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THE ACTION OF ALDOSTERONE AND OUABAIN ON CATION CONCENTRATIONS IN INCUBATED RENAL SLICES

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Abstract—Studies have been made on the effects of aldosterone and ouabain on Na^+ and K^+ concentrations in incubated rat kidney slices. The results indicate that, control slices gained Na^+ over the first 10 min of incubation and subsequently extruded it, with increase in incubation time. In the presence of aldosterone, the same pattern was observed as with control slices, but tissue Na^+ concentrations were significantly higher at all times. In the presence of ouabain, slices accumulated Na^+ continuously, but in the presence of both drugs, tissue Na^+ concentrations were not as high as with ouabain alone. Both aldosterone and ouabain increased the loss of cell K^+ whereas control slices reaccumulated their intracellular K^+ concentration, with increase in incubation time. The results suggest that aldosterone may have an actual mechanism of action in the renal tubule: either by activating two Na^+, K^+ pumps located on the luminal and serosal membranes, respectively, or by enhancing luminal membrane permeability, and at the same time stimulating Na^+, K^+-ATPase on the serosal membrane.

The mechanism of action of aldosterone in enhancing renal tubular Na^+ reabsorption has been puzzling. Several studies suggest that this hormone stimulates Na^+, K^+-ATPase [1, 2] and Na^+-ATPase activities [3], and that this property may be involved in its mechanism of action. The effect on the ATPases is antagonised by spironolactone [1] in the rat kidney tissue and in human renal biopsy samples [4].

It is not clear whether the effect of aldosterone on Na^+, K^+-ATPase is solely responsible for the increased reabsorption of Na^+, induced by the hormone in the kidney or whether other mechanisms are involved.

We have investigated this problem by determining the effects of aldosterone and ouabain on the concentrations of ions in incubated rat kidney slice tissue and in the intracellular water-phase. A preliminary report of this work has already appeared elsewhere [5].

MATERIALS AND METHODS

Sprague–Dawley rats, weighing 250–300 g, were stunned by a blow at the back of the neck, quickly bled and the kidneys decapsulated and placed on ice.

Slices of 0.2 mm thickness were used in all experiments. They were prepared using a McIwain tissue chopper, using maximal blade force to ensure that the blade went right through the tissue, giving neat slices. Slices from 2–3 animals were mixed in a petri-dish, before being distributed into incubation flasks.

Groups of slices weighing approximately 150 mg were lifted with a pointed end pair of forceps and placed in 3 ml of incubation medium in a flask. Paired kidneys from an individual rat were assumed to be identical in composition. The incubation medium contained the following ions (mmoles/l): Na^+ (140), K^+ (5.0), Ca^{2+} (2.5), Mg^{2+} (1.0), Cl^- (144.0), $SO4^{2-}$ (3.0), and glucose (5.5), with or without aldosterone and/or ouabain. In some of the experiments raffinose (10 mmoles/l) was included as an extracellular space marker substance. The medium was modified Robinson's medium [6].

In all experiments incubation was done at 37°, in a Gilson differential respirometer. The flasks were incubated for various time periods from 5 to 120 min, gassed throughout with 100% oxygen and shaken at the rate of about 100 rpm.

Both control and drug-containing slices were incubated in duplicate.

At the end of each desired incubation period, the flasks were removed from the respirometer and the contents poured into a filter funnel with an ashless filter paper to drain off the medium. The slices were carefully removed with forceps and blotted gently without squeezing.

When both tissue and intracellular concentrations were being measured, the slices were transferred to pear-shaped extraction flasks and rapidly weighed to obtain the wet weights of the slices. They were homogenised immediately in 10 ml of deionised water, using an MSE overhead drive homogeniser. Three millilitres of the homogenate were pipetted off, using a bulb-pipette with a sawn-off tip to facilitate uptake of the homogenate. This was transferred into a 10 ml volumetric flask and used for raffinose estimation.

A further 3 ml was pipetted off and placed in a pear-shaped flask. Five millilitres of 1 M nitric acid was added and the digests shaken for at least 12 hr on a flask-shaker to extract the ions.

