Regulation of Collecting Tubule Adenosine Triphosphatases by Aldosterone and Potassium

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Abstract

To examine the precise role of potassium and aldosterone on acid–base composition and on collecting tubule ATPases, glucocorticoid-replete adrenalectomized rats were replaced with zero, physiological, or pharmacological doses of aldosterone and were fed varying potassium diets to produce hypokalemia, normokalemia, or hyperkalemia. Radiochemical measurement of ATPase activities showed that collecting tubule H/K-ATPase changed inversely with potassium and not with aldosterone whereas H-ATPase changed directly with aldosterone but not with potassium. When both enzymes changed in the same direction, alterations in acid–base composition were profound; however, when these two acidifying enzymes changed in opposite directions or when only one enzyme changed, the effect on acid–base balance was modest. Serum bicarbonate was ~ 45 meq/liter when aldosterone was high and potassium was low; it was only 29 meq/liter when aldosterone was high but potassium was normal or when aldosterone was normal and potassium was low. Our observations may help explain the metabolic alkalosis of primary aldosteronism in which aldosterone excess and hypokalemia are combined and the metabolic acidosis of aldosterone deficiency in which hypoaldosteronism and hyperkalemia are paired. The present study also demonstrated that aldosterone plays the major role in controlling Na/K-ATPase activity in cortical collecting tubule. Hypokalemia stimulates Na/K-ATPase activity in the medullary collecting tubule; this stimulatory effect of hypokalemia supports the hypothesis that the enzyme is present on the apical membrane at this site. (*J. Clin. Invest.* 1993. 91:2385–2392.) Key words: renal proton ATPases • acidification mechanisms • hyperaldosteronism • luminal Na/K-ATPase • potassium reabsorption

Introduction

Potassium and aldosterone (Aldo)[1] exert major effects on collecting tubule function (1–3). When potassium depletion is accompanied by aldosterone excess both acidification and sodium transport are stimulated. Conversely, when potassium excess is accompanied by aldosterone deficiency, acidification and sodium transport are inhibited (4, 5).

In the collecting tubule, sodium transport is modulated by the enzyme, Na/K-adenosine triphosphatase (ATPase) (1). This enzyme is thought to be localized solely to the basolateral membrane. Acidification appears to be controlled by two renal proton-translocating ATPases, an electrogenic H-ATPase inhibitable by *N*-ethylmaleimide (NEM) (6) and an electroneutral H/K-ATPase similar to that of gastric mucosa, inhibitable by SCH28080 and vanadate (7, 8). Previous work has shown that aldosterone stimulates collecting tubule H-ATPase activity (9–11), but the effect of aldosterone on the renal H/K-ATPase enzyme is not known. Potassium depletion enhances the activity of the H/K-ATPase; the effect of potassium depletion on H-ATPase activity is controversial (7, 8, 12, 13). In the cortical segment of the collecting tubule, aldosterone enhances Na/K-ATPase activity whereas in the medullary segment, potassium depletion stimulates its activity (14–17). The interrelation between these two variables is not yet clear. Obviously, changing potassium metabolism alters aldosterone release, and a change in aldosterone secretion alters potassium homeostasis (3).

This study was designed to examine the effects of altering potassium and aldosterone metabolism under controlled conditions on both acid–base composition and ATPase activities in rat cortical collecting tubule (CCT) and medullary collecting tubule (MCT). In these experiments we did not allow plasma potassium to change as the plasma aldosterone levels were altered and vice versa. Consequently, the direct effects of potassium and of aldosterone on enzyme activities could be discerned.

Methods

Adrenalectomized rats (*Table I*). 10 experimental groups of male albino Sprague–Dawley rats (150–200 g) were designed, but only nine groups could be studied (Table I). After nembutal anesthesia (50 $mg \cdot kg^{-1} \cdot body\ wt^{-1}$, i.p.), animals underwent either a sham adrenalectomy (group 1) or were bilaterally adrenalectomized (ADX) (groups 2–9). An osmotic minipump (Alzet 2002; Alza Corporation, Palo Alto, CA) for hormone replacement was implanted subcutaneously in the intrascapular region of each rat at the time of adrenalectomy. Each ADX rat received dexamethasone 1.4 $\mu g \cdot 100\ g\ body\ wt^{-1} \cdot d^{-1}$ as maintenance glucocorticoid replacement; polyethylene glycol 400 was used as diluent for the hormone. The daily dose of aldosterone replacement was zero (0 Aldo); 1 μg/100 g body wt (N Aldo); or 50 μg/100 g body wt (↑ Aldo). After recovery from surgery the rats were fed a diet containing varying potassium concentrations (ICN Pharmaceuticals, Inc.,

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1. *Abbreviations used in this paper:* Aldo, aldosterone; ADX, adrenalectomized; ATPase, adenosine triphosphate; CCT, cortical collecting tubule; MCT, medullary collecting tubule; N, normal concentration; NEM, *N*-ethylmaleimide; ↑, high concentration; ↓, low concentration.

Table I. Experimental Groups in Rats

Group	Treatment
1	Control, (sham ADX)
2	ADX, N Aldo, N K
3	ADX, N Aldo, ↓ K
4	ADX, N Aldo, ↑ K
5	ADX, 0 Aldo, N K
6	ADX, 0 Aldo, ↓ K
7	ADX, 0 Aldo, ↑ K
8	ADX, ↑ Aldo, N K
9	ADX, ↑ Aldo, ↓ K
10*	ADX, ↑ Aldo, ↑ K

$n = 6$ in each group; N, normal. * We were unable to induce hyperkalemia in animals replaced with a pharmacological dose of aldosterone (group 10). Thus, we could not study this group.

Costa Mesa, CA) to produce hypokalemia, normokalemia, or hyperkalemia.

