Relation of Na-K-ATPase to Acute Changes in Renal Tubular Sodium and Potassium Transport

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ABSTRACT Renal Na-K-ATPase activity changes adaptively in response to chronic alterations in sodium reabsorption or potassium secretion, but the role of this enzyme in rapid adjustments of renal tubular Na and K transport is not known. To evaluate this question, microsomal Na-K-ATPase specific activity and kinetics were determined in the rat and guinea pig kidney after massive but short-term (3 h) sodium or potassium loading. In other experiments renal sodium handling was evaluated in hydropenic and saline-loaded rats in which enzyme synthesis was prevented by the concurrent administration of actinomycin D or cycloheximide. Saline loading increased net sodium reabsorption in both rats and guinea pigs, but microsomal Na-K-ATPase from the outer medulla (where the reabsorptive increment is greatest) did not change significantly in either species. In vitro [3H]ouabain binding to guinea pig microsomes and apparent $K_m$ for sodium of rat microsomal Na-K-ATPase, both from outer medulla, were also unaltered. Actinomycin D and cycloheximide failed to increase sodium excretion and microsomal Na-K-ATPase remained unchanged. KCl loading resulted in a 10-fold increase in K excretion but again Na-K-ATPase specific activity (in cortex, outer medulla, and papilla), and its apparent $K_m$ for potassium were not affected. Taken together these results suggest that rapid adjustments in renal tubular Na or K transport are mediated by mechanisms that do not involve the Na-K-ATPase enzyme system.

INTRODUCTION

Sodium-potassium-activated adenosine triphosphatase (Na-K-ATPase) is considered to play a major role in cation transport across cell membranes (1-4). This enzyme system is presumed to be identical to the "sodium-potassium pump," since the hydrolysis of ATP catalyzed by it is translated into the vectorial movement of sodium and potassium against their electrochemical gradients (5, 6). In the kidney Na-K-ATPase is present in highest concentrations in the outer medulla (7, 8) and particularly in the thick ascending limbs of Henle's loops (9), and has been demonstrated mainly at the peritubular cell membrane (10, 11), in accord with its postulated role in active cation transport by the renal tubule.
Renal Na-K-ATPase changes adaptively in response to chronic changes in the reabsorption of sodium or secretion of potassium in a variety of physiological and experimental circumstances (12–15). In these studies alterations in cation transport lasted for days or weeks, and the parallel variations in Na-K-ATPase activity lent support to the concept that the enzyme is involved in the renal adaptation to long-term changes in the reabsorption or secretion of sodium and potassium. However, the role of Na-K-ATPase in rapid adjustments of renal tubular sodium and potassium transport is not known.

The present studies were designed to evaluate if Na-K-ATPase participates in the modulation of rapid changes in renal sodium reabsorption and potassium secretion. The specific activity and kinetics of renal Na-K-ATPase were measured in rats and guinea pigs after massive but short-term Na or K loading. In addition, we evaluated renal sodium and potassium handling in antidiuretic and saline-loaded rats in which the formation of new enzyme was prevented by the concurrent administration of the metabolic inhibitors actinomycin D and cycloheximide.

**METHODS**

Adult male Sprague-Dawley rats and albino guinea pigs of the Hartley strain were used in all experiments. Animals were fed a standard chow diet and had free access to tap water. Kidney function was measured in vivo and at the completion of the experiments microsomal Na-K-ATPase was measured in vitro in the same animals. The microsomal fraction was chosen because it contains the highest Na-K-ATPase specific activity of all fractions studied (12).

**Sodium Chloride Loading**

Rats and guinea pigs were anesthetized with Inactin 120 mg/kg body wt intraperitoneally, a tracheostomy was performed and the bladder and a jugular vein were cannulated with polyethylene PE50 tubing. The animals were placed on a heated platform and their rectal temperature was monitored by a thermistor probe and maintained between 37 and 38°C.

