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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^+

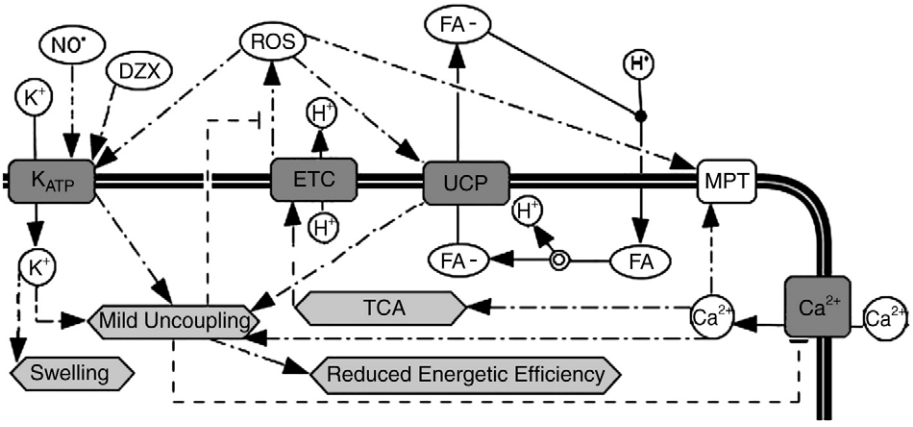

Fig. 1. K^+, H^+ and Ca^{2+} transport in mitochondria — effects on ROS production and energy metabolism. K^+ transport (through mitoK_{ATP} channels), H^+ transport (mediated by UCPs, and involving free fatty acids, FA) and Ca^{2+} transport (through Ca^{2+} uniporters) occurs down the electrochemical 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).

channels on the plasma membrane, and leading to lower insulin release [60,61] (see Fig. 2). As a result, inhibition of UCP2 leads to more efficient insulin secretion in the presence of equal quantities of glucose. This helps explain why UCP2 expression levels were often paradoxically related to body weight, and may be the reason for correlations between UCP2 polymorphisms and type 2 diabetes [62,63].

A recent publication has suggested yet another role for tissue-specific effects of UCP2 in the regulation of energy metabolism: Andrews et al. [64] suggest that UCP2 in arcuate nucleus neurons controls the response to ghrelin and, hence, food intake. However, the UCP2 knockout preparations in this work appear to have lower mitochondrial membrane potentials and capacity to phosphorylate ADP, a point which requires further clarification. Another possible role for uncoupling proteins in energy metabolism has been suggested based on studies in plant mitochondria, which present significant plant uncoupling mitochondrial protein (PUMP) activity [65]: by increasing NADH oxidation, uncoupling allows NAD^+-dependent reactions such as those within the citric acid cycle to occur even in the presence of high ATP levels, thus permitting biosynthesis reactions to occur in the presence of high energy states.

UCP1, the first UCP to be described, is highly abundant and overtly uncoupling in brown adipose tissue, and was for many years believed to be an adaptation to induce thermogenesis under specific conditions, such as arousal after hibernation and body heat maintenance in newborns. The finding that brown adipose tissue is closely related to muscular tissue [66–68] has shed new interest in this protein as a more general metabolic regulator. In a highly insightful study, Needegard's group demonstrated that UCP1 ablation induced obesity in mice housed at thermoneutrality [69]. Indeed, there is convincing, if preliminary, evidence that UCP1 and brown adipose tissue activity may be a factor in the regulation of human weight gain [70–72].

UCPs are not the only fatty acid anion transporters in mitochondria capable of promoting mild uncoupling. In fact, in many tissues, the adenine nucleotide translocator is the main protein responsible for this activity, and other mitochondrial carriers such as the aspartate/glutamate antiporter have also been shown to present this activity (for review, see [73]). Although difficult to evaluate, it would be very interesting to know if the uncoupling activity of these proteins can impact energy metabolism. The adenine nucleotide translocator has been shown to regulate ROS release through its uncoupling activity [74], and is involved in cardiac protection induced by ischemic preconditioning [75,76].

