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MICROSOMAL ($Na^+ + K^+$)-ACTIVATED ATPase FROM FROG SKIN EPITHELIUM

CATION ACTIVATIONS AND SOME EFFECTS OF INHIBITORS*

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SUMMARY

A method is described for the extraction of microsomal ouabain-sensitive ($Na^+ + K^+$)-activated ATPase from separated frog skin epithelium. The method yields a microsomal fraction containing ($Na^+ + K^+$)-stimulated activity in the range of 30–40 nmol · mg^{-1} · min^{-1} at 26 °C. This portion, which is also ouabain sensitive, is about half of the total activity in media containing Mg^{2+}, Na^+ and K^+. These preparations also contain Mg^{2+}-dependent or Ca^{2+}-dependent activities which are not additive and which are not significantly affected by ouabain, Na^+, K^+ or Li^+.

The activations of the ouabain-sensitive ATPase activity by Mg^{2+}, Na^+, and K^+ are similar to those described in other tissues. It is found that Li^+ does not substitute for Na^+ as an activator but in high concentrations does produce partial activation in the presence of Na^+ with no K^+. These results are pertinent to the reported observations of ouabain-sensitive Li^+ flux across frog skin. It is concluded that this flux is not apparently due to a direct activating effect of Li^+ on the sodium pump.

INTRODUCTION

It has been reported [1, 2] that the isolated frog skin transfers Li^+ from Na^+-free external bathing media into sodium-Ringer internal media and that the Li^+ flux is inhibited by ouabain. Since ouabain is an apparently specific inhibitor of the sodium pump and of ($Na^+ + K^+$)-ATPase, it is of interest to determine whether the Li^+ flux might be related to an activating effect of Li^+ on the sodium pump-enzyme complex in frog epidermis. On this assumption, one would expect Li^+ to act at the cytoplasmic or Na^+ site on the sodium pump since the Li^+ is presumably extruded from the epithelial cell cytoplasm to the internal bath in the studies with isolated frog skin.

However, there is little information on frog skin ($Na^+ + K^+$)-ATPase, pre-

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sumably because of difficulty in disrupting the tissue and obtaining batches of enzyme suitable for kinetic studies. There is no previous report of $(Na^+ + K^+)$-ATPase in microsomal fractions of frog skin. This paper reports on a method for preparing ouabain-sensitive $(Na^+ + K^+)$-ATPase in microsomes from separated frog skin epithelium. It is found that Li^+ does not substitute for Na^+ as an activating ion but that it does, in high concentrations, partially activate this enzyme in the presence of Na^+ with no K^+. Therefore, it is considered unlikely that the Li^+ flux across frog skin is due to a direct activation of the sodium pump by Li^+.

MATERIALS AND METHODS

Collagenase type I and the Tris salt of ATP were obtained from Sigma. New England Nuclear supplied [γ-^{32}P]ATP.

Separation of frog skin epithelium

All the experiments were performed using the abdominal skin epithelium isolated from *Rana catesbeiana*. Large animals weighing about 600 g were kept in a container with tap water at room temperature (21–23 °C). The following procedure, derived from that of Aceves and Erlij [3] and of Rajerison et al. [4] was used. The inside of the skin was scraped with a scalpel to remove part of the tela subcutanea. The skin was then mounted on the open side of a cylindrical lucite chamber having a diameter of 5.3 cm. The tissue was secured to the chamber by tying a cord around the tissue with the connective tissue side facing the inside of the chamber. The chamber with the bound skin was immersed in Ringer (103 mM NaCl, 2.5 mM KCl, 1.8 mM calcium gluconate, and 1.2 mM Tris · HCl, pH 7.4) at 37 °C contained in a beaker. Oxygen was bubbled into the beaker. The skin chamber had a 60 cm long plastic tube inserted into its closed side so that the bottom portion of the tube just touched the inside surface of the skin. A 20-ml solution of Ringer containing 7.2 mg of collagenase (Sigma Chemical Co.) was injected through an outlet into the skin chamber. Following this, air was forced into the chamber, using the same syringe, to maintain a 50 cm high column of solution in the plastic tube attached to the chamber. In most cases, after about 2 h, a glossy skin blister was directly observed. A cut was made around the circumference of the blister, and the epithelial layer could then be peeled off the skin surface in one continuous piece. The area of this continuous piece varied between 8 and 19 cm^2. The separated epithelium was transferred to liquid N_2 for storage up to one month. In this way the skin epithelium could be collected from many frogs.

