**A23187: A Divalent Cation Ionophore**

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**SUMMARY**

A23187 is a carboxylic acid antibiotic which simultaneously uncouples oxidative phosphorylation and inhibits ATPase of rat liver mitochondria incubated in a magnesium-free medium. The antibiotic acts as a freely mobile carrier to transport calcium and magnesium, but not potassium, from an aqueous medium buffered at pH 7.4 into a bulk organic phase. A23187 causes a progressive release of endogenous magnesium and inorganic phosphate from mitochondria incubated with substrate, but little change in calcium or potassium content. If EDTA is added, A23187 severely depletes intramitochondrial calcium and magnesium in <30 s and produces loss of intramitochondrial potassium.

Release of State 4 succinate oxidation by A23187 is inhibited by lanthanum chloride, ruthenium red, (ethylene bis)oxyethylene nitrilo)tetraacetic acid (EGTA), EDTA, MgCl₂, and ATP. Inhibition by EGTA (or EDTA) of A23187's uncoupling is reversed readily by calcium but not magnesium. In the presence of A23187 plus EGTA, mitochondria retain only 2 to 3 nmoles of Mg²⁺ per mg of protein but remain coupled and phosphorylate ADP at 80 to 85% of control rates with β-hydroxybutyrate or succinate as substrate. Under these conditions, coupled phosphorylation supported by glutamate, and ATP hydrolysis are severely depressed, but increasing concentrations of magnesium in the incubating medium prevent these inhibitions. It is proposed that a cyclic, energy-dissipating flux of mitochondrial calcium accounts for uncoupling by A23187, whereas inhibition of ATPase and glutamate oxidation and increased potassium permeability of mitochondria result from antibiotic-mediated magnesium efflux.

The ionophorous antibiotics are lipid-soluble molecules that complex alkali metal cations and transport them across a variety of membranes (2–6) or into a bulk organic phase (3, 6, 7). Neutral sequestering agents, such as valinomycin (2, 4, 6), or the neutral sequestering ionophores induce active, electrogenic accumulation of monovalent cations by respiring mitochondria (3, 6, 10–12). The antibiotic A23187, having a molecular weight of 523 and an elemental analysis of C₃₉H₅₃N₃O₁₉, is a new carboxylic acid antibiotic that is crystallized from broth of Streptomyces chartreusensis as the magnesium plus calcium salt and can be converted to and crystallized as the free acid. The free acid has a molecular weight of 523 and an elemental analysis of C₃₉H₅₃N₃O₁₉. A23187 binds divalent, but not monovalent, cations at pH 7.4 and uniquely uncouples oxidative phosphorylation while inhibiting ATP hydrolysis by rat liver mitochondria (1, 15). The data presented in this report suggest that A23187 affects mitochondrial function by perturbing endogenous calcium and magnesium content and indicate that this antibiotic has appreciable potential as a tool to study the role of divalent cations in various biological systems (15).

**EXPERIMENTAL PROCEDURE**

Mitochondria were prepared as described by Johnson and Lardy (16) and suspended in 250 mM mannitol plus 70 mM sucrose. Some sucrose solutions contained appreciable amounts of calcium and these solutions were passed over a Dowex 50 H⁺ cation exchange resin before use. All incubations of mitochondria were at 30°C. Substrates were the free acids (except sodium β-hydroxybutyrate and potassium pyruvate) and nucleotides were the Tris salts; both were brought to pH 7.4 with triethanolamine base. A23187 was the generous gift of Dr. Robert Hamill, Eli Lilly and Co., Indianapolis, Indiana, and the free acid was used in all experiments.

Oxidative phosphorylation (17), ATPase activity (18), inorganic phosphate (19), and protein (20) were assayed by the methods referred to. Respiration was measured manometrically or with a collection covered vibrating platinum electrode fitted to the Gilson Oxygraph (Gilson Medical Electronics, Middleton, Wisconsin). The dissolved oxygen was taken to be 0.445 µg atom per ml of medium at 30°C.

When mitochondrial ion content was examined, incubations were initiated by addition of mitochondria to polypropylene...
tubes containing various media and terminated by rapid centrifugation in a high speed centrifuge. Under the conditions employed, sedimentation was complete in approximately 30 s. After the supernatant solution was decanted, tube insides were wiped dry and the pellets were lysed in deionized water and subsequently extracted with 0.1 N perchloric acid (calcium, magnesium, or inorganic phosphate) or 0.1 N HCl (above ions and potassium). Calcium, magnesium, and potassium were measured with a Perkin Elmer model 303 or 403 atomic absorption spectrophotometer. Strontium chloride (1%) was included in all calcium sample tubes to prevent interference from phosphate.

