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QUARTERLY

Minireview

ATP-sensitive K^+ channel in mitochondria*

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A variety of different ionic channels was found to be present in inner and outer mitochondrial membranes. In the outer membrane a voltage dependent anion channel (VDAC) was identified [1]. On the other hand, in the mitochondrial outer membranes of the VDAC-deficient yeast mutant, an alternative channel, with a single-channel conductance of 0.21 nS, was observed [2, 3]. Moreover, in the outer-mitochondrial membrane the activity of a cationic channel of large conductance, blocked by a mitochondrial addressing peptide and thus called the "peptide sensitive channel", was also measured [4]. In the inner-mitochondrial membrane, activities of a multiple conductance channel, a centum picosiemens channel (MCS) and a channel with the conductance of 40 pS were measured [5 - 7]. In addition, two channels (cation- and anion-selective) induced by alkaline pH were observed in the mitochondrial inner membrane [8, 9].

Recently, the ATP-sensitive potassium (K_{ATP})[1] channel has been described in rat liver mitochondria [10, 11]. It was found that this channel is blocked not only by ATP but also by the antidiabetic sulfonylurea, glibenclamide (Fig. 1), giving evidence that the mitochondrial K_{ATP} channel may belong to the well known family of ATP-dependent potassium channels found in plasma membranes of cardiac, smooth and skeletal muscle cells [12], pancreatic β-cells [13] and in the nervous system [14 - 16]. In pancreatic β-cells K_{ATP} channels have a physiological role in glucose-stimulated insulin secretion. Channel opening blocks insulin secretion, whereas channel closing leads to depolarization of the membrane, activation of voltage dependent calcium channels, activation of exocytotic machinery and finally to insulin secretion. Interestingly, various heart diseases are characterized by alterations in potassium channel activities. For example, during acute cardiac ischemia and anoxia, activation of K_{ATP} channels results in shortening of action potential duration and elevation of extracellular potassium concentration. In brain, K_{ATP}

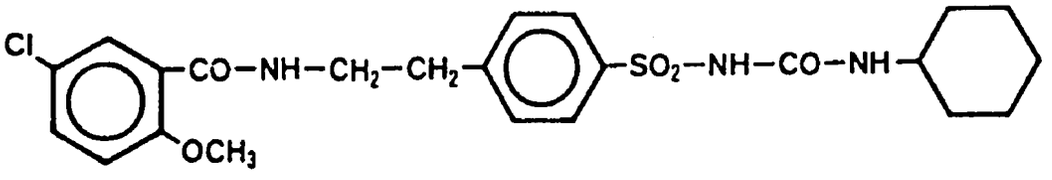

Fig. 1. Chemical structure of glibenclamide.

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[1] Abbreviations: DCCD, dicyclohexylcarbodiimide; K_{ATP}, ATP-sensitive potassium channel; MCS, multiple conductance channel; VDAC, voltage dependent anion channel

channels are believed to be involved in gamma-aminobutyric acid secretion from *substantia nigra* [17].

In β-cells, K_{ATP} channels are specifically blocked by sulfonylureas which are the drugs capable to restore insulin secretion in patients affected by non-insulin-dependent (type II) diabetes mellitus [13]. The K_{ATP} channels are also blocked by some of non-sulfonylurea drugs like 8-methoxypsoralen [18] and 8-(*N,N*-dimethylamino) octyl-3,4,5-trimethoxybenzoate [19]. K_{ATP} channels in pancreatic β-cells are activated by hormones such as somatostatin and galanin [20, 21] and free fatty acids [22]. They are also activated by such drugs as diazoxide, pinacidil and minoxidil sulfate, known as potassium channel openers (for review see [23 - 25]).

Identification of the ATP-sensitive potassium channel in rat liver mitochondria

Using the patch clamp technique, a small conductance channel highly selective for K^+ was identified in the inner mitochondrial membrane [10]. This channel could be reversibly inactivated by ATP applied to the matrix side under inside-out patch configuration. It was also inhibited by 4-aminopyridine and glibenclamide. The slope conductance of the unitary currents measured at negative membrane potentials was 9.7 ± 1.0 pS when the pipette solution contained 100 mM K^+ and the bathing solution 33.3 mM K^+.

Recently, the group of Garlid described a partially purified protein fraction from the inner membrane of rat liver and beef heart mitochondria that was shown to catalyze an electrophoretic K^+ flux after reconstitution into liposomes [11]. This activity was inhibited by low concentrations of ATP and ADP in the presence of divalent cations and by glibenclamide in the absence of divalent cations. A very similar sensitivity to ATP and glibenclamide was originally observed by Inoue *et al.* [10] in intact mitochondria.