Sham-operated controls received normal rat chow. To produce hyperkalemia, the animals, regardless of aldosterone status, were fed a diet that contained 15 times the amount of potassium but was otherwise identical to a normal chow diet (18). Irrespective of aldosterone status, the animals subjected to hypokalemia received a potassium-deficient diet containing 1.5 meq K^+/kg. The 0 Aldo and N Aldo groups were fed a normal diet to produce and maintain plasma potassium. The ↑ Aldo group subjected to normokalemia was fed a high potassium content diet because of a tendency to develop hypokalemia with a normal chow diet. We were unable to induce hyperkalemia in the ↑ Aldo rats (see Table I), thus we could not study this group. All animals were given 0.3% NaCl to drink ad libitum.

The influence of varying aldosterone and potassium levels on acid–base composition and on the activity of microdissected collecting tubule proton ATPases (H-ATPase and H/K-ATPase) and Na/K-ATPase was studied after 7 d.

Adrenal intact rats. These studies examined ATPase activities and systemic acid–base composition during potassium depletion. The rats were divided into two groups control and ↓ potassium. Control rats were fed a normal potassium diet for 7 d (150 meq K^+/kg) whereas the ↓ K group was fed a semisynthetic potassium-deficient diet containing 1.5 meq K^+/kg for 7 d. These animals were allowed free access to tap water.

On the day before each experiment the rats were placed in metabolic cages for a 24-h urine collection. On the day of the experiment, the animals were anesthetized as previously described (19) and blood samples were obtained from the aorta for measurement of pH, P_{CO_2}, electrolytes, and hormones. Arterial pH, P_{CO_2} and urine and plasma electrolytes were measured by standard methods as previously described (20). Muscle potassium concentration was measured by atomic-absorption spectrophotometry as previously described (20). Plasma aldosterone concentration was determined by radioimmunoassay (COAT-A-COUNT®; DPC, Los Angeles, CA).

Tubule microdissection and enzymatic measurements. Cortical and medullary collecting tubules were microdissected as previously described (19, 20). In brief, the left renal artery was cannulated and the kidney was perfused for 15 min in situ at a rate of 0.7 ml/min with a balanced salt solution containing 400 U/ml collagenase, 4°C, pH 7.4. The kidneys were then cut along the cortico-papillary axis and incubated in 3 ml of collagenase–albumin containing Hanks' solution at 35°C for 12 min. The tissues were continuously bubbled with compressed air (3 psi). After incubation, the pyramids were rinsed and segments of CCT and MCT were microdissected. (If H/K-ATPase activity was measured an additional step was performed to remove the extracellular potassium; nephron segments were incubated for 15 min at 37°C [pH 7.4] in potassium-free buffer (7, 20). If the other enzymes were measured, this step was omitted [9, 16, 19]). The nephron segments were then permeabilized using a two-step hypotonic–hypothermic shock and ATPase activities were determined using γ^{32}P-labeled ATP (37°C, 15 min, pH 7.4). H-ATPase, H/K-ATPase, and Na/K-ATPase were measured by the radiochemical method of Doucet and Marcy (7) and Khadouri et al. (11, 12) as subsequently described by us (19, 20).

Na/K-ATPase activity was measured as the ouabain-sensitive Na/K-dependent ATPase. ATPase activity was first measured in buffer with the following composition (mM): 50 NaCl, 5 KCl, 10 $MgCl_2$, 1 EGTA, and 100 Tris·HCl. High specific activity [γ^{32}P]ATP (2–10 Ci/mmol) was added in tracer amounts (10 nCi/μl) to vanadium-free ATP (12 mM). After incubation (15 min), the reaction was stopped by placing the tubules on ice; this was followed immediately by the addition of 7 μl of a 10% activated charcoal suspension in 5% trichloroacetic acid (4°C). Activated charcoal was used to bind the unhydrolyzed ATP, and the labeled inorganic phosphate was separated under vacuum by rapid Millipore® (Millipore Corp., Bedford, MA) filtration coupled with a wash of 0.1% phosphate buffer (1 ml), pH 6.0, at 4°C. To determine the ouabain-sensitive Na/K-ATPase, sodium and potassium were replaced with 50 mM Tris HCl, and 2 mM ouabain was added to the buffer. Na/K-ATPase activity is defined as the difference in activity found in the presence and absence of ouabain (19, 20).

H-ATPase activity was measured as the NEM-sensitive H-ATPase. ATPase was first measured in a sodium- and potassium-free buffer containing 150 mM Tris HCl, 10 mM $MgCl_2$, 1 mM EGTA, 2 mM ouabain, 2 mM sodium azide, 20 μg/ml oligomycin, and 12 mM vanadium-free ATP. After incubation (15 min), the reaction was stopped as described above. To determine NEM-sensitive ATPase, tubules were also incubated in a solution containing 2 mM NEM. H-ATPase activity is defined as the difference in activity found in the presence and absence of NEM (19, 20).

H/K-ATPase activity was measured as the SCH 28080–sensitive H/K-ATPase. ATPase activity was measured first in the sodium-free buffer containing 150 mM Tris HCl, 10 mM $MgCl_2$, 1 mM EGTA, 2 mM ouabain, 2 mM NEM, 2 mM sodium azide, 20 μg/ml oligomycin, 4 mM KCl, and 12 mM vanadium-free ATP. To determine H/K-ATPase activity, the tubules were incubated in a zero potassium buffer containing 200 μM SCH 28080. The concentration of SCH 28080 is greater than the two- to threefold that is needed to inhibit the enzyme under the above conditions. H/K-ATPase activity is defined as the difference in the activity found in the presence and the absence of potassium and SCH 28080 (20).

