Mitochondrial uncoupling proteins

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Abstract

The aim of this review is to provide an overview of current knowledge on the three mammalian uncoupling proteins, UCP-1, UCP-2, and UCP-3.
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Thermogenic uncoupling protein in brown adipose tissue

The concept of mitochondrial uncoupling protein comes from the thermogenic organ of mammals known as brown adipose tissue [1]. This is a specialized type of fat tissue in which the cells (brown adipocytes) contain many more mitochondria, and less lipid, than normal fat (white adipocytes). These mitochondria contain a specialized protein, uncoupling protein (UCP-1). When introduced into artificial membranes, UCP-1 increases the permeability of the membrane to protons [2]. According to the chemiosmotic theory of Mitchell, oxidization of substrate by mitochondrial respiratory chain causes proton pumping and thus creates an electrical and pH gradient across the mitochondrial inner membrane, which is the intermediate that creates a tight link (coupling) between substrate oxidation and production of the energy-rich molecule, ATP. The proton conductance created by UCP-1 dissipates this potential, whereas the oxidation of substrates attempts to maintain it [3]. This greatly accelerates metabolism, and results in an intense production of heat. This raised interest, because the consequence of the activity of brown adipose tissue on energy expenditure is the same as that of exercise (*Figure 1*). However, in humans, active brown adipose tissue was recognized only in newborns. Recently, the use of positron emission tomography has revealed a significant percentage of young adults with active brown fat – which, furthermore, reacts to external temperature [4].

The emergence of ''new'' uncoupling proteins

Uncoupling protein-1 appeared to be a mammalian acquisition linked to regulation of body temperature. When the protein sequences were made available, it became apparent that it and other transporters in the mitochondrial inner membrane share common properties [5]: there is a shared motif of amino acids, which provides a useful criterion for the recognition of a ''mitochondrial carrier'' of this nature; six transmembranous alpha helices are usually present; the sequence length is usually approximately 300 amino acids. The 3-dimensional structure of one member of this family of transporters, the ADP/ATP translocator, has been determined [6].

In the late1990 s, several sequences very similar to that of UCP-1 were discovered [7]. Two were described in mammals (UCP-2 and UCP-3) and one in birds, but ''UCPs'' were also found in plants and cold-blooded animals. Consequently, their participation in thermogenesis was questioned. However, it was relatively easily demonstrated that, under certain conditions, they could transport protons, and they were therefore considered to be new uncoupling proteins. There are reports of UCPs-4 and -5 in mammals; however, the similarity of these two proteins to UCP-1 is much lower.

Molecular mechanisms

The uncoupling activity of UCP-1 is tightly regulated: binding of nucleotides inhibits transport by UCP-1,

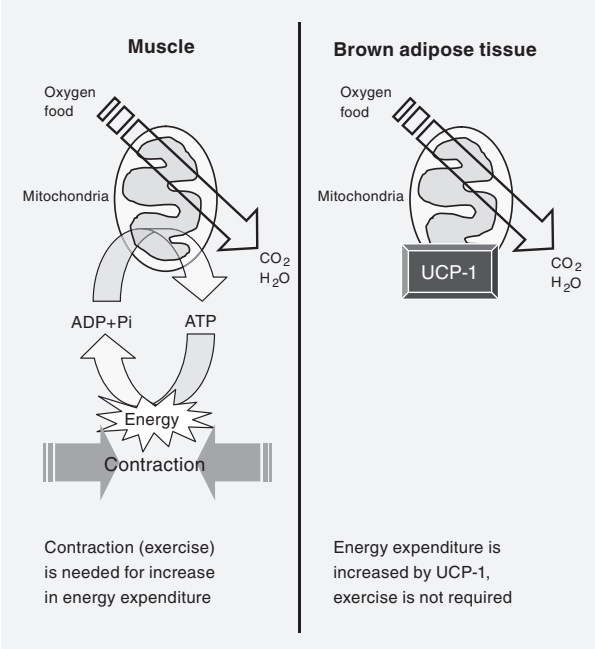

Figure 1. For the mitochondria and the upstream events (catabolism of nutrients), the consequences of exercise and of UCP-1 activation in brown adipose tissue are similar.

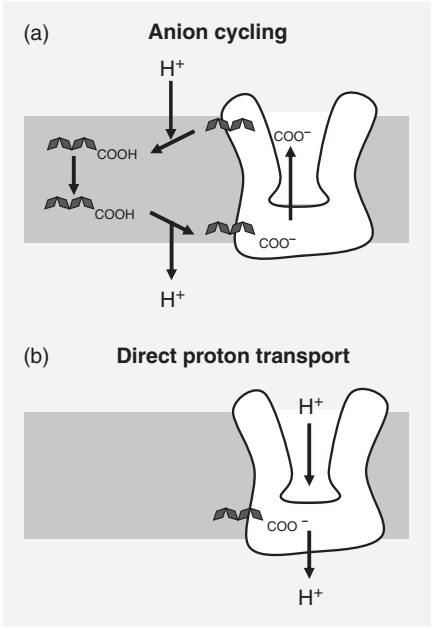

Figure 2. The phospholipidic domain of the inner mitochondrial membrane is depicted as a gray area. Uncoupling protein-1 (UCP-1) is drawn in a shape inspired by Pebay-Peyroula et al [6]. (a) Fatty acid cycling. The fatty acid is depicted as linked diamonds (hydrophobic alkyl chain) and the protonated or ionized carboxylic group. The protonated form diffuses freely in membrane phospholipids, but the ionized needs UCP-1, which behaves as a fatty acid transporter. (b) UCP-1 drives proton transport directly, and fatty acid is an activator.