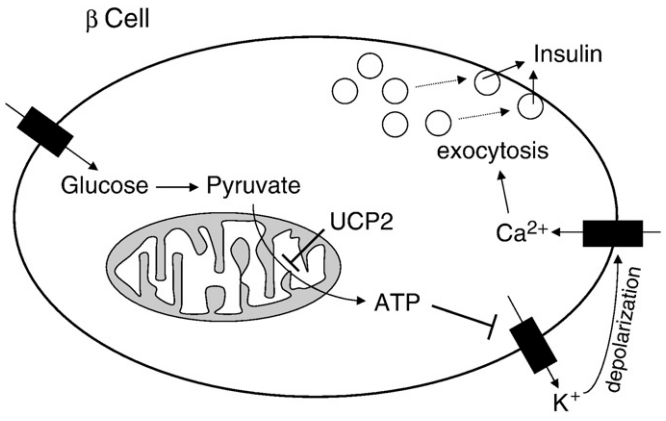

Fig. 2. Regulation of insulin release by UCP2 in pancreatic β cells. Glucose freely enters the cytosol, producing intracellular pyruvate, which is oxidized by mitochondria, generating ATP in a manner regulated by UCP2 activity. High ATP levels close plasma membrane ATP-sensitive K^+ channels, leading to membrane depolarization and activation of voltage-gated Ca^{2+} channels. Increased intracellular Ca^{2+} stimulates both insulin synthesis (not shown) and release through exocytosis.

4. Ca^{2+} uniporters

One of the first characteristics noted in isolated mitochondria was the high capacity to take up Ca^{2+} ions. Indeed, mitochondrial inner membranes posses a highly selective Ca^{2+} uniporter [77]. As is often the case for inner membrane transporters, the identity of mitochondrial Ca^{2+} uniporters is yet undetermined. Trenker et al. [78] suggested the activity was mediated by UCPs, but this concept has been strongly rebuffed by most prominent researchers in the area [79]. Indeed, silencing UCP genes does not alter mitochondrial matrix calcium concentrations [80]. Furthermore, physiological characterizations suggest that more than one Ca^{2+} uptake pathway into mitochondria exists (reviewed in [81,82]).

The quantity and rate of Ca^{2+} uptake into mitochondria are determined not only by the activity of mitochondrial Ca^{2+} uptake pathways but also by the availability of this ion within the mitochondrial microenvironment, since the affinity of mitochondrial Ca^{2+} transporters is in general lower than those present in the endoplasmic reticulum. Indeed, Ca^{2+} signals are closely transmitted between the reticulum and mitochondria, which are functionally and spatially coupled [83].

Ca^{2+} in the matrix has strong effects on mitochondrial metabolism (see Fig. 1 and [82,84] for reviews). Pyruvate, isocitrate, α-glycerophosphate and α-ketoglutarate dehydrogenase are strongly activated by Ca^{2+} ions, leading to enhanced NAD^+ reduction and increased protonmotive force. Ca^{2+} uptake by mitochondria also has an important role in regulating physiological Ca^{2+} transients [85].

Furthermore, Ca^{2+} ions can be determinant for the rates of mitochondrial ROS release (reviewed in [11,86]). Uptake of low concentrations of Ca^{2+} by mitochondria can decrease ROS release due to the temporary decrease in protonmotive force and, possibly, loss of pyrimidine nucleotides [87,88]. On the other hand, uptake of higher Ca^{2+} quantities can significantly increase ROS release from mitochondria [89–92], possibly due to interactions with inner mitochondrial membrane cardiolipin, leading to structural changes in the membrane-inserted electron transport chain [93].