We and others have previously shown that the assays just described are performed under V_{max} conditions (7, 19–21). Enzyme activity is expressed as pmol/mm tubule length per h of ATP hydrolyzed. All samples were run in triplicate or quadruplicate and appropriate corrections were made for blanks and the spontaneous hydrolysis of ATP.

Materials. All chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and were of highest purity. Radiolabeled ATP was obtained from New England Nuclear (Boston, MA).

Statistics. Statistical significance was assessed using the Student's t test or analysis of covariance, where appropriate, with P values of ≤ 0.05 being significant.

Results

Metabolic data for adrenalectomized rats (Tables II and III). Plasma sodium was similar in all experimental groups. As expected, hypokalemia developed in groups 3, 6, and 9 whereas a normal plasma potassium was observed in groups 2, 5, and 8. In groups 4 and 7 hyperkalemia was apparent. The changes in fractional potassium excretion and muscle potassium occurred in the same direction as those seen with the plasma potassium.

Table II. Metabolic Parameters in Adrenalectomized Rats

Group #	Body weight Day 1	Body weight Day 8	P_K	P_{Na}	FE_K	Muscle potassium	CCr
	g		*meq/liter*		*%*	*meq/g dry wt*	*ml·min⁻¹·100 g body wt⁻¹*
1. Sham	149±3	185±3	4.1±0.2	145±2	33±2	0.44±0.01	0.94±0.08
2. N Aldo, N K	147±2	183±3	4.1±0.2	143±6	31±2	0.43±0.01	0.90±0.09
3. N Aldo, ↓ K	152±2	184±2	2.3±0.2‡	142±3	1±0.8‡	0.30±0.02*	0.89±0.06
4. N Aldo, ↑ K	152±2	142±2*	6.0±0.4‡	146±4	104±13‡	0.48±0.01	0.88±0.08
5. 0 Aldo, N K	149±4	160±5*	4.2±0.2	146±2	34±2	0.43±0.01	0.89±0.04
6. 0 Aldo, ↓ K	148±5	160±4*	2.8±0.2‡	147±3	2±0.6‡	0.34±0.02*	0.88±0.05
7. 0 Aldo, ↑ K	152±4	128±3‡	6.4±0.7‡	147±2	85±6‡	0.49±0.01	0.86±0.08
8. ↑ Aldo, N K	149±5	178±2	4.4±0.2	146±3	102±12‡	0.45±0.01	0.89±0.06
9. ↑ Aldo, ↓ K	152±4	181±6	2.2±0.2‡	148±4	2±0.6‡	0.30±0.03*	0.88±0.04

FE_K, fractional potassium excretion; CCr, creatinine clearance. $n = 6$ in each group. * $P < 0.05$, ‡ $P < 0.005$ vs. sham.

There was no significant difference in creatinine clearance in any of the experimental groups.

Plasma aldosterone levels averaged ~ 5 ng/dl in all animals receiving physiological hormone replacement (i.e., groups 2–5), values comparable those measured in normal unstressed animals (Table III). In the 0 Aldo rats (i.e., groups 5–7), plasma aldosterone was undetectable. By contrast, aldosterone levels were markedly elevated in rats receiving with pharmacological doses of the mineralocorticoid (i.e., groups 8 and 9). The moderately high aldosterone level in intact control rats (i.e., group 1) likely reflects the stress of surgery and anesthesia.

H/K-ATPase activity (Fig. 1). In normokalemic rats (groups 2, 5, and 8), regardless of the aldosterone level, H/K-ATPase activity in both CCT and MCT was similar to that of control. In hypokalemic rats (groups 3, 6, and 9), however, enzyme activity was markedly increased compared with control. This increase was noted regardless of the aldosterone status. Compared with control, the percent increase in H/K-ATPase activity in CCT averaged 82±6.8, 88±9.0, and 90±8.9% in the 0 Aldo, N Aldo, and ↑ Aldo groups, respectively ($P < 0.005$); in the MCT, the percent increase averaged 92±8.6, 100±11.7, and 94±9.2% in the 0 Aldo, the N Aldo, and the ↑ Aldo rats, respectively ($P < 0.005$).

In both of the hyperkalemic groups, H/K-ATPase activity was markedly decreased (groups 4 and 7). This decrease was noted regardless of the aldosterone status of the animals. Enzyme activity in CCT and MCT was 69±1.6 and 65±2.1% of control, respectively, in the 0 Aldo group ($P < 0.005$). In the N Aldo group, it was 67±2.1% of control in CCT and 61±3.1% of control in MCT ($P < 0.005$). There was no significant difference in H/K-ATPase activity at different aldosterone levels in those animals having the same potassium concentration.

H-ATPase activity (Fig. 2). In rats with physiological aldosterone replacement (N Aldo groups), H-ATPase activities were not significantly different from the control at any level of

Table III. Plasma Aldosterone Level in Adrenalectomized Rats

Group	↓ K	N K	↑ K
0 Aldo	ND	ND	ND
N Aldo	5.50±0.48	5.28±0.46	5.60±0.38
↑ Aldo	282.00±21.40*	268.00±21.91*	Not done
Sham	—	33.40±1.60	—

Plasma aldosterone is expressed as ng/dl; ↓ K, hypokalemia; N K, normokalemia; ↑ K, hyperkalemia; ND, not detectable. $n = 6$ in each group. * $P < 0.005$ vs. N Aldo with similar potassium level.

