Modulation of Renal Sodium-Potassium-Adenosine Triphosphatase by Aldosterone

Effect of High Physiologic Levels on Enzyme Activity in Isolated Rat and Rabbit Tubules

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Abstract

The purpose of this study was to determine the nephron site, time course, and mechanism of mineralocorticoid action on renal tubular Na-K-ATPase in rats and rabbits, without dietary manipulation and by using the natural mineralocorticoid aldosterone. Sustained, high physiologic levels of circulating aldosterone mimicking those produced endogenously during potassium loading or sodium deprivation were provided by constant delivery of the hormone in doses of 5 or 50 μg/100 g body wt per 24 h, respectively, from osmotic minipumps implanted subcutaneously.

In adrenal-intact rats receiving the 5-μg dose, aldosterone levels were similar to those seen in animals fed a high K diet and produced a time-dependent increase in Na-K-ATPase activity in the cortical-collecting tubule (CCT) to a level 103% higher than in controls after 7 d (2,007±178 vs. 989±72 pmol/mm per h, $P < 0.001$); the enzyme activity in the proximal convoluted tubule, medullary thick ascending limb, and the inner stripe of the medullary-collecting tubule did not change significantly. The increment in CCT Na-K-ATPase was larger (142%) in animals receiving for the same period of time the 50-μg dose, which produced circulating aldosterone levels similar to those of sodium-deprived rats. A significant stimulation of Na-K-ATPase activity was seen in the CCT of adrenalectomized rats after 24 h of treatment with either dose of the hormone, and at 12 h only in animals receiving the 50 μg/100 g per 24 h regimen. To determine whether the enhanced Na-K-ATPase activity produced by aldosterone is due to synthesis of new enzyme units or to alteration in its kinetics, we examined the ouabain-binding capacity and the affinity for Na and K of the enzyme from CCT of rabbits treated with 5 μg/100 g body wt per 24 h aldosterone for 3 d. These experiments revealed a parallel increment on Na-K-ATPase activity and specific [^{3}H]ouabain binding in aldosterone-treated rabbits, while the affinity of the enzyme for either sodium or potassium was unaltered.

The results of this study indicate that high physiologic levels of aldosterone simulating those measured during K loading or Na deprivation lead to a segment-specific increase in Na-K-ATPase activity in the CCT. This effect was time- and dose-dependent and was due to an increase in the number of active enzyme units. The segmental specificity and time course of the increase in enzyme activity suggest that modulation of Na-K-ATPase by aldosterone plays a role in the chronic adaptation of the CCT to altered availability of sodium and potassium, and therefore in the homeostasis of these cations by the kidney.

Introduction

It has been established beyond doubt that renal Na-K-ATPase is under partial corticosteroid control, as its activity invariably decreases after adrenalectomy and is restored by administration of adrenal steroids (reviewed in reference 1). Recent studies with isolated nephron segments using physiologic doses of hormone indicate that the enzyme activity in the cortical-collecting tubule (CCT),[1] the major binding site for both classes of corticosteroids, is normally modulated by mineralocorticoid, rather than glucocorticoid hormones (1, 2).

Chronic changes in mineralocorticoid activity, whether produced by dietary manipulation of endogenous hormone levels (3–7) or administration of exogenous hormone (5, 7, 8), alter the structure and function of the mammalian CCT. Basolateral cell membrane surface area has been found to augment in the principal cells of this nephron segment in animals fed a high K, low Na diet (7) or treated with deoxycorticosterone (7, 8). Schwartz and Burg (6) demonstrated a clear relationship between sodium and potassium transport by the isolated rabbit CCT perfused in vitro and the endogenous plasma aldosterone level (altered by varying Na and K intake) in the animals from which the tubules were obtained. Similarly, Natke and Stoner (9) described enhanced transport of sodium across the basolateral membrane of CCT cells obtained from rabbits in which endogenous aldosterone levels were increased by a low sodium diet.

