Biochimica et Biophysica Acta, 1064 (1991) 205–211

0005-2736/91/$03.50
ADONIS 000527369100188H

BBAMEM 75219

Characterization and localization of ouabain-insensitive Na-dependent ATPase activities along the rat nephron

Ghazi El Mernissi [1], Catherine Barlet-Bas [2], Chakir Khadouri [2], Sophie Marsy [2], Lydie Cheval [2] and Alain Doucet [2]

[1] *Laboratoire des Biomembranes, Faculté des Sciences, Université Cadi Ayyad, Marrakech (Morocco) and* [2] *Laboratoire de Physiologie Cellulaire, CNRS URA 219, Collège de France, Paris (France)*

(Received 12 November 1990)

Key words: Ouabain; ATPase activity; Sodium concentration; (Rat nephron)

Single segments of rat nephron contain two distinct ouabain-insensitive, K-independent, Na-dependent ATPase activities: a Na-stimulated ATPase and a Na-inhibited ATPase. Na-inhibited ATPase activity is found in the proximal tubule and the thick ascending limb of Henle's loop but is absent in the collecting tubule whereas Na-stimulated ATPase is exclusively located in the proximal convoluted tubule. Na-inhibited ATPase, but not Na-stimulated ATPase, is totally abolished in the presence of 100 μM Ca^{2+}. Conversely, Na-stimulated ATPase, but not Na-inhibited ATPase, is curtailed when nephron segments are preincubated at pH 7.2 whereas it is activated at pH 7.8. Finally, Na-stimulated ATPase displays an apparent K_m for Na^+ of ≈ 10 mM, and is dose-dependently inhibited by the diuretic triflocin ($IC_{50} \approx 6 \cdot 10^{-6}$ M).

Introduction

The presence of an ouabain-insensitive, Na-stimulated ATPase activity has been demonstrated in basolateral plasma membranes from guinea pig and rat kidney cortex [1,2] and from rat jejunum [3]. In the kidney cortex of guinea pig, this enzymatic activity was reported to appear after storage of basolateral membrane preparations for several days in the cold [4]. This 'ageing' procedure could be shortened by modifying the composition of the medium, in particular its pH [1] and its calcium concentration [5].

The purposes of the present study were therefore: (1) to assess the presence of this Na-stimulated ATPase in intact cells of rat kidney and to define its kinetical and pharmacological properties; (2) to define its localization along the rat nephron; and (3) to analyze the mechanism of action of pH and calcium on its activity.

For this purpose, we evaluated the effect of increasing Na concentration on ATPase activity determined by a microenzymatic assay [6] on single segments of nephron obtained from collagenase-treated rat kidneys [7].

Correspondence: A. Doucet, Laboratoire de Physiologie Cellulaire, CNRS URA 219, Collège de France, 11 Place Marcelin Berthelot, 75231 Paris cedex 5, France.

Materials and Methods

Isolation of single segments of rat nephron

Experiments were carried out on male Wistar rats weighing 150–200 g and fed the usual laboratory diet ad libitum. After anaesthesia (pentobarbital 5 mg per 100 g body wt., i.p.), the left kidney was quickly perfused by way of the abdominal aorta with 4 ml of perfusion solution (see composition below) containing 0.16% (w/v) collagenase (Worthington, CLS II, 190–250 U/mg) and 0.1% bovine serum albumin. Then, the kidney was rapidly removed and sliced in small pyramids which were incubated at 30°C for 20 min in aerated perfusion solution containing 0.1% (w/v) collagenase. Pyramids were then thoroughly rinsed in ice-cold dissection solution and stored on ice until use.

The perfusion solution was derived from Hank's solution and contained: 137 mM NaCl, 5 mM KCl, 0.8 mM $MgSO_4$, 0.33 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1 mM $MgCl_2$, 10 mM Tris-HCl and 1 mM $CaCl_2$ (pH 7.4). The dissection solution was similar to the perfusion solution except than $CaCl_2$ was 0.25 mM and that pH was adjusted to either 7.2 or 7.8, according to the experimental protocol.

