EFFECT OF Mg^{2+} DEPLETION OF MITOCHONDRIA ON THEIR PERMEABILITY TO K^+: THE MECHANISM BY WHICH IONOPHORE A23187 INCREASES K^+ PERMEABILITY

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SUMMARY: Ionophore A23187 produces rapid swelling of rat liver mitochondria suspended in isotonic KNO_3 if an uncoupler and EDTA are also present. It also produces swelling of mitochondria in isotonic $Mg(NO_3)_2$ in the presence of an uncoupler. Washing with serum albumin removes the ionophore from mitochondria, as indicated by lack of swelling in magnesium nitrate (+ uncoupler). However, such treatment does not abolish rapid swelling in KNO_3 (+ uncoupler). This finding is interpreted in the sense that depletion of mitochondrial magnesium mobilizes K^+/H^+ antiport in the inner mitochondrial membrane.

The carboxylic ionophore A23187 catalyzes an electroneutral exchange of divalent alkali metal cations, in particular Mg^{2+} and Ca^{2+}, for H^+ across biological membranes (1). The ionophore also induces a loss of K^+ from isolated mitochondria (1) and erythrocytes (2). This process has been believed to be secondary to divalent cation depletion (1). However, Pfeiffer and Lardy (3) have recently proposed that A23187 may also directly induce a K^+/H^+ exchange across the mitochondrial membrane.

The aim of the present investigation was to look into the mechanism by which the ionophore A23187 increases the permeability of mitochondrial membranes to potassium ions. A more general aspect of the role of mitochondrial magnesium in maintaining a low permeability of mitochondrial membrane to monovalent cations is also discussed.

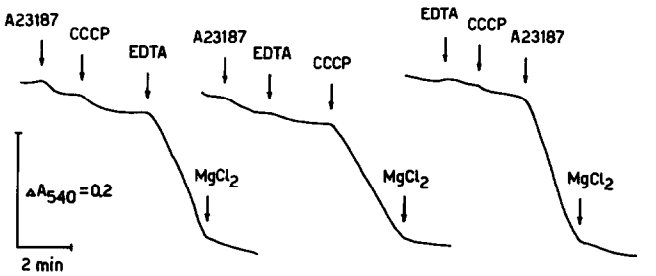

Fig. 1. Swelling of mitochondria in 135 mM KNO_3. Additions: ionophore A23187, 1 /uM; CCCP, 2 /uM; EDTA, 1.3 mM; $MgCl_2$, 10 mM.

MATERIALS AND METHODS

Rat liver mitochondria were isolated in 225 mM mannitol - 75 mM sucrose - 2 mM Tris-HCl (pH 7.4). The permeability of mitochondria to cations was measured by a modification of the swelling technique of Chappell and Crofts (4) in which potassium and magnesium salts of the permeant anion NO_3^- (5) were used. Mitochondria (about 0.4 mg protein) were suspended in 3.0 ml of isotonic solutions of potassium or magnesium nitrates containing 10 mM Tris-HCl (pH 7.4) and 2 /uM rotenone, and the decrease in light absorbance at 540 nm was followed at 23°C. Magnesium content of mitochondria was determined by atomic absorption.

The ionophore A23187 was a generous gift of Eli Lilly Co. (Indianapolis, Ind.). Bovine serum albumin (Sigma, St. Louis, Mo.) was defatted by the method of Chen (6) followed by dialysis.

RESULTS

It is well established that NO_3^- easily penetrates across the inner mitochondrial membrane [cf. (5)]. Therefore, swelling of mitochondria in isotonic solutions of nitrates depends on the penetration of the cation. Fig. 1 shows that swelling of rat liver mitochondria in KNO_3 can be produced by a concerted action of three factors: the A23187 ionophore, the uncoupler CCCP (a protonophore) and a chelator of Mg^{2+}. Neither of these factors alone or in combinations of two was effective. The order of additions was unimportant.

Abbreviation: CCCP, carbonyl cyanide-m-chlorophenylhydrazone.

