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SODIUM- AND POTASSIUM-DEPENDENT ADENOSINE TRIPHOSPHATASE ACTIVITY IN A RAT-KIDNEY ENDOPLASMIC RETICULUM FRACTION

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SUMMARY

ATPase activity has been characterized in a rat-kidney microsomal membrane preparation. Dialyzed preparations have a magnesium-dependent ATPase that is activated by sodium and potassium added in combination. Evidence is presented for separate sites of binding for sodium and potassium in the preparation. This sodium–potassium-dependent ATPase activity is inhibited by ouabain, organic mercurial diuretics and calcium. Ouabain inhibition appears to be on the uptake of cations rather than the ATPase activity. Hydrogen-ion effects on ATPase activity are examined and discussed. It is postulated that the activity observed is a component of the active-transport system for sodium and potassium in the rat kidney.

INTRODUCTION

Electron microscopy studies have revealed the presence of a complex system of membranes which surrounds vesicles and tubules in the interior cytoplasm of a large variety of mammalian cells[1,2]. This intracellular membrane system was given the name of endoplasmic reticulum by PALADE AND PORTER[2]. It was classified as rough endoplasmic reticulum if ribonucleoprotein particles adhered to the membrane and smooth endoplasmic reticulum if these particles were absent. PALADE AND SIEKEVITZ[3,4] have shown that microsomal particles from liver and pancreas, when freed of mitochondria by differential centrifugation, are made up almost exclusively of fragments of the endoplasmic reticulum system. This membrane system has been postulated to play an active role in the transport of substances between the external medium and cytoplasm[5]. In view of the considerable transport activity carried out by kidney tissue, the properties of a similarly prepared endoplasmic reticulum fraction from kidney are of special interest.

ATPase activity has been found in membranes derived from red blood cells[6], crab nerve[7], rat-brain endoplasmic reticulum[8], and bacteria[9,10]. The first three ATPase systems show enhanced activity in the presence of added sodium and potassium in a manner which suggests that they function in cation transport. The present study characterizes ATPase activity of a rat-kidney endoplasmic reticulum fraction.

Evidence is presented that this rat-kidney ATPase system plays a role in cation transport.

MATERIALS AND METHODS

The rats employed were 3–4-month-old male albinos of the Sprague–Dawley strain, obtained from the Holtzman Rat Co., Madison, Wisc. Crystalline pyruvic kinase and sodium salts of ATP and ADP were obtained from the Sigma Chemical Co. The platinum electrode oxygraph used for microoxygen-uptake measurements was made by the Gilson Electronics Co., Middleton, Wisc.

ATPase incubations were carried out at 37° in a Dubnoff metabolic incubator. Flasks were shaken at 80 cycles/min. After incubation, 1-ml aliquots of the 3 ml incubation mixture were deproteinized with 1 ml of 5 % trichloroacetic acid. Phosphate analyses were carried out on aliquots of the deproteinized supernatant. Inorganic phosphate was determined according to the method of LOWRY *et al.*[13] except that phosphate solutions in the presence of magnesium and the trichloroacetic acid supernatant of the endoplasmic reticulum fraction required about 20 min for full color development. The Tris salt of ATP (pH 7)[6] was employed in many of the experiments.

Protein was determined by a modification of the phenol method[14] with crystalline bovine albumin used as protein standard. RNA content of the enzyme was determined by the MEJBAUM orcinol method[15]. For RNA determination, preparations were freed of sucrose by recentrifugation and resuspension in water.

The sodium and potassium content of several preparations was measured with a Baird-Atomic flame photometer employing an internal lithium standard.

EXPERIMENTAL

Preparation of fraction

The method of preparation of the rat-kidney endoplasmic reticulum fraction employed in this study is a modification of the procedure used by PALADE AND SIEKEVITZ[3] in preparing their rat-liver endoplasmic reticulum fraction. Kidneys from freshly killed animals were homogenized at 4° with a teflon pestle Potter homogenizer in 0.25 M sucrose at a concentration of 1 g of tissue per 2 ml of sucrose. Subsequent operations were carried out at 0°. The 0.25 M sucrose used in this preparation contained $2 \cdot 10^{-3}$ M disodium EDTA.

