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The K^+ Conductance of the Inner Mitochondrial Membrane

A STUDY OF THE INDUCIBLE UNIPORT FOR MONOVALENT CATIONS*

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Addition of A23187 plus EDTA to rat liver mitochondria induces a common uniport pathway for monovalent cations. In this study, we have carried out a detailed characterization of the flow/force relationship for K^+ transport along this pathway under steady state conditions. In the presence of EDTA, the K^+ conductance is a linear function of external K^+ in the range 0–20 mM K^+, with a slope of 0.15 nmol of K^+ × mg of protein^{-1} × min^{-1} × mV^{-1}. The K^+ conductance is inhibited by Mg^{2+} in the range 10^{-9}–10^{-6} M, while K^+ flux is stimulated by the sulfhydryl group reagent mersalyl. Uniport activity can be detected in native mitochondria. These findings are compatible with the notion that electrophoretic K^+ flux across the inner membrane takes place via a regulated K^+ uniport with the potential of transporting K^+ at rates in excess of 600 nmol × mg of protein^{-1} × min^{-1}.

As postulated by Mitchell 30 years ago (1, 2), mitochondria possess an endogenous electroneutral H^+-K^+ antiporter (3) catalyzing K^+ efflux against the K^+ electrochemical gradient (4). The role of the H^+-K^+ antiporter is to compensate electrophoretic K^+ uptake, thus providing an essential device for volume homeostasis (5). As a consequence of the chemiosmotic concept of the insulating membrane, it is widely perceived that electrophoretic K^+ uptake is due to K^+ leaks across the inner membrane rather than to the activity of a regulated transport pathway. Garlid (6) has illustrated most clearly this concept in an excellent review on mitochondrial volume control, stating that "...the best defense against wasteful K^+ leaks lies in the intrinsic energy barrier of the membrane itself. That is, a specific K^+ uniport pathway would appear to be contraindicated on physiological grounds. Absent such a pathway, it is difficult to imagine how it could be regulated."

A number of earlier studies, however, indicated that a specific pathway for K^+ uptake may exist. Thus, mitochondria catalyze respiration-dependent $^{42}K^+$-$^{39}K^+$ exchange and the $^{42}K^+$ influx process is dependent on the K^+ concentration, shows saturation kinetics, and is sensitive to uncouplers (7–9). Some of these findings have been used as an argument against the chemiosmotic hypothesis rather than to analyze the basis for K^+ transport itself (10), and have largely been abandoned parallel to the acceptance of Mitchell's hypothesis. Brierley's (11) concept of a regulated K^+ uniport, on the other hand, was conceived in a chemiosmotic framework but did not gain general consensus.

We have recently re-examined the problem of the pathways for electrophoretic cation transport in mitochondria. We have shown that mitochondria possess two transport systems regulated by different pools of Mg^{2+}: the Na^+ (Li^+)-selective uniport, which is activated by depletion of surface Mg^{2+} and does not transport K^+ (12), as suggested in earlier reports (13–15); (ii) the K^+ uniport, which is activated by depletion of matrix Mg^{2+} (16). In this paper, we have characterized the latter pathway further. We show that (i) the uniport induced by depletion of matrix Mg^{2+} is unselective, and transports Rb^+, Na^+, and Li^+ as well; (ii) the K^+ conductance on this pathway is a linear function of $[K^+]_o$, with a slope of 0.15 nmol of K^+ × mg of protein^{-1} × min^{-1} × mV^{-1} × mM^{-1}; (iii) the uniport is inhibited by Mg^{2+} with high affinity, and is stimulated by mersalyl. We conclude that mitochondria are endowed with a uniport for monovalent cations with the potential of transporting K^+ at rates in excess of 600 nmol × mg of protein^{-1} × min^{-1}, and that the K^+ conductance across this pathway is efficiently regulated by matrix Mg^{2+}, as suggested in earlier studies of mitochondrial K^+ transport (11).

MATERIALS AND METHODS

Preparation of mitochondria and measurements of oxygen consumption were performed as described previously (17). The kinetics of mitochondrial absorbance changes were followed at 540 nm with a Hewlett-Packard 8452A diode array spectrophotometer. Membrane potential was measured with a triphenylmethylphosphonium ion-selective electrode as described by Zoratti *et al.* (18). Mg-EDTA buffers were prepared based on an apparent stability constant of 5.65 at pH 7.4, and the value of $[Mg\text{-}EDTA]_{total}$ was taken as being equal to the total Mg^{2+} concentration since $[Mg^{2+}]_{free} \ll [Mg\text{-}EDTA]_{total}$. Incubation conditions are specified in the figure legends. All chemicals were of the highest purity commercially available.

