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PARTIAL CHARACTERIZATION OF THE OUABAIN-INSENSITIVE, Na^+-STIMULATED ATPase ACTIVITY OF KIDNEY BASAL-LATERAL PLASMA MEMBRANES

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The present paper characterizes the Na^+-stimulated ATPase activity present in basal-lateral plasma membranes from guinea-pig kidney proximal tubular cells. These characteristics are compared with those of the ($Na^+ + K^+$)-stimulated ATPase activity, and they are: (A) Na^+-ATPase activity: (1) requires Mg^{2+}; (2) may be activated by μmolar quantities of Ca^{2+}; (3) optimal ratio Mg:ATP = 5:1–2 and K_a for Mg:ATP = 3: 0.60 mM; (4) K_a for Na^+: 8 mM; (5) does not require K^+; (6) is only stimulated by Na^+ and Li^+ (in a lower extent); (7) is similarly stimulated by the Na^+ salt of different anions; (8) hydrolyzes only ATP; (9) optimal temperature: 47°C; (10) optimal pH: 6.9; (11) is ouabain insensitive; (12) is totally inhibited by 1.5 mM ethacrynic acid, 2 mM furosemide and 0.75 mM triflocin. (B) ($Na^+ + K^+$)-ATPase activity: (1) also requires Mg^{2+}; (2) is inhibited by Ca^{2+}; (3) optimal ratio Mg:ATP = 1.25:1 and K_a for Mg:ATP = 0.50: 0.40 mM; (4) K_a for Na^+: 14 mM (data not shown); (5) needs K^+ together with Na^+; (6) K^+ may be substituted by: $Rb^+ > NH_4^+ > Cs^+$; (7) is anion insensitive; (8) hydrolyzes mostly ATP and to a lesser extent GTP, ITP, UTP, ADP, CTP; (9) optimal temperature: 52°C; (10) optimal pH: 7.2; (11) 100% inhibited by 1 mM ouabain; (12) 63% inhibited by 1.5 mM ethacrynic acid, 10% inhibited by 2 mM furosemide and insensitive to 0.75 mM triflocin.

Introduction

Different kinds of ATPase activities have been demonstrated in kidney cell preparations: (1) a basal, Mg^{2+}-dependent activity; (2) a Ca^{2+}-dependent activity; (3) a Mg^{2+}-dependent, $Na^+ + K^+$-stimulated activity and finally (4) a Mg^{2+}-dependent, Na^+-stimulated activity [1–6]. The Mg^{2+}-dependent, Na^+-stimulated ATPase activity, was originally demonstrated in aged microsomal fractions from guinea-pig kidney cortex [4] and from rat whole kidney [7]. Recently [10], an Na^+-stimulated ATPase activity has been shown in basal-lateral plasma membranes from guinea-pig kidney proximal tubular cells. This activity has characteristics that fit into some of the conditions that should be fullfilled by the energy source of the system responsible for the active extrusion of Na^+, accompanied by Cl^- and water, described in guinea-pig kidney cortex slices [3,8,9].

It is still subject to debate whether this second Na^+ pump does exist separately from the classical $Na^+ + K^+$ pump or whether the ouabain-resistant Na^+ extrusion can be accomplished by the classical Na^+ pump or by other ion-transport or exchange mechanisms as could be derived from Na^+-Ca^{2+} exchange, secondary to the function of a Ca^{2+} pump [11–13].

To help ascertain these different mechanisms, we thought it important to study and compare the

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characteristics of the Na^+-stimulated, Mg^{2+}-dependent ATPase activity, with those of the $Na^+ + K^+$-stimulated, Mg^{2+}-dependent activity in kidney cortex basal-lateral plasma membranes. The results of this study are described here.

Materials and Methods

Preparation of fractions enriched in basal-lateral plasma membranes

The outermost slices of kidney cortex (rich in proximal tubules) of healthy adult guinea-pig were obtained as described [14]. Each gram of tissue was immediately homogenized at 4°C, with 8 strokes, at 2500 rpm in an Eberbach homogenizer with a teflon pestle, in three volumes of a solution of 0.25 M sucrose, 20 mM Tris-HCl (pH 7.2) and 2 mM EDTA. The plasma membrane-enriched fractions were prepared according to the described method [10]. The final pellet was resuspended in the sucrose, Tris, EDTA medium, either at pH 7.2 or 7.8, frozen and kept at −20°C. Before the ATPase assays, the membranes were treated with deoxycholate and EDTA for 30 min, following the method of Jørgensen and Skou [15].

