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Cell volume-sensitive Na^+-ATPase activity in rat kidney cortex cell membranes

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A ouabain-insensitive, K^+-independent, sodium pump, has been demonstrated in guinea-pig and rat kidney proximal tubular cells. This pump is thought to be distinct from the ouabain-sensitive Na^+/K^+ pump. We present evidence here indicating the modulation of the biochemical expression of the Na^+ pump, i.e. the ouabain-insensitive Na^+-ATPase, by the cell volume in rat kidney proximal tubular cells. Thus, basolateral plasma membranes from swollen cells show a ouabain-insensitive Na^+-ATPase activity 10-times higher than that in membranes from control cells. If the swollen cells recover their volume, the activity decreases ten times to control values. The ouabain-sensitive Na^+/K^+-ATPase is not affected by changes in the cell volume.

Much work has been done which shows that many kinds of eukaryotic cells can regulate their volume by way of ion transport systems located in the plasma membranes which are activated by cell swelling or shrinkage [1–5]. These volume-sensitive transport systems evidently use alterations in existent ion concentration gradients to change the cell volume.

Two types of active Na^+ transport mechanisms have been demonstrated to occur in guinea-pig and rat kidney proximal tubular cells. One type is the well-known ouabain-sensitive Na^+/K^+ pump, that exchanges intracellular Na^+ for external K^+. The second type, a ouabain-insensitive Na^+ pump, extrudes Na^+ from the cells together with Cl^- and water [6–9]. Also, two types of Na^+-stimulated ATPase activities have been characterized in basolateral plasma membranes of the same proximal tubular cells; the ouabain-sensitive Na^+/K^+-ATPase and the ouabain-insensitive Na^+-ATPase [10–14]. The properties of the ouabain-insensitive Na^+-ATPase led us to propose that this represents the biochemical expression of the second type of Na^+ pump [6,10].

Healthy male Sprague-Dawley rats of 3 months of age were anesthetized with ether and killed by cervical dislocation. The kidneys were immediately removed and decapsulated. Outermost slices (0.2 to 0.3 mm thick) of the kidney cortex, which are rich in proximal tubules [15], were prepared and used for determinations of cell water content or ATPase activities in homogenates of these slices [8,9]. Unless otherwise stated, the slices used to manipulate their cell water contents were suspended in a medium containing (mM): sodium acetate, 9; $NaHCO_3$, 15; NaH_2PO_4, 2.4; $MgSO_4$, 1.2; Na_2SO_4, 0.6, calcium gluconate, 1; glucose, 5; NaCl, 120 ($0K^+$ medium). All the cell water determinations were carried out either with freshly prepared slices or with slices pre-incubated under the specified conditions to vary their cell water content. The cell volume of the slices was

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determined by measuring the tissue water content and the inulin space of the slices as already described [9]. [^{14}C]Inulin and instagel were obtained from New England Nuclear (Boston, MA).

To prepare for ATPase assays the freshly prepared or the pre-incubated slices were washed once in a medium containing (mM): sucrose, 250; Tris-HCl (pH 7.2), 20; dithiothreitol, 0.5; phenylmethylsulfonyl fluoride, 0.2 (sucrose/Tris/DTT/PMSF medium). Then, the slices were homogenized in an Eberbach homogenizer using eight strokes with a tight-fitting Teflon pestle, in 3 volumes per gram of tissue of sucrose/Tris/DTT/PMSF medium. The ouabain-insensitive, Na^+-ATPase and the ouabain-sensitive, Na^+/K^+-ATPase activities were measured according to methods previously described [12,14]. The incubation media contained (final concentration): 50 mM Tris-HCl (pH 7.2); 5 mM $MgCl_2$; 2 mM Na^+-free Tris-ATP and according to the experimental design 100 mM NaCl; 20 mM KCl; 7 mM ouabain; 2 mM furosemide. The ionic strength was kept constant with Tris-HCl (pH 7.2). 180 μl of the incubation medium were preincubated at 37 °C for 5 min and the reaction was started by adding 20 μl of the homogenates (0.4 mg protein/ml) and continued for 10 min. The reaction was stopped and the inorganic phosphate was determined with the phosphomolybdate method as previously described [12,14]. The ATPase activity was expressed as nmoles of P_i per milligram of protein per minute. Each assay point was done in quadruplicate. Protein was determined by the Coomassie blue dye binding assay [16]. Coomassie blue assay kit was purchased from Bio-Rad Laboratories (Richmond, CA). Na^+/K^+-ATPase activity was calculated as the difference between the P_i liberated in the presence of Na^+ and K^+ and that liberated in the presence of Na^+, K^+ and ouabain (7 mM). This concentration of ouabain inhibits completely the Na^+/K^+-ATPase activity of this preparation. Na^+-ATPase activity was calculated as the difference between the P_i liberated in the presence of Na^+ and ouabain (7 mM) and that liberated in the presence of Na^+, ouabain (7 mM) and furosemide (2 mM). This concentration of furosemide was shown to inhibit completely the expression of the Na^+-ATPase (measured as the difference in activity in the presence and absence of Na^+ in the incubation medium) without affecting the Mg^{2+}- or the Na^+/K^+-ATPase (see Refs. 10, 11). Similar values were obtained for the Na^+-ATPase when the assays were carried out in the presence and absence of furosemide or in the presence and absence of Na^+.

