levels. The uncoupling protein UCP2 is found in many animal tissues and organs and its expression levels are often increased in pathological processes in which there is oxidative stress (lipotoxicity, atherosclerosis, chronic inflammation, etc.). Tumour cells have a high intrinsic level of oxidative stress and, in these cells, UCP2 can also play a protective role that has made this protein a target for cancer treatment [4]. Thus, it has been shown that in colon cancer UCP2 expression is increased and that this induction appears linked to NF-κB activation and oxidative stress. Increased UCP2 levels have also been associated with resistance to chemo- and radiotherapy in sublines of leukemia and melanoma. Additionally, it has been shown that overexpression of UCP2 in tumour cells reduces ROS levels and apoptosis when treated with antitumour drugs such as doxorubicin or camptothecin. We present data showing that inhibition of UCP2 in tumour cells causes oxidative stress and that the inhibition acts synergistically with chemotherapeutic agents to reduce cell viability.

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10L.4 Mitochondrial uncoupling proteins in unicellular eukaryotes

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Uncoupling proteins (UCPs) are members of the mitochondrial anion carrier protein family that are present in the mitochondrial inner membrane and mediate free fatty acid (FFA)-activated, purine nucleotide (PN)-inhibited proton conductance. Since 1999, the presence of UCPs has been demonstrated in some non-photosynthesising unicellular eukaryotes, including amoeboid and parasite protists, as well as in non-fermentative yeast and filamentous fungi. In the mitochondria of these organisms, UCP activity is revealed upon FFA-induced, PN-inhibited stimulation of resting respiration and a decrease in membrane potential, which are accompanied by a decrease in membranous ubiquinone (Q) reduction level. UCPs in unicellular eukaryotes are able to divert energy from oxidative phosphorylation and thus compete for a proton electrochemical gradient with ATP synthase. Our recent work indicates that membranous Q is a metabolic sensor that might utilise its redox state to release the PN-inhibition of UCP-mediated mitochondrial uncoupling under conditions of phosphorylation and resting respiration. As this regulatory feature was demonstrated for microorganism UCPs (*A. castellanii* UCP), plant and mammalian UCP1 analogues, and UCP1 in brown adipose tissue, the process could involve all UCPs. We discuss the functional connection and physiological role of UCP and alternative oxidase, two main energy-dissipating systems in the plant-type mitochondrial respiratory chain of unicellular eukaryotes, including the control of cellular energy balance as well as preventive action against the production of reactive oxygen species.

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10L.5 UCP1 ablation increases the production of reactive oxygen species by mitochondria isolated from brown adipose tissue

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We provide evidence that ablation of uncoupling protein 1 increases the rate of reactive oxygen containing species production (as detected by Amplex Red) by mitochondria from brown adipose tissue, no matter what electron transport chain substrate is used (succinate plus rotenone (about 1.5-fold, $p = 0.033$), glycerol-3-phosphate (2-fold, $p = 0.030$), pyruvate plus malate (2-fold, $p = 0.044$). The substrate glycerol-3-phosphate resulted in the greatest amount of reactive oxygen containing species production. Consistent with these data are our observations that (a) the mitochondrial membrane potential is maximal when is uncoupling protein 1 is ablated and (b) oxygen consumption rates in mitochondria from uncoupling protein 1 knock-out mice, are significantly lower than those from wild-type mice. In summary, we show that uncoupling protein 1 can affect reactive oxygen containing species production by isolated mitochondria from brown adipose tissue.

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Posters

10P.1 Absence of mitochondrial uncoupling protein 1 affects apoptosis and T-cell profile in mice

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The thymus is a primary lymphoid organ the progenitor cells of which develop into mature thymocytes. Following antigen selection processes these CD4/CD8 double positive cells develop into naïve single positive (CD4 or CD8) T cells which migrate to the peripheral lymphoid tissue, such as spleen and lymph nodes. This maturation and selection process results in the apoptosis of 90% of thymocytes. UCP1 has been shown to be present in mouse thymocyte but not spleenocyte mitochondria. Flow cytometric analysis of cell profiles in thymus and spleens show that there are (i) more immature double positive thymocytes in thymus [10% increase, $p = 0.04$] and spleen [2-fold increase, $p = 0.02$] and (ii) less single positive CD8 (mature) thymocytes in the thymus [2-fold decrease, $p = 0.002$] and spleen [2-fold decrease, $p = 0.01$] of $UCP1^{-/-}$ mice compared to wild-type C57Bl/6 mice [x-fold, p value]. In an endeavour to explain these cell profile differences, we were able to show that spontaneous apoptosis is less prevalent in thymocytes isolated from $UCP1^{-/-}$ mice compared to those isolated from C57Bl/6 mice [20% decrease, $p = 0.006$], indicating a role for UCP1 in T-cell selection in the thymus.

