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WATER AND ELECTROLYTE CONTENTS OF RAT RENAL CORTICAL SLICES INCUBATED IN POTASSIUM-FREE MEDIA AND MEDIA CONTAINING OUABAIN

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SUMMARY

1. Slices of rat renal cortex were leached anaerobically at 0.5° for 2.5 h in various media, and subsequently reincubated for 60 min at 25° in media of identical composition. Slices were taken for analysis at 1, 2, 4, 8, 16, 30 and 60 min after the beginning of reincubation.

2. Slices in ordinary media containing 1 mM sodium iodoacetate continued to gain H_2O, Na^+ and Cl^- and to lose K^+ throughout 60 min of anaerobic reincubation at 25°.

3. Slices reincubated aerobically lost H_2O, Na^+ and Cl^- in ordinary media, K^+-free media and media containing ouabain. Only in slices reincubated in ordinary media, or ordinary media containing 1 mM ouabain was this recovery in H_2O, Na^+ and Cl^- accompanied by any net uptake of K^+ by the tissue. Slices reincubated in K^+-free media (with and without 1 mM or 10 mM ouabain), and slices reincubated in ordinary media containing 7.5 or 10 mM ouabain showed a net loss of K^+ throughout reincubation.

4. The recovery of H_2O, Na^+ and Cl^- which occurred during aerobic reincubation at 25° even in K^+-free media containing enough ouabain to produce maximal inhibition of a linked Na^+–K^+ cation pump could not be attributed to passive K^+ efflux, or to initial activity of a Na^+–K^+ pump.

5. The results could be explained if rat renal cortical tissue possessed two distinct mechanisms—one inhibited by ouabain and cooling, requiring K^+ in the medium and concerned with the maintenance of intracellular K^+; the other inhibited by cooling but insensitive to ouabain, not requiring K^+ in the medium and concerned with the maintenance of cellular volume.

INTRODUCTION

It is commonly held that the volume and principal ionic composition of mammalian cells are determined by two factors. The colloid osmotic pressure exerted by non-diffusible intracellular substances, mostly proteins, tends to produce swelling

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with the entry of extracellular water and ions. Opposing this is a cation pump which, using energy derived from metabolism, extrudes Na^+, and thereby Cl^- and water, from the cell[1,2]. Extrusion of Na^+ from cells by this cation pump has often seemed to require potassium in the external medium to be accumulated in exchange for sodium, and to be inhibited by lack of available energy and also by cardiac glycosides such as ouabain which appear to interfere with the carrier mechanism[3]. If this cation pump is in fact responsible for the maintenance of cellular volume, then cells should swell when exposed to a K^+-free medium containing a cardiac glycoside. Conversely, once swollen they should fail to recover their water content when incubated under aerobic conditions in such a medium. A number of observations on a variety of tissues from various species support this conclusion[4–7]. However, KLEINZELLER AND KNOTKOVA[7] observed a loss of water and ions from swollen rabbit kidney slices incubated in media containing ouabain, and suggested that some mechanism other than a cation pump was responsible for this recovery. From results which they presented, however, it is not possible to exclude, as explanations for the recovery of cellular volume, (a) incomplete inhibition of the conventional cation pump (since increasing the concentration of ouabain in the medium from 0.3 mM to 1 mM resulted in a smaller loss of H_2O, Na^+ and Cl^- and a greater loss of K^+ after 60 min, and a maximal effect of ouabain was thus not demonstrated), (b) the possibility that ouabain, like some mercurial diuretics[8], takes several minutes to inhibit cation transport at 25°, so that the recovery observed after 60 min could have taken place in the early minutes before inhibition of cation transport had occurred, (c) a passive diffusion of K^+ together with Cl^- and H_2O from the cells during reincubation. This has been suggested as an explanation for the recovery in volume of mouse ascites tumour cells in K^+-free media[9] and might theoretically produce a transient, though not a sustained, recovery of volume.

Experiments were therefore performed to test whether these possibilities could explain a loss of water and electrolytes like that found by KLEINZELLER AND KNOTKOVA[7] by measuring the rates at which water and ions changed when metabolism was restored to rat kidney slices which had been leached at 0.5°. The results excluded all three of the possibilities named, and it seems necessary to postulate that another mechanism, apart from a coupled Na^+–K^+ pump, sensitive to ouabain, regulates cellular volume.

A preliminary account of a portion of this work has been presented[10].

