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Research Article

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Modulation of Na-K-ATPase Activity in the Mouse Medullary Thick Ascending Limb of Henle

Effects of Mineralocorticoids and Sodium

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Abstract

This study investigates the effect of variations in mineralocorticoid as well as cell sodium delivery and uptake on Na-K-ATPase activity in the mouse medullary thick ascending limb of Henle (mTALH). Pharmacologic doses of the mineralocorticoid deoxycorticosterone acetate (DOCA) resulted in a 28% increase of Na-K-ATPase activity. Furosemide-induced inhibition of sodium uptake by the mTALH cell also resulted in Na-K-ATPase activity reduction (45%). Sodium deprivation did not cause a clear change in enzyme activity, either at 3 d or 2 wk, likely reflecting the result of the opposing influences of decreased sodium delivery and increased endogenous aldosterone. Finally, the behavior of Na-K-ATPase activity at 3 d of sodium deprivation in the mTALH contrasted with a 60% increase in activity observed in the cortical collecting tubule, a nephron segment known to be responsive to mineralocorticoid, and this heterogeneity of response may suggest an important role for the mTALH in maintaining salt homeostasis.

Introduction

The distal nephron segments, particularly the distal convoluted tubule and the cortical collecting tubule (CCT),¹ are able to adapt to variations in salt intake and attendant changes in hormonal milieu. The CCT, for example, has been shown to undergo structural (1-3), functional (2, 4-7), and biochemical (4, 8-18) changes in response to alterations in sodium and potassium intake, as well as to alterations in the circulating levels of mineralocorticoid. On the other hand, the medullary thick ascending limb of Henle (mTALH), long known to absorb a substantial fraction of filtered sodium and recently shown to both absorb (19) and secrete (20) potassium, has

received less attention with respect to its ability to respond to alterations in dietary salt load and mineralocorticoids.

Na-K-ATPase is the enzyme responsible for electrogenic sodium and potassium transport in the mTALH as well as in the CCT. Modulation of Na-K-ATPase activity is among the important biochemical alterations observed in the CCT with variation of salt intake (8, 9, 11, 21), and Na-K-ATPase activity in the CCT is stimulated when exposed to mineralocorticoid. Only recently have there been reports that Na-K-ATPase activity in the mTALH may be altered. Trinh-Trang-Tan, Bankir, and their colleagues (22) have demonstrated that mTALH Na-K-ATPase activity increases when antidiuretic hormone is administered to rats with central diabetes insipidus. In addition, these (23) and other (24) investigators have observed that increased protein intake also causes stimulation of Na-K-ATPase activity in the mTALH of rats. Doucet and Katz (9) noted a 25% increase in mTALH Na-K-ATPase activity in mice after chronic potassium adaptation. Finally, Scherzer et al. (25) have demonstrated increases in enzyme activity in rabbit mTALH segments after uninephrectomy and increased salt intake.

In the present studies, we demonstrate that large doses of mineralocorticoid increase Na-K-ATPase activity in the mTALH, and that furosemide-induced inhibition of sodium uptake decreases enzyme activity. It is possible that sodium delivery to, and uptake by, the mTALH participate in the mineralocorticoid-associated modulation Na-K-ATPase in this nephron segment. The response of enzyme activity in the mTALH during sodium deprivation differs from that in the CCT, and this finding suggests an important role for the mTALH in the maintenance of renal salt homeostasis.

Methods

Animals. 3- to 4-wk old male mice (CD₁, Charles River Breeding Laboratories, Wilmington, MA) were maintained on one of several specially modified AIN-76 diets (ICN Nutritional Biochemicals, Cleveland, OH), until the time of experiment, (6-21 d later). The diets varied in sodium and potassium content as follows: standard diet (0.05 meq Na/g, 0.17 meq K/g); sodium replete (0.17 meq Na/g, 0.16 meq K/g); sodium poor (0.01 meq Na/g, 0.16 meq K/g). All diets contained 20% protein by weight. The mice were fed tap water ad lib.

Deoxycorticosterone acetate (DOCA; Sigma Chemical Co., St. Louis, MO) was administered either by daily subcutaneous injection (20 mg/kg, dissolved in sesame oil) or by a subcutaneous tablet (25 mg: 6-mm diam, thickness 2.5 mm; Innovative Research of America, Rockville, MD). Furosemide was also administered by subcutaneous tablet (100 mg: 10-mm diam, thickness 6 mm). These tablets contained a matrix consisting of cholesterol, methyl cellulose, and lactose in addition to the drug being administered.

The tablets were inserted subcutaneously through a small dorsal transverse incision under light ether anesthesia, and the incision closed with 6-0 silk sutures. Immediate and long-term postoperative survival

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1. *Abbreviations used in this paper:* CCT, cortical collecting tubule; DOCA, deoxycorticosterone acetate; mTALH, medullary thick ascending limb of Henle.