When accumulated by mitochondria at high levels, and associated with conditions of oxidative stress, Ca^{2+} ions can lead to extensive changes in mitochondrial function, including a non-selective form of inner membrane permeabilization known as the mitochondrial permeability transition (see Fig. 1, reviewed in [82,86,94]). Ca^{2+}-induced mitochondrial dysfunction has been associated with a wide variety of disorders, including dyslipidemias and diabetes [95–97]. On the other hand, although Ca^{2+} uniporters in the mitochondrial membrane have a large set of elements suggestive that they may be involved in dysfunctions associated with metabolic diseases, the lack of a molecular identity has hampered direct studies indicating if this is indeed the case.

5. K^+ uniporters

K^+ uniporters were first described in inner mitochondrial membranes in the early 1990s [98,99]. The presence of a regulated K^+ entry pathway into mitochondria was surprising, since K^+ is the main intracellular cation and leaks at small but significant rates through the mitochondrial lipid bilayer, reaching the matrix. Today, it is evident that there are many functional advantages in having a regulated K^+ entry pathway in addition to a K^+ leak (reviewed in [14,100–102]).

K^+ uptake into mitochondria is inhibited by ATP and sulfonylureas, leading to the characterization of these K^+ uniporters as ATP-sensitive K^+ channels (mitoK_{ATP}, [99,103,104]). Other more recent findings suggest mitochondria may also have Ca^{2+} activated and/or voltage-gated Kv1.3 potassium channels and the twin-pore domain TASK-3 potassium channels (reviewed in [105]), but the roles of these are still poorly understood.

K^+ uptake is driven by the electrochemical potential and accompanied by phosphate and water, resulting in matrix swelling, which in turn dilutes matrix Mg^{2+} and activates the K^+/H^+ exchanger [14]. The net result is K^+ cycling and lower protonmotive force. The decrease in protonmotive force is, however, dependent on the transport efficiency of the K^+ channel. In keeping with the reality that mitochondria must be able to maintain oxidative phosphorylation, K^+ transport through mitoK$_{ATP}$ channels is very limited, and often undetectable by conventional measurement techniques [106]. MitoK$_{ATP}$ channels are thus a (very) mild uncoupling pathway (Fig. 1).

Over the years, many functions have been proposed for mitochondrial K^+ uniporters. Since their activity results in water uptake by the organelle, they play a role in the regulation of mitochondrial matrix volume, which may be important to maintain the structural relationship between the inner and outer membrane [14,106]. Another possible role for mitoK$_{ATP}$ may be to regulate mitochondrial ΔpH, since the activity of this channel may result in matrix alkalinization [107].

Based on the fact that uncoupling, even to a very mild degree, can prevent mitochondrial ROS release [52,108–111], we proposed that K^+ cycling due to mitoK$_{ATP}$ activity could act as a regulating pathway to control rates of ROS release in mitochondria [100,112–115]. Although this hypothesis has met some resistance [116], the idea is supported by the finding that mitoK$_{ATP}$ channels are strongly activated by oxidants, and inhibited by thiol reductants [100,114,115,117–120]. Furthermore, the redox effects of this channel explain, at least in part, why mitoK$_{ATP}$ activation is protective against acute tissue damage [113,115] (see Fig. 3). Other protective activities of this channel include regulating mitochondrial volume, the physical relationship between the inner and outer membrane and, as a result, transport of metabolites into mitochondria (reviewed in [100]).

Although the strong protective effects of mitoK$_{ATP}$ in situations of acute tissue damage have attracted most of the attention in the area, it is most probable the main role of these channels is not related to acute stimuli. In this sense, the first evidence that these channels could have a more general role regulating energy metabolism was uncovered by Vercesi's group [121], who found that mitoK$_{ATP}$ channel activity was higher in the livers of transgenic hypertriglyceridemic mice. Interestingly, the higher activity of mitoK$_{ATP}$ in these animals results in increased oxidative metabolism and a lower efficiency of energy conversion in these animals, preventing obesity. In view of these results, it is tempting to propose that mitoK$_{ATP}$ channels may act as modulators of animal energy metabolism and, as such, may play a central role in metabolic disorders [95,121]. Further studies have revealed that hypertriglyceridemic mice, while presenting indicators of oxidative stress in cytosolic extracts from their livers, present a protection against mitochondrial oxidation that is dependent on mitoK$_{ATP}$ activity [122]. Thus, the redox role of mitoK$_{ATP}$ channels is important also within metabolic alterations.