Assay of the ATPase activity

The method already described was followed [10]. Briefly, required amounts of the membrane suspensions were preincubated for 5 min at the prescribed incubation temperature (usually 37°C) and then incubated, for 15 min, in the presence of (final concentrations): 50–150 mM Tris-HCl (pH 7), 5 mM $MgCl_2$, and when required, 100 mM NaCl, 20 mM KCl or isosmolar quantities of other salts. According to the experimental design, 25 μM Ca^{2+}, 1 mM ouabain, 1.5 mM ethacrynic acid or the required amount of other agents were added to the incubation medium. The final volume was 1 ml. The reaction was started by adding the ATP (2 mM final concentration) and stopped by adding to the incubation tubes 1 ml of ice-cold 6% $HClO_4$. The samples were chilled, centrifuged and the liberated orthophosphate (P_i) was determined in the deproteinized solution [16]. All samples were run in triplicate. The protein content of the original suspensions was measured by the Folin method [17]. The ATPase activity is expressed as nmol of P_i produced per mg of protein per min, after subtraction of a blank run in parallel without the membrane suspension, which was added after the $HClO_4$. The Mg^{2+}-dependent ATPase activity refers to the liberation of P_i in the presence of Mg^{2+}. The difference in activity of ($Mg^{2+} + Na^+$)-ATPase minus Mg^{2+}-ATPase will be referred to as Na^+-ATPase activity. The difference of ($Mg^{2+} + Na^+ + K^+$)-ATPase minus ($Mg^{2+} + Na^+$)-ATPase will be referred to as ($Na^+ + K^+$)-ATPase. The Ca^{2+}-ATPase activity was assayed as already described for this tissue [6,18], i.e. in the presence of 5 mM Ca^{2+} and without Mg^{2+} in the incubation medium.

Chemicals

ATP, ADP, AMP, and other nucleotides, ouabain (strophanthin-G), EDTA, and deoxycholate, were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Triflocin, furosemide and ethacrynic acid, were generously provided by Cyanamid International (Pearl River, NY); by Medicamentos York S.A. (Caracas) and by Merck, Sharp and Dohme (Rahway, NJ), respectively.

Results

Except for the determination of the ($Na^+ + K^+$)-ATPase activity, all other assays were done in the presence of 1 mM ouabain. The Na^+-stimulated ATPase activity was determined in the presence of 25 μM Ca^{2+} in the incubation medium [10].

Preliminary experiments were carried out to ascertain the linearity of the studied systems as a function of the incubation time and the quantity of protein in the incubation medium. The Mg^{2+}-, ($Mg^{2+} + Na^+$)- and ($Mg^{2+} + Na^+ + K^+$)-ATPase activities are linear, at least for the first 15 min of incubation. This incubation time was chosen for our experiments. The activity of the three systems is linear within a very high range of proteins in the incubation medium (20 μg–150 μg/ml). Usually the protein content of the incubation tubes was 40–60 μg/ml). To avoid vesicle formation, the membranes were treated with 0.1% deoxycholate and 2 mM EDTA at pH 7.8, according to the method of Jørgensen and Skou [15], before the assays.

Effect of isosmolar quantities of cations and anions on the Na^+-stimulated ATPase activity

The enzyme was incubated in the presence of 100 mM of the chloride salt of different monovalent cations. The results are shown in Table I: as already shown for the microsomal fractions [4], from all the tested cations, only Li^+, besides Na^+, significantly stimulated the Mg^{2+}-dependent ATPase activity. K^+, Rb^+ and NH_4^+ were not stimulatory and Cs^+ and choline$^+$ were slightly inhibitory.

The optimal Na^+ concentration, as shown in Fig. 1, is around 50 mM and the apparent K_a, calculated by the Woolf variation of the Lineweaver-Burk equation with the data of Fig. 1, as shown in the insert, is around 8 mM.

