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Na-pump activity in rat kidney cortex cells and its relationship with the cell volume

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The present work was undertaken to evaluate whether changes in cell water content of rat kidney cortex cells can modulate the transport activity of the ouabain-insensitive Na pump as they modulate the ouabain-insensitive Na^+-ATPase. It was found that there is a close relationship between the cell volume and activity of the Na pump, whereas Na,K-pump activity is not affected by variations in cell volume. When the cell water content is low, Na-pump activity (Na^+ transport and Na^+-ATPase activity) is minimal. Increases in cell water content produce a concomitant increase in Na-pump activity.

Na^+ pump; Na^+-ATPase; Cell volume; (Rat kidney cortex)

1. INTRODUCTION

Two types of active Na^+-transport mechanisms have been demonstrated to be present in basolateral plasma membranes of guinea-pig and rat kidney proximal tubular cells: the ouabain-sensitive Na,K pump and ouabain-insensitive Na pump [1–5]. There is also well-supported experimental evidence showing that the biochemical expression of these mechanisms is the ouabain-sensitive Na^+,K^+-ATPase and ouabain-insensitive Na^+-ATPase, respectively [2–6]. Although there are no doubts about the physiological role of the Na,K pump, it is still subject to debate whether the Na pump has any physiological role.

Recent experiments have shown that when the cell volume of rat kidney cortex slices increases, there is a concomitant increase in activity of the Na^+-ATPase (determined in homogenates prepared from these slices) [7,8]. These results have been interpreted as demonstrating that the Na^+-ATPase may play an important role in the regulation of cell volume. If this is the case, changes in the cell volume of rat kidney cortex cells should produce alterations not only in Na^+-ATPase activity, but also in Na^+-transport activity of the Na pump. The present work was undertaken to study this point.

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2. MATERIALS AND METHODS

Healthy male Sprague-Dawley rats (3 months old) were anesthetized with diethyl ether and killed by cervical dislocation. The kidneys were removed and decapsulated. Outermost kidney cortex slices (0.2–0.3 mm thick), which are known to be rich in proximal tubules [9], were prepared. The slices were preincubated for either 120 min in 5 mM K^+ medium ($5K^+$) at 0°C (swollen slices) or 30 min in $5K^+$ medium at 25°C (partially swollen slices) or 30 min in $5K^+$ + 100 mM sucrose medium at 25°C (slices with normal volume). After preincubations, some slices were homogenized for ATPase studies, while the rest were incubated at 25°C for 10 min in $5K^+$ medium, with and without the appropriate inhibitors, to study the active ion fluxes. $5K^+$ medium contained (mM): 9 Na acetate, 15 $NaHCO_3$, 2.4 NaH_2PO_4, 1.2 $MgSO_4$, 0.6 Na_2SO_4, 1 Ca gluconate, 5 glucose, 115 NaCl, 5 KCl. Active ion fluxes were measured as follows: at different incubation times, some slices were removed and assayed for intracellular content of Na^+, K^+, Cl^- and water [1]. In each case, the intracellular contents were plotted as a function of incubation time. The initial slopes of the curves were calculated and taken as the initial net fluxes of the different ions

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Table 1

Na- and Na,K-pump activities in rat kidney cortex slices with different cell water contents

Slices	Cell water content[a]	Na-pump activity		Na,K-pump activity	
		Na^+ transport	Na^+-ATPase	Na^+ transport	Na^+,K^+-ATPase
Freshly prepared	1.82 ± 0.04	0.5 ± 0.4	6 ± 1	7.7 ± 0.5	87 ± 4
Partially swollen	2.15 ± 0.03	2.9 ± 0.3	42 ± 2	8.1 ± 0.3	88 ± 5
Swollen	2.91 ± 0.04	7.4 ± 0.4	85 ± 4	7.8 ± 0.6	91 ± 7

[a] g cell water/g solids

Na^+ transport is expressed as μmol cell Na^+/g solids per min and ATPase activity as nmol P_i/mg protein per min. Values are means ± SE of 5 experiments

or water. The transport activity of the Na,K pump was calculated as the difference between the initial net fluxes measured in the presence and absence of 7 mM ouabain (the Na,K pump is 100% inhibited by this concentration of ouabain while the Na pump is unaffected [3]). The transport activity of the Na pump was calculated as the difference between the initial net fluxes measured in the presence and absence of 2 mM ethacrynic acid (under this condition, this concentration inhibits the Na pump without affecting the Na,K pump [3]). In another set of experiments, swollen slices were incubated for 20 min in $5K^+$ + sucrose medium. Slices were taken at 0 (swollen slices), 10 (partially recovered slices) and 20 min (recovered slices) of incubation and assayed for water content and active ion fluxes or homogenized for ATPase assays. Throughout incubations all media were gassed with O_2/CO_2 (95:5, v/v).

