological temperatures the idle enzyme undergoes conversion into the D-form. This can gradually convert back to the A-form in the presence of substrate (NADH and ubiquinone) during slow turnover(s) of the enzyme. In the D-form of complex I a critical cysteine-39 of the ND3 subunit becomes exposed to the outside of the enzyme and is susceptible to modification and inhibition by peroxynitrite, nitrosothiols or ROS. This cysteine group is not accessible to any covalent modification in the A-form. Using a cultured cell line we have shown that A-to-D transition occurred during anaerobic incubation, when the respiratory chain was reduced. Accumulation of the D-form of complex I may be protective because slow re-activation of the D-form may reduce the burst of damaging ROS that occurs after reoxygenation. We also demonstrated that re-activation of the D-form could be prevented by prolonged incubation with endogenously-generated nitric oxide (NO). It is possible, therefore, that in some circumstances NO-dependent formation of S-nitrosothiols or peroxynitrite may lead to modification of complex I when it is in its D-form and so impede its return to the active state. Indeed, accumulation of the covalently modified D-form is likely to be responsible for the so-called persistent inhibition of cellular respiration that occurs in the presence of NO. The detrimental effect of such irreversible locking of complex I in the D-form could be due to the fact that the modified D-form of the enzyme generates ROS at a higher rate than the A-form. Thus a combination of changes in mitochondrial ROS production, a change in NAD/NADH ratio and a decline in the rate of oxidative phosphorylation could lead to cellular death and might be responsible for ischaemic damage as well as for the early stages of neurodegeneration.

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5P.7 The mechanism of metformin action in 3T3-L1 adipocytes

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Metformin (dimethylbiguanidine) is the most commonly used drug for the treatment of type 2 non-insulin dependent diabetes mellitus. The beneficial effects of the drug include a decrease in blood glucose, without stimulating insulin secretion and a general improvement in peripheral insulin sensitivity. Additionally, treatment of type 2 diabetes with metformin is associated with an overall reduction in circulating lipids and body weight both leading to a lower cardiovascular risk. Metformin action involves AMPK activation although the metabolic consequences will vary in the different target tissues. In white adipose tissue metformin-induced AMPK activation stimulates catabolic pathways that results in the reduction of triglyceride stores as reflected by the smaller size of the adipocytes. Despite fifty years of research, the early steps of metformin action are far from being elucidated. It is known that biguanidine derivatives inhibit mitochondrial respiration and this effect is probably at the basis of their antidiabetic action. There are data suggesting an indirect effect of metformin and, thus, the existence of a cell-signalling pathway targeted to the respiratory chain has been proposed. The inhibition of respiration would be responsible of two important events that would signal or initiate a shift in the cellular metabolism: (1) a decrease in the ATP levels that could lead to AMPK activation and (2) an increase in the production of superoxide by the respiratory chain with the concomitant raise in the levels of reactive oxygen and nitrogen species. We have investigated the early effects of metformin on 3T3-L1 adipocytes. We have observed that metformin rapidly inhibits cellular respiration that leads to an increase in reactive oxygen species levels and to AMPK activation. UCP2 levels raise as part of the antioxidant response of the cell, since the presence of a superoxide scavenger blocks its induction. UCP2 mRNA levels are unchanged and therefore the raise in protein levels must reflect an increased mRNA translation. AMPK activation is rapid, is associated with the expected decrease in fatty acid synthesis and does not require either the induction of UCP2 or a drop in ATP levels. Interestingly, metformin inhibits pyruvate oxidation but does not prevent the oxidation of added fatty acids.

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5P.8 Mitochondria-targeted antioxidants prevented ischemic injury of kidney

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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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