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Review

Mitochondrial ion transport pathways: Role in metabolic diseases

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ABSTRACT

Mitochondria are the central coordinators of energy metabolism and alterations in their function and number have long been associated with metabolic disorders such as obesity, diabetes and hyperlipidemias. Since oxidative phosphorylation requires an electrochemical gradient across the inner mitochondrial membrane, ion channels in this membrane certainly must play an important role in the regulation of energy metabolism. However, in many experimental settings, the relationship between the activity of mitochondrial ion transport and metabolic disorders is still poorly understood. This review briefly summarizes some aspects of mitochondrial H^+ transport (promoted by uncoupling proteins, UCPs), Ca^{2+} and K^+ uniporters which may be determinant in metabolic disorders.

1. Introduction

Mitochondria are the central coordinators and the site of essential biochemical transformations involved in energy metabolism. As such, these organelles have always been focused on within studies involving metabolic diseases. Indeed, a vast array of findings link changes in mitochondrial functions with disorders associated with the metabolic syndrome. In some cases, mitochondrial alterations appear as causes of the metabolic changes observed. For example, enhancement of mitochondrial proliferation improves symptoms associated with the metabolic syndrome, indicating that defective mitochondrial biogenesis leads to these characteristics [1–3]. Indeed, mutations in mitochondrial tRNA promote maternally-inherited symptoms characteristic of the metabolic syndrome [4]. In other studies, the link between mitochondrial dysfunction and metabolic syndrome is correlative, but still highly interesting. As examples, the selection for low aerobic capacity produces animals with metabolic alterations typical of the metabolic syndrome and decreased mitochondrial biogenesis [5]. Insulin resistance induced by early introduction to animal fat in the diet is preceded by altered mitochondrial gene expression and reduced mitochondrial DNA content [6]. Non-alcoholic steatohepatitis and gains in visceral fat are associated with mitochondrial dysfunction [3,7,8]. Furthermore, mitochondria are the most quantitatively relevant intracellular source of reactive oxygen species (ROS) [9–11], and oxidative imbalance is strongly linked to the metabolic syndrome [12].

These studies mostly focus on changes in mitochondrial content, point mutations or changes of respiratory capacity as determinants for alterations in metabolic control. On the other hand, recent results suggest mitochondrial ion carriers may also be important regulators of animal energy metabolism. In this review, we uncover some characteristics of mitochondrial ion transport which may be important in metabolic disorders.

2. Mitochondrial ion transport: general properties

Mitochondria must, at the same time, exchange metabolites and other compounds with the cytoplasm and maintain the high protonmotive force across the inner mitochondrial membrane necessary for oxidative phosphorylation. Most metabolites transported are anions, and are often symported with protons or antiported against hydroxyl anions in order to use protonmotive force to drive the accumulation of these metabolites. Cation exchangers are present in the mitochondrial inner membrane to remove specific ions from the matrix. A small group of cation uniporters allow the regulated entry of selected cations into the matrix. These uniporters must present limited transport rates in order to maintain protonmotive force and oxidative phosphorylation [13,14].

Most mitochondrial ion transporters have been characterized functionally and pharmacologically, but still remain uncharacterized structurally, due to their low abundance. This makes their link with metabolic diseases much harder to study than other properties and biomolecules in mitochondria.

3. Uncoupling proteins

A notable exception to the lack of structural knowledge regarding mitochondrial ion carriers are uncoupling proteins (UCPs), a family of inner membrane carriers that increase proton conductance and are

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the product of well-established genes [15–18]. Interestingly, UCPs are not proton channels, but anion transporters instead. They are believed to transport free fatty acid anions from the mitochondrial matrix to the intermembrane space (see Fig. 1). The fatty acids become protonated due to the electrochemical proton gradient, lose their charge and flip-flop through the inner membrane lipid bilayer, transporting a proton into the matrix (for reviews, see [19,20]). Another proposed mechanism for UCP function [21] suggests UCPs transport H^+ using fatty acids at their active site, in a process mediated by histidines. However, not all UCPs possess histidines in this site [20,22]. The following publications provide overviews of differing proposed mechanisms of uncoupling protein function: [15,23–26].

UCP1, the first such protein described, is present in high quantities in the brown adipose tissue, and promotes overt uncoupling, widely associated with thermogenesis [27–30]. The discovery of a family of proteins with high identities to UCP1 in the 1990s, widely distributed in many tissues, immediately attracted the attention of researchers in energy metabolism, and the idea that UCP content could regulate body weight by determining mitochondrial coupling surfaced [31,32]. Subsequently, a large body of work investigated the expression of UCPs in metabolic alterations, including obesity, diabetes and hyperlipidemias [33–35]. Many correlations were uncovered, including correlations of UCP polymorphisms with obesity and diabetes [35,36], but unfortunately results varied widely, and often showed unexpected correlations (such as increased UCP expression in obesity [37]). Furthermore, most studies quantified mRNA levels for UCP2 or UCP3 and investigated polymorphisms, while few measured protein levels in tissues or looked directly at the activity of these transporters, hampering precise conclusions. Indeed, Yu et al. [38] demonstrated experimentally that significant discrepancies exist between UCP mRNA levels, temperature and mitochondrial proton leak.