Further, the K_{ATP} channel in patch-clamped mitochondria was found to be selective for potassium and unable to transport Na^+ [11]. The unit conductance of the channel at saturating $[K^+]$ is about 30 pS when measured in the planar lipid bilayer [11].

Thus, the mitochondrial K_{ATP} channel seems remarkably similar functionally to K_{ATP} channels of plasma membrane of different cells (for review see [26]).

Properties of the mitochondrial ATP-sensitive potassium channel

Basic properties of the mitochondrial K_{ATP} channel were reported by the group of Garlid [11]. The studies were performed after partial purification and reconstitution of the K_{ATP} channel into liposomes. Purification was performed after solubilization of mitochondria with Triton X-100, followed by chromatography on DEAE-cellulose, and the channel protein was reconstituted into liposomes by detergent removal on BIO-BEADS resin. A profound inhibitory effect of ATP on K^+ transport was observed when measured with a fluorescent potassium-binding dye benzofuran isophthalate. Both Mg^{2+} ions and ATP were required for the inhibition. ATP had no effect in the absence of magnesium, and magnesium had no effect in the absence of ATP. Interestingly, the obseravtion that Mg^{2+} was found ineffective in the absence of ATP differed from the results of studies in intact mitochondria where the potassium channel was inhibited by magnesium ions alone [27, 28]. Similar effects were observed for 3 mM Ca^{2+}; calcium alone had no effect, whereas calcium *plus* ATP inhibited the transport. In the presence of 3 mM magnesium K_i[ATP] was estimated to be 39 μM, and K_i[ADP] was 280 μM. The latter value was found to increase up to 639 mM in the presence of 50 μM ATP. This is consistent with the competition between ATP and ADP for the same binding site. In the presence of 0.5 mM ATP the K_i values, referring to free Mg^{2+} and Ca^{2+}, were estimated to be 80 μM for magnesium and 151 μM for calcium. Glibenclamide was found capable of inhibiting potassium transport in the reconstituted system, with the K_i of 62 nM, in the presence of EDTA. Mg^{2+}, which alone had no effect on potassium transport, was found to reduce the inhibitory potency of glibenclamide (K_i in the presence of 3 mM magnesium was estimated to be 3.1 μM). This result supports the suggestion that magnesium ion may interact directly with the channel protein.

Gauthier & Diwan [29] were the first to show that the alkylating agent dicyclohexylcarbodiimide (DCCD) partially inhibited K^+ transport in intact mitochondria. No activity of the K_{ATP} channel was found after pretreatment

with DCCD of the partially purified potassium channel protein from mitochondria. Potassium transport was unaffected by tetraethylammonium cation, the K_m for potassium uptake being 32 mM. The K_{ATP} channel activity exhibited little dependence on pH (in the range 6.5 - 8.5). No indication was found, either, that mitochondrial K_{ATP} channel could be voltage gated.

Potassium uniport or ATP-dependent potassium channel?

Respiring mitochondria drive the electrophoretic uptake of K^+, a process known as potassium uniport. Generally, it is accepted that in the presence of permeant anions such transport may lead to mitochondrial swelling, if it is not compensated by electroneutral K^+/H^+ exchange mediated by the K^+/H^+ antiporter [30 - 32]. Recently, the properties of K^+ uniport were investigated in more detail [33]. Modulation of the activity of potassium uniport by nucleotides and triazine dyes, as probes of nucleotide binding side, was studied. It was concluded that potassium uniport activity involved in fact the K_{ATP} channel [33].

Further evidence that potassium uniport is catalyzed by the K_{ATP} channel was obtained from the experiments with the Me^{2+} ionophore A23187 [28, 34]. Addition of A23187 to rat liver mitochondria in the presence of EDTA induces K^+ influx into mitochondria *via* the potassium uniport, resulting in mitochondrial swelling [28, 34]. It is believed that A23187 depletes mitochondria of magnesium ions, thus unmasking their permeability for potassium. Figure 2 shows a typical recording of the absorbance changes of respiring mitochondria incubated in an isotonic medium in the presence of potassium ions. Under these conditions, A23187 induced a rapid, low amplitude absorbance decrease. The K_{ATP} channel blocker, glibenclamide, was found to abolish this effect in a dose-dependent manner [35].

