J. Membrane Biol. 85, 169–179 (1985)

The Journal of
Membrane Biology

Sodium-Dependent Modulation of the Renal Na-K-ATPase: Influence of Mineralocorticoids on the Cortical Collecting Duct

Roger G. O'Neil* and Russell A. Hayhurst
Department of Physiology and Cell Biology, University of Texas Medical School, Houston, Texas 77225

Summary. Mineralocorticoids play a major role in the regulation of sodium transport in a variety of tissues, including the cortical collecting duct (CCD) of the mammalian nephron. To assess, in part, the underlying mechanism(s) of this control, the present studies were designed to evaluate, first, the influence of mineralocorticoids on the Na-K-ATPase activity in the rabbit CCD and, secondly, a possible role of sodium entry into the cell at the luminal border on the regulation of the Na-K-ATPase. In the first series of studies, rabbits were maintained on a low sodium diet which raised serum aldosterone levels from 16 to 70 ng/dl after 3–4 days, with further elevations being expressed with treatment for two weeks or more. In CCDs isolated from these animals, the Na-K-ATPase increased from 13 to 40 pmol ADP min^{-1} mm^{-1} after 3–4 days on the low sodium regimen, but then declined, returning to control values after approximately 2 weeks. This decline in activity was preceded by a decrease in the Na^+ concentration of the urine to low levels and hence, likely coincided with a decreased delivery of sodium to, and sodium entry into the cells of, the CCD. If dietary manipulations were used to maintain a high delivery of sodium to the CCD in the animal, elevation of plasma mineralocorticoid levels by treatment with deoxycorticosterone acetate (DOCA) caused a similar elevation in the Na-K-ATPase activity after 3–4 days, which did not decline with continued treatment for up to 2 weeks. Furthermore, it was observed that mineralocorticoids only exerted their effect on the Na-K-ATPase after a latent period of 1 day, well after sodium excretion had fallen, indicating that sodium entry into the CCD cells was already stimulated. If animals were simultaneously treated with DOCA and the sodium channel blocker amiloride for 3–4 days, the effects on the Na-K-ATPase were markedly reduced, whereas amiloride treatment alone had no effect on the enzyme activity. Since others have shown that mineralocorticoids induce synthesis of the Na-K-ATPase subunits in toad bladder cells in an amiloride-insensitive manner, sodium must be exerting its effect on a process after translation. It is concluded that the initial effect of mineralocorticoids in the CCD is on sodium entry with a delayed induction of the Na-K-ATPase, which is regulated by Na-dependent modulation of a posttranslational process.

Key Words Na-K-ATPase · Na^+ pump · aldosterone · mineralocorticoids · Na^+ transport · Na^+ permeability · amiloride · cortical collecting duct · epithelia

* Established Investigator of the American Heart Association.

Introduction

The Na-K-ATPase of biological tissues has been demonstrated to be the enzymatic equivalent of the Na : K exchange pump. In renal epithelia this enzyme is responsible for both sodium and fluid reabsorption by the nephron and hence plays a pivotal role in the regulation of fluid and electrolyte balance (Katz, 1982; Jørgensen, 1980). In view of the importance of this enzyme in epithelial function, the underlying mechanisms regulating its levels have been the subject of intense study in both renal and nonrenal tissue alike (*see* recent reviews by Jørgensen, 1980; Stekhoven & Bonting, 1981; Katz, 1982; Rossier, 1984).

Several hormones appear to be intimately associated with the regulation of the renal Na-K-ATPase of specific nephron segments. Chronic suppression or elevation for several days of the plasma levels of either glucocorticoid or thyroid hormones results in corresponding alterations in the levels of the enzyme and in Na^+ transport in the proximal tubule (Edelman, 1981; Katz, 1982). Similarly, chronic alterations in the plasma levels of mineralocorticoids, such as aldosterone, bring about corresponding alterations in the levels of the Na-K-ATPase and the capacity for Na^+ transport in the collecting duct system (Edelman, 1981; Katz, 1982; Marver & Kokko, 1983). These hormone-induced changes generally occur slowly, requiring 1 to 2 days or more for the effects on the Na-K-ATPase to be fully expressed (Jørgensen, 1968, 1969; Silva, Hayslett & Epstein, 1973; Charney et al., 1974; Lo et al., 1976; Lo & Edelman, 1976; Lo & Lo, 1979; Doucet & Katz, 1981; Mujais et al., 1984). Recent evidence indicates that these chronic effects are due to alterations in the rate of synthesis of the enzyme, and not to alterations in the rate of degradation or to the unmasking of latent enzyme units (Jørgensen, 1968; Lo & Edelman, 1976; Geering et al., 1982). In view of the slow turnover of this enzyme, the dem-

onstration of a rapid (1–3 hr) aldosterone-induced stimulation in the levels of the Na-K-ATPase, which has been reported only in adrenalectomized animals by some investigators (Horster, Schmid & Schmid, 1980; Petty, Kokko & Marver, 1981; el-Mernissi & Doucet, 1983), but not by others (Doucet & Katz, 1981), is likely to fast, if it occurs, to be explained by stimulation in the rate of enzyme synthesis. Hence, this rapid response observed in adrenalectomized animals only may reflect the recruitment of pre-existing latent enzyme units (*see* Discussion).

In recent years it has become apparent that the delivery of sodium to the various nephron segments may also play an important role in the chronic regulation of Na-K-ATPase levels, as stressed previously by Katz and coworkers (Westenfelder et al., 1977) and Jørgensen (1972). Alterations in sodium delivery to the renal tubules have been shown to influence the Na-K-ATPase activity of whole tissue homogenates from both the cortex and the medulla (Jørgensen, 1968, 1972; Westenfelder et al., 1977). Furthermore, the effects of mineralocorticoids on the Na-K-ATPase of cortical tissue homogenates are depressed if the animals are maintained on a low sodium diet (Hendler et al., 1972; Charney et al., 1974; Westenfelder et al., 1977). This has raised the question as to whether the influence of hormones on the Na-K-ATPase reflects a primary action of the hormone, or alternatively, the primary action is on sodium entry into the cell with the effects on the Na-K-ATPase reflecting a secondary or delayed action (Jørgensen, 1972; Katz, 1982).

The purpose of the present study was to assess the underlying role of sodium on the hormonal regulation of the renal Na-K-ATPase in a selected nephron segment. The model used was the mineralocorticoid-dependent regulation of the Na-K-ATPase in individual segments of the cortical collecting duct from animals with intact adrenals (Garg, Knepper & Burg, 1981; LeHir, Kaissling & Dubach, 1982; O'Neil & Dubinsky, 1984). This tubule model was particularly advantangeous for these studies, since sodium delivery to this segment could be controlled in the animal by dietary manipulation, and sodium entry into the cells controlled pharmacologically with the diuretic amiloride. It was found that both exogenous and endogenous mineralocorticoids cause a similar elevation in the levels of the Na-K-ATPase in the cortical collecting duct, but this effect was totally dependent upon sodium delivery to, and entry into, the cells of this nephron segment. Evidence is presented from the results of this and other studies, in support of a general scheme in which sodium acts as a modulator of the hormone-induced Na-K-ATPase activity by regulating a post-translational process. Part of this study has been presented in preliminary form (O'Neil & Dubinsky, 1983; O'Neil & Hayhurst, 1984).