Microdissection was carried out in cold dissection solution under stereomicroscopic observation (×16–25 magnification). Nephron segments were separated from

interstitial tissue with fine steel needles. The different nephron segments were characterized according to topographical and morphological criteria. Proximal convoluted tubules (PCT) were dissected in the superficial cortex, next to their attachment to the glomerulus. Proximal straight tubules, or pars recta (PR), were obtained from the outer zone of the outer medulla, above their junction with the thin descending segment of Henle's loop. Medullary and cortical segments of the thick ascending limb of Henle's loop (MTAL and CTAL) were dissected from the inner zone of the outer medulla and from the cortex, respectively. They were characterized by their diameter, their straight and bright aspect and, in the case of CTAL, by their attachment to the macula densa. Cortical and medullary collecting tubules (CCT and MCT) were isolated from the cortex, beyond the last branching, and from the outer medulla, respectively.

After dissection, nephron segments were individually transferred with ≈ 1 µl of dissection solution into the concavity of a sunken bacteriological slide coated with dried bovine serum albumin, to avoid tissue sticking to the glass. Slides were then tightly covered with another glass slide and stored on ice until the end of the microdissection. The length of each tubular segment was then determined by automatic processing of its microscopic image [8]. The length (about 0.3 to 1.5 millimeter) served as reference for enzymatic activity since the size of each sample is too small (30–300 ng of total protein per sample) to determine their protein content routinely.

Determination of ATPase activity

ATPase activity in individual nephron segments was determined with the radiochemical microassay previously developed in the laboratory for Na,K-ATPase measurement [6]. This assay is based on the quantitation of $[^{32}P]P_i$ released from $[\gamma\text{-}^{32}P]ATP$ by the ATPase present in a single segment of nephron. Briefly, each tubule was successively rinsed in distilled water to remove ions and was then rapidly frozen on dry ice in 0.2 µl of distilled water to permeabilize cell membranes and to allow the access of exogenous ATP to its intracellular sites. After thawing and addition of 1 µl of incubation solution (see composition below), incubation was carried out at 37°C for 15 min. The reaction was stopped by cooling and by adding 5 µl of 5% (w/v) cold trichloroacetic acid. Samples were then transferred into 2 ml of a suspension of 10% (w/v) activated charcoal. After mixing and centrifugation, the radioactivity of 500 µl of supernatant, which contained P_i formed from ATP, was determined by liquid scintillation.

Unless indicated otherwise, the composition of the incubation solution was calculated so as to obtain the following concentrations in the final incubate: 10 mM $MgCl_2$, 5 mM ouabain, 50 mM Tris-HCl, 10 mM Tris-ATP, tracer amounts (≈ 5 nCi/sample) of $[\gamma\text{-}^{32}P]ATP$ (New England Nuclear, Boston, U.S.A., 2–10 Ci/mmol), 50 mM or 100 mM of either choline chloride (for basal, ATPase activity) or NaCl (for total ATPase activity measurement), in the absence or the presence of 100 µM $CaCl_2$ (pH 7.4).

ATPase activity was expressed as picomoles inorganic phosphate liberated per millimeter of tubule length per hour. For each condition, ATPase activity was measured on 4–6 replicate samples. In addition, in each experiment, the non enzymatic breakdown of ATP was determined in 4–6 samples containing no tubular segment. Results are presented either as means ± S.E. of the replicates from a single animal, or as means ± S.E. of the mean values from several animals.

Statistics

Statistical significance was assessed either by Student's *t*-test for unpaired data, or, when necessary, by variance analysis according to Dunnett [9], P values less than 0.05 being considered significant.

Results

pH and calcium sensitivity of Na-dependent ATPase activities

In this first series of experiments, we evaluated the respective influence of Na, Ca and pH on ATPase activity. For this purpose, ATPase activity was determined at different Na concentrations (0 to 100 mM), in the presence or the absence of 100 µM Ca^{2+}, in nephron segments dissected either at pH 7.2 or pH 7.8 (see Methods). This study was restricted to the PCT, MTAL and CCT as representative structures of the proximal tubule, thick ascending limb and collecting tubule, respectively. Results of typical experiments are depicted in Fig. 1. For each structure, the different experimental conditions were tested in nephron segments obtained from the same rat kidney.

In CCT (bottom panels), basal ATPase activity (Na = 0) was similar in the absence (left) or presence (right) of calcium. In addition, this enzyme activity was also similar in tubules dissected at pH 7.2 (closed symbols) and at pH 7.8 (open symbols). Finally, increasing Na concentration from 0 to 100 mM in the incubation medium did not modify Mg-ATPase activity. Thus, in the CCT, the basal Mg-ATPase activity measured in the presence of 5 mM ouabain was insensitive to Ca, Na, and pH.