METHODS

Media

The media had the following compositions in mequiv/l:

1. Ordinary media: Na^+, 146; K^+, 5; Ca^{2+}, 5; Mg^{2+}, 2; Cl^-, 134; SO_4^{2-}, 2; acetate, 10; buffered with phosphate (8 mM) at pH 7.26.

2. Potassium-free media: these had the same composition as the ordinary media except that they contained 150 mequiv/l of Na^+, and no K^+.

When ouabain (obtained from B.D.H.) was to be used it was dissolved in the media to give a final concentration of either 1, 7.5, or 10 mM. Media for anaerobic experiments contained 1 mM sodium iodoacetate. Ouabain and iodoacetate were always present in the stated concentrations in both the leaching and reincubation media.

Procedure

Slices from the renal cortex of adult male hooded rats were cut, immediately transferred to ordinary media at 25° and stirred by oxygen bubbled through the media. It took about 20 min to cut all the slices from the 3 or 4 animals used in any one experiment, and after the last slice had been added to the media another 15 min was allowed for equilibration. Following equilibration 1 or 2 slices were analysed to determined the composition before leaching. The remaining slices were transferred either to ordinary media or to K^+-free media at 0.5° where they remained for up to 150 min, with nitrogen bubbled through the media.

3 or 4 leached slices were taken for analysis, the remainder were transferred to fresh media at 25° and stirred by bubbling oxygen, or in anaerobic experiments, nitrogen, through the medium. The medium for reincubation had a composition identical with that of the leaching medium in the same experiments. This ensured that changes in cellular composition were not masked by changes in extracellular composition upon changing from the leaching to the reincubation media. It also allowed ouabain and iodoacetate, when used, to equilibrate in the tissue during the leaching procedure.

Slices to be analysed after 1, 2 and 4 min reincubation at 25° were removed singly from the leaching media between 135 and 150 min. Slices to be reincubated for 8, 16, 30 and 60 min were removed together from the leaching media at 150 min. This enabled the changes in slices reincubated for 1, 2 and 4 min to be followed accurately to within 5 sec. Slices reincubated for longer periods were reincubated within $\pm$ 1 min of the stated time. In each experiment 2–3 slices were reincubated for each period.

Each group of experiments consisted of at least 3 separate experiments utilising kidneys from not fewer than 12 rats.

Analytical methods

Slices removed from media were immediately blotted on hardened filter paper (Whatman, No. 542), and their water content determined gravimetrically[11]. Each slice was then extracted overnight with 10 ml 0.1 M nitric acid at room temperature.

Na^+ and K^+ were determined in the acid extracts using an EEL flame photometer with external standards made up in 0.1 M nitric acid.

Chloride was determined, using the method of COTLOVE, TRANTHAM AND BOWMAN[12] which has been shown to be satisfactory under the present experimental conditions (J. V. ALLISON, personal communication).

Results

Results are expressed in terms of tissue content of water and ions (in mequiv/kg dry matter) rather than as concentrations in the tissue water (mequiv/l tissue H_2O). All graphs show the mean $\pm$ S.D. of the relevant observations. The "before leaching" values are averages of 30 observations from all groups of experiments. The statistical significance of differences between groups was evaluated by student's t test.

RESULTS

The changes in tissue composition which occurred when leached slices were reincubated aerobically at 25° are shown in Fig. 1. Within the first minute of reincu-

bation there was a substantial recovery in water content, associated with loss of Na^+ and Cl^-, and uptake of K^+. After 4 min reincubation the water content of the tissue was not significantly different from that of the unleached slices, and was maintained over the next 56 min.

The tissue Na^+ content decreased rapidly, although it never quite recovered to the level found in slices which had not been leached. The amount of Na^+ in the tissue remained relatively constant between 4 and 60 min.

In contrast to the rapid recovery in water content, and Na^+ content, K^+ continued to be reaccumulated for the first 30 min of reincubation. The K^+ content of the tissue also returned to a level comparable to that found in slices before leaching.

The recovery of Cl^- in these experiments approximately paralleled the excess of Na^+ lost over K^+ gained. For example, after 30 min reincubation the net ($Na^+ + K^+$) loss from the slices was 70 mequiv/kg dry matter, while the amount of Cl^- lost was 61 mequiv/kg dry matter.