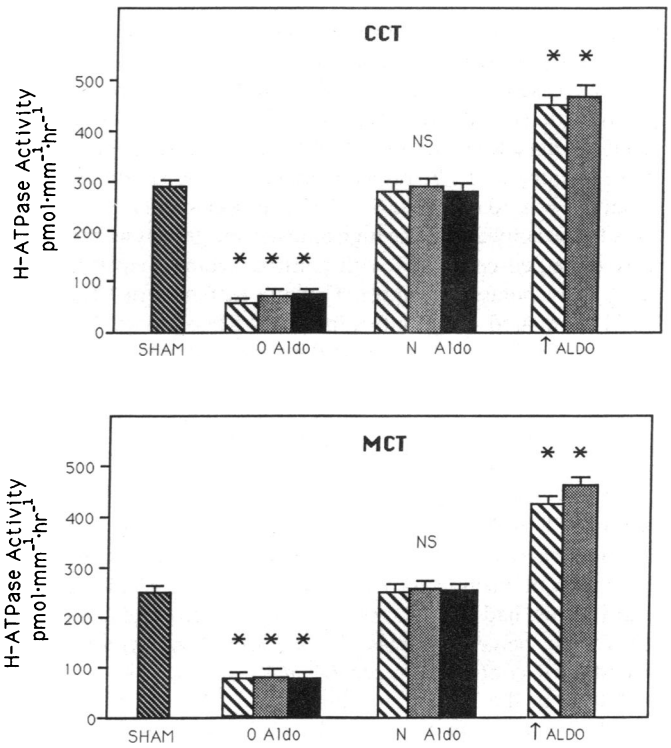

Figure 1. The effect of aldosterone and potassium on H/K-ATPase activity in CCT and MCT in adrenalectomized rats. 0 Aldo, no aldosterone replacement; *N Aldo,* 1 μg aldosterone · 100 g body $wt^{-1} \cdot d^{-1}$; ↑ *Aldo,* 50 μg aldosterone · 100 g body $wt^{-1} \cdot d^{-1}$; * $P < 0.005$ vs. SHAM animals. *NS,* not significant. Hypokalemia, white hatched bars; normokalemia, gray stippled bars; hyperkalemia, black bars.

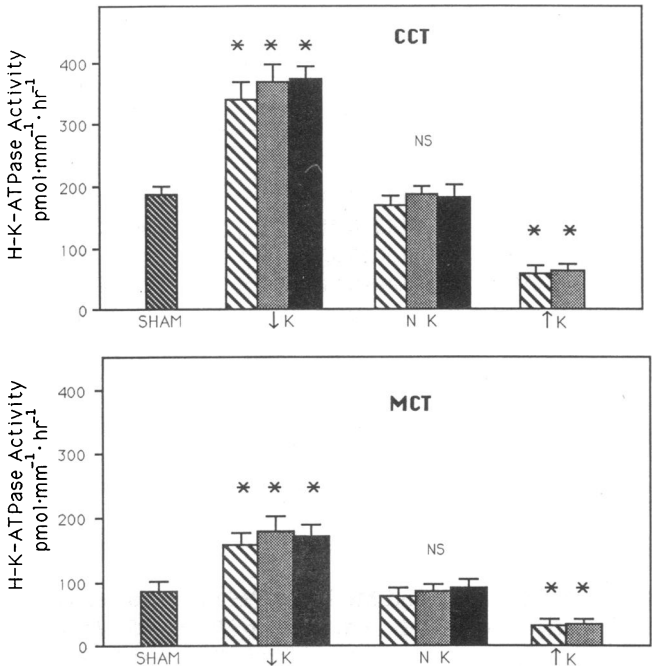

Figure 2. The effect of aldosterone and potassium on H-ATPase activity in CCT and MCT in adrenalectomized rats. ↓ *K*, hypokalemia; *N K*, normokalemia; ↑ *K*, hyperkalemia; $*P < 0.005$ vs. sham animals. *NS*, not significant. 0 Aldo replacement, white hatched bars; 1 μg Aldo · 100 g body $wt^{-1} \cdot d^{-1}$, gray stippled bars; 50 μg Aldo · 100 g body $wt^{-1} \cdot d^{-1}$, black bars.

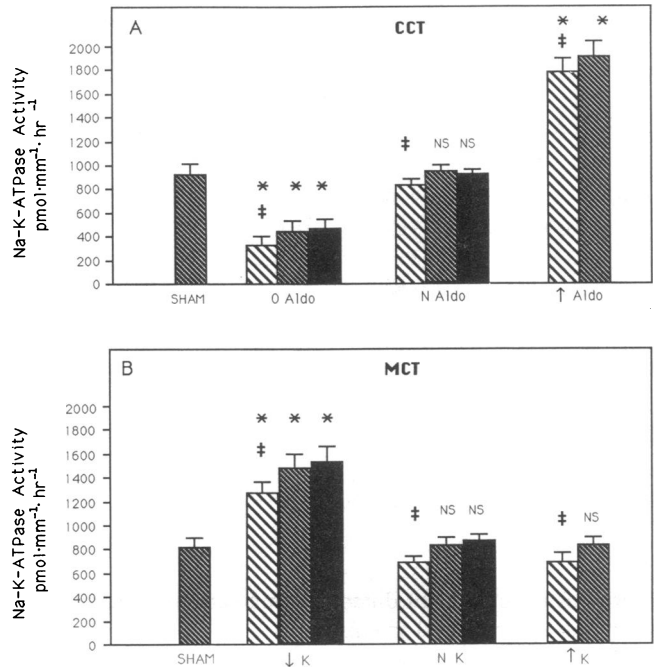

Figure 3. The effect of aldosterone and potassium on Na/K-ATPase activity in CCT (*A*) and MCT (*B*). In CCT Na/K-ATPase activities were studied with different aldosterone levels. Hypokalemia, white hatched bars; normokalemia, gray shaded bars; Hyperkalemia, black bars. $*P < 0.005$ vs. sham animals $‡P < 0.05$ vs. normokalemic animals at same aldosterone status. In MCT, Na/K-ATPase activities were studied with different potassium levels. 0 Aldo replacement, white hatched bars; 1 μg Aldo · 100 g body $wt^{-1} \cdot d^{-1}$, gray shaded bars; 50 μg Aldo · 100 g body $wt^{-1} \cdot d^{-1}$, black bars. $*P < 0.005$ vs. sham animals $‡P < 0.05$ vs. normokalemic animals at same potassium level.

