Potassium Ion-Stimulated and Sodium Ion-Dependent Adenosine Diphosphate–Adenosine Triphosphate Exchange Activity in a Kidney Microsomal Fraction

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1. K+ did not affect the Mg2+-dependent transphosphorylation but markedly increased the Na+-stimulated ADP–ATP exchange rate mediated by a microsomal fraction from guinea-pig kidney. 2. Rb+, Cs+, NH4+ and Li+ were equally effective in stimulating the Na+-dependent ADP–ATP exchange activity. 3. Treatment of the microsomal fraction with N-ethylmaleimide or increased concentrations of Mg2+ prevented stimulation of the Na+-dependent exchange reaction by K+. 4. Ouabain (2.5 μM) inhibited ATP hydrolysis by 33% but did not decrease the K+-stimulated Na+-dependent ADP–ATP exchange rate. 5. A possible mechanism for stimulation of exchange activity by K+ is discussed.

The Na+-dependent [14C]ADP–ATP exchange activity observed in a variety of microsomal preparations from crab nerve, electric organ, brain, erythrocytes and kidney (Skou, 1960; Fahn et al., 1966a; Stahl, 1968; Blostein, 1968; Banerjee et al., 1972b) has been implicated as a part of the (Na++K+)-ATPase† enzyme system. Although, the stimulation of the ADP–ATP exchange reaction by Na+ has been investigated in great detail, little attention has been given to the effects of other univalent cations on this exchange activity. Fahn et al. (1966a) observed that in tris–HCl buffer, pH 7.5, with 0.15 mM-MgCl2, K+ and other univalent cations did not affect the Na+-dependent ADP–ATP exchange rate. However, in 2-amino-2-methylpropane-1,3-diol–HCl buffer, pH 9.0, with 0.34 mM-MgCl2, K+ inhibited 50% of the Na+-dependent ADP–ATP exchange activity. Fahn et al. (1966a) did not discover the reason for the inhibitory effects of K+ on the Na+-dependent exchange rate, which could be caused by either the increment in MgCl2 concentration or the change in pH or both these factors.

Recently we have observed two sets of conditions for Na+-dependent exchange activity in the kidney microsomal fraction. When the concentration of Mg2+ was 0.1 mM, maximal exchange rate was obtained with 1.5 mM-Na+. (Banerjee et al., 1972b). Raising the Mg2+ concentration to 0.4 mM changed the optimum concentration of Na+ to 32 mM. The concentration of ATP was 4 mM under both these conditions. K+ affected the exchange activity differently under these two conditions. K+ did not significantly affect the Na+-dependent exchange rate with 0.1 mM-Mg2+, but stimulated it when the concentration of Mg2+ was increased to 0.4 mM. In the present paper stimulation of Na+-dependent ADP–ATP exchange activity by K+ is described and an attempt has been made to delineate the possible mechanisms for such activation.

Materials and Methods

The microsomal (Na++K+)-ATPase from guinea-pig kidney was prepared by the method of Post & Sen (1967) as described previously (Banerjee et al., 1970a). The microsomal preparation was stored at 4°C in 10 mM-imidazole–0.1 mM-EDTA with the pH adjusted to 6.9 ± 0.1 with HCl. The ATP-hydrolysing activity of the isolated (Na++K+)-ATPase was 1–2.5 units/mg of protein, where one unit is defined as the amount of enzyme that splits 1 μmol of ATP/min at 37°C. [14C]ADP (trilithium salt; specific radioactivity approx. 40 mCi/mmol) was purchased from Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A. Ouabain, N-ethylmaleimide and ATP and ADP (disodium salts) were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. The sodium salts of the nucleotides were converted into tris salts by ion-exchange chromatography with Dowex 50 (tris form).

The Na+-dependent ADP–ATP exchange rate was measured by the method of Fahn et al. (1966a), as described previously (Banerjee et al., 1970b) except that adenylate kinase activity was assayed by measuring the amount of [14C]AMP formed as described by Blostein (1968). Thus the net exchange rate was calculated by subtracting the amount of [14C]AMP...
formed from the total amount of [14C]ATP synthesized in each experimental tube. The usual incubation medium consisted of 4.0 mM-tris–ATP, 1 mM-tris–[14C]ADP, 0.4 mM-MgCl2, ±32 mM-NaCl, 10 mM-imidazole–glycylglycine buffer (pH 7.4±0.1) and about 0.10–0.15 mg of protein in a total volume of 0.5 ml. Since under these experimental conditions the exchange rate remains linear for more than 40 min (Banerjee et al., 1972b), the samples in 10 ml centrifuge tubes were incubated for 15 min at 25°C. Changes in ATP and ADP concentrations owing to the ADP–ATP exchange reaction were less than 1% and the results are expressed as the average specific radioactivity of [14C]ADP. Prior treatment of the kidney microsomal fraction with N-ethylmaleimide was carried out as described by Banerjee et al. (1971). Each experiment was repeated at least once and the results shown are the averages of two experiments.

