S3.O2

In the presence of fatty acid, GDP stimulates respiratory rate and decreases membrane potential of mammalian mitochondria: GDP could not function as a potent inhibitor of mitochondrial uncoupling protein (UCP)

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By creating in vitro physiological-like conditions, i.e., favoring oxidative phosphorylation (omission of carboxyatractyloside and oligomycin, inhibitors of adenine nucleotide translocase and FOF1-ATP synthase, respectively, from the incubation medium) and in the presence of ATP, we found that a high concentration of GDP (1 mM), but not GTP, stimulates respiratory rate and decreases membrane potential of mammalian (rat kidney and human endothelial cells) isolated mitochondria. The stimulatory effect of GDP resembled the state 4 (non-phosphorylating respiration)–state 3 (phosphorylating respiration) transition, which is characteristic of ADP oxidative phosphorylation. What is more, the GDP-induced effect occurred irrespective of the presence of linoleic acid, a potent activator of uncoupling protein (UCP) isoforms. In turn, the addition of 1 mM GTP resulted in a decrease in the respiratory rate accompanied by an increase in the membrane potential, which is characteristic of UCP inhibition. On the contrary, under conditions excluding oxidative phosphorylation, in the presence of carboxyatractyloside and/or oligomycin, the GDP stimulatory effect was completely abolished. The GDP-induced oxidative phosphorylation-like effect could be explained by nucleoside diphosphate kinase (NDPK)-dependent transphosphorylation between GDP and ATP, which generates the ADP pool and subsequently induces oxidative phosphorylation. However, another explanation might be needed, as NDPK was found to be sensitive to increasing concentrations of GDP. The alternative explanation involves the possibility of the direct transport of GDP across the inner mitochondrial membrane and its oxidative phosphorylation in the mitochondrial matrix. Our studies clearly indicate that GDP-dependent inhibition of UCPs could have minor physiological significance but GTP most likely play a role of a strong negative regulator of UCPs thus should be used as a diagnostic inhibitor of UCP function instead of GDP. This work was supported by grants of the Polish Ministry of Science and Higher Education (NN 301 636440 and Iuventus Plus program 2013–2015 IP2012 059172) and partially by the European Union with resources from the European Regional Development Fund under the Innovative Economy Programme (POIG.01.01.02-00-069/09).

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S3.P1

Uncoupling protein-2 dampens palmitate-induced mitochondrial reactive oxygen species in INS-1E insulinoma cells

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In type 2 diabetes, high levels of circulating glucose and non-esterified fatty acids (NEFAs) impair pancreatic beta cell function, and we have recently shown in INS-1E insulinoma cells that mitochondrial dysfunction is involved in this glucolipotoxicity [1]. Specifically, palmitate-induced defects in oxidative phosphorylation attenuate glucose-stimulated insulin secretion, and palmitate-induced reactive oxygen species (ROS) cause INS-1E cell loss. The deleterious effects of palmitate on ROS and INS-1E viability are largely prevented by its monounsaturated counterpart palmitoleate [1]. Here we show that the origin of palmitate-induced ROS is mitochondrial: oxidation of MitoSOX, a mitochondria-targeted superoxide probe, is increased after 24-h palmitate exposure at high glucose, whilst oxidation of the equivalent non-targeted probe (hydroethidine) is unaffected. Moreover, dissipation of the mitochondrial protonmotive force with FCCP annuls the palmitate-induced rise in MitoSOX oxidation, and inhibition of respiratory Complex III with antimycin A increases this rise a little further. Given the mitochondrial nature of palmitate-induced ROS, we next assessed how mitochondrial uncoupling protein-2 (UCP2) activity affects this glucolipotoxic phenotype. We show with RNA interference experiments that UCP2 knockdown amplifies the mitochondrial ROS response of INS-1E cells to palmitate. This augmented response does not cause significant further cell loss and is still fully prevented by palmitoleate.

Reference

[1] J. Barlow, C. Affourtit, Novel insights in pancreatic beta cell glucolipotoxicity from real-time functional analysis of mitochondrial energy metabolism in INS-1E insulinoma cells, Biochem. J. 456 (2013) 417–426.

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S3.P2

UCP4C mediates uncoupled respiration in larvae of *Drosophila melanogaster*

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Larvae of *Drosophila melanogaster* reared at 23 °C and switched to 14 °C for 1 h are 0.5 °C warmer than the surrounding medium. In keeping with dissipation of energy, respiration of *D. melanogaster* larvae cannot be decreased by the F-ATPase inhibitor oligomycin or stimulated by protonophore. Silencing of Ucp4C conferred sensitivity of respiration to oligomycin and uncoupler, and prevented larva-to-adult progression at 15 but not 23 °C. Uncoupled respiration of larval mitochondria required palmitate, was dependent on Ucp4C and was inhibited by GDP. UCP4C is required for development through the pre-pupal stages at low temperatures and may be an uncoupling protein.

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S3.P3

Uncoupling protein-2 does not mediate palmitate-induced glucolipotoxic defects in oxidative phosphorylation in INS-1E insulinoma cells

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Palmitate exposure at high glucose concentrations impairs the ability of INS-1E insulinoma cells to secrete insulin in response to