serum potassium. By contrast, 0 Aldo rats had a marked reduction in enzyme activity regardless of potassium status. The percent decrease in the enzyme activity in CCT and MCT compared with control averaged 70±1.5 and 70±1.1%, respectively, in the ↓ K groups ($P < 0.005$). In the N K groups it was 70±2.0 and 69±2.1%, respectively ($P < 0.005$). In the ↑ K groups it was 70±0.7 and 69±0.8%, respectively ($P < 0.005$). H-ATPase activities in the high aldosterone groups were markedly increased compared with control, again independent of total body potassium stores. Enzyme activities in CCT and MCT compared with control increased 55±6.4 and 86±6.9%, respectively, in the ↓ K groups ($P < 0.005$) whereas in the N K groups it increased by 61±3.0 and 91±3.4%, respectively ($P < 0.005$). There was no significant difference in H-ATPase activity at different potassium levels in those animals having the same aldosterone values.

Na/K-ATPase activities (*Fig. 3*). Na/K-ATPase activities in the CCT of N Aldo rats with N K and ↑ K were similar to control whereas the ↓ K group had a small, but significant reduction in enzyme activity. 0 Aldo rats, regardless of potassium status, had about a 50% reduction in enzyme activity in CCT. The percent decrease in Na/K-ATPase activity compared with control averaged 64±6.1% in the ↓ K group ($P < 0.01$). In the N K group there was a 52±8.3% decrease in Na/K-ATPase activity ($P < 0.01$) and in the ↑ K group there was a 50±8.1% decrease ($P < 0.01$). Thus, enzyme activity in CCT of the ↓ K group was significantly lower than in the N K or the ↑ K groups ($P < 0.05$).

In the CCT of the ↑ Aldo rats, irrespective of potassium status, Na/K-ATPase activities were enhanced by ~ 100% of control (91±8.5% in the ↓ K group and 108±8.1% in the N K group) ($P < 0.01$). Again, the enzyme activity in the ↓ K group was significantly lower than in the N K group ($P < 0.05$).

In MCT, hypokalemia, irrespective of aldosterone activity, enhanced Na/K-ATPase activity. The percent increase was 67±4.5% in the 0 Aldo group, 81±8.9% in the N Aldo group, and 84±8.5% in ↑ Aldo ($P < 0.005$). In the ↑ K animals, however, enzyme activity was similar to that of control if the animals were replaced with physiological amounts of aldosterone. It should be noted that Na/K-ATPase activity in 0 Aldo rats, irrespective of potassium level, was lower than those of the N Aldo group and the ↑ Aldo group at the same potassium level.

Alterations in acid–base composition (*Table IV*). When the relationship between acid–base parameters and the changes in aldosterone and potassium were assessed (*Table IV*), interesting correlations were observed. When both aldosterone excess and hypokalemia were present simultaneously, a condition under which both renal proton ATPases (H-ATPase and H/K-ATPase) were markedly stimulated, severe metabolic alkalosis occurred (plasma HCO_3^- = 45.9±2.0 vs. 23.9±1.1 meq/liter in sham controls, $P < 0.005$). By contrast, metabolic acidosis resulted only when both enzymes were inhibited; this occurred only when aldosterone deficiency (0 Aldo) was associated with hyperkalemia (plasma HCO_3^- = 16.8±1.0 vs. 23.9±1.1 meq/liter in sham controls, $P < 0.005$). When one enzyme fell and the other remained unchanged (as in N Aldo ↑ K or 0 Aldo N K), modest changes in bicarbonate were ob-

Table IV. The Effect of Aldosterone and Potassium on Plasma Bicarbonate in Adrenalectomized Rats

Group	0 Aldo	N Aldo	↑ Aldo
↓ K	20.8±0.6*	29.3±1.6*	45.9±2.0*
N K	20.3±0.5*	24.2±1.0	29.7±1.5*
↑ K	16.8±1.0‡	22.4±0.8	Not done
Sham	—	23.9±1.1	—

Plasma bicarbonate is expressed as meq/liter; 0 Aldo, no aldosterone replacement; N Aldo = 1 μg aldosterone · 100 g body $wt^{-1} \cdot d^{-1}$; ↑ Aldo = 50 μg aldosterone · 100 g body $wt^{-1} \cdot d^{-1}$; ↓ K, hypokalemia; N K, normokalemia; ↑ K, hyperkalemia. * $P < 0.05$, ‡ $P < 0.005$ vs. sham control animals.

served. In the former plasma HCO_3^- was 22.4±0.8 meq/liter whereas in the latter it was 20.3±0.5 meq/liter. In the 0 Aldo N K group there was a greater fall in plasma bicarbonate than there was in the high N Aldo ↑ K group. When one enzyme fell and the other increased, as in 0 Aldo ↓ K, the change in bicarbonate concentration was not significant. When one enzyme increased, but the other was unchanged (as in N Aldo ↓ K and ↑ Aldo N K), the bicarbonate concentration increased by only 6 meq/liter rather than the 22 meq/liter that was seen when both enzymes were stimulated (i.e., in the ↑ Aldo ↓ K group). Thus, when both enzymes change in the same direction, alterations in acid–base composition are profound. When only one enzyme changes (or if both enzymes change, but in the opposite direction), the effect on acid–base balance is modest.