Fig. 1 shows a scheme for K^+ and H^+ fluxes in energized mitochondria treated with A23187 in the presence of EDTA. Under these conditions both the H^+-K^+ antiporter (4) and the K^+ uniport (16) are activated. In K^+-based medium, uniport-mediated K^+ influx (pathway 4) collapses the membrane potential (16) with compensatory increase of H^+ ejection (pathway 2) and therefore of respiration. As long as its activity is in excess, antiport-mediated H^+-K^+ exchange (pathway 3) prevents net K^+ accumulation, and futile K^+ and H^+ cycling is established. If H^+ pumping by the respiratory chain is not rate-limiting, respiration is a function of the K^+ concentration (see Fig. 4). The K^+ conductance, C_MK, is given by the following.

$$C_MK = J_K/\Delta\tilde{\mu}K \quad (1)$$

The K^+ flux, J_K, was determined as follows. In sucrose medium total steady state H^+ flux in the presence of A23187 is given by

$$J_H \text{ total} = J_H \text{ leak} = nJO_2 \text{ (S)} \quad (2)$$

where JO_2 (S) is the rate of oxygen consumption and n the stoichiometry of the H^+ pumps, taken as 8 H^+ extruded/nanoatom of oxygen reduced with succinate as the electron donor (19). In KCl medium,

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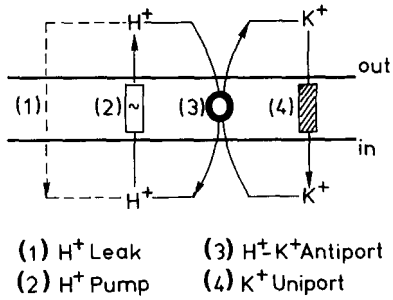

FIG. 1. **Scheme for K^+ and H^+ fluxes in energized mitochondria at steady state.** For explanation see text.

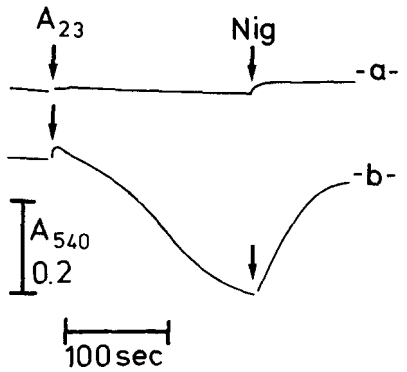

FIG. 2. **Quinine-dependent volume changes induced by A23187 and nigericin in energized mitochondria.** The incubation medium contained 0.16 M sucrose, 20 mM KCl, 10 mM Tris-Mops,[1] pH 7.4, 5 mM succinate Tris, 5 mM P_i-Tris, 5 mM EDTA-Tris, 0.2 mM EGTA-Tris, 2 μM rotenone, 5 μM cytochrome *c*, and 0.5 μg of oligomycin × ml^{-1}. The experiment was started by the addition of 0.5 mg of mitochondria to a final volume of 2 ml (not shown). When indicated, 1 nmol of A23187 × mg of $protein^{-1}$ (*A23*) and 0.28 nmol of nigericin × mg of $protein^{-1}$ (*Nig*) were added. In *trace b*, the medium was supplemented with 1 mM quinine.

total steady state H^+ flux in the presence of A23187 is given by

$$J_H \text{ total} = J_H \text{ leak} + J_K = nJO_2 \text{ (K)} \quad (3)$$

where JO_2 (K) is the rate of oxygen consumption at the given K^+ concentration. Therefore, K^+ flux is as shown below.

$$J_K = nJO_2 \text{ (K)} - nJO_2 \text{ (S)} \quad (4)$$

Thus, K^+ flux can be calculated from measurements of oxygen consumption in the presence of A23187.