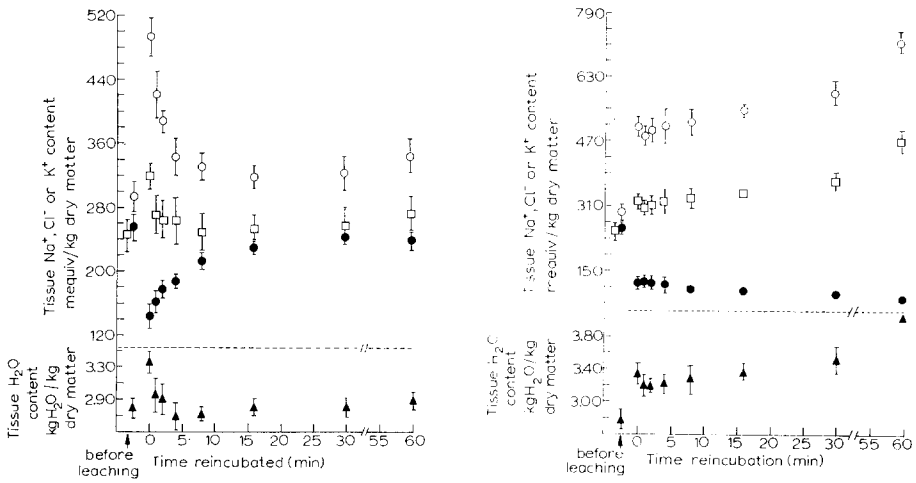

Fig. 1. Composition of rat renal cortical slices leached anaerobically at 0.5° for 150 min and then reincubated aerobically at 25° in ordinary media. Each point represents mean ±S.D. of 7–13 separate observations. Composition of slices at end of leaching plotted at 0. ▲, H_2O; ●, K^+; □, Cl^-; ○, Na^+.

Fig. 2. Composition of rat renal cortical slices leached anaerobically at 0.5° for 150 min, and then reincubated anaerobically at 25° in ordinary media containing 1 mM sodium iodoacetate. Each point represents mean ±S.D. of 7–11 separate observations. Composition of slices at end of leaching plotted at 0. ▲, H_2O; ●, K^+; □, Cl^-; ○, Na^+.

In contrast to this observed recovery in water and electrolytes when slices were reincubated aerobically, swelling continued, with uptake of water, Na^+, and Cl^- and a continual slow loss of K^+ when slices were reincubated at 25° anaerobically in ordinary media containing 1 mM sodium iodoacetate (Fig. 2). Thus the recovery was due to the restoration of metabolism rather than to the change in temperature as such.

Fig. 3 permits comparisons of the changes in water, Na^+, K^+ and Cl^- during reincubation in K^+-free media alone, and in K^+-free media containing 1 mM and 10 mM ouabain. The changes in water content only, of slices reincubated in ordinary medium, are also plotted.

In the 4 experiments the water contents after leaching were not significantly different, and the recoveries of water contents during reincubation followed common patterns. In all 4 groups the water content recovered to a value not significantly different from that of slices before leaching, and this recovery was sustained during much of the reincubation.

However, the behaviour of Na^+ and K^+ in slices incubated in ordinary media (Fig. 1) was in strong contrast to that in slices incubated in the K^+-free media. The latter showed no net uptake of K^+ during reincubation; there was instead a continued steady loss of K^+ from the slices throughout reincubation, and this loss of K^+ did not parallel the rapid initial loss of H_2O, Na^+ and Cl^-.

The tissue reincubated in the K^+-free media initially lost both Na^+ and Cl^-, but this loss was followed later by a steady gain of both ions, so that by the end of 60 min the slices contained more Na^+ and more Cl^- than they had done immediately after leaching. Some of the Na^+ gained presumably replaced K^+ lost from the cells, and some perhaps balanced losses of other positively charged groups, *e.g.*, on macromolecular polyions. Similarly, the Cl^- gained probably replaced negatively charged groups lost from the cells.

Fig. 3, contrasted with Fig. 1, shows that incubation in a K^+-free media with 1 mM ouabain had a profound effect upon the Na^+ and K^+ contents of the tissue,