The homogenate was centrifuged at 37000 × *g* for 40 min in a Servall RC-2 refrigerated centrifuge. Intact cells, nuclei, mitochondria and a fluffy layer were sedimented. The supernatant was decanted leaving behind enough of the supernatant to avoid visible contamination from the fluffy layer at the bottom of the centrifuge tube. The supernatant was centrifuged at 105000 × *g* for 120 min in a Spinco preparative ultracentrifuge. This centrifugation forms a pellet and a clear pink supernatant fraction. The pellet formed is the endoplasmic reticulum fraction employed in this study. The pellet was rehomogenized in a volume of 0.25 M sucrose equal in volume to the clear pink supernatant, divided into 1- and 2-ml aliquots in small plastic tubes and frozen in a dry ice–ethanol bath (—60°). The frozen aliquots of the endoplasmic reticulum fraction were stored at —20°. The clear pink supernatant fraction contained the soluble glycolytic enzymes and was similarly divided into small aliquots, frozen and stored. The procedure of instantaneous freezing at low temperature is

essential for the maintenance of DPNH oxidase activity by the endoplasmic reticulum fraction. These stored preparations maintain high levels of ATPase activity in the endoplasmic reticulum fraction and high levels of glycolytic activity in the soluble supernatant fraction for many weeks.

The centrifugation in our rat-kidney preparative procedure differs from that employed by PALADE AND SIEKEVITZ[3,4] in their preparation of endoplasmic reticulum fractions from liver and pancreas in that our initial centrifugation was at 37900 × *g* for 40 min in 0.25 M sucrose while their initial centrifugation was at 20000 × *g* for 30 min in 0.88 M sucrose. Therefore the microsomal particles which formed as a pellet in the second centrifugation and which make up the kidney endoplasmic reticulum fraction employed in this study are of lower average density than the particles in the preparations from liver and pancreas reported by PALADE AND SIEKEVITZ[3,4]. The initial high-speed centrifugation insured complete removal of mitochondria and large mitochondrial fragments, but resulted in the loss of a large fraction of endoplasmic

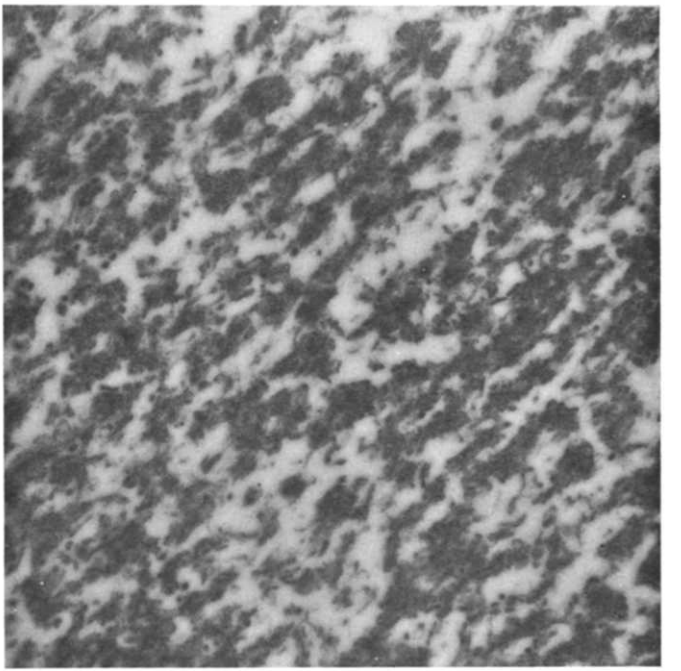

Fig. 1. Electron micrograph of rat-kidney endoplasmic reticulum fraction. Pellet consisting of material sedimenting between 38000 and 105000 × *g* was fixated in buffered 1 % osmium tetroxide in 0.25 M sucrose. Magnification × 25500.

reticulum material. Using glucose-6-phosphatase activity as an index of distribution of the endoplasmic reticulum fraction, about two-thirds of the endoplasmic reticulum fraction was discarded in the sediment of the initial centrifugation.