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was excellent (> 90%) in mice receiving the DOCA tablets. While immediate recovery was also > 90% in the mice receiving furosemide tablets, only 20% generally survived for > 24 h. This may have been the result of the marked diuresis and natriuresis which occurred during the initial 24 h after furosemide tablet insertion. This is in accordance with the claim of the manufacturer that furosemide could be detected in plasma within the same time period. It is likely that DOCA also was absorbed rapidly from the DOCA tablets, and that significant blood levels of the drug were achieved within 24 to 48 h. Delivery of these drugs to the circulation should persist for up to 14 d for furosemide and up to 21 d for DOCA (Innovative Research of America). The average daily dose of DOCA, assuming complete and steady absorption, was between 4 and 5 mg/100 g body weight.

Metabolic studies. 24-h food and water intakes and urine outputs were determined from two or three mice placed in a metabolic cage (Lab Products). Preliminary studies demonstrated that having more than one mouse in a cage improved the ability of the mice to adapt to the cage environment, providing for rapid attainment of a stable metabolic state. Total food and water intakes and urine outputs from the two to three mice in each cage were normalized to 100 g mouse body weight. At the end of the 24-h period, the mice were anesthetized with ether, and plasma was obtained by exsanguination via the abdominal aorta for determination of sodium, potassium, and aldosterone. Plasma sodium and potassium concentrations were determined by flame photometer (model 443; Instrumentation Laboratories). Plasma aldosterone concentration was measured by radioimmunoassay (Diagnostic Products).

Microdissection. The mice were anesthetized with ether and their kidneys perfused *in vivo* via the abdominal aorta with 3–5 ml of chilled (4°C) dissection solution (see below) containing collagenase (type 1A; Sigma Chemical Co., 65–90 U/ml). The kidneys were then removed, quartered, and incubated in the collagenase-containing solution for 10 min at 37°C. Preliminary studies demonstrated that collagenase had no effect on Na-K-ATPase activity. Collagenase treatment was not used to obtain CCT segments.

Individual mTALH segments were obtained by freehand dissection of the inner stripe of the outer medulla, using the lower extent of the proximal straight tubule as the demarcation between inner and outer stripes. CCT segments were dissected from the medullary rays, and morphology was confirmed by the typical appearance under differential interference-contrast microscopy (26). Because of the recognized tubule-to-tubule variation within a given animal of both transport characteristics and enzyme activity, six tubule segments were obtained from each animal and assayed separately to increase the accuracy of enzyme activity determinations. Each mTALH tubule segment was measured with a calibrated eyepiece reticle. This method of measurement proved unsatisfactory for CCT segments because of their considerable tortuosity. Instead, video images of CCT segments were digitized (Ikgame video camera interfaced to an IBM-AT-based Imaging Technologies digitizing board) and length measured using Media Cybernetics interactive image processing software. The shortest tubule segments accepted for assay were 0.3 mm, which is above the lower limit of sensitivity for this assay reported by O'Neil and Dubinsky (12).

ATPase assay. The ATPase activities of the mTALH and CCT segments were determined by a modification of the microfluorometric assay described by O'Neil and Dubinsky [(12), see Fig. 1]. The assay links the hydrolysis of ATP to the formation of NAD, which then fluoresces after heating in the presence of strong alkali.

Several methods (12) were tested to permeabilize the tubule before enzyme assay, including treatment with several nonionic detergents [lubrol WX and polyoxyethylene ether W-1 (Sigma Chemical Co.)], freeze-thaw shock, and osmotic shock. The easiest, most reliable and most reproducible method proved to be a combination of osmotic and freeze-thaw shocks. Thus, 5 μ l distilled deionized water was added to a reactivial (Pierce Chemical Co., Rockford, IL) containing an individual tubule segment in 2.5 μ l dissection solution, and then the reactivial was alternately submersed in liquid nitrogen and water at 37°C three times.

After permeabilization of the tubule, 7.5 μ l of incubation medium (see below) was added to the vial containing the tubule, and 7.5 μ l silicone oil (Dow Corning 200; Corning Glassware, Corning, NY) was layered on top to prevent evaporation. The vials were then placed in a water bath at 37°C for 30 min. After incubation, the reaction was terminated and residual NADH destroyed by adding 15 μ l of 0.5 N HCl. The condensation of NAD requires darkness, thus the acidified incubation medium (20 μ l) was placed in a foil-wrapped testtube, 750 μ l of 6 N NaOH was added, and the combination mixed thoroughly. The mixture was heated to 60°C in a water bath for 20 min to condense the NAD, and thereby enhance fluorescence. To stabilize the fluorescence signal, 1.0 ml of distilled deionized water was added to each sample (27), and the fluorescence was determined in arbitrary units with a standard manual fluorometer (Farrand Optical, Valhalla, NY) at an excitation of 340 nm, and an emission of 460 nm.