A similar mode of action of glibenclamide on the mitochondrial potassium uniport and the plasma membrane K_{ATP} channel was observed. In pancreatic β-cells, using electrophysiological techniques, it was observed that raising of pH of bath solution, in the presence of a constant concentration of tolbutamide, diminished the degree of inhibition of the ATP-dependent K^+ channel activity by sulfonylurea [36]. The conclusion was that the effective undissociated forms of sulfonylurea gained access to their specific binding site(s) on the receptor molecule from the lipid phase of the β-cell plasma membrane [36]. To verify the possibility that a similar mechanism of action of sulfonylureas operates in the case of the mitochondrial K_{ATP} channel, effects of these drugs on the A23187-induced swelling at dif-

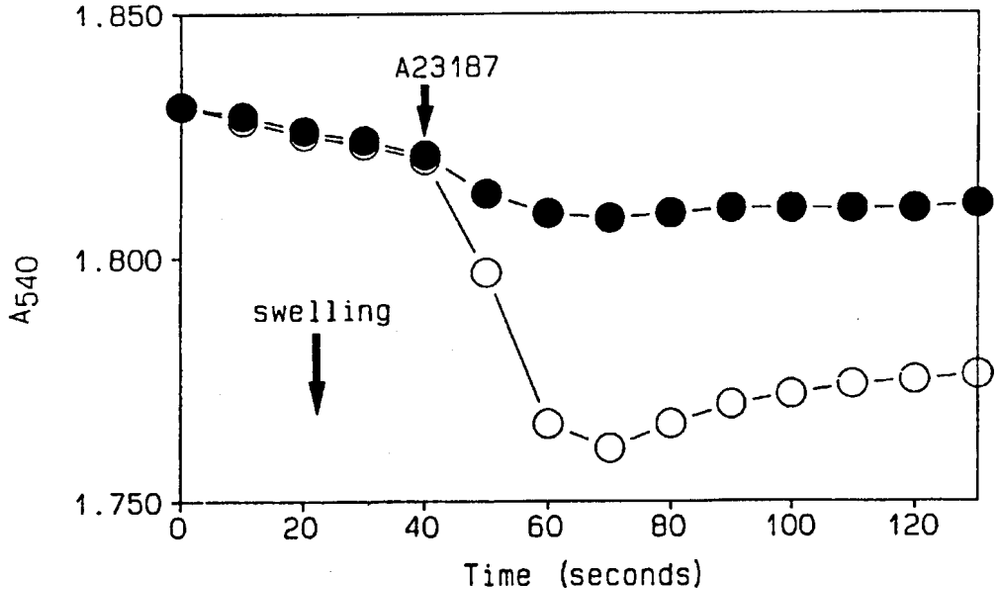


Fig. 2. Effect of glibenclamide on A-23187 induced mitochondrial swelling.
Energy-dependent swelling of mitochondria induced by A-23187 was measured in the absence (open circles) or presence (closed circles) of 50 μM glibenclamide. The light scattering changes, reflecting mitochondrial volume changes, was measured at 540 nm.

ferent pH values were measured [35]. It was found that the pH of bath solution has no effect on the A23187-induced swelling of mitochondria, while the inhibitory effect of glibenclamide observed at pH 7.4 was completely abolished at pH 8.6 [35]. This points to a similarity of interaction of antidiabetic drugs with the K_{ATP} channel both in rat liver mitochondria and in the plasma membrane of β-cell.

Despite low permeability of the mitochondrial inner membrane to charged and uncharged molecules, it has been frequently reported that a variety of agents or conditions may alter the permeability of mitochondrial inner membrane, leading to swelling of mitochondria and to leakiness to K^+, Mg^{2+}, Ca^{2+} (for review see [37]). Cyclosporin A, a cyclic peptide, is known to block permeability transitions in mitochondria but was shown to be without effect on mitochondrial swelling induced by A23187 [35]. This indicates that the glibenclamide-sensitive, A23187-induced swelling of mitochondria does not involve the permeability transition mechanism.

The results described in this part suggest that the activity known previously as the mitochondrial potassium uniport could, in fact, be identical with the activity of the ATP-sensitive potassium channel. It is still a matter of dispute where the nucleotide binding site is located on the channel molecule, i.e. whether it may face the outer- and/or the inner-leaflet of the mitochondrial inner membrane.