Microsome preparations

The frozen, separated epidermis, 4–6 g wet weight, was thawed and minced with scissors in a Petri dish containing an ice-cold solution of 30 mM Tris · HCl (pH 7.4), 2 mM ethyleneglycolbis-(β-aminoethylene)-*N*,*N*′-tetraacetic acid (EGTA), and 0.1 mM Tris/EDTA. The minced tissue was transferred to a VirTis flask with 10 volumes of the above solution and dispersed with the turbo-shear assembly for 1 min at medium speed to lessen foaming. The homogenizing flask was packed in ice. If small shreds of skin remained, the mixture was further homogenized by hand in a Ten Broeck glass tissue grinder until the suspension was uniform.

The homogenate, including foamy material, was sedimented at $2000 \times g$ for 5 min. The supernatant fraction was removed and sedimented at $20\,000 \times g$ for 30 min. The $2000 \times g$ pellet (PI) was re-suspended in half the original volume using the same homogenizing solution and again sedimented at $2000 \times g$ for 5 min. The supernatant portion was removed and sedimented at $20\,000 \times g$ for 30 min. The $20\,000 \times g$ pellets were combined (PIIa) and re-suspended in 1–2 ml of cold glass-distilled water in a small glass tissue grinder. All procedures were performed at 0–2 °C.

In some experiments, the first $20\,000 \times g$ supernatant fraction was sedimented at $50\,000 \times g$ for 1 h and the resulting pellet re-suspended in cold glass-distilled water (PIIIa).

Initial experiments showed that pellet PI had ATPase activity that was not sensitive to Na^+ or Li^+ in the presence of 10 mM K^+ and was not inhibited by ouabain. The final $50\,000 \times g$ supernatant fraction had little measureable ATPase activity with or without Na^+ plus K^+.

Pellets were treated with NaI reagent [5] according to a published method with minor modifications [6]. The freshly made reagent contained 40 ml of 5 M NaI, 2.5 ml of 1 M Tris base, 0.25 ml of 1 M $MgCl_2$, 1.25 ml of 0.2 M Tris/EDTA, 90 mg of ATP, and 30 mg of L-cysteine free base in a volume of 50 ml with water. An equal volume of this reagent was added slowly with stirring to the microsome suspension obtained from 4–6 g of starting tissue (without adjusting the protein concentration) kept cold in an ice bath. A volume of cold water three times that of the NaI reagent was added after 30 min and the mixture was then sedimented at $20\,000 \times g$ for 30 min. The supernatant fraction was discarded. The pellet was washed three times by re-suspension in the Tris/EGTA/EDTA solution used for homogenization and sedimentation at $20\,000 \times g$. The final washed pellet was suspended in 1 ml of water and stored in liquid N_2. Except where indicated, experimental results are with the NaI-treated PII pellet. Protein contents of suspensions were measured by a published method [7].

Assay of ATPase activity

Enzyme was diluted at the time of assay in 0.2 mM 2,3-dimercaptopropanol, 0.3 mM Tris/EDTA, and 150 mM Tris · HCl (pH 7.4). In the usual assay, 20 μl of diluent, containing 5–8 μg of protein, were incubated in 40 μl of media containing final concentrations of 3 mM $MgCl_2$, 3 mM [γ-^{32}P] ATP ($5 \cdot 10^5$ cpm/μmol), 80 mM NaCl, and 10 mM KCl at 26 °C for 30 or 60 min. The ionic composition was changed where indicated. Enzymatic reactions were stopped by the addition of 10 μl of 5 % ammonium molybdate in 2 M H_2SO_4. Phosphomolybdate was extracted into isobutanol and radioactivity was measured by scintillation counting as described [8].

