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Cation transport in mitochondria – the potassium cycle

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Abstract

The existence in mitochondria of separate, highly regulated pathways for K^+ influx and efflux strongly implies that mitochondrial volume is subject to regulation in vivo. Volume, in turn, has been shown to regulate activity of the electron transport chain. Thus, the mitochondrial K^+ cycle appears to play a key signalling role in regulating cellular bioenergetics, including the metabolic fate of fatty acids. Consistent with this role, the channel is inhibited by long-chain acyl-CoA esters and activated by GTP, and these ligands interact with sites that face the cytosol. The work to be summarized shows that K_{ATP} channels from mitochondria and plasma membranes are regulated by the same biochemical and pharmacological ligands. We hypothesize that the mitochondrial K_{ATP} channel, like its counterparts in the plasma membrane, is heteromultimeric, consisting of a regulatory sulfonylurea receptor (mitoSUR) and an inward-rectifying K^+ channel (mitoKIR).

Keywords: Potassium transport; Potassium/proton antiporter; Potassium ion channel opener; Sulfonylurea receptor; Potassium ion channel; Mitochondrion

1. Introduction

The mitochondrial K^+ cycle consists of electrophoretic K^+ uptake and electroneutral K^- efflux across the inner membrane. The redox energy consumed by the cycle is the cost of regulating matrix volume [1]. Two transporters have been identified that catalyze K^+ transport, and both are highly regulated. Efflux is mediated by the K^+/H^+ antiporter, whose existence was predicted by Mitchell [2] and first demonstrated nearly 20 years later [3]. A reconstitutively active K^+/H^+ antiporter from liver and heart mitochondria has been identified as an 82 kDa inner membrane protein [4]. Influx is mediated by the mitochondrial K_{ATP} channel (mitoK_{ATP}). K_{ATP} channels were identified in plasma membranes of cardiac myocytes in 1984 [5], and mitoK_{ATP} were discovered in 1991. Inoue et al. [6] reported evidence from patch clamp studies of fused mitoplasts at the same time that we began describing reconstitution of a highly purified mitoK_{ATP}. Although it possesses unique properties, mitoK_{ATP} is regulated by every ligand that regulates the plasma membrane K_{ATP} channels (cellK_{ATP}); consequently, we infer that it belongs to the same gene family [7–10].

A primary role of the regulated K^+/H^+ antiporter is to compensate for unregulated K^+ leakage into the matrix, driven by the high voltages required for oxidative phosphorylation. Thus, the K^+/H^+ antiporter is responsible for *volume homeostasis* and is essential for maintaining vesicular integrity in the face of high ionic traffic across the inner membrane.

The discovery of mitoK_{ATP} has profound new implications for the physiological role of the mitochondrial K^+ cycle, because the existence of a regulated K^+ influx pathway now permits *volume regulation*. For example, opening mitoK_{ATP} will transiently shift the balance between K^+ uniport and K^+/H^+ antiport until the antiport catches up with the higher rate of K^+ influx. This will cause transient swelling and will result in a higher steady-state volume for as long as mitoK_{ATP} remains open. Such a 'regulated interplay' between K^+ uniport and K^+/H^+ antiport was correctly postulated many years ago by Brierley [11].

2. Materials and methods

We have studied mitoK_{ATP} reconstituted from beef heart, rat liver and brown adipose tissue mitochondria. We have also studied cellK_{ATP} reconstituted from beef heart sarcolemmal vesicles. MitoK_{ATP} channel activity has been

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quantitated using three techniques: electrical currents were measured in lipid bilayer membranes following fusion of vesicles containing purified mitoK$_{ATP}$ or cellK$_{ATP}$. K^+ flux in proteoliposomes was measured using steady-state spectroscopy of the fluorescent probe, PBFI. K^+ flux in intact mitochondria was measured using light scattering. These protocols are fully described in our publications and reviewed in Ref. [12].