In this study, membrane vesicles were made by rewarming 100 μl aliquots (approx. 30 mg protein per ml) of control homogenates at 37 °C for 15 min. The homogenates were then assayed for the presence of vesicles, regardless of their sidedness, by measuring their ouabain-sensitive Na^+/K^+-ATPase activity before and after treatment with sodium dodecyl sulfate (SDS, Bio-Rad Laboratories, Richmond, CA), using optimal ratios of SDS/protein (for Na^+/K^+-ATPase 1 SDS per protein and for Na^+-ATPase 0.2 SDS/protein) [12,14]. This procedure allowed us to obtain homogenates with $70 \pm 5\%$ of basolateral plasma membrane vesicles (assuming 100% vesicles when there is no Na^+/K^+-ATPase activity and 0% vesicles when the Na^+/K^+-ATPase activity is maximal).

Changes of the intravesicular volume were achieved as follows: aliquots of control homogenates (100 μl), with an without pretreatment to make vesicles (see above), were incubated at 0 °C for 15 min with 400 μl of 10 mM Tris-HCl (pH 7.2) in order to increase the intravesicular volume (in the fraction with vesicles). At the end of this period, all the samples were diluted with the sucrose/Tris/DTT/PMSF solution, to give a protein concentration of about 0.2–0.4 mg/ml. The samples were then treated with SDS and assayed for Na^+- and Na^+/K^+ATPase activities as indicated above.

Table I shows the cell water content of slices incubated under different conditions, as well as the Na^+- and the Na^+/K^+-ATPase activities of their homogenates. Control slices refer to slices prepared and utilized immediately after extraction of the kidneys. Cell swelling could be induced by inhibition of the Na^+ pumps achieved by incubation of the slices for 2 h at 0 °C in an isotonic K^+-free medium. Under these conditions, the cells lose K^+ and increase their volume by gaining Na^+, Cl^- and water. Partial reversal of cell swelling occurs by activation of the Na^+ pumps by

TABLE I

CELL WATER CONTENT OF SLICES INCUBATED UNDER DIFFERENT CONDITIONS AND Na^+-STIMULATED ATPase ACTIVITIES OF THEIR HOMOGENATES

Control slices are freshly prepared slices washed once in the $0K^+$ medium (see text for details). Swollen slices are slices pre-incubated for 2 h at 0 °C in the $0K^+$ medium. Partially reversed slices are swollen slices rewarmed for 15 min at 25 °C in the same incubation medium but adding 16 mM KCl in place of an equal amount of NaCl. Reversed slices are swollen slices incubated under the same conditions to those of the partially reversed slices but with the addition of 100 mM sucrose (final concentration) to the incubation medium. The slices were used either for determinations of cell water content or homogenized for ATPase activity assays. The homogenates were pretreated with 0.2 μg SDS/μg protein for Na^+-ATPase assays and with 1 μg SDS/μg protein for Na^+/K^+-ATPase assays. Values are means ± S.E. of five experiments. The values for the Na^+- and the Na^+/K^+-ATPase activities were calculated by paired data. M1: 5 mM Mg + 100 mM Na + 7 mM ouabain; M2: M1 + 2 mM furosemide. M3: 5 mM Mg + 100 mM Na + 20 mM K; M4: M3 + 7 mM ouabain.