Materials and Methods

Treatment Groups

New Zealand white female rabbits were used for all studies. The animals were divided into four groups and maintained on one of the following diets (Purina chow): (1) control group: standard chow (0.4% Na^+, 1.4% K^+) plus tap water for at least one week; (2) high Na^+ group: high Na^+ chow (0.5% Na^+, 1.3% K^+) plus 0.9% NaCl drinking water for at least one week; (3) high Na^+, low K^+ group: high Na^+, low K^+ chow (0.5% Na^+, 0.2% K^+) plus 0.9% NaCl drinking water for at least one week; (4) low Na^+ group: low Na^+, normal K^+ chow (0.05% Na^+, 1.3% K^+) plus tap water for 1 to 17 days. Other than the standard Purina chow (control group), synthetic Purina chows were used which were identical in composition except for the Na^+ and K^+ content as indicated. All drinking solutions were administered *ad libitum*. Unless otherwise indicated, the animals were of the control group.

In addition to the above dietary regimens, animals from selected diet groups, as outlined in the Results section, were treated with the following steroids and drugs: (i) the mineralocorticoid deoxycorticosterone acetate, DOCA (Organon Inc., Atlanta, GA), 2 mg/kg/day, i.m.; (ii) the glucocorticoid dexamethasone, DEXA (DEXATE—Burns-Biotec Laboratories, Inc., Omaha, NE), 0.5 mg/kg/day, s.c.; (iii) the diuretic amiloride (Merck Sharp and Dohme, Rahway, NJ) in isotonic saline, 10 mg/kg/twice daily, s.c.; and (iv) DOCA (2 mg/kg/day) plus amiloride (10 mg/kg/twice daily). In addition, to ensure that animals that were maintained on a low Na^+ diet (low Na^+ group) were rapidly Na^+ depleted (and volume depleted), furosemide (Lasix—Hoechst-Roussel, Somerville, NJ) was administered (1.0 mg/kg, i.v.), on day 1 and 4 after switching to the low Na^+ diet, in a manner similar to that of Schwartz and Burg (1978).

Isolation and Permeabilization of Tubule Segments

At the end of each treatment specified above, the animal was sacrificed and the right kidney removed. The kidney was quickly sliced sagitally (approx. 1 mm thick), and the slices were placed in dissection medium on ice. The medium contained (in mM): 148 NaCl, 5 KCl, 1.2 $MgSO_4$, 1.0 $CaCl_2$, 0.25 Na_2HPO_4, 1.0 NaH_2PO_4, pH 7.4. Fragments (0.5 to 2.0 mm length) of cortical collecting duct were dissected free-hand from the slices under stereoscopic observation without enzymatic treatment, as described previously (O'Neil & Dubinsky, 1984).

Isolated cortical collecting duct segments were permeabilized using combined osmotic and temperature shock as before (O'Neil & Dubinsky, 1984). Individual segments were deposited into a droplet of distilled water containing 0.1% BSA in the well of a concave microscope slide. After a few minutes, the tubule was transferred along with 5 μl of the water into a V-shaped Delrin well embedded in an aluminum plate (*see* O'Neil & Dubinsky, 1984). In order to rapidly freeze the tubule, the aluminum plate was placed on dry ice and then removed, covered, and placed on crushed ice to allow the tubule to thaw slowly. Whereupon, the tubule was assayed for Na-K-ATPase activity.

Table 1. Influence of various dietary maneuvers, designed to reduce plasma aldosterone levels, on the ATPase activities of the cortical collecting duct

Diet/treatment	*n*	Total ATPase	Mg-ATPase	Na-K-ATPase	Fractional Na-K-ATPase
		(pmol ADP min^{-1} mm^{-1})			(%)
Normal Na^+, normal K^+ diet (control diet)	12	31.0 ± 5.4	15.3 ± 3.0	15.7 ± 2.6	52.6 ± 4.1
High Na^+, normal K^+ duet	8	23.8 ± 0.8	10.6 ± 1.4	13.2 ± 2.1	54.0 ± 7.1
High Na^-, low K^- diet	6	33.5 ± 4.2	16.5 ± 3.1	17.0 ± 2.4	51.4 ± 6.3

Microassay for Determination of Na-K-ATPase Activity

The following microassay has been discussed at length previously (O'Neil & Dubinsky, 1984), and will therefore only be presented briefly here. This assay quantitatively determines ATPase activity by stoichiometrically linking ATP hydrolysis and the production of ADP to a highly fluorescent condensed form of NAD. This coupling was initiated enzymatically, using pyruvate kinase and lactate dehydrogenase to link ATP hydrolysis with the oxidation of NADH. The resultant NAD was then condensed to its highly fluorescent form for quantitation by incubation with strong base (*see* Lowry & Passonneau, 1972).

Following permeabilization of the tubule, 10 μl of concentrated incubation medium was added to the 5 μl of distilled water containing the tubule in the Delrin well. The final composition of the 15 μl of medium in mM was: 60 NaCl, 60 choline Cl, 30 KCl, 50 imidazole (low fluorescence blank), 5 $MgCl_2$, 0.5 EGTA, 5 Na_2ATP (equine muscle, vanadium-free), 10 phosphoenolpyruvate (tri-monocyclohexylammonium salt), 1 ascorbic acid, 1 NADH, plus 1.4 U/ml pyruvate kinase and 2.0 U/ml lactate dehydrogenase, pH 7.0. The NaCl, KCl, and $MgCl_2$ were obtained from MCB Manufacturing Chemists, Inc.; all other components were from Sigma.

The tubules were covered with oil to prevent evaporation, and the ATP hydrolysis reaction was initiated by placing the aluminium plate in a 37°C water bath. After a 30-min incubation period, the reaction was stopped by the addition of 15 μl of 0.1 N HCl and cooling of the aluminium plate. After several minutes, 20-μl aliquots were removed from the wells and placed in 6 × 50 mm test tubes to which 750 μl of 6 N NaOH was added. The tubes were immediately and thoroughly mixed, then incubated for 20 min at 60°C, removed, and cooled to room temperature in the dark. The resulting converted flourescent form of NAD was then quantitated using a Turner Model 430 Spectrofluorometer (excitation, 340 nm; emission, 460 nm).

In order to determine the quantity of ADP in a sample, a standard curve was produced by adding various quantities of ADP (di-monocyclohexylammonium salt, Sigma) to incubation media in test tubes and treating these samples in a manner similar to that for the tubules. The quantity of ADP produced is directly related to the amount of ATP hydrolyzed, and, therefore, the rate of ADP appearance can be used as an estimate of the total ATPase activity. The resulting ATPase activities were expressed as pmol ADP per min incubation per mm of tubule length.

The ouabain-sensitive fraction of the total ATPase activity was used as an estimate of the Na-K-ATPase activity (O'Neil & Dubinsky, 1984). Therefore, for each study, two tubules were analyzed, one as described above to obtain the total ATPase activity, and one in the additional presence of maximally inhibiting doses of ouabain (0.1 or 1.0 mM, Sigma) to obtain the ouabain-insensitive or Mg-ATPase activity. The difference in activities, i.e., the ouabain-sensitive fraction, was used as an estimate of the Na-K-ATPase activity.

Serum and Urine Analysis

Just prior to sacrificing an animal, a blood sample was obtained by directly puncturing the ventricle using an 18-gauge hypodermic needle. The sample was allowed to clot for collection of the serum. Subsequently, immediately after sacrifice a sample of urine was collected directly from the bladder. Both samples were analyzed for Na^+ and K^+ content on a Corning Model 450 flame photometer.

Some serum samples were stored (−20°C) for later evaluation of aldosterone levels. Serum aldosterone concentrations were determined by a modified radioimmunoassay procedure (New England Nuclear, Boston, MA) and performed by the laboratory of Dr. Merrill L. Overturf, University of Texas Medical School at Houston.