Control and experimental animals were handled identically and studied simultaneously. Guinea pigs received isotonic NaCl at the rate of 0.42 ml/min (±1.0 ml/min/kg body wt) and rats at the rate of 0.58 ml/min (±1.3 ml/min/kg body wt) for 3 h, while the controls of both groups received the same infusion at the rate of 0.02 ml/min for the same length of time. Fluid was delivered with a Harvard constant infusion pump (model 975, Harvard Apparatus Co., Inc., Millis, Mass.). The 3-h period permits adequate measurement of renal function and at the same time allows experimental manipulations producing large changes in Na and K transport.

**Potassium Chloride Loading**

Rats were prepared as described above. Experimental animals received 0.02 ml/min of a 1 M KCl solution (±70 μmol/min/kg body wt) for 3 h while their controls were infused with isotonic saline at the same rate. Na-K-ATPase was measured in the
entire kidney in one series of experiments, and separately in the cortex, outer medulla, and papilla in another.

Studies with Metabolic Inhibitors

The effect of actinomycin D and cycloheximide on sodium handling by the rat kidney was evaluated in antidiuretic and saline-loaded animals. Experimental rats received at the beginning of infusion either 2 mg/kg body wt cycloheximide (Sigma Chemical Co., St. Louis, Mo.) or 1 mg/kg body wt actinomycin D (Merck, Sharp & Dohme, West Point, Pa.) intravenously, while controls received an equal volume of isotonic saline. One hour later (2 h before sacrifice) all animals in the cycloheximide experiments received 25 μCi/100 g body wt of [3H]leucine (New England Nuclear, Boston, Mass.).

At the completion of the study whole kidneys were homogenized and Na-K-ATPase specific activity assayed (see below). To measure [3H]leucine incorporation into protein, 2-ml aliquots of the microsomal fraction were precipitated with 10% trichloroacetic acid. The precipitate was washed three times with the homogenizing medium used to prepare the tissue suspensions, and resuspended in 2 ml Protosol (New England Nuclear) for digestion. When the solution became clear it was added to 15 ml of a scintillation mixture (Aquasol, New England Nuclear) and counted for 10 min in a Packard Tri Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) [3H]leucine incorporation was expressed as dpm/mg protein.

Renal Function

Glomerular filtration rate (GFR) was measured by the clearance of inulin. After a priming dose of 20 mg, a sustaining infusion sufficient to maintain plasma inulin levels between 40-80 mg/100 ml was administered throughout the experiment. After an equilibration period of 60 min, urine was obtained under mineral oil in preweighed plastic tubes for four consecutive 30-min collection periods and the volumes determined by weighing to the nearest 0.1 mg. Blood from the cut end of the tail was collected in capillary tubes at the beginning and end of each collection period and plasma concentrations calculated as the average of the two determinations. Inulin was measured by a semimicro modification of the anthrone method (16), and sodium and potassium by flame photometry. Fractional and absolute sodium and potassium excretion and net tubular sodium reabsorption were calculated from standard equations.

Enzyme Studies

All determinations were done simultaneously in experimental and control animals. At the end of 3 h of infusion animals were rapidly exsanguinated from the aorta and the kidneys immediately chilled in an ice-cold buffered sucrose solution used subsequently for tissue homogenization. Kidneys were either used whole or cut longitudinally, placed in Petri dishes on filter paper moistened with cold saline, and the outer medulla dissected with iris scissors from the cortex and papilla. The entire kidney or its component zones separately were homogenized in a 10/1 (vol/wt) solu-
tion containing 0.25 mol sucrose, 5 mmol Na$_2$EDTA, 30 mmol histidine buffer per liter, and 0.2% sodium deoxycholate at pH 6.8. The homogenate was centrifuged at 10,800 $g$ for 30 min in a refrigerated Spinco model L preparative ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) to sediment cell debris, nuclei, and mitochondria. The supernate was carefully removed to avoid contamination and centrifuged at 108,000 $g$ for 90 min. The resulting sediment (“microsomal fraction”) was gently resuspended in 0.5-2.5 ml of the original homogenizing solution without deoxycholate and frozen overnight at $-20^\circ$C.