As indicated in the reaction sequence diagrammed in Fig. 1, the total activity of the tubule ATPases (Na-K plus Mg) is directly proportional to the rate of ATP hydrolysis to ADP. Standard curves of fluorescence were obtained for ADP (0.25 to 4.0 nmol) treated in the same manner as for tubules. The standard curves were then used to calculate the rate of ATP hydrolysis of individual mTALH tubule segments. Thus, ATPase activity is reported in picomoles ADP generated normalized to a millimeter of tubule length per hour of incubation.

To determine specific Na-K-ATPase activity, three of the mTALH segments obtained from each mouse were incubated separately in the absence of ouabain (total ATPase activity), and three in the presence of ouabain (Mg ATPase activity). Na-K-ATPase activity (ouabain-sensitive ATPase) was then calculated as the difference between the average activities (mean of three separate determinations) in the absence and presence of ouabain.

Initial studies indicated that at concentrations of ouabain above one millimolar, quenching of fluorescence occurred. As a result, when using concentrations of ouabain higher than 1 mM, a separate standard curve was generated in the presence of ouabain, and this was used to calculate the tubule ATPase activity in the presence of this inhibitor.

The inhibition of total ATPase activity in the mouse mTALH with increasing concentrations of ouabain is shown in Fig. 2. All activities were corrected for the "quenching" effect noted above. Maximal ouabain-mediated inhibition of ATPase activity occurred at a concentration between 1 and 5 mM (Fig. 2), and thus, the latter concentration was used to determine the ouabain-independent (i.e., Mg-dependent) ATPase activity in all studies described in this paper. It should be noted that this concentration of ouabain is higher than that needed to inhibit completely Na-K-ATPase activity in the rabbit (12). However, the requirement for a higher ouabain concentration is consistent with the known relative insensitivity of Na-K-ATPase to this inhibitor in some rodents and is similar to that recently used by Garg et al. (10) and by Doucet et al. (28) to inhibit renal Na-K-ATPase activity in the rat and mouse, respectively.

Solutions. The dissection solution contained (in millimolar): 148 NaCl, 5 KCl, 1.2 MgSO₄, 1.0 CaCl₂, 0.25 Na₂HPO₄, 1.0 NaH₂PO₄, pH 7.4. The incubation medium, made daily, contained (in millimolar): 120 NaCl, 120 choline Cl; 60 KCl, 100 imidazole (low fluorescence blank), 10 MgCl₂, 1.0 EGTA, 10 Na₂ATP (equine muscle, vanadium free), 20 phosphoenolpyruvate (PEP, tri-monocyclohexylammonium salt), 2 ascorbic acid, 2.0 NADH; plus 2.8 U/ml pyruvate kinase and 4.0 U/ml lactate dehydrogenase, pH 7.0 at 37°C. All reagents, enzymes, and nucleotides were obtained from Sigma Chemical Co. The concentrations of substrates, enzymes, and ions in the final

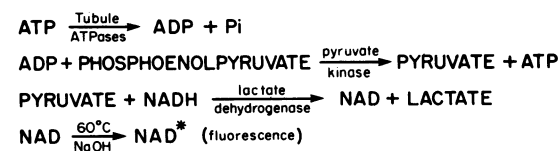


Figure 1. ATPase assay reaction sequence.

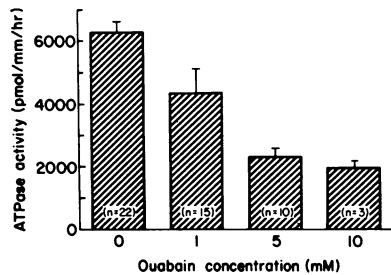


Figure 2. Ouabain inhibition of total ATPase activity in the mouse mTALH. A range of concentrations of ouabain were employed to determine the concentration at which maximal inhibition of total ATPase activity would occur to quantitate accurately the Na-K-dependent (ouabain-inhibited) portion of total ATPase activity. Maximal inhibition occurred between 1 and 5 mM.

reaction mixture were approximately one-half of those in the incubation medium as a result of dilution of the medium with dissection solution and deionized water. However, these final concentrations remained far in excess of that required for the enzymatic reactions to proceed at maximal velocity (12).

Statistical analysis. Total and Mg ATPase activities were obtained by averaging the activities of four to six tubule segments in each mouse, half in the absence and half in the presence of ouabain, respectively. Na-K-ATPase activity was calculated by taking the difference between average total and average Mg ATPase activity for each mouse. Food intake and urine output values were calculated per 100 mg body weight of the two or three mice in the metabolic cage. Plasma sodium, potassium, and aldosterone values were treated separately for each mouse. The results of the study are summarized as mean values \pm SEM. The unpaired Student's *t* test was used for comparison of untreated animals on a standard diet (group 1) to those treated with DOCA (group 2), and for comparison of Na-K-ATPase activities of CCT segments from sodium replete and sodium deprived (3–4 d) animals. When more than two groups were compared (groups 3–6), one-way analysis of variance and the Bonferroni *t* test were applied (29, 30). *P* values < 0.05 were considered significant.