Sustained changes in Na-K-ATPase activity in the CCT have also been produced by administration of exogenous mineralocorticoids (10, 11) or by dietary modulation of their endogenous production (10, 12). However, interpretation of such experiments is often complicated by the following considerations: first, in most studies with exogenous steroid, investigators used deoxycorticosterone acetate (DOCA) rather than

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1. *Abbreviations used in this paper:* ADX, adrenalectomized; CCT, cortical-collecting tubule; DOCA, deoxycorticosterone acetate; MAL, medullary thick ascending limb of Henle's loop; MCT, medullary-collecting tubule (inner stripe); PCT, proximal convoluted tubule.

the main natural mineralocorticoid aldosterone, and the hormone was usually given in pharmacologic doses. Second, during dietary manipulations factors other than aldosterone (e.g., altered delivery of Na and K to the CCT) may modify Na-K-ATPase activity in this nephron segment. Relevant in this respect are observations that in kidney homogenates the rate of decline in Na-K-ATPase activity after adrenalectomy is dependent on the intake of sodium (13), and that the supply of sodium takes precedence over administration of DOCA in the restoration of enzyme activity in adrenalectomized rats (14). Moreover, Na-K-ATPase activity in the collecting tubule varies directly with the potassium content of the diet (15, 16).

The present study was designed to assess the effects of sustained increments in circulating aldosterone on Na-K-ATPase activity of individual nephron segments. The hormone was given by constant infusion in doses calculated to obtain levels similar to those produced endogenously during potassium loading or sodium deprivation, but without changing the dietary supply of these cations. We have examined the segment localization and the time course of the stimulation of Na-K-ATPase activity by aldosterone in tubules microdissected from rat and rabbit kidneys, and have explored possible mechanisms underlying this phenomenon.

Methods

Studies were performed in male albino Sprague Dawley rats (Laboratory Supply Co., Inc., Indianapolis, IN) weighing 180–230 g, and male New Zealand White rabbits (Thompson Research Foundation, Monee, IL) weighing 0.8–1.5 kg. All animals were fed a normal chow diet and drank tap water ad lib., except adrenalectomized rats (groups IV–VI), which received 0.3% NaCl as drinking fluid.

Studies in rats

Aldosterone was given by constant subcutaneous delivery from osmotic minipumps to achieve high physiologic levels simulating potassium loading (~125 ng/dl) or sodium depletion (>400 ng/dl) (17). Rats were distributed into the following groups.

Adrenal-intact rats. Group I (n = 27) underwent a sham surgical procedure and constituted the control group.

In group II (n = 24), osmotic minipumps (ALZET model 2001; Alza Corp., Palo Alto, CA) containing aldosterone in concentrations calculated to deliver 5 μg/100 g body wt per 24 h at the rate of 1 μl/h were implanted subcutaneously in the interscapular region.

In group III (n = 10) the aldosterone content of the osmotic minipumps was calculated to provide 50 μg/100 g body wt per 24 h at the same delivery rate.

Na-K-ATPase activity in CCT was determined in all three groups 7 d after initiation of hormone administration. In addition, in rats from groups I and II, enzyme activity in the proximal convoluted tubule (PCT), thick medullary ascending limb (MAL), and outer medullary-collecting tubule (inner stripe) (MCT) was measured after 7 d. Na-K-ATPase activity in CCT was also determined 1 and 3 d after start of hormone administration in rats from group II.

Adrenalectomized rats. To evaluate short-term effects of aldosterone while avoiding the increased endogenous production of the hormone due to the stress of surgery and anesthesia, we studied rats adrenalectomized (ADX) 7 d before study. Rats in groups IV–VI underwent bilateral adrenalectomy under pentobarbital anesthesia (25 mg/kg i.p.) using a lumbar approach.

Group IV rats (n = 12) received no hormone (ADX control).

In group V rats (n = 13), osmotic minipumps delivering 5 μg/100 g body wt per 24 h of aldosterone were implanted subcutaneously.

In group VI (n = 12), aldosterone was infused by osmotic minipumps at a rate of 50 μg/100 g body wt per 24 h.

In these last two groups, Na-K-ATPase activity in CCT was measured 12 and 24 h after pump implantation with appropriate time controls from group IV.

Metabolic balance study. Rats of groups I–III were individually placed in metabolic cages (Nalge Co., Div. of Sybron Corp., Rochester, NY) for two separate periods of 24 h (6th and 7th d of hormone administration). On the first day, they were allowed to acclimate to the cage; food and fluid intake and the urinary excretion of sodium and potassium were determined during the second study period (7th d).

