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S3.P6

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

Yang Lee, Edmund R. Kunji, Paul G. Crichton
MRC Mitochondrial Biology Unit, UK
E-mail address: pgc@mrc-mbu.cam.ac.uk

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

Assunta Lombardi[a], Rosa Anna Busiello[b], Sabrina Savarese[c], Lillà Lionetti[a], Fernando Goglia[b]
[a]*Department of Biology, University of Naples Federico II, Italy*
[b]*Università del Sannio, Italy*
[c]*Università degli Studi di Napoli Federico II, Italy*
E-mail address: assunta.lombardi@unina.it

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

Elia López-Bernardo[a], M. Saadeh Suleiman[b], Susana Cadenas[a]
[a]*Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM) and Dpto. Biología Molecular UAM, Spain*
[b]*Bristol Heart Institute, University of Bristol, UK*
E-mail address: scadenas@cbm.csic.es

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