Effect of Potassium on Sodium-dependent Adenosine Diphosphate-Adenosine Triphosphate Exchange Activity in Kidney Microsomes*

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SUMMARY
Maximal activation of a Na+-dependent adenosine diphosphate-adenosine triphosphate exchange reaction mediated by kidney microsomes was achieved at 0.1 mM and 0.4 mM Mg++ in the presence of 1.5 mM and 32 mM Na+, respectively. K+ inhibited the Na+-dependent exchange rate when lower concentrations of Na+ and Mg++ were used and stimulated it in the presence of 0.4 mM Mg++ and 32 mM Na+. The inhibitory effect of K+ could be reversed either by increasing the Na+ concentration or decreasing that of K+. N-Ethylmaleimide treatment of microsomes abolished the stimulatory effect of K+. Ouabain, alkaline pH, and lower temperatures less effectively decreased stimulation of Na+-dependent exchange by K+. Mg++ and K+ appeared to exhibit competitive antagonism on (Na+ + K+)-sensitive transphosphorylation activity. It is proposed that the stimulation of Na+-dependent adenosine diphosphate-adenosine triphosphate exchange reaction by K+ may be mediated, in part, by slowing the conversion of one form of the phospho-enzyme E1-P into another E2-P.

Several studies indicate that the Na+-activated ADP-ATP exchange activity observed in microsomal preparations derived from electric organ, brain, red blood cells, and kidney may be a manifestation of the (Na+ + K+)-ATPase enzyme system (1-5). Although, K+ (1) is believed to have little effect on the Na+-dependent ADP-ATP exchange reaction, results of Fahn et al. (1) indicate that K+ may significantly influence this exchange activity depending upon the concentration of Mg++ or pII of the incubation medium, or both.

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1 The abbreviations used are: (Na+ + K+)-ATPase, sodium and potassium ion-stimulated adenosine triphosphatase; NEM, N-ethylmaleimide.

By modifying the incubation medium a stimulatory as well as an inhibitory effect of K+ on the Na+-dependent ADP-ATP exchange activity may be observed. In this paper we describe and attempt to explore the possible mechanisms for two different effects of K+ on the Na+-dependent transphosphorylation reaction.

MATERIALS AND METHODS
The microsomal (Na+ + K+)-ATPase from guinea pig kidney was prepared by the method of Post and Sen (6) as described previously (7). The microsomal preparation was stored at 4° in a solution containing 10 mM imidazole and 0.1 mM EDTA (free acid) with the pH adjusted to 6.9 ± 0.1 with hydrochloric acid. The ATP-hydrolyzing activity of the isolated (Na+ + K+)-ATPase was 1 to 2.5 units per mg of protein where 1 unit splits 1 μ mole of ATP per min at 37°. Universally labeled [14C]ADP was purchased from Schwarz BioResearch Inc., Orangeburg, N.Y., as the trilithium salt (specific activity was about 40 mCi per mmole). Ouabain, N-ethylmaleimide, and disodium salts of ATP and ADP were obtained from Sigma. The sodium salts of the nucleotides were converted to Tris salts by ion exchange chromatography.

The Na+-dependent ADP-ATP exchange rate was estimated by the method of Fahn et al. as described previously (8) except that adenylate kinase activity was estimated by measuring the amount of [14C]AMP formed as described by Blostein (9). Thus the net exchange rate was calculated by subtracting the amount of [14C]AMP formed from the total [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 (pH 7.4 ± 0.1) and about 0.1 to 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 (4), the samples in 10-ml centrifuge tubes were incubated for 15 min at 25°. Changes in ATP and ADP concentration due to 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 microsomes with NEM was carried out as described for ethacrynic acid by Banerjee et al. (9). A microsomal suspension containing 1 to 2 mg of protein was incubated with 5 mM NEM in the presence of 100 mM Na+.
plus 3 mM Tris-ATP and 0.25 mM ouabain, in a volume of 2 ml at 37° for 60 min. The treated microsomes were washed three times and resuspended in 10 mM imidazole buffer as described before (7, 9).

Unless otherwise stated, all results described in the present paper are the average of two experiments.

RESULTS

Previously we have reported that in the kidney microsomes the Na+-dependent activation of ADP-ATP exchange reaction may occur in two sets of conditions (4). With 0.4 mM Mg++, the maximal activation of the exchange reaction was obtained when the concentration of Na⁺ was 32 mM or more. However, the exchange rate could be further improved by lowering the concentration of Mg++ to 0.1 mM, in which case the optimum concentration of Na⁺ shifted to 1.5 mM.