Na-K-ATPase specific activity was determined as follows: 0.1 ml of tissue suspension (final protein concentration 40-100 μg/ml) was used in all assays. ATPase activity was determined in 5 ml of a reaction mixture prewarmed at 37$^\circ$C containing 100 mmol NaCl, 20 mmol KCl, 10 mmol imidazole buffer, 5 mmol MgCl$_2$, and 5 mmol disodium adenosine triphosphate (ATP)/liter at pH 7.8, and in an identical solution containing in addition, 2 mmol ouabain/liter. The reaction was carried out for 5 min at 37$^\circ$C in a shaking water bath, and terminated by the addition of 1 ml ice-cold 35% (wt/vol) trichloroacetic acid. The precipitated protein was discarded after centrifugation and the inorganic phosphate in the supernate determined by the method of Fiske and SubbaRow (17). The Na-K-ATPase was defined as the difference between the inorganic phosphate liberated in the absence and presence of 2 mM ouabain in the incubation mixture. Correction was made for the spontaneous, nonenzymatic breakdown of ATP, measured as the inorganic phosphate liberated under the same experimental conditions in the absence of enzyme, which usually accounted for less than 10% of the total inorganic phosphate present. The protein content of tissue suspensions was determined by the method of Lowry et al. (18), using crystalline bovine albumin as standard. Sodium and potassium activation curves of the microsomal Na-K-ATPase were determined by increasing concentrations of the cation studied (Figs. 1 and 2) in assay media in which all other components were kept constant and the ionic strength maintained with Tris (hydroxymethyl) aminomethane.

Binding of [3H]ouabain to guinea pig microsomes was measured by the method of Matsui and Schwartz (19). Briefly, microsomes were incubated for 3 min at 37$^\circ$C in the presence of 3 × 10$^{-7}$ M [3H]ouabain (specific activity 0.5 mCi/μmol, New England Nuclear) in a 2-ml mixture containing 100 mmol NaCl, 50 mmol Tris-HCl, 5 mmol MgCl$_2$, and 1 mmol EDTA-Tris per liter. Tubes were then centrifuged in a prewarmed rotor (37$^\circ$C) for 3 min at 108,000 $g$, and the pellet dissolved in boiling 0.2 M NaOH. The solution was added to 15 ml of a scintillation mixture and counted in a Packard Tri Carb liquid scintillation counter.

Results are presented as mean ±1 SEM. The statistical significance of the differences between group means was assessed by Student's "t" test; $P$ values less than 0.05 were considered significant.

RESULTS

Sodium Chloride Loading

Despite the considerable natriuresis induced by volume expansion, saline loading in the rat led to an increase in absolute sodium reabsorption (Table I)
TABLE I
EFFECT OF NaCI LOADING ON RENAL SODIUM REABSORPTION AND
MICROSOMAL Na-K-ATPase IN THE RAT

| Sodium reabsorption | Absolute | Fractional | Na-K-ATPase, outer medulla |
|---------------------|----------|------------|--------------------------|
|                     | µg/min   | % of filtered | µmol P<sub>i</sub>/mg protein/h |
| Control             | 483.4±30.1* (14) | 99.88±0.03 (14) | 92.5±4.8 (18) |
| NaCl Loading        | 566.0±21.1 (18)   | 93.21±0.66 (18)   | 98.0±4.2 (21)   |
| * = ±SEM.           |           |      |                          |
| * Not significant.  |           |      |                          |

In parentheses = number of animals.

because the increase in filtered sodium load exceeded the decline in fractional reabsorption. Although overall sodium reabsorption increased only 17%, sodium reabsorption in the loop of Henle probably increased to twice (20) or three times (21) the level in control animals. This assumption is based on micropuncture studies which demonstrated that saline loading in the rat markedly increases distal delivery of filtrate, and that the bulk of this increment is reabsorbed in the ascending limb of Henle's loops (20-22). In spite of the large increase in sodium chloride reabsorption, microsomal Na-K-ATPase specific activity in the outer medulla (where the thick ascending limbs of Henle's loops predominate) remained unchanged (98.0 ± 4.2 µmol P<sub>i</sub>/mg protein/h, P = 0.40).

