Acta Biochimica Polonica

Vol. 43 No. 4/1996

713–720

QUARTERLY

Minireview

The ATP-regulated K^+ channel in mitochondria: five years after its discovery*

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Received: 3 October, 1996

Key words: mitochondria, K^+ channel, sulfonylurea, ATP

Mitochondria contain a potassium specific channel (mitoK$_{ATP}$ channel) sensitive to ATP and antidiabetic sulfonylureas. The mitochondrial K$_{ATP}$ channel plays an important role in the mitochondrial volume control and in regulation of the components of protonmotive force. This minireview describes the properties and current hypotheses concerning the function of mitoK$_{ATP}$ channel.

DISCOVERY OF THE MITOCHONDRIAL ATP-REGULATED POTASSIUM CHANNEL

Five years ago an ionic channel highly selective for K^+ was found in the inner membrane of rat liver mitochondria [1]. This channel is blocked by ATP (hence the abbreviation "mitoK$_{ATP}$ channel" is used throughout the text) and by the antidiabetic sulfonylurea derivative, glibenclamide. Later on, the mitoK$_{ATP}$ channel was partly purified from the inner membrane of rat liver and beef heart mitochondria and was shown to catalyze electrophoretic K^+ transport when reconstituted into phospholipid liposomes [2]. In addition to liver and heart mitochondria, glibenclamide-sensitive potassium transport has recently been discovered in yeast mitochondria [3].

The mitoK$_{ATP}$ channel shares some pharmacological properties with the ATP-regulated K^+ channel (K$_{ATP}$ channel) found in plasma membranes of various cells, including endocrine, smooth muscle, and skeletal muscle cells as well as in neurons [4, 5]. Plasma membrane K$_{ATP}$ channels constitute an important class of ionic channels linking cellular bioenergetics to plasma membrane potential, playing the role of a cytosolic ATP sensor [4].

This overview summarizes five years of investigations on the mitoK$_{ATP}$ channel. Essential properties and current hypotheses concerning the function of this channel are described. Additionally, I would like to draw the reader's attention to future prospects in the mitoK$_{ATP}$ channel field. Detailed information on the mitoK$_{ATP}$ channel pharmacology was presented previously [6].

ISOLATION, PURIFICATION AND RECONSTITUTION OF THE MITOCHONDRIAL K$_{ATP}$ CHANNEL

The K$_{ATP}$ channel from liver and heart mitochondria was isolated, partially purified and reconstituted into liposomes and "black" lipid membranes by the group of Garlid [2]. The purification was performed after solubilization

*This work was supported by a grant No. 6 P203 003 04 from the State Committee for Scientific Research.
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of mitochondrial inner-membrane with Triton X-100 and chromatography on DEAE-cellulose. On SDS-polyacrylamide gel electrophoresis the preparation gave several minor protein bands and a major protein band corresponding to 54 kDa. Reconstitution of this preparation into liposomes conferred the electrophoretic transport of K^+, measured with the fluorescent probe: potassium-binding benzofuran isophtalate (PBFI), into vesicles [2]. Typically for K_{ATP} channels, the transport was reduced by ATP and by antidiabetic sulfonylureas. Interestingly, Mg^{2+}, which alone had no effect on the channel activity, was found to reduce drastically the inhibitory potency of glibenclamide (K_i increased from 0.062 μM to 3.1 μM, in the presence of 3 mM Mg^{2+}).

Reconstitution of the partially purified mitoK_{ATP} channel into planar phospholipids membranes ("black" lipid membranes) was also performed and enabled to estimate single channel conductance as 30 pS at saturating K^+ concentration [2].

PROPERTIES OF THE MITOCHONDRIAL K_{ATP} CHANNEL

Regulation by ATP and guanine nucleotides

The mitoK_{ATP} channel is reversibly inactivated by ATP [1]. The channel activity was half inhibited at 0.8 mM ATP and almost completely blocked at > 2 mM. By contrast, neither ADP nor GTP had a significant effect on the channel activity. ATP sensitive currents ran down gradually in inside-out patches, reactivation of the channel was observed after addition of ATP to, and its removal from, the bathing solution during the run-down period [1]. Similarly to the plasma membrane K_{ATP} channel, run-down of the mitoK_{ATP} channel involves dephosphorylation of ionic channel protein.