The K^+ electrochemical gradient, $\Delta\tilde{\mu}K$, is given by

$$\Delta\tilde{\mu}K = \Delta\psi - 60 \log [K^+]_{in}/[K^+]_{out} \quad (5)$$

where $\Delta\psi$ denotes the membrane potential in millivolts. Since $[K^+]_{in}$ is constant (see Fig. 2) and $[K^+]_{out}$ is known, $\Delta\tilde{\mu}K$ can be calculated with precision from measurements of membrane potential at steady state. The feasibility of this approach is confirmed by the finding that in the range 4–20 mM $[K^+]_{out}$, the $\Delta\tilde{\mu}K$ stays constant at 110 ± 3.1 mV, indicating that the changes in the electrical and chemical terms of the gradient are balanced, as expected for a steady state where H^+ pumping and H^+-K^+ exchange activities are in excess and electrophoretic K^+ flux is rate-limiting.

This fundamental point is supported by the experiment of Fig. 2, showing a continuous recording of mitochondrial absorbance at 540 nm, which is a sensitive indicator of mitochondrial volume. Addition of A23187 to energized mitochondria incubated in isotonic medium containing 20 mM K^+ did not cause any major change in absorbance (*trace a*). Addition of A23187 caused a decrease in absorbance, indicative of mitochondrial swelling, only when the experiment was performed in the presence of quinine, which inhibits the H^+-K^+ antiporter (20) (*trace b*, see also, Ref. 16). The absorbance decrease observed in *trace b* was reversed by the exogenous H^+-K^+ exchanger nigericin, proving that swelling depends on electrophoretic K^+ uptake. Since under the conditions of Fig. 2, *trace a*, respiration is stimulated (see Fig. 4), we conclude that no net K^+ movements take place under these conditions, and therefore that $[K^+]_{in}$ remains constant throughout our measurements.

Under the experimental conditions used to determine the K^+ conductance we ascertained that (i) the rate of respiration was linear with time, was not stimulated by nigericin, and could always be increased by uncouplers; (ii) the membrane potential was stable throughout the experiments; and (iii) no net K^+ accumulation occurred.

A similar approach has been used by Heaton and Nicholls (21) to determine the Ca^{2+} conductance across the inner mitochondrial membrane.

RESULTS

The experiments depicted in Fig. 3 report on the effect of increasing concentrations of Na^+, Li^+, and K^+ on mitochondrial respiration. In the absence of A23187 (*panel A*) the rate of respiration increased in a dose-dependent fashion with Na^+ and Li^+ but not K^+, consistent with the opening of a Na^+- and Li^+-selective uniport upon removal of surface Mg^{2+} (12). When A23187 was added together with EDTA (*panel B*) the increase in respiration was observed with K^+ (and Rb^+, not shown) as well, consistent with the induction of an unselective uniport pathway for monovalent cations (16). It is noteworthy that in the case of Na^+ and Li^+ flux through the unselective uniport was faster, since at any cation concentration tested the rate of respiration in the presence of A23187 was faster than that observed with EDTA alone (compare *panels A* and *B*). To avoid complexities arising from the overlap of the two pathways, we have carried out a detailed study of the unselective uniport for monovalent cations induced by A23187 plus EDTA with K^+ ions.

Fig. 4 shows a typical determination of the respiratory rate and membrane potential maintained by energized mitochondria incubated in the presence of EDTA. In the absence of A23187, the rate of respiration remained constant at about 20 nanoatoms of oxygen × mg of $protein^{-1}$ × min^{-1} as K^+ was increased from 0 to 20 mM (*upper panel*), while the membrane potential decreased slightly (*lower panel*). Addition of A23187 doubled the basal rate of respiration with a minor change of membrane potential in K^+-free medium. This indicates that an increase of the steady state H^+ leaks has been induced, which is being fully compensated by the increase of respira-

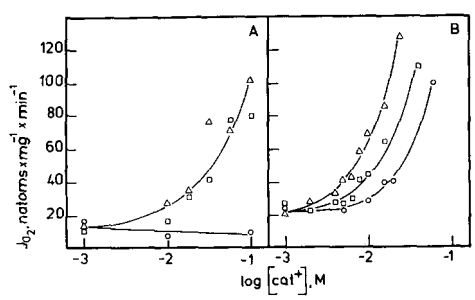

FIG. 3. **Cation-dependent increase of mitochondrial respiration: effects of EDTA and A23187.** The incubation medium contained NaCl (△), LiCl (□), or KCl (○) as indicated plus sucrose to give 200 mOsm, 10 mM Tris-Mops, pH 7.4, 5 mM succinate Tris, 5 mM P_i-Tris, 0.2 mM EGTA-Tris, 2 μM rotenone, 2.5 μM cytochrome *c*, and 0.5 μg × ml^{-1} oligomycin. Final volume 2 ml, 25 °C. The experiments were started by the addition of 1 mg × ml^{-1} of mitochondria. In *panel A*, 5 mM EDTA-Tris was added 2 min after mitochondria and values on the *ordinate* refer to the difference in respiration before and after EDTA. In *panel B*, 5 mM EDTA-Tris was present, 1 nmol of A23187 × mg of $protein^{-1}$ was added 2 min after mitochondria, and values on the *ordinate* refer to the difference in respiration before and after A23187.