Sodium and potassium were measured by flame photometry with lithium as the internal standard (model IL 343; Instrumentation Laboratory, Inc., Lexington, MA). Serum aldosterone was measured with a commercial aldosterone radioimmunoassay kit (no. Kald-2; Diagnostic Products Corp., Los Angeles, CA).

Tubule microdissection and ATPase assay. The procedures for tubule microdissection and Na-K-ATPase assay have been reported in detail previously (18, 19) and are described only briefly here. The animals were killed by exsanguination from the abdominal aorta, serum was separated for electrolyte and hormone determinations, and the left kidney was perfused with a collagenase-containing solution. Renal tubules were dissected in the cold under stereomicroscopic observation in a medium containing (in millimolars): NaCl, 137; KCl, 5; $MgSO_4$, 0.8; Na_2HPO_4, 0.44; $MgCl_2$, 1; $CaCl_2$, 0.25; Tris-HCl, 10; pH 7.4. No attempt was made to distinguish between segments from superficial or juxtamedullary nephrons. The tubules were individually transferred to a concave bacteriological slide and photographed to determine their length. ATPase activity was measured after a two-step permeabilization procedure, by incubation of each tubule segment for 15 min at 37°C in a 1-μl droplet of the following solutions: For the determination of total ATPase activity (in millimolars), NaCl, 50; KCl, 5; $MgCl_2$, 10; EGTA, 1; Tris-HCl, 100; Na_2ATP (grade II, vanadate-free; Sigma Chemical Co., St. Louis, MO), 10; and [γ-^{32}P]ATP (Amersham Corp., Arlington Heights, IL) in tracer amounts (~5 nCi/μl). For determination of Mg-dependent ATPase activity, NaCl and KCl were omitted, Tris-HCl was 150 mM, and 1 mM ouabain was added. Phosphate liberated by the hydrolysis of [γ-^{32}P]ATP was separated by filtration through a Millipore filter (Millipore Corp., Bedford, MA) after absorption of the unhydrolyzed nucleotide on activated charcoal, and the radioactivity was counted in a liquid scintillation spectrophotometer (Packard Instrument Co., Inc., United Technologies, Downers Grove, IL).

Total and Mg-dependent ATPase activities were determined on five replicate samples (each consisting of two to three pieces of tubule from a given segment) from individual animals, and were expressed as picomoles of inorganic phosphate liberated per millimeter of tubule length per hour. Sodium- and potassium-dependent, ouabain-inhibitable ATPase was taken as the difference between the means of each group of measurements, and thus represents a single data point in each animal; the results show mean values of these determinations in all animals within a given experimental group. To minimize the variability between experiments, one appropriate control and one animal from each experimental group were studied simultaneously.

Studies in rabbits

This species was chosen because rat tissues are less adequate for ouabain binding measurements. Control rabbits (n = 23) underwent a sham operation and those in the experimental group (n = 20) received aldosterone (5 μg/100 g body wt per 24 h) for 3 d by constant delivery from an osmotic minipump implanted subcutaneously in the interscapular region. At the end of the study tubules were microdissected and Na-K-ATPase activity in CCT was determined as described. Affinity of the enzyme for Na and K was determined in separate experiments from activation curves obtained with increasing concentrations of each cation, while the concentrations of all other ligands were kept constant (18). In these experiments osmolality was also kept constant by varying the concentration of Tris-HCl, and Tris ATP was used instead of Na_2ATP. [^{3}H]Ouabain binding to CCT was determined

Table I. Metabolic Balance Study

Group	n	Body weight Day 1	Body weight Day 8	Food intake	Water intake	Urine vol	$U_{Na} \cdot V$	$U_K \cdot V$	S_{Na}	S_K	S_{ALDO}
		g	*g*	*g/24 h*	*ml/24 h*	*ml/24 h*	*μeq/24 h*	*μeq/24 h*	*meq/liter*	*meq/liter*	*ng/dl*
Sham*	5	216±9	254±8	23.4±0.7	41.5±2.5	16.0±0.9	3,413±363	4,420±458	147.7±0.5	4.2±0.2	34.8±5.8
Aldosterone (5 μg/100 g per 24 h)‡	7	210±4	245±9	20.2±2.3	40.6±5.6	20.5±2.4	2,982±407	3,691±469	145.7±1.9	3.3±0.1‖	125.3±15.7¶
Aldosterone (50 μg/100 g per 24 h)§	8	214±5	262±8	22.6±1.2	52.4±4.4	26.0±3.1	3,480±362	4,211±339	147.5±0.7	3.3±0.2‖	263.7±56.7¶**