Three important effects of K⁺ on Na⁺-dependent transphosphorylation reaction in native and NEM-treated kidney microsomes were observed (Table I). (a) In agreement with previous workers (1, 2) K⁺ in the absence Na⁺ did not stimulate the exchange rate. (b) When the concentration of Mg++ was 0.1 mM, K⁺ inhibited the Na⁺-dependent exchange rate by about 25%. When the concentrations of Mg++ and Na⁺ were increased to 0.4 mM and 16 mM, respectively, K⁺ markedly stimulated the Na⁺-dependent exchange rate. (c) NEM-treatment of kidney microsomes prevented the stimulation of the Na⁺-dependent transphosphorylation reaction by K⁺ and further increased its inhibitory effect.

For the stimulatory effect of K⁺ the ratio of Na⁺:K⁺ was 10, which was 10 times higher than the ratio of Na⁺:K⁺ employed in the incubation medium when an inhibition of Na⁺-dependent exchange activity by K⁺ was observed (Table I). Therefore, we examined the effect of lower concentrations of K⁺ on the Na⁺-dependent transphosphorylation reaction with 0.1 mM Mg++ and 1.5 mM Na⁺ (Fig. 1). As the concentration of K⁺ was lowered, its inhibitory effect on Na⁺-dependent exchange rate slowly disappeared and was replaced by a stimulatory effect at 0.2 mM K⁺ (Fig. 1).

The stimulation of Na⁺-dependent exchange activity by 0.2 mM K⁺ in the presence of 0.1 mM Mg++ and 1.5 mM Na⁺ was only one and a half times control values in contrast to a 1500% increase in the Na⁺-dependent exchange activity elicited by K⁺ in the presence of 0.4 mM Mg++ and 16 mM Na⁺. The marked enhancement of the Na⁺-dependent transphosphorylation reaction by K⁺ under the latter conditions may be due to the increased concentration of Na⁺. When the concentration of Mg++ was maintained at 0.1 mM, increasing concentrations of Na⁺ produced a gradual decline in the exchange rate in the absence of K⁺ (Fig. 2), in agreement with our previous finding (4). However, when 1 mM K⁺ was included in the incubation medium, there was a marked stimulation of the exchange rate with the increment of Na⁺ concentration and at 10 mM Na⁺.

Table I

Effect of Na⁺ and K⁺ on ADP-ATP transphosphorylation reaction

The microsomes were treated with NEM, and ADP-ATP exchange rate was determined as described in the text. Data are the averages of three separate experiments.

<table>
<thead>
<tr>
<th>Microsomal preparation</th>
<th>ADP-ATP exchange rate</th>
<th>Na⁺-stimulated exchange</th>
<th>(Na⁺ + K⁺)-stimulated exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monovalent cations added</td>
<td>[μmol [14C]ATP/mg protein/min]</td>
<td>[μmol [14C]ATP/mg protein/min]</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>Na⁺</td>
<td>K⁺</td>
</tr>
<tr>
<td>Native</td>
<td>9.1</td>
<td>20.4</td>
<td>4.2</td>
</tr>
<tr>
<td>NEM-treated</td>
<td>9.0</td>
<td>38.0</td>
<td>3.0</td>
</tr>
<tr>
<td>1D</td>
<td>25.4</td>
<td>28.5</td>
<td>24.5</td>
</tr>
<tr>
<td>Native</td>
<td>31.3</td>
<td>143.8</td>
<td>12.6</td>
</tr>
<tr>
<td>NEM-treated</td>
<td>31.3</td>
<td>143.8</td>
<td>12.6</td>
</tr>
</tbody>
</table>

- At concentrations of 0.1 mM Mg++, 1.5 mM Na⁺, and 1.5 mM K⁺.
- At concentrations of 0.4 mM Mg++, 16 mM Na⁺, and 1.6 mM K⁺.
there was a 900% increase in the Na⁺-dependent exchange activity.