[1] The abbreviations used are: Mops, 4-morpholinepropanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

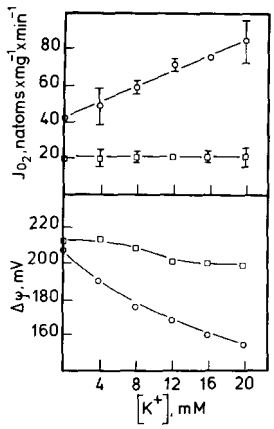

FIG. 4. **K^+-dependent changes of mitochondrial respiration and membrane potential: effect of A23187.** The incubation medium contained the indicated concentrations of KCl plus sucrose to give 200 mOsm, and was otherwise identical to that of Fig. 2, *trace a*, except that 5 μM triphenylmethylphosphonium ion was added. The experiments were started by the addition of 1 mg × ml^{-1} of mitochondria, and respiratory rates (*upper panel*) and membrane potential (*lower panel*) were determined in the absence (□) or presence (○) of 1 nmol of A23187 × mg of protein^{-1}. For measurements of respiration, the values shown are average of triplicate samples ± S.D.

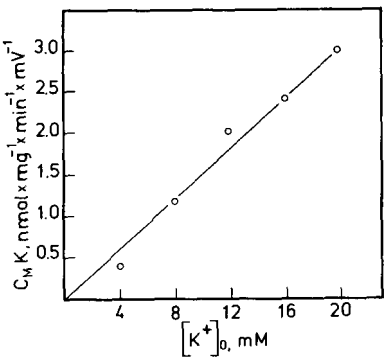

FIG. 5. **Dependence of K^+ conductance on K^+ concentration in mitochondria treated with A23187.** K^+ conductance values were calculated from the data of Fig. 4 as described under "Materials and Methods."

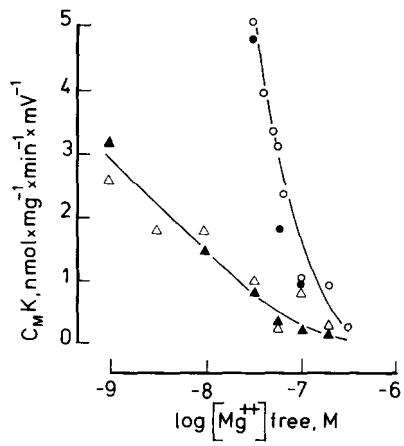

FIG. 6. **Effect of Mg^{2+} on K^+ conductance in A23187-treated mitochondria.** The incubation medium contained 0.1 M KCl (*circles*) or 20 mM KCl plus 0.16 M sucrose (*triangles*), and was otherwise identical to that of Fig. 2, *trace a*, except that $MgCl_2$ was added to give the indicated concentrations of free Mg^{2+}, and 5 μM triphenylmethylphosphonium ion was present. Conductance values were determined as described under "Materials and Methods" from measurements of respiration and membrane potential. *Filled* and *open symbols* refer to data obtained from different mitochondrial preparations.

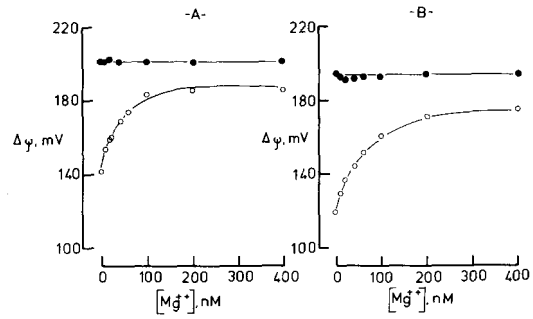

FIG. 7. **Effect of Mg^{2+} on membrane potential in mitochondria treated with A23187.** The incubation medium contained 20 mM (*panel A*) or 40 mM (*panel B*) KCl plus sucrose to give 200 mOsm, and experimental conditions were otherwise identical to those of Fig. 6. Membrane potential was determined in the absence (*closed symbols*) or presence (*open symbols*) of 1 nmol of A23187 × mg of protein^{-1}.

tion. When the K^+ concentration was increased in the presence of A23187, a linear increase of the respiratory rate took place (*upper panel*), which was matched by a K^+-dependent decrease of membrane potential (*lower panel*).