Characterization of the fraction

Electron micrographs revealed homogeneous fields of fragmented membrane material (Fig. 1). On close scrutiny oval shaped and elongated membrane vesicles are discernible throughout the fields. No intact mitochondria or large mitochondrial fragments are present. At higher magnification vesicles appear to be primarily of the smooth type devoid of ribosome particles. Fig. 1 may be compared with electron micrographs of intact tubule cells at similar magnification[11].

When reduced with dithionite the preparation showed a conspicuous cytochrome spectrum. The probable identity of the cytochrome as b_5 is based on the prominent Soret absorption peak of the reduced preparation observed at 423 mμ. 68 preparations averaged 5.7 mg protein per ml with a standard deviation of $\pm$ 1.5 mg. 7 preparations were analyzed for RNA and results ranged from 7 to 16 % of the protein content. Electron micrographs of kidney cells[11] have shown that they contain very few ribonucleoprotein particles.

Enzyme activities found in the kidney endoplasmic reticulum fraction included DPNH oxidase, glucose-6-phosphatase and ATPase. The preparation was tested enzymically for contamination by intact mitochondria. α-Ketoglutarate or pyruvate was added to the preparations with the appropriate levels of adenine nucleotides, phosphate, cytochrome *c* and magnesium as employed by LARDY AND WELLMAN[12] in studies of mitochondria. The platinum electrode oxygraph used to measure oxygen uptake detects changes as small as a fraction of a μmole of oxygen. There was no detectable oxygen uptake with the two substrates employed. This biochemical evidence substantiated the observation with the electron microscope that no intact mitochondria were present.

RESULTS

There is a liberation of inorganic phosphate when ATP is incubated with the endoplasmic reticulum fraction. The endoplasmic reticulum fraction splits ATP into inorganic phosphate and ADP. The formation of ADP has been demonstrated directly by coupling ATP and the endoplasmic reticulum fraction with the crystallized pyruvic kinase assay, and by analyzing the deproteinized incubation mixture for product ADP using the same enzyme assay[16]. Cleavage of ATP to AMP or pyrophosphate could not account for the phosphate liberation from ATP since phosphate liberation from AMP or pyrophosphate is negligible as compared to phosphate liberation from ATP. The ATPase activity has an absolute requirement for magnesium ions with a 1:1 ratio of magnesium to ATP giving maximal activity. The optimal substrate concentration is $4 \cdot 10^{-3}$ M Mg–ATP. The splitting of ATP is markedly affected by changes in pH of the incubation medium. Between pH 6 and pH 8, ATPase activity rises rapidly with increasing pH (Table V). ADP is also split by the endoplasmic reticulum fraction but at a rate less than half of that for ATP. This reaction is not significantly affected by pH.

At pH 7.5 the specific activity of the kidney endoplasmic reticulum ATPase

ranged from 0.3 to 0.5 μmole/min/mg protein. This high level of ATPase activity is also found in bacterial membranes[9] and crystalline myosin[17].

ATPase activity of the endoplasmic reticulum fraction is not changed by sodium and potassium added in combination. However, if the fraction is first dialyzed against distilled water at 4° for 10–20 h the preparation can than be stimulated by sodium and potassium in combination as has been reported for red blood cell and crab nerve membrane preparations[6,7].

