SHORT COMMUNICATION

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Regulation of cellular volume during anaerobic incubation of rat renal cortical slices

It is well established that slices of adult rat renal cortex which have lost K^+ during leaching are unable to reaccumulate K^+ during anaerobic reincubation[1]. If the reaccumulation of K^+ provides an indication of the activity of a coupled Na^+–K^+ pump, and if such a coupled pump is responsible for the regulation of renal cellular volume, it might be expected that anaerobic metabolism would provide insufficient energy for the recovery of cellular volume; and it has frequently been observed that slices incubated anaerobically at 25° or 37° do become swollen[2]. Since recent work[3–6] has indicated that a mechanism other than a K^+-dependent, ouabain-sensitive Na^+ pump may be responsible for the regulation of cellular volume in renal cortical tissue, the changes in volume and in ion contents of leached rat renal cortical slices have been followed during anaerobic reincubation at 25° for up to 60 min. A preliminary report of a portion of this work has appeared elsewhere[7].

The methods have been described previously[4]. The ordinary medium used in these experiments contained, in addition, 5 mM glucose. In some media sodium iodoacetate (10 mM) replaced sodium acetate (10 mM) and glucose was omitted. After equilibration in oxygenated ordinary medium at 25°, slices were leached either in ordinary medium, in ordinary medium containing 10 mM ouabain or in medium containing 10 mM sodium iodoacetate where they remained at 0.5° for 150 min. Nitrogen was bubbled through the media throughout. Some slices were taken for analysis, the remainder were transferred to medium of identical composition to that in which they had been leached and were reincubated at 25° for up to 60 min. Nitrogen was again bubbled through the media continuously.

Each result shown in Fig. 1 represents the mean ± S.D. of observations on 7 slices from 7 separate experiments utilising the kidneys of 28 rats. After 150 min at 0.5°, all slices had swollen to the same extent with the uptake of water, Na^+ and Cl^- and loss of K^+. After reincubation at 25° for 60 min, all slices were more swollen than they had been after leaching, containing more water, Na^+ and Cl^- and less K^+. Slices in ordinary medium had, however, shown initial significant losses of water, Na^+ and Cl^-, and this initial recovery in volume was not affected by the presence of ouabain in the medium. It was as great as that previously observed[4] initially in slices reincubated in oxygenated medium at 25° (0.41 kg water/kg tissue dry matter was lost from slices after 1-min reincubation in each case). This initial recovery was, however, prevented entirely by the presence of 10 mM iodoacetate. In fact, the composition of slices incubated anaerobically in medium containing 10 mM iodoacetate did not differ appreciably from that of slices incubated anaerobically in medium containing 1 mM iodoacetate reported earlier[4]. This suggests that 10 mM iodoacetate produced a maximal inhibition of metabolism under these experimental conditions.

The recovery of cellular volume previously observed[4] when leached slices were reincubated in oxygenated medium at 25° was accompanied by a significant uptake of K^+, 19 mequiv/kg tissue dry matter being accumulated in the first minute of reincubation. In contrast, slices reincubated anaerobically in the present experiments showed a net loss of 7 mequiv K^+/kg tissue dry matter in the first minute and continued to lose K^+ throughout reincubation. The presence of 10 mM ouabain, shown

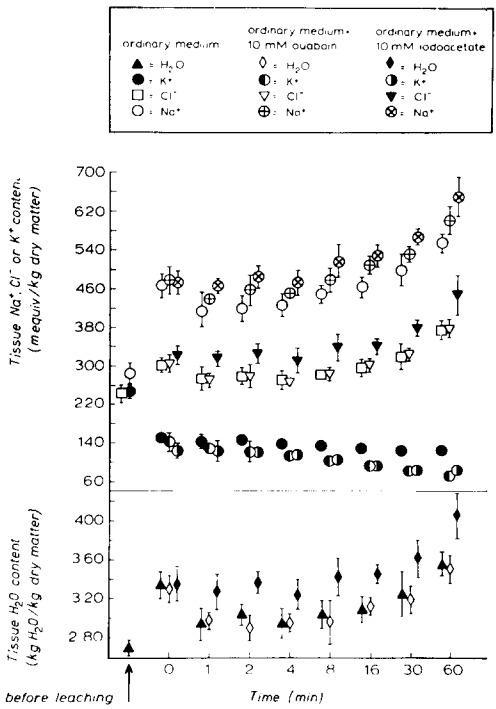

Fig. 1. Composition of rat renal cortical slices leached anaerobically at 0.5° for 150 min and then reincubated anaerobically at 25° in ordinary medium, ordinary medium containing 10 mM ouabain or medium containing 10 mM iodoacetate. Each point represents mean ± S.D. of 7 observations. Composition of slices at end of leaching plotted at 0.

previously[4] to inhibit net K^+ uptake in slices reincubated in oxygenated medium without affecting the regulation of cellular volume, also had no significant effect upon the loss of tissue water from slices reincubated anaerobically. It did, however, produce a greater net loss of K^+ from the tissue. After 60-min reincubation, slices in medium containing ouabain had lost 67 mequiv K^+/kg tissue dry matter (or 46% of their K^+ content after leaching) compared with the net loss of 24 mequiv K^+/kg tissue dry matter (16%) from slices reincubated in ordinary medium alone. The K^+ content in slices reincubated with ouabain was of the same order as that found in slices reincubated in the presence of iodoacetate. This suggests, that under anaerobic conditions, 10 mM ouabain completely suppressed active K^+ uptake.

It is concluded that sufficient metabolic energy was available to the cells for initial recovery of cellular volume when slices leached anaerobically at 0.5° were reincubated anaerobically at 25°. There was apparently insufficient energy available

to allow net K^+ accumulation, though it is probable that some active K^+ uptake was occurring since the presence of ouabain in ordinary medium caused a further decrease in tissue K^+ content during reincubation. However, insufficient energy was available to maintain this initial recovery in cellular volume, and swelling subsequently occurred, though the water and Cl^- contents of the tissue reincubated anaerobically in ordinary medium or ordinary medium containing ouabain were always appreciably less than those found in slices reincubated for the same length of time in medium containing iodoacetate, in which both aerobic and anaerobic metabolism were suppressed. Finally, the suggestion that cellular volume is maintained in this tissue by a mechanism other than a linked, cardiac glycoside-sensitive, Na^+–K^+ pump is supported both by the initial recovery in cellular volume in ordinary medium in the absence of net K^+ uptake and by the similarity of the water and Cl^- contents of slices reincubated in ordinary medium and ordinary medium containing 10 mM ouabain, in spite of the very great differences in K^+ content which ouabain produced.

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