ATP-sensitive Potassium Channel in Mitochondria of the Eukaryotic Microorganism Acanthamoeba castellanii*

Received for publication, February 20, 2007, and in revised form, April 11, 2007 Published, JBC Papers in Press, April 12, 2007, DOI 10.1074/jbc.M701496200

Anna Kicinska, Aleksandra Swida, Piotr Bednarczyk, Izabela Koszela-Piotrowska, Katarzyna Choma, Krzysztof Dolowy, Adam Szewczyk, and Wieslawa Jarmuszkiewicz

From the 1Laboratory of Bioenergetics, Adam Mickiewicz University, 61-614 Poznan, Poland, 5Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, 02-093 Warsaw, Poland, and the 4Department of Biophysics, Agriculture University (SGGW), 02-766 Warsaw, Poland

We describe the existence of a potassium ion transport mechanism in the mitochondrial inner membrane of a lower eukaryotic organism, Acanthamoeba castellanii. We found that substances known to modulate potassium channel activity influenced the bioenergetics of A. castellanii mitochondria. In isolated mitochondria, the rate of resting respiration is increased by about 10% in response to potassium channel openers, i.e. diazoxide and BMS-191095, during succinate-, malate-, or NADH-sustained respiration. This effect is strictly dependent on the presence of potassium ions in an incubation medium and is reversed by glibenclamide (a potassium channel blocker). Diazoxide and BMS-191095 also caused a slight but statistically significant depolarization of the mitochondrial membrane potential (ΔΨ). Additionally, the mitochondrial membrane permeability to K+ ions improves in response to potassium channel openers (KCOs) (14, 15). Diazoxide is an especially potent activator of the mitoK_ATP channel (16). The molecular identity of the mitoK_ATP channel is unknown. Several observations on the pharmacological profile and immunoreactivity with specific antibodies suggest that the mitoK_ATP channel belongs to the inward rectifier K⁺ channel family Kir6.x (17, 18). Recently, it has been hypothesized that succinate dehydrogenase forms part of a structure that constitutes the mitoK_ATP channel (19). The primary function of the mitoK_ATP channel is to allow K⁺ transport into the mitochondrial matrix. This can lead to an increase in matrix volume and matrix alkalization as well as an increase in reactive oxygen species production by mitochondria (for review see Ref. 20). Additionally, in mammalian cells, it is now well established that mitoK_ATP channels play an important role in protecting cells against ischemia-reperfusion-induced injury. It has been found that an increase in the inner mitochondrial membrane permeability to K⁺ ions improves cellular tolerance to ischemia-reperfusion injury in various tissues, including liver, gut, brain, kidney, and heart (for review see Ref. 2).

Although we now have quite a breadth of knowledge about ion transport in mammalian mitochondria, thus far nothing has been elucidated about cation-transporting systems in the mitochondria of unicellular eukaryotes. Acanthamoeba castellanii, a non-photosynthesizing amoeboid protozoon, is an especially interesting example of eukaryotic microorganisms, as in molec-

* This work was supported by a grant from the Polish Ministry of Education and Science (PBZ-MIN-012/P04/06) and by the Polish Mitochondrial Network. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed: Laboratory of Bioenergetics, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland. Tel.: 48-61-8295881; Fax: 48-61-8295636; E-mail: wiesias@amu.edu.pl.

2 The abbreviations used are: mitoK_ATP channel; mitochondrial ATP-sensitive potassium channel; BLM, black lipid membrane; BSA, bovine serum albumin; CATR, carboxyatractyloside; 5-HD, 5-hydroxydecanoic acid; KCO, potassium channel opener; pS, picosiemens; SMP, submitochondrial particles; TPP⁺, tetraphenylphosphonium.
Mitochondrial Potassium Channel of Acanthamoeba castellanii

ular phylogeny appears on a branch basal to the divergence points of the animal, plant, and fungal kingdoms (21).

