EFFECT OF ALDOSTERONE ON RENAL Na,K-ACTIVATED ADENOSINE-TRIPHOSPHATASE ACTIVITY IN NON-ADRENALECTOMIZED RATS

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Accepted September 11, 1978

It has been reported that the activity of Na, K-activated adenosinetriphosphatase (Na,K-ATPase) in the kidney is reduced by adrenalectomy and that the activity is restored by administration of aldosterone (1, 2). However, with the adrenalectomized rats, the restoration of the enzyme by aldosterone has been shown to take much longer time (i.e. 6–24 hr) as compared with the time (1–2 hr) for the maximal effect of aldosterone on the sodium reabsorption (3). We attempted to determine whether or not aldosterone stimulates Na,K-ATPase in correlation with the enhancement of Na reabsorption in non-adrenalectomized rats.

Male Wistar rats weighing 170–250 g were used. Each animal was given water (3 ml p.o.) and pitressin tannate in oil (0.5 U i.m.), and then anesthetized 1 hr later with pentobarbital sodium (30 mg/kg i.p.). Administration of 5% mannitol through the femoral vein was started at a rate of 0.1 ml/min and continued during the experiment. After a 30 min equilibration period, one 30-min urine collection was made and then aldosterone (10 μg per animal) was given i.v. to animals of the experimental group. The control animals were infused with 5% mannitol through the experiment. The bladder was catheterized and sodium and potassium in the urine were determined by flame photometry (Hitachi 205), and the chloride content by a chloridemeter (Hiranuma, Chloride Counter CL-3). To determine the activities of Mg- and Na,K-ATPase, animals were sacrificed and the kidney

was removed quickly. The tissue was dissected into three sections (cortex, medulla and papilla) and homogenized in ice-cold 0.25 M sucrose containing 25 mM imidazole-HCl (pH 7.0) and 1 mM EDTA-Tris (pH 7.0). The homogenate was diluted adequately and passed through two layers of gauze. Incubation for ATPase assay was carried out for 10 min at 37°C in the medium containing 50 mM Tris-HCl (pH 7.4), 3 mM MgCl₂, 100 mM NaCl, 10 mM KCl, 1 mM EDTA-Tris, 3 mM ATP-Na₂ and an aliquot of the tissue homogenate with or without 1 mM ouabain. Reactions were terminated by the addition of 1 mM of 10% trichloroacetic acid and inorganic phosphate released during the incubation was determined colorimetrically by the method of Chen et al. (4). Mg-ATPase or Na,K-ATPase activity was calculated as μmoles Pi/mg protein/hr of ouabain insensitive or ouabain sensitive ATPase activity. Nonenzymic release of inorganic phosphate from ATP during the incubation was negligible. Protein concentration was determined by the method of Lowry et al. (5), using crystalline bovine serum albumin as a standard.

As shown in Fig. 1, in the control animals given 5% mannitol, urine volume, excretion of sodium, potassium and chloride was not altered during three 30 min periods of the experiments. In the aldosterone-treated animals, no change was observed in urine volume. However, excretion of sodium and chloride decreased and excretion of potassium increased significantly in the second 30 min period after administration of aldosterone as compared to increases in the control animals. The activities of Mg- and Na,K-ATPase of the renal cortex, medulla and papilla were measured in all animals at the end of the second 30 min period after administration of aldosterone to the experimental animals (Table 1). Na,K-ATPase activity in the cortex of the aldosterone-treated animals significantly increased as compared with increases in the control animals, while Mg-ATPase activity was not altered with administration of aldosterone.

