conditions. These results demonstrate the presence of iPLA2 in lung mitochondria and support the hypothesis that the activation of mitochondrial phospholipases by mild oxidative stress can provide free fatty acids as cycling substrates for UCP2. However, attenuation of ROS production by UCP2 is not significant.

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S10.21 Quinones inhibit the mitochondrial permeability transition pore at two sites

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We have studied the quinone structural features that confer modulatory properties on the mitochondrial permeability transition pore (PTP). Reduced derivatives of ubiquinone (Ub) 0 with acetoxy or methoxy substitutions of the carbonyl groups became ineffective at PTP inhibition. Consistent with a key role of the Ub0 oxidation-reduction state for its interactions with the PTP, DTT prevented the inhibitory effects on the pore when added before but not after Ub0. Of note, the addition of DTT after Ub0 prevented the toxic effects of Ub0 on respiration. The combination of Ub0 and DTT thus allowed inhibition of the PTP without mitochondrial toxicity, which in the absence of DTT reveals itself with a bell-shaped curve where the Ub0-dependent increase of mitochondrial Ca^{2+} retention capacity (a measure of PTP inhibition) is superceded by a decrease as the Ub0 concentration is raised above about 50 μM. The PTP inhibitory effects of decylUb were instead unaffected by reducing agents, and Ub0 and decylUb displayed additive effects on PTP inhibition, indicating that they act at different inhibitory sites on the PTP.

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S10.22 Novel mitochondria-targeted antioxidants are capable to defense cells from the oxidative damage

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Synthesized in the Skulachev's laboratory new type of mitochondria-targeted antioxidants — plastoquinone derivatives (SkQs) were investigated. The aim of our study was to estimate of antioxidant properties of the SkQ family members plastoquinonyl decyltriphenylphosphonium (SkQ1) and plastoquinonyl decylrhodamin 19 (SkQR1), a fluorescent derivative. These compounds combined with a positively-charged penetrating cations were selectively targeted to mitochondria, being accumulated inside, and can be reduced by the respiratory chain. Using confocal microscope we observed that SkQR1 was accumulated by mitochondria. Uncoupling action of FCCP, leading to mitochondrial depolarization, prevented the SkQR1 staining. In human cell cultures HeLa and K562 very low concentrations (nanomolar) of antioxidants were found to prevent ROS-induced apoptosis and necrosis. Oxidative stress initiated by addition of small amount H_2O_2 to living cells, caused secondary generation of endogenous ROS and lowered the level of reduced glutathione. SkQ1 and SkQR1 prevented oxidative damage as well as glutathione oxidation, C1/2 being around 2nM and 0.5 nM respectively. It is concluded that cationic plastoquinone derivatives are rechargeable, mitochondria-targeted antioxidants of very high efficiency.

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S10.23 Variegate porphyria induces higher H_2O_2 production in stimulated lymphocytes due to an impaired respiratory function

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Our aim was to analyse the effects of VP on the production of ROS by lymphocytes and determine the possible mitochondrial sources of these ROS. Twelve women affected by VP and twelve control healthy women participated in the study. H_2O_2 production was measured using 2,7-dichlorofluorescin-diacetate as indicator in basal conditions and after stimulation with PMA. In addition three treatments with allopurinol, rotenone or myxothiazol were performed in PMA-stimulated lymphocytes. No differences were observed between porphyric and control women in the basal production of H_2O_2. The stimulation with PMA increased H_2O_2 production in both groups but lymphocytes from porphyric women produced higher levels of H_2O_2 than controls. The treatments with allopurinol and rotenone did not modify H_2O_2 production but after treatment with myxothiazol H_2O_2 production decreased back to basal levels in both groups. In conclusion, lymphocytes from women affected by VP produce the same amount of H_2O_2 in basal conditions than control women, but after stimulation lymphocytes from porphyric women produce higher levels of H_2O_2. This increased H_2O_2 production is due to an impaired function of the mitochondrial respiratory chain rather than to other ROS sources such as xanthine oxidase.

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S10.24 Involvement of p38 in camptothecin induced expression of UcP2 in rat neonatal cardiomyocytes

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Camptothecin (CPT), a topoisomerase I inhibitor, is used for treatment of certain types of malignancies. Uncoupling protein 2 (UcP2) is proposed to protect cardiomyocytes against oxidative stress. Modulation of UcP2 level appears important because the protein increases in failing human heart. $p38^{MAPK}$ belongs to a group of protein serine/threonine kinases that become activated in response to extracellular stimuli and mediate signal transduction in cell growth, differentiation and apoptosis. We found that CPT treatment induces UcP2 expression on both mRNA and protein level in cardiomyocytes. This induction is accompanied by short-term increase in production of ROS (<1 h) preceded by activation of $p38^{MAPK}$ (<30 min). Activation of $p38^{MAPK}$ by CPT was comparable to anisomycin, a protein synthesis inhibitor that activates stress-related MAPKs, namely $p38^{MAPK}$ in mammalian cells. Pretreatment of cardiomyocytes with $p38^{MAPK}$ inhibitor, SB203580, blocked activation of $p38^{MAPK}$ by both compounds and abolished the camptothecin-mediated UcP2 induction. Our results

suggest that activation of $p38^{MAPK}$-regulated signaling cascade leads to increased expression of UcP2 in cardiomyocytes as seen on protein as well as mRNA level offering a protective mechanism against CPT-related apoptosis.