A. castellanii and higher plants share several common features at the level of the respiratory chain of the inner mitochondrial membrane, such as the presence of an alternative cyanide-resistant ubiquinol oxidase and nonphosphorylating rotenone-insensitive internal (matrix face) and external (cytosolic face) NADH dehydrogenases (22–24). On the contrary, recent studies on the translocase of the outer membrane (TOM) complex of A. castellanii mitochondria suggest that it could be rather similar to the animal-fungal TOM proteins (25). As mitochondrial potassium channels are present in higher plant mitochondria and animal mitochondria, it is of special interest to explore its existence in ameboid protozoon. Identification and characterization of the mitoK-ATP channel in A. castellanii mitochondria, the subject of the present study, indicate that the K+ transporting system emerged early during phylogenesis, prior to the divergence of eukaryotes into animals and plants.

EXPERIMENTAL PROCEDURES

Chemicals—1,α-Phosphatidylcholine (asolecint), diazoxide, glibenclamide, and n-decane were from Sigma-Aldrich. All other chemicals were of the highest purity available commercially.

Cell Culture and Isolation of Mitochondria—The soil amoeba A. castellanii, strain Neff, was cultured as described by Jarmuszewicz et al. (22). Trophozoites of the amoeba were collected between 44 and 48 h following inoculation at the mid-log phase. The suspension was centrifuged at 200,000 × g for 30 min to pellet the unbroken mitochondria. The supernatant was sonicated five times for 30 s and centrifuged at 12,000 × g. The supernatant was centrifuged at 34,000 × g (30 min) to collect fraction 2 of the submitochondrial particles (SMP, larger particles). Fractions of SMP, which are the mitochondrial inner membrane-enriched fractions, were resuspended in 0.3M sucrose and 10 mM Hepes-KOH, pH 7.2, at a concentration of 5 mg of protein/ml. Mitochondrial potassium channel into the BLM was usually observed within a few minutes. The studied compounds were added to the cis and trans compartments. All measurements were carried out at 24°C. The formation and thinning of the bilayer were monitored by capacitance measurements and optical observations. Final accepted capacitance values ranged from 110 to 180 pico-farads. Electrical connections were made by Ag/AgCl electrodes and agar salt bridges (3 M KCl) to minimize liquid junction potentials. Voltage was applied to the cis compartment of the chamber, and the trans compartment was grounded (Fig. 4C). The current was measured using a bilayer membrane amplifier (BLM-120, BioLogic). Signals were filtered at 500 Hz. The current was digitized at a sampling rate of 100 kHz (A/D converter PowerLab 2/20, ADInstruments) and transferred to a PC for off-line analysis by Chart version 5.2 (PowerLab, ADInstruments) and pCLAMP 8.1 (Axon Laboratory). The pCLAMP 8.1 software package was used for data processing. The channel recordings presented are representative of the most frequently observed conductance values under the given conditions. The conductance was calculated from the current-voltage relationship. The permeability ratios for K+ and Cl− were calculated according to the Goldman-Hodgkin-Katz voltage equation (28),

\[
E_{rev} = -\frac{RT}{2F} \ln \left[ \frac{\left[Cl^-\right]_{trans}}{\left[Cl^-\right]_{cis}} + \frac{\left[P_i/\left[P_O\right]\right]}{\left[K^+\right]_{trans}} \right]
\]

where \(E_{rev}\) is the potential at which the current is zero, \(R\) is the gas constant, \(T\) is temperature in Kelvin, \(z\) is equal to 1 (chloride anion charge), \(F\) is Faraday constant, \(P_{ion}\) is the permeability of the ion, and \([Cl^-]\) and \([K^+]\) are the respective concentrations of the ion in the cis and trans chambers.

Mitochondrial Oxygen Consumption—Oxygen uptake was measured polarographically with a Clark-type oxygen electrode (Rank Brothers, Cambridge, United Kingdom) in 3 ml of incubation medium (25°C), as described in a given figure legend (Figs. 1–3), with 1–2 mg of mitochondrial protein. Benzohydroxymate was used to inhibit the alternative oxidase activity. Succinate (5 mM) plus rotenone (4 μM), malate (5 mM), or NADH (1 mM) was used as the respiratory substrate. Measurements of the respiratory rate were performed in the absence of added ADP, i.e. in the resting state (state 4). To exclude the activity of an ATP/ADP antiporter, 1.8 μM carboxyatractyloside (CATR) was used. State 3 (phosphorylating) respiration measurements were performed (in the high KCl medium with 0.65 MgCl2 (23)) to check the coupling parameters. Only high quality mitochondria preparations, i.e. with an ADP/O value of around 1.40 and a respiratory control ratio of around 3 (with succinate), were used in all experiments. Values of O2 uptake are presented in nmol of oxygen × min−1 × mg−1 protein.