3. Results and discussion

3.1. Matrix volume is determined by net potassium flux

When K^+ influx and efflux are out of balance, the resulting net K^+ flux will be accompanied by electroneutral flux of anions and osmotically obligated water [13]. Because matrix [K^+] is about 180 mM, net K^+ transport will have little effect on the matrix concentration of K^+, but it will have a profound effect on matrix volume. Any departure from zero net K^+ flux will cause a change of matrix volume. The importance of regulating K^+ flux to zero in the steady state is illustrated by the following: uncompensated K^+ uptake amounting to as little as 10% of H^+ pumping would double matrix volume within 1–2 min [14]. The K^+/H^+ antiporter is inhibited by matrix Mg^{2+} ($K_i \approx 300$ μM) as well as by matrix protons, and the concentrations of these inhibitors decrease with uptake of K^+ salts, causing compensatory activation of K^+ efflux [13].

3.2. Matrix volume regulates electron transport

Fatty acids are the fuel for thermogenesis by brown adipose tissue mitochondria, and their rate of oxidation is strictly controlled by matrix volume [15]. A thorough characterization of this phenomenon by Halestrap and coworkers [16] has led to the following conclusions: (1) Increased matrix volume, over the narrow range thought to obtain in vivo, greatly stimulates activity of the respiratory chain in both heart and liver mitochondria. β-Oxidation of fatty acids is particularly sensitive to matrix volume. (2) The site of activation has been localized to membrane enzymes that feed electrons to ubiquinone. (3) Matrix volume changes have been observed in vivo during respiratory stimulation secondary to hormonal activation of liver [16] and brown adipose tissue [17]. (4) The molecular mechanism of volume activation is not known, but may involve a stretch receptor.

These findings point to a signalling role for mitoK$_{ATP}$ in determining the metabolic fate of fatty acids and regulating cellular bioenergetics, as has also been suggested by Halestrap [18] and Szewczyk et al. [19].

3.3. Basic properties of mitoK$_{ATP}$

K^+ flux through reconstituted mitoK$_{ATP}$ is highly selective for K^+ and unaffected by Na^+ or tetraethylammonium. MitoK$_{ATP}$ is not voltage-gated, and the flux-voltage dependence is consistent with a channel containing a single energy well near the center of the membrane. The open channel conductance of mitoK$_{ATP}$ is about 16 pS in symmetric 150 mM KCl [10,20]. MitoK$_{ATP}$ activity has also been demonstrated in intact mitochondria [19,21]. To control for the unavoidable coexistence of K^+ diffusion, we compared the effects of ATP on fluxes of K^+ and TEA^+. K^+ flux was inhibited by ATP to the level of TEA^+ flux. TEA^+ flux was unaffected by ATP [21].

3.4. Regulation of mitoK$_{ATP}$ by nucleotides and long-chain acyl-CoA esters

MitoK$_{ATP}$ is subject to complex regulation by metabolites, in keeping with the hypothesis that this channel is involved in regulating electron transport. Following is a summary of $K_{1/2}$ values for inhibitors and openers [7,9,10]:

3.4.1. Inhibition of K^+ flux

ATP:	$K_{1/2} = 22–30$ μM
ADP:	$K_{1/2} = 160–200$ μM
Palmitoyl CoA:	$K_{1/2} = 0.25$ μM
Free Mg^{2+}:	$K_{1/2} = 80$ μM in 500 μM ATP.
Free Ca^{2+}:	$K_{1/2} = 150$ μM in 500 μM ATP.

3.4.2. Activation of inhibited K^+ flux

	500 μM ATP	500 μM ADP	1 μM palm CoA
GTP: $K_{1/2}$ =	3–7 μM	0.12 μM	230 μM
GDP: $K_{1/2}$ =	140 μM	3 μM not	done

ATP and ADP are mutually competitive inhibitors of K^+ flux through mitoK$_{ATP}$. Very careful studies demonstrate that the Hill slope for ATP inhibition is 1.0 and increases to 2 in the presence of 20 μM GTP. K^+ flux through mitoK$_{ATP}$ is inhibited by palmitoyl CoA in both mitochondria and proteoliposomes. The Hill slope for palmitoyl CoA inhibition is 2.