**Results**

The ADP–ATP exchange reaction was not stimulated by K+ in the absence of Na+ (Fig. 1). However, when Na+ was also included in the incubation medium K+ further increased the exchange rate (Fig. 1). K+ (32 mM) induced about a ninefold increase in the Na+-dependent exchange rate. Since K+ is believed to increase dephosphorylation rate without any effect on the phosphorylation step (Whittam & Wheeler, 1970), this appears to be an unexpected finding.

Prior treatment of the microsomal preparation with N-ethylmaleimide stimulated the Na+-dependent exchange rate (Fahn et al., 1966b; Banerjee et al., 1972b). Further, addition of K+ or Rb+ in the presence of Na+ produced inhibition of the N-ethylmaleimide-induced exchange rate (Fahn et al., 1966b). We therefore decided to examine the effect of K+ on the Na+-dependent exchange activity catalysed by N-ethylmaleimide-treated kidney microsomal preparations. After prior treatment of the kidney microsomal preparations with N-ethylmaleimide for shorter time-periods, there was a dual effect of K+ on the Na+-dependent ADP–ATP exchange rate (Fig. 2). At 2 mM-K+ there was more than a 100% increase in the exchange rate, but this stimulatory

![Fig. 1. Effect of K+ on Mg2+-dependent and Na+-dependent ADP–ATP exchange rates](image1)

![Fig. 2. Effect of K+ on the Na+-stimulated ADP–ATP exchange reaction in a N-ethylmaleimide-treated microsomal fraction](image2)
effect rapidly disappeared with increasing concentration of K+ (Fig. 2). Since the rate of ATP hydrolysis was inhibited by only 30%, the N-ethylmaleimide-treated microsomal preparations contained inhibited and uninhibited enzyme molecules. One possible explanation for the dual effect of K+ may be that the stimulation of the exchange rate was catalysed by uninhibited enzyme molecules in the treated microsomal fractions and inhibition of the exchange rate was catalysed by the part of the (Na++K+)-ATPase that had already reacted with N-ethylmaleimide. This possibility was tested and the results are shown in Table 1. With increasing inhibition of the enzyme activity by N-ethylmaleimide the stimulatory effects of low concentrations of K+ was replaced by an inhibitory effect on the Na+-dependent exchange rate (Table 1).

The ability of K+ to stimulate the ADP-ATP exchange rate showed little specificity, as a variety of univalent cations were equally effective (Table 2). Ouabain decreases (Na++K+)-ATPase activity more selectively, with little inhibition of the Na+-dependent exchange reaction (Fahn et al., 1966a). Similarly, 2.5 μM-ouabain inhibited the rate of ATP hydrolysis by about 35%, with no decrease in the K+-stimulated Na+-dependent ADP-ATP exchange rate.

The results of Fahn et al. (1966a) show that the effect of K+ on the Na+-dependent exchange reaction changes depending on the concentration of Mg2+. This was more critically examined in the kidney microsomal preparation. Fig. 3 shows that maximal stimulation by K+ of the Na+-dependent exchange rate was observed at 0.4 mM-Mg2+. There was a rapid decrease in the stimulatory effect of K+ with either an increase or decrease of Mg2+ concentration. However, N-ethylmaleimide-induced stimulation of the exchange rate was less affected by the changes in Mg2+ concentration (Fig. 3).

**Discussion**

There is considerable agreement on the reaction sequence for the hydrolysis of ATP by (Na++K+)-ATPase, which may be described as follows (see Whittam & Wheeler, 1970, for general references):

\[
E_1 \xrightleftharpoons{[\text{ATP} + \text{Na}^+] / \text{Mg}^{2+}} E_1 - \text{P} + \text{ADP} 
\]

(1)

\[
E_1 - \text{P} \xrightarrow{\text{Mg}^{2+}} E_2 - \text{P} 
\]

(2)

\[
E_2 - \text{P} \xrightleftharpoons{\text{K}^+ / \text{Mg}^{2+}} E_2 + \text{P}_1 
\]

(3)

\[
E_2 - \text{P} + \text{Mg}^{2+} \xrightarrow{\text{K}^+} E_1 
\]

(4)

**Table 1. K+-stimulated Na+-dependent ADP-ATP exchange reaction in a N-ethylmaleimide-treated microsomal fraction**

Kidney microsomal fraction was preincubated with 2.5 mM-N-ethylmaleimide in the presence of 16 mM-Na+, 0.25 mM-ouabain and 3 mM-tris-ATP at 37°C either for 30 min or for 60 min. The treated microsomal fraction was washed free of all added ligands by the procedure described by Banerjee et al. (1971).

<table>
<thead>
<tr>
<th>Concentration of K+ (mM)</th>
<th>Incubation time (min)</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>44.8</td>
<td>93.1</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>53.3</td>
<td>85.0</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>57.6</td>
<td>66.2</td>
</tr>
</tbody>
</table>

**Table 2. Effect of univalent cations on Na+-dependent ADP-ATP exchange rate**

For experimental details see the text.