Mitochondrial Membrane Potential Measurements—The mitochondrial transmembrane electrical potential (ΔΨ) was measured simultaneously with oxygen uptake using a tetraphenylphosphonium (TPP+)–specific electrode according to Kamo et al. (29). Measurements were performed in the presence of 1.3 μM TPP+. For calculation of the ΔΨ value, the matrix volume of amoeba mitochondria was assumed to be 2.0 μl × mg−1 pro-
tein. Calculation assumes that TPP\(^+\) distribution between mitochondria and medium followed the Nernst equation. Corrections were made for the binding of TPP\(^+\) to mitochondrial membranes. Values of \(\Delta \Psi\) are presented in mV.

**SDS-PAGE and Immunoblotting**—Protein content was determined by the Bradford method (Bio-Rad). Samples (150 \(\mu\)g) of isolated mitochondrial proteins or SMP were solubilized in sample buffer containing 2% (w/v) SDS, 50 mM Tris/Cl, pH 6.8, 10% glycerol, 0.004% (w/v) bromphenol blue, and 8% mercaptoethanol and subsequently were boiled for 4 min. Proteins were separated on 12% SDS-polyacrylamide gels and, after separation, electrotransferred to a nitrocellulose membrane. Membranes were then hybridized with anti-Kir6.1 and anti-Kir6.2 antibodies (at dilutions of 1:100) (Santa Cruz Biotechnology) in the presence or absence of blocking peptide. Protein bands were visualized using the GE Healthcare ECL system.

**RESULTS**

Activation of \(K^+\) Flux through the *A. castellanii* Mitochondrial Membrane by Potassium Channel Openers—The addition of a mitochondrial potassium channel opener, 100 \(\mu\)M diazoxide, increases the rate of mitochondrial state 4 oxygen uptake (with succinate) by 10.0 ± 1.6% \((n = 9, \text{ S.D.})\). This effect is reversed to the control value by a potassium channel blocker, 10 \(\mu\)M glibenclamide. At the same time, \(\Delta \Psi\) decreases after addition of diazoxide by 1.2 ± 0.4 mV \((n = 9, \text{ S.D.})\) and afterward is restored to the resting state value by glibenclamide. An example of simultaneously performed measurements of the resting respiratory rate and \(\Delta \Psi\) under the conditions described is shown in Fig. 1A. These results suggest that diazoxide stimulates the \(K^+\) ion flux into *A. castellanii* mitochondria, decreasing the steady state \(\Delta \Psi\) and thus causing the acceleration of mitochondrial respiration rate. To further test this hypothesis, we performed the same experiments in media deprived of \(K^+\) ions. In a sucrose (0.25M) medium (data not shown) and in NaCl (0.12 M) medium (Fig. 2, A and B) the addition of either diazoxide or glibenclamide did not change the control values of resting respiration and \(\Delta \Psi\) in *A. castellanii* mitochondria.

Another potassium channel opener, described previously as specific for the mitoK\(_{ATP}\) channel (30), BMS-191095, also was applied in our studies. Similar to diazoxide, 3 \(\mu\)M BMS-191095 stimulated mitochondrial resting oxygen uptake by 8.9 ± 2.5% \((n = 4, \text{ S.D.})\) and decreased \(\Delta \Psi\) by 1.2 ± 0.1 mV \((n = 4, \text{ S.D.})\). Both effects were reversed by the addition of 10 \(\mu\)M glibenclamide. A representative experiment is shown in Fig. 1B.

Fig. 1C shows that another previously described potassium channel blocker, 5-hydroxydecanoic acid (5-HD) (16), is not active when used with amoeba mitochondria. The addition of 500 \(\mu\)M 5-HD did not change the mitochondrial respiration rate and \(\Delta \Psi\) accelerated by diazoxide (Fig. 1C).
Mitochondrial Potassium Channel of Acanthamoeba castellanii

