Effects of aldosterone and spironolactone on renal ATPase activities

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Two main theories have been advanced to account for the action of aldosterone of increasing the reabsorption of sodium by the kidney. One proposes that it is the movement of sodium into the tubular cells from the mucosal side which is rate-limiting and which is stimulated by aldosterone; the other theory proposes that it is the active transport of sodium out of the tubular cell at the serosal edge which is rate-limiting and stimulated by aldosterone. This second possibility implies that aldosterone increases the activity of a sodium pump at the serosal surface. The operation of a sodium pump implies involvement of an ATP-hydrolysing system activated by sodium and potassium ions in the presence of magnesium ions (Na^+,K^+-ATPase). It is also possible that the pump involves a sodium-activated, magnesium-dependent ATPase (Na^+-ATPase) (Gilbert & Wyllie, 1975).

We report here the effects of aldosterone and its antagonist spironolactone on ATPase activities of microsomal fractions prepared from rat kidney. The fractions were prepared by centrifuging 10% kidney homogenates (in 0.32 M sucrose containing 1 mM EDTA) at 1,000 *g* for 10 min to remove nuclei and cell debris followed by centrifugation of the supernatant at 22,000 *g* for 20 min to remove the mitochondria and the final supernatant was centrifuged at 100,000 *g* for 60 minutes. ATPase activities were determined by measuring the release of inorganic phosphate from ATP (4 mM) in imidazole/HCl buffer (50 mM) pH 7.4 containing sodium (100 mM), potassium (20 mM) and magnesium (4 mM), sodium and magnesium, or magnesium ions alone, as the chloride salts by methods described previously (Gilbert & Wyllie, 1975). Protein was determined by the method of Lowry, Rosenbrough, Farr & Randall (1951).

Aldosterone at concentrations of 0.01 to 0.5 mM stimulated the Na^+,K^+-ATPase activity of whole kidney microsomal fractions. It also stimulated the activity of Na^+-ATPase but not the activity of Mg^{++}-ATPase. The sodium dependence curves of both the Na^+,K^+-ATPase and the Na^+-ATPase activities were markedly increased by the hormone at sodium concentrations of 25–50 mM.

A similar but much more pronounced effect of aldosterone was detected in microsomal fractions from the renal medulla but no such effect was detected in preparations from the renal cortex. Spironolactone, at a concentration of 0.01 mM, antagonised the stimulatory effect of aldosterone (0.1 mM) on the Na^+,K^+-ATPase of the medulla without itself exerting any significant effect upon the activity of the enzyme.

In other experiments it was possible to assess effects of the drugs on human renal biopsy samples. The samples, commonly 2 mg in weight were homogenised in imidazole/HCl buffer and samples of the suspensions used for ATPase assays. Aldosterone (0.1 mM) significantly stimulated Na^+,K^+-ATPase. The effect was again antagonised by spironolactone (0.01 mM) which did not itself significantly influence the activity of the enzyme.

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References

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