Results

Effect of chronic DOCA administration on Na-K-ATPase activity in the mTALH. Initial studies assessed whether DOCA modulated Na-K-ATPase activity in the mTALH as it does in the mineralocorticoid-responsive CCT. Two groups of mice were studied: group 1 mice were fed the standard diet and group 2 mice were administered DOCA and fed the standard diet.

Metabolic study. The metabolic data collected from groups 1 and 2 are summarized in Table I. There was no difference in the food intake between the groups 1 and 2. Urinary sodium and potassium excretions paralleled intakes in groups 1 and 2, indicating that both groups were in balance and that the mice receiving DOCA had achieved mineralocorticoid "escape." Plasma potassium in the DOCA-treated animals was significantly lower compared with group 1 (4.0 vs. 5.2, respectively). This reduction in plasma potassium is consistent with mineralocorticoid-mediated increases in potassium secretory processes in distal nephron segments (31).

ATPase activity. Na-K-ATPase and Mg-ATPase activities of mTALH segments in groups 1 and 2 are shown in Table II. Na-K-ATPase activities in mTALH segments from mice in group 1 averaged 4,000 pmol ADP \cdot mm⁻¹ \cdot h⁻¹, a value comparable to that observed by Doucet and Katz (4,063 pmol \cdot mm⁻¹ \cdot h⁻¹) (9) in this same species. 10–21 d of DOCA treatment increased Na-K-ATPase activity in the mTALH \sim 30% to 5120 pmol ADP \cdot mm⁻¹ \cdot h⁻¹ (*P* < 0.05). The effect of DOCA on Na-K-ATPase activity reached a maximal value by the earliest sampling point (10 d) of the experimental period. Thus, DOCA administration results in enhanced Na-K-ATPase activity in the mouse mTALH.

Effects of varying sodium intake and of furosemide administration on Na-K-ATPase activity in the mTALH. During chronic mineralocorticoid administration, glomerular filtration rate tends to increase (32, 33) and as a result, sodium delivery to the distal nephron may be enhanced. Salt intake and delivery to sodium-transporting distal nephron segments has been shown to modulate the Na-K-ATPase activity in these segments (4, 11, 21, 34, 35). It is possible, therefore, that the increase in enzyme activity following DOCA administration (Table II) could have resulted from a mineralocorticoid-induced increase of sodium delivery to this nephron segment rather than a direct cellular effect of the hormone. Thus, we investigated whether changes in Na-K-ATPase activity in the mTALH occurred in response to the inhibition of cell sodium uptake with furosemide as well as in response to decreased sodium intake. Four groups of mice were studied: group 3 mice were fed a sodium-replete diet, group 4 mice received furosemide and were fed the sodium-replete diet, group 5 mice were fed a sodium-poor diet for 3–4 d, and group 6 mice were fed the sodium-poor diet for 10–15 d.

Metabolic study. The metabolic data for groups 3, 4, and 6 are summarized in Table III. Sodium, potassium, and water intakes paralleled outputs in all three groups. The mice administered furosemide (group 4) excreted 32% more sodium and potassium and had a fivefold increase in urine output

Table I. Daily Metabolic Data for Groups 1 and 2

Diet	Treatment	No. of days	n	Intake			Urine		Plasma				
				Food	Na	K	Volume	Na	K	Na	n	K	
				g/100 gBW	meq/100 gBW	cm ³ /100 gBW	cm ³ /100 gBW	meq/100 gBW		meq/liter			
Standard (group 1)	—	6–21	(6)	11.5 \pm 1.6	0.58 \pm 0.08	1.96 \pm 0.28	14.4 \pm 1.4	5.3 \pm 0.8	0.38 \pm 0.05	1.25 \pm 0.11	140.7 \pm 0.9	(17)	5.2 \pm 0.3
Standard (group 2)	DOCA	6–21	(4)	10.8 \pm 1.0	0.54 \pm 0.05	1.84 \pm 0.18	19.2 \pm 4.2	7.0 \pm 0.5	0.54 \pm 0.06	1.32 \pm 0.13	138.6 \pm 1.3	(6)	4.0 \pm 0.2*

* *P* vs. group 1 < 0.05.

Table II. Influence of DOCA on ATPase Activities in the mTALH

Diet	Treatment	n	Total ATPase	Mg-ATPase		Na-K ATPase
				<i>pmol ADP mm⁻¹ h⁻¹</i>		
Standard (group 1)	—	(22)	6,270±340	2,270±150		4,000±230
Standard (group 2)	DOCA	(12)	7,590±560*	2,470±260		5,120±430*

* *P* < 0.05.

compared with group 3. Thus, group 4 manifested marked natriuresis and diuresis, the hallmarks of furosemide administration, and consistent with the inhibition of sodium uptake in the mTALH by furosemide. Sodium intake and urinary sodium excretion of the sodium-poor group (group 6) was 5–10% of that of the control group.

