EFFECT OF EXOGENOUS ADENOSINE TRIPHOSPHATE ON THE EXTRUSION AND RETENTION OF ION IN THE KIDNEY CORTEX MITOCHONDRIA

MUNEKAZU GEMBA, KENJIRO YAMAMOTO AND JURO UEDA
Department of Pharmacology, Osaka City University Medical School, Abeno-ku, Osaka
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Mitochondrial ion transport has been studied by many investigators since the early work of Bartley and Davies (1). Passive ion extrusion was observed on aged mitochondria or on fresh mitochondria with valinomycin (2-5). On the other hand, metabolism dependent ion retention (6-8), ion uptake (9-17) and ion extrusion (18-20) have been found on mitochondria. It is suggested that there are some unknown factors which control ion movement in the mitochondria.

Presently, in order to clarify the factor, movement of potassium and magnesium was studied in the mitochondria of the kidney cortex and liver of rats. A metabolism dependent potassium and magnesium ion extrusion was observed in the presence of a respiratory substrate, and this extrusion was converted to retention by further addition of 1.0 mM of ATP. This effect of exogeneous ATP was discussed.

METHODS

Preparation of mitochondria

Male Wistar rats weighing 120 to 160 g were employed.

Mitochondria were prepared from the kidney cortex and liver at low temperature of from 0°C to 4°C (21). Cold sucrose (0.25 M) was used in order to homogenize tissue with a glass-Teflon homogenizer. Centrifugation was performed at 0°-4°C by Hitachi 20P. Special care was taken to obtain a pure preparation of mitochondria neglecting better yield.

Incubation

Incubation was done at 30°C for 10 minutes in a basal medium containing 15 mM NaCl, 5 mM KCl, 10 mM tris- HCl Buffer (pH 7.4), 31.2 mM tris-succinate and 105 mM sucrose.

In some experiments, succinate was replaced with pyruvate or glutamate, and, ATP and several metabolic inhibitors were added alone or together combined to the basal medium. Total volume was 6.0 ml. Mitochondrial suspension containing about 3.0 mg as protein was added to the medium which had been kept at 30°C.

Sampling

At the end of the reaction period, the reaction mixture was filtrated through a mil-
lipore filter (DAWP 02500) to rapidly separate mitochondria from reaction medium. Separation was carried out less than 15 seconds. Potassium and magnesium in mitochondria trapped on the filter were extracted with 3 ml of 85% of formic acid for 20 minutes at room temperature (11). The extract diluted with 6 ml of distilled water was used for analysis.

**Analysis**

Potassium was measured by a lithium internal standard flame photometer (I.L. model 143) and magnesium by an atomic absorption apparatus (Hitachi 207). Mitochondrial protein was determined by the method of Lowry et al. (22) with crystalline bovine serum albumin as a standard.

Potassium and magnesium in mitochondria were quantitatively expressed as mmol/mg of mitochondrial protein.

A net movement between mitochondria and the medium was expressed as \( \Delta K \) or \( \Delta Mg \); mmol of K or Mg/mg protein/10 min, which was calculated from the difference between the initial content and that after reaction.

The data were averaged of 6-8 experiments. Antimycin A (Kyowa Fermentation Ind. Co., Tokyo), oligomycin (Sigma Chemical Co.), Tris-ATP (Sigma Chem.), Rotenone (a gift of Dr. C.E. Wenner of Roswell Park Memorial Institute) were used. All reagents were neutralized with tris-base. Antimycin A and oligomycin were dissolved in ethyl alcohol.

**RESULTS**

1) **Time course of potassium and magnesium flux and effect of ATP on it**

Changes of potassium and magnesium concentration in the kidney cortex mitochondria during incubation at 30°C are shown in Fig. 1. Initial contents of potassium and magnesium were 81.7 and 27.7 mmol/mg protein respectively.

Initial extrusion rate until 3 minutes was low (4.0 mmol of K and 5.0 mmol of Mg/mg protein) but a marked decrease occurred hereafter, 57.4 mmol of K and 22.0 mmol of Mg/mg protein at 10 minutes after incubation. These values were almost continued at 20 minutes after incubation. Thereafter, following experiments were done for 10 minutes, and the effect of ATP on this extrusion was observed.