On the other hand, we can see in Table II, that the addition of 100 mM of the Na^+ salt of different anions to the incubation medium, was always stimulatory. Thus Na^+ stimulation was, for all the tested anions, independent of the anion used. This observation is similar to that made with the microsomal fractions [4].

Effect of the temperature and pH of the incubation medium

Fig. 2 shows the optimal and the 50% stimulation and inhibition temperatures of the incubation medium for the three ATPase activities. It can be seen that while the Mg^{2+}- and the $(Na^+ + K^+)$-ATPase activities reach their optimum at 52°C, the Na^+ activity, is strongly inhibited at this tem-

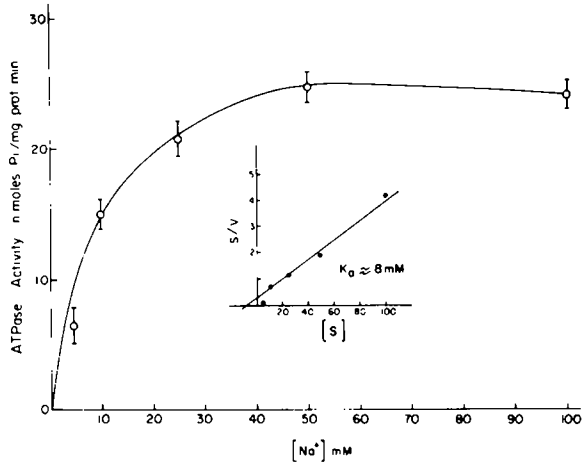

Fig. 1. Effect of increasing Na^+ concentration (as NaCl) on the Mg^{2+}-dependent ATPase activity of basal-lateral plasma membranes. Assays performed in the presence of 1 mM ouabain. Mg^{2+} concentration, 5 mM. In this and in the following figures, the values are expressed as the mean ± S.E. In the present figure, $n = 10$. The apparent K_a was calculated by means of the Woolf derivative of the Lineweaver-Burk transformation of the Michaelis-Menten equation of the form

$$S/V = K_a/V_{max} + S/V_{max}$$

where $K_a = -S$ on the S axis (see insert).

TABLE I

EFFECT OF 100 mM CHLORIDE SALT OF DIFFERENT CATIONS ON THE Mg^{2+}-DEPENDENT ATPase ACTIVITY OF BASAL-LATERAL PLASMA MEMBRANES

Assays performed in the presence of 1 mM ouabain. Mg^{2+} concentration, 5 mM. In this and in the following tables, the values are expressed as the mean ± S.E. In the present table, $n = 10$. The ATPase activity is expressed in nmol P_i liberated per min per mg protein. ns., not significant.

Added cation	ATPase activity	Δ cation	P
Tris	254 ± 2	-	
Na^+	285 ± 3	+31 ± 4	<0.001
Li^+	276 ± 3	+22 ± 4	<0.001
K^+	247 ± 3	−7 ± 4	n.s.
Rb^+	251 ± 4	−3 ± 4	n.s.
NH_4^+	253 ± 3	−1 ± 4	n.s.
Cs^+	236 ± 5	−18 ± 5	<0.01
Choline$^+$	212 ± 4	−42 ± 4	<0.001

TABLE II

EFFECT OF 100 mM SODIUM SALT OF DIFFERENT ANIONS ON THE Mg^{2+}-DEPENDENT ATPase ACTIVITY OF BASAL-LATERAL PLASMA MEMBRANES

Assays performed in the presence of 1 mM ouabain. Mg^{2+} concentration, 5 mM ($n = 4$). The ATPase activity is expressed in nmol P_i liberated per min per mg protein.