To prepare for ATPase assays, slices were homogenized in an Eberbach homogenizer using 8 strokes with a tight-fitting Teflon pestle, in 250 mM sucrose/20 mM Tris/0.5 mM dithiothreitol/0.2 M phenylmethylsulfonyl fluoride medium. The ouabain-insensitive, Na^+-ATPase and ouabain-sensitive, Na^+,K^+-ATPase activities were measured according to [6].

3. RESULTS AND DISCUSSION

The transport activity of the Na pump can be measured as the active Na^+ extrusion that is independent of K^+, insensitive to ouabain, inhibited by ethacrynic acid, and accompanied by Cl^- and water. On the other hand, the transport activity of the Na,K pump can be measured as the active Na^+ extrusion that is K^+-dependent, inhibited by ouabain, not affected by ethacrynic acid during the first few minutes of incubation, and exchanged by extracellular K^+ (reviews [1,3,8]). Table 1 lists the transport activities of the Na and Na,K pumps of slices of differing cell water contents. This table also shows the Na^+- and Na^+,K^+-ATPase activities of homogenates prepared from these slices. Note that there is a close relationship between the cell water content of slices at the beginning of incuba-

Table 2

Na- and Na,K-pump activities in rat kidney cortex slices incubated at 0°C in $5K^+$ medium for 120 min and then rewarmed at 25°C in $5K^+$ + sucrose medium

Slices	Cell water content[a]	Na-pump activity		Na,K-pump activity	
		Na^+ transport	Na^+-ATPase	Na^+ transport	Na^+,K^+-ATPase
Swollen	2.91 ± 0.04	7.1 ± 0.3	83 ± 4	7.7 ± 0.5	93 ± 7
Partially recovered	2.13 ± 0.02	3.1 ± 0.4	48 ± 3	7.6 ± 0.4	85 ± 5
Recovered	1.85 ± 0.03	0.4 ± 0.3	7 ± 2	7.9 ± 0.4	90 ± 6

[a] g cell water/g solids

Na^+ transport is expressed as μmol cell Na/g solids per min and ATPase activity as nmol P_i/mg protein per min. Values are means ± SE of 5 experiments

tion and Na-pump activity (Na^+-transport and Na^+-ATPase activity). Freshly prepared slices display the lowest Na-pump activity (Na^+-transport and Na^+-ATPase activity). Slices with slight increases in cell water content (partially swollen slices), show Na-pump activity greater than that observed with freshly prepared slices. When the cell volume of the slices is strongly increased (swollen slices), the Na-pump activity is maximal. The swelling effect on the Na pump is reversible, as shown in table 2. Slices that were previously subjected to the swelling process and then incubated to recover their cell water content (partially recovered and recovered slices) show lower Na-pump activity (Na^+-transport and Na^+-ATPase activity) than that of swollen slices. In contrast to the Na pump, the Na,K-pump activity (Na^+-transport and Na^+,K^+-ATPase activity) is not affected by changes in the cell volume of slices (tables 1,2). These results represent a clear demonstration that the Na-pump activity is modulated by changes in cell volume. The Na,K pump, on the other hand, does not appear to be modulated by changes in cell volume.

Considering the present results, a straightforward model for the physiological role of the Na and Na,K pumps in these cells can be proposed: the Na,K pump controls the intracellular Na^+ and K^+ concentrations while the Na pump functions to readjust the cell volume to normal when perturbed by transport stress. Since the proximal tubular cell is exposed to a tremendous transcellular movement of water, these cells require efficient mechanisms to readjust continuously their volume. The Na pump might be one of these mechanisms.

REFERENCES

[1] Whittembury, G. and Proverbio, F. (1970) Pflügers Arch. 316, 1–25.
[2] Marín, R., Proverbio, T. and Proverbio, F. (1985) Biochim. Biophys. Acta 814, 363–373.
[3] Marín, R., Obando, M.A., Proverbio, T. and Proverbio, F. (1986) Kidney Int. 30, 518–523.
[4] Marín, R., Proverbio, T. and Proverbio, F. (1983) Acta Cient. Venez. 34, 46–55.
[5] Marín, R., Proverbio, T. and Proverbio, F. (1985) Biochim. Biophys. Acta 817, 299–306.
[6] Proverbio, F., Proverbio, T. and Marín, R. (1986) Biochim. Biophys. Acta 858, 202–205.
[7] Proverbio, F., Duque, J.A., Proverbio, T. and Marín, R. (1988) Biochim. Biophys. Acta, in press.
[8] Proverbio, F., Marín, R. and Proverbio, T. (1988) Curr. Top. Membranes Transp., in press.
[9] Russo, M.A., Ernst, S.A., Kapoor, S.C. and Van Rossum, G.D.V. (1985) J. Membrane Biol. 85, 1–24.