Table I demonstrates the effect of sodium and potassium on the ATPase activity of 5 of a series of 14 dialyzed endoplasmic reticulum preparations. After dialysis there is a considerable drop in ATPase activity. Addition of either sodium or potassium alone fails to stimulate the dialyzed preparation. However, when sodium and potassium are added in combination to the dialyzed preparation there is marked stimulation of the ATPase activity. ATPase activity stimulated by sodium and potassium in the dialyzed preparation may approach or attain the level of activity found in the nondialyzed preparation. Table II which is representative of 6 similar experiments demonstrates a competitive inhibition by potassium of the sodium activation in the sodium–potassium stimulation of ATPase activity. In the presence of 33 mM

TABLE I

THE EFFECT OF SODIUM AND POTASSIUM ON THE ATPase ACTIVITY OF THE ENDOPLASMIC RETICULUM FRACTION

The incubation mixture contained 300 μmoles Tris buffer (pH 7.1), 12 μmoles of $MgCl_2$, 12 μmoles of Tris–ATP and 0.2 ml of the endoplasmic reticulum fraction. The incubation time was 20 min. The incubation volume was 3 ml.

Preparation	*μmoles phosphate liberated*				
	A	*B*	*C*	*D*	*E*
Nondialyzed	7.8	9.8	7.0	7.1	6.1
Dialyzed					
no addition	2.5	4.1	3.4	3.4	3.9
133 mM Na^+	2.6	3.8	2.9	4.1	3.5
16.7 mM K^+	2.6	4.2	3.9	3.2	4.0
133 mM Na^+ and 16.7 mM K^+	5.9	5.9	5.7	6.5	6.5

TABLE II

COMPETITIVE INHIBITION BY POTASSIUM OF SODIUM ACTIVATION OF ATPase ACTIVITY

Incubations were carried out as described in Table I.

Preparation	*μmoles phosphate liberated*					
mM KCl added to the incubation:	*none*	*3.3*	*6.7*	*16.7*	*33*	*67*
Nondialyzed	6.8					
Dialyzed						
no addition	4.6					
33 mM NaCl		6.4	6.4	5.7	5.6	4.8
67 mM NaCl		6.4	5.9	5.6	6.4	5.3
133 mM NaCl		5.9	5.6	6.2	6.4	5.9
200 mM NaCl		5.8	5.7	6.2	7.0	6.4

NaCl, increasing levels of KCl depress the ATPase activity of the endoplasmic reticulum fraction. This inhibition by KCl is reversed if increased amounts of NaCl are added to the incubation medium. K_I values calculated for potassium inhibition of sodium in these 6 experiments are approx. 5 mM. Half-maximal stimulation of ATPase activity is obtained with 0.67 mM potassium when the sodium level is maintained at 33 mM and with 8 mM sodium when the potassium level is maintained at 3.3 mM. These values indicate that their exist separate sites of binding for sodium and potassium activating the ATPase. The sodium binding is inhibited competitively by potassium.

10–20 h of dialysis appears to be optimal. Prolonged dialysis or excessively vigorous stirring during dialysis partially inactivates the preparation. Dialyzed preparations lose activity rapidly when frozen and stored. The effect of dialysis on ATPase activity of the endoplasmic reticulum suggests that much of the ATPase activity found in the preparation before dialysis can be attributed to sodium and potassium already present in the membrane fraction. Dialysis removes this sodium and potassium, and subsequent restoration of these ions is accompanied by restoration of the ATPase activity. To test this concept 3 preparations were analyzed for sodium

TABLE III

THE EFFECT OF DIALYSIS ON THE ENDOPLASMIC RETICULUM FRACTION

Incubations were carried out as described in Table I. Determination of sodium and potassium from aliquots of the endoplasmic reticulum preparation were carried out after deproteinization with an equal amount of 5% trichloroacetic acid.

Fraction	*ATPase (μmoles phosphate liberated)*	*Na^+ (mequiv/l)*	*K^+ (mequiv/l)*
Nondialyzed	9.6	6.6	1.2
Dialyzed	4.6	2.5	0.67
Nondialyzed	7.1	5.2	0.8
Dialyzed	1.9	0.88	0.2
Nondialyzed	7.0	5.0	0.7
Dialyzed	2.3	1.5	0.32

and potassium content before and after dialysis. Table III indicates that in each dialyzed preparation there was a loss of ATPase activity and a considerable loss of sodium and potassium. The observed loss in sodium from the preparations could readily correspond to the 4 mequiv/l of sodium added with the EDTA in the preparation of the fraction and does not necessarily indicate sodium loss from the membrane. Fractions prepared without EDTA show similar loss of ATPase activity upon dialysis which is not restored by adding sodium and potassium. It was also observed that replacing the sucrose lost on dialysis did not alter the stimulation by sodium and potassium in our EDTA preparations.