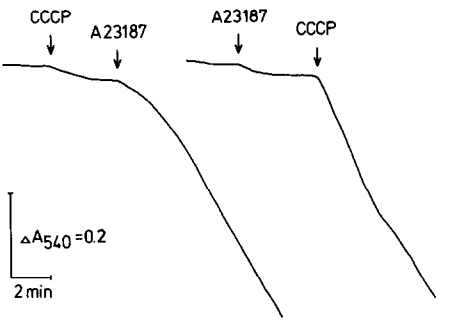

Fig. 2. Swelling of mitochondria in 90 mM $Mg(NO_3)_2$. Additions as in Fig. 1.

This experiment can be interpreted in accordance with the assumption of Pfeiffer and Lardy (3) that the ionophore catalyzes the exchange of K^+ for H^+ under conditions when Mg^{2+} is absent in the medium. However, it does not exclude the interpretation in line of a secondary increase of the K^+/H^+ antiport in the mitochondrial membrane as result of Mg^{2+} depletion.

Fig. 2 shows that swelling of mitochondria in magnesium nitrate is a good indicator of the permeability of mitochondrial membranes to Mg^{2+}. This experiment also confirms that the ionophore A23187 catalyzes an electroneutral exchange of Mg^{2+} for H^+ (1, 3, 7, 8), since a recycling of protons (induced by addition of an uncoupler) is required in order to enable Mg^{2+} to accumulate and the swelling to proceed.

To differentiate between the primary and the secondary effects of A23187 on K^+ permeability, mitochondria were pretreated with the ionophore and EDTA and subsequently washed with a sucrose solution containing serum albumin, followed by washing with sucrose alone. This treatment removed essentially all A23187, as indicated by the lack of swelling in $Mg(NO_3)_2$ upon addition of uncoupler (Fig. 3, trace A). Nevertheless, mito-

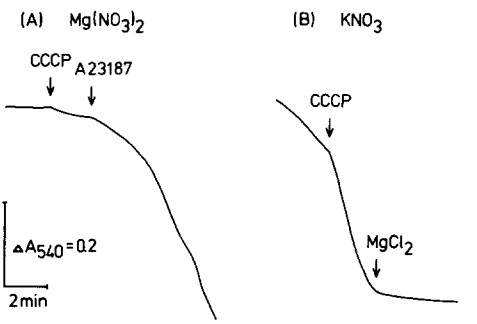

Fig. 3. Swelling of mitochondria pretreated with A23187 and washed with serum albumin. Mitochondria (20 mg protein) were suspended in 3.0 ml of 225 mM mannitol - 75 mM sucrose containing 2 mM EDTA (potassium salt), and 8 nmoles of A23187 was added. After 5 min at 0°C, the mixture was diluted by adding 10 ml of the mannitol-sucrose medium containing 0.5% defatted serum albumin, and mitochondria were sedimented by centrifugation. The resulting pellet was washed twice with mannitol-sucrose without albumin. A, Swelling in 90 mM $Mg(NO_3)_2$; B, swelling in 135 mM KNO_3. Additions as in Fig. 1.

chondria responded by swelling to the addition of the ionophore in the same way as did untreated mitochondria. This indicates that the impermeability of the mitochondrial membrane to Mg^{2+} was not altered by a temporary exposure to A23187 followed by its removal, although such mitochondria contained 4 ng atoms magnesium/mg protein as compared to 20 - 30 ng atoms/mg protein in untreated particles (8, 9).

In a sharp contrast to this, A23187-pretreated mitochondria underwent a spontaneous swelling when suspended in isotonic KNO_3 (Fig. 3, trace B). This swelling was further potentiated by uncouplers, but was inhibited by addition of Mg^{2+}.

Fig. 4 illustrates schematically ion translocation as occurring during mitochondrial swelling shown in Figs. 1 - 3. The left side of the scheme represents the $Mg^{2+}/2H^{+}$ exchange as induced by the ionophore A23187. Net accumulation of Mg^{2+} together with NO_3^- is possible only when the influx of protons is facil-