Thus in native microsomes, inhibition of the Na⁺-dependent exchange rate by 1.5 mM K⁺ (Table I) may be related either to the low concentration of Na⁺ used or to a lower ratio of Na⁺:K⁺ concentrations in the incubation medium. Neither of these two possibilities is applicable to NEM-treated microsomes, because even at a higher concentration of Na⁺ and a higher concentration ratio of Na⁺:K⁺ there was no stimulation of the Na⁺-dependent exchange rate by K⁺ in NEM-treated microsomes (Table I). Another possibility is that when 0.4 mM Mg++ was employed with NEM-treated microsomes, the (Na⁺ + K⁺)-ATPase was catalyzing ADP-ATP exchange at a maximal possible rate (4) and so could not be further stimulated by K⁺. Accordingly, we determined the exchange rate in NEM-treated microsomes in the presence of 0.1 mM Mg++ and 1.5 mM Na⁺ with or without 0.2 mM K⁺ (Table II). Under these conditions, (Na⁺ + K⁺)-ATPase did not catalyze ADP-ATP exchange at a maximal rate but 0.2 mM K⁺ still inhibited the Na⁺ dependent transphosphorylation reaction even when the concentration ratio of Na⁺:K⁺ was high (Table II). However, with 1.5 mM Na⁺ some Na⁺ sites will remain free and the occupancy of these sites by K⁺ may cause inhibition of exchange rate. Therefore the effect of 1.6 mM K⁺ on the Na⁺-dependent exchange rate in the presence of 0.1 mM Mg++ and 16 mM Na⁺ was determined in NEM-treated microsomes. There was no significant effect of K⁺ on the exchange rate under these conditions (Table II).

Effect of pH and Temperature on the K⁺-stimulated Na⁺-dependent ADP-ATP Exchange Reaction—Fahn et al. (1) have reported that incubation at pH 9 inhibits ATP hydrolysis with little effect on the exchange reaction. Similarly the K⁺-stimulated Na⁺-dependent exchange reaction was less affected than ATP hydrolysis by changes in pH (Table III). The (Na⁺ + K⁺)-stimulation ADP-ATP exchange rates were similar at 25° as well as 35° but the rate of ATP hydrolysis was markedly smaller at the lower temperature (10) (Fig. 3).

Effect of Ouabain on the Activation of the Na⁺-dependent Exchange Reaction by K⁺—Although 0.1 mM ouabain inhibits more than 85% of (Na⁺ + K⁺)-sensitive ATP hydrolysis, about 50% of Na⁺-dependent exchange activity remains unaffected in electrophal microsomes (1). Furthermore, since ouabain more selectively inhibits K⁺-sensitive dephosphorylation (11, 12) it was of interest to study its effects on Na⁺ and (Na⁺ + K⁺) stimulated exchange activity. Both of these activities were inhibited to a similar extent by ouabain (Fig. 4). Since K⁺ is known to inhibit ouabain binding (13, 14), the present result may appear surprising. Therefore we measured the binding of [³H]ouabain to the kidney microsomes under conditions identical with those of the exchange reaction's incubation medium. With 2.5 mM radiolabeled cardiac glycoside present in the incubation medium, 299 and 157 pmolecules of [³H]ouabain were bound per mg of microsomal protein in the absence and presence of 1 mM K⁺.

The inability of ouabain to selectively inhibit K⁺-sensitive stimulation of the Na⁺-dependent exchange rate may thus be

<table>
<thead>
<tr>
<th>Table II</th>
<th>Effect of low concentrations of Na⁺ and K⁺ on ADP-ATP exchange reaction in native and NEM-treated microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomal preparation</td>
<td>Additions</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>1.5 mM Na⁺</td>
</tr>
<tr>
<td></td>
<td>1.5 mM Na⁺ + 0.2 mM K⁺</td>
</tr>
<tr>
<td>B</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>10 mM Na⁺</td>
</tr>
<tr>
<td></td>
<td>16 mM Na⁺ + 1.6 mM K⁺</td>
</tr>
<tr>
<td>II</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>1.5 mM Na⁺</td>
</tr>
</tbody>
</table>

* Mg⁺⁺ concentration was 0.1 mM.
* Mg⁺⁺ concentration was 0.4 mM.
Fig. 4. Inhibition of ADP-ATP exchange activity by ouabain. Incubation medium contained 0.4 mM Mg++ plus 32 mM Na+ with (○) or without (●) 1 mM K+ and the indicated concentration of ouabain. The rest of the procedure is the same as described in the text. Each point is the average of three experiments.

TABLE IV

<table>
<thead>
<tr>
<th>Prior incubation time</th>
<th>ADP ATP exchange activity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na-dependent (μmol [3H]ATP/mg protein/min)</td>
<td>(Na+ + K+)-dependent</td>
<td></td>
</tr>
<tr>
<td>No ouabain</td>
<td>6.7</td>
<td>45.3</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>5.4</td>
<td>43.8</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>5.6</td>
<td>41.9</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>5.3</td>
<td>35.7</td>
<td></td>
</tr>
</tbody>
</table>

related to decreased binding of the inhibitor to the microsomal protein. Therefore ouabain was permitted to bind to the enzyme in the absence of K+ (Table IV). Subsequently K+ and [3H]ADP were added and the exchange rate was determined. Although in the presence of ATP, Mg++, and Na+, complete ouabain binding is achieved within 60 s (15), prior incubation of kidney microsomes with ouabain for 1 min did not prevent stimulation of Na+-dependent exchange activity by K+ (Table IV). However, there was about 21% inhibition of the (Na+ + K+)-stimulated exchange rate after 3 min prior incubation of the microsomes with ouabain.