In MTAL (middle panels), the situation is quite different since basal ATPase activity (Na $\approx$ 0) was higher in the absence of Ca (left) than in its presence (right). Furthermore, increasing intracellular sodium concentration inhibited in a dose-dependent fashion (apparent $K_i \approx 25$ mM) the ATPase activity determined in the absence of Ca down to the activity determined in the

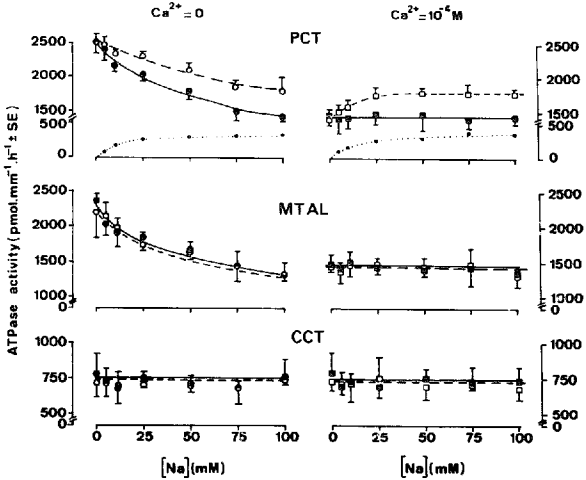

Fig. 1. Effect of increasing NaCl concentration on ouabain-insensitive ATPase activity in rat proximal convoluted tubules (PCT, top), medullary thick ascending limbs (MTAL, middle) and cortical collecting tubules (CCT, bottom). ATPase assay was performed either in the absence of added calcium (left panels) or in the presence of 10^{-4} M $CaCl_2$ (right). Nephron segments were dissected either at pH 7.2 (closed symbols) or at pH 7.8 (open symbols), whereas ATPase assay was performed at pH 7.4 in all conditions. Changes in NaCl concentrations were performed by substituting choline chloride for NaCl so as to maintain a constant osmolality. For each structure, the different conditions were tested in tubular segments microdissected from the same rat kidney. ATPase activity is expressed as pmol mm^{-1} h^{-1} and values are means ± S.E. from 4–6 replicates. Dotted curves displayed in the top panels represent the difference in ATPase activities between tubules dissected at pH 7.8 and those dissected at pH 7.2.

presence of Ca. Conversely, Na had no effect on basal ATPase measured in the presence of 10^{-4} M Ca (right). Finally, changing the pH of the dissection medium from 7.2 (closed symbols) to 7.8 (open symbols) did not modify ATPase activity whatever the concentrations of Na and Ca were. These findings suggest that the MTAL contains an ATP hydrolytic activity which is: (1) totally inhibited by 10^{-4} M calcium; (2) inhibited in a dose-dependent fashion (apparent $K_i \approx 25$ mM) by sodium; and (3) independent of the pH of the microdissection solution, at least in the 7.2–7.8 range.

In PCT (top panels) pretreated at pH 7.2 (closed symbols) results were similar to those obtained in MTAL: Increasing Na concentration did not modify ATPase activity in the presence of Ca, whereas in the absence of Ca (left) it inhibited dose-dependently (apparent $K_i \approx 25$ mM) ATPase activity. However, PCT dissected at pH 7.8 (open symbols) behaved differently than those obtained at pH 7.2. Indeed, in the presence of Ca in the incubation medium (right), ATPase activity increased dose-dependently with sodium concentration (apparent $K_{1/2} \approx 10$ mM) and reached a plateau at 50 mM Na. In the absence of Ca (left), ATPase activity still decreased as Na concentration rose, but whatever Na concentration was, ATPase activity was higher in tubules pretreated at pH 7.8 than in those pretreated at pH 7.2. Furthermore, the difference between ATPase activities determined in PCT pretreated at pH 7.8 and pH 7.2 (dotted curves) displayed the same dependency towards Na in the absence (left) and presence of calcium (right). These results are consistent with the hypothesis that, in addition to a Na- and Ca-inhibited ATPase activity similar to that observed in MTAL cells, PCT contains a Na-stimulated ATPase activity which is: (1) insensitive to Ca (up to 10^{-4} M); (2) inhibited in tubules pretreated at pH 7.2 and activated after pretreatment at pH 7.8; and (3) is half-maximally stimulated by 10 mM Na.

