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S3.P6

Uncoupling protein 1 binds one nucleotide per monomer and is stabilised by tightly bound cardiolipin

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Brown adipose tissue oxidises fatty acids to produce heat for thermoregulation in the cold and has been identified in adult humans where it could, when activated, combat obesity and the metabolic syndrome. BAT thermogenesis relies on Uncoupling protein-1 (UCP1), a mitochondrial carrier protein that transports protons across the mitochondrial inner membrane, decoupling electron transfer from ATP synthesis to generate heat. The direct targeting and activation of UCP1 is a possible strategy to induce thermogenesis therapeutically. However, the molecular nature of UCP1 transport, activation by fatty acids and inhibition by purine nucleotides has not been resolved. The protein is thought to bind one nucleotide molecule per protein dimer and, unlike other mitochondrial carriers, is not believed to bind cardiolipin. Here, we have developed a novel method to purify UCP1 from native sources that, unlike conventional hydroxyapatite methods, allows the protein to be prepared in defined conditions, free of excess detergent and lipid. Assessment of purified preparations by thin-layer chromatography reveals that UCP1 co-purifies tightly bound cardiolipin. This lipid stabilises the protein over other phospholipid species, as demonstrated by thermal stability measurements, and is essential to successfully reconstitute UCP1 into liposomes. The stabilised protein is monomeric in size exclusion experiments and has a ligand titration profile in isothermal calorimetric measurements that clearly indicates one GDP molecule binds per UCP1 monomer. These findings clarify several fundamental properties of UCP1, indicating that previous conclusions are incorrect.

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S3.P7

Role of uncoupling protein-3 in energy metabolism and in the prevention of high fat induced overweight of mice housed at themoneutrality

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Despite that the physiological role of uncoupling protein-3 (UCP3) is still under debate, the protein seems to mediate mitochondrial mild uncoupling under specific condition. Due to its expression in skeletal muscle (SkM), a tissue that significantly contribute to metabolic rate of the whole animal, the thermogenic contribution of UCP3, even if mild, could influence metabolic rate and could contrast the onset of high fat diet induced overweight. Studies on UCP3 KO mice do not support this hypothesis. However, they were performed on mice living at standard housing temperature of 22–24 °C, a temperature significantly below thermoneutrality (30 °C for mice), thus mice were under constant thermal stress and extra energy was required to maintain their body temperature. Aim: In the present study we evaluate if UCP3 could influence metabolic rate and predisposition to fat accumulation and overweight, by using UCP3 KO mice and their control WT housed at thermoneutrality. Experimental Design: On adult WT and KO mice, we detected resting metabolic rate, respiratory quotient and energy expenditure by indirect calorimetry as well as SkM and brown adipose tissue (BAT) mitochondrial thermogenesis. To evaluate if UCP3 could affect the onset of high fat diet induced overweight, at the weaning WT and KO mice were fed a high fat diet (30% carbohydrate, 25% protein, 45% fat) for 12 weeks. At the end of treatment, we evaluated mice body composition (in terms of water, lipid and protein percentage), energy gain and the energy partitioning, i.e the fraction of energy gained that is stored as lipid and as protein. Results: KO mice displayed lower metabolic rate (−20%) and energy expenditure (−25%) than WT ones. In addition KO mice showed a reduced mitochondrial thermogenesis both in SkM and BAT. When mice were grown under high fat diet regime, the absence of UCP3 i) did not affect energy intake, ii) enhanced body weight gain (+15% vs WT), iii) affected mice body composition [KO mice presented higher fat percentage (+17%) and lower protein percentage (−22%) vs. WT], iv) enhanced the fraction of energy gained that is stored as lipid rather than as protein, and v) enhanced gross energy efficiency (+20% vs WT). Conclusions: Data reported suggest that in the absence of thermogenic stimuli it is possible to shed in light the role played by UCP3 in energy homeostasis, in the predisposition to gain weight and to accumulate lipids under a high fat diet regime.

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S3.P8

Uncoupling protein 3 reduces myocardial ischemia–reperfusion injury in the intact mouse heart

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Uncoupling proteins 2 and 3 (UCP2, UCP3) might be involved in controlling the production of mitochondrial reactive oxygen species (ROS) and protecting against oxidative stress, although the mechanism is unclear. Oxidative damage contributes to ischemia–reperfusion (IR) injury in myocardial infarction. We aimed to determine the expression levels of UCP3 after IR and its potential cardioprotective role against IR damage, as well as the protein involvement in ischemic preconditioning (IPC). We also examined the activation of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2), a master regulator of the cellular antioxidant response, in the heart after IR. We determined UCP3 and Nrf2 protein expression in preconditioned and non-preconditioned hearts from UCP3 knockout and wild-type mice subjected to IR. Hearts were cannulated via the aorta and perfused retrogradely with warm (37 ºC) Krebs buffer using a Langendorff perfusion system to apply ischemia, IR or IPC protocols. Control hearts were perfused for 120 min with standard oxygenized Krebs solution at 37 ºC; ischemia hearts were allowed to stabilize for 20 min before the flow was completely stopped to generate global normothermic ischemia for 40 min; IR hearts were allowed to stabilize for 20 min before generating global normothermic ischemia for 40 min, and then flow was restored and hearts were reperfused for 120 min; preconditioned hearts were allowed to stabilize for 20 min and then subjected to two cycles of 5 min ischemia