Statistical Analysis

The results of the study are summarized as mean values ±SEM. Differences between two groups were assessed with the paired or unpaired *t*-test as appropriate. If more than two treatment groups were considered, differences among mean values were determined using the one-way analysis of variance in combination with the Newman-Keuls test.

Results

Suppression of Endogenous Aldosterone Levels

In initial studies, attempts were made to reduce the cortical collecting duct Na-K-ATPase activity to low basal levels by using diet to depress endogenous aldosterone levels. Animals were maintained on either a high Na^+ diet (high Na^+ group) or a high Na^+, low K^+ diet (high Na^+, low K^+ group) for one week, conditions known to depress plasma aldosterone levels in the rabbit (Schwartz & Burg, 1978), although the hormone levels were not measured in this first series of experiments. The results are summarized in Table 1. In the control group of animals the ouabain-sensitive Na-K-ATPase activity and ouabain-insensitive Mg-ATPase activity of the cor-

tical collecting duct averaged 15.7 and 15.3 pmol ADP min^{-1} mm^{-1}, respectively. Neither the high Na^+ diet nor the high Na^+, low K^+ diet significantly reduced the basal enzyme activities.

Elevation of Endogenous Aldosterone Levels

The influence of elevating the endogenous plasma aldosterone levels on the Na-K-ATPase of the cortical collecting duct was assessed using dietary maneuvers to stimulate aldosterone secretion. Animals were initially maintained on a high Na^+, normal K^+ diet for one week. At the end of this period, the animal diet was switched to a low Na^+, normal K^+ diet for up to 17 days. In order to both sodium and volume deplete the animals, and thereby insure stimulation of aldosterone secretion by the adrenal glands, the animals were also injected with furosemide on day 1 and day 4 of the low Na^-, normal K^+ regimen, similar to that done by Schwartz and Burg (1978).

The effect of the low Na^+, normal K^+ diet on the serum aldosterone levels of the rabbit is shown in Fig. 1. At day 0, prior to switching to the low Na^+ regimen, the serum aldosterone levels averaged near 16 ng/dl. After 3–4 days on the low Na^+ diet, the serum aldosterone levels were markedly increased, averaging near 70 ng/dl. The serum aldosterone levels continued to increase modestly with continued maintenance on this diet until after 2 weeks when the aldosterone levels appear to undergo another marked stimulation. The reason for this latter stimulation is not known but may reflect a compensatory response to combined volume and sodium depletion as evidenced by a significant reduction in the serum sodium concentration during this period (Table 2).

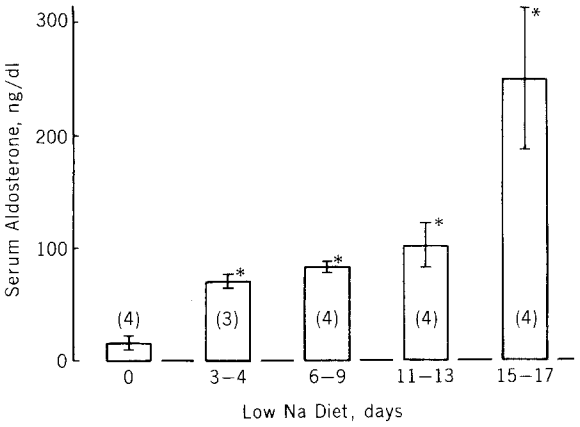

Fig. 1. Time course of the effects of a low Na^+, normal K^+ diet on the serum aldosterone levels of rabbits. The number of determinations at each of the indicated time periods is given in brackets (n). *Significantly different ($P < 0.05$) from the control "0 day" treatment group

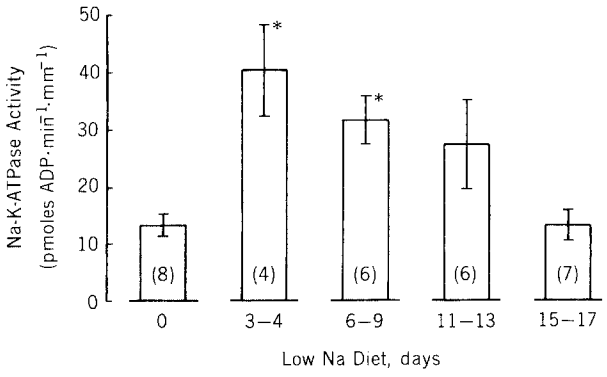

Fig. 2. Time course of the effects of a low Na^-, normal K^+ diet on the Na-K-ATPase activity of the cortical collecting duct. The sample size is given in brackets (n). *Significantly different ($P < 0.05$) from the control "0 day" treatment group

Table 2. Influence of low Na^+, normal K^+ diet on urine and serum electrolytes

Low Na^+ diet treatment	Urine electrolytes				Serum electrolytes			
Time (days)	n	$[Na^+]$ (mM)	$[K^+]$ (mM)	$[Na^+]/[K^+]$	n	$[Na^+]$ (mM)	$[K^+]$ (mM)	$[Na^+]/[K^+]$
0[a]	6	151 ± 40	65 ± 18	3.2 ± 1.1	3	146 ± 3	5.9 ± 0.6	25 ± 0.1
3–4	5	18[b] ± 11	148[b] ± 33	0.4 ± 0.4	3	144 ± 2	5.8 ± 0.1	25 ± 1
6–9	6	2.8[b] ± 0.3	184 ± 54	0.05[b] ± 0.03	7	142 ± 2	6.2 ± 0.1	24 ± 1
11–13	7	4.7[b] ± 1.7	103 ± 26	0.06[b] ± 0.02	4	152 ± 2	6.4 ± 0.2	24 ± 1
15–17	6	3.8[b] ± 2.4	78 ± 14	0.1[b] ± 0.1	4	136[b] ± 0.3	5.6 ± 0.7	26 ± 3

[a] Animals were initially maintained on a high Na^+, normal K^+ diet prior to switching to the low Na^+, normal K^+ diet on day 1.

[b] Significantly different ($p < 0.05$) from the 0 day, high Na^+ diet group.

After treatment with the low Na^+, normal K^+ diet as noted above, CCDs were isolated to determine the effect of this regimen on the Na-K-ATPase activity. As shown in Fig. 2, the Na-K-ATPase activity of the CCDs averaged near 13 pmol ADP min^{-1} mm^{-1} at day 0 and increased to near 40 pmol ADP min^{-1} mm^{-1} after only 3–4 days on the low Na^+ regimen. Surprisingly, however, this activity was not sustained, but declined with continued exposure to this regimen, returning to near control levels after approximately two weeks. Because the serum aldosterone levels were markedly elevated at this point, it is evident that the Na-K-ATPase activity of the CCD is not solely dependent upon this hormone, but that other factors may play an important role in the regulation of this enzyme.

While the reason for the decline in the Na-K-ATPase activity of the CCD with prolonged low Na^+ diet treatment is not known, it may be related to a decline in sodium entry into the cells of the CCD. Considering only the urine sodium concentration for the moment, it is apparent from the data in Table 2, that the urine sodium concentration fell dramatically, as expected, after only 3–4 days on the low Na^+ regimen. The Na^+ levels continued to fall and reached a minimal level of near 3 mM after 6–9 days on this diet. This decline in the urine Na^+ levels precedes the decline in the aldosterone-stimulated Na-K-ATPase activity (Fig. 2). Furthermore, urinary K^+ concentration was elevated after 3–4 days (*see* Table 2) but returned to control levels with continued dietary treatment—even though urinary volume was likely markedly reduced in these Na^+ (and volume) depleted animals—indicating that potassium secretion by the cortical collecting duct (and late distal tubule) was depressed. Because K^+ secretion is dependent upon active Na^+ absorption, the decline in K^+ excretion likely occurred as a secondary consequence of reduced sodium absorption. It seems probable, therefore, that sodium delivery to the CCD in the whole animal was reduced, which, in turn, may result in reduced entry of sodium into the cell across the apical cell border. Hence, a reduced rate of Na^+ entry may be responsible for the decline in the Na-K-ATPase activity (*see* below).