<table>
<thead>
<tr>
<th>Univalent cation added</th>
<th>Na+-dependent ADP-ATP exchange rate (nmol of [14C]ATP/min per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>12.2</td>
</tr>
<tr>
<td>K+ (2 mM)</td>
<td>39.9</td>
</tr>
<tr>
<td>Rb+ (2 mM)</td>
<td>34.4</td>
</tr>
<tr>
<td>Cs+ (2 mM)</td>
<td>37.4</td>
</tr>
<tr>
<td>NH4+ (2 mM)</td>
<td>41.0</td>
</tr>
<tr>
<td>Li+ (16 mM)</td>
<td>44.9</td>
</tr>
</tbody>
</table>
Fig. 3. Effect of Mg\(^{2+}\) on the Na\(^{+}\)-dependent exchange rate stimulated by K\(^{+}\)

The incubation medium for each experimental point consisted of 0.12 mg of microsomal protein, 10 mM imidazole–glycylglycine buffer (pH 7.4), 4 mM-tris–ATP, 1 mM-tris–[\(^{14}\)C]ADP and the indicated concentration of Mg\(^{2+}\). The exchange rate was determined either in the absence of any added univalent cation (●, □) or in the presence of 20 mM-Na\(^{+}\) (○, ■) or 20 mM-Na\(^{+}\) plus 20 mM-K\(^{+}\) (▲), in native (●, ○, ▲) and N-ethylmaleimide-treated (□, ■) microsomal fractions. Treatment with N-ethylmaleimide was done as described in Fig. 2 except that the preincubation time was 45 min.

According to the above scheme, when the [Mg\(^{2+}\)]/[ATP] ratio is low, the membrane fragments in the presence of Na\(^{+}\) predominantly form E\(_{1}\)–P and, on addition of an appropriate amount of ADP to this system, an ADP–ATP exchange reaction occurs (Fahn et al., 1966a). This exchange activity shows an absolute specificity for Na\(^{+}\). However, in the presence of Na\(^{+}\), K\(^{+}\) and other univalent cations markedly stimulated the Na\(^{+}\)-dependent ADP–ATP exchange activity by the order of 800–900% (Fig. 1). The mechanism for stimulation of the Na\(^{+}\)-dependent ADP–ATP exchange rate by K\(^{+}\) may involve one or more of the four steps described for the hydrolysis of ATP in the above scheme.

N-Ethylmaleimide is known to inhibit ATP hydrolysis by blocking either step (1) or step (2) (Banerjee et al., 1972a). When N-ethylmaleimide prevents conversion of E\(_{1}\)–P into E\(_{2}\)–P (by affecting
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K+-stimulated Na+-dependent ADP-ATP exchange step 2) markedly stimulates the exchange rate (Fahn et al., 1966b). K+ was unable to stimulate the Na+-dependent ADP-ATP exchange in N-ethylmaleimide-treated microsomal preparations (Fig. 2 and Table 1), suggesting that the stimulation of the exchange activity by K+ is not mediated through step (1).

K+ can promptly dephosphorylate E2-P (Whittam & Wheeler, 1970). Therefore, another explanation for the stimulation of the exchange rate by K+ may be rapid dephosphorylation of E2-P, which regenerates more and more E1, and thus increases the concentration of the reactant in the forward direction of the exchange reaction. This possibility seems unlikely because ouabain, which preferentially inhibits dephosphorylation (Post et al., 1969; Sen et al., 1969), did not affect K+-induced stimulation of the exchange rate in spite of more than 30% inhibition of ATP hydrolysis.

Lastly, K+-induced stimulation of the exchange rate may be mediated by preventing conversion of E1-P into E2-P (step 2). This possibility is supported by two separate observations. First, N-ethylmaleimide treatment, which blocks conversion of E1-P into E2-P, prevented the stimulation of the exchange rate by K+ (Table 1). Secondly, increasing the Mg2+ concentration in the incubation medium effectively antagonized the K+-induced stimulation of the ADP-ATP exchange reaction (Fig. 3). These observations lead us to conclude that K+-induced stimulation of the Na+-dependent ADP-ATP exchange is probably due to a decrease in the proportion of E2-P formed in an exchange reaction medium or to conversion of E2-P into E1-P.

The present work may further help in finding a molecular basis for the transport of univalent cations. The possible ability of K+ to catalyse conversion of E2-P into E1-P and similarly of E2 into E1 (step 4) would suggest that after dephosphorylation K+ may contribute in driving the Na+ pump from E2 to E1 by antagonizing the effects of Mg2+ (which tends to convert the E1 form into E2) to help normal translocation of ions. Again, reversal of steps (2) and (3) in the above scheme has not been shown with fragmented membranes. Stimulation of the Na+-dependent exchange activity by K+ suggests that step (2) can be reversed in fragmented membranes. This is consistent with the ouabain-sensitive labelling of ATP with 32P in intact erythrocyte 'ghosts' (Glynn et al., 1970; Glynn & Lew, 1970; Lant et al., 1970).

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References

Stahl, W. L. (1968) J. Neurochem. 15, 499