Results are mean±SE. S_{ALDO}, serum aldosterone; S_K, serum K; S_{Na}, serum Na; $U_{Na} \cdot V$, urinary sodium excretion; $U_K \cdot V$, urinary potassium excretion. * Group I. ‡ Group II. § Group III. ‖ Significantly ($P < 0.01$) different from group I. ¶ Significantly ($P < 0.001$) different from group I. ** This value is a minimum estimate, as in three of the seven animals in which they were measured aldosterone concentrations were outside the range of the assay (upper limit = 400 ng/dl).

by the method of El Mernissi and Doucet (20), which is summarized below.

Ouabain binding. Individual tubules were placed on small pieces of aluminum foil in wells of a chilled aluminum plaque and covered by 1 μl incubation medium containing (for total binding) the following: 250 mM sucrose, 3 mM $MgSO_4$, 3 mM Na_2HPO_4, 1 mM $NaVO_3$, 10 mM Tris-HCl, and 7.5 μM [^{3}H]ouabain (33 Ci/mmol; New England Nuclear, Boston, MA), pH 7.2; for nonspecific binding we used an identical solution containing 100-fold excess unlabeled ouabain. Incubation (30 min) was started by placing the plaques in a water bath at 37°C and stopped by replacing them on ice. Tubules were rinsed four times by adding and then suctioning off 3 μl microdissection solution; they were then left on ice for 60 min, rinsed twice more as above, and transferred to counting vials containing 0.5 ml of 1% deoxycholic acid. After addition of scintillation liquid, bound radioactivity was counted in a Packard liquid scintillation spectrophotometer. Specific [^{3}H]ouabain binding was taken as the difference between total and nonspecific binding, the latter averaging only 5–9% of the total.

Statistical analysis. The *t* test was used for nonpaired samples. When more than two groups were compared (data in Tables I and II, and Fig. 3), Bonferroni inequalities were considered (21) and a level of significance of $0.05/n$ was used, *n* being the number of groups. All results are expressed as mean±SEM.

Results

Chronic effects of aldosterone (adrenal-intact rats)

Metabolic balance study (Table I). Control rats gained on average 6 g/d; after 7 d of aldosterone administration, body weight of animals in groups II and III was similar to control. Food, water, and sodium intakes were also comparable in the control and aldosterone-treated rats, as were the serum sodium and the urinary sodium and potassium excretion. Aldosterone in either dose led to significant hypokalemia. Aldosterone levels were markedly elevated by both treatment regimens, and corresponded to levels previously reported for K-loaded and Na-depleted rats respectively (17). (Plasma aldosterone in control rats reflects the fact that they were under the stress of surgery and anesthesia.)

ATPase activity (Table II). Aldosterone administration with both doses led to a marked increase (103% and 142%, respectively) in Na-K-ATPase activity of CCT. Mg-dependent ATPase activity was not affected by the lower dose of the hormone, but administration of 50 μg/100 g per 24 h resulted in a slight (20%) but significant increment in the activity of this enzyme.

The effect of aldosterone (5 μg/100 g per 24 h) on Na-K-ATPase activity in different nephron segments is shown in Fig. 1. Aldosterone doubled the enzyme activity in CCT (2,007±178 vs. 989±72 pmol/mm per h), whereas Na-K-ATPase activity in the PCT, MAL, and MCT (2,189±178, 3,600±423, and 965±70 pmol/mm per h, respectively), did not change significantly.

The time course of the stimulation of enzyme activity by aldosterone is illustrated in Fig. 2. Enzyme activity measured 24 h after initiation of aldosterone supplementation was higher than in control rats sham operated on 7 d earlier, but was not significantly different from levels measured in rats stressed by surgery and anesthesia when the sham procedure was done 24 h before (1,367±119 pmol/mm per h). After 3 d, however, enzyme activity in CCT was clearly augmented, and it increased further by 7 d. Serum potassium declined progressively during aldosterone administration in reciprocal fashion with the increment in Na-K-ATPase activity (1 d, 4.1±0.1; 3 d, 3.7±0.1; 7 d, 3.3±0.1 meq/liter).