Effect of Exogenous Mineralocorticoids

Elevation of exogenous plasma mineralocorticoid levels can cause an elevation in the Na-K-ATPase activity of CCD similar to that observed within a few days of elevation of endogenous plasma aldosterone levels. As shown in Fig. 3, if control group animals are treated with high doses of deoxycorticosterone acetate (DOCA, 2 mg/kg/day, i.m.) for 3–4 days, the Na-K-ATPase activity of the CCD increases from 15.7 to 35.4 pmol ADP min^{-1} mm^{-1}, which is similar to that observed upon elevation of endogenous aldosterone levels (*compare* with Fig. 2).

It has been shown recently that chronic alterations in potassium balance may significantly influence the Na-K-ATPase levels in the CCD (Doucet & Katz, 1980; Garg, Mackie & Tisher, 1982). It follows that an alteration in potassium balance may itself influence the response to mineralocorticoids. Hence, studies were performed to assess whether or not potassium depletion, a condition known to depress plasma K^+ levels (Table 3), may influence the effect of mineralocorticoids on the Na-K-ATPase activity of the CCD.

If animals were maintained on a high Na^+, low K^+ diet for one week and then treated with DOCA for 3–4 days, an increase in the Na-K-ATPase activity was observed similar to that with animals maintained on a control diet (Fig. 3). This parallelism exists despite the fact that the plasma potassium concentration is depressed from the normal levels of 5.2 mM to near 4 mM (Table 3). Therefore, the mineralocorticoid-dependent regulation of the Na-K-ATPase does not appear to be influenced by a negative potassium balance, at least not within the range studied.

Effect of Amiloride

As noted above, the effects of chronic mineralocorticoid elevation on the Na-K-ATPase of the CCD may be dependent upon sodium entry into the cell

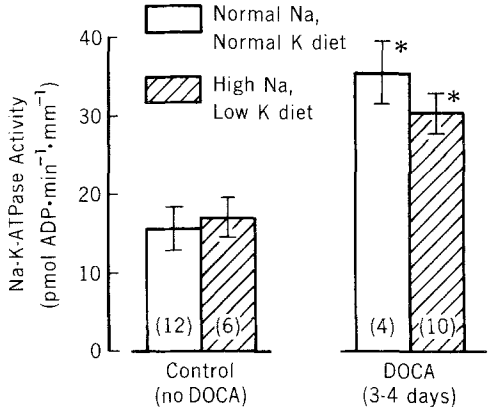

Fig. 3. The effect of deoxycorticosterone acetate (DOCA) treatment on the Na-K-ATPase activity of the cortical collecting duct in animals maintained on either a normal Na^+, normal K^+ diet, or a high Na^+, low K^+ diet. Animals were maintained on the diets for at least 1 week prior to determination of the Na-K-ATPase activity. The sample size is given in brackets (*n*). *Significantly different ($P < 0.05$) from the corresponding control group

Table 3. Effect of DOCA, DEXA, and amiloride treatment on urine and serum electrolytes in animals maintained on either a normal Na^+, normal K^+ or a high Na^+, low K^+ diet

Treatment	Urine electrolytes				Serum electrolytes			
	n	$[Na^+]$, mM	$[K^+]$, mM	$[Na^+]/[K^+]$	*n*	$[Na^+]$, mM	$[K^+]$, mM	$[Na^+]/[K^+]$
A. Normal Na^+, normal K^+ diet								
Untreated, control	10	65 ± 11	85 ± 17	0.94 ± 0.19	7	143 ± 2	5.2 ± 0.1	28 ± 1
DOCA, 1 day	4	10[a] ± 4	175 ± 59	0.06[a] ± 0.03				
DOCA, 2 days	6	53 ± 27	131 ± 51	0.66 ± 0.22				
DOCA, 3–4 days	4	75 ± 22	61 ± 13	1.2 ± 0.1				
DOCA, 12–15 days	4	13[a] ± 5	27 ± 12	0.66 ± 0.21				
B. High Na^+, low K^+ diet								
Untreated, control	6	138 ± 15	8.1 ± 1.4	21 ± 5	4	151 ± 1	3.7 ± 0.1	41 ± 2
DOCA, 1 day	6	71[a] ± 24	84 ± 36	7.1[a] ± 3.6				
DOCA, 3–4 days	10	149 ± 35	54[a] ± 12	5.1[a] ± 2.5	5	150 ± 2	4.5 ± 0.5	35 ± 4
DOCA + amiloride, 3–4 days	12	192 ± 20	8.9 ± 1.2	26 ± 4	4	146 ± 2	3.8 ± 0.6	42 ± 7
Amiloride, 3 days	6	178[a] ± 9	18[a] ± 3	12 ± 2	6	143[a] ± 1	4.8 ± 0.6	32 ± 4
DEXA, 3 days	6	176 ± 36	9.9 ± 1.6	22 ± 7				

[a] Significantly different ($p < 0.05$) from the untreated control group.

across the luminal cell border. To study this notion more directly, the effects of the sodium channel blocker amiloride on the mineralocorticoid-dependent Na-K-ATPase activity were investigated. However, since treatment of animals with amiloride—a potassium-sparing diuretic—reduces potassium excretion, such treatment could effectively result in potassium loading, a condition known to cause an increase in the Na-K-ATPase activity of the CCD (Doucet & Katz, 1980; el-Mernissi & Doucet, 1984).[1] To negate such an indirect effect of amiloride treatment on the Na-K-ATPase activity, the studies were performed on animals that were maintained on a high Na^+, low K^+ diet, thereby initially potassium depleting the animal as evidenced by a reduction in the potassium plasma concentration, but not affecting the response to mineralocorticoids as demonstrated above.

Animals were maintained on a high Na^+, low K^+ diet for at least one week prior to study. At the end of this period, the animals were treated for 3–4 days with DOCA alone, with DOCA and amiloride, or with amiloride alone. As summarized in Fig. 4, DOCA treatment alone caused a significant elevation in the Na-K-ATPase activity from 17.0 to 30.4 pmol ADP min^{-1} mm^{-1}. This effect of DOCA treatment on the Na-K-ATPase activity was significantly reduced with the simultaneous treatment with amiloride. Further, since treatment with amiloride alone did not appear to affect the Na-K-ATPase activity of the CCD, it can be inferred that sodium entry into the cells of the CCD plays a significant role in regulating the mineralocorticoid-induced Na-K-ATPase activity only.

Mineralocorticoid *versus* Glucocorticoid Activity of DOCA

The mineralocorticoid DOCA has significant glucocorticoid activity. To ascertain whether the effects of DOCA treatment on the Na-K-ATPase of the CCD reflect mineralocorticoid or glucocorticoid activities, animals were maintained on a high Na^+, low K^+ diet and either treated with a high dose of the glucocorticoid dexamethasone (0.5 mg/kg/day, i.m.) for 3–4 days, or DOCA (2 mg/kg/day, i.m.) for the same time period. As shown in Fig. 4, the Na-K-ATPase activity of CCDs from animals treated with DEXA averaged near 17 pmol ADP min^{-1} mm^{-1} and did not differ from the control values. Therefore, the effects of DOCA treatment on the Na-K-ATPase activity of the CCD likely reflect the mineralocorticoid activity of DOCA.