The ability of various antibiotics to transfer cations from an aqueous medium to an organic phase (3, 6, 7) was studied by vortexing metal salt, buffer, and antibiotic with an organic phase of 30% 1-butanol-70% toluene. An aliquot of the organic phase was evaporated to dryness in a platinum crucible and residues were reaccumulated by mitochondria in the absence of chelator. The addition of EDTA did not affect the efflux of mitochondrial calcium and magnesium and resulted in a small decrease in magnesium content of mitochondria. When mitochondria were incubated in the presence of EDTA plus A23187 (Fig. 1, open triangles), endogenous calcium and magnesium were depleted markedly during the first 30 s of incubation. Intracellular levels and thiocyanate markedly potentiated transfer of the charged K+-valinomycin complex (6). Neither nigericin nor valinomycin were divalent cation ionophores under these conditions.

Since A23187 was an ionophore for the two predominant divalent cations of rat liver mitochondria (21), the effect of the carboxylic acid on endogenous mitochondrial ions was examined. Mitochondria retained endogenous calcium and magnesium during a 10-min incubation under the conditions described in the legend to Fig. 1 (closed circles). A slow loss of intramitochondrial calcium and a more rapid efflux of inorganic phosphate were observed. Addition of A23187 (closed triangles) produced a time-dependent loss of endogenous magnesium while mitochondrial calcium content was not affected. Potassium and inorganic phosphate efflux occurred more rapidly in the presence of the antibiotic.

When EDTA was added to bind divalent cations (open circles), the calcium content of mitochondria slowly decreased, suggesting that calcium which leaked to the external medium was reaccumulated by mitochondria in the absence of chelator. The addition of EDTA did not affect the efflux of mitochondrial potassium or inorganic phosphate and resulted in a small decrease in magnesium content of mitochondria. When mitochondria were incubated in the presence of EDTA plus A23187 (Fig. 1, open triangles), endogenous calcium and magnesium were depleted markedly during the first 30 s of incubation. Intracellular levels and thiocyanate markedly potentiated transfer of the charged K+-valinomycin complex (6). Neither nigericin nor valinomycin were divalent cation ionophores under these conditions.

<table>
<thead>
<tr>
<th>pH</th>
<th>Antibiotic</th>
<th>KCl</th>
<th>KSCN</th>
<th>MgCl₂</th>
<th>CaCl₂</th>
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</thead>
<tbody>
<tr>
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<td>None</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>7.4</td>
<td>475 µM A23187</td>
<td>2</td>
<td>6</td>
<td>218;</td>
<td>100;</td>
</tr>
<tr>
<td>7.4</td>
<td>500 µM nigericin</td>
<td>343</td>
<td>423</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>7.4</td>
<td>500 µM valinomycin</td>
<td>19</td>
<td>100</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>9.8</td>
<td>None</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>9.8</td>
<td>475 µM A23187</td>
<td>52</td>
<td>66</td>
<td>229</td>
<td>187</td>
</tr>
<tr>
<td>9.8</td>
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<td>422</td>
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</tr>
<tr>
<td>9.8</td>
<td>500 µM valinomycin</td>
<td>15</td>
<td>110</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

* KSCN (20 mm) was present in addition to 20 mm divalent metal chloride.

FIG. 1. The effect of A23187 on ion content of mitochondria incubated in the presence or absence of EDTA. Mitochondria were prepared in the absence of EDTA to produce elevated calcium content and were incubated (21.1 mg of protein) in 2 ml of a medium which contained 4 mM chloride-(triethanolamine), pH 7.4, 8 mM succinate, plus 1.5 µM rotenone, 122 mM mannitol, and 144 mM sucrose. Ion contents at zero time (□) were, in nmoles per mg of protein: calcium, 14; magnesium, 34; potassium, 108; and inorganic phosphate, 23. •, no additions; ○, 0.6 mM EDTA-(Tris), pH 7.4; ▲, 0.3 n mole of A23187 per mg of protein; △, EDTA + A23187.
mitochondrial potassium and phosphate fell rapidly during the first 5 min of incubation under these conditions. Half maximal release of mitochondrial calcium and magnesium during a 30-s incubation in the presence of A23187 plus EDTA occurred at a concentration of 0.1 nmole of A23187 per mg of protein (15).