Interactions of potassium channel openers with mitochondria

Modulation of potassium channel activity by drugs, especially by those known as potassium channel openers (Fig. 3), is a rapidly growing area of pharmacology. Recently, the attention of many laboratories [12, 38] became focused on studies on the ability of various openers to influence K^+ channels activity.

It was shown that some of potassium channel openers are able to stimulate matrix volume changes of rat liver mitochondria and that this effect may be reversed by glibenclamide [39]. The so-called passive swelling in KSCN medium, and the energy-dependent swelling in CH_3COOK medium, of the mitochondria energized by the addition of succinate were measured. Under such conditions, swelling of mitochondria reflects influx of K^+ into the mitochondrial matrix. Figure 4A shows that two potassium channel openers, namely diazoxide and RP 66471, are able to stimulate passive

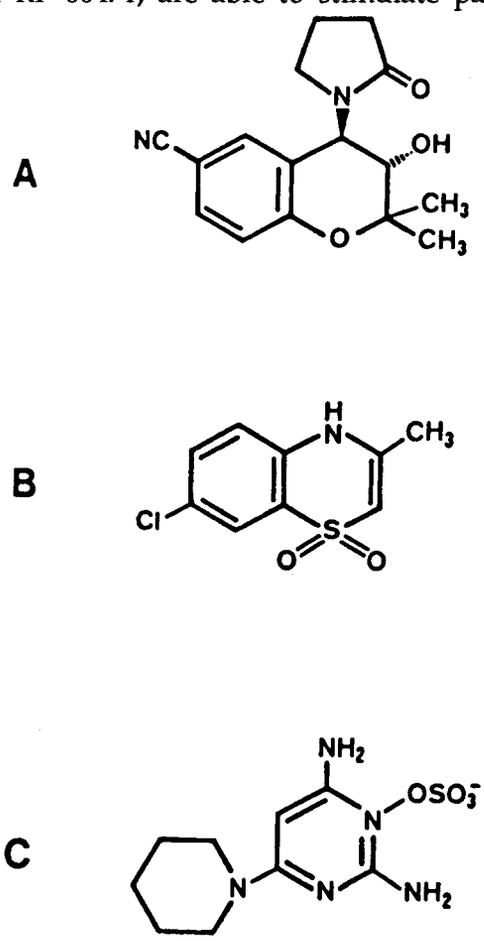
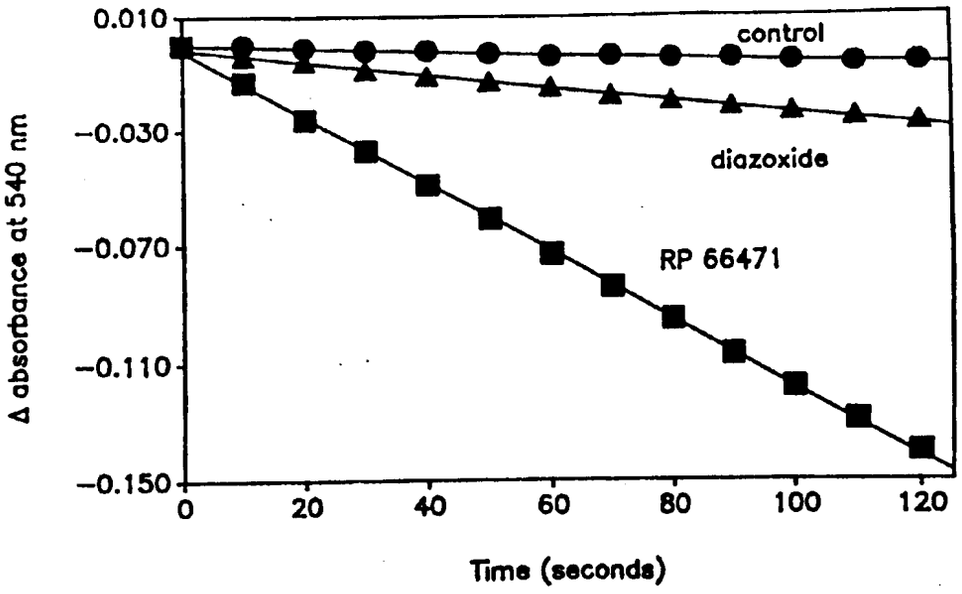