Thus, even in non-adrenalectomized rats, there was an increase in Na,K-ATPase activity with no effect on Mg-ATPase activity, and this increase in Na,K-ATPase activity occurred concomitantly with change in the urinary excretion of ions after administration of aldosterone. The specific activity of

![Figure 1](image-url)
TABLE 1. Activity of Mg or Na, K-ATPase in kidneys of control and aldosterone treated rats

<table>
<thead>
<tr>
<th>Activity</th>
<th>Cortex (μmoles Pi/mg protein/hr)</th>
<th>Medulla (μmoles Pi/mg protein/hr)</th>
<th>Papilla (μmoles Pi/mg protein/hr)</th>
<th>Cortex (μmoles Pi/mg protein/hr)</th>
<th>Medulla (μmoles Pi/mg protein/hr)</th>
<th>Papilla (μmoles Pi/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.43 ± 0.40</td>
<td>8.35 ± 0.39</td>
<td>3.13 ± 0.18</td>
<td>2.94 ± 0.11</td>
<td>3.37 ± 0.11</td>
<td>1.56 ± 0.11</td>
</tr>
<tr>
<td>Aldosterone treated</td>
<td>7.61 ± 0.32</td>
<td>8.60 ± 0.31</td>
<td>3.35 ± 0.14</td>
<td>4.87 ± 0.55</td>
<td>4.03 ± 0.39</td>
<td>1.29 ± 0.23</td>
</tr>
</tbody>
</table>

Values are given as means ± SE of 5 determinations. *: P (Aldosterone treated vs. Control)<0.01. Activities of Mg- and Na,K-ATPase were determined in all experimental animals 60 min after administration of aldosterone (10 μg/animal).

Na,K-ATPase was highest in the medulla before the animals were given aldosterone. However, as increase in cortical Na,K-ATPase activity was more evident than the increases in the medulla and papilla, the cortical tubules may be regulated by aldosterone to a greater extent than are other sections of the renal tubules. Discrepancy between our results and those reported (1, 2) concerning the time course of change in Na,K-ATPase activity may be explained by any one of the following factors: 1) the rats used in our experiments were non-adrenalectomized. 2) the animals used were given pitressin tannate to eliminate factors variable with change in the level of intrinsic antidiuretic hormone. 3) ATPase preparation was not treated with detergents to minimize artificial changes in the membrane structure. The mechanism of increase by aldosterone in renal Na,K-ATPase activity of adrenalectomized rats has been suggested to be due to increased synthesis or decreased degradation of the enzyme protein (6), increased synthesis of an unknown protein that unmasks the enzyme (7) or increase of specific activity of the enzyme secondary to stimulation of absolute tubular sodium transport by aldosterone (8). The data obtained in the present experiments suggest that, in a physiological state, aldosterone regulates functional renal Na,K-ATPase activity in connection with a renal cation transport.

REFERENCES

7) SCHMIDT, U., SCHMIDT, J., SCHMIDT, H. AND DUBACH, U.C.: Sodium- and potassium-
DIFFERENCES IN SPEED AND DEGREE OF MYOSIN B SUPERPRECIPITATION IN THE PRESENCE OF DIVALENT CATIONS

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Accepted October 2, 1978

Efforts have been made to acquire precise quantitative evaluation for the superprecipitation of contractile protein myosin B. Ebashi first reported a method for evaluating the degree of superprecipitation by turbidimetry at 660 m\(\mu\) (1). Watanabe used two parameters for study of superprecipitation; the time required to reach the half maximum increase in turbidity \(t_{0.5}\) and the maximum increase in turbidity \(DOD\) (extent) (2). In the present experiments, we introduced the velocity of superprecipitation at \(t_{0.5}\) \(Vt_{0.5}\) in addition to the above two parameters (Fig. 1). The objective was to obtain information concerning the effects of Ca, Mg and Mn on these three parameters.

Myosin B was extracted from the leg muscle of rabbit (1). The superprecipitation of myosin B was continuously measured using a spectrophotometer at 660 m\(\mu\) (Hitachi 100-01) and the findings were displayed on paper (National pen recorder VP-654B). The reaction mixture in a total volume of 3.0 ml, contained 0.6 mg/ml myosin B, 0.06 M Tris-maleate

![Graphical representation of the time course of superprecipitation of myosin B](image)

**Fig. 1.** Parameters in time course of the superprecipitation of myosin B. Parameters \(t_{0.5}, F_{0.5}\) and \(Vt_{0.5}\) are illustrated on the curve in case of b. Three representative cases in the time course of superprecipitation are shown: a, control (no added divalent cations); b, presence of \(10^{-4}\) M MnCl\(_2\); c, presence of \(10^{-4}\) M MgCl\(_2\).

* We termed this parameter \(F_{\text{max}}\).