Inhibition of mitoK$_{ATP}$ exhibits an absolute requirement for divalent cations. ATP or palmitoyl CoA have no effect in the absence of Mg^{2+}, and Mg^{2+} has no effect in the absence of ATP or palmitoyl CoA. Mg^{2+} also prevents glyburide inhibition. Since glyburide and palmitoyl CoA are not Mg^{2+} chelators, these findings indicate that Mg^{2+} interacts independently with mitoK$_{ATP}$ and not as a MgATP complex.

The high affinity of mitoK$_{ATP}$ for ATP raised the conundrum of how this channel can be opened under physiological conditions. We hypothesized that endogenous activators overcome the high affinity for ATP, and

we have now confirmed this hypothesis by showing that guanine nucleotides reverse the inhibition by ATP, ADP or palmitoyl CoA in both mitochondria and proteoliposomes.

Guanine nucleotides are competitive with ATP. GTP appears to react with a high affinity (0.2 μM) and a low affinity (15–20 μM) site, whereas GDP appears to react with two low affinity sites (20 μM). ATP is unable to inhibit in the presence of physiological GTP concentrations; for example, 20 μM GTP increased the $K_{1/2}$ for ATP inhibition from 21 μM to 6 mM. On the other hand, the $K_{1/2}$ for GTP activation of palmitoyl CoA inhibition is in the physiological range. Our thinking is that ATP does not regulate mitoK_{ATP} at all, because the nucleotide-binding sites are always occupied by the high affinity ligands. We suggest that the open/closed state of the channel is determined in vivo by the relative occupancy of the sites by GTP or long-chain acyl CoA esters. We speculate that the 2 binding sites correspond to the nucleotide binding folds on mitoSUR.

3.5. Orientation of mitoK_{ATP} in the inner membrane

In order to understand the signalling role of mitoK_{ATP} in cell bioenergetics, it is necessary to know where its regulatory sites are located. Do they face the matrix, as suggested by Inoue [6] and Halestrap [18], or do they face the cytosol? Do they all coexist on the same pole of the protein? Experiments with proteoliposomes, mitochondria and BLM provided an unambiguous answers to these questions: the mitoK_{ATP} regulatory sites for Mg^{2+}, ATP, GTP and palmitoyl CoA face the cytosol [10].

3.6. MitoK_{ATP} is an intracellular receptor for K^+ channel openers

K^+ channel openers (KCOs) activate ATP-inhibited K_{ATP} channels and exhibit a rich and clinically important pharmacology [22]. It is important to establish whether these drugs, which distribute throughout the cell, also act on mitochondrial K_{ATP} channels in their therapeutic range. We have found that KCOs reverse inhibition by ATP, ADP, palmitoyl CoA and glyburide, and that the $K_{1/2}$ values (below) are well within the range observed with cellK_{ATP} from various tissues [22]. Similar results were obtained in intact mitochondria.

KCOs reverse inhibition by 500 μM ATP:

Cromakalim: $K_{1/2} = 1$ μM

Diazoxide: $K_{1/2} = 0.4$ μM

EMD 60480: $K_{1/2} = 1$ nM

EMD 57970: $K_{1/2} = 6$ nM

(The EMD drugs are experimental benzopyranyl derivatives (cromakalim analogues) from E. Merck and Co.)

It is important to stress that KCOs cannot open an open channel – they have no effect on uninhibited K^+ flux, which is also characteristic of cellK_{ATP}. Our finding that KCOs in the therapeutic range do not open the uninhibited channel is consistent with the results of Szewczyk et al. [23], who did not include Mg^{2+} and ATP in the assay medium used for their studies.