Short-term effect of aldosterone (adrenalectomized rats)

Because the evaluation of short-term effects of aldosterone in intact animals is complicated by the increased endogenous production of this hormone due to the stress of surgery and anesthesia (17, 22), the following studies were done in adrenalectomized rats.

Administration of aldosterone by osmotic minipump led to a rapid increase in body weight after 24 h with both the 5-μg (11.3±7.5 g) and the 50-μg (15.9±8.1 g) doses, as compared with the modest weight gain observed in adrenalectomized controls (1.8±1.8 g). The response of Na-K-ATPase activity in CCT (451±72 pmol/mm per h in untreated ADX rats) was time- and dose-dependent (Fig. 3). Aldosterone at the lower dose had an insignificant effect at 12 h but produced a moderate stimulation at 24 h. When given in the larger dose

Table II. ATPase Specific Activity in the CCT

Group	*n*	Mg-ATPase	Na-K-ATPase
		pmol/mm per h	*pmol/mm per h*
Sham*	8	782±51	989±72
Aldosterone (5 μg/100 g per 24 h)‡	8	904±79	2,007±178‖
Aldosterone (50 μg/100 g per 24 h)§	10	937±28¶	2,393±193‖

Results are mean±SE.
* Group I.
‡ Group II.
§ Group III.
‖ Significantly ($P < 0.001$) different from group I.
¶ Significantly ($P < 0.01$) different from group I.

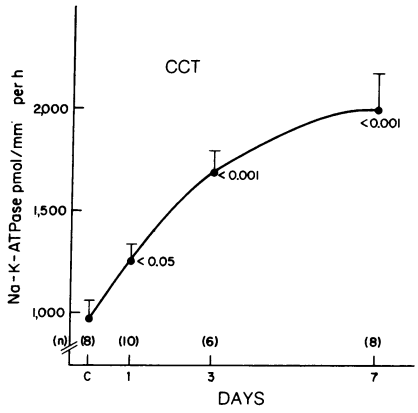

Figure 2. Time course of the stimulation of CCT Na-K-ATPase activity by aldosterone (5 μg/100 g per 24 h) in adrenal-intact rats. Controls (C) were sham-operated 7 d before (see text for explanation). Number of rats (*n*) is given in parentheses.

of 50 μg/100 per 24 h, aldosterone increased CCT Na-K-ATPase activity moderately at 12 h and at 24 h caused a marked enhancement in enzyme activity. Mg-ATPase (449±43 pmol/mm per h in untreated ADX rats) was not altered by aldosterone in either dose, both 12 and 24 h after administration. Again, as in the chronic experiments, serum potassium changed in a reciprocal fashion with Na-K-ATPase activity.

Mechanism of Na-K-ATPase stimulation by aldosterone

To determine whether the enhanced Na-K-ATPase activity produced by aldosterone is due to synthesis of new enzyme units or to alteration in its kinetics, we examined the activation curves of CCT Na-K-ATPase by Na and K and measured the ouabain-binding capacity of this nephron segment. CCT were obtained from adrenal-intact rabbits treated for 3 d with a high physiologic dose (5 μg/100 g body wt per 24 h) of aldosterone, and from control, untreated rabbits. The shape of the activation curves by either Na (Fig. 4) or K (Fig. 5) was similar in aldosterone-treated rabbits and in controls. The apparent $K_{½}$ (~16 mM Na and ~0.65 mM K), derived from Lineweaver-Burk double reciprocal plots (*insets*) were also similar, indicating an unaltered affinity of the enzyme for either cation as a result of aldosterone treatment. Ouabain-binding capacity was next measured to determine whether the increased Na-K-ATPase activity (V_{max}) observed in both rats and rabbits is due to an increased number of active enzyme units or to increased turnover of the pump. These experiments (Fig. 6) showed a similar increment (by a factor of ~2.2) in Na-K-ATPase activity and in the specific binding of ouabain, which strongly suggests that aldosterone stimulates the enzyme activity of CCT by augmenting the number of active pump sites.

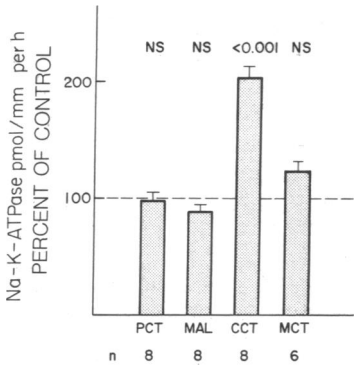

Figure 1. Effect of aldosterone (5 μg/100 g per 24 h for 7 d) on Na-K-ATPase activity of several nephron segments from adrenal-intact rats. Selective stimulation of enzyme activity was observed only in the CCT. *N* is number of rats from which segments were obtained.