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10P.2 Characterization of the sensitivity of 4-hydroxynonenal-activated proton conductance to GDP and carboxyatractylate in skeletal-muscle and heart mitochondria

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The lipid peroxidation product 4-hydroxynonenal (HNE) is an important mediator of free radical damage [1]. HNE specifically induces uncoupling of mitochondria through the uncoupling proteins (UCPs) and the adenine nucleotide translocase (ANT) although the relative contribution of the two carriers to these effects is unclear [2,3]. To clarify this we studied the sensitivity of HNE-activated proton conductance to GDP (UCPs inhibitor) and carboxyatractylate (ANT inhibitor) in skeletal-muscle and heart mitochondria from mice expressing different amounts of UCP3. Mitochondria were isolated from wild-type and *Ucp3* knockout mice. To increase UCP3 expression, some mice were i.p. injected with LPS (12 mg/kg body weight). HNE activated proton conductance in skeletal-muscle and heart mitochondria. In skeletal muscle, this increase correlated with UCP3 expression levels: it was lower in *Ucp3* knockout mice and higher in LPS-treated wild-type mice. GDP partially abolished HNE effects whereas carboxyatractylate or addition of both inhibitors completely abolished it. In contrast, GDP had no effect on HNE-induced proton conductance in heart mitochondria, but carboxyatractylate or administration of both inhibitors had a partial effect. In skeletal muscle mitochondria, GDP-mediated inhibition of HNE-activated proton conductance was specific for UCP3 since it was not observed in *Ucp3* knockout mice. Carboxyatractylate was able to inhibit UCP3 through an unknown mechanism. We conclude that, in skeletal muscle, HNE-induced increase in proton conductance is mediated by UCP3 (30%) and ANT, whereas in the heart the increase is mediated by ANT and other carriers, possibly including UCP3.

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10P.3 Regulation of H_2O_2 generation by uncoupling protein 1 in thymus mitochondria

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It has been proposed that uncoupling proteins can attenuate mitochondrial production of free radicals and therefore protect against oxidative damage, degenerative diseases and aging. Recently it has been established that uncoupling protein 1 (UCP1) is located in the thymus. We tested a hypothesis that UCP1 can regulate ROS production in rat mitochondria from thymus using an Amplex red/H_2O_2 assay. Our data show that inhibition of UCP1 by GDP caused an increase in ROS production by non-phosphorylating thymus mitochondria respiring on a) succinate and rotenone (1.5 fold increase) and b) glycerol-3-phosphate and rotenone (1.2 fold increase). In parallel with H_2O_2 production measurements, the effect of GDP on membrane potential was monitored by uptake of the fluorescent probe safranine, while the inhibitory effect of GDP on oxygen consumption was measured using an oxygraph respirometer. The observed increase in ROS production upon GDP addition was accompanied by a relative decrease in oxygen consumption. We are currently performing equivalent experiments using thymus mitochondria isolated from *UCP1*$^{-/-}$ mice and their control littermates, to establish that our observations are indeed due to UCP1 activity in the thymus.

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10P.4 Are the novel uncoupling proteins acutely regulated by fatty acids and nucleotides?

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In brown adipose tissue (BAT) mitochondria, uncoupling protein 1 (UCP1) dissipates the protonmotive force to generate heat. UCP1 is activated by fatty acids and inhibited by nucleotides such as GDP, but the precise mechanisms involved remain controversial. Even less is known about the physiological role and regulation of the novel uncoupling proteins, UCP2 and UCP3. Here we present the first demonstration of a conformational change induced by fatty acids for both UCP1 and UCP3 in rat BAT mitochondria. Conformational changes were inferred from the kinetics of proteolysis when isolated mitochondria were treated with exogenous trypsin. Palmitate increased the rate of proteolysis for both proteins, showing that palmitate binds and affects their conformation. Trypsinolysis of UCP1 could be fully rescued by GDP, consistent with its ability to compete functionally with fatty acids. UCP3 degradation, however, was GDP-independent, suggesting that GDP interacts differently (or not at all) with UCP3. Experiments to determine the acute regulation of UCP2 as diagnosed by trypsinolysis are currently being conducted.

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10P.5 *In vivo* gene silencing of uncoupling protein-2 in kidney cortex of diabetic rats results in increased uncoupling, decreased oxidative stress and reduced membrane potential Implications for the adenine nucleotide transporter

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Mitochondria uncoupling *via* uncoupling protein (UCP) 2 may be an important mechanism to reduce oxidative stress and preserve mitochondria function in the diabetic kidney. Short interference (si) RNA to knockdown UCP2 or a non-functional scrambled equivalent (100 μg/rat) was administered to healthy and diabetic (streptozotocin; 60 mg/kg b.w.) Sprague–Dawley rats and the mitochondria isolated 48 h thereafter. Glutamate-stimulated QO_2 in the presence of ATP-synthase inhibitor oligomycin was used to estimate mitochondria uncoupling since proton release across over the inner membrane with subsequent increase in QO_2 will not occur in coupled mitochondria. Diabetes increased UCP2 (192 ± 34% of control corrected for β-actin) and siRNA decreased UCP2 (control + siRNA 70 ± 11 and diabetes + siRNA 88 ± 8% of control). Glutamate-stimulated QO_2 was significantly higher in control + siRNA, untreated diabetics and diabetes + siRNA compared to control (1.6 ± 0.5, 1.5 ± 0.5 and 3.8 ± 0.5 vs. 0.0 ± 0.1 nmol O_2/s/mg protein). The UCP2 inhibitor guanosine diphosphate (GDP) inhibited QO_2 in diabetics (0.9 ± 0.5 vs. 1.6 ± 0.5 nmol O_2/s/mg protein) but not in diabetes + siRNA (4.6 ± 0.6 vs. 3.8 ± 0.5 nmol O_2/s/mg protein). ADP in the presence of oligomycin reduced QO_2 in diabetes + siRNA (2.0 ± 0.5 vs. 3.8 ± 0.5 nmol O_2/s/mg protein) but had no effect in any of the other