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Effect of a high Na^+ diet on cell volume and Na^+-stimulated ATPase activities of rat kidney membranes

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Proximal tubular cells from kidneys of male rats chronically fed with an isotonic NaCl solution, show a volume increase which is dependent on the length of the treatment with NaCl, when compared with control rats. Parallel to the cell volume increase, there is an increase of the ouabain-insensitive Na-ATPase activity, whereas the ouabain-sensitive Na,K-ATPase activity remains unchanged. These results establish a clear relationship between a chronic Na-diet, kidney cell volume and Na-ATPase activity.

Na-pump; Na-ATPase; High Na^+-diet; Cell volume; Na,K-ATPase; Rat kidney cortex

1. INTRODUCTION

The transepithelial re-absorption of Na^+ through the proximal tubular cell of mammalian kidney consists basically of two steps: (1) passive Na^+ entry from the tubular lumen into the cell cytoplasm (through the luminal membrane) following its electrochemical gradient, and (2) active Na^+ extrusion from the cell cytoplasm (through the basolateral plasma membrane), to the interstitial space against the electrochemical gradient. This active Na^+ extrusion occurs via two mechanisms: Na^+ extrusion in exchange for K^+ and Na^+ extrusion along with Cl^- and water [1-5]. A ouabain-sensitive, Na,K-stimulated ATPase activity, responsible for the Na^+ extrusion in exchange for K^+, and a ouabain-insensitive, Na-stimulated ATPase activity, responsible for the Na^+ extrusion along with Cl^- and water, have been demonstrated in basolateral plasma membranes of proximal tubular cells from guinea-pig and rat kidney [6-8,11].

In previous works [12,13] we have found that the transport activity catalyzed by the Na-ATPase, as well as the specific ATP hydrolysis associated with it, in rat kidney proximal tubular cells, increase in about twice their normal values when the rats are fed for 4 months with a high Na^+-diet. Since we have also shown [14,15] that the Na-ATPase activity increases when the cell volume of rat kidney cortex slices increases, we decided to study whether, or not, the effect of the high Na-diet on the Na-ATPase activity of proximal tubular cells is mediated by an increase of their volume.

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2. MATERIALS AND METHODS

Sprague-Dawley rats of 25 days old were separated into two groups: a control group, which was given tap water to drink and an experimental group, which was given a 0.9% NaCl solution to drink. Every two months, control and experimental rats were anesthetized with diethyl-ether and killed by cervical dislocation. The kidneys were removed and decapsulated. Some of the kidneys were collected in a medium containing 250 mM sucrose/20 mM Tris-HCl (pH 7.2)/0.5 mM dithiothreitol/0.2 mM phenylmethylsulfonyl fluoride, at 4°C. Outermost kidney cortex slices (which are rich in proximal tubules [16], were homogenized at 4°C in 3 vols/g of tissue of sucrose/Tris/DTT/PMSF medium, with 8 strokes at 2500 rpm in an Eberbach homogenizer with a tight-fitting Teflon pestle. The ouabain-insensitive Na-ATPase and the ouabain-sensitive Na, K-ATPase activities of the homogenates were measured as described elsewhere [17]. Cortex slices from the rest of the kidneys, in each case, were analyzed for Na^+, K^+, Cl^- and water content. The slices were dried on filter paper, weighed, desiccated for 24 h at 100°C, and reweighed. They were then shaken for 24 h in 2 ml 1 N HNO_3, and the Na^+, K^+ and Cl^- concentrations of the resulting extracts were determined [4].

3. RESULTS AND DISCUSSION

Fig. 1 shows the time course of the Na- and Na,K-ATPase activities of homogenates of kidney slices from rats drinking a 0.9% NaCl solution during 11 months (experimental rats), or drinking tap water for the same length of time (control rats). After 3-4 months of a high Na^+ diet, the Na-ATPase activity began to rise, reaching values of almost 10 times higher than those of control rats, after 11 months of high Na^+ diet. On the other hand, the Na-ATPase activity of control rats, did not change during the 11 months. The Na,K-ATPase activity, in both cases, control and experimental rats, remained unchanged during the 11 months.

Similar results can be obtained by incubating kidney slices from control rats at 0°C in a hypotonic solution,

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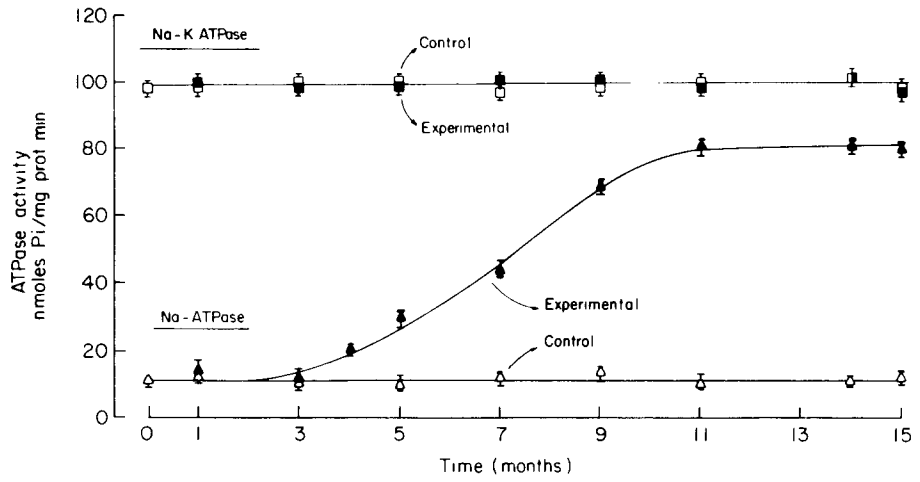

Fig. 1. Time course of the Na- and Na,K-ATPase activities of kidney slice homogenates from control (drinking tap water) and experimental (drinking 0.9% NaCl solution) rats. The values are expressed as the means ± SE for $n = 6$. The standard errors for the ATPase activities were calculated from paired data.

