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A Na^+-ACTIVATED Mg^{2+}-DEPENDENT RENAL ADENOSINE TRIPHOSPHATASE (Na^+-ATPase) SENSITIVE TO ALDOSTERONE

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It is now well recognised that kidney tissue possesses an ouabain-sensitive Na^+K^+-stimulated Mg^{2+}-dependent ATPase, which is believed to be linked to the mechanism of Na^+ and K^+ exchange in renal epithelial cells (1,2).

Thus renal slices incubated aerobically show a decrease in Na^+ concentration and an increase in K^+ concentration (3,4). This extrusion and uptake of Na^+ and K^+ respectively, can be inhibited by ouabain (5) and this seems to be due to the inhibition of the Na^+K^+-ATPase activity, since the dose-response curves of the inhibitory action of ouabain on this enzyme closely parallel its inhibitory action on Na^+-K^+ exchange (6).

However, such a mechanism is not sufficient to explain the experimental finding that Na^+ can be extruded out of renal slices despite the presence of concentrations of ouabain which inhibit the Na^+-K^+-ATPase completely (7,8).

Consideration has therefore been given to the possible existence of other pumps in the renal tubule (9,10).

Proverbio et al (6) tested for the presence of an ouabain-insensitive Na^+-stimulated Mg^{2+}-dependent ATPase system, but could only demonstrate activity in microsomal preparations of guinea-pig kidney which had aged by storage at 4°C for 10-15 days.

The study presented here reports on the presence of a Na^+-stimulated Mg^{2+}-dependent ATP-hydrolysing enzyme system in fresh microsomal fractions of rat kidney.

The fractions were prepared by centrifuging 10% kidney homogenates (in 0.32M sucrose containing 1mM EDTA) at 1,000g for 10 minutes to remove nuclei and cell debris followed by centrifugation of the supernatant at 22,000g for 20 minutes to remove the mitochondria and the final supernatant was centrifuged at 100,000g for 60 minutes, to sediment the microsomes.

ATPase activities were determined by measuring the release of inorganic phosphate from ATP (4mM) in imidazole/HCl buffer (50mM) pH 7.4 containing sodium (100mM) and magnesium (4mM) or magnesium ions alone, as the chloride salts, by methods described previously (11). Protein was determined by the method of Lowry et al (12). The ATPase activity measured in the presence of 100mM NaCl and 4mM $MgCl_2$ (Na^+Mg^{2+}-ATPase) was significantly higher than the activity obtained in the presence of 4mM $MgCl_2$ only (Mg^{2+}-ATPase). The results are summarised in Table 1.

Table 1

ATPase ACTIVITY OF FRESH MICROSOMAL FRACTIONS PREPARED FROM RAT KIDNEY n = 10

Incubation Medium	ATPase activity (umol P.img Protein$^{-1}h^{-1}$)
Mg^{2+}	41.34 ± 5.4 *
Mg^{2+}+Na^+	45.31 ± 5.7 *
Na^+-ATPase Activity	3.97 ± 0.8

The values are expressed as the mean ± S.E.

Paired Students 't' test was used, for paired Mg^{2+} and Mg^{2+}-Na^+ Observations * P 0.005

In some of the experiments 1mM ouabain was included and had no marked effect on the Na^+-ATPase activity as previously observed (Proverbio et al, 1975) despite the fact that Na^+K^+-ATPase was completely inhibited. The Na^+-ATPase activity was stimulated by low concentrations of sodium (Fig. 1) and maximal activity was obtained at 50mM sodium. Between 50 and 200mM, Na^+, the activity steadily declined. This enzyme system was stimulated by Aldosterone (0.5mM), maximal activity still occurring at 50mM medium, Na^+ concentration (Fig. 1). This stimulation was significantly different from the controls up to 150mM Na^+ concentration.

These results show that the residual ATPase activity that is not sensitive to ouabain in the kidney consists of a Mg^{2+}-ATPase and a Mg^{2+} dependent Na^+-stimulated ATPase activity (Na^+-ATPase) This enzyme may well play a crucial role in the reabsorption of Na^+ in the renal tubules, in view of the fact that it is stimulated by aldosterone. It is therefore possible that two Na^+ pumps may well be involved in the mechanism of action of Aldosterone, one involving Na^+K^+-ATPase (13) and the other involving Na^+-ATPase.

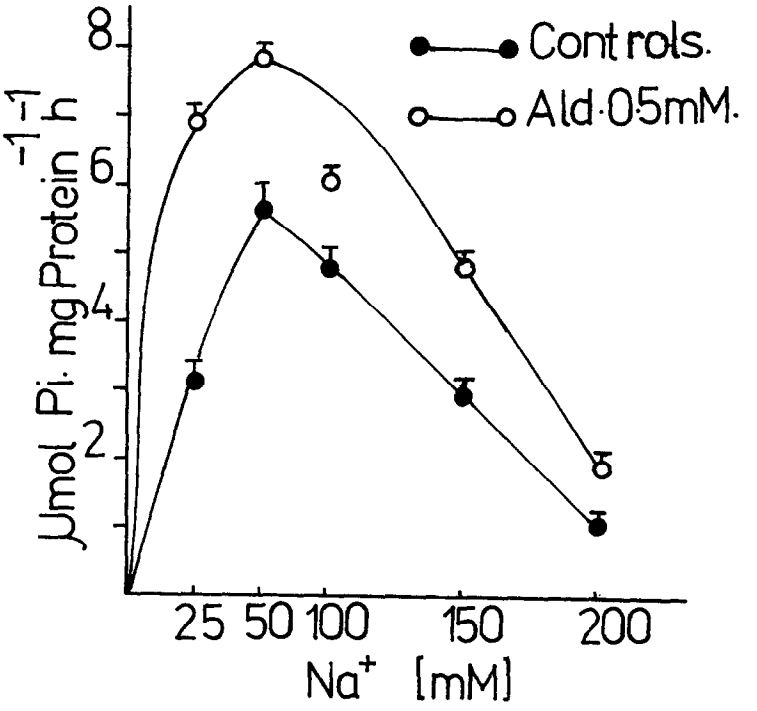

Fig. 1: Stimulation of renal microsomal Na^+-ATPase by Aldosterone.

The incubation medium consisted of 4mM $MgCl_2$, 50mM imidazole HCl buffer pH 7.4, 4mM Tris-ATP and varying concentrations of NaCl, from 25mM-200mM. Na^+-ATPase was obtained by subtracting the activity in the presence of $MgCl_2$ only, from the activity in the presence of both $MgCl_2$ and NaCl.

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