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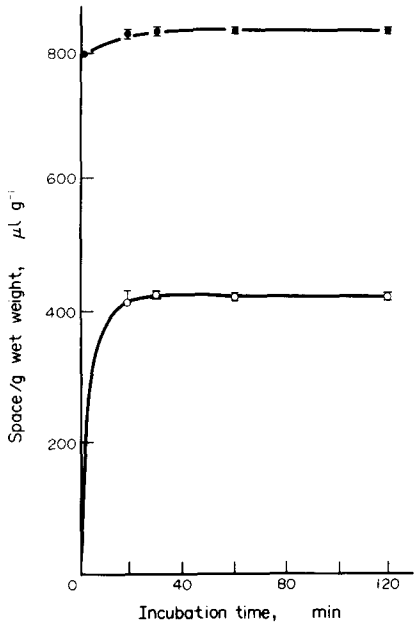

Fig. 1. Effect of incubation time on total cell water and raffinose space of renal slices. Ordinate: total cell water (filled symbols) and raffinose space (open symbols) expressed as μl/g wet wt. Each point is the mean ± S.E.M. of 3 experiments. The mean raffinose space over the 2 hr incubation period was 405.33 ± 1.5 μl/g wet wt.

The digests were filtered using an ashless filter paper and the filtrates diluted with either a 4000 μg/ml solution of potassium chloride or a 2000 μg/ml solution of caesium chloride for the assay of Na^+ and K^+, respectively, using the Varian Techtron 1100 Atomic Absorptiometer.

Raffinose was estimated by the method described by Gilbert [7] and all calculations for fractional penetration of intracellular water by the two cations were based upon considerations in that paper.

D-Aldosterone and ouabain (strophanthin-G) were supplied by Sigma Chemical Co. (London, U.K.). Statistical analysis was made using the Student's *t*-test.

RESULTS

Measurement of raffinose (extracellular) space

Figure 1 shows the effect of incubation time on the penetration of the extracellular water phase by raffinose. The raffinose space increased rapidly with time over the first few minutes of incubation, reaching a value of about 400 μl/g wet wt within the first 5 min or so of incubation. Similarly, the total cell water of the slices rose rapidly and remained constant after reaching a value of about 825 ml/g wet wt. Subsequently, the increase in raffinose space was parallelled by the increase in total cell water, and the intracellular water did not change appreciably during the incubation phase.

Effects of D-*aldosterone and ouabain on tissue Na^+ and K^+ concentrations*

Figure 2 shows that control slices incubated in ordinary media increased their Na^+ concentration rapidly reaching a maximum at 10 min incubation. This was followed by a decrease, reaching a value similar to the initial value, at the end of 2 hr of incubation.

In the presence of 0.1 mM aldosterone tissue Na^+ concentration increased pronouncedly reaching a significantly higher value than the control by the end of 10 min incubation.

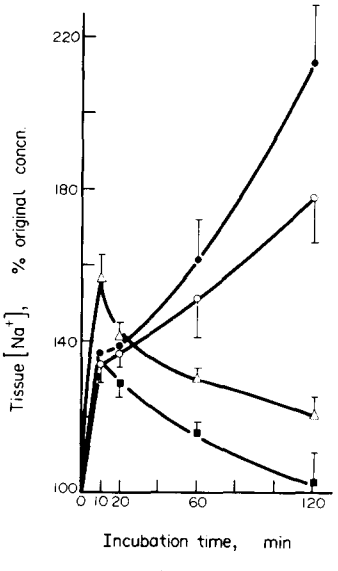

Fig. 2. changes in tissue Na^+ concentrations in slices incubated in control media (■), media with 0.1 mM aldosterone (△), media with 1 mM ouabain (●) and media with aldosterone, 0.1 mM, plus ouabain, 1 mM (○). Each point represents mean ± S.E.M. of 6–10 experiments. Student's *t*-test, ouabain vs ouabain/aldosterone (120 min), $P < 0.05$.