[1] Recently, el-Mernissi and Doucet (1984) reported that treatment of rats on a normal diet with low doses of amiloride for 3–8 days can result in an increase in the Na-K-ATPase activity of the CCD. While the authors made no attempt to assess the origin of this response, it is apparent from their data that treatment with amiloride resulted in a small decrease in the net rate of potassium excretion by approximately 9% per day. It can be inferred from these data, that the animals were retaining potassium, and hence effectively becoming potassium loaded, a condition shown by Doucet and Katz (1981) to increase the Na-K-ATPase activity of the CCD. The authors, for unknown reasons, chose not to address this problem. In the present study, a possible potassium-loading effect of amiloride treatment was purposedly avoided by maintaining the animals on a low K^+ diet. Under these conditions, amiloride treatment for 3–4 days had no influence on the basal Na-K-ATPase activity of the CCD and, in addition, abolished the mineralocorticoid-induced elevation of the enzyme activity.

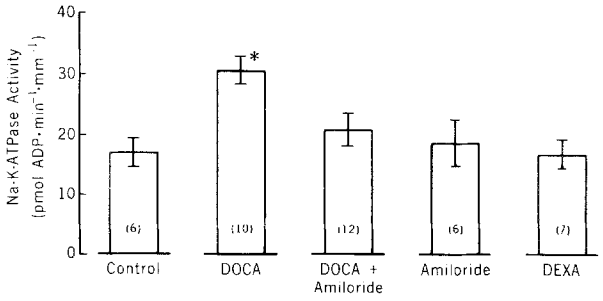

Fig. 4. The influence of treatment with DOCA, dexamethasone (DEXA), and/or amiloride for 3–4 days on the Na-K-ATPase of the cortical collecting duct. In all cases, the animals were maintained on a high Na^+, low K^+ diet. The sample size is given in brackets (*n*). *Significantly different ($P < 0.05$) from the untreated control group

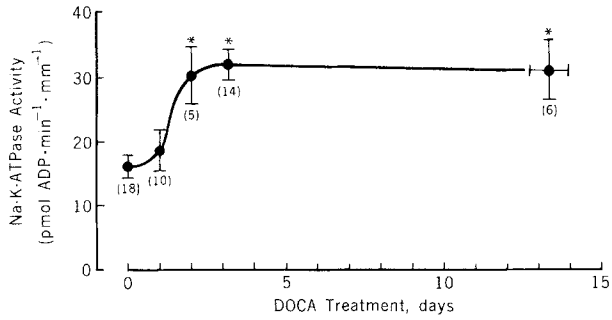

Fig. 5. Time course of the effect of DOCA treatment on the Na-K-ATPase activity of the cortical collecting duct. The animals were maintained on either a normal Na^+, normal K^+ diet or a high Na^+, low K^+ diet. The sample size is given in brackets (*n*). *Significantly different ($P < 0.05$) from the untreated control group (0 day)

Time-Dependent Effects of Mineralocorticoids on the Na-K-ATPase Activity

To gain a better understanding of the role of mineralocorticoids in regulating the Na-K-ATPase activity of the CCD, the time-dependence of the hormone effects were studied. This was accomplished by evaluating the effects of DOCA treatment for varying periods of time on the Na-K-ATPase of the CCD. Since the effects of DOCA treatment in animals maintained on a control diet were similar to those observed with a high Na^+, low K^+ diet, the results were combined to better delineate the time-dependence of the mineralocorticoid effects.

As shown by the data in Fig. 5, chronic elevation in the plasma mineralocorticoid levels was accompanied by a latent period of at least one day followed by an increase in the Na-K-ATPase activity to a maximum plateau value of near 31 pmol ADP min^{-1} mm^{-1} after approximately 3 days. Further, now that sodium delivery to the distal nephron was maintained at a higher level than when animals were exposed to the low sodium regimen (*compare* urine Na^+ levels, Tables 2 and 3), the Na-K-ATPase remained elevated with prolonged mineralocorticoid treatment for up to two weeks. It would appear, therefore, that in the presence of elevated Na^+ delivery to the CCD, and hence an elevated gradient for Na^+ entry into the cell, increased mineralocorticoid levels result in a sustained elevation of the Na-K-ATPase activity of the cells.

The notion that the effects of mineralocorticoid treatment on the Na-K-ATPase are secondary to an increase in sodium entry into the CCD cells is supported also from the observed changes in the sodium and potassium excretion patterns. Within one day of DOCA administration the urine sodium concentration appears to be declining and the urine potassium concentration rising. This is particularly apparent from the decrease in the ratio of the sodium to potassium concentration ratio in animals maintained on either the normal Na^+, normal K^+ diet (Table 3*A*) or the high Na^+, low K^+ diet (Table 3*B*). This response reflects an increased reabsorption of sodium and secretion of potassium by the nephron, due in part to the CCD, and occurs prior to any effect on the Na-K-ATPase activity (Fig. 5).

Discussion

Relation between Na-K-ATPase Activity and Sodium Transport in CCD

The mammalian cortical collecting tubule plays a major role in the regulation of sodium and potassium electrolyte balance by controlling sodium absorption and potassium secretion by the epithelial cells. Since this segment is a target site for mineralocorticoids (Kaissling, 1982; Katz, 1982; Marver & Kokko, 1983), aldosterone and other mineralocorticoids are intimately involved in this regulation. It has been established that elevation of plasma mineralocorticoid levels causes a stimulation in the ability of the CCD to absorb sodium and secrete potassium, while the converse is observed upon depression of these levels (Koeppen, Biagi & Giebisch, 1983; Natke & Stoner, 1982; O'Neil & Helman, 1977; Schwartz & Burg, 1978; Stokes, 1981; Wingo, Seldin & Kokko, 1982). The underlying mechanism responsible for this regulation appears to be related, in part, to the Na-K-ATPase levels of the cells. As shown previously (Garg et al., 1981; Le Hir et al., 1982; O'Neil & Dubinsky, 1984), and confirmed in the present study, chronic eleva-

tion of plasma mineralocorticoids for several days in animals with intact adrenal glands causes an increase in the Na-K-ATPase activity of the CCD.

We were surprised to observe in the present study that attempts to reduce the Na-K-ATPase activity below the normal basal levels, using dietary measures to reduce the plasma aldosterone levels, were not successful. It is possible that exposure of the animals to these modified diets for only one week was not long enough to cause a sufficient and/or rapid enough reduction in plasma aldosterone levels for a change in the Na-K-ATPase activity to be expressed. Indeed, Garg et al. (1982) have noted that reducing plasma aldosterone levels in rabbits by maintaining the animals on a low K diet for 8 weeks does result in a reduction in the Na-K-ATPase levels of the CCD. Further, if more drastic measures are used to reduce plasma aldosterone levels, such as adrenalectomy, the Na-K-ATPase activity of the CCD is reduced after several days (Doucet & Katz, 1981). Hence, the prevailing evidence is consistent with the view that chronic alterations in plasma mineralocorticoid levels cause proportional changes in both the Na-K-ATPase activity of the cells and the capacity of the CCD to transport sodium and potassium.