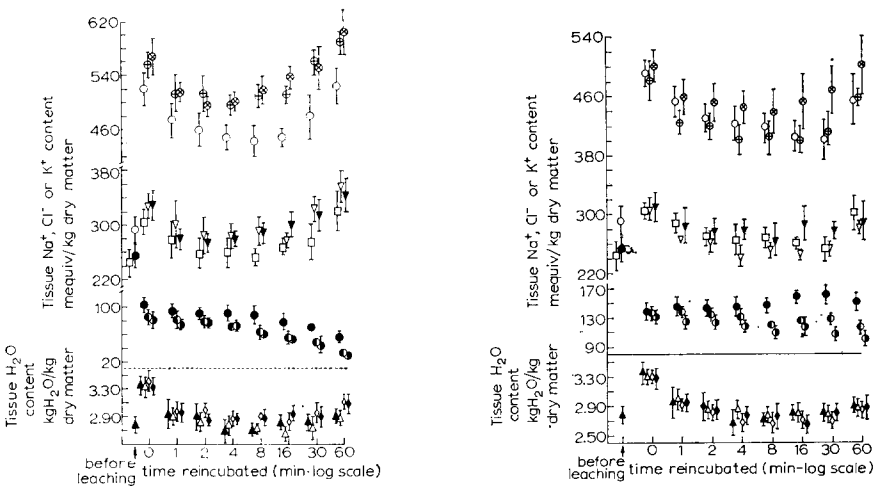

Fig. 3. Composition of rat renal cortical slices leached anaerobically at 0.5° for 150 min and then reincubated aerobically at 25° in ordinary media (water content only shown here) and in K^+-free media alone or with 1 mM or 10 mM ouabain. Each point represents mean ±S.D. of 6–16 separate observations. Composition of slices at end of leaching plotted at 0. Ordinary media: ▲, H_2O. K^+-free media: △, H_2O; ●, K^+; □, Cl^-; ○, Na^+. K^+-free media containing 1 mM ouabain: ◇, H_2O; ◐, K^+; ▽, Cl^-; ⊕, Na^+. K^+-free media containing 10 mM ouabain: ◆, H_2O; ◑, K^+; ▼, Cl^-; ⊗, Na^+.

Fig. 4. Composition of rat renal cortical slices leached anaerobically at 0.5° for 150 min, and then reincubated aerobically in ordinary media alone (water contents only are shown), or in ordinary media containing either 1 mM, 7.5 mM or 10 mM ouabain. Each point represents mean ± S.D. of 6–23 separate observations. Composition of slices at end of leaching plotted at 0. Ordinary media: ▲, H_2O. Ordinary media containing 1 mM ouabain: △, H_2O; ●, K^+; □, Cl^-; ○, Na^+. Ordinary media containing 7.5 mM ouabain: ◇, H_2O; ◐, K^+; ▽, Cl^-; ⊕, Na^+. Ordinary media containing 10 mM ouabain: ◆, H_2O; ◑, K^+; ▼, Cl^-; ⊗, Na^+.

and also that there was no significant further effect upon the behaviour of water, Na^+, K^+ and Cl^- when the concentration of ouabain in the K^+-free medium was increased tenfold. Hence it is probable that ouabain was exerting its maximal action upon transport of cations in renal cortical tissue under these conditions, yet despite this the recovery of cellular volume was not impaired.

Fig. 3 also shows that ouabain affected the retention of K^+ in slices reincubated in K^+-free media. Both after leaching and after 60 min reincubation the slices contained significantly less K^+ when ouabain had been present in the media. This difference could be explained if ouabain inhibited the reaccumulation into the cells of K^+ from intercellular spaces and allowed it to be lost from the slices to the medium. It may be noted that the increased loss of K^+ produced by ouabain in K^+-free media was not accompanied by an appreciable change in the water content of the tissue. The results for the leached slices thus provide further evidence for active K^+ uptake by renal tissue even at 0.5° (refs. 13, 14). They also support the conclusion that cardiac glycosides affect ion transport at 0.5° (ref. 13).

Fig. 4 shows the effects of increasing concentrations of ouabain on tissue water, Na^+, K^+ and Cl^- in slices leached and reincubated in ordinary media. Ouabain had no significant effect on the water content of the tissue, and the recovery of water content was in each case associated with loss of Na^+ and Cl^- from the slice. In the presence of 1 mM ouabain there was a slight net uptake of K^+ over the 60-min reincubation, whereas with 7.5 mM and 10 mM ouabain there was a net loss of K^+ from the first minute of reincubation onwards.

DISCUSSION

Although the extracellular space was not measured and the results are shown in terms of tissue content, they must reflect largely intracellular changes unless the extracellular space decreased significantly as a proportion of the total tissue with the rise in temperature.