RESULTS

ATPase activity was found in the crude homogenate and in the first $2000 \times g$ pellet but without dependency on added Na^+ and K^+ or reproducible sensitivity to ouabain. Table I shows that, in media containing Na^+ and K^+, ATPase activity is found in the $20\,000 \times g$ and $50\,000 \times g$ pellets. Although the specific activities and proportions of ouabain-sensitive activities are similar, pellet PIIa contains about twice

TABLE I

ATPase ACTIVITY IN FRACTIONS OF FROG EPIDERMIS

Separated epidermis (2.25 g wet weight) of *Rana catesbiana* was disrupted as described in Materials and Methods. PII is the pellet sedimented at 20 000 × *g* and PIII is the pellet sedimented at 50 000 × *g* from the 20 000 × *g* supernatant portion. Subscripts (a) and (b) indicate the pellets before and after treatment with NaI as described in Materials and Methods. ATPase activity in 5 μg protein per tube was measured in the presence of 3 mM $MgCl_2$, 3 mM ATP, 100 mM NaCl, and 10 mM KCl with and without 0.5 mM ouabain at 26 °C for 30 min. Total activities and the ouabain-inhibited portions are listed. All values are referred to the total pellet.

Fraction	Protein (mg)	ATPase activity		Ouabain inhibition (%)
		Total	Ouabain	
		(μmol · min⁻¹)		
PIIa	5.90	0.8610	0.1710	19.9
PIIb	0.61	0.0211	0.0122	57.8
PIIIa	2.46	0.3880	0.0812	22.1
PIIIb	0.77	0.0217	0.0029	13.8

as much total activity as does pellet PIIIa. When the pellets are treated with NaI, the effects are to reduce the total protein and specific activity recovered from either of the fractions. However, the ouabain sensitivity of ATPase in the NaI-treated PIIb fraction is increased from 20 % to about 50–60 % of the total activity in different trials. On the other hand, ouabain sensitivity in the 50 000 × *g* pellet is reduced by this treatment. Pellet PIIb, which had the highest proportion of ouabain-sensitive activity, was used in all subsequent experiments.

It is necessary to characterize the properties of the enzyme activity in this microsomal fraction of frog skin. Table II shows that either Mg^{2+} or Ca^{2+}, but not Mn^{2+}, can stimulate the ATPase in the absence of univalent cations. The effect of Mg^{2+}, however, is increased by the presence of Na^+ and K^+ while the effect of Ca^{2+} is not. In the absence of any divalent cation, Na^+ and K^+ have no significant effect. Thus, the ($Na^+ + K^+$)-stimulated portion is dependent on Mg^{2+}.

TABLE II

Mg^{2+} REQUIREMENT FOR FROG EPIDERMAL ($Na^+ + K^+$)-ATPase

ATPase activity was measured as described in Materials and Methods in media containing 3 mM ATP plus divalent cations as indicated with and without 80 mM NaCl plus 10 mM KCl. Incubations were at 26 °C for 60 min.

Divalent cation (3 mM)	Added univalent cations (nmol · mg⁻¹ · min⁻¹)		
	None	($Na^+ + K^+$)*	Δ($Na^+ + K^+$)
None	6.15	8.10	2.0
$MgCl_2$	36.00	59.30	23.3
$MnCl_2$	1.77	1.96	0.2
$CaCl_2$	54.00	59.00	5.0

* 80 mM NaCl plus 10 mM KCl.

It was found that the Mg^{2+}-dependent and Ca^{2+}-dependent activities in the absence of added Na^+ and K^+ could be inhibited by 0.5 mM ouabain to various extents from zero to 10 % in different experiments. The effects on ATPase activity of adding 3 mM $MgCl_2$ plus 3 mM $CaCl_2$ together to incubation media were not additive. The Ca^{2+}-dependent activity was not significantly affected by Na^+ alone and with Li^+, or by K^+ alone and with Li^+, or by Li^+ alone. Concentrations tested were 100 mM Na^+, 10 mM K^+, and 100 mM Li^+.

Fig. 1 shows the Mg^{2+}-dependency of the $(Na^+ + K^+)$-activated ATPase at two concentrations of ATP. The optimum concentration of Mg^{2+} is equal to the concentration of ATP present.

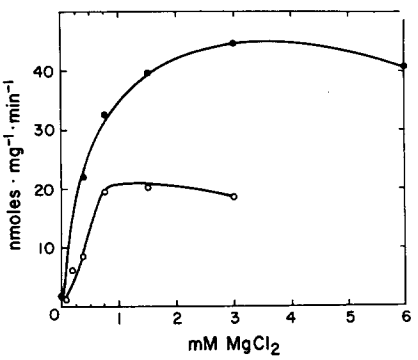

Fig. 1. Effects of Mg^{2+} on $(Na^+ + K^+)$-stimulated ATPase of frog epidermis. ATPase was measured as described in Materials and Methods with and without 100 mM NaCl plus 10 mM KCl for each concentration of $MgCl_2$. Values obtained with Mg^{2+} alone were subtracted from those obtained in the complete media. Incubations were at 26 °C for 30 min. Values for zero Mg^{2+} concentration were obtained in the presence of 0.1 mM Tris/EDTA. ● — ●, 3 mM ATP; ○ — ○, 0.75 mM ATP.