Sodium-Dependent Actions of Mineralocorticoids

It is apparent from the results of the present study that the effects of mineralocorticoids on the CCD Na-K-ATPase are dependent upon sodium entry into the cell. If sodium entry is reduced or abolished, the effects of mineralocorticoids on the Na-K-ATPase activity are likewise reduced or abolished. This view is supported by three lines of evidence. First, when endogenous aldosterone levels are elevated by maintaining the animal on a low Na^+ diet, the Na-K-ATPase activity of the CCD is elevated within a few days. However, with continued maintenance on this diet, sodium delivery to the collecting duct system, and hence sodium entry into the cells, appears to be markedly depressed. This is accompanied by a decline in the Na-K-ATPase activity of the CCD which returns to near control levels after approximately two weeks on this regimen (Fig. 2). Secondly, if sodium delivery to the collecting duct system is held at a relatively high level by maintaining the animals on either a normal Na^+ or high Na^+ diet, and the plasma mineralocorticoid levels elevated by treatment with DOCA, the Na-K-ATPase activity of the CCD again increases, but shows no evidence of a decline after approximately two weeks (Fig. 5). Thirdly, if sodium entry into the CCD cells was reduced by treating the animals with the sodium channel blocker amiloride, the effects of DOCA on the Na-K-ATPase were markedly reduced (Fig. 4).[1]

A final argument in support of the notion that the initial effect of mineralocorticoids is on the Na^+ permeability of the luminal cell membrane comes from consideration of the initial response of the tubule. It was observed that the effects of mineralocorticoids on the Na-K-ATPase of the CCD were evident only after a latent period of approximately one day (Fig. 5). However, the sodium and potassium excretion patterns had already changed within the first day (Table 3), indicating that sodium absorption and potassium secretion in the CCD and other distal nephron segments had already been altered, supposedly reflecting an increase in the ion permeabilities of the luminal cell membrane. This view was directly verified recently using microelectrode techniques to quantitate the amiloride-sensitive sodium conductance of the luminal (apical) cell border of the rabbit CCD (Sansom & O'Neil, 1985). Within one day of elevating plasma mineralocorticoid levels, the sodium conductance of the apical cell border had doubled. Considering these data collectively, it seems reasonable to conclude that the initial effect of mineralocorticoids in stimulating Na^+ transport is on the Na^+ permeability of the apical cell membrane, and that the effects of the hormone on the Na-K-ATPase activity are secondary or delayed actions.

The actions of mineralocorticoids in the mammalian CCD appear to be similar to those observed in the toad urinary bladder where it is well known that sodium absorption is stimulated within 1–2 hr after exposure to the hormone (Civan & Hoffman, 1971; Crabbé, 1963). This initial effect can be attributed to an increase in the sodium conductance of the apical cell membrane (Civan & Hoffman, 1971; Palmer et al., 1982; Park & Edelman, 1982*a*; Spooner & Edelman, 1975) without an effect on the Na-K-ATPase activity (Hill, Cortas & Walser, 1973; Park & Edelman, 1984*b*). However, using polyclonal antibody techniques, Geering et al. (1982) demonstrated that the synthesis of the two subunits of the Na-K-ATPase of this tissue was stimulated in the presence of mineralocorticoids, but only after a delay of 6 hr. These investigators also noted that the two subunits could be immunoprecipitated separately, even after 18 hr, indicating they were not likely packaged into a functional Na-K-ATPase enzyme at that point. Consequently, it would appear that any effect of mineralocorticoids on the Na-K-ATPase activity of the total urinary bladder would only be expressed after a latent period of many hours, such as observed in the CCD, long after the initial effect on the apical cell membrane sodium permeability. By analogy of the toad

urinary bladder, therefore, it seems most likely that the mineralocorticoid-induced increase in the Na-K-ATPase activity of the CCD reflects increased synthesis of the enzyme, although other possibilities cannot be excluded.

Site of Sodium-Dependent Modulation of Na-K-ATPase Activity

As noted above, reduction of sodium entry into the CCD cells abolished the mineralocorticoid-induced increase in the Na-K-ATPase activity. This may be a common phenomenon in all aldosterone-sensitive epithelia since Handler and coworkers (1981) have also noted that aldosterone treatment of a toad kidney cell line (A6 cells) for 18 hr caused an increase in the number of Na^+ pump sites (ouabain-binding sites), which was abolished by blocking sodium entry into the cell with amiloride. Most importantly, Geering et al. (1982) noted that, while aldosterone treatment of toad bladder cells stimulated the rate of synthesis of the Na-K-ATPase subunits, the response was not influenced by blocking sodium entry into the cells with amiloride. It can be inferred from these studies, that Na^+ entry must regulate a posttranslational process. Explicitly, sodium entry must act as a positive modulator of a posttranslational process which regulates the expression of the enzyme as a functional Na-K-ATPase molecule at the basolateral membrane.

The notion of a sodium-dependent modulation of a posttranslational process in regulating the mineralocorticoid-induced stimulation of Na-K-ATPase activity is also apparent in studies of the CCD from adrenalectomized animals. In mammals, the Na-K-ATPase activity of the CCD declines to relatively low levels over several days after adrenalectomy (Doucet & Katz, 1981). It has been shown by some laboratories (Horster et al., 1980; Petty et al., 1981; el-Mernissi & Doucet, 1983), but not by others (Doucet & Katz, 1981), that if plasma aldosterone levels are now elevated, the Na-K-ATPase activity of the CCD is rapidly restored to near control values within 1 to 3 hr. Such a rapid recovery, if it occurs, is in marked contrast to the slow rate of stimulation of activity observed in animals with intact adrenal glands as reported in the present study. In view of the usual latent periods for mineralocorticoid-induced synthesis and expression of the Na-K-ATPase as noted above, and since the usual turnover of this enzyme is on the order of days (Jorgensen, 1972; Lo & Edelman, 1976), this rapid increase in the Na-K-ATPase activity in adrenalectomized animals likely does not reflect increased synthesis of the enzyme. That is, it most likely reflects regulation of a posttranslational process—enzyme subunit assembly, glycosylation, activation of latent pump sites, exocytosis, etc. Furthermore, this rapid mineralocorticoid-induced elevation in the Na-K-ATPase activity of CCD from adrenalectomized animals was shown by Petty et al. (1981) to be abolished by treatment with amiloride. This effect again points to Na^+-dependent regulation of a posttranslational process as previously suggested by Marver and Kokko (1983) for the adrenalectomized animal. It is probable, therefore, that there is a common Na^+-dependent posttransitional process modulating both acute and chronic mineralocorticoid-induced alteration in the Na-K-ATPase activity, at least for the CCD.

There is increasing evidence that if Na^+ entry into cells acts as a positive modulator of the Na-K-ATPase activity, it exerts this control on the mineralocorticoid-induced fraction of the Na-K-ATPase only. In the present study, for example, it was noted that amiloride treatment had no influence on the Na-K-ATPase activity of the CCD from animals not stimulated with mineralocorticoids.[1] Further, in animals maintained on a low Na diet, there was an initial stimulation in the Na-K-ATPase activity, which was slowly abated, returning to, but not below, its initial basal levels after approximately two weeks on this regimen. This supposedly occurred as a result of reduction in sodium entry into the cells of the CCD. Likewise, amiloride did not affect the basal Na-K-ATPase activity in CCD from adrenalectomized rabbits (Petty et al., 1981), the basal number of Na^+ pumps in the toad kidney cell line (Handler et al., 1982), or the basal rate of synthesis of the Na-K-ATPase subunits (Geering et al., 1982). It can be inferred from these data that under the normal control conditions, sodium entry into the cell likely does not exert, either directly or indirectly, any influence on the synthesis or posttranslational processing of the Na-K-ATPase molecule. The sodium-dependent modulation of the Na-K-ATPase must therefore reflect regulation of a mineralocorticoid-induced posttranslational process only.