Altogether, these results suggest the presence of two distinct Na-dependent ATPase activities in rat nephron

segments: a Na-inhibited ATPase present in PCT, MTAL but not CCT, and a Na-activated ATPase present in PCT.

Localization of Na-activated and Na-inhibited ATPase activities along the rat nephron

To localize more precisely these two ATPases along the rat nephron, we determined their maximal activities in the successive segments of nephron using assay conditions which allow their segregation. Thus, Na-activated ATPase activity was determined in the presence of 10^{-4} M calcium (which totally inhibits Na-inhibited activity), in tubules pretreated at pH 7.8 (which demasks Na-activated activity), and was taken as the difference between the activities measured in the presence of 50 mM Na (which allows maximal stimulation) or in the absence of Na. Conversely, Na-inhibited ATPase activity was determined in the absence of calcium on tubules pretreated at pH 7.2 (which inhibits Na-activated ATPase), and was taken as the difference between the activities measured in the absence of Na (maximal stimulation) or in the presence of 100 mM Na (maximal inhibition).

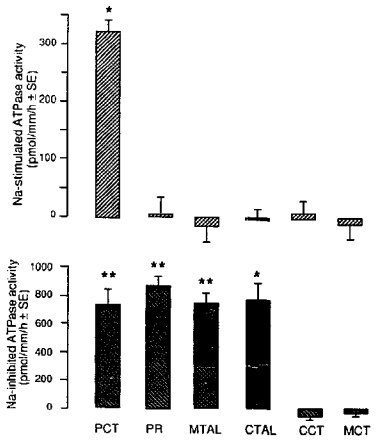

Fig. 2. Distribution of Na-stimulated, ouabain-insensitive ATPase (top) and Na-inhibited, ouabain-insensitive ATPase (bottom) along the rat nephron. PCT, proximal convoluted tubule; PR, pars recta; MTAL, medullary thick ascending limb; CTAL, cortical thick ascending limb; CCT, cortical collecting tubule; MCT, medullary collecting tubule. Na-stimulated ATPase activity was taken as the difference between ATPase activities measured in the presence of 50 mM NaCl and in the absence of Na, on nephron segments dissected at pH 7.8. Na-inhibited ATPase activity was taken as the difference between ATPase activities measured in the absence of NaCl and in the presence of 100 mM NaCl, on nephron segments dissected at pH 7.2. Besides NaCl (or choline chloride), the assay medium contained 5 mM ouabain, 10 mM $MgCl_2$, 10 mM Tris-HCl, 10 mM ATP and either 0.1 mM $CaCl_2$ (Na-stimulated ATPase) or no $CaCl_2$ (Na-inhibited ATPase). Data are means ± S.E. from five (top) or four (bottom) rats. Values statistically different from zero were determined by Student's *t*-test; *, $P < 0.01$; **, $P < 0.005$.

Results in Fig. 2 clearly indicate that Na-activated ATPase was present in the PCT exclusively, as its activity was undetectable in both PR, MTAL, CTAL, CCT and MCT. In PCT, it averaged 325 ± 20 pmol $mm^{-1} h^{-1}$ (mean ± S.E., $n = 5$), that is around 15% of Na,K-ATPase activity present in that same nephron segment [10].

Conversely, Na-inhibited ATPase activity was found along the whole proximal tubule (PCT and PR) and thick ascending limb (MTAL and CTAL), but was undetectable in the cortical and medullary collecting tubule (CCT and MCT). Na-inhibited ATPase activity was almost constant along the proximal tubule and thick ascending limb (750–850 pmol $mm^{-1} h^{-1}$) and represented 20–30% (PCT, MTAL, CTAL) to 100% (PR) of Na,K-ATPase activity determined in the corresponding segments of the rat nephron [10]. Finally, in PCT, Na-inhibited ATPase activity was 2-fold higher than Na-activated ATPase activity (731 ± 111, $n = 4$, vs. 325 ± 20, $n = 5$, $P < 0.005$).

Kinetical and pharmacological properties of Na-activated ATPase

The last part of this study was designed to further characterize the kinetical and pharmacological properties of the Na-activated ATPase activity present in rat PCT.

TABLE I

Ionic selectivity of sodium-activated ATPase in proximal convoluted tubule

Values are means ± S.E. of 5–7 replicates. ATPase activity of rat proximal convoluted tubules (PCT) pretreated at pH 7.8 was measured under basal conditions (Base) or after addition of 50 mM of either NaCl, LiCl, KCl, RbCl, NH_4Cl, CH_3COONa, $CH_3COCOONa$ or 25 mM of Na_2SO_4. Δ is the difference between each value and the basal activity. Values statistically different from basal activity were determined by variance analysis.