The addition of ATP to the basal medium suppressed the extrusion of mitochondrial potassium and magnesium (Fig. 2). In the presence of 1.0 mM of ATP in the medium, 17.4 mmol of K/mg protein and 7.6 mmol of Mg/mg protein were extruded from mitochondria to the medium during a period of 10-minute incubation. Decreasing concentration of ATP decreased the suppressive effect of ion extrusion. In the presence of 1.0 mM of ATP, 57.3 mmol of K and 18.8 mmol of Mg/mg protein were extruded, similar to that without ATP as shown in Fig. 1. In order to clarify the mode of action of ATP on ion movement, respiratory inhibitor, antimycin A (1 \( \times 10^{-3} \) M), was added to the incubation medium. Antimycin A increased the ion efflux with ATP in high concentration, and decreased that with ATP in low concentration. That
Fig. 1. Time course of potassium and magnesium content of kidney cortex mitochondria during incubation at 30°C.

The incubation was carried out in the basal medium containing 15 mM NaCl, 5 mM KCl, 10 mM tris-HCl (pH 7.4), 31.2 mM tris-succinate, 105.0 mM sucrose, and 2.94 mg protein of mitochondria in a final volume of 6.0 ml.

Fig. 2. Effect of ATP and ATP plus antimycin A on extrusion of potassium and magnesium ions from kidney cortex mitochondria.

The indicated concentration of ATP and 1 x 10^{-5} M of antimycin A (dotted lines) was added to the basal medium.

The incubation was carried out for 10 minutes at 30°C.

--- K extrusion, Basal medium

--- K extrusion, Basal medium + antimycin A

--- Mg extrusion, Basal medium

--- Mg extrusion, Basal medium + antimycin A
is, following respiratory inhibition by antimycin A, the effect of ATP on ion efflux was disappeared. It was observed that exogenous ATP, in high concentration, showed a retentive effect on both ions.

2) Effect of various agents on the flux of ions

The effect of various agents on the ion flux of mitochondria was studied. In the absence of ATP the extrusion rate of potassium from mitochondria was reduced to 54.7% with antimycin A (1 x 10^{-5} M) and 34.8% with 2, 4-DNP (5 x 10^{-4} M) and the extrusion of magnesium was also reduced to 81.1% with antimycin A and 49.4% with 2, 4-DNP.

Rotenone (1 x 10^{-6} M) had little effect on the extrusion of potassium but reduced the extrusion of magnesium by 34.4%. Oligomycin (10 \mu g/ml) was ineffective to extrude both ions. Neither ion was extruded at 0°C as shown in Table 1. It may be concluded that the extrusion of potassium and magnesium is closely related with the mitochondrial metabolism in the presence of succinate which was an energy source in the basal medium.

Table 1. Effect of various agents on potassium and magnesium extrusion from the kidney cortex mitochondria.

<table>
<thead>
<tr>
<th></th>
<th>K (\Delta K/Initial)</th>
<th>Mg (\Delta Mg/Initial)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>70.9</td>
<td>29.5</td>
</tr>
<tr>
<td><strong>Antimycin A 1 x 10^{-5} M</strong></td>
<td>64.8</td>
<td>23.8</td>
</tr>
<tr>
<td><strong>Rotenone 1 x 10^{-6} M</strong></td>
<td>72.3</td>
<td>24.4</td>
</tr>
<tr>
<td><strong>2,4-DNP 5 x 10^{-5} M</strong></td>
<td>60.5</td>
<td>24.3</td>
</tr>
<tr>
<td><strong>Incubating at 0°C</strong></td>
<td>57.9</td>
<td>28.0</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>53.4</td>
<td>13.7</td>
</tr>
<tr>
<td><strong>Oligomycin 10 \mu g/ml</strong></td>
<td>90.2</td>
<td>25.8</td>
</tr>
</tbody>
</table>

The reaction was carried out in the basal medium described in Fig. 1 for 10 minutes at 30°C. The indicated concentration of the inhibitors and uncoupler was added to the basal medium (control).

* Initial content, \text{mmoles/mg protein}.