**Fig. 2.** The effect of diazoxide concentration on resting respiratory rate and membrane potential ($\Delta \Psi$). Mitochondria were incubated in medium containing 20 mM Tris-HCl, 120 mM KCl, 0.5 mM MgCl$_2$, 3 mM KH$_2$PO$_4$, 1.5 mM EGTA, 1.8 $\mu$M CATR, 0.2% BSA, and 5 mM benzohydroxamate (A) or 20 mM Tris-HCl, 120 mM NaCl, 0.5 mM MgCl$_2$, 3 mM Na$_2$HPO$_4$, 1.5 mM EGTA, 0.2% BSA, and 5 mM benzohydroxamate (B). A and B, with 5 mM succinate (SA) (plus 4 $\mu$M rotenone) as a respiratory substrate. C and D, with 5 mM malate (MA) as a respiratory substrate. Under certain conditions, 10 $\mu$M glibenclamide (glib) was applied additionally. The data deal with three different mitochondrial preparations. S.D. is shown.

Fig. 2, A and B, shows the diazoxide concentration-dependent effects on resting respiratory rate (stimulation) and $\Delta \Psi$ (depolarization) with succinate-oxidizing A. castellanii mitochondria in the KCl-based medium. The apparent 50% maximal effect of diazoxide ($K_{0.5}$) is reached at 43–45 $\mu$M. Concentrations of diazoxide higher than 100 $\mu$M were described to have nonspecific effects on mitochondria (31) and therefore have not been used in this study. Moreover, it is clear from Fig. 2, A and B, that the effects of diazoxide action are strictly dependent on the K$^+$ ion presence in the incubation medium (no effects in a NaCl-based medium) and reversed by 10 $\mu$M glibenclamide. Furthermore, the dose-dependent and glibenclamide-blocked influence of diazoxide on mitochondrial bioenergetics is also observed during malate-sustained respiration (Fig. 2, C and D). In the case of malate, mitochondrial respiration is stimulated up to about 11%, whereas $\Delta \Psi$ is maximally decreased by 1.5 mV. The apparent value of $K_{0.5}$ is observed at 52–53 $\mu$M diazoxide.

The inhibitory effect of ATP on K$^+$ transport into A. castellanii mitochondria has been studied with external NADH as a respiratory substrate in order to exclude the influence of the nucleotide on malate and succinate dehydrogenase activities (23). With mitochondria oxidizing external NADH, 100 $\mu$M diazoxide stimulates respiration by 15.7 $\pm$ 2.5% ($n = 9$, S.D.) and decreases $\Delta \Psi$ by 1.30 $\pm$ 0.45 mV ($n = 9$, S.D.). The apparent value of $K_{0.5}$ is estimated at around 50 $\mu$M diazoxide (Fig. 3C). The effect of diazoxide is abolished by the addition of 1 mM ATP (Fig. 3, A and C). Moreover, diazoxide was not able to activate potassium transport (no effect on either respiratory rate or $\Delta \Psi$ was observed) when added after ATP (Fig. 3B). These results indicate that the nucleotide is an inhibitor of potassium transport in A. castellanii mitochondria. Moreover, in the K$^+$ ion-deprived medium (0.25 M sucrose), the inhibitory effect of ATP was not observed (data not shown). The presence of 1.8 $\mu$M CATR in experiments where ATP was applied is especially important to exclude the adenine nucleotide translocator action and thereby ATP uptake into mitochondrial matrix. The same results were also observed in the presence of oligomycin (1 $\mu$g/mg of protein), a F$_{1}$F$_{0}$-ATP synthase inhibitor.

The above functional characterization, drawn from experiments performed with isolated mitochondria, suggests that the potassium transport mechanism, similar to that observed in mitochondria of higher eukaryotes (plants and mammals), is present in A. castellanii mitochondria. To support these results, a further characterization of potassium transport with the inner mitochondrial membrane-enriched fractions (SMPs) reconstituted in a planar lipid bilayer was performed.