Since intracellular insulin responses involve Akt-dependent pathways (for a review see [123]), and mitoK$_{ATP}$ can be activated by Akt, at least in the ischemic heart [124,125], it is tempting to propose that mitoK$_{ATP}$ channels may have interesting functions under conditions of insulin resistance, although this possibility is largely unexplored. An indirect indication that mitoK$_{ATP}$ channels have changes in their activity in diabetes is the finding that protection against ischemic damage conferred by preconditioning, a process mediated by mitoK$_{ATP}$ (for reviews see [100,126]), is abrogated in models of this disease [127–130]. Another often overlooked point of relevance in diabetes is that treatment of the disorder with sulfonylureas such as glybenclamide (reviewed in [131]) not only increases insulin secretion due to the inhibition of β-cell K$_{ATP}$ channels (see Fig. 2) but may also inhibit mitoK$_{ATP}$ channels, with yet unknown metabolic and redox consequences (see Fig. 3).

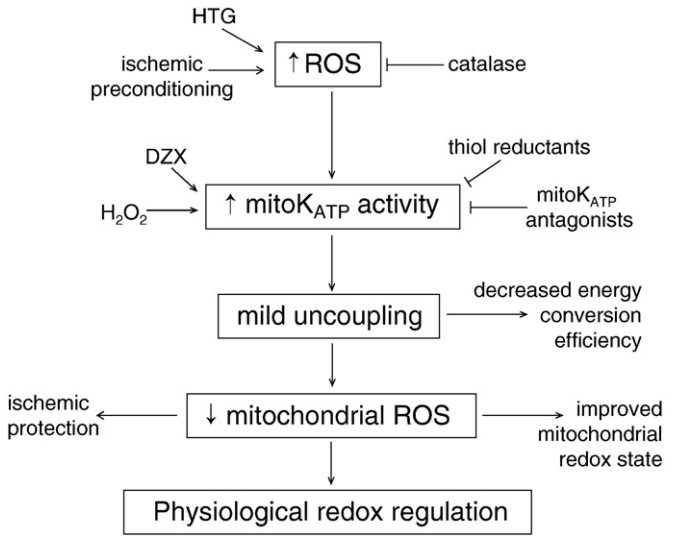

Fig. 3. MitoK$_{ATP}$ channels: redox-sensitive pathways that control physiological and pathological ROS release. Physiological increases in ROS levels in the mitochondrial microenvironment lead to the activation of mitoK$_{ATP}$ channels, resulting in mild uncoupling and controlling the production of oxidants in mitochondria. Specific pathological conditions also alter redox state, resulting in the activation of this pathway: ischemic preconditioning in the heart increases ROS release, resulting in mitoK$_{ATP}$ activation and ischemic protection associated with decreased ROS formation during reperfusion. Preconditioning is mimicked by oxidants such as H_2O_2 and mitoK$_{ATP}$ agonists (including diazoxide, DZX), and is inhibited by antioxidants and mitoK$_{ATP}$ antagonists. Hypertriglyceridemia (HTG) increases ROS and activates mitoK$_{ATP}$, resulting in decreased efficiency of energy conversion and an improvement in the mitochondrial redox state. Thiol reductants, which inhibit mitoK$_{ATP}$, prevent these effects.

6. Final remarks

Altogether, many different approaches support the idea that ion transport rates across the inner mitochondrial membrane may be determinant in the regulation of energy metabolism. As a result, changes in activities of these transporters are certainly important as causes and response mechanisms in metabolic diseases. Unfortunately, studies in the field are limited by methodological difficulties regarding measuring the activities of ion channels *in vivo*. We believe the relationship between mitochondrial ion transport and metabolic disorders is an area that should be explored more intensely in the near future.

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