These data suggested that A23187 acted to equilibrate endogenous mitochondrial divalent cations with the external medium. Rat liver mitochondria actively accumulate calcium (21-29) but not magnesium (21, 28-30) or potassium (30, 31) by an energy-dependent process. Thus, calcium discharged by A23187 could not be reaccumulated unless EDTA was present in the medium to sequester it, whereas magnesium would remain in the extramitochondrial medium. Since A23187 did not appear to act as a potassium ionophore, loss of intramitochondrial potassium appeared secondary to severe magnesium depletion (31-33).

Inhibition of Mitochondrial ATPase by A23187—A23187 inhibited the ATPase activity of rat liver mitochondria induced by a variety of agents (Fig. 2). Half-maximal inhibition was observed at a concentration of 0.1 to 0.2 nmole of A23187 per mg of protein. Effective inhibition of all ATPase of intact mitochondria by A23187 contrasts to the selective inhibition by nigericin of ATP hydrolysis associated with active ion uptake by mitochondria (4, 9, 34). Neither the endogenous nor the magnesium-stimulated ATPase (9, 35, 36) of submitochondrial particles prepared by sonic disruption contained magnesium chloride, high concentrations of A23187 inhibited ADP phosphorylation associated with oxidation of all substrates (Fig. 5, C and D). Under these conditions, however, respiration was inhibited in a substrate-dependent manner by A23187 (Fig. 5, A and B). Thus, in contrast to other inhibitors of ATPase, A23187 uncoupled oxidative phosphorylation with various substrates, and magnesium chloride tended to prevent uncoupling.

Inhibition by A23187 of State 3 phosphorylation by mitochondria incubated in the absence of added magnesium was not due to impaired hexokinase activity. Magnesium released by mitochondria during thermal equilibration (approximately 30 μM) and added with the crude hexokinase (1 to 10 μM) was sufficient to support conversion of ATP to ADP by hexokinase so that State 3 phosphorylation rates were only slightly decreased in the absence of added 3 mM magnesium chloride and antibiotic. The effective uncoupling by A23187 in a medium free of added magnesium but containing inorganic phosphate was not a result of mitochondrial swelling, for ATP or ADP prevents this phosphate-dependent swelling and uncoupling (41). Since magnesium bound the antibiotic (Table I) and increasing concent-

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1 P. W. Reed and H. A. Lardy, to be published.
FIG. 4. The effect of A23187 on oxidative phosphorylation by mitochondria incubated in the presence of magnesium chloride. Mitochondria (10 mg of protein) were equilibrated for 10 min in 3 ml of a medium which contained 13 mM PO₄ (triethanolamine), pH 7.4, 15 mM KCl, 3 mM MgCl₂, 2 mM ATP, 42 mM mannitol, and 102 mM sucrose. State 3 was initiated by addition of hexokinase (2 mg), glucose (final concentration 18 mM), and substrate. A antibiotic from a side arm and allowed to proceed for 10 min. A and C: ⊙, 10 mM succinate + 1 µm rotenone; ⊙, 20 mM sodium DL-β-hydroxybutyrate; ▲, 10 mM proline. B and D: △, 10 mM glutamate; ●, 5 mM glutamate + 5 mM malate; □, 5 mM malate + 5 mM potassium pyruvate; ▽, 10 mM α-ketoglutarate.

FIG. 5. Substrate dependent uncoupling of mitochondrial oxidative phosphorylation by A23187 in the absence of added magnesium chloride. Mitochondria were incubated exactly as described in the legend to Fig. 4, except that the incubation medium contained no added magnesium chloride.

Table II

Effect of A23187 on oxidative phosphorylation by mitochondria incubated in the presence or absence of EGTA