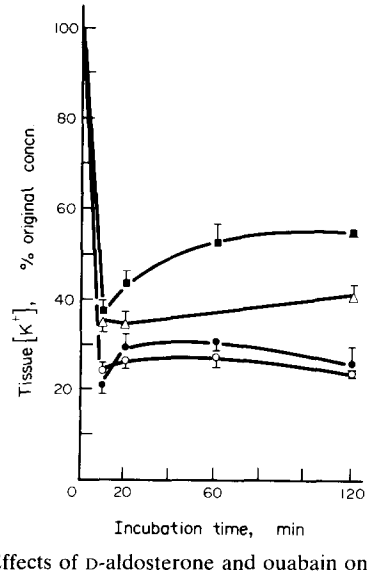

Fig. 3. Effects of D-aldosterone and ouabain on tissue K^+ concentrations of slices incubated for different times. The diagram shows the changes in tissue K^+ concentration in control slices (■) and slices incubated in media with 0.1 mM aldosterone (△), 1 mM ouabain and 1 mM aldosterone (○).The concentrations are expressed as percentage of the original fresh tissue concentration. Each point represents mean ± S.E.M. of 4–11 experiments. Control vs aldosterone (120 min), $P < 0.05$.

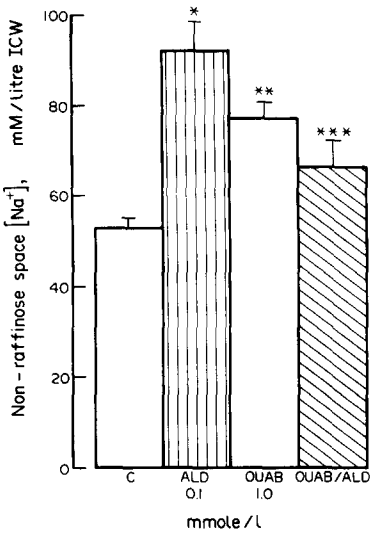

Fig. 4. The influence of aldosterone and ouabain on non-raffinose (intracellular) space sodium concentrations of slices incubated for 10 min. The non-raffinose space Na^+ concentrations (ordinate) are expressed as mmoles/l of intracellular water. The concentrations of aldosterone and ouabain used are shown on the abscissa. Each column represents mean ± S.E.M. of 7–13 experiments. * $0.005 < P < 0.01$, ** $0.001 < P < 0.005$, *** $0.2 < P < 0.3$, c = control.

Slices incubated in media containing 1 mM ouabain on the other hand accumulated increasing amounts of Na^+ throughout the incubation period, so that at the end of 2 hr they contained 212.9 ± 31.4% of the initial tissue Na^+ concentration. This was significantly higher than the concentration in control and aldosterone treated slices, incubated for the same time period.

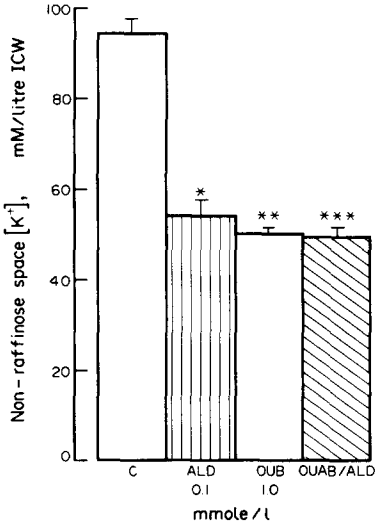

Fig. 5. The influence of aldosterone and ouabain on non-raffinose (intracellular) space K^+ concentrations of slices incubated for 10 min. The non-raffinose space K^+ concentrations (ordinate) are expressed as mmoles/l of intracellular water. The concentrations of aldosterone and ouabain used are shown on the abscissa. * $0.001 < P < 0.005$, ** $0.001 < P < 0.005$, *** $0.001 < P < 0.005$.

When aldosterone (0.1 mM) and ouabain (1 mM) were both present in the incubation medium, it was clear from the results obtained that slices had a tendency to accumulate less Na^+ than when ouabain was present alone, and this effect was more noticeable during longer incubation times (60–120 min).

In the case of K^+, control slices lost considerable amounts of this ion (Fig. 3) reaching the lowest level by the end of 10 min, and thereafter the slices tended to reaccumulate K^+, so that by the end of 2 hr, control slices had about 55% of the original tissue K^+ concentration.