Incubation medium	ATPase activity	Δ Na^+	P
Mg^{2+}	264 ± 3	-	
Mg^{2+} + NaCl	293 ± 3	+29 ± 4	<0.001
Mg^{2+} + NaBr	291 ± 2	+27 ± 4	<0.001
Mg^{2+} + $NaHCO_3$	294 ± 4	+30 ± 5	<0.001
Mg^{2+} + Na_2SO_4	294 ± 3	+30 ± 4	<0.001
Mg^{2+} + sodium lactate	295 ± 3	+31 ± 4	<0.001
Mg^{2+} + sodium acetate	293 ± 3	+29 ± 4	<0.001

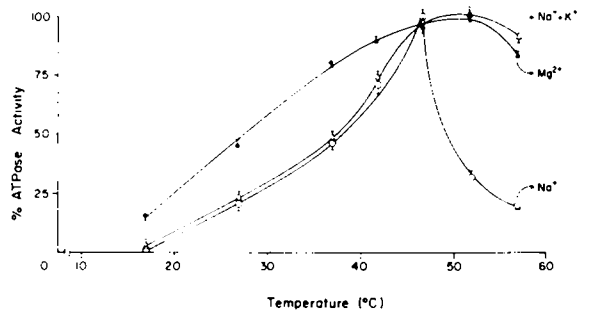

Fig. 2. Effect of the incubation temperature on the Mg^{2+}-, Na^+- and ($Na^+ + K^+$)-ATPase activities of basal-lateral plasma membranes. Mg^{2+} concentration, 5 mM; Na^+, 100 mM; K^+, 20 mM. Except for the ($Na^+ + K^+$)-ATPase activity, all other assays were carried out in the presence of 1 mM ouabain. (The pH of the incubation media was adjusted to 7 for any given temperature.) $n = 8$.

perature, having its maximum at 47°C. The half stimulation and inhibition temperatures are 28 and over 57°C for the Mg^{2+}-ATPase activity; 38 and 49°C for the Na^+-stimulated activity and 37°C and over 57°C for the $Na^+ + K^+$-stimulated activity.

The effect of the pH of the incubation medium is shown in Fig. 3: the optimal pH is 8.5 for the Mg^{2+} activity; 6.9 for the Na^+-stimulated activity and 7.2 for the $Na^+ + K^+$-stimulated activity. The half activity pH values are: 6.75 and higher than 9 for the Mg^{2+}; 6.55 and 7.55 for the Na^+; and 5.65 and 8.1 for the ($Na^+ + K^+$)-ATPase activity.

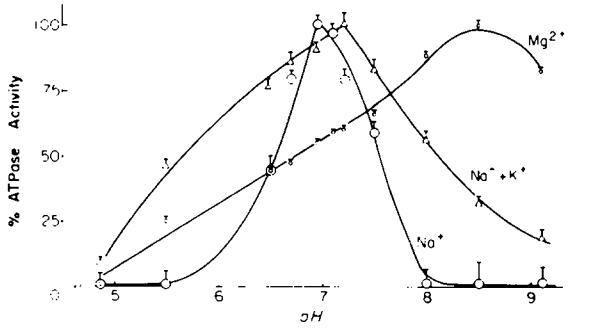

Fig. 3. Effect of the pH of the incubation medium on the Mg^{2+}-, Na^+- and ($Na^+ + K^+$)-ATPase activities of basal-lateral plasma membranes. Mg^{2+} concentration, 5 mM; Na^+, 100 mM; K^+, 20 mM. Except for the ($Na^+ + K^+$)-ATPase activity, all other assays were carried out in the presence of 1 mM ouabain. (The pH values of the incubation media were adjusted at 37°C, the temperature at which the assays were carried out.) $n = 8$.

Quality and quantity of substrate

The capacity of the Mg^{2+}-, Na^+- or ($Na^+ + K^+$)-ATPases to hydrolyze other substrates, is compared in Table III: while the Mg^{2+}-ATPase system is able to hydrolyze, to a higher or lesser degree, all the tested substrates, including different nucleotides as well as ATP derivatives, the ($Na^+ + K^+$)-ATPase hydrolyzes almost exclusively ATP and the Na^+-ATPase hydrolyzes only ATP.

The optimal Mg:ATP ratios for the three Mg^{2+}-dependent ATPase activities were determined. Previous experiments have shown that the higher values for each of the three studied ATPase systems are obtained at 5 mM Mg^{2+}, at concentrations of 4–6 mM ATP for the Mg^{2+}-ATPase; 1–4 mM ATP for the Na^+-ATPase and 3–6 mM ATP for the ($Na^+ + K^+$)-ATPase. Following these results, the effect of varying the Mg^{2+} as well as the ATP concentrations at fixed ratios of Mg:ATP of 1.25:1 and 5:1 was studied. The results are shown in Fig. 4.