Further studies on ATP inhibition of the mitoK_{ATP} channel were performed after partial purification and reconstitution of the channel into phospholipid liposomes [2]. A strong inhibitory effect of ATP on K^+ transport, similar to that described by Inoue *et al.* [1], was observed ($K_{1/2}$ = 22–30 μM). Mg^{2+} was required to obtain inhibition by ATP: neither ATP nor Mg^{2+} alone was effective. In contrast, in intact mitochondria potassium transport was inhibited by Mg^{2+} alone. It is not clear why inhibition of the mitoK_{ATP} channel by ADP was observed after reconstitution into proteoliposomes [2] but not in patch-clamp studies on mitoplasts [1].

It has been shown that guanine nucleotides reverse the inhibition of mitoK_{ATP} channel by ATP and ADP [7]. GTP and GDP are able fully to activate the channel in the presence of 500 μM ATP with K_a of 7 μM and 140 μM, respectively. It has been proposed that the endogenous activators (GTP or GDP) overcome the high affinity binding for ATP and play a role of physiological regulators of the mitoK_{ATP} channel [7].

An important point concerns orientation of the mitoK_{ATP} channel in the inner-membrane. According to Garlid [7], regulatory sites for ATP and GTP face the cytosol. There are also some observations suggesting that the ATP binding site faces the matrix compartment [1]. This problem, important for understanding the role of the mitoK_{ATP} channels in signal transduction, needs further clarification.

Blockers of the mitochondrial K_{ATP} channel

The majority of information about the effects of various chemical substances on the electrophoretic K^+ transport in mitochondria comes from studies on intact (isolated) mitochondria. Experiments of this kind are complicated because the mitochondrial inner membrane contains several K^+ (or other monovalent cation) transporting systems [8, 9] and the differentiation between them is not always simple. It is well known that respiring mitochondria exhibit electrophoretic K^+ uptake, the process known as K^+ uniport. Modulation of the activity of this uniport by adenine nucleotides has been studied and it was demonstrated that K^+ influx was inhibited by adenine nucleotides with low IC_{50} values of 0.5 and 2.3 μM for ADP and ATP, respectively [10]. The K^+ transport was also inhibited by AMP and by a nucleotide analog belonging to the triazine dye family, Cibacron Blue F3GA [10]. It was concluded that K^+ uniport activity involved at least partly the mitoK_{ATP} channel [10].

Recently, it has been shown that the K^+ uniport induced by magnesium depletion is blocked by glibenclamide in a concentration-dependent manner [11]. Additionally, other antidiabetic sulfonylureas are also able to block the K^+ uniport induced in this way [12].

Surprisingly, glibenclamide also inhibits Na^+ and Ca^{2+} uniport in rat liver mitochondria with a similar affinity [11]. There are several possibilities for the interpretation of the mechanism of glibenclamide parallel action on K^+, Na^+, and Ca^{2+} uniports in mitochondria. The equilibrium binding studies point to the presence of a single class of binding sites for sulfonylurea in the inner mitochondrial membrane [11]. Hence, one cannot exclude the possibility that a sulfonylurea-binding protein (mitochondrial sulfonylurea receptor) interacts with the proteins forming in membrane pores for K^+, Na^+ and Ca^{2+}. It is difficult to draw any conclusions concerning the mitochondrial receptor for glibenclamide on the basis of studies performed on the plasma membrane sulfonylurea receptor. But it is worth mentioning that the sulfonylurea receptor cloned from β-cells is unable to form a K^+ channel [13] and, only after co-expression with the channel protein, it builds a functional complex having the same properties as the K_{ATP} channel in native membranes [14]. It will be the subject of further investigations to determine whether a similar mechanism concerns the mitochondrial sulfonylurea receptor and monovalent cation uniports.