As shown in Fig. 2, the rate of K^+ uptake via the unselective uniport is rate-limiting in this range of K^+ concentrations, allowing an accurate calculation of the flow/force relationship for K^+ transport. Fig. 5 shows the changes of K^+ conductance as a function of the K^+ concentration calculated from the data of Fig. 4 as described under "Materials and Methods." The K^+ conductance increased linearly with $[K^+]_o$, reaching a value of 3 nmol of K^+ × mg of protein^{-1} × min^{-1} × mV^{-1} at 20 mM K^+. If the linearity range extends to physiological K^+ concentrations, this value extrapolates to the surprisingly high figure of 22.5 nmol of K^+ × mg of protein^{-1} × min^{-1} × mV^{-1} at 150 mM K^+, with predicted flux rates of 4500 nmol of K^+ × mg of protein^{-1} × min^{-1} at 200 mV. As shown in Fig. 6, such high values are unlikely to be ever attained under physiological conditions due to the presence of Mg^{2+}. Indeed, both at 20 and 100 mM $[K^+]_o$ submicromolar Mg^{2+} concentrations decreased the K^+ conductance, which dropped to very low values at $[Mg^{2+}]_{free} > 0.3$ μM.

Determinations of K^+ flux from measurements of steady state respiration become less reliable when the absolute rate of respiration in the presence of K^+ is close to the basal rate in sucrose. In our hands, this occurs when $[K^+]_o$ is lower than 4 mM in EDTA-treated mitochondria or when $[Mg^{2+}]_{free}$ is higher than about 0.5 μM at any K^+ concentration. Since cytosolic Mg^{2+} is in the millimolar range (22), it is difficult to make predictions on uniport activity under physiological conditions with this approach. For this reason, we have also studied the uniport-mediated K^+ current indirectly from measurements of the steady state membrane potential.

Fig. 7*A* shows that energized mitochondria incubated in isotonic medium containing 20 mM K^+ maintained a steady state membrane potential of 200 mV irrespective of the concentration of external free Mg^{2+} (*closed symbols*). In the presence of A23187, however, depletion of Mg^{2+} with EDTA led to activation of K^+ uptake with membrane depolarization (*open symbols*, 0 Mg^{2+}). When the concentration of free Mg^{2+} was raised, the membrane repolarized up to a maximal value of about 180 mV but did not recover the level preceding the addition of A23187. When the experiment was performed at 40 mM K^+ (Fig. 7*B*), steady state membrane potential in the absence of A23187 was lower and still independent of external free Mg^{2+} concentration (*closed symbols*). As expected, A23187-induced depolarization with excess EDTA was more

marked (*open symbols*, 0 Mg^{2+}). However, the maximal membrane potential that could be attained by increasing external free Mg^{2+} was now less than 180 mV, suggesting that uniport inhibition may not be complete in the presence of physiological Mg^{2+} concentrations.

Some support for this contention comes from the experiments of Fig. 8, showing a determination of the steady state membrane potential maintained by energized mitochondria incubated in isotonic media containing increasing concentrations of K^+. Addition of EDTA plus A23187 induced the expected depolarization. Interestingly, however, a K^+-dependent decrease of membrane potential could be detected even in the absence of the ionophore both with 1 mM Mg^{2+} and 1 mM EDTA. Since under these conditions mitochondria do not swell (not shown) but take up $^{42}K^+$ in an energy-dependent process (7–11), it is likely that the depolarization is due to increased K^+ cycling via coupling of K^+ uniport and H^+-K^+ antiport.