Adrenal-intact hypokalemic rats (Figs. 4 and 5). Fig. 4 shows the relationship between plasma potassium and aldosterone in adrenal-intact hypokalemic rats with dietary potassium restriction. After 3 d of potassium deprivation, plasma potassium was significantly reduced (4.1±0.1 vs. 3.5±0.1 meq/liter, $P < 0.05$); plasma aldosterone levels fell from 35.0±1.2 to 11.6±1.7 ng/dl ($P < 0.005$). After 7 d, both parameters fell further. During this same time interval, plasma HCO_3^- concentration was not significantly different from control animals (Table V). When collecting tubules were microdissected from these animals and ATPase activities were measured, the following results were obtained (Fig. 5): H/K-ATPase activity was increased 71±6.0 and 111±5.6% in CCT and MCT, respectively, compared with controls ($P < 0.005$). By contrast, potassium depletion significantly decreased H-ATPase activity, with a 42±2.0 and 32±1.8% reduction in CCT and MCT, respectively, compared with controls ($P < 0.005$). Na/K-ATPase activity was decreased 35±2.2% in the CCT but in the MCT a 66±9.1% increase was noted ($P < 0.005$).

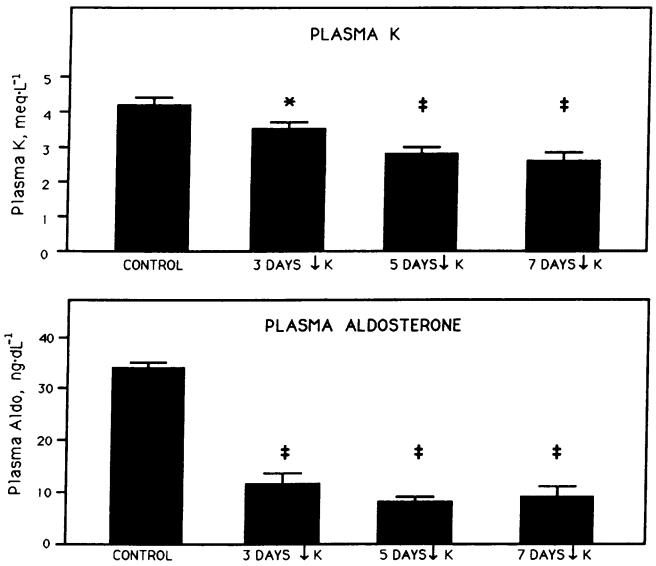

Figure 4. The effect of potassium depletion on plasma potassium and plasma aldosterone in adrenal intact rats. * P 0.05, ‡ $P < 0.005$ vs. control animals.

Discussion

Potassium depletion has ambiguous effects on acid–base metabolism (5). When accompanied by aldosterone excess, it is invariably associated with metabolic alkalosis. In contradistinction, potassium depletion alone is associated with a tendency to mild metabolic acidosis or alkalosis depending on the animal species studied (22–25). Potassium depletion enhances proximal acidification and ammoniagenesis, both of which increase plasma bicarbonate concentration. On the other hand, potassium depletion inhibits aldosterone release, which decreases distal acidification and, hence, plasma bicarbonate concentration (2–4).

Recently, two proton ATPases have been found in the mammalian nephron. The first is an electrogenic proton-translocating ATPase, the H-ATPase, inhibitable by NEM (6). The H-ATPase is currently recognized as the major kidney proton pump (2). The second is an electroneutral proton-translocating ATPase, an H/K-ATPase similar to that of gastric mucosa, recently reported to reside in the mammalian kidney with the highest activity detected in the CCT (7, 8). The H/K-ATPase is inhibited by vanadate and SCH28080. In potassium-depleted animals H/K-ATPase activity markedly increases and appears to play an important role in distal potassium reabsorption. Recent studies suggest that the H/K-ATPase may influence distal acidification in both potassium-depleted and normokalemic animals as well as during metabolic acidosis and respiratory acidosis (26–28).

Several lines of evidence have clearly demonstrated that the mineralocorticoid, aldosterone, plays a major role in regulating the renal H-ATPase. In adrenalectomized animals, H-ATPase activity is markedly reduced in mineralocorticoid sensitive nephron segments, especially in the collecting tubule (29). Indeed, chronic administration of aldosterone enhances H-ATPase activity in normal rats (9) as well as adrenalectomized rabbits (10). Acute aldosterone administration to adrenalectomized rats also stimulates H-ATPase activity (11). On the other hand, there are scarce data about the effect of aldosterone on H/K-ATPase. A single, recent study suggested that a high physiological dose of aldosterone enhances H/K-ATPase activity in rabbit connecting tubule and CCT, but that it has no effect on enzyme activity in the MCT (30). Whether aldosterone deficiency or pharmacological doses of aldosterone affect H/K-ATPase activity is not yet known.

H/K-ATPase activity is stimulated by potassium deprivation (7, 8) and is inhibited by hyperkalemia (31). Studies of potassium depletion on H-ATPase activity, however, have