** \text{mmoles of K or Mg extruded from mitochondria/mg protein/10 minutes}.

*** The extent of the extrusion of mitochondrial potassium or magnesium.

In the presence of 1.0 mm of ATP the retention of potassium and magnesium in mitochondria was inhibited by 2, 4-DNP, or oligomycin as well as by antimycin A (Table 2). Of these three inhibitors, 2, 4-DNP proved to be most potent. Following 10-minute incubation, the retention of potassium and magnesium in mitochondria was 93.7% and 81.7% but it was reduced to 52.7% and 57.1% respectively following the addition of 2, 4-DNP.

The addition of 5 mm of MgCl_2 to the basal medium suppressed the extrusion of mitochondrial potassium by 30.4% and completely prevented the extrusion of magnesium (Fig. 3(a)). Therefore, it may be assumed that potassium and magnesium are transported with the common mechanism in the mitochondrial membrane as reported by previous investigators (23, 24).
TABLE 2. Effect of inhibitors and uncoupler on the retention of potassium and magnesium in kidney cortex mitochondria.

<table>
<thead>
<tr>
<th></th>
<th>Initial* (A)</th>
<th>After incubation* (B)</th>
<th>(B)** (A)</th>
<th>Initial* (C)</th>
<th>After incubation* (D)</th>
<th>(D)** (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.4</td>
<td>80.0</td>
<td>0.937</td>
<td>26.1</td>
<td>21.3</td>
<td>0.817</td>
</tr>
<tr>
<td>Antimycin A 1×10⁻⁵M</td>
<td>74.1</td>
<td>32.5</td>
<td>0.709</td>
<td>25.0</td>
<td>18.4</td>
<td>0.737</td>
</tr>
<tr>
<td>Oligomycin 10 μg/ml</td>
<td>81.8</td>
<td>71.8</td>
<td>0.878</td>
<td>25.3</td>
<td>15.0</td>
<td>0.593</td>
</tr>
<tr>
<td>2,4-DNP 5×10⁻³M</td>
<td>82.4</td>
<td>43.4</td>
<td>0.527</td>
<td>26.5</td>
<td>15.1</td>
<td>0.571</td>
</tr>
</tbody>
</table>

The incubation carried out with the addition of 1.0 mM of ATP to the basal medium for 10 minutes at 30°C. The indicated concentration of inhibitors and uncoupler was added to the reaction medium.

* : mmoles of K or Mg/mg protein.

** : The extent of the retention of the mitochondrial potassium or magnesium.

Fig. 3. Effects of addition of MgCl₂ (a) or omission of NaCl (b) on potassium and magnesium extrusion from kidney cortex mitochondria.

(a) Five millimolars of MgCl₂ were added to the basal medium.
(b) Fifteen millimolars of NaCl were replaced by choline chloride.

The reaction was carried out for 10 minutes at 30°C.

Since potassium and magnesium account for the most part of the metal-ions of freshly isolated mitochondria (25), most of the extruded ions are likely to be replaced by other cations in the medium through a compensatory uptake.

When sodium chloride in the medium was replaced with choline chloride, no effect was observed on the potassium and magnesium flux (Fig. 3(b)). From this result and the previous data that the content of sodium ion of mitochondria did not change during
incubation (4, 7, 8, 23, 26), it was safely assumed that sodium ion was not taken up by mitochondria in compensatory process of the extrusion of potassium and magnesium.

Substitution of tris-glutamate or tris-pyruvate for tris-succinate as a substrate gave no effect on the extrusion of ions which were dependent upon metabolism (Table 3).

The extrusion and retention of ion in the rat liver mitochondria were compared to those in the kidney cortex mitochondria. Similar results were obtained from the experi-

<table>
<thead>
<tr>
<th>Addition</th>
<th>K</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial*</td>
<td>ΔK**</td>
</tr>
<tr>
<td>Succinate</td>
<td>31.2</td>
<td>107.1</td>
</tr>
<tr>
<td>Glutamate</td>
<td>21.5</td>
<td>107.4</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>30.5</td>
<td>112.3</td>
</tr>
</tbody>
</table>

The indicated concentration of substrate was added to the basal medium and the reaction was carried out for 10 minutes at 30°C.

*: Initial content, μmoles/mg protein.