Energy-dependent influx of $^{42}K^+$ in mitochondria is stimulated by the SH group reagents *N*-ethylmaleimide (8) and mersalyl (9). Table I reports an experiment designed to test whether activity of the inducible K^+ uniport is affected by

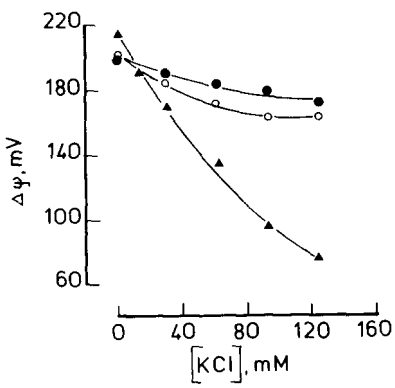

FIG. 8. **K^+-dependent changes of membrane potential: effect of Mg^{2+}, EDTA, and A23187.** The incubation medium contained the indicated concentrations of KCl plus sucrose to give 250 mOsm, 1 mM Tris-HCl, 5 mM succinate Tris, 5 mM P_i-Tris, 0.2 mM EGTA-Tris, 5 μM triphenylmethylphosphonium ion, 2 μM rotenone, and 1 mM $MgCl_2$ (●), 1 mM EDTA (○), or 1 mM EDTA plus 1 nmol of A23187 × mg of protein^{-1} (▲). The experiments were started by the addition of 1 mg × ml^{-1} of mitochondria, and values on the *ordinate* refer to membrane potential at steady state.

TABLE I

Activation of K^+ uniport by mersalyl

The incubation medium contained 0.1 M KNO_3, 10 mM Tris-Mops, pH 8.0, 5 mM EDTA-Tris, 0.2 mM EGTA-Tris, 2 μM rotenone. The mitochondrial stock solution was pretreated with 10 μM rotenone and 0.5 μg/ml of antimycin A. Final volume 2 ml, 25 °C. The experiments were started by the addition of 0.5 mg of mitochondria followed by the ionophore listed in the first column, and values on the ordinate refer to the rate of swelling. When present, mersalyl was added at 30 nmol × mg of protein^{-1} to the mitochondrial stock solution on ice, and incubated for 10 min. Ionophores were added at the following doses: A23187, 1 nmol × mg of protein^{-1}; nigericin, 1.12 nmol × mg of protein^{-1}; valinomycin, 0.72 nmol × mg of protein^{-1}; carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), 0.8 nmol × mg of protein^{-1}.

Ionophore	Swelling rate	
	No additions	Mersalyl
	ΔA_{540nm} × *mg protein*$^{-1}$ × *min*$^{-1}$	
A23187	0.652	0.958
A23187 + valinomycin	1.958	2.068
A23187 + nigericin	0.632	0.886
A23187 + FCCP	0.716	0.944

mersalyl. To avoid complexities arising from mersalyl interaction with the respiratory complexes and the phosphate carrier, we used a passive swelling assay in KNO_3 at pH 8.0. Table I shows that addition of A23187 induced a swelling process, which could be increased by addition of valinomycin. When nigericin or carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone were added together with A23187 the rate of swelling did not increase. As discussed in detail elsewhere (16), these findings indicate that mitochondria treated with A23187 in K^+ nitrate at alkaline pH exhibit: (i) high permeability to NO_3^- (maximal rate of swelling induced by valinomycin); (ii) low permeability to H^+ and HNO_3 (no stimulation of swelling rate by nigericin); (iii) low H^+-K^+ antiport activity (little stimulation of swelling rate by carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone). Thus, the basal swelling rate induced by A23187 is due to activation of K^+ uniport (16). The novel finding described in Table I is that treatment of mitochondria with 30 nmol of mersalyl × mg of protein^{-1} induced a 50% increase in the swelling rate induced by A23187. The increase was due to increase of K^+ transport on the uniport since: (i) anion transport rate was unaffected (maximal rate with valinomycin was unchanged); (ii) H^+ and HNO_3 diffusion were unaffected (nigericin did not increase the basal rate); (iii) no induction of H^+-K^+ antiport was apparent (the rate of swelling was not stimulated by uncoupler).

DISCUSSION

A general concept emerging from a number of studies of mitochondrial ion transport is that the inner mitochondrial membrane possesses both overt and inducible ion transport systems. The *overt* transport systems include the Ca^{2+} uniporter (23, 24), the Na^+-Ca^{2+} (25, 26) and H^+-Ca^{2+} antiporters (27–29), and the H^+-Na^+ antiporter (5, 20); the *inducible* transport systems include: (i) the electroneutral H^+-K^+ (Na^+) antiporter (3), activated by depletion of matrix Mg^{2+} (4, 17, 30), hypotonic swelling (17, 31), and alkaline pH (17); (ii) the inner membrane anion channel, activated by depletion of matrix Mg^{2+} (32–34) and alkaline pH (35, 36); (iii) the Na^+ (Li^+)-selective uniport, activated by depletion of surface Mg^{2+} (12–15); (iv) the unselective uniport for monovalent cations, activated by depletion of matrix Mg^{2+} (16) and by mersalyl (Table I).