Slices	Cell water content (g/g solids)	ATPase activity (nmol P_i/mg protein per min)					
		incubation media				differences	
		M1	M2	M3	M4	M1 − M2 Na^+-ATPase	M3 − M4 Na^+/K^+-ATPase
Control	1.75 ± 0.06	233 ± 7	227 ± 8	159 ± 8	75 ± 4	6 ± 1	84 ± 5
Swollen	2.90 ± 0.04	314 ± 7	232 ± 6	149 ± 9	69 ± 6	82 ± 3	80 ± 7
Partially reversed	2.25 ± 0.06	270 ± 8	224 ± 7	154 ± 8	70 ± 6	46 ± 6	84 ± 6
Reversed	1.80 ± 0.07	237 ± 5	230 ± 9	154 ± 9	68 ± 5	7 ± 1	86 ± 7

incubation of the slices for 15 min at 25 °C and by adding K^+ to the medium. Under these circumstances, the cells regain K^+ and extrude Na^+, Cl^- and water, reaching a water content intermediate between those of the control and swollen slices. Essentially complete reversal of water content of swollen slices can be achieved by increasing the tonicity of the medium used for partial reversal by adding 100 mM sucrose (final concentration). Under these conditions, the cell water content reaches values similar to those of the control slices (Table I). It is evident that while the Na^+/K^+-ATPase activity is not affected by variations in cell water content, the ouabain-insensitive Na^+-ATPase is activated by increasing cell volume. The Na^+-ATPase activity of homogenates prepared from control slices is low and representing perhaps 10% of the Na^+/K^+-ATPase activity. When the cells are swollen, the Na^+-ATPase reaches values similar to those of the Na^+/K^+-ATPase. Partial reversal of cell volume produces an intermediate level of Na^+-ATPase activity. When total recovery of the cell volume is achieved (reversed slices), the Na^+-ATPase activity is diminished to control values.

While the foregoing results show that the Na^+-ATPase activity is sensitive to the relative volume the cells were at when the tissue was homogenized, it is not clear whether the Na^+-ATPase contained in homogenates prepared from control slices would also respond to variations in medium tonicity.

TABLE II

ACTIVATION OF THE Na^+-ATPase IN VESICLES OF CONTROL HOMOGENATES

Values are means ± S.E. of five experiments. The membrane vesicles were prepared by rewarming 100-μl aliquots of control homogenates (approx. 30 mg protein/ml) at 37 °C for 15 min. The preincubation in a hypotonic medium was performed by adding 400 μl of 10 mM Tris-HCl (pH 7.2) to 100 μl of control homogenates (with or without membrane vesicles). The preincubation lasted 15 min at 0 °C. The ATPase activities were measured after treatment with SDS, using optimal ratios of SDS/protein.

Presence of vesicles in homogenates	Preincubation in a hypotonic medium	ATPase activity (nmol P_i/mg protein per min)	
		Na^+-ATPase	Na^+/K^+-ATPase
No	No	8 ± 2	85 ± 6
No	Yes	10 ± 2	80 ± 8
Yes	No	9 ± 3	83 ± 7
Yes	Yes	74 ± 2	87 ± 7

Preliminary experiments indicated that only homogenates that contained vesicles showed alterations in Na^+-ATPase activity with changes in the medium tonicity. Therefore homogenates were prepared in such a way that the only differences between them were whether or not plasma membrane vesicles had been induced to form as described above. From the results presented in Table II it is apparent that the Na^+-ATPase activity is only stimulated under the conditions when vesicles were present and when the vesicles had presumably been swollen by exposure to a hypotonic medium prior the Na^+-ATPase assay. The results presented in Table II are similar to those presented in Table I showing the insensitivity of the Na^+/K^+-ATPase to these manipulations.

In summary, we have shown that changes in ouabain-insensitive Na^+-ATPase activity are associated with changes in cell volume. This, of course, is not the case with the Na^+/K^+-ATPase. The question then remains: what is the mechanism underlying the volume sensitivity of the Na^+-ATPase? If we assume that an activating or modulating factor is present, it must interact or be associated with the basolateral plasma membrane in order to exert its effect. Changes in membrane conformation or architecture that necessarily accompany changes in cell volume (shape) must underlie the effector efficacy. An important point is that it is the swelling per se that induced the stimulation in Na^+-ATPase activity, since the assays were all carried out in the same medium under isotonic conditions. The induction of Na^+-ATPase activity upon swelling is reversed with shrinking, but it is not reversed with disruption of the cells. Both manipulations of the cells exert mechanical effects, but obviously of different kinds. Nevertheless it would be worth considering what kind of mechanism could be sensitive to cell volume change, but is neither activated nor inhibited by disrupting the cells, which would cause a drastic conformational change. Current experiments are being carried out to try to clarify this point. It is clear that the physiological importance of this ouabain-insensitive Na^+ transport system has yet to be established. This ouabain-insensitive Na^+ pump may be important in the regulation of cell volume following swelling secondary to Na^+ uptake.

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