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S10.25 Effect of targeted quinones on ROS production and lipid peroxidation in mitochondria: Mitochondrial DNA polymerase mutant mice exibit high sensitivity

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Oxidative damage of mitochondrial compartments contribute to a range of degenerative diseases. Here protective effect of quinones and their derivatives on mitochondrial proteins and lipids under oxidative stress was analysed. The most effective quinone analogues appear to be conjugates of lipophilic cations ("Skulachev cations", by D. Green) with ubiquinone (Murphy et al., 2007) or plastoquinone (Skulachev et al., 2008, in press), so called "mitochondrially targeted antioxidants" (MTAO). MTAO effectively inhibit lipid peroxidation and protect membrane cardiolipin from oxidation. Using dihydroethidium and Amplex Red as a probes for ROS detection, we found that MTAO effectively suppress ROS production from different sites of the mitochondrial respiratory chain. In succinate-energised mitochondria there is a lag-period for the MTAO effect, but not in pyruvate/malate-energised mitochondria. When mitochondria isolated from the heart of a mouse strain mutated on mitochondrial DNA polymerase PolgA (Trifunovic et al., 2004) were used, we found that the mitochondria of PolgA mice did not produce more ROS than wild type, but the inhibitory effect of MTAO was stronger and more pronounced. Taken together, these data provided evidence that MTAO could be useful for treatment of genetic disorders, manifested degenerative diseases and aging complications.

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S10.26 Nerolidol disturbs mitochondrial bioenergetics but delays the permeability transition pore due a membrane antioxidant protective effect

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Nerolidol is a naturally occurring sesquiterpenol found in the essential oils of many types of plants and flowers. The aim of this study was to assess the impact of a racemic mixture of nerolidol on the oxidative phosphorylation of mitochondria isolated from rat liver. We examined the effect of nerolidol on respiratory indexes, membrane potential and opening of the mitochondrial permeability transition pore, all the assays were performed with 0.5 mg mitochondrial protein/mL. Our results showed a complex array of effects on liver mitochondrial bioenergetics. With nerolidol (1.2 μM) we observed an increase in state 4 respiration rate (170±5.4% of control), a depression in state 3 (64±9.7% of control) and a decreased uncoupled respiration rate (53±10,1% of control). Mitochondrial membrane potential was decreased (1.2 μM; 27±6.4% of control) by nerolidol in a concentration manner. ATP synthase was not significantly affected in the concentration range of study (0–1.2 μM). Nerolidol seems to increase the mitochondrial ability to accumulate calcium by decreasing the susceptibility of mitochondria to the opening of the transition pore. Nerolidol (0.2 μM) protect against *tert*-buthylhydroperoxide mitochondrial membrane lipid peroxidation, however H_2O_2 produced by mitochondria with blocked respiratory complexes was increased by nerolidol. From our data it is concluded that concentrations of nerolidol lower than 0.4 μM don't affect mitochondrial bioenergetics and could probably used to prevent the deleterious effect of some oxidative events occurring in mitochondria.

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S10.27 Deletion of mitochondrial uncoupling protein 2 gene enhances ischemic brain damage by suppressing cell cycle gene and anti-oxidative gene

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Mitochondrial uncoupling proteins (UCPs) are inner mitochondrial membrane proteins that dissipate the mitochondrial proton gradient by transporting H^+ across the inner membrane, thereby stabilizing the inner mitochondrial membrane potential ($\Delta\Psi_m$) and reducing the formation of ROS (Nicholls 1977; Stuart et al., 2001). Previous studies have shown that UCP2 protects neurons against oxidative stress and cerebral stroke (Bechmann et al., 2002; Mattiasson et al., 2003). However, a recent study reported opposite effects of UCP2 by showing that ablation of UCP2 reduced stroke infarct area in the brain (de Bilbao et al., 2004). The objectives of this study are to clarify the effects of UCP2 on ischemic brain damage and to explore whether deletion of UCP2 gene alters expression profile of other genes after transient cerebral ischemia. Middle cerebral artery occlusion (MCAO) of 1 h duration was induced in UCP2 knockout (UCP-KO) and wild type mice. Animals were sacrificed 24 h after reperfusion. The infarct volume was depicted using the TTC staining. The integrity of the circle of Willis of UCP2-KO and wild type mice was examined by carbon black injection. Transcript levels of 84 genes in the cortical ischemic penumbra area were detected using a Mouse Stress Toxicity PCR array (Super Array). The results were normalized against housekeeping genes Hprt1 and beta actin. The results showed that deletion of UCP2 gene significantly increased infarct size and there was no obvious vascular abnormalities observed. The SuperArray study demonstrated that knocking out UCP2 gene significantly suppressed DNA repair gene cyclin G1 (van Lookeren Campagne and Gill 1998), antioxidative gene GSTM1 (McBride et al., 2005) and neuroprotective gene MDM2 (Saito et al., 2005). These results were further verified by immunohistochemistry. It is concluded that knocking out of UCP2 gene exacerbates neuronal death after cerebral ischemia and reperfusion and that deletion of UCP2 gene suppresses genes associated with cell survival.

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