Ouabain-insensitive, Na-ATPase activity in pure suspensions of rat kidney proximal tubules

Reinaldo Marín, Daniela C. Gómez, Gloria A. Rodríguez, Teresa Proverbio and Fulgencio Proverbio

Centro de Biofisica y Bioquímica, Instituto Venezolano de Investigaciones Cientificas (IVIC), A.P. 21827, Caracas 1020A, Venezuela

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The present work was undertaken to evaluate the distribution of the Na-ATPase activity in the different components of the rat kidney cortex. Suspensions of glomeruli, proximal and distal tubules were prepared following a collagenase digestion of outermost kidney cortex slices and a separation on a Percoll gradient. It was found that the Na-ATPase activity is higher in the fraction enriched in proximal tubules. The fraction enriched in glomeruli and in distal tubules show also a Na-ATPase activity, but it is lower.

1. INTRODUCTION

Ouabain-insensitive, Na^+-stimulated ATPase (Na-ATPase), has been shown to play an important role in active Na^+ extrusion accompanied by Cl^- and water in cells from mammalian outermost kidney cortex slices (for review see [1]). Since these slices are a rich source of proximal tubules [2], the Na-ATPase has been ascribed as an active mechanism responsible for Na^+ extrusion accompanied by Cl^- and water from the proximal tubular cells to the interstitial space, and therefore associated with Na^+ reabsorption by the proximal tubule. However, in addition to proximal tubules, the slices contain a mixture of different nephron segments [3–5] which could also have the same mechanism. This fact does not allow more meaningful correlations between Na-ATPase activity and functional measurements that might be obtained with micropuncture or single tubule perfusion techniques. The study of the Na-ATPase activity in dispersions of the rat kidney cortex, purified in glomeruli, in proximal tubules and in distal tubules, is the subject of this report.

2. MATERIALS AND METHODS

Tubule suspensions were prepared following a modification of the methods of Scholer and Edelman [6] and Vinay et al. [7]. In brief, 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), were prepared and placed in 30 ml of ice-cold 5 mM K^+ medium ($5K^+$) previously gassed at room temperature with 95% O_2/5% CO_2 for 30 min. The 5 mM K^+ medium contained (mM): Na-acetate 9, $NaHCO_3$ 15, NaH_2PO_4 2.4, $MgSO_4$ 1.2, Na_2SO_4 0.6, Ca-gluconate 1, glucose 5, NaCl 115, KCl 5. The slices were washed 3 times with 30 ml of $5K^+$ medium and resuspended in 15 ml of the same medium containing 75 mg of collagenase type I-A (Sigma Chemical Co., St. Louis, MO). The slices were then gassed for 70 min with 95% O_2/5% CO_2 with gentle magnetic stirring. The slices were then removed and resuspended in 10 ml of $5K^+$ medium (collagenase-free) and passed 3–4 times through a tygon tube (25 mm i.d., 25 cm length) attached to a 20 ml syringe. This suspension was filtered to remove slice fragments, centrifuged at 30 × *g* for 30 s and resuspended in 70 ml of $5K^+$ medium. This washing procedure was repeated three times. The resulting pellet of the final washing was incubated for 5 min in the $5K^+$ + 100 mM sucrose medium and then, for another 5 min with the $5K^+$ + 5% albumin medium. The suspension was then centrifuged at 30 × *g* for 30 s and resuspended in 5 ml of $5K^+$ medium (approx. 20 mg protein/ml) and separated on a Percoll gradient (density varying between 1.05 and 1.35). This resulted in three distinct bands (F1–F3, from top to bottom). The bands were carefully removed from the Percoll gradient with a 5 ml volumetric pipette. Each fraction was then resuspended in 10 ml of $5K^+$ medium and washed three times at 30 × *g* for 30 s. After the last centrifugation, the fractions were resuspended in 5 ml of an ice-cold solution of 250 mM sucrose, 20 mM Tris-HCL (pH 7.2 at 4°C), 0.5 mM dithiothreitol (DTT), 0.2 mM phenylmethylsulphonyl fluoride (PMSF). Aliquots were removed for microscopic examination of the fractions. The fractions were then homogenized in an Eberbach homogenizer using 8 strokes with a tight-fitting Teflon pestle. The ouabain-insensitive, Na-ATPase and ouabain-sensitive, Na,K-ATPase activities were measured as described elsewhere [8]. Alkaline phosphatase activity was determined according to [9].