The lack of an effect of reducing Na^+ entry on the basal Na-K-ATPase activity is not to imply that an increase in sodium entry into the cell, and hence the sodium load to the pump, may not, in and of itself, bring into play regulatory processes that increase the synthesis and/or posttranslational processing of the Na-K-ATPase. Indeed, as shown for the HeLa cells, elevation of intracellular sodium results in an increase in the number of sodium pumps in the plasma membrane within several hours (Boardman et al., 1974; Pollack, Tate & Cook, 1981). Whether an increase in the intracellular sodium concentration of the CCD results in a similar elevation in the number of functional sodium

pumps remains to be assessed directly for this tissue.

Finally, there is increasing evidence that the final step in insertion of a functional Na-K-ATPase enzyme into the plasma membrane may involve an exocytic process. It was noted for HeLa cells that insertion of the Na-K-ATPase likely occurs via an exocytic process, while removal of the enzyme likely occurs via an endocytic process (Cook, Tate & Shaffer, 1982). For the CCD, it has been observed that upon chronic mineralocorticoid stimulation of the Na-K-ATPase activity, the basolateral membrane area of the principal cell increases in equal proportion (approximately twofold) to the increase in Na-K-ATPase activity (Wade et al., 1979; Rastegar et al., 1980; Stanton et al., 1981; Kaissling & Le Hir, 1982; Stanton, 1985). These parallel changes likely reflect simultaneous addition of both enzyme and membrane, thereby implicating an exocytic process in the insertion of the Na-K-ATPase molecule into the plasma membrane.

If a functional Na-K-ATPase molecule is inserted into the plasma membrane via an exocytic process, this final step may be a candidate for sodium-dependent modulation of the Na-K-ATPase activity. Support for this view was obtained from the demonstration that some exocytic processes, such as amylase release by the pancreatic acinar cells, are modulated by Na^+ entry into the cells (Williams, 1975; Kanno, Saito & Sato, 1977). This Na^+-dependent modulation does not appear to be a direct effect of Na^+, but rather an indirect action of Na^+ entry via is effects on intracellular calcium levels, which in turn is thought to be the second messenger influencing the exocytic process (Cochrane et al., 1975; Poulsen & Williams, 1977). Hence, if the Na-K-ATPase is inserted into the plasma membrane by an exocytic mechanism, it could underly the Na^+-dependent modulation of the Na-K-ATPase activity observed in the present study, although other possibilities are viable. It remains for future studies to evaluate directly the role of sodium entry into the cell on exocytic insertion of the Na-K-ATPase into the basolateral membrane of the renal tubule.

The project was supported by a Grant-in-Aid from the American Heart Association (83 782) and with funds contributed in part by the Texas Heart Association. The authors express appreciation to Dr. William P. Dubinsky for assistance in setting up the Na-K-ATPase assay, and for critical reading of the manuscript. Amiloride was a generous gift of Merck, Sharp and Dohme (Rahway, NJ). This work was done during tenure of an Established Investigatorship (awarded to R.G. O'Neil) of the American Heart Association and with funds contributed in part by the Texas Heart Association (82 224).

References

Boardman, L., Huett, M., Lamb, J.F., Newton, J.P., Polson, J.M. 1974. Evidence for the genetic control of the sodium pump density in Hela cells. *J. Physiol. (London)* **241**:771–794

Charney, A.N., Silva, P., Besarab, A., Epstein, F.H. 1974. Separate effects of aldosterone, DOCA, and methylprednisolone on renal Na-K-ATPase. *Am. J. Physiol.* **227**:345–350

Civan, M.M., Hoffman, R.E. 1971. Effect of aldosterone on electrical resistance of toad bladder. *Am. J. Physiol.* **220**:324–328

Cochrane, D.E., Douglas, W.W., Mouri, T., Nakazato, Y. 1975. Calcium and stimulus-secretion coupling in the adrenal medulla: Contrasting stimulating effects of the ionophores X-537A and A23187 on catecholamine output. *J. Physiol. (London)* **252**:363–378

Cook, J.S., Tate, E.H., Shaffer, C. 1982. Uptake of [^{3}H] ouabain from the cell surface into the lysosomal compartment of Hela cells. *J. Cell Physiol.* **110**:84–92

Crabbé, J. 1963. Site of action of aldosterone on the bladder of the toad. *Nature (London)* **200**:787–788

Doucet, A., Katz, A.I. 1980. Renal potassium adaptation: Na-K-ATPase activity along the nephron after chronic potassium loading. *Am. J. Physiol.* **238**:F380–F386

Doucet, A., Katz, A.I. 1981. Short-term effect of aldosterone on Na-K-ATPase in single nephron segments. *Am. J. Physiol.* **241**:F273–F278

Edelman, I.S. 1981. Receptors and effectors in hormone action on the kidney. *Am. J. Physiol.* **241**:F333–F339

el-Mernissi, G., Doucet, A. 1983. Short-term effects of aldosterone and dexamethasone on Na-K-ATPase along the rabbit nephron. *Pfluegers Arch.* **399**:147–151

el-Mernissi, G., Doucet, A. 1984. Stimulation of Na-K-ATPase in the rat collecting tubule by two diuretics: Furosemide and amiloride. *Am. J. Physiol.* **247**:F485–F490

Garg, L.C., Knepper, M.A., Burg, M.B. 1981. Mineralocorticoid effects on Na-K-ATPase in individual nephron segments. *Am. J. Physiol.* **240**:F536–F544

Garg, L.C., Mackie, S., Tisher, C.C. 1982. Effect of low potassium-diet on Na-K-ATPase in rat nephron segments. *Pfluegers Arch.* **394**:113–117

Geering, K., Girardet, M., Bron, C., Kraehenbuhl, J.-P., Rossier, B.C. 1982. Hormonal regulation of (Na^+, K^+)-ATPase biosynthesis in the toad bladder. *J. Biol. Chem.* **257**:10338–10343

Handler, J.S., Preston, A.S., Perkins, F.M., Matsumura, M., Johnson, J.P., Watlington, C.O. 1981. The effect of adrenal steroid hormones on epithelia formed in culture by A6 cells. *Ann. N.Y. Acad. Sci.* **372**:442–454

Hendler, E.D., Torretti, J., Kupor, L., Epstein, F.H. 1972. Effects of adrenalectomy and hormone replacement on Na-K-ATPase in renal tissue. *Am. J. Physiol.* **222**:754–760

Hill, J.H., Cortas, N., Walser, M. 1973. Aldosterone action and sodium- and potassium-activated adenosine triphosphatase in toad bladder. *J. Clin. Invest.* **52**:185–189

Horster, M., Schmid, H., Schmidt, U. 1980. Aldosterone *in vitro* restores nephron Na-K-ATPase of distal segments from adrenalectomized rabbits. *Pfluegers Arch.* **384**:203–206

Jørgensen, P.L. 1968. Regulation of the (Na^+ + K^+)-activated ATP hydrolyzing enzyme system in rat kidney: II. The effect of adrenalectomy and the supply of sodium on the enzyme system. *Biochim. Biophys. Acta* **151**:212–224

Jørgensen, P.L. 1969. Regulation of the (Na^+ + K^+)-activated ATP hydrolyzing enzyme system in rat kidney: I. The effect of aldosterone on the activity in kidneys of adrenalectomized rats. *Biochim. Biophys. Acta* **192:**326–334

Jørgensen, P.L. 1972. The role of aldosterone in the regulation of (Na^+ + K^+)-ATPase in rat kidney. *J. Steroid Biochem.* **3:**181–191

Jørgensen, P.L. 1980. Sodium and potassium ion pump in kidney tubules. *Physiol. Rev.* **60:**864–917