Mitochondria (13 to 15 mg of protein) were incubated as described in the legend to Fig. 5. A23187 was added at a concentration of 0.4 n mole per mg of protein with glutamate and β-hydroxybutyrate as substrates and 0.7 n mole per mg of protein with succinate as substrate. EGTA (Tris), pH 7.4, when added, was present in the main compartment at 0.2 mM concentration.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>A23187</th>
<th>EGTA</th>
<th>A23187 + EGTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>O</td>
<td>0.60</td>
<td>0.26</td>
<td>0.56</td>
</tr>
<tr>
<td>P</td>
<td>1.48</td>
<td>0.14</td>
<td>1.33</td>
<td>0.38</td>
</tr>
<tr>
<td>P/O</td>
<td>2.5</td>
<td>0.6</td>
<td>2.4</td>
<td>2.1</td>
</tr>
<tr>
<td>β-Hydroxybutyrate</td>
<td>O</td>
<td>0.34</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>P</td>
<td>0.85</td>
<td>0.29</td>
<td>0.85</td>
<td>0.65</td>
</tr>
<tr>
<td>P/O</td>
<td>2.5</td>
<td>0.9</td>
<td>2.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Succinate</td>
<td>O</td>
<td>1.06</td>
<td>0.99</td>
<td>1.06</td>
</tr>
<tr>
<td>P</td>
<td>1.47</td>
<td>0.71</td>
<td>1.40</td>
<td>1.20</td>
</tr>
<tr>
<td>P/O</td>
<td>1.4</td>
<td>0.7</td>
<td>1.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* * O, µg atom of oxygen taken up per mg of protein in 10 min; P, µmoles of P₁ taken up per mg of protein in 10 min.

Of magnesium chloride in the incubation medium restored State 3 glutamate oxidation to control levels (data not shown). Thus, impaired glutamate metabolism appeared to result from the magnesium loss produced by A23187, rather than from uncoupling per se.

In contrast to results with glutamate, State 3 phosphorylation...
with succinate or β-hydroxybutyrate was inhibited only 15 to 25% by A23187 when EGTA prevented uncoupling by the antibiotic (Table II). Low concentrations of EGTA were sufficient to restore β-hydroxybutyrate-supported ADP phosphorylation by mitochondria incubated with A23187. Exogenous calcium chloride inhibited the ability of EGTA to restore phosphorylation (Fig. 6). A23187 decreased magnesium content of mitochondria incubated in the oxidative phosphorylation medium containing EGTA to 2 to 3 nmoles per mg of protein in less than 30 s. Thus, it appeared that inhibition of State 3 phosphorylation by A23187 resulted from a calcium-dependent uncoupling by the antibiotic when magnesium loss did not markedly inhibit substrate oxidation.

The oxidation of 10 mM succinate, NADH, or β-hydroxybutyrate plus 1 mM NAD⁺ by submitochondrial particles (8, 9) and the oxidation of 10 mM glutamate plus 1 mM NAD⁺ by particles plus supernatant not sedimenting with intact mitochondria were unaffected by A23187 (0.6 to 2.5 nmoles per mg of protein) whether or not substrate oxidation was coupled to phosphorylation by 3 mM magnesium chloride (36).

Role of Calcium in Release of State 4 Respiration by A23187—Release of State 4 respiration⁴ by A23187 showed the same pattern of substrate specificity as was observed with stimulation of uncoupled respiration by the antibiotic under State 3 conditions. Release of State 4 succinate oxidation by A23187 was a function of both antibiotic and calcium concentration (Fig. 7).

The same maximal respiration per mg of mitochondrial protein was achieved in the presence of three different protein concentrations by increasing concentrations of A23187 alone or less than optimal amounts of the carboxylic acid plus exogenous calcium chloride. The composite data of Fig. 7 show that 4 or 8 μM calcium chloride reduced the concentration of A23187 required for half-maximal respiratory stimulation from 0.15 to 0.1 or less nmoles per mg of protein and even more strikingly decreased the antibiotic concentration required for maximal uncoupling. Low concentrations of EGTA (or EDTA) prevented the release of State 4 succinate oxidation by A23187, and small amounts of calcium reversed this inhibition (15). In addition, ATP depressed A23187-stimulated respiration, presumably by binding the small amounts of calcium necessary for uncoupling by the antibiotic.

Previous observations indicated that A23187, in contrast to other uncouplers (26, 27, 44), did not necessarily deplete intramitochondrial calcium unless EDTA was present (see Fig. 1) and uncoupling was prevented. This suggested that energy-dependent reaccumulation of calcium discharged by A23187 might play a role in uncoupling by the antibiotic. Low concentrations of both lanthanum chloride and ruthenium red, two inhibitors of active calcium uptake by mitochondria (29, 45-49), inhibited the release of State 4 succinate oxidation by A23187 (Fig. 8). Although lanthanum appears to bind A23187, addition of a large molar excess of antibiotic after maximal inhibition by either lanthanum or ruthenium red (Fig. 8B) did not restore uncoupling. In contrast, carbonyl cyanide m-chlorophenylhydrazone or 2,4-dinitrophenol promptly accelerated respiration (Fig. 8), indicating an unimpaired capacity for electron transport.
endogenous calcium across the mitochondrial membranes and mitochondria to establish an energy-dissipating cyclic flux of thus releases substrate oxidation from ADP control. acts in concert with the high affinity, divalent cation carrier of competitively with calcium to bind A23187. Apparently, A23187 uncoupling by preventing mitochondrial energy-dependent reaccumulation of discharged calcium rather than acting communally.