Effects of D-*aldosterone and ouabain on non-raffinose space (intracellular)* Na^+ *and* K^+ *concentrations*

Figure 4 shows the non-raffinose Na^+ concentrations expressed as millimoles per litre of intracellular water (ICW) for slices incubated over a 10 min period.

Slices incubated in ordinary control media attained a non-raffinose compartment Na^+ concentration of 52.75 ± 4.3 (13 experiments) mmoles/l ICW, whereas those incubated in media with 0.1 mM aldosterone attained a Na^+ concentration of 92.34 ± 13.6 (10 experiments) mmoles/l ICW—a concentration which was significantly higher than the control value.

The non-raffinose compartment Na^+ concentrations of slices in media with 1 mM ouabain were also elevated, but remained relatively less than those of slices incubated in media with aldosterone.

When both drugs were present in the medium less Na^+ tended to accumulate in the slices than whether either drug along was present, but the effect of aldosterone was not nullified. The same effects were observed at 60 min incubation.

As for whole kidney slice tissue, the same general pattern of K^+ distribution was observed in the non-raffinose compartment (Fig. 5).

Slices incubated in media with aldosterone had a more substantial net loss of K^+, compared with control slices.

The non-raffinose compartment K^+ concentrations are illustrated in Fig. 5. Control slices had an intracellular K^+ concentration of 94.54 ± 5.8 (14 experiments) mmoles/l ICW, whereas slices incubated in media with aldosterone experienced a substantial loss of intracellular K^+. Taking the theoretical normal intracellular fluid K^+ concentration as 150 mmoles/l, calculations show that normal media slices had a net loss of about 37% of their intracellular K^+, whereas those in media with aldosterone lost 65%.

DISCUSSION

The results presented above clearly demonstrate that aldosterone has differential effects on Na^+ and K^+ ions in renal tissue. It appears therefore that the mechanisms by which aldosterone accelerates Na^+ transport in the kidney epithelial cells could be the net result of rather complex effects upon different components of the Na^+ transport system.

We have already demonstrated [1] that aldosterone stimulates the Na^+, K^+-ATPase activity, located on the peritubular side of the renal epithelial cell. Therefore the fact that this hormone initially

enhanced both tissue and intracellular Na^+ concentrations in the present studies, would tend to suggest that it is also acting on components of the Na^+ transport system, located on the luminal membrane.

Subsequently the levels of cell Na^+ go down, suggesting that the stimulation of the peritubular pump by aldosterone results in extrusion of this cation from the intracellular water-phase, and that this activity probably outweighs the effects of the hormone on the luminal membrane.

Increased intracellular Na^+ concentrations could be the result of enhanced permeability of the luminal membrane by aldosterone, resulting in diffusion of Na^+ into and K^+ out of the cells. This is compatible with the low of levels of K^+ observed in the presence of aldosterone.

It is also possible that, a pumping mechanism situated at the luminal membrane could account for the changes observed in Na^+ and K^+ distribution in the first 10 min of incubation. Thus, the pump could cause an initial rise in intracellular Na^+ concentrations and the pumping of K^+ out of the cell into the mucosal solution. Such a pump would be a coupled Na^+–K^+ pump like the one situated on the serosal membrane of the cell, but pumping cations in opposite directions.

In this connection, the demonstration of two distinct Na^+–K^+-ATPases [8] on either side of an epithelial cell, makes the above suggestion tenable.

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REFERENCES

1. J. C. Gilbert, A. T. Lambie and H. Osore, *Br. J. Pharmac.* **64**, 408P (1978).
2. U. Schmidt, J. Schmid, H. Schmid and U. C. Dubach, *J. clin. Invest.* **55**, 655 (1975).
3. H. Osore, *Biochem. Pharmac.* **28**, 2865 (1979).
4. H. Osore, *Kenya J. Sci. Tech.* **2**, 25 (1982).
5. H. Osore, *Proceedings of the 8th International Congress of Pharmacology, Tokyo, July* 19–24 (1981).
6. J. R. Robinson, *Biochem. J.* **45**, 68 (1949).
7. J. C. Gilbert, *J. Neurochem.* **13**, 729 (1966).
8. M. Gachelin and F. Bastide, *C. R. Acad, Sci., Paris* **267** (1968).