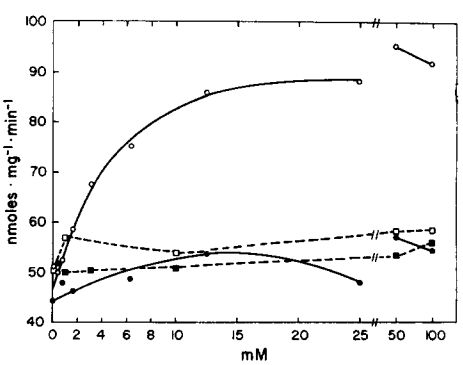

Fig. 2. Effects of Na^+ or Li^+ on frog epidermis ATPase in the presence of K^+ with and without ouabain. ATPase was measured as described in Materials and Methods in media containing 3 mM $MgCl_2$, 3 mM ATP, and 10 mM KCl at 26 °C for 30 min. ○ — ○, mM NaCl without ouabain; ● — ●, mM NaCl with 0.5 mM ouabain; □- - -□, mM LiCl without ouabain; ■- - -■, mM LiCl with 0.5 mM ouabain.

Activation of the ouabain-sensitive ATPase by Na^+ in the presence of K^+ is shown in Fig. 2. Na^+ addition produces a doubling of the total activity with an optimum concentration of about 50–100 mM Na^+ in the presence of 10 mM added KCl. The $[Na^+]_{0.5}$ value is 4 mM. At least 85 % of the Na^+ stimulation is abolished by ouabain. Thus, of the total ATPase activity, 43 % in this experiment is both dependent on added Na^+ and is inhibited by ouabain. At 100 mM Na^+ and 10 mM K^+, the ouabain-sensitive activity is 40 nmol · mg^{-1} · min^{-1} at 26 °C. In the absence of added Na^+, ouabain inhibition is about 10 %. If this activity is subtracted, then the upper limit for the $(Na^+ + K^+)$-ATPase is obtained which, in this experiment, is 48 nmol · mg^{-1} · min^{-1} under the same conditions.

In this experiment (Fig. 2), Li^+, on the other hand, at a concentration of 1 mM produces an activation of about 15 % which was independent of further Li^+ increments up to 100 mM. This effect was apparently blocked by ouabain. In other experiments, however, no significant Li^+ activation in the presence of K^+ without Na^+ could be demonstrated (Table III) and the small effects sometimes seen appear to be within experimental error. In addition, 50 mM Li^+ had no significant effect on the activation of ATPase by Na^+ plus K^+ (Table IV).

TABLE III

COMPARISON OF Na^+ AND Li^+ EFFECTS ON OUABAIN-SENSITIVE ATPase

ATPase was measured as described in Materials and Methods in media containing 3 mM $MgCl_2$, 3 mM ATP, 10 mM KCl, plus other additions as indicated. Incubations were for 30 min at 26 °C. Standard errors for three determinations are shown in parentheses.

	Second cation (100 mM) (nmol · mg^{-1} · min^{-1})		
	None	LiCl	NaCl
Control	60.8 (3.8)	63.5 (2.7)	104.0 (2.8)
0.5 mM ouabain	54.3 (2.6)	61.3 (2.6)	60.2 (2.0)
Percent ouabain inhibition	10.7	3.5	42.1

TABLE IV

$(Na^+ + K^+)$-ACTIVATION OF FROG EPIDERMAL ATPase IN THE PRESENCE OF Li^+

ATPase was measured as described in Materials and Methods in the presence of 3 mM $MgCl_2$, 3 mM ATP, and various concentrations of NaCl. 10 mM KCl and 50 mM LiCl were added where indicated. Incubations were for 30 min at 26 °C. Activity measured in the presence of 0.5 mM ouabain, 50 mM NaCl, and 10 mM KCl (51.7 nmol · mg^{-1} · min^{-1}) was subtracted from total values.