	ATPase activity	Δ	P vs. basal
Base	731 ± 94		
+ NaCl	1106 ± 53	375 ± 45	< 0.001
+ LiCl	970 ± 36	239 ± 45	< 0.001
+ KCl	724 ± 44	− 7 ± 40	n.s.
+ RbCl	729 ± 68	− 2 ± 49	n.s.
+ NH_4Cl	711 ± 86	− 20 ± 52	n.s.
+ Na-acetate	1070 ± 74	339 ± 48	< 0.001
+ Na-pyruvate	1074 ± 70	343 ± 52	< 0.001
+ Na_2SO_4	1085 ± 74	354 ± 48	< 0.001

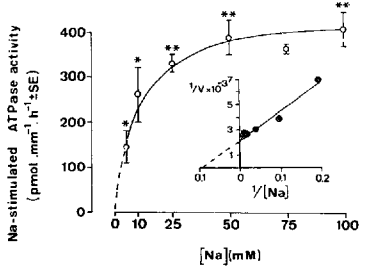

Fig. 3. Sodium dependency of Na-activated, ouabain-insensitive ATPase in rat proximal convoluted tubule. PCT were dissected at pH 7.8 and ATPase assay was performed in the presence of 10^{-4} M $CaCl_2$. Na-activated ATPase activity was taken as the difference between the activity measured at a given NaCl concentration and that measured in the absence of Na. Data are means ± S.E. from three animals. Values statistically different from zero were determined by variance analysis: *, $P < 0.05$; **, $P < 0.001$. Inset: Lineweaver and Burk plot indicates a K_m value of 10.3 mM and a V_{max} value of 461 pmol $mm^{-1} h^{-1}$.

First, we evaluated the ionic specificity of ATPase activity. Data in Table I indicate that Na-stimulated ATPase was insensitive to chloride since: (1) NaCl induced the same stimulation of the basal ATPase activity as Na-acetate, Na-pyruvate and Na-sulfate did; and (2) neither KCl, RbCl nor NH_4Cl stimulated basal ATPase. They also indicate that this ATPase activity is quite specific for Na since only Li could partially mimick its stimulatory action whereas other cations such as K^+, Rb^+ or NH_4^+ had no effect.

Fig. 3 shows the sodium dependency of the rate of ATP hydrolysis by Na-activated ATPase in rat PCT preincubated at pH 7.8. Na-activated ATPase follows a Michaelis-Menten kinetics which, after linearization in a Lineweaver-Burk plot indicates a V_{max} value of 461 pmol $mm^{-1} h^{-1}$ and a K_m value of 10.3 mM.

Na-stimulated ATPase activity is insensitive to ouabain since it was determined in the presence of 5 mM ouabain, which confirms that it is different from Na,K-ATPase. Conversely, Na-stimulated ATPase in PCT was inhibited in a dose-dependent fashion (Fig. 4) by the diuretic triflocin [11], as previously described for basolateral membranes [12,13]: maximal and half-maximal inhibition of Na-stimulated ATPase were observed in the presence of 10^{-3} M and $6 \cdot 10^{-6}$M triflocin, respectively.

Discussion

In 1975, Proverbio and colleagues [4] reported that 100 mM NaCl partially inhibited Mg-dependent ATPase activity from microsomal fractions of the cortex of guinea pig kidney. However, when the microsomal preparations were stored at 4°C for 10–15 days (a procedure they named 'ageing') Mg-dependent ATPase showed stimulation by Na^+ instead of inhibition [4]. This Na-stimulated ATPase was clearly different from Na,K-ATPase since it was independent of K^+ and insensitive to ouabain [4]. Similar results were latter reported in basolateral membranes from rat kidney cortex [2].

Our hypothesis has been that such results could be explained by the presence of two distinct Na-dependent ATPase activities in the rat kidney: a Na-inhibited ATPase activity and a Na-stimulated ATPase activity, the former being labile and disappearing in the course of ageing. Thus, in aged preparations, only the Na-activated ATPase would be detected whereas in fresh preparations, the observed effect of Na^+ would result from the algebraic sum of Na^+ induced stimulations and inhibitions. If this hypothesis were correct, the fact that Na^+ inhibits Mg-dependent ATPase activity in fresh preparations would also suggest that, in absolute values, the Na-inhibited ATPase activity is greater than the Na-activated ATPase.