Plasma sodium did not differ among groups. Plasma potassium was slightly, but significantly, lower in group 6 than in the sodium replete group (4.1 vs. 4.9 meq/liter, respectively). Since aldosterone levels in group 6 were significantly elevated (from 22 ng · dl⁻¹ in group 3, to 191 ng · dl⁻¹ in group 6, see Table IV), the reduction in plasma potassium in group 6 mice likely reflects mineralocorticoid effects on distal potassium secretory processes (31).

ATPase activity and plasma aldosterone. ATPase activities of mTALH segments as well as aldosterone levels in plasma from the sodium-replete (group 3), furosemide-treated (group 4), and sodium-poor (both short- and long-term, groups 5 and 6, respectively) animals are summarized in Table IV. Total ATPase activities in the experimental groups (4–6) differed significantly from the sodium replete control group, and Mg-ATPase activities did not differ among the four groups of mice. Yet, while there was a significant decrease in Na-K-ATPase activity in the furosemide-treated group (group 4), the Na-K-ATPase activities of the sodium-poor groups (groups 5 and 6) did not differ significantly from the control sodium-replete group (group 3). The pronounced decrease in Na-K-ATPase activity observed in the furosemide-treated mice suggests that inhibition of sodium entry to the mTALH cell results in inhibition of pump activity. When sodium intake, and presumably sodium delivery to the mTALH, was decreased either for 3–4 d or for 2 wk, no significant change in Na-K-ATPase activity was observed. In this regard, it should be noted that the differences in Na-K-ATPase activities do approach statistical significance

(*P* < 0.09 at 3–4 d, and *P* < 0.14 at 2 wk) using the relatively rigorous Bonferroni *t* test, and are nearly significant applying the less rigorous Neuman-Keuls test. Since Na-K-ATPase activity is a calculated value, the error associated with each individual value will be larger than that of either of the measured ATPase values. Thus it is possible that the decreases in the total ATPase activities observed in the experimental groups may represent reductions in Na-K-ATPase activity.

Whether or not Na-K-ATPase remains unchanged or decreases during sodium deprivation, this behavior occurs in the face of significant increases in plasma aldosterone. Plasma aldosterone after 3–4 d on the sodium-poor diet was 107 ng · dl⁻¹ (vs. 22 ng · dl⁻¹ in group 3), and after 10–15 d was 191 ng · dl⁻¹. Thus it appears that while furosemide-induced blockade of cell sodium uptake results in a decrease in mTALH Na-K-ATPase activity, potential decreases in this enzyme activity following sodium deprivation appear to be counterbalanced, completely or nearly completely, by increases in plasma aldosterone.

Comparison of the effect of short-term sodium deprivation (3–4 d) on Na-K-ATPase activity in the mTALH vs. the CCT. It is well recognized that the CCT is a target site for mineralocorticoids (36) and that Na-K-ATPase activity in this nephron segment is regulated by aldosterone or other mineralocorticoids, including exogenously administered DOCA (10, 12–14). Recently, O'Neil and Hayhurst (11) examined the effects of sodium deprivation on Na-K-ATPase activity in the rabbit CCT. These workers observed an increase in enzyme activity in animals on a low sodium diet for 3–4 d, and a subsequent reduction in enzyme activity to control levels at 2 wk despite an early and sustained elevation of plasma aldosterone. In light of the behavior of Na-K-ATPase activity in the mouse mTALH after 3–4 d of sodium deprivation noted above (unchanged or slightly decreased from sodium replete animals) that may influence sodium delivery to the CCT

Table III. Daily Metabolic Data for Groups 3, 4, and 6

Diet	Treatment	No. of Days	n	Intake				Urine			Plasma		
				Food	Na	K	Water	Volume	Na	K	Na	K	
				<i>g/100 g</i>	<i>meq/100 g</i>		<i>cm³/100 g</i>	<i>cm³/100 g</i>	<i>meq/100 g</i>	<i>meq/100 g</i>	<i>meq/liter</i>		
Sodium replete (group 3)	—	10–21	(9)	13.9 ±1.8	2.37 ±0.18	2.22 ±0.17	19.9 ±1.2	8.2 ±0.7	1.95 ±0.16	1.86 ±0.14	148.2 ±1.5	(16)	4.9 ±0.1
Sodium replete (group 4)	Furosemide	9–11	(5)	16.1 ±0.9	2.73 ±0.16	2.57 ±0.15	74.0 ±5.7 [‡]	44.4 ±5.0 [‡]	2.57 ±0.20 [‡]	2.45 ±0.20 [‡]	146.5 ±1.1	(3)	4.3 ±0.3
Sodium poor (group 6)	—	10–15	(6)	12.2 ±0.7	0.15 ±0.01*	1.95 ±0.11	14.2 ±1.1*	5.2 ±0.3	0.09 ±0.01*	1.53 ±0.10	144.1 ±0.9	(9)	4.1 ±0.1 [§]

* *P* < 0.05 vs. groups 3 and 4. [‡] *P* < 0.05 vs. groups 3 and 6. [§] *P* < 0.05 vs. group 3.