NaCl (mM)	Added cations (nmol · mg^{-1} · min^{-1})		
	None	K^+	$K^+ + Li^+$
None	0	7.3	10.0
0.78		14.1	9.7
3.13		17.1	17.5
50.00	9.9	35.3	40.1

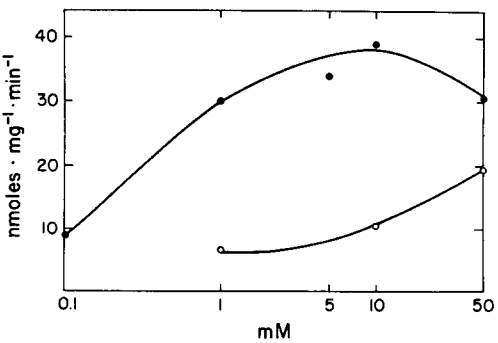

Fig. 3. Effects of K^+ or Li^+ on ouabain-sensitive frog epidermis ATPase in the presence of Na^+. ATPase was measured as described in Materials and Methods in media containing 3 mM $MgCl_2$ 3 mM ATP, and 100 mM NaCl at 26 °C for 30 min. The ouabain-insensitive portion as measured in 10 mM K^+, 100 mM Na^+, and 0.5 mM ouabain (63 nmol · mg^{-1} · min^{-1}) was subtracted. ●—●, mM KCl; ○—○, mM LiCl.

TABLE V

OUABAIN EFFECT ON ($Na^+ + Li^+$)-ACTIVATION OF ATPase

ATPase was measured as described in Materials and Methods in media containing 3 mM $MgCl_2$, 3 mM ATP, 0.1 M NaCl, and the indicated concentrations of LiCl, with and without 0.5 mM ouabain. Incubations were for 60 min at 26 °C. Activity obtained in the absence of LiCl is subtracted from the total to give the Li^+ increment.

LiCl (mM)	Control (nmol · mg^{-1} · min^{-1})		Ouabain (nmol · mg^{-1} · min^{-1})
	Total	ΔLi^+	Total
Zero	47.2		47.2
50	55.1	7.9	45.4
100	66.2	19.0	45.0
150	48.6	1.4	40.8

K^+ activates the ATPase and the $[K^+]_{0.5}$ is 0.8 mM in the presence of 0.1 M NaCl (Fig. 3). In the same concentration of Na^+, 50 mM Li^+ produces about half the maximum activation given by K^+ (Fig. 3). Table V shows that the stimulation produced by Na^+ plus Li^+ is inhibited by ouabain. Thus it appears that Li^+, if anything, can act as a poor replacement for K^+.

The effects of various agents on the Mg^{2+}-ATPase and ($Na^+ + K^+$)-stimulated ATPase are shown in Table VI. The only significant effect observed was inhibition of the $Na^+ + K^+$ increment by $2.4 \cdot 10^{-6}$ M oligomycin.

DISCUSSION

The frog skin and the separated skin epithelium have been extensively utilized for investigations of transepithelial cation and water transport. There is evidence for the hypothesis that the sodium pump located in the epithelial cell membranes within

TABLE VI

OLIGOMYCIN INHIBITION OF $(Na^+ + K^+)$-ATPase

ATPase was measured as described in Materials and Methods in media containing 3 mM $MgCl_2$, 3 mM ATP, with and without 100 mM NaCl plus 10 mM KCl. Where indicated, enzyme was pretreated with collagenase, 0.36 mg/ml, at 23 °C for 60 min prior to assay. Other additions to the media as indicated were made prior to the enzyme. Incubations were at 26 °C for 60 min. Column I, media contain Mg^{2+}; column II, media contain Mg^{2+}, Na^+, and K^+; column III, increment in activity due to $Na^+ + K^+$.

	($nmol \cdot mg^{-1} \cdot min^{-1}$)		
Addition	I	II	III
None	36.0	59.3	23.3
Furosemide, 10^{-5} M	42.8	74.8	32.0
Amiloride, 10^{-5} M	39.3	72.5	33.2
Na azide, 10^{-4} M	40.9	70.5	29.6
Oligomycin, $2.4 \cdot 10^{-7}$ M	41.7	70.4	28.7
Oligomycin, $2.4 \cdot 10^{-6}$ M	42.5	57.8	15.3
Collagenase	63.0	92.5	29.5

the epidermis participates in the transcellular movement of Na^+ by actively extruding this ion once it enters the cell [9]. The main evidence is that ouabain, a potent inhibitor of the sodium pump and of $(Na^+ + K^+)$-ATPase in other tissues, also inhibits this enzyme in frog skin and reduces the transepithelial Na^+ flux and short-circuit current across the skin [9, 10].