**Characterization of Potassium Channel from A. castellanii Mitochondrial Membrane in BLMs**—The particular inner mitochondrial membrane-enriched fractions (SMP fractions 1 and 2) from the amoeba A. castellanii were reconstituted into BLMs as described under “Experimental Procedures.” The results obtained upon reconstitution indicate no functional distinction between both fractions of particles. A variety of channel-like activities has been observed, both anion- and cation-selective ($n = 117$). Among them, we have focused on the K$^+$ ion transport. Fig. 4A shows representative current-time traces and current-voltage relationship for potassium channel opening at different voltages in the 50/450 mM KCl (cis/trans) gradient solutions. The calculated channel conductance is equal to 166 $\pm$ 10 pS for potential from $-30$ to $+30$ mV and $90 \pm 7$ pS for potential from 50 to 110 mV. The reversal potential of 47 mV (Fig. 4B) has been calculated from measurements in the 50/450 mM KCl gradient solutions and curve fitting to the experimental data. This value indicates that the examined ion channel is a cation-selective. The calculated permeability ratio for K$^+$ and Cl$^-$ is equal to 20.4 according the Goldman-Hodgkin-Katz voltage equation (28) (see Equation 1 under “Experimental Procedures”).

Substances known to modulate the mitoK$_{ATP}$ channel activity have been used to examine the properties of the K$^+$ ion channel observed in our experiments. Fig. 5A shows the single...
channel recordings in the 50/450 mM KCl (cis/trans) gradient solutions at 0 mV, before and after the addition of 1 mM ATP/Mg²⁺ to both chambers (cis/trans). ATP/Mg²⁺ causes a complete inhibition of the channel. The effect has usually been observed after about 5 min following ATP/Mg²⁺ addition. The number of open state events changes from 50% to zero after glibenclamide addition, as illustrated in the histogram (Fig. 5B). The last trace on Fig. 5C shows that the inhibitory effect of ATP/Mg²⁺ is reversed by the addition of 30 μM diazoxide (cis/trans) in the presence of ATP/Mg²⁺ (cis/trans). The effect of diazoxide was usually evident within a few seconds after its application. Diazoxide causes the transition into an open state in 67% of events (histogram below the trace, Fig. 5C).

Interestingly, the activity of a potassium-selective channel with a larger conductance has also been recorded in the A. castellanii inner mitochondrial membrane-enriched fractions. Some of the channel pharmacological properties, such as no sensitivity to ATP/Mg²⁺ or glibenclamide, indicate the existence of another K⁺/H⁺-selective channel in addition to the mitoKATP channel in A. castellanii mitochondria.

Immunological Detection of A. castellanii Mitochondrial Potassium Channel Protein—Immunoblotting of the total mitochondrial protein, as well as SMP, has allowed immunological detection of the A. castellanii mitochondrial potassium channel. We have used antibodies raised against the mammalian ATP-sensitive potassium channel pore (Kir6.1 and Kir6.2 subunits). These antibodies have been shown to cross-react with the mammalian mitoKATP channel proteins (17). With A. castellanii mitochondrial and SMP fractions, a protein band with a molecular mass slightly higher than 48 kDa is detected using anti-Kir6.1 antibodies (Fig. 6A). The band is not visible in a lane containing an homogenate of A. castellanii cells, likely because of the too low amount of mitochondrial protein in this case. Fig. 6B presents immunodetection with both anti-Kir6.1 antibodies and the commercially available blocking peptide. The blocking peptide blocks antibody-antigen binding, demonstrating the specificity of reaction in Western blot analysis. As there is no detectable protein band when the Kir6.1 antibody epitope binding site is blocked (Fig. 6B), compared with no blocking peptide conditions (Fig. 6A), we can conclude that the A. castellanii mitochondrial potassium channel may contain subunits similar to Kir6.1. On the other hand, no cross-reactivity was observed with anti-Kir6.2 antibodies (data not shown).
Mitochondria of the soil, free-living, non-photosynthesizing, amoeboid protozoan *A. castellanii*, share many bioenergetic properties with the mitochondria of plants, animals, and fungi (32, 33). Moreover, on the basis of ribosomal RNA analysis, *A. castellanii* is located in the molecular phylogenetic tree on a branch basal to the divergence points of the above kingdoms (21, 34). As little is known about monovalent cation-transporting systems in the inner mitochondrial membrane of the unicellular microorganisms and, on the other hand, as potassium channels have been thus far described only in animal and plant cells, it was considered of utmost importance to study the mitochondrial potassium transport mechanism of *A. castellanii* mitochondria and to compare its properties with those described for other organisms.