whereas free fatty acids stimulate proton transport by UCP-1. This has the consequence that mitochondria could be fully ''prepared'' for thermogenesis, primed with a large supply of UCP-1 that remains fully inhibited by endogenous nucleotides and the absence of fatty acids; when activation occurs, however, thermogenesis starts within seconds [1]. The process of activation involves adrenergic stimulation that triggers lipolysis, which provides fatty acids for mitochondrial oxidation that in turn simultaneously stimulate proton transport by UCP-1 [3]. Moreover, adrenergic stimulation is the last step necessary for a maximal transcription of the *Ucp1* gene, which is also activated by nuclear receptors such as peroxisome proliferator activated receptors (PPARs), retinoic acid, and thyroid hormone receptors [8], with the participation of the coactivator, proliferator-activated receptor-gamma coactivator-1 (PGC-1). The transcription of *Ucp2* and *Ucp3* genes, although not sensitive to adrenergic stimulation, is also under the control of nuclear receptors, in particular PPARs that mediate the transcriptional response to fatty acids [9]. The regulation of mRNA translation plays a significant role in the case of UCP-2 [10].

The mechanism of proton transport by UCPs remains a matter of debate [11]. One proposal is that proton transport is the indirect consequence of the transport of an anionic hydrophobic ''activator'' (*Figure 2a*). This mechanism is shared with other proteins not known to be UCPs [12], and the new UCPs could probably act similarly with activators that might be different than those involved with UCP-1. The physiological relevance of this cycle has been questioned, and the alternative proposal put forward that UCP-1 uses a specific proton transport mechanism [5] (*Figure 2b*). If the latter is the case, it remains to be determined whether or not ''new UCPs'' possess a similar property. It has also been suggested that only half of the cycle is relevant, and that the new UCPs drive fatty acids out of mitochondria [13,14]. All these mechanisms of transport are much less efficient than that afforded by transport channels: the amount of transporter present is clearly a limiting factor, and a quantitatively significant uncoupling is associated with a high level of expression of UCP-1. In contrast, the new UCPs are much less abundant, which sometimes renders their detection questionable.

Physiology

Uncoupling increases energy expenditure and leads to weight loss. Accordingly, the chemical uncoupler, dinitrophenol (now completely banned because of side effects), was, for a period, used to trigger weight loss. Conversely, UCP knockout was expected to decrease energy expenditure, with obesity as a consequence. Although overexpression of UCPs provided resistance against obesity in animal models, knockout of individual UCP genes failed to produce obese

animals: (1) UCP-1 appeared to be required for cold-induced thermogenesis [15]; (2) no major defect has been associated with the disappearance of UCP-3, which is expressed essentially in muscle [16] (notwithstanding the observation that loss of UCP-3 in mice was associated with disappearance of the metamphetamin induced hyperthermia [17]); (3) inactivation of UCP-2 has led to two observations: a modification of insulin secretion by pancreatic β cells [18] and an increase in immunity [19]. Genipin, a molecule from a plant used in Chinese medicine, appears to be an inhibitor of UCP-2 and could thus be used to improve insulin secretion [20]. There appears to be a consensus of opinion that inactivation of UCP leads to increased oxidative stress. Overexpression of UCPs has produced remarkable examples of protection against ischemic or traumatic shock in which oxidative stress contributes heavily to the damage sustained. Modifications observed in transgenic animals (or cellular models) could be accounted for by variation of mitochondrial activity and eventual ''uncoupling''. However, this uncoupling has been difficult to demonstrate. It may be because it is of modest degree, which would be in agreement with the level of expression of UCP-2/3. Alternatively, uncoupling is not the explanation, and the observed phenotypes are to be explained by a different transport activity of UCP-2/3 leading to subtle modification of mitochondrial metabolism, not yet understood at the molecular level [21].

Conclusion and perspectives

To summarize the present situation, the well defined physiological role of UCP-1 contrasts with a more confused situation with regard to the other UCPs discovered 20 years later. It may be considered that there is consensus over the following points in the case of the ''new'' UCPs: (1) they are not the explanation for the ''basal proton leak'' observed in all mitochondria, but, in the opinion of several authors, constitute a supplementary inducible proton leak; (2) their level of expression is orders of magnitude lower than that of UCP-1; (3) their relevance appears to be related to metabolism, rather than to energy expenditure; (4) a link exists between UCPs and the production/handling of reactive oxygen species.

Other aspects of mitochondrial uncoupling proteins that are already under study or deserving consideration include: (1) the question whether or not expression of UCP-1 is strictly restricted to brown adipocytes, which remains a matter of controversy; (2) the relationships between UCP-2, cellular division, and cancer; (3) examining whether any interaction with other proteins occurs and has a physiological relevance, or whether it is valid that the UCPs continue to be considered ''solitary proteins'' operating a transport system in the mitochondrial inner membrane; (4) the mechanisms by which UCPs are degraded. ■

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