Open questions are: (i) how these transport systems are regulated; (ii) whether each transport activity is mediated by independent carriers, or rather the inducers modulate the ion selectivity of one or more "common" transport pathways; (iii) whether a basal activity mediated by the inducible pathways is present in unmodified mitochondria.

To date, only the inducible H^+-K^+ antiporter has been isolated in active form (3). The reconstituted protein catalyzes electroneutral H^+-K^+ exchange, indicating that the monovalent cation uniports must be different molecular species. While a final answer to these problems will eventually come from isolation and reconstitution of the carrier proteins, we have obtained further information on the regulation of electrophoretic membrane permeability to monovalent cations in mitochondria.

Electrophoretic Permeability to Monovalent Cations in Mg^{2+}-depleted Mitochondria—The evidence supporting the suggestion that depletion of matrix Mg^{2+} does not lead to a generalized permeability increase of the inner mitochondrial membrane, and that K^+ transport is not the consequence of unspecific leaks may be summarized as follows: (i) addition of A23187 to energized mitochondria in sucrose medium leads to membrane hyperpolarization (16), and the membrane po-

tential remains high despite some stimulation of the respiration (Ref. 17 and Fig. 4); the opposite would be expected in case of a generalized permeability increase; (ii) inhibition of the K^+ current by Mg^{2+} is in the nanomolar range (Figs. 6 and 7), suggesting a high specificity; even if it is difficult to predict the concentration of matrix Mg^{2+} causing inhibition, under our experimental conditions the "K_i" for Mg^{2+} corresponds to about 50 pmol × mg of protein^{-1}, which is in line with a specific interaction with a membrane protein; (iii) K^+ transport is inhibited by ruthenium red with a K_i of 40 nM (37). Besides inhibition of the Ca^{2+} uniporter (38) and of the cation uniports (37), ruthenium red does not affect any mitochondrial function that is not linked to Ca^{2+} recycling (39).

In this paper, we have extended our previous observation that matrix Mg^{2+} regulates the activity of the latent uniport for K^+ transport (16). From the data of Fig. 3, it appears that this pathway is not selective, but transports Na^+ and Li^+ as well. The data of Fig. 3*B* seem to support a $Na^+ > Li^+ > K^+$ selectivity, but conclusions based solely on flux rates may be misleading. Indeed, since K^+ is the main intramitochondrial cation, the lower rate of K^+ transport relative to Na^+ and Li^+ may be a reflection of a lower K^+ electrochemical gradient.

Na^+ and Li^+ flux along the unselective cation uniport is faster than Na^+ and Li^+ flux along the Na^+ (Li^+)-selective uniport activated by removal of surface Mg^{2+} (Ref. 12 and Fig. 3*A*). For example, at 10 mM Na^+ flux through the Na^+-selective pathway accounts for a respiration of about 25 nanoatoms of oxygen × mg of protein^{-1} × min^{-1}, while flux through the unselective conductance pathway corresponds to a respiration of about 70 nanoatoms of oxygen × mg of protein^{-1} × min^{-1} (Fig. 3*B*). Since up to 20 nanoatoms of oxygen × mg of protein^{-1} × min^{-1} are due to A23187 induced H^+ leaks (Fig. 4), at this Na^+ concentration flux through the unselective pathway is twice as fast as flux through the Na^+ (Li^+)-selective one. This finding was confirmed in measurements of initial rate of energy-dependent swelling in NaCl media (not shown). These data make it unlikely that removal of matrix Mg^{2+} with A23187 plus EDTA modifies the Na^+ (Li^+)-selective pathway making it available for K^+ transport. If this were the case, the increased Na^+ flux in the presence of A23187 should be explained by a decreased K_m for external Na^+ induced by removal of internal Mg^{2+}, which we consider less likely.