In this study, for the first time (to our knowledge) we have shown the presence and described the functional properties of the mitoK<sub>A,T,P</sub> channel-like protein in mitochondria from protozoan *A. castellanii*. Potassium channel openers diazoxide and BMS-191095 were able to modulate resting respiratory rate (stimulation) and ΔΨ (slight depolarization) in isolated *A. castellanii* mitochondria. These effects were strictly dependent on potassium ion presence in the incubation medium and were not observed when potassium ions were replaced with sodium ions or sucrose. Thus, we have provided evidence that the observed effects of KCOs are due to activation of electrogenic potassium transport through the inner mitochondrial membrane, likely mediated by a potassium channel that belongs to the family of mitochondrial potassium channels previously described in mammalian and plant mitochondria (5, 12).

It is worth noting that the discrete mitochondrial ΔΨ changes (up to 2 mV) observed with isolated *A. castellanii* mitochondria in the presence of KCO could not be measured other than with the TPP<sup>+</sup>-selective electrode applied in these studies. In contrast to other studies (35), we did not observe the inhibition of *A. castellanii* mitochondrial potassium transport by TPP<sup>+</sup> in the applied concentration (data not shown). In isolated *A. castellanii* mitochondria, the extent of the increase in respiratory rate (up to 15%) and decrease in ΔΨ (by as much as 2 mV) observed after KCO addition is in good agreement with previous studies on mammalian mitochondria (for review see Ref. 20) and conclusion that potassium channels present in the mammalian inner mitochondrial membrane are low copy number proteins, and thereby their activity must be very strictly regulated to avoid uncoupling. This is in contrast to higher plant mitochondria that seem to possess a highly active K<sup>+</sup> transport that could lead to total mitochondrial depolarization in the presence of 25 mM KCl (12). Moreover, *A. castellanii* mitochondria do not swell significantly in the K<sup>+</sup> ion containing isotonic medium (data not shown) in contrast to plant mitochondria (12, 13).

In *A. castellanii* mitochondria, the ΔΨ depolarization due to diazoxide addition was not dependent on the applied respiratory substrate and thus could not be attributed to succinate dehydrogenase inhibition, as suggested by others, for mammalian mitochondria (36). Moreover, we did not observe any respiratory...
rate inhibition caused by diazoxide. In this work, for the first time, quantitative properties based on precise and rigorous oxygen consumption and membrane potential measurements are shown for the mitoK\(_{\text{ATP}}\) in the case of any isolated mitochondria (including mammalian mitochondria).

In *A. castellanii* mitochondria, the potassium channel blocker sulfonylurea glibenclamide reversed the effects of KCOs by decreasing the respiratory rate and restoring \(\Delta \Psi\) to the values observed before the addition of activators. Thus, the decrease in respiratory rate caused by glibenclamide is not due to the inhibition of the respiratory chain complexes but to prevention of uncoupling \(\mathrm{K}^+\) transport into mitochondria. The specificity of the glibenclamide effect was additionally confirmed, as the drug did not reverse the \(\Delta \Psi\) depolarization caused by the potassium ionophore valinomycin, which was used in small concentrations to mimic mitochondrial potassium channel opening (data not shown). Another potassium channel blocker, 5-HD, was not effective with *A. castellanii* mitochondria. Thus, in this respect, potassium transport in *A. castellanii* mitochondria exhibits pharmacological properties similar to skeletal muscle rather than heart mitochondria (9). Neither of these blockers, glibenclamide or 5-HD, affect \(\mathrm{K}^+\) transport in plant mitochondria (12, 13).

In *A. castellanii* mitochondria, the \(\Delta \Psi\) depolarization by diazoxide was reversed by 1 mM ATP. Moreover, the addition of ATP to the respiring mitochondria completely prevented activation of potassium transport by diazoxide (when added after ATP). This seems to be in contrast to other studies on mammalian mitochondrial potassium transport, which report that diazoxide is able to overcome channel blockage by ATP (26, 37). However, the electrophysiological studies suggest that this effect (at least in the case of *A. castellanii* potassium channel) could be observed but is probably related to specific experimental conditions (see below). In the case of the plant mitochondrial potassium channel, there are controversial results concerning the sensitivity of the channel to ATP (12, 13) related to the use of BSA during experiments with isolated mitochondria. In *A. castellanii* mitochondria, the inhibitory effect of ATP was independent of the presence of BSA in the medium.