Ouabain, a known inhibitor of active cation-transport systems[18–22], inhibits the sodium–potassium-stimulated ATPase of the endoplasmic reticulum. It has no effect on the nondialyzed preparation or on the dialyzed preparation in the absence of added sodium and potassium. The inhibitory effects of ouabain on sodium–potassium stimulation are shown in Table IV.

TABLE IV

THE EFFECT OF OUABAIN ON THE SODIUM–POTASSIUM-STIMULATED ATPase ACTIVITY OF THE DIALYZED ENDOPLASMIC RETICULUM FRACTION

Incubations were carried out as described in Table I. In several experiments $6.9 \cdot 10^{-3}$ M ouabain was shown to be identical in effect as $2.3 \cdot 10^{-3}$ M ouabain. ATPase activity at this maximally effective ouabain level was approximately that obtained when no sodium and potassium were added.

Incubation	Ouabain concn. (M)	µmoles phosphate liberated	
		Expt. A	Expt. B
200 mM Na^+ and 33 mM K^+	none	5.9	8.2
	$9.2 \cdot 10^{-6}$	5.9	
	$2.3 \cdot 10^{-5}$	5.5	7.6
	$4.6 \cdot 10^{-5}$	5.4	6.1
	$9.2 \cdot 10^{-4}$	4.8	5.3
	$2.3 \cdot 10^{-3}$	3.7	4.3
No addition	none	3.8	4.8

TABLE VI

CATIONS STIMULATING ATPase ACTIVITY IN DIALYZED RAT-KIDNEY ENDOPLASMIC RETICULUM PREPARATIONS

Incubations were as described in Table I except that 100 µmoles of Tris buffer (pH 7.4) were employed.

Cation	In presence of	Concn. required for half-maximal activation of ATPase activity (mM)
Sodium	3.3 mM K^+	8
Potassium	33 mM Na^+	0.67
Ammonium	33 mM Na^+	3.3
Lithium	33 mM Na^+	1.3
Cesium	33 mM Na^+	2
Rubidium	33 mM Na^+	1

The effect of pH on sodium–potassium-stimulated ATPase activity is shown in Table V. The dialyzed endoplasmic reticulum fraction with or without added sodium and potassium shows increased ATPase activity with increasing pH. The stimulation by sodium and potassium addition is slight at pH 6.1 and becomes maximal at about pH 7.3. Ionization of Mg–ATP does not appear to explain the pH effects. Increasing the Mg–ATP from 4 to 10 mM at pH 6.6 does not increase the ATPase activity. The data in Table VI indicate that there are several cations which can replace potassium but not sodium ions in cation stimulation of ATPase activity. The cation levels giving half-maximal stimulation are shown for pH 7.4. An attempt was made with several cations to determine the amount required for half-maximal stimulation at pH 6.6. The lower level of stimulation at pH 6.6 made this very difficult to evaluate for cations other than potassium. In 3 experiments the values for potassium shifted consistently upward from 0.67 mM at pH 7.4 to about 2.5 mM at pH 6.6 suggesting a lowered affinity for potassium ions at lower pH.

Calcium ions can substitute for magnesium in the ATPase system. Activity is

TABLE V

EFFECT OF MERCURIAL DIURETICS ON THE ATPase OF ENDOPLASMIC RETICULUM PREPARATIONS

Appropriate imidazole and Tris buffers were employed. Incubations were otherwise as described in Table I.