Comparisons of results expressed in terms of tissue contents depend upon the assumption that incubation in different media did not alter the ratio, cell matter lost: water and ions lost[15]. There would be an apparent loss of tissue water and ions if slices incubated in K^+-free media or in media containing ouabain lost less cellular matter upon reincubation than slices incubated in ordinary medium alone. But there is no reason to expect that this occurred and it has been reported that the losses of soluble protein from rabbit renal cortical slices at 25° were the same in the presence and absence of ouabain[7].

Aerobic reincubation at 25° resulted in the loss of water, Na^+ and Cl^- from the cells in all experiments. In some of these K^+ was reaccumulated at the same time, in others it continued to be lost from the tissue. Unlike the changes in water content found in mouse ascites tumour cells incubated in a K^+-free medium[9] the loss of water in these experiments could not be explained by passive diffusion of K^+ from the cells. During reincubation in K^+-free media or in media containing ouabain (Figs. 3 and 4) the loss of water occurred in the first minutes of reincubation and was associated with loss of Na^+ and Cl^-, whereas K^+ diffused slowly and continuously from the tissue throughout, as it did also when metabolism was suppressed (Fig. 2) and no loss of water was observed.

The possibility that initial activity of a coupled Na^+–K^+ pump was responsible

for the loss of Na^+, Cl^- and H_2O during early reincubation when ouabain was present in the media can also be excluded. In ordinary media without ouabain there was a significant uptake of K^+ in the first minute (Fig. 1), whereas in the presence of 7.5 mM and 10 mM ouabain there was a net loss of K^+ from the first minute of reincubation onwards (Fig. 4). Yet in each case the initial loss of water and Cl^- from the tissues occurred at the same rate and to the same extent. This conclusion is supported by the observations in K^+-free media at 0.5° (Fig. 3), which seemed to show that ouabain was active at this temperature during leaching. BURG AND ORLOFF[13] also observed an effect of the related cardiac glycoside, strophanthidin, at 0°.

Rat tissue is known to be relatively insensitive to cardiac glycosides, but the concentrations of ouabain used were sufficient to produce maximal inhibition of K^+ uptake with a very large loss of K^+ from the tissue in K^+-free media (Fig. 3). It is possible, however, that the Na^+–K^+ pump was not maximally inhibited in ordinary media where, during aerobic reincubation at 25°, the tissue K^+ was lower when the concentration of ouabain was higher (Fig. 4). This observation suggests that the coupled cation pump was progressively inhibited as the concentration of ouabain was raised. Yet, in spite of this, the changes in water content during reincubation were not significantly different, either one from another, or from those observed in ordinary medium without any inhibitor (also shown in Fig. 4).

It seems unlikely that a Na^+–K^+ pump regulating and determining cell volume would work at four different rates (as judged by net K^+ uptake) and yet produce the same recovery of water content at the same rate. Moreover there is no experimental evidence to support an alternative explanation that the rate at which the cation pump worked in the 4 experiments was the same but that the rate of passive efflux of K^+ from the cells increased as the concentration of ouabain in the medium rose[6,16]. Neither can the recovery of volume in the present experiments be ascribed to a decrease in rate of passive Na^+ influx, the rate of Na^+ efflux being unaffected as the concentration of ouabain was raised, since there is no evidence to suggest that cardiac glycosides affect Na^+ influx in the presence of K^+ in the medium[3,6].

Hence in those experiments in which the conventional cation pump should have been inhibited by ouabain, it appears reasonable to ascribe the observed recovery to another active process moving water, Na^+ and Cl^- from the cell. The results thus support and extend the observations of KLEINZELLER AND KNOTKOVA[7] on rabbit kidney slices and are in agreement with those outlined recently in a preliminary report by WHITTEMBURY[17], who found that renal cortical slices from guinea pigs also recovered their volume with extrusion of Na^+ and Cl^- even in the presence of ouabain. Although the precise mechanism determining cellular volume remains to be elucidated, WHITTEMBURY[17] has suggested that renal tissue may possess a second Na^+ pump. It certainly appears reasonable to postulate that rat renal tissue possesses two distinct mechanisms. One requires K^+ in the medium, is sensitive to ouabain, and to chilling, is similar to that found in other tissues, and may be concerned, in the kidney, with the maintenance of intracellular K^+. The other does not require K^+ in the medium, it is also inhibited by chilling, but is insensitive to ouabain, and is concerned with the maintenance of cellular volume. How these two possible mechanisms are related to the major task of conveying glomerular filtrate across the tubular epithelium at a rate approximately equivalent to half the volume of the cells every minute, remains to be discovered.

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