Our purpose has therefore been to verify this hypothesis by localizing these two ATPase activities at the level of single segments of rat nephron, and by characterizing some of their properties. However, ageing is impossible in whole cells, because of the presence of endogenous proteolytic enzymes. Since Proverbio and colleagues also reported [2,3] that ageing could be replaced by changing the assay conditions (in particular by altering medium pH and Ca^{2+} concentration) we evaluated the actions of these parameters on Na-depen-

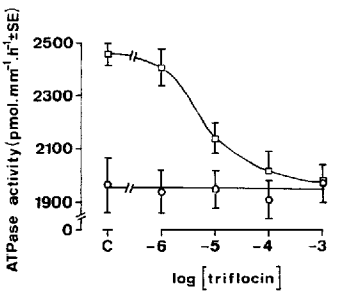

Fig. 4. Inhibition by triflocin of Na-activated ATPase in rat proximal convoluted tubules dissected at pH 7.8 and assayed in the presence of 10^{-4} M $CaCl_2$. ATPase activity was measured either in the presence of 50 mM NaCl (□) or in its absence (○).

dent ATPase activities in the successive segments of the rat nephron.

Our results indicate that the proximal convoluted tubule (the major origin of kidney cortex membrane preparations) displays the two Na-dependent ATPase activities: a Na-inhibited ATPase activity which is inhibited by low concentrations of Ca^{2+} and a Na-stimulated ATPase insensitive to these concentrations of Ca^{2+} but inhibited at pH 7.2 (Fig. 1, top). Furthermore, and as predicted, the Na-inhibited ATPase activity is higher than the Na-activated one (compare PCT values in Fig. 2).

That these two ATPase activities reflect the presence of two distinct molecular complexes is attested first by their relative sensitivity towards Ca^{2+} and pH and, second, by their different localization along the nephron. Thus, Na-stimulated ATPase activity is exclusively found in the proximal convoluted tubule (Fig. 2, top) whereas Na-inhibited ATPase activity is located along the whole proximal tubule but also in the thick ascending limb (Fig. 2, bottom).

The functional significance of these two ATPase activities is not known. It is likely, however, that due to its high sensitivity to Ca^{2+}, Na-inhibited ATPase activity is not functional under normal physiological conditions. Concerning Na-stimulated ATPase activity, it is important to note that it is different from any other ATPase activity previously found in nephron segments. Indeed, it is insensitive to ouabain, K^+ (Table I) and Ca^{2+} (Fig. 1), which distinguishes it from Na,K-ATPase, K-ATPase and Ca-ATPase, respectively. Furthermore, it is independent of anions (Table I) which distinguishes it from anion-stimulated ATPase [14]. Finaiiy, its specific localization in the proximal convoluted tubule is unique among other ATPases such as Na,K-ATPase [10], Ca-ATPases [15,16], K-ATPase [17], anion-ATPase [14] and H-ATPase [18].

The kinetical properties of tubular Na-stimulated ATPase activity are quite similar to those previously described in basolateral membranes of kidney and jejunum. Thus, its K_m of 10.3 mM for Na (Fig. 3) is close to those previously reported for kidney [4,13] and jejunum enzyme [3]. Like in kidney membranes [13], but unlike the jejunum enzyme [3], Na-stimulated ATPase activity in PCT is also stimulated by Li^+, although to a lower extent (Table I). Finally, as previously reported by Del Castillo et al. [13], Na-stimulated ATPase activity is dose-dependently inhibited by the diuretic triflocin in the proximal convoluted tubule (Fig. 4).

In rat proximal convoluted tubule, active reabsorption of Na^+ measured by stop-flow microperfusion averaged $1.7 \cdot 10^{-11}$ mol $cm^{-1} s^{-1}$ [19], that is about 6000 pequiv. $mm^{-1} h^{-1}$. In that same segment, the activity of Na,K-ATPase (2400 pmol of ATP $mm^{-1} h^{-1}$, [10]) could account for an active reabsorption of Na^+ of 7200 pequiv. $mm^{-1} h^{-1}$ assuming a $3Na^+/1ATP$ stoichiometry and a 100% efficiency. However, due to the low intracellular Na concentration, Na,K-ATPase rather works around 50% its V_{max} under physiological conditions, and can therefore account for the active reabsorption of no more Na^+ than 3600 pequiv. $mm^{-1} h^{-1}$, i.e. 60% of the flux actually measured. Even if we assume that some Na^+ can be actively reabsorbed through the basolateral Na^+/HCO_3^- symport [20] primarily energized by Na,K-ATPase, it appears that the activity of the pump is not sufficient to account for the whole active Na^+ reabsorption in the proximal tubule. Na-stimulated ATPase activity could be an alternative pathway for proximal reabsorption of Na^+.

