groups. Uptake of tetramethylrhodamine methyl ester (TMRM), measurement of membrane potential, was decreased in administration of siRNA in both groups (control − 14% and diabetes − 36% compared to corresponding untreated group). GDP only increased the membrane potential in untreated diabetics. Thiobarbituric acid reactive substances (TBARS), marker of oxidative stress, were increased in kidney cortex of control + siRNA and untreated diabetics compared to controls (both + 220%), and siRNA given to diabetics completely normalized the TBARS levels. Paradoxically, *in vivo* gene silencing of UCP-2 increases the mitochondria uncoupling in both control and diabetic animals, measured both as glutamate-stimulated QO_2 and altered membrane potential in the absence of a functional ATP production. However, this uncoupling is not inhibited by GDP, excluding a role of UCP2. Excessive amounts of ADP in the absence of a functional ATP production (inhibited by oligomycin) results in reduced uncoupling, indicating an uncoupling role of the adenine nucleotide transporter in the absence UCP2 uncoupling in both control and diabetic kidneys.

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10P.6 Cardioprotective activity of dehydrosilybin is linked to its uncoupler-like behavior

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Uncoupling protein 2 (UCP2) is a carrier protein located in the inner mitochondrial membrane, which is thought to confer tolerance of cardiac myocytes to oxidative stress *via* diminished mitochondrial Ca^{2+} overload, reduced generation of ROS by mild uncoupling of mitochondrial respiration, and perhaps other means. Silibinin, also known as silybin (SB), is the major active constituent of silymarin, the mixture of flavonolignans extracted from seeds of milk thistle (*Silybum marianum*). It is used in the treatment and prevention of liver diseases because of its hepatoprotective (antihepatotoxic) properties. We used silybin and its derivative, 2,3-dehydrosilybin (DHSB), to evaluate their effect on UCP2 expression and mitochondrial bioenergetics in neonatal rat cardiomyocytes. Both of the compounds affected thyroid hormone-mediated UCP2 up-regulation possibly attenuating the cardioprotective effects of UCP2. Investigating the effect on UCP2 level, we noted the ability of DHSB to de-energize the cells by monitoring JC-1 fluorescence. The same phenomenon was confirmed in isolated rat heart mitochondria. Further experiments evaluating oxygen consumption revealed that DHSB uncouples the respiration of isolated rat heart mitochondria albeit with much lower potency than FCCP. Furthermore, DHSB showed very high potency to suppress ROS formation in isolated rat heart mitochondria with $IC_{50} = 1.5$ μM. It is more effective than its effect in a purely chemical system generating superoxide or in cells capable of oxidative burst. DHSB also attenuated ROS formation caused by 7 μM rotenone in primary cultures of neonatal rat cardiomyocytes. We infer that the apparent uncoupler-like activity of DHSB is the basis of its ROS modulation effect in neonatal rat cardiomyocytes and may also be the culprit in DHSB diminishing the thyroid hormone-mediated UCP2 up-regulation.

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10P.7 Nitrolinoleic acid is a cycling substrate of mitochondrial uncoupling protein UCP2

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Nitrated derivatives of fatty acids (FA-NO_2) are pluripotent cell-signaling mediators that exhibit anti-inflammatory properties. Experimental evidence suggests that nitrolinoleic acid (LNO_2) stimulates mitochondrial uncoupling *via* adenine nucleotide translocase (ANT) and UCP2-dependent mechanism and that LNO_2 nitroalkylates ANT and possibly UCP2 (Nadtochiy SM *et al.*, 2009, *Cardiovasc Res.* **82**, 333–340). Here we tested the hypothesis that LNO_2 acid induces the UCP2-mediated proton transport and that LNO_2 is transported *via* UCP2 by the FA-protonophore cycling mechanism. Using protein-free liposomes, we determined that the addition of synthesized LNO_2 caused fast acidification of liposomal interior, indicating spontaneous transport of protonated LNO_2 across the phospholipid bilayer. Using *E. coli*-expressed UCP2 reconstituted into liposomes, we found that LNO_2 induced purine nucleotide-sensitive H^+ uniport. In parallel experiments, LNO_2 induced K^+ influx balanced by anionic charge transfer in UCP2-proteoliposomes, indicating that LNO_2 was transported as an anion. Additional experiments using isolated lung mitochondria from *Ucp2*-WT and *Ucp2*-KO mice verified UCP2-dependent, LNO_2-induced uncoupling. Taken together, these data are consistent with LNO_2 being transported by UCP2 as a cycling substrate by the FA-protonophore mechanism. The data further indicate an active role of UCP2 in mediating the FA-NO_2 signal transduction.

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10P.8 Investigation into the role of uncoupling protein 3 in the thymus and spleen

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UCP3 is predominantly associated with skeletal muscle and brown adipose tissue (BAT). Our laboratory has also demonstrated the presence of UCP3 protein in thymus and spleen mitochondria of rats [1]. The aim of this study was to determine the level of apoptosis in cells isolated from these tissues and whether the absence of UCP3 affected the degree of apoptosis. To that end, we utilized propidium iodide and annexin V staining of thymocytes and splenocytes from wild type and *Ucp3* knockout ($UCP3^{-/-}$) mice followed by FACS analysis to determine the levels of apoptosis. Thymocytes and splenocytes were treated for 18 h with dexamethasone (0.1 μM) or vehicle (EtOH, to measure spontaneous apoptosis). We were able to detect a significant increase in spontaneous apoptosis in thymocytes isolated from $UCP3^{-/-}$ mice compared to those from wild type mice (33% increase, $p = 0.04$) We detected no significant difference in spontaneous apoptosis in splenocytes or in apoptosis due to dexamethsone in splenocytes and thymocytes in a comparison of $UCP3^{-/-}$ and wild-type mice. These preliminary data suggest that UCP3 plays a role in protection against apoptosis in thymocytes but not in splenocytes.

Reference

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