Kaissling, B. 1982. Structural aspects of adaptive changes in renal electrolyte excretion. *Am. J. Physiol.* **242:**F211–F226

Kaissling, B., LeHir, M. 1982. Distal tubular segments of the rabbit kidney after adaptation to altered Na-and K-intake: I. Structural changes. *Cell Tissue Res.* **224:**469–492

Kanno, T., Saito, A., Sato, Y. 1977. Stimulus-secretion coupling in pancreatic acinar cells: Influences of external sodium and calcium on responses to cholecystokinin-pancreozymin and ionophore A23187. *J. Physiol. (London)* **270:**9–28

Katz, A.I. 1982. Renal Na-K-ATPase: Its role in tubular sodium and potassium transport. *Am. J. Physiol.* **242:**F207–F219

Koeppen, B.M., Biagi, B.A., Giebisch, G.H. 1983. Intracellular microelectrode characterization of the rabbit cortical collecting duct. *Am. J. Physiol.* **244:**F35–F47

LeHir, M., Kaissling, B., Dubach, U.C. 1982. Distal tubular segments of the rabbit kidney after adaptation to altered Na- and K-intake: II. Changes in Na-K-ATPase activity. *Cell Tissue Res.* **224:**493–504

Lo, C.-S., August, T.R., Liberman, U.A., Edelman, I.S. 1976. Dependence of renal (Na^+ + K^+)-adeonosine triphosphatase activity on thyroid status. *J. Biol. Chem.* **251:**7826–7833

Lo, C.-S., Edelman, I.S. 1976. Effect of triiodothyronine on the synthesis and degradation of renal cortical (Na^+ + K^+)-adenosine triphosphatase. *J. Biol. Chem.* **251:**7834–7840

Lo, C.-S., Lo, T.N. 1979. Time course of the renal response to triiodothyronine in the rat. *Am. J. Physiol.* **236:**F9–F13

Lowry, O.H., Passonneau, J.V. 1972. Flexible System of Enzymatic Analysis. Academic Press, New York

Marver, D., Kokko, J.P. 1983. Renal target sites and the mechanism of action of aldosterone. *Min. Electrol. Metab.* **9:**1–18

Mujais, S.K., Chekal, M.A., Jones, W.J., Hayslett, J.P., Katz, A.I. 1984. Regulation of renal Na-K-ATPase in the rat: Role of the natural mineralo- and glucocorticoid hormones. *J. Clin. Invest.* **73:**13–19

Natke, E., Stoner, L.C. 1982. Na^+ transport properties of the peritubular membrane of cortical collecting tubule. *Am. J. Physiol.* **242:**F664–F671

O'Neil, R.G., Dubinsky, W.P. 1983. Na-dependent mineralocorticoid regulation of cortical collecting duct (CCD) Na-K-ATPase. *Fed. Proc.* **42:**475

O'Neil, R.G., Dubinsky, W.P. 1984. Micromethodology for measuring ATPase activity in renal tubules: Mineralocorticoid influence. *Am. J. Physiol.* **247:**C314–C320

O'Neil, R.G., Hayhurst, R.A. 1984. Time course of mineralocorticoid effects on the renal cortical collecting duct Na-K-ATPase. *In:* Proceedings of the 8th International Biophysics Congress. 29 July–4 August, 1984, Bristol. p. 296

O'Neil, R.G., Helman, S.I. 1977. Transport characteristics of renal collecting tubules: Influences of DOCA and diet. *Am. J. Physiol.* **233:**F544–F558

Palmer, L.G., Li, J.H.-Y., Lindemann, B., Edelman, I.S. 1982. Aldosterone control of the density of sodium channels in the toad urinary bladder. *J. Membrane Biol.* **64:**91–102

Park, C.S., Edelman, I.S. 1984*a*. Effect of aldosterone on abundance and phosphorylation kinetics of Na-K-ATPase of toad urinary bladder. *Am. J. Physiol.* **246:**F509–F516

Park, C.S., Edelman, I.S. 1984*b*. Dual action of aldosterone on toad bladder: Na^+ permeability and Na^+ pump modulation. *Am. J. Physiol.* **246:**F517—F525

Petty, K.J., Kokko, J.P., Marver, D. 1981. Secondary effect of aldosterone on Na-K-ATPase activity in the rabbit cortical collecting tubule. *J. Clin. Invest.* **68:**1514–1521

Pollack, L.R., Tate, E.H., Cook, J.S. 1981. Turnover and regulation of Na-K-ATPase in HeLa cells. *Am. J. Physiol.* **241:**C173–C183

Poulsen, J.H., Williams, J.A. 1977. Effects of the calcium ionophore A23187 on pancreatic acinar cell membrane potentials and amylase release. *J. Physiol. (London)* **264:**323–339

Rastegar, A., Biemesderfer, D., Kashgarian, M., Hayslett, J.P. 1980. Changes in membrane surfaces of collecting duct cells in potassium adaptation. *Kidney Int.* **18:**293–301

Rossier, B.C. 1984. Biosynthesis of Na^+, K^+-ATPase in amphibian epithelial cells. *In:* Molecular Approaches to Epithelial Transport. J.B. Wade and S.A. Lewis, editors. pp. 125–145. Academic Press, New York

Sansom, S.C., O'Neil, R.G. 1985. Mineralocorticoid regulation of cell membrane Na and K pathways in rabbit cortical collecting dust (CCD). *Kidney Int.* **27:**319

Schwartz, G.J., Burg, M.B. 1978. Mineralocorticoid effects on cation transport by cortical collecting tubules *in vitro*. *Am. J. Physiol.* **235:**F576–F585

Silva, P., Hayslett, J.P., Epstein, F.H. 1973. The role of Na-K-activated adenosine triphosphatase in potassium adaptation: Stimulation of enzymatic activity by potassium loading. *J. Clin. Invest.* **52:**2665–2671

Spooner, P.M., Edelman, I.S. 1975. Further studies on the effect of aldosterone on electrical resistance of toad bladder. *Biochim. Biophys. Acta* **406:**304–314

Stanton, B.A. 1985. Role of adrenal hormones in regulating distal nephron structure and ion transport. *Fed. Proc. (in press)*

Stanton, B.A., Biemesderfer, D., Wade, J.B., Giebisch, G. 1981. Structural and functional study of the rat distal nephron: Effects of potassium adaptation and depletion. *Kidney Int.* **19:**36–48

Stekhoven, F.S., Bonting, S.L. 1981. Transport adenosine triphosphatases: Properties and functions. *Physiol. Rev.* **61:**1–76

Stokes, J.B. 1981. Potassium secretion by cortical collecting tubule: Relation to sodium absorption, luminal sodium concentration, and transepithelial voltage. *Am. J. Physiol.* **241:**F395–F402

Wade, J.B., O'Neil, R.G., Pryor, J.L., Boulpaep, E.L. 1979. Modulation of cell membrane area in renal collecting tubules by corticosteroid hormones. *J. Cell Biol.* **81:**439–445

Westenfelder, C., Arevalo, G.J., Baranowski, R.L., Kurtzman, N.A., Katz, A.I. 1977. Relationship between mineralocorticoids and renal Na^+-K^+-ATPase: Sodium reabsorption. *Am. J. Physiol.* **233:**F593–F599

Williams, J.A. 1975. Na^+ dependence of *in vitro* pancreatic amylase release. *Am. J. Physiol.* **229:**1023–1026

Wingo, C.S., Seldin, P.W., Kokko, J.P. 1982. Dietary modulation of active potassium secretion in the cortical collecting tubule of adrenalectomized rabbits. *J. Clin. Invest.* **70:**579–586

Received 16 October 1984