Na^+ flux through the selective uniport and K^+ flux through the unselective uniport are inhibited by ruthenium red with a K_i of about 40 nM (37), which is close to the K_i reported for inhibition of the Ca^{2+} uniporter (40, 41). Based on this finding, Kapus *et al.* (37) have proposed that Mg^{2+} modifies the cation specificity of the Ca^{2+} uniporter, and that Ca^{2+}, Na^+, and K^+ fluxes occur through a common pathway. Inhibition by ruthenium red strongly supports the view that Na^+ and K^+ fluxes are mediated by specific transport systems rather than by leak pathways (12, 16). On the other hand, identification of these pathways with the Ca^{2+} uniporter requires some caution since ruthenium red also inhibits the Ca^{2+} channel of the skeletal muscle sarcoplasmic reticulum (42) and binds to other proteins such as calsequestrin (43) and troponin C (44). At present, however, we consider the proposal of Kapus *et al.* (37) as an intriguing possibility.

The K^+ conductance across the unselective cation uniport is surprisingly high, and the measured value of 3 nmol of K^+ × mg of protein^{-1} × min^{-1} × mV^{-1} at 20 mM K^+ translates into a flux of 600 nmol of K^+ × mg of protein^{-1} × min^{-1} at 200 mV. Since intracellular K^+ is about 150 mM, this value represents a lower limit, and flux on the fully activated uniport is expected to be higher even if the increase of conductance with external [K^+] is not linear above 20 mM K^+. When compared to a H^+ conductance of 0.2 nmol of H^+ × mg of protein^{-1} × min^{-1} × mV^{-1} (45), it is clear that this pathway is endowed with a vast excess capacity. The high sensitivity to Mg^{2+} indicates that it is highly inhibited under physiological conditions, consistent with Brierley's (11) concept of a regulated K^+ uniport.

Electrophoretic Permeability to Monovalent Cations in Native Mitochondria—Freshly isolated mitochondria take up $^{42}K^+$ in an energy-dependent reaction, and $^{42}K^+$ uptake has some of the features of a carrier-mediated process (7–11). We think that the $^{42}K^+$ transport activity measured in unmodified mitochondria could be mediated by the same pathway activated by depletion of matrix Mg^{2+}. Support for this view comes from three lines of evidence: (i) typical rates of $^{42}K^+$ uptake in native mitochondria at 4.8 mM $[K^+]_o$ are 1–2 nmol × mg of protein^{-1} × min^{-1} (calculated from Fig. 1 of Ref. 8), while Jung *et al.* (9) extrapolated a V_{max} of 7.5 nmol × mg of protein^{-1} × min^{-1} at saturating K^+ concentrations. Even if at physiological [Mg^{2+}] the unselective uniport were operating at less than 1% of its capacity, this would account for the flux measured with $^{42}K^+$ as a tracer in earlier studies of K^+ transport (7–10); (ii) energy-dependent $^{42}K^+$ uptake in native mitochondria is inhibited by uncouplers (8) and under phosphorylating conditions (9), when the membrane potential decreases following H^+ influx on the F_1-F_0-ATPase (1, 2); similarly, net K^+ uptake on the unselective uniport is inhibited by uncoupler (16), suggesting that physiological changes of membrane potential can modulate electrophoretic K^+ fluxes both in native and Mg^{2+}-depleted mitochondria; (iii) K^+ uptake on the unselective uniport and $^{42}K^+$ uptake in native mitochondria are activated by the same concentrations of mersalyl (Ref. 9 and Table I).

If native mitochondria possess a monovalent cation uniport, a puzzling observation by Diwan and Tedeschi (10) remains to be explained. These authors measured unidirectional $^{42}K^+$ fluxes in energized mitochondria, and showed that both $^{42}K^+$ influx and $^{42}K^+$ efflux are inhibited by uncouplers and respiratory inhibitors (10). Since inhibition of $^{42}K^+$ efflux by deenergization is inconsistent with a passive electrophoretic K^+ flux, they concluded that the electrochemical potential gradient could not be the only driving force for K^+ flux (10). This conclusion is correct, and we now realize that energized K^+ efflux largely occurs on the electroneutral H^+-K^+ antiporter (46), which is inhibited by uncouplers and respiratory inhibitors (17, 30), rather than on the K^+ uniport. This is even more likely at pH 8.0, used by Diwan and Tedeschi (10), since activity of the antiporter increases at increasing pH (17).

Taken together, these observations support the concept that K^+ cycling and volume regulation in intact energized mitochondria occur through an interplay of *regulated* K^+ uniport and H^+-K^+ antiport, first proposed by Brierley in the late 1970s (11). After isolation of the H^+-K^+ antiporter (3) the challenge appears to be the isolation of the uniporters for monovalent cations.

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