Indeed, the following findings may suggest that ouabain-insensitive Na-activated ATPase activity works as a Na^+ pump involved in Na^+ reabsorption in the proximal convoluted tubule: (1) this ATPase activity is associated with active Na^+ uptake in basolateral membrane vesicles from rat kidney [21]; (2) triflocin inhibits Na^+ reabsorption [11] as well as Na-stimulated ATPase activity in the proximal tubule (Fig. 4); (3) Na-stimulated ATPase activity is exclusively found in the proximal convoluted tubule (Fig. 2) where a deficit of Na,K-ATPase exists, as compared to active Na^+ reabsorption. Further studies will be necessary to confirm this hypothesis; in particular, it would be important to determine the Na/ATP stoichiometry of this system.

Acknowledgments

The authors are grateful to L. Bonnet-Lericque for her help during the preparation of the manuscript. Triflocin was kindly provided by T.M. Romano from Lederle International Division. This work was supported in part with a grant for international cooperation from the Centre National de Coordination et de Planification de la Recherche Scientifique et Technique (Morocco) and the Centre National de la Recherche Scientifique (France).

References

1 Proverbio, F. and Del Castillo, J.R. (1981) Biochim. Biophys. Acta 646, 99–108.
2 Marín, R., Proverbio, T. and Proverbio, F. (1985) Biochim. Biophys. Acta 817, 299–306.
3 Orsenigo, M.N., Tosco, M., Esposito, G. and Faelli, A. (1988) Int. J. Biochem. 20, 1411–1415.
4 Proverbio, F., Condrescu-Guidi, M. and Whittembury, G. (1975) Biochim. Biophys. Acta 394, 281–292.
5 Proverbio, F., Proverbio, T. and Marín, R. (1982) Biochim. Biophys. Acta 688, 757–763.
6 Doucet, A., Katz, A.I. and Morel, F. (1979) Am. J. Physiol. 237, F105–F113.
7 Morel, F., Charbardès, D. and Imbert-Teboul, M. (1978) Methods Pharmacol. 4B, 297–323.
8 Khadouri, C., Marsy, S., Barlet-Bas, C. and Doucet, A. (1987) Am. J. Physiol. 253, F495–F499.

9 Dunnett, C.W. (1955) J. Am. Stat. Assoc. 50, 1096–1121.
10 Katz, A.I., Doucet, A. and Morel, F. (1979) Am. J. Physiol. 237, F114–F120.
11 Gussin, R.Z., Cummings, J.R., Stokey, E.H. and Ronsberg, M.A. (1969) J. Pharmacol. Exp. Ther. 167, 194–206.
12 Pérez-Gonzalez de la Mantia, M., Proverbio, F. and Whittembury, G. (1980) Curr. Top. Membr. Transp. 13, 315–335.
13 Del Castillo, J.R., Marin, R., Proverbio, T. and Proverbio, F. (1982) Biochim. Biophys. Acta 692, 61–68.
14 Ben Abdelkhalek, M., Barlet C. and Doucet, A. (1986) J. Membr. Biol. 89, 225–240.
15 Katz, A.I. and Doucet, A. (1980) Int. J. Biochem. 12, 125–129.
16 Doucet, A. and Katz, A.I. (1982) Am. J. Physiol. 242, F346–F352.
17 Doucet, A. and Marsy, S. (1987) Am. J. Physiol. 253, F418–F423.
18 Ait-Mohamed, A.K., Marsy, S., Barlet, C., Khadouri, C. and Doucet, A. (1986) J. Biol. Chem. 261, 12526–12533.
19 Frömter, E. (1974) in Kidney and Urinary Tract Physiology (Thurau, K., ed.), pp.1–38, Butterworths, London.
20 Yoshitomi, K., Burckhardt, B.C. and Frömter, E. (1985) Pflügers Arch. 405, 360–366.
21 Marin, R., Proverbio, T. and Proverbio, F. (1985) Biochim. Biophys. Acta 814: 363–373.