

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-induced 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 (UCP₃) (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 UCP₃^{+/+} and UCP₃^{-/-} mice, we investigated on the role of UCP₃ in (1) LOOH export across the MIM and (2) LOOH-induced mitochondrial uncoupling. To address point 1, we tested the ability of UCP₃^{+/+} and UCP₃^{-/-} 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 O₂⁻ release at the matrix side, UCP₃^{+/+} mitochondria (energized with succinate without rotenone) released significantly more LOOH respect to UCP₃^{-/-} ones. This difference was abolished both when UCP₃ 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-UCP₃^{-/-} and UCP₃^{+/+} mitochondria when assessed in the absence of endogenous free fatty acid and rotenone, was enhanced by the addition of AA both in UCP₃^{+/+} and UCP₃^{-/-} mitochondria, the first showing greater enhancement. The inhibition either of the release of O₂⁻ in the matrix (by rotenone) or the formation of LOOH (by phenylbutylnitron) significantly abolished the uncoupling effect of AA in UCP₃^{+/+} mitochondria while being ineffective in UCP₃^{-/-} ones. As a whole, these data demonstrate that UCP₃ 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 (VO₂) 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