and 5 min reperfusion, before generating global normothermic ischemia for 40 min followed by 120 min reperfusion. Samples were processed to extract total and nuclear proteins. In addition, myocardial infarction was studied by staining with triphenyltetrazolium chloride (TTC), and creatine kinase activity in the coronary effluent was measured to determined reperfusion damage. Nrf2 and UCP3 expression levels increased significantly during reperfusion in the nuclear and total tissue extracts, respectively, from both preconditioned and non-preconditioned hearts, suggesting that Nrf2 might regulate UCP3 expression in the heart under oxidative stress, as previously reported in cells. Hearts from UCP3 knockout mice had increased infarct size and creatine kinase activity compared to those from wild-type mice. These results suggest that the Nrf2/UCP3 signalling pathway can be targeted to protect the heart against the damaging effects of IR.

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S3.P9

Hepatic mitochondrial function and efficiency in rats simultaneously exposed to chronic high-fat diet and low doses of persistent organic pollutant

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Introduction: The physiological impact associated with chronic simultaneous exposure to both high-fat diet and low doses of persistent organic pollutants, such as p,p′-diphenyldichloroethene (DDE) (DDT's major metabolite with the highest persistence), is poorly understood. Given that liver is one of the main organs involved in response to both toxic injury and high-fat feeding, the aim of the present work was to investigate the effect of chronic simultaneous exposure to low doses of DDE and high-fat feeding on hepatic mitochondrial function and efficiency, reactive oxygen species (ROS) production and endoplasmic reticulum (ER)-stress in rats. Methods: Three groups of 8 rats were so treated for 4 weeks: 1) standard diet (10% fat J/J) (N rats); 2) high-fat diet (45% fat J/J) (D rats); and 3) high-fat diet plus DDE (10 mg/kg b.w. by gavage) (D + DDE rats). In isolated liver mitochondria, oxygen consumption rates (OCR) in the presence of FADH2-dependent (succinate + rotenone) and lipid (palmitoylcarnitine + malate) substrates were determined polarographically. To test mitochondrial efficiency, we measured the OCR oligomycin/OCR FCCP ratio. ROS and lipid peroxide production was analyzed by determining H2O2 production and TBARs content. Glucose-regulated protein (GRP) 78 expression, as ER-stress marker, was determined in liver homogenates. Results: D rats showed higher fatty acid oxidation rate, ROS production and GRP78 expression vs. N rats. D + DDE rats showed a further increased fatty acid oxidation rate, higher succinate state 4 rates and OCR oligomycin/OCR FCCP ratio suggesting an increased mitochondrial uncoupling compared to D groups. D + DDE groups also showed increases in ROS production and GRP78 expression similar to those found in D rats vs. N. Conclusion: Compared to high-fat diet treatment, the simultaneous chronic exposure to low doses of DDE and high fat diet elicited further increase in lipid utilization that may be useful to cater to energy requests for detoxification processes. In addition, this simultaneous exposure also elicited an increase in the degree of mitochondrial uncoupling that may counteract a possible further increase in ROS production and ER stress caused by toxic injury.

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S3.P10

Activation of UCP and mitoKATP channel efficiently decreases superoxide anion production in insect mitochondria

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Uncoupling proteins (UCPs) and ATP-regulated channels (mitoKATP channels) are proteins located in inner mitochondrial membrane. It was evidenced, that these energy-dissipating systems are present in insect tissues, such as trophic tissue of fat body and leg muscle. In cockroach Gromphadorhina coqereliana, GcUCP4 as well as mitoKATP channel decrease superoxide anion production ($O2^-$) [1,2]. In the present study, we elucidated, whether GcUCP4 and mitoKATP channel collaborate in the modulating of reactive oxygen species (ROS) level in fat body and muscle tissue of cockroach. In isolated mitochondria, UCP was activated by palmitic acid and mitoKATP channel was stimulated by diazoxide or pinacidil and $O2^-$ production was measured by nitroblue tertazolim method (NBT). Simultaneous activation of both proteins resulted in a very efficient decrease of $O2^-$ level, approximately 2.5 times higher than when the proteins were activated separately. Moreover, after the addition of the UCP and mitoKATP channel inhibitors, such as GTP or ATP, the level of $^-$ increased by approximately 25%, but it was still almost twice lower compared to control conditions, where activators and inhibitors were not added. When both UCP and mitoKATP channel were activated in the presence of GTP (an UCP inhibitor), the increase in $O2^-$ production was higher compared to measurements with ATP (a mitoKATP channel inhibitor). These results suggest a bigger amount of UCP protein in insect inner mitochondrial membrane and/or a more significant role of this protein in modulation of mitochondrial ROS formation. The cumulative effects of GcUCP4 and mitoKATP channel activation on mitochondrial $^-$ formation indicate the physiological role of both proteins in ROS formation in fat body and muscle of cockroach. We hypothesize, that GcUCP4 and mitoKATP channel might be implicated in cellular protection against metabolic stress in insect tissues during energy demand events such as molting or flight.

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