Recent studies [1] have shown that Li^+ flux across frog skin is inhibited by ouabain and exhibits a number of other features in common with Na^+ flux [2]. These observations suggest the possibility, among others, that Li^+ can replace Na^+ at the intracellular site as an activator of the sodium pump and of $(Na^+ + K^+)$-ATPase. As one effort in the study of this problem, it is necessary to obtain biochemical data on the ATPase activity in frog skin. In these experiments, we have been interested in developing a convenient method for obtaining ouabain-sensitive ATPase from frog epidermis and in determining the effects of Li^+ on this activity.

There is very little biochemical data available on $(Na^+ + K^+)$-ATPase activity in frog skin despite the considerable use of this tissue in the study of transepithelial transport. One of the main reasons is the great difficulty in homogenizing the skin and obtaining uniform microsomal enzyme suspensions that yield reproducible results. Bonting and his colleagues [10, 11], in the first report on frog skin ATPase, demonstrated the presence of $(Na^+ + K^+)$-activated ATPase in whole homogenates of individual skin samples. Results thus obtained, however, showed high variability [10]. Another study on fractions separated by sedimentation from skin homogenates showed ATPase activity dependent on either Mg^{2+} or Ca^{2+} but no activation by Na^+ and K^+ [12]. Finally, in a study of ATPase activity in whole homogenates of separated epidermis [13], $(Na^+ + K^+)$-stimulated activity of 1 $\mu mol \cdot mg^{-1} \cdot h^{-1}$ at 37 °C was found. This corresponds to a rate of 8 $nmol \cdot mg^{-1} \cdot min^{-1}$ at 26 °C, assuming a Q_{10} of 2.

The method reported here yields a microsomal fraction with $(Na^+ + K^+)$-ATPase activities in the range of 30–40 $nmol \cdot mg^{-1} \cdot min^{-1}$ at 26 °C which is almost

completely inhibited by ouabain. This ouabain-sensitive portion is about half the total activity in this tissue fraction in the presence of Mg^{2+}, Na^+, and K^+. The activations of the ouabain-sensitive enzyme by Mg^{2+}, Na^+, and K^+ are similar to results obtained with many other tissue preparations [14].

The preparations also contain Mg^{2+}-dependent and Ca^{2+}-dependent activities which are not additive and which are not substantially inhibited by ouabain. The Ca^{2+}-dependent portion is not significantly effected by Na^+, K^+, and Li^+ under the conditions tested. The significance of these activities is not understood.

Inhibition of the $(Na^+ + K^+)$-ATPase activity by $2.4 \cdot 10^{-6}$ M oligomycin (Table VI) is consistent with effects in other tissues [15, 16]. Lack of inhibition by 10^{-4} M azide (Table VI) suggests the absence in this fraction of significant mitochondrial ATPase [17].

It is important to study the effects of epithelial-transport inhibitors on the enzyme activity but available information is sparse. The only data available regarding amiloride effects on enzyme activity showed no inhibition in rat kidney but the conditions were not described [18]. Amiloride did not inhibit active or ouabain-sensitive ion flux in red cells [19] from which it may be assumed that erythrocyte $(Na^+ + K^+)$-ATPase was not affected. Failure of amiloride to inhibit the frog skin enzyme (Table VI) is consistent with the presumed mode of amiloride action on membrane components other than the sodium pump [20].

Furosemide, on the other hand, evidently has multiple actions on ion flux [21, 22], which involve both ouabain-sensitive and -insensitive components. The only available data regarding effects directly on $(Na^+ + K^+)$-ATPase activity in vitro showed less than 25% inhibition by 10^{-4} M furosemide in a kidney homogenate [23]. In vivo administration produced much greater inhibition of activity in the kidney ascending and distal tubules, specifically [23]. Table VI shows no significant effect of 10^{-5} M furosemide but this question reguires further investigation.

It is found that Li^+ does not replace Na^+ as an activator of this enzyme in frog epidermis (Results). This is consistent with the original observations of Skou [24] on crab nerve enzyme. It is also consistent with the failure of Li^+ to substitute for Na^+ in either (a) enzyme phosphorylation [25] or (b) ATP-ADP transphosphorylation [15] in studies using electroplax $(Na^+ + K^+)$-ATPase.