The optimal values for the Mg^{2+}-ATPase are obtained at the Mg:ATP ratio of 1.25:1 at 2.5 mM Mg^{2+}. The apparent K_a of activation, for Mg^{2+} and ATP, at this ratio, calculated by the Woolf variation of the Lineweaver-Burk equation is 0.40 and 0.32 mM, respectively. At the higher ratio, 5:1 (Mg:ATP), the apparent K_a for ATP is 0.32 mM, while the K_a for Mg^{2+} is increased by a factor of 4.

The optimal values for the $Na^+ + K^+$-ATPase are obtained at the Mg:ATP ratio of 1.25:1 at 5 mM Mg^{2+}. The K_a for Mg^{2+} and ATP is 0.50 and 0.40 mM, respectively. At the higher ratio, 5:1 (Mg:ATP), the apparent K_a for ATP is 0.40 while the K_a for Mg^{2+} is increased by a factor of 4.

The optimal values for the Na^+-ATPase are obtained either at the Mg:ATP ratio of 1.25:1 at 5 mM Mg^{2+} or at the ratio of 5:1 at 10 mM Mg^{2+}. The K_a for Mg^{2+} and ATP is 2.5:1.92 mM (Mg:ATP) in the first case and 3:0.60 mM in the second. Notice that for this system, the apparent K_a for Mg^{2+} remains almost the same for the two different Mg:ATP ratios. It is the K_a for ATP that changes, being about 3 times lower for the 5:1 Mg:ATP ratio. It is interesting to point out that while the V_{max} for the Na^+-ATPase activity is the same for the two Mg:ATP ratios, this is not the case for the Mg^{2+}- and ($Na^+ + K^+$)-ATPase ac-