Clues regarding the functional activities of UCP family members were also expected to be uncovered using knockout animal models. Interestingly, knockouts of either UCP2 or UCP3 have little or no phenotype [34,39–41]. Overexpression of UCP3 generated leaner mice in one model [42], but the levels of overexpression required were very high, and can lead to uncoupling simply due to protein misfolding [43]. These results increasingly made it clear that the role of UCP family members in energy metabolism was more subtle and complex: The simplistic hypothesis for the function of these proteins did not completely account for their actions. Indeed, the degree of uncoupling promoted by these transporters varies largely with their abundance, and generalized uncoupling leading to whole body increases in energy expenditure does not seem to the primary function of UCP2 and UCP3 [43–45].

Since the metabolic syndrome involves a complex network of pathophysiological changes, tissue-specific activation of UCPs could be involved in the metabolic responses. Indeed, obesity and a pro-inflammatory state can induce the expression of UCP2 in the liver, where its expression is normally low [46–49]. UCP2 could be an adaptation to oxidize excessive lipids in mitochondria by increasing respiratory rates and the NAD^+ pool. However, UCP2 null mice submitted to hyperlipidemic diets do not exhibit any differences in non-alcoholic steatohepatitis development [34], possibly due to the compensatory effect of increasing other uncoupling pathways that mitigate the steatotic phenotype, such as K^+ channels (discussed below). Indeed, UCP2 overexpression measured in the livers of obese animals could be due to Kupffer cells (liver resident macrophages), without a relevant hepatocyte-related change in metabolic function [34].

A well-established function for UCP family members is the control of the intracellular redox state, by limiting mitochondrial production of ROS [19,45,50,51], a necessary byproduct of energy metabolism [10,11]. Indeed, mild mitochondrial uncoupling is often an effective manner to control the generation of mitochondrial oxidants in isolated mitochondria [10,11,22,52], and systemic mild uncoupling is associated with strong improvements in redox state [53,54]. In this general line, many publications have shown that UCP activation effectively prevents mitochondrial ROS release, under physiological and pathological conditions [19,45,50,51,55,56]. While ROS control contributes toward tissue protection under many conditions, mild uncoupling can be lethal for cerebellar cultures [57]. In these cells, mild uncoupling decreases ATP generation leading to a decreased capacity to exchange Na^+ for K^+, resulting in cell death. Thus, the protection caused by mild uncoupling via ROS regulation is probably dependent on the ability of the cell to maintain levels of ATP despite the decrease in coupling.

Other results suggest UCPs may also have a role transporting ROS anion fatty acid hydroperoxides, thus further contributing toward redox control [58]. A strong indicator that the redox role of UCP proteins is indeed physiologically relevant is the finding that the activity of these proteins is increased by oxidants [59].

Another clear metabolic role for a specific member of the UCP family, UCP2, is the control of glucose-stimulated insulin release by pancreatic β-cells (for a review see [43]). UCP2 activity in these cells decreases the quantity of ATP produced in the presence of a set concentration of glucose, increasing the activity of ATP-sensitive K^+

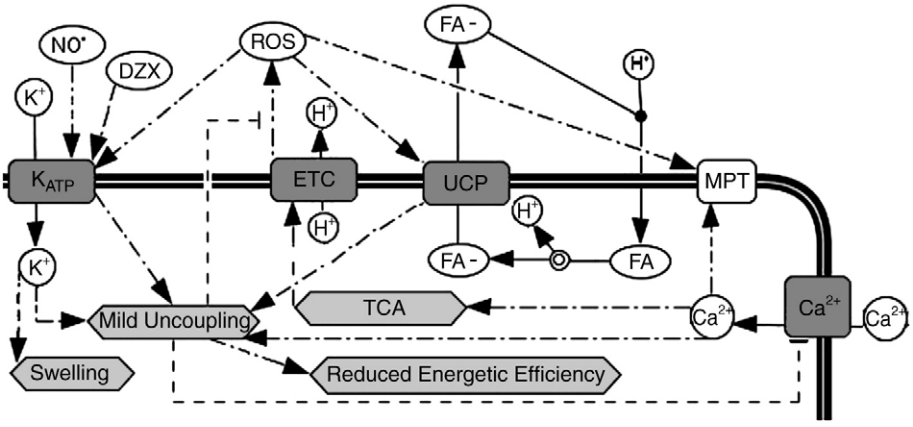

Fig. 1. K^+, H^+ and Ca^{2+} transport in mitochondria — effects on ROS production and energy metabolism. K^+ transport (through mitoK_{ATP} channels), H^+ transport (mediated by UCPs, and involving free fatty acids, FA) and Ca^{2+} transport (through Ca^{2+} uniporters) occurs down the electrochemical gradient generated by the electron transport chain (ETC), using electrons collected in the tricarboxylic acid cycle (TCA). The activity of these pathways promotes uncoupling, which prevents the formation of mitochondrial reactive oxygen species (ROS), which in turn, are activators of mitoK_{ATP} and UCPs. MitoK_{ATP} is also activated by agonists such as diazoxide (DZX) and the reactive nitrogen species $NO^{\bullet}$. Uncoupling decreases energetic efficiency. Excessive ROS and Ca^{2+} uptake into mitochondria can lead to non-selective inner membrane permeabilization, due to the activation of the mitochondrial permeability transition (MPT).