On the other hand, 50 mM Li^+, or higher, does produce partial activation in the presence of Na^+ with no K^+ and this activation is ouabain-sensitive (Fig. 3, Table V). Thus Li^+ may have an action similar to K^+, although it is much less potent. This effect of Li^+ is similar to that obtained in crab nerve homogenates [24]. The addition of 50 mM Li^+ in the presence of an optimum K^+ concentration does not produce a significantly additive effect (Table IV) in contrast to results with rat kidney enzyme [26]. However, it has been previously pointed out by other authors [27] that Li^+ may partially substitute for either Na^+ or K^+ in certain tissues as red cells and squirrel kidney. But, demonstration in any system that Li^+ stimulation of ATPase activity is paralleled by stimulation of ion flux is lacking.

It is evident from the data reported here that Li^+ is not a substitute for Na^+ as an activator of frog skin $(Na^+ + K^+)$-ATPase. It is, therefore, unlikely that Li^+ flux across the frog skin ensues from a direct activation of the sodium pump by Li^+. However, since both Li^+ influx and Na^+ influx are similarly inhibited by addition of ouabain to or removal of Na^+ or K^+ from the internal bath [2], the Li^+ influx

may be a secondary effect of the Na^+ and K^+ pump. The nature of this relationship will be the subject of a future report.

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REFERENCES

1 Candia, O. A. and Chiarandini, D. J. (1973) Biochim. Biophys. Acta 307, 578–589
2 Candia, O. A., Reinach, P., Tormay, A. and Siegel, G. J. (1974) Fed. Proc. 33, 240
3 Aceves, J. and Erlij, D. (1971) J. Physiol. Lond. 212, 195–210
4 Rajerison, R. M., Montegut, M., Jard, S. and Morel, F. (1972) Pflügers Arch. 332, 302–312
5 Nakao, T., Tashima, Y., Nagano, K. and Nakao, M. (1965) Biochem. Biophys. Res. Commun. 19, 755–758
6 Albers, R. W., Koval, G. J. and Siegel, G. J. (1968) Mol. Pharmacol. 4, 324–336
7 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
8 Siegel, G. J. and Albers, R. W. (1967) J. Biol. Chem. 242, 4972–4979
9 Ussing, H. (1965) Harvey Lect. 59, 1–30
10 Bonting, S. L. and Caravaggio, L. L. (1963) Arch. Biochem. Biophys. 101, 37–46
11 Bonting, S. L., Caravaggio, L. L. and Hawkins, N. M. (1962) Arch. Biochem. Biophys. 98, 413–419
12 Dargent-Salée, M. L. (1970) Arch. Int. Physiol. Biochem. 78, 253–258
13 Kawada, J., Taylor, Jr, R. E. and Barker, S. B. (1969) Comp. Biochem. Physiol. 30, 965–975
14 Albers, R. W. (1967) Annu. Rev. Biochem. 36, 727–756
15 Fahn, S., Koval, G. J. and Albers, R. W. (1966) J. Biol. Chem. 241, 1882–1889
16 Wheeler, K. P. and Whittam, R. (1964) Biochem. J. 93, 349–363
17 Vigers, G. and Ziegler, F. (1968) Biochem. Biophys. Res. Commun. 30, 83–88
18 Baer, J. E., Jones, C. B., Spitzer, S. A. and Russo, H. F. (1967) J. Pharmacol. Exp. Ther. 157, 472–485
19 Aceves, J. and Cereijido, M. (1973) J. Physiol. Lond. 229, 709–718
20 Bentley, P. F. (1968) J. Physiol. Lond. 195, 317–330
21 Rettori, O. (1972) Medicina 32, 242–246
22 Candia, O. A. (1973) Biochim. Biophys. Acta 298, 1011–1014
23 Schmidt, U. and Dubach, U. C. (1970) Nephron 7, 447–458
24 Skou, J. C. (1960) Biochim. Biophys. Acta 42, 6–23
25 Fahn, S., Koval, G. J. and Albers, R. W. (1968) J. Biol. Chem. 243, 1993–2002
26 Gutman, Y., Hochman, S. and Wald, H. (1973) Biochim. Biophys. Acta 298, 284–290
27 Willis, J. S. and Fang, L. S. T. (1970) Biochim. Biophys. Acta 219, 486–489