In order to characterize the interactions of glibenclamide with the inner mitochondrial membrane, equilibrium binding studies were performed using ^3H-labeled glibenclamide [11]. As described by Garlid and co-workers [2], in the reconstituted system, inhibition of the mitochondrial K_{ATP} channel by glibenclamide occurs at its nanomolar concentration range, with IC_{50} of 62 nM. This suggests the presence of high-affinity binding sites in the inner mitochondrial membrane. In contrast, in intact rat liver mitochondria the presence of only a single class of low-affinity binding sites for glibenclamide in the inner mitochondrial membrane, with K_d of 4 μM, was found [11]. The same results were obtained with the use of ^{125}I-glibenclamide of higher specific radioactivity than that of the tritiated compound (Szewczyk, unpublished observation). Moreover, according to Garlid and co-workers [2], the properties of the rat liver and beef heart mitoK_{ATP} channels are the same in terms of interactions with glibenclamide. In intact beef heart mitochondria K_d for glibenclamide binding is by one order of magnitude lower, 300 nM, than that in rat liver mitochondria [11].

Some discrepancy between the results obtained with intact mitochondria and proteoliposome, concerning glibenclamide concentration, was observed (see [6]). Probably the interaction of glibenclamide with its target protein in the reconstituted system may differ from that in the inner mitochondrial membrane because of differences in their lipid composition. In fact, in all studies performed on the inner mitochondrial membrane preparations (mitoplasts and intact mitochondria) glibenclamide has been used at concentrations ranging from 5 to 150 μM in order to inhibit K^+ transport [1, 15, 16]. The relatively high concentration of glibenclamide required to inhibit the K^+ and Na^+ uniport in energized mitochondria can be explained by the fact that the mitochondria are known to maintain a high concentration of adenine nucleotides which can, by themselves, lower the affinity of glibenclamide to its putative receptor [17].

The pH dependence of the inhibition of K^+ uniport activity by glibenclamide, i.e. the observation that acidic pH raises the potency of glibenclamide to inhibit these processes [11], suggests that the blocking property of glibenclamide might be restricted to the undissociated form of the drug (pK_a of glibenclamide 6.3, [18]). This property resembles the mode of action of glibenclamide on the plasma membrane K_{ATP} channel [18]. A binding study [19] showed, that in the cardiac muscles, acidification led indeed to an increase of glibenclamide binding to its receptor, while alkalization caused its decrease. Observations on mitochondria may suggest that the interaction of sulfonylurea with its target (sulfonylurea receptor) occurs within the mitochondrial membrane [11]. Probably, thiol groups of mitochondrial sulfonylurea receptor are also important for glibenclamide binding. Treatment of mitochondrial membranes with mersalyl or *N*-ethylmaleimide, substances reacting with SH groups, affects both B_{max} and K_d of glibenclamide binding (Szewczyk, Wójcik, unpublished observation).

Similarities in the properties of plasma membrane K_{ATP} channels and mitoK_{ATP} channels concern also their interaction with non-sulfonylurea inhibitors. Plasma membrane K_{ATP} are

blocked by some non-sulfonylureas (for example, α-adrenoreceptor blockers, phentolamine and clonidine, could inhibit K_{ATP} channels in β-cells). In insulinoma cells the K_{ATP} channel was also blocked by 8-methoxypsoralen [20] and calcium antagonist TMB-8 [21]. Recently, it has been shown that a guanidine derivative U-37883A acts as a vascular K_{ATP} channel antagonist [22, 23]. This compound was also shown to inhibit the K^+ influx into rat liver mitochondria [24] thus suggesting that it is active against the mitoK_{ATP} channel as well. Moreover, U-37883A binds in mitochondria to a different binding site than does sulfonylurea, similarly as in the case of the plasma membrane sulfonylurea receptor [25].

Activators of the mitochondrial K_{ATP} channel

Plasma membrane K_{ATP} channels are specifically activated by such drugs as diazoxide, pinacidil and minoxidil sulfate, known as potassium channel openers (for review see [26]). Potassium channel openers constitute a chemically diverse group of compounds which are able to increase the permeability to potassium ions of various cells containing K_{ATP} channels [26].