The relation of microsomal Na-K-ATPase activity to substrate sodium concentration was examined in separate experiments in which the entire outer medulla of three animals was pooled to yield sufficient tissue for assay. The results from 13 experiments in salt-loaded and control rats including 38 animals in each group are summarized in Fig. 1. As described by others (23) we also found that sodium activation curves of Na-K-ATPase differ from those predicted by the Michaelis-Menten equation, probably due to the allosteric interaction of sodium with the enzyme (24). The activation curves with increasing sodium concentrations showed a sigmoidal pattern and were similar in salt-loaded and in control rats. Furthermore, the apparent K<sub>m</sub> of the enzyme from salt-loaded animals was not appreciably different from that of controls.

To evaluate further the effect of large increments in sodium reabsorption on outer-medullary microsomal Na-K-ATPase, we repeated the same experiments in guinea pigs (Table II). In this species, the binding of radiolabeled ouabain to microsomes offers another way of evaluating Na-K-ATPase since it represents an indirect estimate of the active sites present on the enzyme. As in the rats, fractional sodium reabsorption decreased and absolute sodium reabsorption increased after saline loading. Again, the
FIGURE 1. Sodium activation curves of microsomal Na-K-ATPase from rat outer medulla. Shape of the curves and apparent $K_m$ were similar in NaCl-loaded and control animals.

**TABLE II**

| Sodium reabsorption | Na-K-ATPase, outer medulla | [H]Ouabain binding |
|---------------------|---------------------------|-------------------|
|                     | Absolute | Fractional | $a^*$ | $b^*$ | pmol/$U$ Na-K-ATPase$±$ |
| Control             | 333.4±16.9 | 99.48±0.08 | 60.2±3.9 | 45.7±5.3 | 112.9±5.7 | 2.08±0.23 |
| NaCl Loading        | 422.7±22.5 | 94.66±0.54 | 65.1±4.1 | 46.7±8.3 | 129.9±8.4 | 2.01±0.24 |
| $P$                 | <0.005 | <0.001 | NS | NS | NS |

* Assay medium Na concentration = 100 mM ($a$), 20 mM ($b$).

specific activity of the microsomal Na-K-ATPase from the outer medulla was not significantly different in salt-loaded and control guinea pigs. The lack of effect of salt loading on the enzyme was apparent both when the in vitro assay was performed under standard conditions ($a$) and when the sodium concentration of the medium was kept at 20 mmol/liter ($b$) to simulate more closely the intracellular conditions in which the enzyme probably operates. This concentration is also close to the apparent $K_m$ for Na demonstrated in the rat (Fig. 1).

[3H]Ouabain binding to the microsomal Na-K-ATPase was also similar in the salt-loaded and control animals, whether expressed in picomoles ouabain bound per milligram microsomal protein or per unit of Na-K-ATPase activity (micromoles $P_i$ per hour), suggesting that the number of active enzyme
sites was not different in the two groups. The amount of \[^{[4}\text{H}]\text{ouabain bound, approximately 2 pmol} / \text{U enzyme, agrees closely with results obtained in other tissues (19, 25), although it is somewhat higher than that reported in guinea pig outer medulla by Hendler, et al. (7).}\]

**Potassium Chloride Loading**

After KCl loading, potassium excretion (12.3 \(\mu\text{eq/min}\)) was 10-fold higher than that of controls (1.2 \(\mu\text{eq/min}\)). Despite this enormous increase in K excretion, Na-K-ATPase specific activity in the whole kidney or in the cortex and outer medulla assayed separately was practically identical in the two groups (Table III). Papillary Na-K-ATPase assayed in three pooled samples from three animals each was very low and averaged 4.8 and 2.8 \(\mu\text{mol} \text{P}_i / \text{mg protein/h}\) in the control and KCl-loaded rats, respectively.