Table IV. Influence of Dietary Sodium Intake, Furosemide, and Endogenous Aldosterone on ATPase Activities in the mTALH

Diet	Treatment	Plasma aldosterone <i>ng dl⁻¹</i>	<i>n</i>	Total ATPase	Mg-ATPase <i>pmol ADP mm⁻¹ h⁻¹</i>	Na-K ATPase	<i>n</i>
Sodium replete (group 3)	—	22±8	(5)	7,240±430	2,830±260	4,410±290	(10)
Sodium replete (group 4)	Furosemide	76±23*	(3)	5,410±240 [‡]	2,940±630	2,470±660 [‡]	(4)
Sodium poor (3–4 d) (group 5)	—	107±17 [§]	(5)	5,830±270 [‡]	2,350±150	3,480±240	(9)
Sodium poor (10–15 d) (group 6)	—	191±10	(3)	6,230±220 [‡]	2,530±150	3,700±190	(22)

* $P < 0.05$ vs. group 6. [‡] $P < 0.05$ vs. group 3. [§] $P < 0.05$ vs. groups 3 and 6. ^{||} $P < 0.05$ vs. groups 3 and 5.

downstream, we were interested to determine what the short-term effect of sodium deprivation was in the CCT of the mouse. Thus, Na-K-ATPase activity was measured in CCT segments from mice fed the sodium-poor diet for 3–4 d.

The results from this study are summarized in Fig. 3. Enzyme activity increased by 60% in the mouse CCT, from 1,610±340 to 2,570±260 pmol ADP·mm⁻¹·h⁻¹, $P < 0.05$, ($n = 5$, both groups). The rise in CCT enzyme activity occurred in conjunction with a fivefold increase in plasma aldosterone from 22±8 to 107±17 ng·dl⁻¹, $P < 0.05$, ($n = 5$, both groups; see Fig. 3, inset).

Discussion

This study demonstrates that Na-K-ATPase activity in the mouse mTALH is modulated by exogenous mineralocorticoid (Table II) and by reducing mTALH cell sodium uptake with furosemide (Table IV). Enzyme activity varied from 2,470 pmol ADP·mm⁻¹·h⁻¹ in the furosemide-treated mice (Table IV) to 5,120 pmol ADP·mm⁻¹·h⁻¹ in mice administered DOCA (Table II), spanning a greater than twofold range of activities. Na-K-ATPase activity during sodium deprivation remains little changed and may represent a combination of the enhancing influence of elevated plasma aldosterone levels and the depressing influence of decreased sodium delivery to, and uptake by, the mTALH.

Mineralocorticoid-associated modulation of Na-K-ATPase activity in the mTALH. Our results (Table II) demonstrate for the first time that chronic DOCA administration to mice with intact adrenal function increases Na-K-ATPase activity in the mTALH. Since food intakes were similar in group 1 and 2 animals, it is unlikely that differences in protein (23, 24), sodium or potassium [Tables I and III] intakes could have accounted for the modulation of enzyme activity in the DOCA-treated mice. However, at least one of two other possible explanations might account for this finding: a direct action on the mTALH cell mediated either via mineralocorticoid receptors [type I: (37)] or via glucocorticoid receptors [type II: (36, 38, 39)] resulting from nonphysiologic levels of DOCA; and/or an indirect action related to corticosteroid effects on hemodynamics (glomerular filtration rate or cardiac output) which would alter sodium delivery to the loop of Henle (32, 33).

While the present studies do not permit us to distinguish

unequivocally between these two possibilities, several lines of evidence suggest that DOCA may have exerted a direct action on the mTALH. First, both aldosterone-specific (37) and dexamethasone-specific (36, 38, 39) binding sites have been identified in the mTALH using autoradiography and classic binding techniques. In addition, a number of laboratories (13, 40–42) have reported that Na-K-ATPase activity in the rat mTALH is reduced by 25–50% after adrenalectomy. However, it is unclear whether this effect of adrenalectomy is due to loss of type I receptor effects or type II receptor effects, since either aldosterone (42) or dexamethasone (40, 41) has been shown to restore Na-K-ATPase activity to normal or near normal in the mTALH. Moreover, Marver and co-workers (16, 36) observed a 42% reduction in the activity of another enzyme, citrate synthase (a putative marker of mineralocorticoid action), in the rabbit mTALH following adrenalectomy, and demonstrated that the activity of this enzyme could be restored to normal with aldosterone, but not dexamethasone, administration.