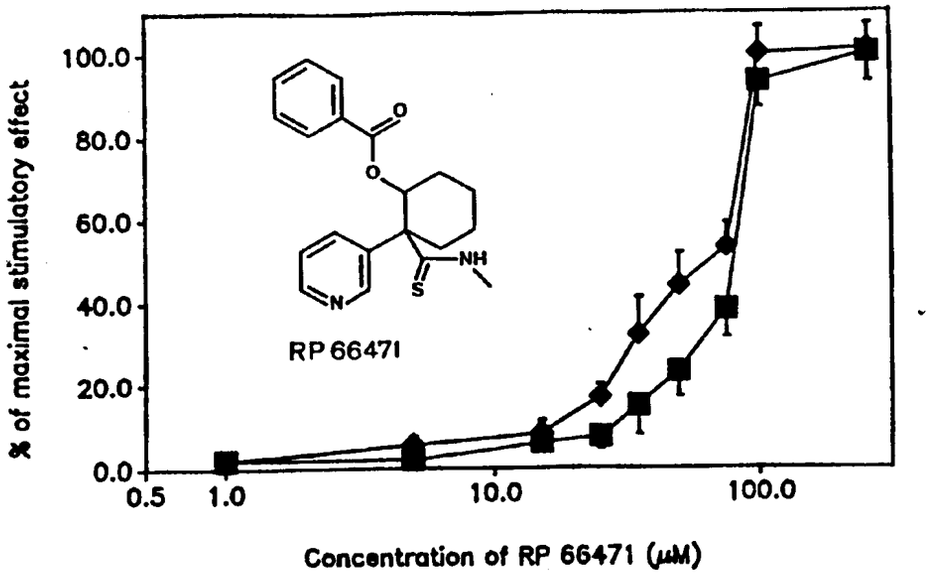
Fig. 3. Potassium channel openers: A, kromakalim; B, diazoxide; C, minoxidil sulfate.

Fig. 4. Effects of potassium channel openers on changes of mitochondrial matrix volume.
A. Efects of potassium channels openers on passive swelling of rat liver mitochondria in KSCN medium. The following substances were added: none (circles), 700 μM diazoxide (triangles), or 250 μM RP 66471 (squares). B. Concentration dependence of the stimulatory effect of RP 66471 on mitochondrial swelling under non-respiring (squares) and respiring (diamonds) conditions. The results are expressed in % of the effect caused by 250 μM RP66471 as potassium channel opener. C. Effect of glibenclamide on potassium channel opener-induced swelling of mitochondria under non-respiring and respiring conditions. Empty bars represent values obtained in the presence of 250 μM RP 66471 or 700 μM minoxidil sulfate, with no glibenclamide added; filled bars represent values after preincubation of mitochondria in the presence of 100 μM glibenclamide, followed by incubation with a potassium channel opener. The results are expressed as percentage of the rate of swelling observed with no additions.

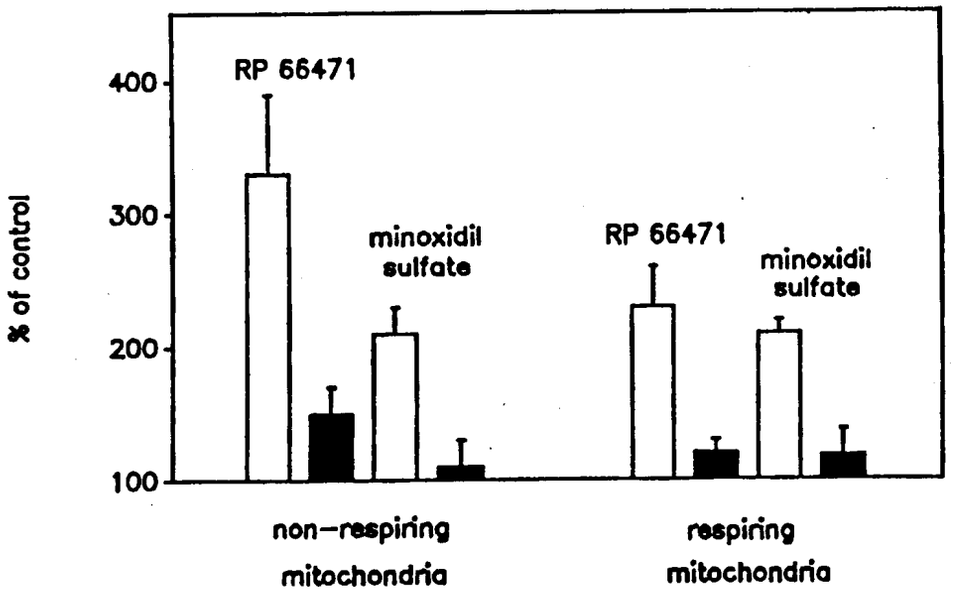
A


0.010
−0.030
−0.070
−0.110
−0.150
Δ absorbance at 540 nm
control
diazoxide
RP 66471
0
20
40
60
80
100
120
Time (seconds)