The potassium activation curves of microsomal Na-K-ATPase were similar, and its apparent \(K_m\) for potassium was identical (0.5 mmol/liter) in the two groups (Fig. 2), indicating that massive, acute KCl loading also does not alter the affinity of the enzyme for potassium.

**TABLE III**

| EFFECT OF KCl LOADING ON RENAL POTASSIUM EXCRETION AND MICROSONAL Na-K-ATPASE IN THE RAT |
|--------------------------------------------------------------------------------------------|
| Absolute | Fractional | Na-K-ATPase | Whole kidney | Cortex | Outer medulla |
|------|------|---------|-------------|--------|-------------|
| \(\mu\text{eq/min}\) | % of filtered | \(\mu\text{mol P}_i / \text{mg protein/h}\) | \(\mu\text{mol P}_i / \text{mg protein/h}\) | \(\mu\text{mol P}_i / \text{mg protein/h}\) | \(\mu\text{mol P}_i / \text{mg protein/h}\) |
| Control | 1.22±0.17 (21) | 9.95±4.40 (21) | 76.4±2.5 (6) | 98.4±0.7 (9) | 98.1±7.0 (9) |
| KCl Loading | 12.26±0.49 (20) | 50.32±3.06 (20) | 75.4±2.4 (6) | 99.7±7.4 (8) | 99.7±7.4 (8) |
| \(P\) | <0.001 | <0.001 | NS | NS | NS |

**CONTROL**

\(n=10\)

**K\(^+\) LOADED**

\(n=10\)

**Figure 2.** Potassium activation curves of microsomal Na-K-ATPase from rat kidneys. Shape of the curves and apparent \(K_m\) were identical in KCl-loaded and control animals.
**Inhibitors of Protein Synthesis**

The role of Na-K-ATPase in the modulation of acute changes in renal sodium transport was also studied in experiments in which enzyme synthesis de novo was inhibited by actinomycin D (Table IV) and cycloheximide (Table V). It was postulated that if enhanced sodium reabsorption is dependent on

### Table IV

**EFFECT OF ACTINOMYCIN D ON RENAL SODIUM EXCRETION AND MICROSOMAL Na-K-ATPase IN THE RAT**

| Sodium excretion          | Absolute | Fractional |
|---------------------------|----------|------------|
|                           | µeq/min  | % of filtered | µmol Pi/mg protein/h |
| (A) Antidiuresis          |          |             |
| Control                   | 1.46±0.28 (8) | 0.28±0.04 (8) | 58.8±3.2 (8) |
| Actinomycin D             | 1.43±0.46 (9) | 0.23±0.07 (9) | 60.3±2.3 (9) |
| *P*                       | NS       | NS         |
| (B) NaCI loading          |          |             |
| Control                   | 45.01±2.85 (8) | 7.57±0.58 (8) | 57.5±0.6 (8) |
| Actinomycin D             | 23.58±2.39 (9) | 4.08±0.31 (9) | 58.4±1.1 (9) |
| *P*                       | <0.001   | <0.001     |

### Table V

**EFFECT OF CYCLOHEXIMIDE ON RENAL SODIUM EXCRETION AND MICROSOMAL Na-K-ATPase IN THE RAT**

| Sodium excretion          | Absolute | Fractional | Na-K-ATPase | [3H]leucine incorporation |
|---------------------------|----------|------------|-------------|--------------------------|
|                           | µeq/min  | % of filtered | µmol Pi/mg protein/h | dpm/mg protein |
| (A) Antidiuresis          |          |             |             |                          |
| Control                   | 1.10±0.35 (11) | 0.19±0.06 (11) | 58.6±1.9 (11) | 5915±345 (11) |
| Cycloheximide             | 0.20±0.04 (10) | 0.05±0.02 (10) | 60.5±2.3 (10) | 2092±373 (10) |
| *P*                       | <0.025   | NS         | NS          | <0.001                   |
| (B) NaCI loading          |          |             |             |                          |
| Control                   | 39.80±6.19 (12) | 7.16±0.58 (12) | 56.3±1.1 (12) | 6545±353 (12) |
| Cycloheximide             | 5.90±0.75 (12) | 1.47±0.18 (12) | 56.5±1.5 (12) | 1804±187 (12) |
| *P*                       | <0.001   | <0.001     | NS          | <0.001                   |