Discussion

The present study demonstrates that circulating levels of aldosterone simulating those achieved during chronic potassium loading and sodium deprivation led to a segment-specific increase in Na-K-ATPase activity in the CCT. This effect was time- and dose-dependent and was due to an increase in the number of active pump units rather than to a modification of the enzyme's affinity for sodium and potassium. These results suggest that modulation of Na-K-ATPase by aldosterone plays a role in the chronic adaptation of the CCT to altered availability of sodium and potassium, and therefore in the homeostasis of these cations by the kidney.

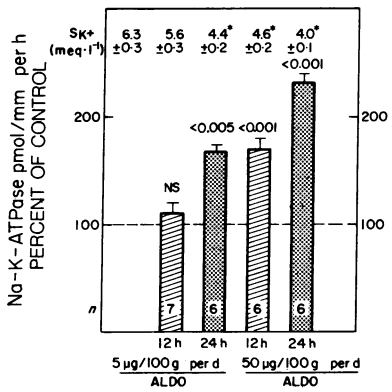

Figure 3. Effect of 12- and 24 h administration of aldosterone on CCT Na-K-ATPase and serum (S) potassium in adrenalectomized rats. A significant increment in enzyme activity and decrease in serum K was observed after 24 h with both doses, and after 12 h with the higher dose (50 μg/100 g per 24 h) only. Asterisks denote $P < 0.01$ compared with values in control, untreated rats; *n* is number of animals in bars (six control rats were used for each time period). Aldo, aldosterone.

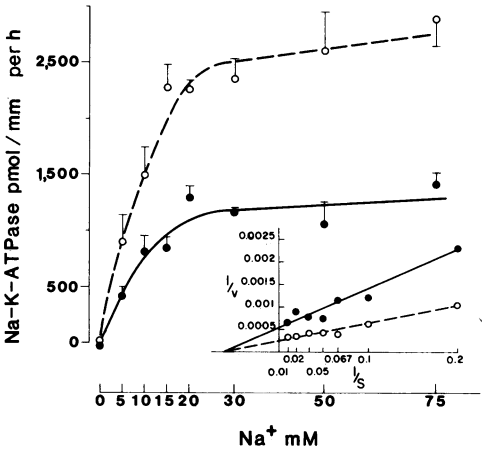

Figure 4. Dependence of rabbit CCT Na-K-ATPase activity on Na concentration. Activation curves in adrenal-intact rabbits receiving 5 μg/100 g per 24 h for 3 d (open circles) and untreated controls (closed circles) were similar in shape; Lineweaver-Burk plot (*inset*) shows that the apparent $K_{½}$ was ~16 mM Na in both groups. Each point represents results from three animals.

Our results are consistent with the structural changes observed by other investigators in response to variations in cation availability and mineralocorticoid administration. Kaissling and Le Hir (7) have recently evaluated the changes that occur in the structure of distal nephron segments after adaptation of rabbits to low sodium and high potassium intake. In the CCT they found a 4.4-fold amplification of the basolateral cell membrane area of the principal cell that was partially blunted by treatment with the aldosterone antagonist canrenoate-K. They further documented a direct effect of exogenous mineralocorticoid (DOCA) in animals receiving a high Na and low K diet, which showed amplification of principal cell basolateral membrane as did those fed a low Na and high K diet. Similarly, Wade and co-workers (8) found that chronic

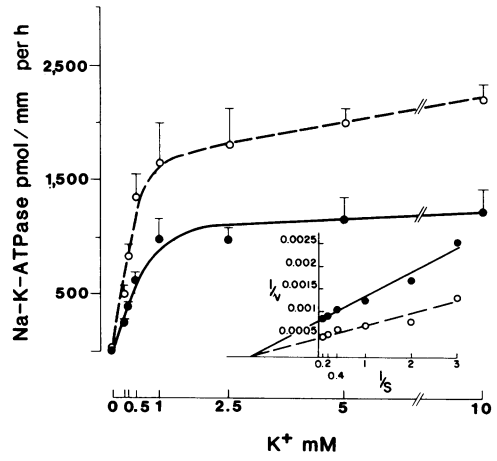

Figure 5. Dependence of rabbit CCT Na-K-ATPase activity on K concentration. Activation curves in adrenal-intact rabbits receiving 5 μg/100 g per 24 h for 3 d (open circles) and untreated controls (closed circles) were similar in shape, and apparent $K_{½}$ was ~0.65 mM K in both groups (*inset*). Each point represents results from four animals.

