The possibility that 'mild' uncoupling could be protective against oxidative damage by diminishing ROS (reactive oxygen species) production has attracted much interest. It is indeed well established that ROS production from the complex-II-linked substrate succinate under reverse electron-flow conditions is sensitive to membrane potential fluctuations. However, not even other complex-II-linked substrates (glycerol-3-phosphate or acyl-CoAs) do generally show such sensitivity, and complex-I-linked substrates do not display membrane potential sensitivity at all. Even concerning succinate-supported ROS production, it only occurs during the rather unphysiological reverse electron-flow conditions and with succinate concentrations that are supraphysiological.

It is sometimes implied that there is a kind of "grace space" where a decrease in membrane potential could diminish ROS production without affecting respiration. However, according to the Mitchellian respiratory control concepts, any decrease in membrane potential, even 'mild uncoupling', must necessarily lead to large increases in respiration, i.e. it must be markedly thermogenic and would thus be costly to the organism.

It has been suggested that members of the uncoupling protein family (UCP1, UCP2 and UCP3) may mediate a mild uncoupling. However, present evidence does not unequivocally support such an effect. For instance: the absence of the truly uncoupling protein UCP1 is not associated with increased oxidative damage.

In a broader cellular context, it must also be counted in that mitochondria within cells are normally ATP-producing and thus already have a diminished membrane potential. Correspondingly, treatment of cells, organs or animals with small amounts of artificial uncoupler does not seem to have beneficial effects that are explainable via reduced ROS production. Thus present evidence does not support mild uncoupling as a physiologically relevant alleviator of oxidative damage.

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13P1

Identification of generator-specific biomarkers and targets of mitochondrial ROS

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The mitochondrial respiratory chain is a major contributor to cellular reactive oxygen species (ROS) production. Within the respiratory chain complexes I (NADH:ubiquinone oxidoreductase) and III (cytochrome bc_1 complex) are considered as the main ROS generators. Besides a well established role in various pathophysiological processes and neurodegenerative diseases, increasing evidence suggests mitochondrial ROS as part of cellular signal transduction. The directed release of ROS to the mitochondrial intermembrane space by complex III and to the matrix space by complex I may be responsible for their remarkable dual role as 'signaling' and 'damaging' agents. In this study we investigated this possible bipartite role of the mitochondrial ROS generators by RedoxDIGE analysis. We addressed the question of whether a putative dual role of mitochondrial ROS is reflected in generator-specific protein thiol oxidation. Hence we selectively induced ROS generation at complexes I and III by supplying intact and highly coupled rat heart mitochondria with different substrates and inhibitors. Further we distinguished between complex I ROS generated during forward mode or reverse electron transfer. The use of an internal standard allowed direct comparison between the different samples and biological variance analysis. Remarkably, complexes I and III derived ROS oxidized a very distinct subset of thiol targets in a highly generator-specific manner. Furthermore, all identified target proteins of the intermembrane space were only oxidized by complex III ROS supporting the view that complex III releases superoxide mainly into the intermembrane space while complex I ROS are released completely into the matrix space.

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13P2

Brain age-dependent effects of acute endotoxemia on oxidative damage and mitochondrial function

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Endotoxemia is a systemic inflammatory state secondary to the activation of the innate immune system. Its pathogenic mechanism is still incompletely understood. Impaired mitochondrial function and increased oxidative damage have been proposed as responsible for the septic final state: multi-organ failure. The incidence of endotoxemia and sepsis is increased in elderly adults, and age is an independent predictor of mortality. The mitochondrial theory of aging hypothesizes that mitochondria are the pacemakers of tissue aging due to the continuous production of oxygen and nitrogen free radicals and related reactive species. Endotoxemia and aging are situations characterized by an increased damage to cellular structures due to increased or cumulative production of reactive oxygen species. Thus, the aim of the present work was to study the occurrence of oxidative stress and mitochondrial contribution to the response in an acute model of endotoxemia, analyzing the effect in animals grouped by age (young adults: 3 mo.; old adults: 12 mo.). O_2 utilization was assessed by measuring O_2 consumption in whole tissue and mitochondria. Whole tissue O_2 consumption was found invariable in young animals (control: $3.88 \pm 0.42 \cdot 10^3$ nmol O_2/min·g tissue), being the non-mitochondrial O_2 consumption increased by 45% ($p<0.01$) in endotoxemic young mice. The percentage of O_2 used by the mitochondria (KCN sensitive) was decreased by 17% ($p<0.01$) in LPS-treated animals. Furthermore, complex I activity was found 10% decreased in LPS-treated young animals ($p<0.05$) and the same effect was observed due to the aging process itself. Organ chemiluminescence (CL) was measured as a marker of steady-state concentration of oxidants and occurrence of oxidative stress. Age was found to be a source of variation, old control animals present a two-fold increase in the CL value (31.0 ± 2 cps/cm^2; $p<0.0001$). LPS treatment produced a 65% increase in CL only in young animals (control value: 15.0 ± 2 cps/cm^2; $p<0.001$). Stable oxidized products, measured as TBARS in brain tissue homogenates, were also found increased by 66% in young animals due to the treatment with LPS (control: 0.210 ± 0.03 nmol MDA/mg protein). These results suggest that endotoxemia induces changes in the cellular utilization of oxygen, generating increased production of oxidative species and decreasing mitochondrial function. The young mice group demonstrated to be more sensitive to the endotoxemic challenge than the old mice group.

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