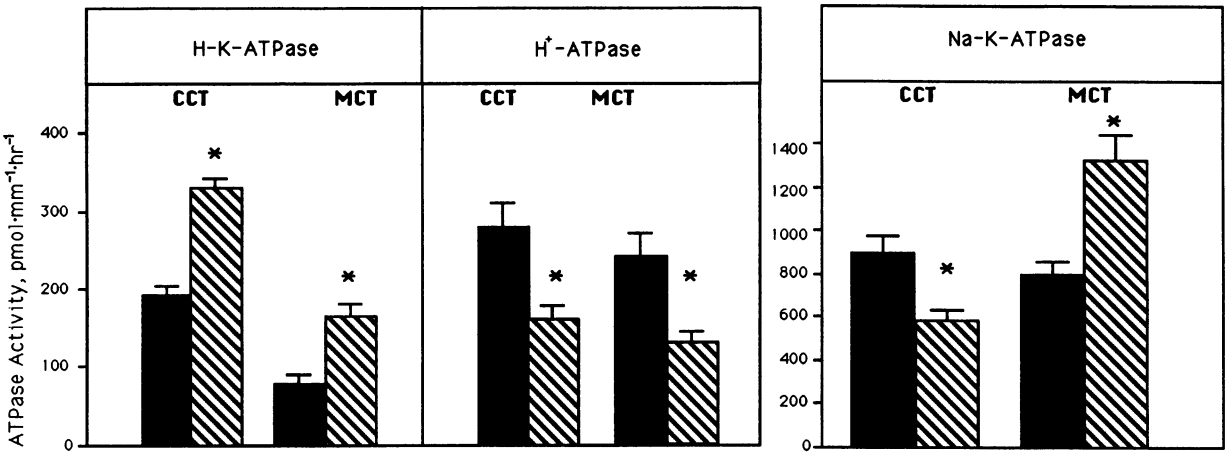

Figure 5. The effect of 7 d of dietary potassium depletion on H/K-ATPase, H-ATPase, and Na/K-ATPase activities in CCT and MCT in adrenal intact rats. $*P < 0.005$ vs. control animals.

yielded conflicting results, reporting either inhibitory or stimulatory effects (12, 13). Changing potassium balance is complicated by profound secondary changes in plasma aldosterone, which in turn can modify H-ATPase activity. For example, the curtailment of dietary potassium intake markedly decreases serum aldosterone (reference 32 and this study). Thus, potassium depletion could decrease H-ATPase activity secondarily because of the decreased plasma aldosterone.

Aldosterone and potassium also affect Na/K-ATPase activity. Previous studies showed that Na/K-ATPase activity and ouabain binding were decreased in CCT and increased in MCT in potassium-depleted animals (14, 15). These alterations in enzyme activity could also be due to a direct effect of hypokalemia; they could also be due to an indirect effect secondary to changes in plasma aldosterone. Indeed, animals receiving high doses of exogenous aldosterone or having high endogenous aldosterone induced by potassium loading show an increase in Na/K-ATPase activity in CCT (16, 33). Zero aldosterone has been reported to decrease Na/K-ATPase activity in rat CCT (17) but to increase it in rabbit CCT (34). Again, there were concomitant alterations in potassium levels in these studies that might also have affected enzyme activity.

Table V. Metabolic Parameters in Control and Adrenal-Intact Rats after 7 d of Dietary Potassium Deprivation

Parameter	Control	P	7-d dietary potassium deprivation
P_K (meq/liter)	4.14±0.09	0.005	2.57±0.08
UV (ml/24 h)	20.0±1.3	0.005	36.7±3.4
FE_K (%)	32.7±1.6	0.005	0.26±0.1
FE_{Na} (%)	0.65±0.04	0.005	0.33±0.05
P_{Cr} (mg %)	0.38±0.04	NS	0.37±0.04
C_{Cr} (ml · min^{-1} · 100 g · body wt^{-1})	0.98±0.07	NS	0.96±0.04
Muscle potassium (meq/g dry wt)	0.44±0.03	0.05	0.35±0.10
pH	7.38±0.02	NS	7.36±0.01
P_{CO_2} (mM)	47.0±1.9	NS	52.8±1.4
[HCO_3^-] (mM)	23.8±0.5	NS	21.7±1.0
Total CO_2 (mM)	26.0±0.4	NS	23.6±0.8

UV, urine volume; FE_K, fractional potassium excretion; FE_{Na}, fractional sodium excretion. $n = 6$ in each group.

Therefore, the present study was conducted to explore the precise role of potassium and aldosterone on both acid–base and electrolyte composition and on collecting tubule transport enzyme activities. In adrenalectomized rats, when serum potassium was normal, H/K-ATPase activity in both CCT and MCT was similar to control regardless of aldosterone level (Fig. 1). When plasma potassium was low, enzyme activity was increased irrespective of aldosterone level. In contradistinction, when plasma potassium was high, enzyme activity was decreased, again regardless of aldosterone status. Thus, H/K-ATPase changed with plasma potassium and not with aldosterone.

With regard to H-ATPase activity in adrenalectomized rats, enzyme activity in both CCT and MCT was similar to control at any potassium level when aldosterone was replaced physiologically (Fig. 2). When aldosterone level was low, H-ATPase activity was decreased regardless of potassium status. On the other hand, when aldosterone level was high, enzyme activity was increased irrespective of potassium level. Therefore, H-ATPase changed with aldosterone and not with potassium.

That aldosterone-controlled H-ATPase activity is consistent with studies reported previously (9–11, 29). Our study also shows that potassium has no effect on the H-ATPase. That potassium inversely modifies H/K-ATPase activity is in agreement with previous studies (7, 8). In addition, we show that aldosterone had no direct effect on H/K-ATPase. This latter finding is at variance with a recent study reported in abstract form that showed stimulation of H/K-ATPase activity in CCT, but not in MCT, of rabbit (30). Whether this effect is due to aldosterone directly or secondarily to changes in potassium cannot be determined from the data in the abstract.

In our adrenal intact hypokalemic rats, which had low aldosterone levels, H/K-ATPase activity was increased while H-ATPase activity was decreased in both CCT and MCT (Fig. 5). The inhibitory effect of potassium depletion on H-ATPase is in agreement with the results reported by Khadouri et al. (12). However, our results are different from those reported by Garg and Narang (13), who showed that the enzyme was stimulated

by potassium depletion. This discrepancy is difficult to explain because all the studies were conducted in the same animal species but may be due to technical differences in enzyme measurement. Serum aldosterone was not measured in these latter studies.