Since salt transport in the mTALH is a secondary active transport process that is dependent on basolateral membrane Na-K-ATPase activity, the modulation of enzyme activity by corticosteroids may provide a means for controlling the transport function of the mTALH. In fact, several recent studies suggest that corticosteroids, particularly mineralocorticoid, directly affect salt transport by the mTALH. Work and Jamison (43), using isolated perfused tubule techniques, and Stanton (44), using microperfusion techniques, demonstrated that adrenalectomy decreased the rates of sodium reabsorption by the rat loop of Henle and that these transport processes were restored to normal by chronic aldosterone, but not dexamethasone, replacement. Since sodium and potassium concentrations and fluid flow rates were held constant in the in vivo microperfusion studies, alterations in salt delivery or tubule fluid composition could not have accounted for the aldosterone-associated enhancement of salt absorption. Moreover, Green et al. (45) showed that adrenalectomy decreased, and subsequent aldosterone (but not prednisolone) administration restored, free water generation by the rat kidney, a process dependent upon NaCl absorption by the virtually water-impermeable TALH. A similar finding was noted with respect to urinary concentrating ability in rats (46), rabbits (47), and dogs (48). Our results (Table II) are, however, at variance with those

of Garg et al. (10), who failed to note any change in Na-K-ATPase activity in the rabbit mTALH after DOCA administration for 12–18 d. The differences between our study and that of Garg et al., which include the dosage of DOCA administered, the salt contents of the diets, and the species studied, may explain this discrepancy. Furthermore, it is possible that tubule-to-tubule variation in enzyme activity may require that a larger number of animals be studied ($n = 4$ vs. $n = 12$ in the present study) and a larger number of tubule segments per animal be assayed ($n = 2$ vs. $n = 6$ in the present study), to appreciate modest fractional difference in enzyme activity. Finally, it should be noted that our results are also at variance with those of Mujais et al. (14) who failed to note an increase in Na-K-ATPase activity in the mTALH of intact rats to whom a smaller dose of aldosterone ($5 \mu\text{g}/100 \text{ g body wt}/24 \text{ h}$) was administered by osmotic minipump. It is likely that our group of 2 animals received a much greater daily delivery of mineralocorticoid (see Methods).

Sodium-dependent modulation of Na-K-ATPase activity. The present studies employed two maneuvers to decrease sodium delivery/uptake to the mTALH: furosemide-induced blockade of sodium uptake and decreased sodium intake. Directly blocking cell sodium uptake with furosemide (Table IV) resulted in a 45% decrease in Na-K-ATPase activity. This decrease in Na-K-ATPase activity is consistent with the finding that sodium loading is associated with an increase in Na-K-ATPase activity in the mTALH of rabbits (25). On the other hand, El Mernissi and Doucet (49) and Scherzer et al. (50) were not able to detect a change in Na-K-ATPase activity in the mTALH of rats following furosemide administration. Scherzer et al. attributed the absence of any change in rat mTALH Na-K-ATPase activity to increased sodium delivery resulting from an increase in glomerular filtration rate after furosemide administration. The El Mernissi and Doucet study resembled ours in that furosemide was administered chronically (3–8 d) and continuously (osmotic minipump), thus providing for a tonic inhibition of Na-K-2Cl cotransport. However, the diuretic effect obtained by El Mernissi and Doucet ($\sim 166\%$ of control) was far below that obtained in the present study ($\sim 500\%$ of control, Table III), suggesting that complete inhibition of sodium entry may not have been achieved by El Mernissi and Doucet.

Sodium deprivation failed to elicit a statistically significant decrease in Na-K-ATPase activity at either 3–4 d or 2 wk. Varying dietary sodium intake in order to alter sodium delivery to the distal nephron has been employed with apparent success to study the modulation of Na-K-ATPase activity in two other distal nephron segments—the mineralocorticoid-responsive CCT (11) and the mineralocorticoid-unresponsive distal convoluted tubule (21), although actual delivery of sodium was not measured. If sodium delivery to the mTALH decreased during sodium deprivation, it is likely that any resulting decrease in Na-K-ATPase activity (in light of the decrease in enzyme activity with furosemide-induced blockade of sodium uptake) was counterbalanced, to a great extent, by five- to ninefold increases in plasma aldosterone (Table IV).