Addition of up to 100 nmoles of calcium chloride per mg of protein, respectively. B, mitochondria (1.1 mg of protein) were incubated with 1.2 nmoles of A23187 per mg of protein before addition of ruthenium red (0.2 to 1.0 n mole per mg of protein). 2,4-Dinitrophenol (DNP) was added at a concentration of 44 \mu M and the second addition of A23187 (*) was at a concentration of 8.8 nmoles per mg of protein. Mitochondrial calcium and magnesium contents were 8 and 29 nmoles per mg of protein, respectively.

Inhibition of A23187 uncoupling by either ruthenium red or lanthanum chloride was complete at all concentrations of the inhibitors but was visibly time-dependent at less than maximally effective inhibitor concentrations (Fig. 8). If calcium chloride (up to 50 nmoles per mg of protein) was added to mitochondria prior to a concentration of A23187 that was maximally effective in the absence of exogenous calcium, the inhibitors required a longer time to decrease succinate oxidation to the State 4 rate. Addition of up to 100 nmoles of calcium chloride per mg of protein following maximal inhibition of A23187 uncoupling by lanthanum or ruthenium red did not restore antibiotic-stimulated respiration. These results were most consistent with the suggestion that lanthanum and ruthenium red inhibited A23187 uncoupling by preventing mitochondrial energy-dependent reaccumulation of discharged calcium rather than acting competitively with calcium to bind A23187. Apparently, A23187 acts in concert with the high affinity, divalent cation carrier of mitochondria to establish an energy-dissipating cyclic flux of endogenous calcium across the mitochondrial membranes and thus release substrate oxidation from ADP control.

**DISCUSSION**

A23187 is a carboxylic acid ionophore that transfers calcium and magnesium, but not potassium, from an aqueous medium at pH 7.4 into a bulk organic phase. A23187 alters the magnesium and calcium content of rat liver mitochondria apparently as a result of the antibiotic's ability to enter mitochondrial membranes and act as a freely mobile carrier to equilibrate divalent cation concentrations across these membranes. It appears that all of the effects of A23187 on intact mitochondria can be accounted for by this property of the antibiotic.

We propose that A23187 initially catalyzes an electroneutral exchange of extramitochondrial protons for intramitochondrial calcium and magnesium. Reaccumulation of calcium on the high affinity carrier in response to the electrochemical potential across the inner mitochondrial membrane (47, 50, 51) would tend to collapse this potential and would be accompanied, therefore, by respiration-driven outward translocation of protons (50). Alternatively, a high energy intermediate could be expended to "pump" calcium back into the mitochondrion in exchange for protons (24, 25). Repetition of this process at a rapid rate would result in accelerated electron transport and uncoupled substrate oxidation. Since rat liver mitochondria do not actively accumulate magnesium (21, 28-30) under these conditions, this cation would remain in the extramitochondrial medium.

The following observations may be considered as supporting the suggested mechanism of A23187 uncoupling. Release of State 4 succinate oxidation by the antibiotic is a function of both calcium and A23187 concentration and is irreversibly inhibited by low concentrations of the calcium transport inhibitors, lanthanum chloride and ruthenium red (29, 45-49). Lanthanum chloride prevents the high affinity binding of calcium to mitochondria, submitochondrial particles (47, 52), and a water-extractable mitochondrial protein (53). ATP effectively inhibits uncoupling by A23187, whereas ADP does not, in accordance with their respective affinities for calcium. Stimulation of State 4 respiration by A23187 is inhibited by low concentrations of EGTA and EDTA and this inhibition is effectively reversed by small amounts of calcium chloride. Finally, A23187 has been shown to catalyze a net exchange of protons for calcium across several biological membranes (rat erythrocyte, bovine spermatozoa (15)).