It has been found that the potassium channel opener RP66471 induces a decrease of the mitochondrial membrane potential [27]. Since neither the inhibition of mitochondrial respiration nor the uncoupling of mitochondria was observed concomitantly, a specific effect on the mitochondrial membrane potential was postulated. It has been therefore concluded that this effect is caused by the increase of permeability of the inner mitochondrial membrane to potassium ions. Interestingly, the effect of RP66471 on mitochondria was found to be specific for this compound. All other potassium channel openers applied, such as Ro 31-6930, KRN 2391, aprykalim and nicorandil, were unable to collapse the membrane potential of energized mitochondria. Comparison of RP66471-induced depolarization in the presence of various monovalent cations: Li^+, Na^+, K^+ and Rb^+, showed that the amplitude of depolarization in the presence of K^+ was significantly larger than that in the presence of Li^+ or Na^+. However, in the presence of rubidium ions RP66471 induced a significant depolarization of the membrane, similar to that observed in the presence of K^+. Recently, other potassium channel opener were shown to activate potassium transport in mitochondria. ATP-inhibited K^+ flux was restored by diazoxide ($K_{1/2}$ = 0.4 μM), cromakalim ($K_{1/2}$ = 1 μM) and two developmental cromakalim analogues, EMD60480 and EMD57970 ($K_{1/2}$ = 6 nM) [28]. Cu^+ ions at micromolar concentrations were also shown to induce glibenclamide sensitive K^+ transport in mitochondria [29].

POSSIBLE FUNCTIONS OF THE MITOCHONDRIAL K_{ATP} CHANNEL

Physiological functions of the mitoK_{ATP} channels are not clear. This is because of confusing regulation of this channel. In the view of the physiological concentration of matrix ATP the mitoK_{ATP} channel should be closed constantly. Only a dramatic lowering of the matrix ATP level should activate the channel and this would only lead to membrane depolarization followed by permanent inhibition of mitochondrial metabolic activity. It may be speculated that, in spite of the fact that ATP inhibits the mitoK_{ATP} channel activity, "ATP depletion" is not the only physiological process leading to channel activation. Probably other efectors, like GTP or GDP, are involved in fine regulation of the mitoK_{ATP} channel activity [30].

According to the present knowledge, the mitoK_{ATP} channel may have a dual physiological function. Firstly, a concerted action of the electrophoretic K^+ uniport and the electroneutral K^+/H^+ exchange is believed to be the main mechanism responsible for maintaining potassium homeostasis within the mitochondrion and thus for controlling intramitochondrial osmotic pressure and mitochondrial volume. Regulatory volume changes are regarded as one of the important mechanisms of metabolic control at the mitochondrial level (for review see [31]). It is of particular importance to establish whether the mitoK_{ATP} channel activity could be, at least partly, responsible for the regulation of mitochondrial metabolism [32]. Observations that glibenclamide and ATP inhibit mitochondrial swelling whereas potassium channel openers potentiate the swelling make it likely that this channel, perhaps together with other potassium pathways, is involved in mitochondrial regulatory volume changes [33–35].