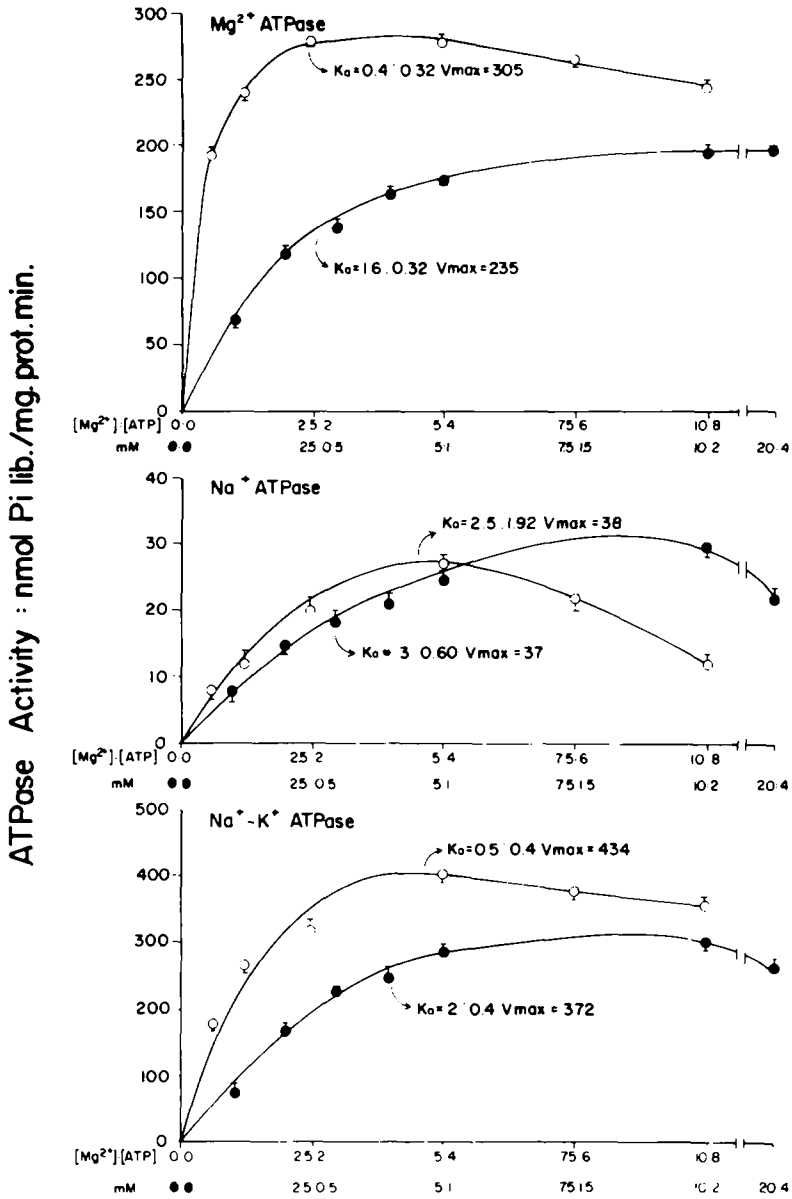

Fig. 4. Effect of increasing the Mg^{2+} : ATP concentrations at fixed ratios of 1.25 : 1 (○——○) and 5 : 1 (●——●) in the incubation medium, on the Mg^{2+}-, Na^+- and ($Na^+ + K^+$)-ATPase activities of basal-lateral plasma membranes. Na^+ concentration 100 mM; K^+, 20 mM. Except for the ($Na^+ + K^+$)-ATPase activity, all other assays were carried out in the presence of 1 mM ouabain. $n = 10$.

tivities, both of which have a higher V_{max} for the 1.25 : 1 ratio.

Effect of inhibitors

Ethacrynic acid, furosemide and triflocin, three known inhibitors of the Na^+-ATPase activity, demonstrable in aged microsomal fractions [4,13], were tested in our preparation. The results are compared with the effects of these agents on the other ATPase activities present in this tissue; namely, on the Mg^{2+}-, ($Na^+ + K^+$)- and Ca^{2+}-ATPases.

TABLE III

HYDROLYSIS OF DIFFERENT NUCLEOTIDES AND ATP DERIVATES BY BASAL-LATERAL PLASMA MEMBRANES DETERMINED IN THE PRESENCE OF Mg^{2+} OR $Mg^{2+}+Na^+$ OR $Mg^{2+}+Na^++K^+$ IN THE INCUBATION MEDIUM

Mg^{2+} concentration, 5 mM; Na^+, 100 mM; K^+, 20 mM; ouabain, 1 mM; nucleotides or derivatives, 2 mM. $n = 10$. The activities are expressed in nmol P_i liberated per min per mg protein.

Substrate	Nucleotidase activity			Δ Na^+	Δ $Na^+ + K^+$
	Mg^{2+} + ouab.	$Mg^{2+} + Na^+$ + ouab.	$Mg^{2+} + Na^+ + K^+$		
ATP	253 ± 3	284 ± 3	719 ± 10	+ 31 ± 4	+ 435 ± 10
GTP	245 ± 4	247 ± 4	267 ± 7	+ 2 ± 6	+ 20 ± 8
UTP	93 ± 4	90 ± 3	127 ± 6	− 3 ± 5	+ 37 ± 7
ITP	149 ± 4	149 ± 4	172 ± 5	0 ± 6	+ 23 ± 6
CTP	103 ± 6	102 ± 4	119 ± 6	− 1 ± 7	+ 17 ± 7
ADP	43 ± 2	41 ± 3	60 ± 3	− 2 ± 4	− 19 ± 4
AMP	41 ± 1	38 ± 2	37 ± 3	− 3 ± 2	− 1 ± 4

Fig. 5 shows the effect of increasing concentrations of ethacrynic acid. As already shown for the aged microsomes [4], 1.5 mM of this agent, totally inhibits the Na^+-stimulated activity. The $Na^+ + K^+$ activity is inhibited by 63%; the Mg^{2+} by 38% and the Ca^{2+} by 45%.

In Fig. 6, we can see that 2 mM furosemide inhibits the Na^+-stimulated ATPase activity by 82%; the Ca^{2+} activity by 50%; the Mg^{2+} activity by 14% and the $(Na^+ + K^+)$-ATPase activity only by about 10%.