The present results (Table IV and Fig. 3) also indicate that the responses of the mTALH and the CCT to sodium deprivation are heterogeneous. O'Neil and Hayhurst (11) recently found that Na-K-ATPase activity in the rabbit CCT increases at 3–4 d on a low sodium diet, but falls to control levels by 2 wk. They suggested that the early increase in CCT enzyme

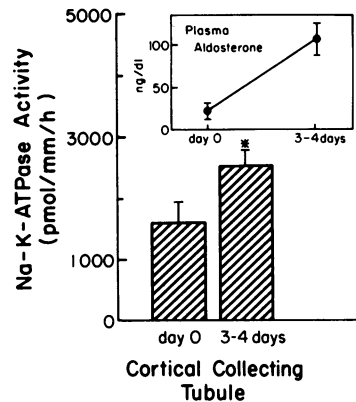


Figure 3. Acute (3–4 d) effects of sodium deprivation on Na-K-ATPase activity in the CCT, and concurrent plasma aldosterone levels (inset). Sodium deprivation for 3–4 d resulted in an increase in both CCT enzyme activity (60%) and in circulating plasma aldosterone levels (fivefold, $P < 0.05$). * $P < 0.05$ vs. day 0.

activity is the result of a rise in circulating aldosterone before sodium delivery to that segment declines, and the fall in enzyme activity seen at two weeks is the result of diminished sodium delivery. The present results (Fig. 3) demonstrate that Na-K-ATPase activity is also increased in the CCT from the mouse and that this enhanced activity coincides with a fivefold increase in plasma aldosterone. In contrast, Na-K-ATPase activity in the mouse mTALH remained the same or slightly decreased (20–25%, $P < 0.09$) at 3–4 d of sodium restriction (Table IV).

Possible conclusions from the heterogeneity of Na-K-ATPase activity response to sodium deprivation in the distal nephron. The heterogeneity of response to sodium intake and, presumably, sodium delivery exhibited by the mTALH and the CCT may aid the kidney in maintaining salt homeostasis. It is possible that maintaining (or slightly decreasing) Na-K-ATPase activity in the mTALH during the early (3–4 d) phase of sodium deprivation may allow distal sodium delivery to the CCT to remain at normal or near normal levels for a longer period of time. O'Neil and Hayhurst (11) suggest that maintenance of distal sodium delivery is required for a maximal effect of aldosterone on enhancing Na-K-ATPase activity in the rabbit CCT. Since potassium excretion by the CCT is both flow- and sodium-dependent (31), maintenance of sodium delivery to the CCT in the early stages of sodium deprivation may also be important in preventing significant reductions in potassium excretion. As a result, the heterogeneity of response exhibited by Na-K-ATPase activity in the mTALH and the CCT may help to maintain potassium homeostasis and prevent hyperkalemia in the early stages of sodium deprivation.

The magnitude of Na-K-ATPase activity changes: mTALH vs. CCT. 2–3 wk of DOCA administration to intact animals causes a two- to fourfold increase in Na-K-ATPase activity in the CCT (10, 12, 14), while only a 30% increase in enzyme activity is observed in the mTALH (Table II). It is important to note, however, that the magnitude of the increases of Na-K-ATPase activity in the mouse mTALH (Table II, $\Delta = 1120 \text{ pmol ADP} \cdot \text{mm}^{-1} \cdot \text{h}^{-1}$) and in the rabbit CCT by O'Neil and co-workers [(11, 12); $\Delta = 814\text{--}1086 \text{ pmol ADP} \cdot \text{mm}^{-1} \cdot \text{h}^{-1}$], using a similar method of enzyme activity determination, are virtually identical. Both kinetic and tritiated ouabain binding studies have shown that mineralocorticoid-mediated increases in enzyme activity in the CCT can be entirely accounted for by an increase in the number of 3Na:2K pump units rather than a change in the kinetic properties of the enzyme. Given that it is likely that Na-K-ATPase activity in the mTALH is also directly proportional to the number of

cation pump units (51), then DOCA treatment results in a similar increase in the number of biochemically identifiable basolateral membrane enzyme units in the mTALH and the CCT.

This study demonstrates that large doses of mineralocorticoid and furosemide-induced inhibition of cell sodium uptake modulate Na-K-ATPase in the mouse mTALH, encompassing greater than a twofold difference from the lowest to the highest values observed. DOCA administration produces an elevation of Na-K-ATPase activity in the mTALH, and furosemide administration results in a decrease in enzyme activity. The maintenance of enzyme activity in the mTALH after 3–4 d or 2 wk of reduced sodium intake is probably secondary to decreases in both sodium delivery and sodium entry to the mTALH cell counter-balanced by a concomitant rise in plasma aldosterone. Clearly, further studies will be required to evaluate the separate influences of mineralocorticoid and sodium delivery on Na-K-ATPase activity in this segment. In contrast, the same 3 d period of sodium deprivation resulted in a 60% enhancement of enzyme activity in the mineralocorticoid-responsive CCT. The heterogeneity of response exhibited by Na-K-ATPase activity in the mTALH and CCT during early sodium deprivation may contribute to the maintenance of sodium delivery to, and thus potassium excretion by, distal nephron segments.

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