the formation of new enzyme but the amount is too small to be detected in the specific activity assay, more sodium should be excreted when enzyme synthesis is blocked. The effect of each inhibitor was evaluated both under basal conditions of sodium reabsorption (antidiuresis) and during saline administration when the reabsorptive mechanism was stressed by the large sodium load.
The specific activity of Na-K-ATPase was not altered by either inhibitor. This observation, besides indicating that no new enzyme was formed, also suggests that the preformed enzyme has a slow degradation rate so that no appreciable decrease in its activity is apparent within 3 h. The inhibition of enzyme synthesis by actinomycin D can only be inferred, but was evident in the cycloheximide study from the striking drop in $[^3H]$leucine incorporation into protein to 20–30% of the control value. Surprisingly, sodium excretion not only did not rise, but usually decreased in the presence of either inhibitor. This decrease was due to a reduction in glomerular filtration rate probably secondary to a renal vasoconstrictor effect. Nonetheless, when normalizing for the different filtration rates by expressing sodium excretion per unit sodium filtered, fractional sodium excretion was the same in hydropenic animals receiving either actinomycin D or cycloheximide. It appears therefore that inhibition of Na-K-ATPase synthesis does not reduce the ability of the kidney to reabsorb sodium, at least over a period of several hours. Moreover, the specific activity of Na-K-ATPase did not decrease during this time interval even though absolute sodium reabsorption was markedly reduced in animals treated with actinomycin D and cycloheximide.

**DISCUSSION**

By analogy to events occurring in chronic adaptation, the initial postulate tested in this study was that the number of transporting units should increase in parallel with the number of ions transported, if Na-K-ATPase modulates rapid adjustments in sodium and potassium movement across the renal tubule. This change would be manifest as an increase in the specific activity of the enzyme (Fig. 3 B), and could result either from de novo synthesis, or from activation or decreased degradation of preexisting enzyme. The enzyme from the outer medulla was studied for several reasons: First, the outer medulla is very rich in Na-K-ATPase (8, 26) and contains the thick ascending limbs of Henle’s loops in which the highest Na-K-ATPase specific activity in the nephron has been demonstrated with microchemical techniques (9). Second, during saline loading the combination of increased glomerular filtration and decreased proximal reabsorption results in a massive increase in absolute sodium chloride reabsorption in the loop of Henle (20–22), an increment larger than can be achieved in any other nephron segment in the intact kidney in vivo. Furthermore, it has been recently suggested that the increase in Na-K-ATPase and the associated accelerated potassium secretion after chronic K loading also occur in the outer medulla (27).

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1 Although fractional sodium excretion actually decreased in saline-loaded animals treated with actinomycin D or cycloheximide, the interpretation of these results is complicated by the hemodynamic effects of the two inhibitors which probably interfered with the natriuresis by decreasing renal plasma flow and glomerular filtration.
Figure 3. Diagrammatic representation of a conceptual model of a renal tubular cell and the postulated pump responsible for active Na and K movement. (A) Resting state. The pumps are located on the basal side of the cell, with the units occupied by cations in the process of being transported depicted as solid circles. Na-K-ATPase specific activity in vitro is assumed to reflect the total number of transporting units in this model. B through D represent three hypothetical alternatives caused by doubling the number of cations transported by the cell: (B) Total enzyme doubles while saturation of its active sites remains the same; (C) total enzyme and its saturation remain the same, but its affinity for either cation doubles; (D) total enzyme and its kinetics remain the same, but the saturation of previously unused active sites doubles. These theoretical considerations are further discussed in the text.