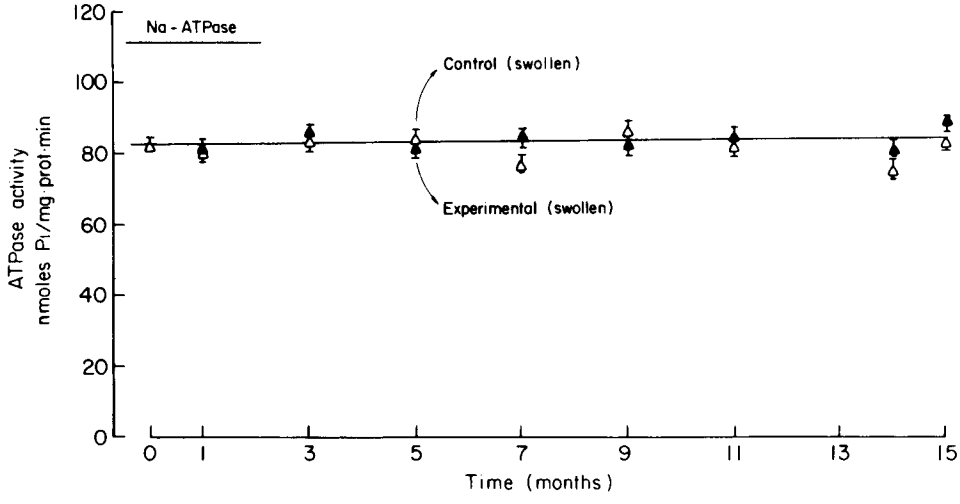

Fig. 2. Na-ATPase activity of kidney slice homogenates swollen in vitro, from control and experimental rats. The values are expressed as the means ± SE for $n = 6$. The standard errors for the ATPase activities were calculated from paired data.

in order to increase the cell volume [14]. A clear relationship between the water content of the cells (cell volume) and the activity of the Na-ATPase (in the slice homogenates) can be seen, in the sense that, for a higher cell water content, the Na-ATPase activity is higher. On the contrary, the activity of the Na,K-ATPase is not modulated by changes in the cell volume. If the effect of the high Na^+-diet on the Na-ATPase activity is produced via a similar mechanism, it could be expected then that the swelling in vitro of slices from experimental rats would increase the Na-ATPase activity only when this activity had not reached maximal values, i.e. between 0 and 11 months of a high Na^+ diet. This possibility was tested and the results are shown in Fig. 2. As expected, the Na-ATPase activity of the slices swollen in vitro was maximal and similar at any time, either for control or experimental rats.

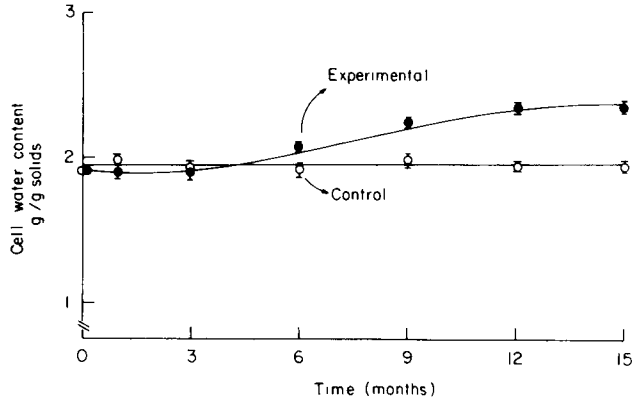

Fig. 3. Time course of the cell volume of kidney slices from control and experimental rats. The values are expressed as the means ± SE for $n = 6$. The standard errors for the ATPase activities were calculated from paired data.

If the cell volume modulates in vivo the activity of the Na-ATPase, it can be expected that the cell volume of the slices from rats on a high Na^+ diet increases with time. This can be seen in Fig. 3: the intracellular water content of the slices from experimental rats increases with time. The higher cell volume would explain then the higher Na-ATPase activity. The slices from control rats, on the other hand, maintained a constant cell volume.

The presented results can be explained according to the following hypothesis: under normal conditions, the cell volume is low and the activity of the Na-ATPase is low. The Na,K-ATPase, on the other hand, works actively to maintain the intracellular Na^+ and K^+ contents. The chronic high Na^+-diet drives the proximal tubular cells to gain Na^+, together with Cl^- and water, and hence, to increase their volume. The increased cell volume activates the Na-ATPase, stimulating in this way the extrusion of Na^+, Cl^- and water, to try to compensate for the increased cell volume.

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