**Effect of Mg++ on (Na+ + K+)-dependent Exchange Rate**—The results described so far show that with an ATP:Mg++ ratio of 10, K+ stimulates the Na+-dependent exchange reaction. This would decrease the rate of ATP hydrolysis. However, when the ATP:Mg++ ratio is lowered toward 1, K+ is known to stimulate ATP hydrolysis (16). Therefore, depending upon the ATP:Mg++ ratio, K+ may retard or accelerate ATP hydrolysis by the kidney microsomes. Thus concentrations of ATP, Mg++, and K+ may determine which direction the enzyme reaction will proceed. We evaluated the effect of varying concentrations of Mg++ on the (Na+ + K+)-dependent exchange rate (Fig. 5). Higher concentrations of Mg++ rap-

**DISCUSSION**

K+ may either inhibit or stimulate the Na+-dependent ADP-ATP exchange reaction catalyzed by (Na+ + K+)-ATPase. In native microsomes, inhibition of Na+-dependent exchange activity was observed when the concentration of Na+ was insufficient to saturate the "Na+-sites" (Table I). Since such inhibition could be reversed either by increasing the concentration of Na+ (Fig. 2) or by lowering that of K+ (Fig. 1), we suggest that K+-dependent inhibition of the exchange rate was due to the occupancy of "Na+-sites" by K+.

The reaction sequence for the hydrolysis of ATP by (Na+ + K+)-ATPase is believed to involve: (a) Na+-dependent formation of E1-P. (b) Conversion of E1-P to E2-P in the presence of Mg++. (c) K+-dependent dephosphorylation to E1. (d) Regeneration of E1 from E2 (for references see 5).

Stimulation of the Na+-dependent exchange reaction by K+ could be due to rapid dephosphorylation of E2-P. If this is true, then ouabain, which preferentially deaccelerates K+-dependent dephosphorylation (11, 12), must inhibit the stimulation of Na+-dependent exchange rate by K+. But rates of
inhibition for Na+-dependent and (Na+ + K+)-dependent exchange activity were of the same magnitude (Fig. 4). However, K+ caused a 50% decrease in the binding of [3H]ouabain to the microsomes. Therefore, it may be argued that ouabain inhibited more selectively K+-sensitive stimulation of the Na+-dependent exchange rate. This conclusion may not be correct in view of recent observations that K+ slows the dissociation of enzyme ouabain complex (17).

More important for the present discussion is the effect of ouabain on ATP hydrolysis and (Na+ + K+)-stimulated exchange rate. In guinea pig kidney microsomes, 25 µM ouabain completely inhibited ATP hydrolysis (18) with only 36% inhibition of (Na+ + K+)-dependent exchange rate (Fig. 4). Furthermore, alkaline pH and lower incubation temperatures decreased ATP hydrolysis significantly with little effect on (Na+ + K+)-stimulated exchange rate (Fig. 3 and Table III). Hence it is unlikely that stimulation of Na+-dependent exchange rate by K+ may be entirely due to rapid dephosphorylation of \( E_2-P \).

K+ failed to stimulate the Na+-dependent exchange rate in NEM-treated microsomes (Tables I and II). Therefore (Na+ + K+)-stimulated transphosphorylation activity could not be due to acceleration of Step a by K+ in the reaction sequence for ATP hydrolysis.

Finally K+ may stimulate the Na+-dependent exchange reaction by affecting Step b. This possibility is supported by the inability of K+ to stimulate Na+-dependent exchange activity in NEM-treated microsomes. Moreover, increasing concentrations of Mg++ inhibited (Na+ + K+)-dependent transphosphorylation and this effect of Mg++ could be antagonized by higher concentrations of K+ (Fig. 5). This observation supports the possibility that K+ stimulated Na+-dependent transphosphorylation activity may be mediated, in part, by the prevention of transformation of the \( E_1-P \) form of the enzyme to the \( E_2-P \) by K+.

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REFERENCES
2. STAHL, W. L. (1968) J. Neurochem. 15, 511
17. AKERS, T., and BRODY, T. M. (1971) J. Pharmacol. Exp. Ther. 175, 179
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