To further confirm the functional observations obtained with isolated mitochondria, studies in reconstituted systems of black lipid membranes were conducted. Submitochondrial par-
Mitochondrial Potassium Channel of Acanthamoeba castellanii

![Western blot analysis of A. castellanii samples with antibodies raised against the Kir6.1 subunit of the mammalian ATP-regulated potassium channel in the absence (A) or presence (B) of the specific blocking peptide. Mito, mitochondria; H, crude cell homogenate; SMP, sub-mitochondrial particles (fraction 1). 150 μg of protein was loaded into each lane. An example of each of six immunoblots (using samples from different preparations, SMP fractions 1 or 2, permutable) is shown.](image)

In conclusion, the findings presented in this study provide functional and electrophysiological data showing that KCO-activated potassium transport in A. castellanii mitochondria could modulate K⁺ mitochondrial distribution due to the presence of the mitoK_ATP channel in the inner mitochondrial membrane. Our data suggest that the A. castellanii mitoK_ATP is similar to the channel of mammalian mitochondria and differs from that of plant mitochondria. The function of the potassium channel in protozoan mitochondria is still a field open to speculation. We know that the primary function of the mitoK_ATP channel to allow K⁺ transport into the mitochondrial matrix. This can lead to an increase in matrix volume and matrix alkalization and modulate reactive oxygen species production by mitochondria (20). In plant mitochondria, where a highly active mitochondrial K⁺ transport has been observed, the activation of potassium transport decreases mitochondrial reactive oxygen release (13). The physiological role of potassium channel in the mitochondria of unicellular microorganisms such as A. castellanii still awaits exploration.

Taking into account the location of A. castellanii in phylogenesis (21, 34) and, on the other hand, the functional and electrophysiological evidence that the mitochondrial potassium channel exists in protozoan mitochondria (this study), we suggest that the protein is much more widespread than previously believed and could occur in the whole eukaryotic world.

Acknowledgment—We thank Karolina Kubiak for technical assistance.