Finally, Fig. 7 shows the effect of Triflocin. At

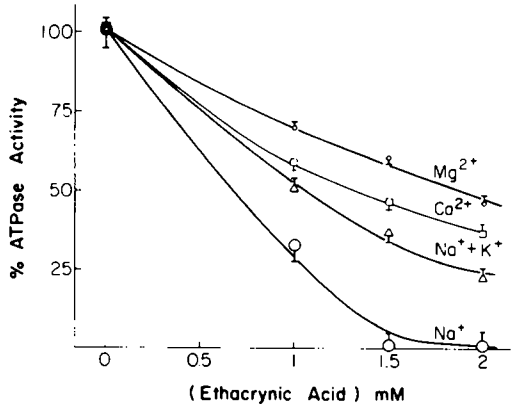

Fig. 5. Effect of increasing concentrations of ethacrynic acid on the Mg^{2+}-, Ca^{2+}-, $(Na^+ + K^+)$- and Na^+-stimulated ATPase activities. Except for the $Na^+ + K^+$ activity, all experiments carried out in the presence of 1 mM ouabain. Assays realized as indicated under Methods. $n = 8$.

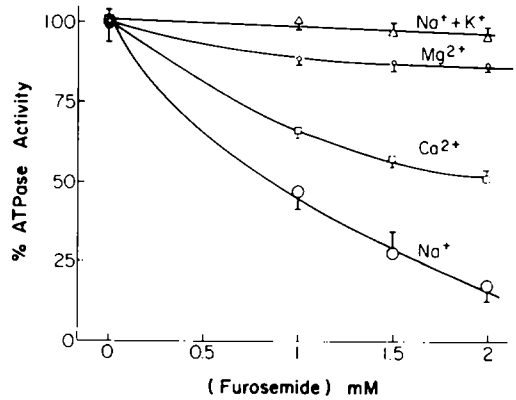

Fig. 6. Effect of increasing concentrations of furosemide on the Mg^{2+}-, Ca^{2+}-, $(Na^+ + K^+)$-, and Na^+-stimulated ATPase activities. Except for the $(Na^+ + K^+)$-ATPase activity, all experiments carried out in the presence of 1 mM ouabain. Assays performed as indicated under Methods. $n = 8$.

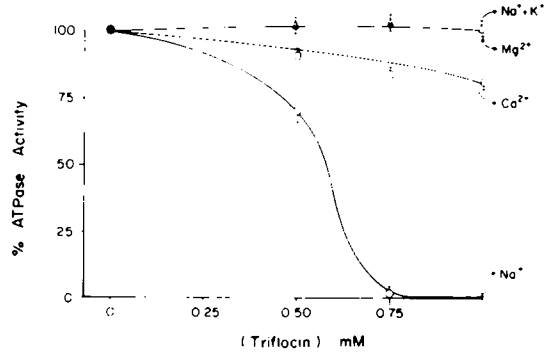

FIg. 7. Effect of increasing concentrations of triflocin on the Mg^{2+}-, Ca^{2+}-, $(Na^+ + K^+)$-, and Na^+-stimulated ATPase activities. Except for the $(Na^+ + K^+)$-ATPase activity, all experiments carried out in the presence of 1 mM ouabain. Assays performed as indicated under Methods. $n = 8$.

concentrations of 0.75 mM, it already inhibits totally the Na^+-ATPase activity, while it has no effect on the Mg^{2+}- and the $(Na^+ + K^+)$-ATPase activities and only inhibits 20% the Ca^{2+}-ATPase.

Discussion

Several characteristics of the Mg^{2+}-dependent ATPase activities, present in basal-lateral plasma membranes from guinea-pig kidney proximal tubular cells, were investigated. As shown in the Results (Tables I–III and Figs. 1–7) the Na^+-ATPase system presents specific characteristics that clearly differentiate it from the other ATPase activities. This system, found in the basal-lateral membrane preparations confirm previous observations in microsomal fractions [4]. Some of the characteristics fulfill the requirements of the energy source of the system responsible for the second mode of active extrusion of Na^+ (accompanied by Cl and water) described in guinea-pig kidney cortex slices [3,8,9]. Briefly, both mechanisms (Na^+-ATPase and active Na^+ extrusion with Cl^- and water) are: (a) stimulated by Na^+; (b) stimulated, to a lesser degree, by Li^+ [19]; (c) do not require a particular anion; (e) are insensitive to the presence of ouabain and (f) are totally inhibited by 1.5 mM ethacrynic acid; 2 mM furosemide and 1 mM triflocin [13], which inhibit, at most, some of the $(Na^+ + K^+)$-ATPase and transport mechanisms.

