10P.15 Identification and characterization of uncoupling protein in fat body and muscle mitochondria isolated from cockroach *Gromphadorhina coquereliana*

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Uncoupling proteins (UCPs) are members of the mitochondrial anion carrier family dissipating the electrochemical proton gradient across the inner mitochondrial membrane. Homologues including closely related UCP1–UCP3 and more diverged UCP4 and UCP5 were found in animals, plants, fungi and protists. The first insect uncoupling protein, dmUCP was identified in *Drosophila melanogaster* and characterized in the heterologous yeast system. The aim of our study was to characterize UCP in mitochondria of cockroach *Grompadorhina coquereliana* isolated from a trophic tissue of fat body (insect analogue of mammalian liver) and skeletal muscle (a high metabolic energy tissue). Bioenergetic studies support evidence for the functioning of UCP in both tissues. UCP activity was subsequently stimulated by addition of micromolar concentrations of fatty acids (palmitic acid and linoleic acid) and inhibited by the purine nucleotide GTP. Cockroach UCP was immunodetected by polyclonal antibodies raised against mouse UCP2, human UCP3, UCP4 and UCP5. All detections showed a single around 36 kDa band. Expression of UCP in cockroach muscle mitochondria was approximately three times higher than that in mitochondria isolated from fat body. LC–MS/MS analysis revealed peptides with high sequence identity of the purified protein to previously characterized UCP4 in human and *D. melanogaster*. The discovery of UCP protein in cockroach *G. coquereliana* might have implications for understanding the bioenergetics, metabolism and function of fat body and muscle in insects.

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10P.16 Mitochondrial uncoupling protein 4 (UCP4) expression in rat inner ear during development and after oxidative stress induction

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UCP4 belongs to the mitochondrial anion transporter family and is proposed to participate in the regulation of free radical production, calcium homeostasis, thermogenesis and apoptosis in mammalian brain. Using a new designed antibody we have previously shown that UCP4 appears in fetal murine brain tissue as early as on embryonic days 12–14 and suggested that it may be involved in neuronal cell differentiation (Smorodchenko A et al., 2009). Here we proved the hypothesis that UCP4 contributes to the regulation of oxidative stress, using the model from our previous work (Gross J et al., 2007). For this we compared UCP4 expression level in freshly isolated explants of organ of Corti (OC), modiolus (MOD) and stria vascularis (SV) from neonatal rats with explants cultured under normoxic or hypoxic conditions. Western Blot analysis showed that protein level is decreased in MOD both at normoxic and hypoxic conditions but was not changed in OC and spiral limbus. UCP4 down-regulation coincided with the decrease of VDAC content and possibly reflected the mitochondrial damage during oxidative stress. Comparing further the UCP4 level at different postnatal stages we found that protein was developmentally regulated and its expression was region-specific. We hypothesized that UCP4 may play an important role in functional maturation of rat inner ear.

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10P.17 Involving of 4-hydroxy-2-nonenal in the activation of UCP1

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Uncoupling proteins (UCPs) is a subfamily in a family of mitochondrial membrane anion carriers. One of the most controversially discussed UCP function is their ability to regulate mitochondrial ROS production and/or to be regulated by ROS or ROS derivatives. Here, we investigated whether the aldehydic product of lipid peroxidation 4-hydroxy-2-nonenal (HNE) activates UCP1. For that we used a well-defined model of lipid membranes reconstituted with recombinant UCP1 and arachidonic acid, AA [1, 2]. The comparison of protein-free and protein-containing membranes in the presence of HNE demonstrated no direct activating effect of aldehyde on UCP1. However, the addition of HNE led to the strong potentiation of the AA-mediated increase of the membrane conductance, Gm, both in UCP-containing and in UCP-free membranes. The application of high potentials [3] additionally stimulated the Gm increase. Alteration of lipid membrane properties due to addition of HNE is proposed to be responsible for the observed protein-independent HNE activity.

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