B


100.0
80.0
60.0
40.0
20.0
0.0
% of maximal stimulatory effect
O
O
NH
S
N
RP 66471
0.5
1.0
10.0
100.0
Concentration of RP 66471 (μM)

C


400
300
200
100
% of control
RP 66471
minoxidil sulfate
RP 66471
minoxidil sulfate
non−respiring mitochondria
respiring mitochondria

mitochondrial swelling. Other K^+ channel openers, i.e. pinacidil, KRN 2391, P1060, and minoxidil sulfate, were also found effective. Ro 31-6930 and nicorandil at the same concentration have no effect. The most potent opener turned out to be RP 66471, with $K_{0.5}$ of 50 μM (Fig. 4B) [39].

The stimulatory effect of RP 66471 and minoxidil sulfate was almost completely abolished by 100 μM glibenclamide, both in respiring and non-respiring mitochondria [39]. Moreover, activation of mitochondrial swelling caused by 700 μM KRN 2391 or pinacidil was also observed to be blocked by 100 μM glibenclamide. However, the inhibition by glibenclamide of the reconstituted K_{ATP} channel was described to occur at a much lower concentration range, i.e. with IC_{50} of 62 nM [11]. This apparent discrepancy between our results [39] and those of Garlid's group [11] could be explained as follows. As shown [11], magnesium ions reduce the inhibitory potency of glibenclamide. Thus, the relatively high concentration of glibenclamide required to abolish the effect of potassium channel openers on mitochondrial swelling could be ascribed to the presence of Mg^{2+} in the mitochondrial preparation, used. In addition, the potency of glibenclamide, a highly hydrophobic compound, in inhibiting potassium channel opener-induced swelling must be limited by its surface concentration (see e.g. [40]). Indeed, on lowering protein concentration, we observed an increase in effectiveness of glibenclamide to reverse the effect of potassium channel openers. Last but not least, mitochondria are known to maintain a high concentration of adenine nucleotides which can by itself lower the affinity of glibenclamide to its putative receptor [41].

Functional role of the K_{ATP} channel

It is still a matter of dispute how the mitochondrial K_{ATP} channel may be engaged in the function of mitochondria. Generally, it is accepted that the K^+ influx into mitochondrial matrix produces changes in water content evoking mitochondrial swelling. Some of these changes are related to the action of hormones which cause an increase of cAMP (glucagon) or of calcium concentration (vasopressin and α-adrenergic agonists), and to the activity of mitochondrial respiratory chain. Since changes of mitochondrial volume are sufficient to modulate metabolic processes such as citruline synthesis, pyruvate carboxylation and fatty acid oxidation, it seems to be of particular importance to establish whether the mitochondrial K_{ATP} channel activity could be, at least partially, responsible for the regulation of mitochondrial metabolism (for review see [39]).

It has been recently shown that K^+ uptake into energized mitochondria is slowed down by glibenclamide and accelerated by the potassium channel opener P1060 [42]. The electrophoretic K^+ uptake (*via* K_{ATP} channel), when accompanying mitochondrial energization, would be able to partly neutralize the transmembrane potential and promote formation of the pH gradient [42]. This would also suggest possible involvement of the K_{ATP} channel in regulation of other processes driven by the mitochondrial transmembrane potential, e.g. adenine nucleotide transport or calcium uptake into mitochondria, and by pH gradient, e.g. phosphate and pyruvate transport. The use of well characterized pharmacological agents as activators or blockers of the K_{ATP} channel could serve as a tool to characterize the role of this channel in mitochondrial bioenergetics.

Final remarks and perspectives

Studies of the mitochondrial ATP-sensitive potassium channel have started only recently. Hence, much of basic information concerning this channel is missing. The mode of interaction of sulfonylureas with the mitochondrial K_{ATP} channel has not yet been characterized. Similarly, the activation induced by potassium channel openers is not clear. Apart from adenine nucleotides and magnesium ions, no other physiological effectors of this channel have been described, and the physiological role of this channel is still under debate.

Recently, the plasma membrane ATP-regulated channel from kidney was cloned and expressed [43]. It can be expected that this will facilitate and accelerate investigations of not only plasma membrane K_{ATP} channels but also of the mitochondrial channel.

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