Uncoupling by A23187 is not simply the result of loss of endogenous magnesium, since mitochondria incubated with the antibiotic plus EDTA show the most rapid and severe depletion of magnesium, yet remain coupled. Magnesium chloride (3 mM) inhibited the uncoupling of State 3 oxidation of various substrates by A23187 as well as the release of State 4 succinate oxidation by the antibiotic. When stimulation of State 4 respiration by A23187 was inhibited by 1 mM magnesium chloride, 5 to 25 \mu M calcium chloride restored uncoupling by the antibiotic. Since A23187 binds magnesium as well as calcium, high concentrations of Mg\(^{2+}\) in the extramitochondrial medium might be expected to compete effectively with intramitochondrial calcium to bind the antibiotic and thus interrupt the suggested mechanism of A23187 uncoupling. Alternatively, since magnesium inhibits the low affinity binding (54, 55) and active accumulation (29, 55) of calcium by rat liver mitochondria, magnesium could inhibit uncoupling by A23187 if low affinity surface binding of calcium is required for uncoupling by the antibiotic. Finally, it is possible that magnesium prevents A23187 uncoupling in a yet unidentified manner, perhaps similar to magnesium restoration of coupled function in mitochondria with slightly elevated calcium content from dystrophic hamster muscle (56) or bovine adrenal cortex (57). Mitochondria incubated with A23187 plus EGTA contained only 2 to 3 nmoles of Mg\(^{2+}\) per mg of protein yet were able to phosphorylate ADP at 80 to 85% of the control rate with suc-
cinate or \(\beta\)-hydroxybutyrate as substrate. In sharp contrast, ATP hydrolys and glutamate-supported phosphorylation remained severely inhibited in these mitochondria and were restored only by conditions of incubation that would be expected to minimize the antibiotic-mediated efflux of endogenous magnesium, i.e. incubation of mitochondria in medium containing 1 to 25 mM magnesium chloride. Magnesium remaining in mitochondria after treatment with A23187 is most likely tightly bound to the inner and outer mitochondrial membranes (58-61). Removal of the majority of mitochondrial magnesium (matrix and intermembrane space, (61)) would decrease intramitochondriol MgATP\(^{\text{-}}\) concentration and could possibly lead to inhibition of the ATPase enzyme by free ATP\(^{\text{-}}\). The low levels of magnesium remaining in the inner membrane would appear sufficient for near optimal phosphorylation of ADP. The marked inhibition of glutamate oxidation in mitochondria depleted of magnesium (58-60, 62, and present results) most likely results from the requirement of \(\alpha\)-ketoglutarate oxidase (62) and succinyl-CoA synthase for Mg\(^{2+}\). It should be noted that carboxyl cyanide \(m\)-chlorophenylhydrazone and 2,4-dinitrophenol are able to release, maximally, State 4 succinate oxidation by mitochondria severely depleted of magnesium by A23187 when uncoupling due to the antibiotic is prevented by lanthanum chloride, ruthenium red, or EGTA. While the foregoing observations do not exclude participation of magnesium in energy transduction phenomena, they do question the adequacy of data cited to support a hypothetical high energy intermediate, X \(\sim\) Mg\(^{2+}\) (58-60).

Alterations in mitochondrial permeability to potassium, inorganic phosphate, and substrate anions may occur as a result of the effect of A23187 on endogenous magnesium, or calcium, or both. Release of intramitochondrial potassium by A23187 was greatest following maximal divalent cation loss under conditions where the antibiotic could not uncouple. Increased permeability to potassium has been observed previously in magnesium-depleted mitochondria (31-33). A rapid efflux of endogenous inorganic phosphate accompanied cation release from mitochondria treated with A23187. Internal acidification of mitochondria with subsequent loss of substrate anions and inorganic phosphate (63, 64) has been suggested to explain the substrate specificity of nigericin's effects on rat liver mitochondria (42). An analogous exchange of extramitochondrial protons for endogenous divalent cations probably occurs with A23187 and may in part account for substrate selectivity observed with this antibiotic. Studies of anion-dependent swelling of non-respiring mitochondria (65, 66) indicate that A23187 may slightly inhibit substrate anion or inorganic phosphate uptake by mitochondria, but further experiments are needed to clarify this point. Although adenine nucleotide exchange is activated under certain conditions by calcium (67, 68) and magnesium (68), mitochondria severely depleted of these divalent cations by incubation with A23187 plus EDTA show unpimpaired adenine nucleotide exchange activity and thus appear to retain endogenous adenine nucleotide anions.

By virtue of its ability both to deplete and selectively restore divalent cations in various biological systems (15), A23187 should serve as a useful investigative tool in a wide variety of tissue, cellular, and subcellular systems.

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