Figure 4. Specific activity of microsomal Na-K-ATPase from rat and guinea pig outer medulla. Acute NaCl or KCl loading did not affect the enzyme from either species.
The results (summarized in Fig. 4) indicate that Na-K-ATPase-specific activity did not change after either acute NaCl or acute KCl loading, in both rats and guinea pigs. Large increments in sodium reabsorption also did not change the number of active enzyme sites since ouabain binding to microsomal Na-K-ATPase was similar in saline-loaded and control guinea pigs. Furthermore, inhibiting new enzyme synthesis did not interfere with the ability of the kidney to reabsorb sodium, since fractional sodium excretion in antidiuresis was not different in actinomycin D- or cycloheximide-treated animals and controls.

Na-K-ATPase could participate in the mediation of the observed increments in sodium reabsorption or potassium secretion without an increase in specific activity if its affinity for these cations increased (Fig. 3 C). This possibility was not substantiated in the present studies in which the sodium and potassium activation curves and the apparent $K_m$ for these two cations were essentially identical in experimental and control animals (Figs. 1 and 2).

Finally, it is theoretically possible that Na-K-ATPase does play a role in acute alterations in cation transport yet no change in its specific activity or kinetics is detectable when the enzyme is studied in vitro. Such a situation might obtain if the in vivo concentration of the enzyme were substantially higher than that required under basal conditions, in which case cation transport might be enhanced by increasing saturation of unused active sites with Na or K (Fig. 3 D). Indeed, it has been suggested that tissue concentrations of some enzymes are much higher than those used in studies in vitro (28), and that Na-K-ATPase is present in excess in the kidney (29). While this possibility cannot be ruled out from the present experiments, and since Na-K-ATPase in vivo cannot be adequately measured it may not lend itself to experimental verification, the fact that such large increments in Na and K transport could occur without changes in the enzyme argues against this hypothesis.

The conclusion emerging from these studies, expressed with the reservation that the enzyme measured in vitro might not reflect accurately its activity in vivo, is that Na-K-ATPase does not mediate acute changes in renal tubular sodium and potassium transport. Consistent with this concept is the demonstration that this enzyme turns over slowly, its half-life being probably measured in days (23, 30, 31). In this respect it is of interest to note that renal Na-K-ATPase is not decreased even 24 h after death (32). Several studies which dealt with this question only peripherally also failed to demonstrate changes in Na-K-ATPase that would suggest its participation in acute alterations in cation transport. Renal Na-K-ATPase was not affected by acute reduction in sodium reabsorption produced by renal artery constriction in the dog (33, 34) or after 24 h of complete ureteral obstruction in the rat (35). Hill et al. (36) could not demonstrate significant changes in the specific
activity or the kinetics of Na-K-ATPase from toad bladders in which sodium transport (measured by the short-circuit current) increased markedly for 2½ h under the influence of aldosterone. Finally, in experiments somewhat similar to ours, infusion of large amounts of isotonic NaCl for 1–4 h did not alter renal Na-K-ATPase in the dog (37).

While very little is known about the biochemical basis of renal tubular K transport besides the fact that Na-K-ATPase probably participates in the chronic adaptation to increased K excretory loads (15), several mechanisms of active Na transport in the kidney have been proposed. Whittentbury and colleagues (39, 40) have postulated the existence of a second pump refractory to ouabain but sensitive to ethacrynic acid, and suggested that it mediates Na extrusion accompanied by Cl efflux rather than by exchange with external K as does the pump deriving its energy from Na-K-ATPase. Moreover, several studies have demonstrated that a substantial part of the tubular reabsorption of sodium continues when Na-K-ATPase is nearly completely inhibited by digitalis glycosides (29, 37, 41), indicating that the enzyme is required only for a portion of the total sodium reabsorption. Thus, the suggestion has been made that not two, but possibly three mechanisms of sodium reabsorption operate in the kidney (42). Finally, recent evidence indicates that at least in the ascending limb of Henle's loop the reabsorption of sodium may be passive (43, 44). The results of the studies reported here, while not excluding it unequivocally, do not support the concept that Na-K-ATPase modulates rapid changes in renal sodium or potassium transport. Whether such changes are mediated by an alternative mechanism of active transport or through a nonactive step in the sequence which results in the translocation of these ions remains to be determined.

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