REFERENCES

1. Bernardi, P. (1999) *Physiol. Rev.* 79, 1127–1155
2. O’Rourke, B. (2007) *Annu. Rev. Physiol.* 69, 23.1–23.31
3. Tsujimoto, Y., Nakagawa, T., and Shimizu, S. (2006) *Biochim. Biophys. Acta* 1757, 1297–1300
4. Ardehali, H., and O’Rourke, B. (2005) *J. Mol. Cell. Cardiol.* 39, 7–16
5. Inoue, I., Nagase, H., Kishi, K., and Higuti, T. (1991) *Nature* 352, 244–247
6. Paucek, P., Mironova, G., Mahdi, F., Beavis, A. D., Woldegiorgis, G., and Garlid, K. D. (1992) *J. Biol. Chem.* 267, 26062–26069
7. Balch, R., Seetharaman, S., Kowaltowski, A. J., Garlid, K. D., and Paucek, P. (2001) *J. Biol. Chem.* 276, 33369–33374
8. Degbok, G., May, R., Kicinska, A., Szewczyk, A., Elger, C. E., and Kunz, W. S. (2001) *Brain Res.* 892, 42–50
9. Degbok, G., Kicinska, A., Skalska, J., Szewczyk, A., May, R., Elger, C. E., and Kunz, W. S. (2002) *Biochim. Biophys. Acta* 1556, 97–105
10. Cancarini, G. V., Trabuc, L. G., Rebovs, N. A., and Kowaltowski, A. J. (2003) *Am. J. Physiol.* 285, F1291–F1296
11. Dahlem, Y. A., Horn, T. F., Buntinas, L., Goni, T., Wolf, G., and Siemen, D. (2004) *Biochim. Biophys. Acta* 1656, 46–56
12. Pastore, D., Stoppelli, M. C., Di Fonzo, N., and Passarella, S. (1999) *J. Biol. Chem.* 274, 26683–26690
13. Ruy, F., Vercesi, A. E., Andrade, P. B., Bianconi, M. L., Chaimovich, H., and Kowaltowski, A. J. (2004) *Bioenerg. Biomembr.* 36, 195–202
14. Szewczyk, A., Skalska, J., Glab, M., Kulawiak, B., Malinska, D., Koszela-Piotrowska, I., and Kunz, W. S. (2006) *Biochim. Biophys. Acta* 1757,
15. Mannhold, R. (2006) *Curr. Top. Med. Chem.* 6, 1031–1047
16. Garlid, K. D., Paucek, P., Yarov-Yarovoy, V., Sun, X., and Schindler, P. A. (1996) *J. Biol. Chem.* 271, 8796–8799
17. Suzuki, M., Kotake, K., Fujikura, K., Inagaki, N., Suzuki, T., Gono, T., Seino, S., and Takata, K. (1997) *Biochem. Biophys. Res. Commun.* 241, 693–697
18. Zhou, M., Tanaka, O., Sekiguchi, M., Sakabe, K., Anzai, M., Izumida, I., Inoue, T., Kawahara, K., and Abe, H. (1999) *Science* 283, 1476–1481
19. Ardehali, H., Chen, Z., Ko, Y., Mejia-Alvarez, R., and Marban, E. (2004) *Proc. Natl. Acad. Sci. U. S. A.* 101, 11880–11885
20. Garlid, K. D., and Paucek, P. (2003) *Biochim. Biophys. Acta* 1606, 23–41
21. Grey, M. W., Burger, G., and Lang, B. F. (1999) *Science* 283, 1476–1481
22. Jarmuszkiewicz, W., Wagner, A. M., Wagner, M. J., and Hryniewiecka, L. (1997) *FEBS Lett.* 11, 110–114
23. Jarmuszkiewicz, W., Sluse-Goffart, C. M., Hryniewiecka, L., Michejda, J., and Sluse, F. E. (1998) *J. Biol. Chem.* 273, 10174–10180
24. Mackenzie, S., and McIntosh, L. (1999) *Plant Cell* 11, 571–585
25. Wojtkowska, M., Szczech, N., Stobienia, O., Jarmuszkiewicz, W., Budzinska, M., and Kmita, H. (2005) *J. Bioenerg. Biomembr.* 37, 261–268
26. Bednarczyk, P., Kicinska, A., Kominkova, V., Ondrias, K., Dolowy, K., and Szewczyk, A. (2004) *J. Membr. Biol.* 199, 63–72
27. Hordejuk, R., Lobanov, N. A., Kicinska, A., Szewczyk, A., and Dolowy, K. (2004) *Mol. Membr. Biol.* 21, 307–313
28. Hille, B. (2001) *Selective Permeability: Independence*, pp. 441–470, Sinauer Associates Inc., Sunderland, MA
29. Kamo, N., Muratsugu, M., Hongoh, R., and Kobatake, Y. (1979) *J. Membr. Biol.* 49, 105–121
30. Grover, G. J., D’Alonzo, A. J., Garlid K. D., Baigar, R., Lodge, N. I., Sleph, P. G., Darbenzio, R. B., Hess, T. A., Smith, M. A., Paucek, P., and Atwal, K. S. (2001) *J. Pharmacol. Exp. Ther.* 297, 1184–1192
31. Kwiatkowski, A. J., Seetharaman, S., Paucek, P., and Garlid, K. D. (2001) *Am. J. Physiol.* 280, H649–H657
32. Jarmuszkiewicz, W., Sluse-Goffart, C. M., Vercesi, A. E., and Sluse, F. E. (2001) *Biosci. Rep.* 21, 213–222
33. Sluse, F. E., and Jarmuszkiewicz, W. (2002) in *Handbook of Plant Growth: pH as the Master Variable* (Rengel, Z., ed) pp. 173–209, Marcel Dekker, Inc., New York
34. Wainright, P. O., Hinkle, G., Sogin, M. L., and Stickel, S. K. (1993) *Science* 260, 340–342
35. Garlid, K. D., Puddu, P. E., Pasdois, P., Costa, A. D., Beauvoit, B., Criniti, A., Tariosse, L., Dielez, P., and Dos Santos, P. (2006) *Am. J. Physiol.* 291, H152–H160
36. Drose, S., Brandt, U., and Hanley, P. J. (2006) *J. Biol. Chem.* 281, 23733–23739
37. Jaburek, M., Yarov-Yarovoy, V., Paucek, P., and Garlid, K. D. (1998) *J. Biol. Chem.* 273, 13578–13582
38. Kopustinskaie, D. A., Toleikis, A., and Saris, N. E. (2003) *J. Bioenerg. Biomembr.* 35, 141–148