10P.9 Examination of UCPs involvement in fatty acid-dependent superoxide-activated uncoupling

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Uncoupling proteins (UCPs) mediate proton conductance induced by fatty acid, stimulated by superoxide and inhibited by GDP. At the same time, mitochondrial adenine nucleotide translocase (ANT) also is able to transport fatty acid anions, and uncouple oxidation and phosphorylation. In present work an attempt was made to reveal protonophore activity of UCP2 and UCP3, using inhibitory effect of GDP (generally considered specific for UCPs) and carboxyatractylate (cAtr), considered specific for ANT. Skeletal muscle mitochondria of Yakutian hibernating ground squirrels were chosen basing on significant level of *UCP3* mRNA. Rat kidney and liver mitochondria were chosen as objects, since literature data indicate the activation of UCP2 by superoxide in kidney unlikely to liver. We found that GDP in millimolar concentrations had a slight recoupling effect on respiration rate and membrane potential in skeletal muscle mitochondria of hibernating animals. GDP had no effect, if cAtr in micromolar concentration was added previously. Moreover, GDP and ADP demonstrated competitive kinetic relative to ANT. Evaluated parameters of kinetics revealed affinity to ANT decreasing in order: ADP>GDP>UDP>CDP. Skeletal muscle mitochondria failed to show chloride permeability unlikely to brown fat mitochondria. In brown fat cAtr was not able to prevent UCP1-iduced chloride permeability, and inhibitory effect of GDP. Superoxide increased the initial respiration rate, decreased the maximal respiration velocity and membrane potential in kidney. Slow decrease of respiration rate and restoration of membrane potential were observed after addition of ADP or GDP, being intensified upon subsequent treatment by cAtr. However, addition of ADP or GDP after cAtr had no effect on recoupling of respiration, even in the presence of superoxide. No crucial differences were found in kidney and liver mitochondria. Evaluation of the mRNA level for *UCP2* and *ANT* genes indicates that the expression of *UCP2* mRNA in kidney was even lower than in liver mitochondria. The stronger effect of ADP in kidney apparently is correlated with a higher level of ANT. The data obtained allow us to conclude that recoupling effect of purine nucleotides both in the presence and in the absence of superoxide in the mitochondria of rat liver and kidneys, and skeletal muscle mitochondria of hibernating ground squirrels may be explained by their interaction with ANT rather than by functioning of UCP2 and UCP3.

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10P.10 Uncoupling protein 3 (UCP3) participates in the translocation of lipid hydroperoxides (LOOH) across the mitochondrial inner membrane and in the LOOH-dependent mitochondrial uncoupling

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Despite many hypotheses on the function of uncoupling protein 3 (UCP_3) (thermogenesis, attenuation of ROS production, mitochondrial calcium uptake, export of free-fatty acid/lipid hydroperoxides (LOOH)/ pyruvate) its role as well as its uncoupling activity is still debated. It has been hypothesized that UCP3 may mediate LOOH translocation across the mitochondrial inner membrane (MIM) thus protecting mitochondria from these very aggressive molecules (Goglia & Skulachev, 2003, *FASEB J.* **17:** 1585-1591). Evidence supporting this possibility comes from experiments on UCP-2 reconstituted liposomes, showing that UCP2 could catalyse the extrusion of LOOHs and their ability to re-entry by flip-flop. However data supporting the previous hypothesis on UCP-3 function, obtained in a more physiological system are still lacking. In this study, by using mitochondria isolated from $UCP_3^{+/+}$ and $UCP_3^{-/-}$ mice, we investigated on the role of UCP_3 in (1) LOOH export across the MIM and (2) LOOH-induced mitochondrial uncoupling. To address point 1, we tested the ability of $UCP_3^{+/+}$ and $UCP_3^{-/-}$ mitochondria to release LOOH when either high or low levels of this compound were formed at the matrix side of MIM. In the presence of arachidonic acid (AA) and high O_2^- release at the matrix side, $UCP_3^{+/+}$ mitochondria (energized with succinate without rotenone) released significantly more LOOH respect to $UCP_3^{-/-}$ ones. This difference was abolished both when UCP_3 was inhibited by GDP and in a condition in which there was a reduced LOOH formation on the matrix side of MIM (due to the addition of rotenone). These data support the involvement of UCP3 in the extrusion of LOOH across the MIM. Concerning point 2, we detected the ability of AA to induce proton conductance both when it undergoes the peroxidative process and when it is inhibited. Proton conductance, not differing between succinate energized-$UCP_3^{-/-}$ and $UCP_3^{+/+}$ mitochondria when assessed in the absence of endogenous free fatty acid and rotenone, was enhanced by the addition of AA both in $UCP^{+/+}$ and $UCP_3^{-/-}$ mitochondria, the first showing greater enhancement. The inhibition either of the release of O_2^- in the matrix (by rotenone) or the formation of LOOH (by phenylbutylnitrone) significantly abolished the uncoupling effect of AA in $UCP_3^{+/+}$ mitochondria while being ineffective in $UCP_3^{-/-}$ ones. As a whole, these data demonstrate that UCP_3 participates both in the extrusion of LOOH across the MIM and in the LOOH-mediated uncoupling.