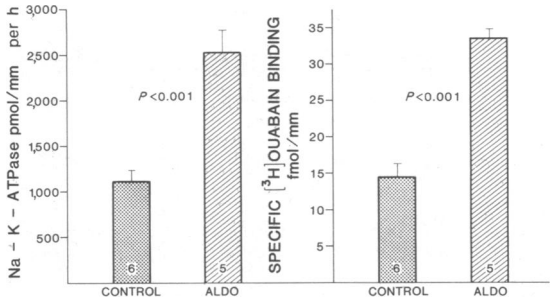

Figure 6. Na-K-ATPase and ouabain binding in CCT of adrenal-intact rabbits receiving aldosterone (5 μg/100 g per 24 h) for 3 d, and of untreated controls. Both measurements increased significantly and comparably after aldosterone treatment. (Number of animals in bars.) Aldo, aldosterone.

DOCA administration to rabbits results in a marked increase in the surface area of the basolateral cell membrane of the principal cell. These changes are the morphologic counterpart of the increased number of Na-K-ATPase enzyme units demonstrated in this study, since the enzyme has been shown to be located in the basolateral membrane of tubular cells. Because no change in cell number in the CCT is observed (10), the increased enzyme activity suggests an increase in active units per cell. Similar conclusions regarding the mechanism of Na-K-ATPase stimulation in rat colonic mucosa were reached by Hayslett et al. (23) who reported a parallel increment in the specific [^{3}H]ouabain binding and Na-K-ATPase activity after both Na deprivation and K loading.

Inasmuch as Na-K-ATPase constitutes the primary component of electrogenic transport in the CCT, it is also of interest to compare the findings of enhanced enzyme activity in this report with the results of cation transport studies. Natke and Stoner (9) examined the sodium transport properties of the basolateral membrane of CCT. They found that the sodium efflux rate across this membrane in CCT dissected from rabbits on a low Na diet was 2.3 times greater than that of tubules from animals on a high Na diet. This increment is in excellent agreement with our finding of a 2.4-fold increase in Na-K-ATPase activity with the 50 μg/100 g per 24 h dose of aldosterone, which simulates levels achieved by Na depletion. Similarly, at endogenous aldosterone levels comparable to the target levels in our study, Schwartz and Burg (6) have found a threefold increase in voltage, and a 2.2-fold increase in net potassium secretion by rabbit CCT studied in vitro, while the net lumen to bath Na flux increased by 1.8-fold. In chronically DOCA-treated rabbits, Stokes et al. (24) found a twofold increase in net sodium absorption and potential difference and a threefold increase in net potassium secretion in CCT studied in vitro. While we recognize that such comparisons should be interpreted with caution,[2] the remarkable concordance between changes in transport rates and Na-K-ATPase, observed under quite different experimental conditions, suggests that modulation of enzyme activity by mineralocorticoids does play a role in the chronic adaptation to altered cation availability. For example, the enhancement in enzyme activity found by us

2. For example, besides their effect on the pump, mineralocorticoids may influence the Na and K conductance of both the basolateral and apical membrane of the CCT (25).

with 50 μg/100 g per 24 h aldosterone is comparable to that seen during Na deprivation (2.3- to 3.1-fold increase), which results in similar elevations of circulating aldosterone; these observations suggest that stimulation of Na-K-ATPase in the latter studies could be due to the increased levels of aldosterone. Furthermore, aldosterone levels similar to those obtained during K adaptation (17) doubled the enzyme activity in the CCT. This increment is comparable to that measured after feeding a high K diet to adrenal-intact rats (26).