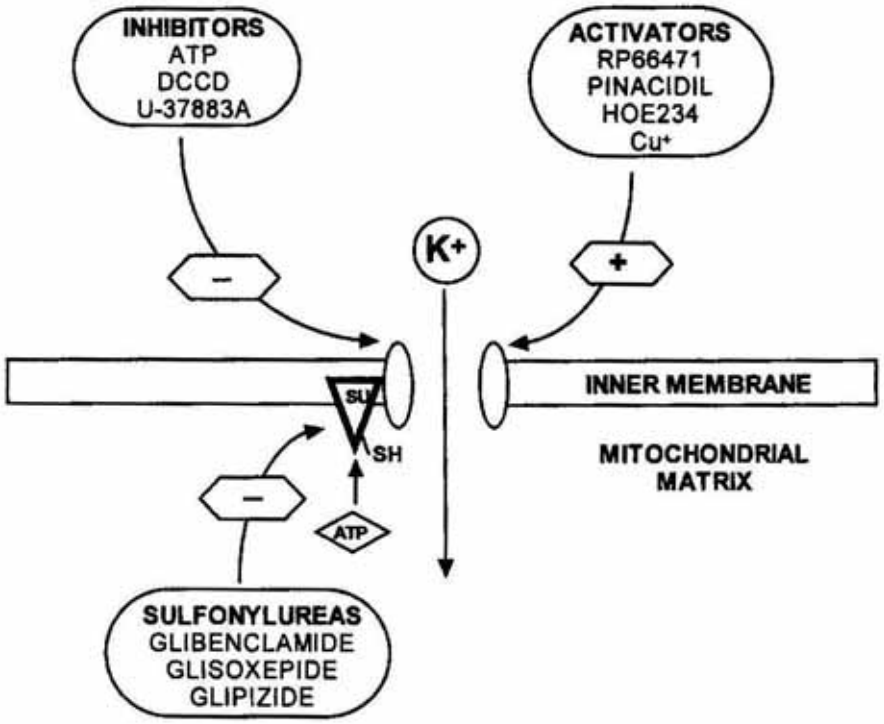

Fig. 1. Interaction of the mitochondrial K_{ATP} channel with various efectors. **SU,** *mitochondrial sulfonylurea receptor.*

Secondly, energization of mitochondria is accompanied by a net uptake of K^+, partly inhibited by glibenclamide and activated by the well known openers of the plasma membrane potassium channel, pinacidil and P1060 [36]. This is compatible with the hypothesis that potassium uptake upon energization partly compensates the electric charge transfer produced by the proton pump and thus enables the formation of ΔpH along with ΔΨ. This was further substantiated by the observation that the rate of ΔpH formation increases with increasing K^+ concentration in the external medium and thus with increasing rate of K^+ influx [37]. The final steady-state value of ΔpH also increases whereas that of ΔΨ decreases at increasing K^+ concentration so that the resultant protonmotive force remains practically unchanged. The assumption that K^+ transport accounts for the formation of ΔpH is also supported by the observation that both the rate of ΔpH formation and its steady-state level in energized mitochondria are increased by the potent opener of ATP-regulated K^+ channel, RP66471 [37]. As shown previously [27], this compound decreases ΔΨ of energized liver mitochondria by increasing the permeability of the inner mitochondrial membrane to K^+.

All this would also suggest possible involvement of the mitochondrial K_{ATP} channel in regulation of processes driven by the mitochondrial transmembrane potential, e.g., adenine nucleotide transport or calcium uptake, and pH, e.g., phosphate and pyruvate transport.

CONCLUSIONS AND PERSPECTIVES FOR FURTHER RESEARCH

Studies on the mitoK_{ATP} channel started only five years ago. Hence, our knowledge on this channel is far from being complete. Additionally, contradictory data obtained in different laboratories working on the mitoK_{ATP} channel pose problems to be solved in near future, in particular:

- where on the channel molecule is located the nucleotide-binding site, i.e., whether it does face the outer or the inner leaflet (matrix side) of the mitochondrial inner membrane;
- what is the specificity of interactions of adenine nucleotides with the mitoK_{ATP} channel; in other words why, depending on the experimental procedure, different nucleotides affect channel activity with different potency;

- what is the mode of action of potassium channel openers on the mitoK$_{ATP}$ channel,
- how do sulfonylureas interact with mitoK$_{ATP}$?
- is ATP a physiological modulator of the mitoK$_{ATP}$ channel or is the channel activity controlled by GTP and/or GDP in the presence of ATP;
- why only the ATP-Mg complex and not free ATP affects channel activity in reconstituted system; does this mean that some phosphorylation events are involved in the mitoK$_{ATP}$ channel inhibition;
- are there physiological modulators of this channel other than adenine and guanine nucleotides;
- are the so called extrapancreatic effects [38] of antidiabetic sulfonylureas due to the interaction of these compounds with the mitoK$_{ATP}$ channel.

The interest in the mitoK$_{ATP}$ channel will grow because of its possible role in mitochondrial energetics. Additionally, this channel could be an important intracellular target for drug design to act on the plasma membrane K$_{ATP}$ channel. The latter point seems to be of great importance for medical application of activators and inhibitors of potassium channels.

The author thanks Professor Lech Wojtczak and Professor Maciej J. Nałęcz for discussions on the subject of K$_{ATP}$ channels during preparation of this manuscript.

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