The Na^+-ATPase activity is about 7 to 10% of the $(Na^+ + K^+)$-ATPase activity. This is a very small (although statistically significant) activity, and it could be thought to be due to incomplete inhibition of the $(Na^+ + K^+)$-ATPase by 1 mM ouabain. This is not the case, however, since the Na^+-ATPase activity is not only insensitive to 1 mM ouabain but also to 10 mM ouabain.

The Na^+-ATPase activity could also be due to a partial insensitivity of the $(Na^+ + K^+)$-ATPase to ouabain because of proteolytic damage to ouabain binding sites. This is not the case either, since the ATPase activity in the presence of $Mg^{2+} + Na^+ + K^+ +$ ouabain is the same as the activity in the presence of $Mg^{2+} + Na^+ +$ ouabain [10]. To explain these results, it is necessary to postulate that we have proteolytic damage to ouabain-binding sites and to K^+-binding sites. Accordingly, what we call the Na^+-stimulated ATPase would be an expression of the $(Na^+ + K^+)$-ATPase, which is insensitive to K^+ and to ouabain. But, if this were the case, how could we explain the results with furosemide or triflocin, which totally inhibit the 'Na^+ expression of the $(Na^+ + K^+)$-ATPase', but do not inhibit at all the full expression of this system? Ouabain (1 to 10 mM), a specific inhibitor of the $(Na^+ + K^+)$-ATPase, does not produce any inhibition on the 'Na^+ expression of the $(Na^+ + K^+)$-ATPase', but triflocin, an agent which does not inhibit the $Na^+ + K^+$-ATPase, totally inhibits the ouabain-insensitive, Na^+-stimulated ATPase activity.

Furthermore, we have found that if the membranes are kept for periods of 3 to 4 h with 2 mM EDTA or EGTA, the Na^+-stimulated ATPase activity disappears. It can be detected again if micromolar quantities of Ca^{2+} are added to the ATPase assay medium or if the membranes are resuspended at pH 7.8 after the treatment with the chelators. In all these cases, the membranes bind specifically equal quantities of [^{3}H]ouabain (data not shown). These results are an indication that the ouabain-insensitive, Na^+-stimulated ATPase activity is not due to proteolytic damage to ouabain binding sites. It is very difficult, in the light of these results, to explain how the Na^+-ATPase activity can be due to an incomplete inhibition of the $(Na^+ + K^+)$-ATPase activity by ouabain.

Even though we have not yet been able to separate the Na^+-ATPase from the $(Na^+ + K^+)$-ATPase, we have found the way to affect differentially the activities of the two enzymes. Thus, membrane fractions from rats maintained under a high Na^+ diet showed, when compared with control rats, an increased ouabain-insensitive, Na^+-stimulated ATPase activity without any change in the $(Na^+ + K^+)$-ATPase activity. In this case, the Na^+-ATPase activity represents about 20% of the $(Na^+ + K^+)$-ATPase activity (data not shown).

Finally, we have been able to show that inside-out vesicles prepared with these membranes can transport Na^+ against the gradient in two ways: one in the absence of K^+, and another in the presence of K^+. In both cases, there is a concomitant hydrolysis of ATP. The two Na^+ transports and their concomitant ATP hydrolysis are specifically and differentially inhibited by ethacrynic acid (absence of K^+) or by ouabain inside the vesicles (presence of K^+) [12,20].

Some of the characteristics of the Na^+-ATPase, namely the activation by micromolar quantities of Ca^{2+} and the high sensitivity of this system toward changes of pH, temperature, Mg^{2+}-ATP concentrations, could indicate that this Na^+-stimulated mechanism is a control mechanism which could be activated under very specific conditions, derived from specific necessities of the kidney cells. Much work remains to be done to learn about the possible physiological role of this system on the control of intracellular Na^+, as well as of the volume of the kidney proximal tubule cells.

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