Recognition of mitoK_{ATP} as an intracellular receptor for KCOs adds a new dimension to KCO pharmacology, which has been focused exclusively on plasma membrane K_{ATP} channels. A particularly exciting development in KCO pharmacology is the finding by Grover and colleagues [24] that KCOs are cardioprotective during experimental ischemia. KCO-treated hearts maintain higher ATP levels and exhibit reduced infarct size and enhanced post-ischemic recovery upon reperfusion. All of these effects are blocked by glyburide. These pharmacological effects point to a role of K_{ATP} channels in myocardial protection; but the receptor for these effects has not been identified, and a mitochondrial site of action cannot be excluded [25].

We have now demonstrated that cardiac mitoK_{ATP} and cellK_{ATP} can be distinguished pharmacologically [8]. We compared drug sensitivities of cardiac mitoK_{ATP} and cellK_{ATP} reconstituted from beef heart mitochondria and sarcolemma, respectively. MitoK_{ATP} and cellK_{ATP} exhibit similar sensitivities to cromakalim and other benzopyran derivatives; however, they differ strikingly in their sensitivity to diazoxide. Cardiac mitoK_{ATP} is 2000 times more sensitive to diazoxide than cardiac cellK_{ATP}. In the ischemic model, diazoxide was cardioprotective over the same dose range as cromakalim and at doses that had no effect on cellK_{ATP} (G. Grover, personal communication). These findings raise the strong and exciting possibility that mitoK_{ATP} is the receptor for the cardioprotective effects of KCOs.

3.7. MitoK_{ATP} is an intracellular drug receptor for glyburide

Glyburide is an antidiabetic sulfonylurea that triggers insulin release from pancreatic β-cells, presumably by inhibiting β-cellK_{ATP} [22]. Glyburide is a potent inhibitor of K^+ flux through reconstituted mitoK_{ATP}, with $K_{1/2} =$ 60 nM in the absence of Mg^{2+} [7]. We were unable to detect inhibition of K^+ flux in intact mitochondria [21], and we have observed profound inhibition of TEA^+ flux at the high doses used by Belyaeva et al. [26]. We have recently reinvestigated this phenomenon and found that glyburide is very potent in mitochondria ($K_{1/2} = 24$ nM) when the assay is preceded by preincubation with the drug. These preliminary results suggest that the kinetics of glyburide binding are slow, but this remains to be confirmed.

3.8. Is mitoK_{ATP} a heteromultimeric complex?

Each of the metabolic and pharmacological ligands that modify cellK_{ATP} also modify mitoK_{ATP}, and they do so in

the same dose range. MitoK_{ATP} is distinctive in its absolute requirement for divalent cations for nucleotide inhibition. A second distinction is found in the effects of ADP and long-chain acyl-CoA esters. Most cellK_{ATP} are opened by MgADP, and Corkey and coworkers [27] have recently demonstrated that the β-cellK_{ATP} is opened by oleoyl CoA in the 200–300 nM range. The parallel, albeit opposite, effects of ADP and long-chain acyl-CoA esters are consistent with the fact that the CoA moiety is an ADP analogue.

These distinctions must reside in the communication between the nucleotide regulatory domain and the transport domain. This communication has been clarified by the recent achievements of the Bryans' laboratory [28–30], who have succeeded in the molecular cloning of β-cellK_{ATP}. Their work shows that cellK_{ATP} consists of two separate proteins, a sulfonylurea receptor (SUR) and a separate inward-rectifying K^+ channel (KIR). SUR is a member of the ATP-binding cassette (ABC) family and contains two nucleotide-binding folds. When SUR was co-expressed with Kir6.2, full K_{ATP} channel activity was observed, including regulation by ATP, sulfonylureas, and diazoxide.

Preliminary studies have led us to a working hypothesis [10] that mitoK_{ATP} is also a heteromultimer consisting of an inward rectifying K^+ channel, mitoKIR, and a sulfonylurea receptor, mitoSUR. This hypothesis is under active investigation in our laboratory.

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