Addition	*μmoles phosphate liberated*				
Nondialyzed preparations *pH*	*6.1*	*6.6*	*7.1*	*7.9*	*8.2*
No addition	7.2	7.7	8.6	10.4	10.7
3.4 mM sodium mercaptomerin	4.1	4.4	5.8	8.3	8.9
13.7 mM sodium mercaptomerin	4.8	4.8	4.8	4.9	5.4
pH	*6.1*		*7.1*		*7.9*
No addition	4.3		8.1		11.1
12.1 mM sodium meralluride	4.3		6.3		6.9
Dialyzed preparations *pH*	*6.3*	*6.7*	*7.3*		*8.0*
No addition	3.5	4.6	5.5		6.0
200 mM Na^+ and 33 mM K^+	4.1	5.6	8.0		8.6
200 mM Na^+ and 33 mM K^+ plus 13.7 mM sodium mercaptomerin	2.9	3.6	4.3		4.4
13.7 mM sodium mercaptomerin	2.8	3.2	4.5		4.7
pH	*6.1*		*7.1*		*7.9*
No addition	1.6		2.8		3.5
200 mM Na^+ and 33 mM K^+	2.0		4.1		5.1
200 mM Na^+ and 33 mM K^+ plus 12.1 mM sodium meralluride	1.4		1.9		2.0

60–70% of that obtained with magnesium. Optimal activity is achieved with a Ca:ATP ratio of 1:1. If present at equal levels, calcium ions inhibit the magnesium activation 10–20%. Dialyzed preparations show no sodium–potassium stimulation of the calcium-activated ATPase. At pH 7.4 no sodium–potassium stimulation occurs in dialyzed preparations if calcium and magnesium are present in equal concentration.

Organic mercurial diuretic agents inhibit ATPase activity in both dialyzed and nondialyzed endoplasmic reticulum preparations (Table V). These agents inhibit the sodium–potassium-stimulated ATPase activity in the dialyzed preparation. At pH 7.3 there is 50% inhibition of this stimulation by 0.8 mM sodium mercaptomerin and 100% inhibition by 1.7 mM or higher levels of sodium mercaptomerin and 0.7 mM or higher levels of sodium meralluride. There is a partial inhibition of the increase in ATPase activity associated with increase in pH. There is a component of the ATPase activity which is not affected by the mercurials even at the high levels employed in the experiments in Table V. This insensitive or less sensitive component represents most of the activity found after dialysis of the preparation.

DISCUSSION

The endoplasmic reticulum membrane system is thought to be continuous with the external cell membrane[5]. The identity of the endoplasmic reticulum fractions of liver and pancreas prepared by PALADE AND SIEKEVITZ[3,4] has been fully established by the conspicuous ribonucleoprotein granules attached to much of the endoplasmic reticulum membrane in the whole cell and found on most of the isolated membrane vesicles (microsomes). SJOSTRAND[23] coined the term "cytomembranes" for the endoplasmic reticulum system. α and γ represent the rough and smooth endoplasmic reticulum, respectively. The convoluted tubule cells in the kidney contain deep visible infoldings of the cell membrane into the cytoplasm[11] which SJOSTRAND labelled β-cytomembranes. It seems probable that fragments of these latter membranes constitute a major portion of the kidney fraction employed in our studies.

PALADE AND SIEKEVITZ[3] could not be as certain of the origin of their smooth membrane vesicles as they were of the rough granular vesicles in their preparation. They stated that ruptured fragments of the plasma (external cell) membrane may be included among the smooth membrane vesicles. Since kidney-membrane fragments are devoid of ribonucleoprotein particles there is no label to identify specifically internal cytoplasmic membranes. The term endoplasmic reticulum fraction is applied to this kidney-membrane preparation since it is prepared in the same manner as the liver and pancreas preparations. It is reasonable to assume that the quantitatively larger internal cytoplasmic membrane system of kidney must contribute the major portion of membrane fragments on homogenization. In kidney the visible continuity of the invaginated β-cytomembranes with the plasma membrane makes this distinction almost academic. The term endoplasmic reticulum is currently in use in biochemical studies of cytoplasmic membrane fragments from liver[24].