Correspondence address: F. Proverbio, CBB–IVIC, A.P. 21827, Caracas 1020A, Venezuela

3. RESULTS AND DISCUSSION

Microscopic examination of the fractions F1, F2 and F3 obtained with the Percoll gradient revealed that they were composed of approximately 92% glomeruli, 81% distal tubules and 98% proximal tubules, respectively. The different components were identified on the basis of their appearance and relative diameter. This distribution was confirmed (Table I) on the basis of the relative distribution of the alkaline phosphatase, which has been demonstrated to be associated preferentially with proximal tubules [6], and the Na,K-ATPase activity, that is present in a higher density in distal tubules than in proximal tubules [10]. The three fractions were tested for the ouabain-insensitive , Na-stimulated ATP-

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Table I

Na,K-stimulated ATPase and alkaline phosphatase activities in homogenates of the different fractions obtained with the Percoll separation

Fraction	Na,K-ATPase	Alkaline phosphatase
F1	57 ± 3	22 ± 2
F2	173 ± 18	34 ± 1
F3	44 ± 3	68 ± 3

ATPase and alkaline phosphatase activities are expressed as nmol P_i/mg protein per min. Values are means ± SE of 5 experiments.

Table II

Na-stimulated ATPase activity in homogenates of the different fractions obtained with the Percoll separation

Fraction	Na-ATPase activity (nmol P_i/mg prot. min)	
	Control	+ 2 mM Furosemide
F1	16 ± 1	2 ± 3
F2	32 ± 2	1 ± 2
F3	76 ± 3	3 ± 3

Values are means ± SE of 5 experiments.

ase activity. The results of this experiment are shown in Table II. Notice that the activity of the Na-ATPase is higher for fraction F3. This is the fraction enriched in proximal tubules. It can also be seen in Table II that the Na-ATPase activity of the different fractions is totally inhibited by 2 mM furosemide, which is an important characteristic of the Na-ATPase activity described in kidney cortex slice homogenates and basolateral plasma membranes isolated from these homogenates [1]. It was also tested, for the three fractions, the modulation of the Na-ATPase activity by changes in the cellular volume, which is a described characteristic of this ATPase [11]. The results are shown in Table III. The different fractions were preincubated for 15 min at 0°C, in the homogenization medium with different osmolarities (20, 300 and 400 mOsm) and then homogenized as described above. When the cell volume of the tubules is strongly increased (tubules incubated in a 20 mOsm medium), the Na-ATPase activity is maximal for the three fractions, while preincubation under hyperosmotic conditions (400 mOsm) produces, also for all the fractions, a clear diminution of the Na-ATPase activity. In contrast to the Na-ATPase activity, the Na,K-ATPase and the alkaline phosphatase activities were not affected by changes in the cell volume of the different cells (data not shown). The present results indicate clearly that the cells of the different components of the rat kidney cortex, i.e. glomeruli, distal tubules and proximal tubules, show a cell volume-sensitive, Na-stimulated ATPase activity with similar characteristics as those described for outermost kidney cortex homogenates [11]. It is also clear that the Na-ATPase activity is higher for proximal tubules than for glomeruli and distal tubules. Since the Na-ATPase is responsible for the activity of the Na-pump, which moves Na^+ along with Cl^- and water out of the proximal tubular cell, it is evident that this ATPase is strongly involved in the active N^+ reabsorption and cell volume regulation at the proximal tubular level.

Table III

Effect of the preincubation of the different fractions in media with different osmolarities, on the Na-ATPase activity

Fraction	Medium osmolarity		
	20 mOsm	300 mOsm	400 mOsm
F1	19 ± 1	15 ± 1	6 ± 2
F2	40 ± 3	30 ± 1	10 ± 2
F3	92 ± 4	72 ± 3	25 ± 2

ATPase activity is expressed as nmol P_i/mg protein per min. Values are means ± SE of 5 experiments.

REFERENCES

[1] Proverbio, F., Marín, R. and Proverbio, T. (1989) Curr. Top. Membr. Transp. 34, 105–120.
[2] Russo, M.A., Ernst, S.A., Kapoor, S.C. and van Rossum, G.D.V. (1985) J. Membr. Biol. 85, 1–24.
[3] Burg, M. and Green, N. (1976) Kidney Int. 10, 221–228.
[4] Burg, M. and Stoner, L. (1974) Fed. Proc. 33, 31–36.
[5] Giebisch, G. (1969) Nephron 6, 260–281.
[6] Scholer, D.W. and Edelman, I.S. (1979) Am. J. Physiol. 237, F350–F359.
[7] Vinay, P., Gougoux, A. and Lemieux, G. (1981) Am. J. Physiol. 241, F403–F411.
[8] Proverbio, F., Proverbio, T. and Marín, R. (1986) Biochim. Biophys. Acta 858, 202–205.
[9] Hübscher, G. and West, G.R. (1965) Nature 205, 799–900.
[10] Katz, A.I., Doucet, A. and Morel, F. (1979) Am. J. Phsyiol. 237, F114–F120.
[11] Proverbio, F., Proverbio, T., Matteo, R.G., Perrone, T.M. and Marín, R. (1988) FEBS Lett. 236, 318–320.