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10P.11 Uncoupling protein 3 is upregulated in mice with impaired glucagon secretion

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Synaptotagmin-7 (Syt7) was recently demonstrated to regulate calcium-dependent insulin and glucagon secretion in mouse pancreas [1, 2]. The knockout of Syt7 reduced glucagon secretion by 80% which consequently resulted in slower recovery from hypoglycaemia [2]. An unexplained phenotype of Syt7 knockout is their lower body weight and body fat content, and this prompted us to investigate whether these mice have an altered energy expenditure. Mitochondria from the skeletal muscle of Syt7 knockout mice had approximately 2 fold higher levels of Uncoupling protein 3 (UCP3), which is widely believed to facilitate fatty acid oxidation. Expressions of other uncoupling proteins, UCP1 in brown adipose tissue and adenine nucleotide translocase (ANT) in liver are unchanged. Basal oxygen consumption (VO_2) based on indirect calorimetry was almost 20% higher in the Syt7 knockout mice compared to their wild type littermates. Respiratory exchange ratio (RER) was significantly lower in the Syt7 knockout mice, suggesting a more efficient use of fat in their energy production than the wild type mice. Mitochondria isolated from the skeletal muscle of Syt7 knockout mice also

displayed increased state 4 respiration accompanied by a decreased membrane potential, a diagnostic of mitochondrial uncoupling. The increased UCP3 expression and activity could be a physiological adaptation to perturbed cellular energy balance, leading to an increase in fatty acid oxidation and hence, the leaner phenotype observed in these Syt7 knockout mice.

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10P.12 Reduced cytotoxic $CD8^+$ lymphocytes in heterozygous UCP2-KO mice in experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) affects over 2.5 million people worldwide and the human uncoupling protein 2 (UCP2) promoter polymorphism −866G/A has been associated with susceptibility to multiple sclerosis [1]. Previous studies have also shown a protective function for UCP2 in Experimental Autoimmune Encephalomyelitis (EAE), a murine model of MS, with higher clinical scores in $UCP2^{-/-}$ mice [2]. We undertook a pilot study using the EAE model with 5 control and 5 $UCP2^{+/-}$ mice. The study showed reduced $CD8^+$ lymphocytes in brain (3.6-fold, $P<0.001$) and spleen (1.2-fold, $P<0.05$) of $UCP2^{+/-}$ mice after 21 days post immunization compared to wild-type controls while the percentage of $CD4^+$ lymphocytes remained unaffected. Our observation is not necessarily consistent with that of Vogler *et al.* (2006) who saw no effect of EAE on peripheral $CD4^+$ to $CD8^+$ ratios in a comparison of $UCP2^{-/-}$ and wild-type mice. Furthermore, $UCP2^{+/-}$ mice showed reduced clinical scores and therefore seemed to be less vulnerable to EAE when compared to wild-type mice. Future work will focus on a larger study comparing various parameters, including T-cell profiling, from *UCP2* deficient and wild-type mice.

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10P.13 UCP2 expression pattern in mouse tissue

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Uncoupling protein 2 (UCP2) is an inner mitochondrial membrane protein, which transports protons in the presence of fatty acids similarly to UCP1 [1]. Although the protein was discovered 1997 its function is still unclear. One reason for this is the obscurity of UCP2 protein expression among different tissues and cell types, caused by a discrepancy between mRNA and protein level [2]. Here, we applied a new designed antibody against UCP2 with proved specificity to the recombinant UCP2. Using this antibody we re-evaluated the protein expression pattern among various tissues of young, mature and adult mice. Additionally we measured the mRNA levels in these tissues and collated them with the protein expression levels. To evaluate the UCP2 expression in nervous system we analysed different brain regions, neuronal cell types and development stages. The obtained results are compared to the expression pattern of the brain specific UCP4 [3] and discussed in view of the hypothesized functions.

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10P.14 Thermogenically competent recruitment of uncoupling protein 1 in brown preadipocytes and in a subset of cell precursors from epididymal white adipose tissue by a PPARγ agonist

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Peroxisome proliferator-activated receptor-γ (PPARγ) activation pathway can competently recruit *brown* adipose tissue (BAT), independently of sympathetic stimulation. Here we studied the thermogenic capacity of brown pre-adipocytes chronically treated with the potent PPARγ agonist rosiglitazone. Mitochondriogenesis, an essential part of BAT recruitment, was significantly enhanced in treated brown adipocytes. Most importantly, these mitochondria were capable of thermogenesis, as rosiglitazone-treated brown adipocytes responded to the addition of norepinephrine with a large increase in oxygen consumption. This thermogenic response was not observable in rosiglitazone-treated brown adipocytes originating from *UCP1*-ablated mice; hence, it was UCP1 dependent. Thus the PPARγ pathway represents an alternative, potent, and fully competent mechanism for BAT recruitment, which may be the cellular explanation for the enigmatic recruitment in prehibernation and prenatal states. We also examined the cell precursors from the purest *white* adipose tissue depot (epididymal), and demonstrate that a similar chronic treatment with the rosiglitazone promotes not only the expression of PGC-1α and mitochondriogenesis in these cells but also *UCP1* gene expression in a significant subset of the cells, providing these cells with a genuine thermogenic capacity. Indeed the addition of norepinephrine to these white adipose tissue-derived cells induced high oxygen consumption. This thermogenic response to norepinephrine was significantly abolished in white adipocytes from *UCP1*-ablated mice, indicating true UCP1-dependent thermogenesis, which previously was considered as being unique brown adipocytes characteristic. However, although functional thermogenic genes are expressed, the cells are devoid of transcripts for the novel transcription factors now associated with classic brown adipocytes (Zic1, Lhx8, Meox2, and characteristically PRDM16) or for myocyte-associated genes (myogenin and myomirs (muscle-specific microRNAs)) and retain white fat characteristics such as Hoxc9 expression. These cells therefore constitute a subset of adipocytes ("brite" adipocytes) with a developmental origin and molecular characteristics distinguishing them as a separate class of cells.

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