POST *et al.*[6] compared the magnesium-activated ATPase activity of the isolated red blood cell membrane with the active-transport system for sodium and potassium in the intact red blood cell. The following characteristics were common to the active transport and ATPase systems: both were located in the membrane; both utilized ATP and required sodium and potassium together; potassium was competitively inhibited by sodium in both systems; ammonium ion could substitute for potassium in both systems; the levels of ions for half-maximal stimulation and the level of ouabain for half-maximal inhibition were the same in both systems.

SKOU[7,25] found a magnesium-activated ATPase in crab nerve membrane which required both sodium and potassium and was inhibited by ouabain. The data indicated a separate binding site for potassium and sodium with competitive inhibition by potassium of the sodium site.

Both of the above authors assume that ATPase systems with these characteristics are components of sodium- and potassium-linked active cation-transport systems. The kidney preparation has essentially all the properties of the above ATPase systems. There is a magnesium-activated ATPase system localized in the membrane. When the preparation is dialyzed, much of the ATPase activity, along with potassium and sodium, is lost. When sodium and potassium are added together the ATPase activity is restored. The sodium–potassium-stimulated ATPase activity is inhibited by ouabain. There is a site of binding for potassium and a site of binding for sodium with inhibition of the sodium binding site by potassium. Ammonium ion and several other cations

will substitute for potassium. It can, therefore, be assumed that this ATPase activity also represents a component of a sodium–potassium-linked active cation-transport system. In addition, the specific inhibition of the sodium–potassium-activated component of the ATPase by mercurial diuretic compounds further suggests that this ATPase system is involved in cation transport. The ATPase activity of the endoplasmic reticulum membrane fraction appears to behave as a magnesium-activated system containing maximal levels of sodium and potassium in the membrane. As a consequence of dialysis this sodium and potassium is lost and can be replaced by sodium and potassium from the medium. Under these conditions manifestations of an ATPase system associated with the active transport of sodium and potassium are clearly visible.

Ouabain and calcium completely inhibit the stimulation of ATPase activity by added sodium and potassium. They have little or no effect on the nondialyzed preparation. This implies that their action is on uptake of cations rather than the ATPase activity itself and further supports the concept that the nondialyzed preparation already contains maximal levels of sodium and potassium. Similar effects of calcium were reported by DUNHAM AND GLYNN[26] for ATPase activity of red blood cell ghosts.

The inhibition by mercurial diuretics of a kidney enzyme system linked to sodium and potassium transport could explain their therapeutic action. In the dog- and rat-kidney cortex mercurial diuretics are concentrated several hundred fold over plasma levels attaining tissue concentrations of the mercurial similar to the levels of mercurials that were inhibitory in these experiments[27]. Studies with the dialyzed preparations indicate that it is the ATPase activity activated by sodium and potassium which can be completely inhibited. Most of the remaining ATPase activity is unaffected at the level of mercurial employed although there appears to be a reduced response of the ATPase activity to an increase of pH when the mercurial is present. The decreased mercurial inhibition of ATPase activity found in the nondialyzed preparation suggests that the ATPase activity may be protected by sodium and potassium already present in the preparation. This would imply that the cation binding sites are sensitive to mercurials.

The increase in ATPase activity with increasing pH in the physiological pH range is striking. It is possible that hydrogen ions compete directly with sodium and potassium for the cation binding sites. Similar sensitivity to pH was seen in the ATPase activity of red blood cell ghosts by DUNHAM AND GLYNN[26]. SANUI *et al.*[28,29] have reported competition between hydrogen ions and sodium and potassium in binding sites of rat-liver microsomes and erythrocyte ghosts.

The microsomal fraction from liver or pancreas when separated by centrifugation from mitochondria has been shown to contain membranes and ribonucleoprotein particles[3,4]. The present study shows that a membranous submicrosomal fraction from kidney has properties associated with the active transport of sodium and potassium. A study of a similar brain microsomal fraction, with many findings similar to those reported in this paper, has been reported recently by ALDRIDGE[30].

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