**: μmoles of K or Mg extruded from mitochondria/mg protein/10 minutes.

***: The extent of the extrusion of mitochondrial potassium or magnesium.

Fig. 4. Potassium and magnesium flux in liver mitochondria.
(a) The reaction was carried out in the basal medium added 5 mM of MgCl₂.
(b) One millimolar of ATP was further added to the medium described in Fig. 4(a).
The final concentration of antimycin A was 1 × 10⁻⁵ M and of 2,4-DNP was 5 × 10⁻⁵ M respectively. The reaction was carried out for 10 minutes at 30°C.
ment using liver mitochondria. The extrusion of mitochondrial potassium and magnesium was suppressed with antimycin A or 2, 4-DNP (Fig. 4(a)).

Further addition of 1.0 mM of ATP resulted in the retention of potassium of mitochondria (Fig. 4(b)), and this retentive effect was decreased by antimycin A and 2, 4-DNP. In the presence of magnesium (5 mM), magnesium was accumulated in mitochondria by ATP as reported by other authors (11, 23), and this effect was also decreased by antimycin A and 2, 4-DNP.

Some difference was observed in magnesium flux between liver and kidney cortex mitochondria. Magnesium was extruded from liver mitochondria, but was slightly accumulated in kidney cortex mitochondria. Thus, the difference in the flux of magnesium of liver and kidney cortex mitochondria may be due to the difference in the concentration of endogenous magnesium in mitochondria.

**DISCUSSION**

The uptake of ion in mitochondria has been reported by many investigators (9-17), but there are only a few reports about the active extrusion of ion in mitochondria (19, 20). Johnson et al. reported that histone enhanced both mitochondrial swelling and potassium efflux consuming energy provided not only by ATP but also by electron transport system (20). Azzi et al. reported the efflux of rubidium associated with the shrinkage using the mitochondria treated with alkali (19).

The present experiment showed slow extrusion of potassium and magnesium from kidney cortex mitochondria in the presence of a substrate, revealing 50% of the extrusion within 5 minutes and reaching a maximum within 10 minutes, accompanying a slight swelling of mitochondria. The extrusion of both ions in the presence of a substrate was reduced by metabolic inhibitors such as antimycin A and 2, 4-DNP. The present result confirmed that the extrusion of ion was dependent on the metabolism in mitochondria without any special treatment reported by previous investigators (19, 20).

Brieley et al. have reported that high energy intermediate is used for the uptake of ions in mitochondria. In the present experiment, it is assumed that the same energy which should be supply from electron transport system is used for the extrusion of ions from mitochondria, because the extrusion is inhibited by antimycin A or 2, 4-DNP but not inhibited by oligomycin.

While without substrate exogeneous ATP (1.0 mM) had no effect on ion extrusion (31), the same amount of ATP suppressed the extrusion in the presence of substrate, that is, exogeneous ATP showed retentive effect on both ions with substrate. Oligomycin slightly reduced the retentive effect of ATP. From these two findings the retentive effect of ATP was not explained only by the high energy intermediate-theory.

It is supported that in the presence of substrate exogeneous ATP controls ion movement in some way on mitochondrial membrane other than as an energy source of ion transport.

Probably, a compensatory uptake of sodium did not occur with the extrusion of potas-
sium and magnesium, because the above extrusion was also occurred in the sodium free medium (Fig. 3).

Relations between the ion permeability and ATPase of mitochondria have been studied by G-Puyon et al. (29, 30). Further study on relations between the direction of ion flux and ATPase in kidney cortex mitochondria is in progress in the authors’ laboratory.

**SUMMARY**

Extrusion and retention of potassium and magnesium were studied with rat kidney cortex mitochondria.

During incubation at 30°C, potassium and magnesium were extruded from mitochondria in the presence of a substrate.

At 0°C, the extrusion was not observed. The extrusion of both ions was suppressed with antimycin A or 2, 4-DNP, and not influenced by oligomycin. Exogeneous ATP reduced the extrusion of both ions, and this effect was markedly reduced by metabolic inhibitors and uncoupler.

It is concluded that the extrusion of mitochondrial potassium and magnesium depended upon metabolism, and exogeneous ATP controlled both ions’ movement.

Similar phenomena were observed in liver mitochondria.

**REFERENCES**

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2) Amoore, J.E.: Biochem. J. 76, 438 (1950)


31) Gemba, M.: Personal communication