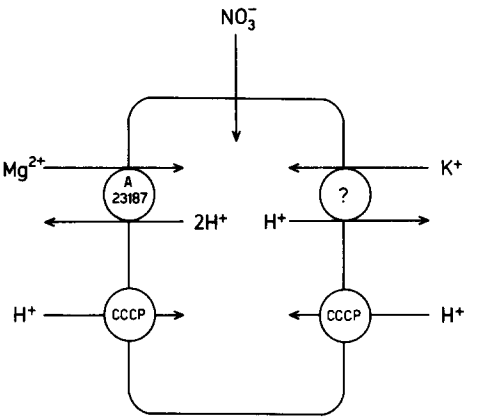

Fig. 4. Scheme for mitochondrial swelling in isotonic magnesium (left) and potassium (right) nitrates.

itated by the uncoupler. The same is true when the K^+/H^+ exchange is operating (right side of the scheme). The latter exchange occurs not only in the presence of A23187 but also when the ionophore is removed by serum albumin. It can be therefore concluded that the presence of A23187 is not necessary for the K^+/H^+ exchange to occur. A prerequisit for this exchange is thus the absece of Mg^{2+} on both sides of the inner mitochondrial membrane. Addition of 10 mM $MgCl_2$ to the external medium immediately blocks the K^+/H^+ antiport (Fig. 3, trace B).

DISCUSSION

The present study shows that an increased permeability of the inner mitochondrial membrane to K^+ and possibly to other monovalent metal cations as well) may be secondary to the ionophoretic action of A23187 on magnesium permeability, as originally suggested by Reed and Lardy (1). It is therefore not necessary to assume a direct ionophoretic mechanism of this compound on monovalent cations, as recently proposed by Pfeif-

fer and Lardy (3). In a similar way, we have recently demonstrated (Bogucka and Wojtczak, in preparation) that an increased permeability of mitochondrial membranes to K^+, induced by mercurials (10 - 12), may also be secondary to the depletion of mitochondrial magnesium.

This study also points to an essential role of intramitochondrial magnesium in maintaining the high impermeability of the inner mitochondrial membrane to potassium ions. This is in line with previous reports by several investigators on a controlling effect of magnesium on the permeability of mitochondrial (13 - 16) and erythrocyte (17, 18) membranes. An increased permeability of mitochondria to potassium, produced by chelating agents, has already been demonstrated (14, 19, 20). It has been also observed that massive loading of mitochondria with calcium produces a large-scale swelling in isotonic saline media (21). Since a loss of mitochondrial magnesium has been observed during Ca^{2+} accumulation (22, 23), this finding also supports the concept of a magnesium-controlled impermeability of mitochondrial membranes to monovalent cations.

The mechanism by which Mg^{2+} maintaints a high impermeability of the inner mitochondrial membrane to monovalent alkali metal cations may be subject for speculation. Firstly, magnesium can stabilize the membrane by forming ternary complexes with anionic groups of membrane proteins and phospholipids [cf. (16)]. By neutralizing the negative surface charge of the membrane and immobilizing anionic groups, it can thus control the natural low permeability of the mitochondrial membrane to monovalent cations (15, 24, 25). Secondly, a possibility of a natural ionophore for monovalent cations present in mitochondria should also be taken into consideration. Blondin et al. (26, 27) reported on the

isolation of an ionophoretic peptide from beef heart mitochondria. On the other hand, one of us (28) demonstrated that long-chain fatty acids greatly increased the permeability of the inner mitochondrial membrane to monovalent cations. Long-chain fatty acids seem to be a good candidate for the natural ionophore (see question mark in Fig. 4), since they are present at low concentrations in freshly isolated mitochondria from a variety of tissues and since their ionophoretic effect is also inhibited by Mg^{2+} (28).

In connection with the role of Mg^{2+} in maintaining a low permeability of the inner mitochondrial membrane to K^{+}, it is worthy to note that high amounts of magnesium are present not only in the mitochondrial matrix but also in the compartment between the outer and the inner membranes (9). Whether or not magnesium-binding proteins, recently detected in the intermembrane compartment (29), play a role in this mechanism is not yet clear.

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Note added in print

After submission of this manuscript we became acquainted with the recent article by J.P. Wehrle, M. Jurkowitz, K.M. Scott and G.P. Brierley [Arch. Biochem. Biophys., 174, 312-323 (1976)]. These authors also demonstrate that depletion of mitochondrial Mg^{2+}, including removal of membrane-bound magnesium, greatly potentiates the permeability of the inner membrane of heart mitochondria to monovalent cations.