The data from the adrenal-intact potassium-depleted animals resemble those from adrenalectomized rats with 0 Aldo ↓ K. Both groups of animals showed low H-ATPase activity and high H/K-ATPase activity although the more profound changes were observed in the latter group. When aldosterone levels were increased to the physiological range while serum potassium was still low (i.e., in the N Aldo ↓ K group), H-ATPase activity increased to normal whereas H/K-ATPase activity was still high (Fig. 2). Thus, it is apparent that, in the adrenal-intact hypokalemic rat, H-ATPase is decreased because of the reduction in aldosterone.

In rats with aldosterone excess and hypokalemia, profound metabolic alkalosis occurred (Table IV). In rats with aldosterone deficiency and hyperkalemia, metabolic acidosis developed. Thus, aldosterone excess with hypokalemia was associated with stimulation of both renal proton ATPases whereas aldosterone deficiency with hyperkalemia was associated with inhibition of both enzymes. These changes may help explain the metabolic alkalosis of primary aldosteronism in which aldosterone excess and hypokalemia are combined; these changes may also explain the metabolic acidosis of aldosterone deficiency in which hypoaldosteronism and hyperkalemia are paired (5). In the first condition both renal proton ATPases are likely turned on whereas in the latter the enzymes are probably turned off. Indeed, when the changes in potassium balance and aldosterone alter the two acidifying enzymes in the opposite direction or when only one enzyme changes, the effect on acid–base balance is modest (Table IV). Consider the adrenal-intact hypokalemic rats in which both renal proton ATPases were altered in opposite directions. Plasma bicarbonate concentration was unchanged (Table IV). Previous studies under this condition, despite differing data on renal proton ATPases, also report small alterations in acid–base composition (12, 13).

The stimulation of H-ATPase activity by aldosterone does not require the presence of triiodothyronine (11). It does depend on protein synthesis for it is totally abolished by actinomycin D and cycloheximide (11). At the present time, there are no data available about the intracellular signaling that regulates the renal H-ATPase. With regard to H/K-ATPase, although potassium directly controls enzyme activity, the signal transduction mechanisms again are not yet known.

The effects of aldosterone and potassium on Na/K-ATPase are different from those on the H/K-ATPase and H-ATPase. Two distinct patterns emerge. In CCT, aldosterone controls Na/K-ATPase activity (Fig. 3). Regardless of potassium status, pharmacological aldosterone enhanced enzyme activity whereas aldosterone deficiency caused a marked reduction. Potassium depletion did have a minor inhibitory effect on Na/K-ATPase in CCT. In CCT of adrenal-intact hypokalemic rats, which had low aldosterone levels, Na/K-ATPase activity was decreased. This result is in agreement with previous studies (14, 15), although it was not clear from them whether the alterations in enzyme activity were due to a direct effect of potassium depletion or a secondary effect of decreasing aldosterone release resulting from potassium depletion. From the results in both adrenalectomized and adrenal-intact hypokalemic rat models, it is apparent that the reduced Na/K-ATPase activity in CCT in adrenal-intact hypokalemic rats mainly is due to a reduction in plasma aldosterone and in a minor part due to a direct effect of potassium depletion.

The stimulatory effect of aldosterone on Na/K-ATPase in CCT could indirectly enhance acidification in this tubule segment (2, 4). Increased Na/K-ATPase activity stimulates sodium transport, which leads to an increased potential difference and, hence, stimulation of H-ATPase. Increased Na/K-ATPase activity also leads to urinary potassium wastage and potassium depletion, which in turn activates the H/K-ATPase.

In MCT, potassium plays the major role in controlling Na/K-ATPase. Regardless of aldosterone activity, potassium depletion enhances enzyme activity (Fig. 3). Aldosterone deficiency, irrespective of potassium status, had a minor inhibitory effect on Na/K-ATPase. In adrenal-intact hypokalemic rats, Na/K-ATPase activity was also enhanced (Fig. 5). This result is in agreement with those reported by Hayashi and Katz (14) and by Imbert-Teboul et al. (15) but is different from those reported by Garg et al. (35), who found no effect of potassium depletion in the MCT of rabbit. Our data from both adrenalectomized and adrenal-intact animals show that potassium depletion directly stimulates Na/K-ATPase in the MCT. The effect of hypokalemia on the MCT enzyme activity supports the proposal of Hayashi and Katz (14) that the enzyme is present on the apical membrane at this site. Alternatively, hypokalemia may change enzyme affinity for potassium in this segment of the nephron. From our studies it is not possible to discern which of these two mechanisms is operative.

Previous studies in potassium-loaded rats showed Na/K-ATPase activity was increased in the CCT while it remained unchanged in the MCT (33, 35, 36). This was mediated by an increase in aldosterone. Administration of an aldosterone antagonist was found to blunt the stimulatory effect of potassium loading on the enzyme (33). Furthermore, potassium loading had no effect on both CCT and MCT enzymes activity in adrenalectomized rats with a physiological replacement dose of aldosterone (33). The results in the 0 Aldo ↑ K group and in the N Aldo ↑ K group (Fig. 3) in the present study confirm these previous findings and indicate that potassium loading stimulates Na/K-ATPase in CCT via increased aldosterone release. This study does not agree with the report in adrenalectomized rabbits that potassium loading has a mineralocorticoid-independent stimulatory effect on CCT Na/K-ATPase activity (34).

In conclusion, the present study demonstrates that collecting tubule H/K-ATPase changes with potassium and not with aldosterone whereas H-ATPase changes with aldosterone and not with potassium. When both enzymes change in the same direction, alterations in acid–base composition are profound, however, when the two acidifying enzymes change in opposite directions or when only one enzyme changes, the effect on acid–base balance is modest. The study also shows that aldosterone plays the major role in controlling Na/K-ATPase activity in CCT whereas hypokalemia exerts an aldosterone-independent stimulatory effect on enzyme activity in MCT.

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