It is of interest that the stimulatory effect on enzyme activity in the CCT, as well as that on transport in this segment (5, 6) are evident at a time when the animals are presumably in the state of "mineralocorticoid escape." These observations indicate that the mechanism of "escape" from the salt-retaining action of mineralocorticoids is not one of interference with the tubular effects of aldosterone in the CCT, but involves regulatory processes acting at more proximal nephron sites (27). In addition, the unchanged levels of Na-K-ATPase activity in other nephron segments (Fig. 1) implies that escape does not occur through inhibition of the sodium pump per se, at least in the segments examined.

The segmental specificity of the effect of aldosterone on rat Na-K-ATPase (Fig. 1), which was confined to the CCT, is in agreement with observations of others using low salt diet and DOCA treatment (10, 12) and suggests a receptor-mediated, direct tubular effect of aldosterone. Not unexpectedly, we could not detect an effect on the enzyme from PCT and MAL, these two segments being devoid of specific aldosterone receptors (28). That the mild increment in the Na-K-ATPase activity seen in the inner stripe of the MCT was not statistically significant, despite the relative abundance of aldosterone binding sites in this segment, is consistent with the hypothesis that the effect of the hormone in this portion of the nephron subserves proton secretion rather than Na or K transport (24, 29).

Na-K-ATPase activity in CCT increased over several days during exposure of adrenal-intact rats to high physiologic levels of aldosterone (Fig. 2). Additional experiments, performed in previously adrenalectomized rats to avoid the aldosterone surge incident to the surgical procedure (22), examined the early effects of the hormone on CCT Na-K-ATPase. In these animals both doses of aldosterone employed produced a definite stimulation of enzyme activity after 24 h, but only the higher dose elicited a significant increment after 12 h (Fig. 3). The reasons for this difference are not clear.

The occurrence of an early stimulation of Na-K-ATPase by aldosterone is controversial (reviewed in references 30 and 31). An increase in enzyme activity in CCT has been observed within several hours after hormone administration to adrenalectomized rabbits (2, 32) and rats (33, 34), and in rabbit CCT exposed to aldosterone in vitro (35). In one study the increment in Na-K-ATPase activity in CCT was accompanied by a parallel increase in tritiated ouabain binding after acute administration of aldosterone to adrenalectomized rabbits (36). In contrast, we found no change in Na-K-ATPase activity of CCT 3 h after intravenous administration of 10 or 50 μg aldosterone to adrenalectomized rats, in spite of high circulating levels and a significant effect on cation transport (37), or in CCT obtained from adrenalectomized mice that received 10 μg/100 g body wt aldosterone (38). Similarly, no effect on the enzyme has been detected in rabbit and mouse CCT treated with physiologic or pharmacologic doses (10^{-10}–10^{-6} M) of aldosterone for 90 min in vitro (38), and in toad bladder exposed for 2½ h to 10^{-6} M aldosterone (39). Geering et al. (40), using monospecific antibodies to the α- and β-subunits of Na-K-ATPase found no change in the rate of enzyme synthesis in toad bladder incubated with aldosterone up to 3 h; a statistically significant increase in the synthesis rate of both enzyme proteins was observed only after 6 h of hormone treatment. The reasons for the discrepancy between the studies cited are not readily apparent, and while in some cases species differences may be suspected, in other studies the discrepancy persists even with the use of same species (34–38)[3] and analytical methods (34, 36–38). Park and Edelman (30) detected a modest (13%) increment in the abundance of the enzyme in deoxycholate-treated cell membranes of toad bladder exposed to aldosterone for 5 h but none in the absence of activation by deoxycholate, whereas transepithelial Na transport increased two- to threefold. These authors postulated that an "aldosterone-induced protein(s)" may modulate the turnover rate (but not the abundance) of the pump, and that an increased activity of Na-K-ATPase (after short-term exposure to aldosterone) may or may not be detected depending upon the experimental conditions, i.e., whether the aldosterone-induced protein(s) is or is not associated with the pump at any given time. Clearly, the mechanism underlying the immediate response of transporting epithelia to aldosterone remains to be elucidated.

The present study examined the long-term effect of aldosterone on renal tubular Na-K-ATPase activity, and defined its time course and location in the nephron. We have determined that sustained, stimulated levels of the hormone mimicking those seen after dietary manipulation enhanced Na-K-ATPase activity of CCT obtained from intact rats and rabbits; this effect was due to an increase in the number of active pump sites rather than to alterations in their kinetics. The results underscore the importance of aldosterone in the regulation of CCT function, and point to this nephron segment as a major target site for the hormone.

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