

Active proton secretion and potassium absorption in the rabbit outer medullary collecting duct. Functional evidence for proton-potassium-activated adenosine triphosphatase.

C S Wingo

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Research Article

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Active Proton Secretion and Potassium Absorption in the Rabbit Outer Medullary Collecting Duct

Functional Evidence for Proton-Potassium-activated Adenosine Triphosphatase

Charles S. Wingo

With the technical assistance of Scott G. Straub

Division of Nephrology and Hypertension, University of Florida, and Veterans Administration Medical Center, Gainesville, Florida 32602

Abstract

We examined the hypothesis that proton-potassium-activated adenosine triphosphatase (H-K-ATPase) mediates K absorption and acidification in the inner stripe of the outer medullary collecting duct (OMCD_i). Rabbits were fed a low-K diet (0.55% K) for 7–14 d because we have demonstrated previously that this low-K diet stimulates K-absorptive flux by the OMCD_i. Proton secretion was measured as net total CO₂ flux (J_{TCO_2}) by microcalorimetry. After basal collections, either vehicle or an inhibitor of gastric H-K-ATPase, omeprazole (0.1 mM), was added to the perfusate during the second period. Addition of vehicle to the perfusate changed neither the transepithelial voltage (V_T , in millivolts) nor the J_{TCO_2} . In contrast, the addition of omeprazole (0.1 mM) to the perfusate abolished J_{TCO_2} (from 14.5 ± 5.6 to -0.1 ± 3.1 $\text{pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$) without significantly affecting V_T . In additional experiments, in 16 tubules there was significant net K absorption (J_K) of 5.0 ± 1.0 $\text{pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$ during the basal period, which exceeded the rate of K absorption that could be attributed to a paracellular voltage-mediated pathway ($J_K^P = 1.0 \pm 0.4$ $\text{pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$, $P < 0.01$). Administration of vehicle did not significantly affect either V_T or J_K . However, omeprazole abolished J_K (from 5.1 ± 1.0 to 0.1 ± 2.5 $\text{pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$) without affecting V_T or J_{Na} .

The present results demonstrate that the OMCD_i possesses an active, omeprazole-sensitive acidification and K-absorptive mechanism. These findings are consistent with the presence of H-K-ATPase activity in this nephron segment.

Introduction

There is morphologic, physiologic, and biochemical evidence that the outer medullary collecting duct (OMCD)¹ is an important site of K reabsorption² when K intake is reduced. The

first renal morphologic changes of K depletion occur in the collecting duct (CD) (1, 2). Specifically, the intercalated cells of the inner stripe of the outer medullary collecting duct (OMCD_i) undergo hypertrophy and ultrastructural changes that suggest these cells are involved in K absorption (3, 4). Moreover, dietary K restriction enhances the absorptive K rate coefficient in the OMCD_i (5). At physiologic flow rates these changes in K-absorptive flux would significantly influence the rate of renal K excretion (5). Moreover, the recent demonstration of ouabain-insensitive, omeprazole-sensitive, potassium-activated adenosine triphosphatase (K-ATPase) activity in the CD (6–8) that is increased in activity in the OMCD_i with K depletion (7) provides further indirect evidence that K absorption may proceed via a primary active, luminal K-absorptive pump. Thus, the present study was designed to examine the role of proton-potassium-activated adenosine triphosphatase (H-K-ATPase) on proton secretion and K absorption in the OMCD_i. Omeprazole, a potent inhibitor of gastric H-K-ATPase, significantly inhibited both H secretion and K absorption without affecting transepithelial voltage (V_T) or Na absorption.

Methods

Dietary pretreatment. Female New Zealand white rabbits were maintained on a low-K diet (0.55% K) (TD82075, Teklad, Madison, WI) for 7–14 d. This diet was selected because we found it enhances the absorptive (lumen-to-bath) K rate coefficient in the OMCD_i (5).

In vitro methods. The microperfusion methods have been described in detail previously (9, 10). In brief, rabbits were decapitated, one kidney was quickly removed, and 1–2-mm slices were placed in a chilled petri dish containing an artificial ultrafiltrate of plasma. The bath and dissection solution was composed of the following (in millimolar): NaCl 105, KCl 20, NaHCO₃ 25, CaCl₂ 1.8, Na₂HPO₄ 2.5, MgSO₄ 1.0, Na acetate 10, glucose 8, alanine 5, mannitol 150, urea 150, and 5% vol/vol fetal calf serum. Mannitol and urea were used to approximate the osmolality and urea concentration expected in vivo without producing large transcellular Na gradients (11). The perfusate and the bath solution were identical with the following exceptions: (a) the bath and dissection solutions contained 5% vol/vol fetal calf serum; (b) the perfusate Na₂HPO₄ concentration was 0.5 mM to minimize fixed buffer content and facilitate the measurement of changes in bicarbonate concentration due to acidification; and (c) the perfusate contained 50 μCi of [*methoxy*-³H]inulin exhaustively dialyzed according to the method of Schafer et al. (12). All solutions were gassed to a pH of 7.4 with 95% O₂ and 5% CO₂.

Dissection proceeded superficially from the junction of the inner and outer medulla. Care was taken to dissect only the portion of the OMCD found adjacent to thin descending limbs of Henle (inner stripe of the outer medulla). Tubules were transferred to a thermostatically controlled chamber, and the two ends of the tubule were aspirated into

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1. Abbreviations used in this paper: CD, collecting duct; H-K-ATPase, proton-potassium-activated adenosine triphosphatase; OMCD, outer medullary collecting duct; OMCD_i, outer medullary collecting duct from the inner stripe.

2. The term K reabsorption is used within the context of whole-kidney physiology to denote the absorption of K by the OMCD that has been previously secreted in the initial collecting tubule and cortical CD.

holding pipettes. The perfusing pipette was advanced $\sim 100 \mu\text{M}$ beyond the holding pipette. V_T was recorded continuously using a KS-700 electrometer and Ag/AgCl electrodes (World Precision Instruments, Inc., New Haven, CT), and the bath was exchanged continuously at 0.64 ml/min. After perfusion at 37°C for 30 min or until V_T was stable, samples of the effluent fluid were collected into a constant volume pipette for analysis of total CO_2 content³ (TCO_2) or $[\text{Na}]$ and $[\text{K}]$ as described below.

The bath was collected for analysis of $[\text{H}^3]\text{inulin}$ before and after each set of collections to determine there were no mechanical leaks. Tubules were not used when $> 5\%$ of the volume marker was present in the bath. In most tubules the leakage rate was $< 2\%$.

Net TCO_2 flux (J_{TCO_2} , in picomoles per millimeter per minute [$\text{pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$]) was calculated by the following formula: $J_{\text{TCO}_2} = V_0/L([\text{TCO}_2]_i - [\text{TCO}_2]_o)$, where V_0 is the collected fluid rate (in nanoliters per minute), L is the length (in millimeters), and $[\text{TCO}_2]_i$ and $[\text{TCO}_2]_o$ are the perfused and collected fluid TCO_2 concentrations (in millimoles per liter), respectively. Similar equations were used to calculate net sodium flux (J_{Na}) and net potassium flux (J_{K}). Positive numbers denote net absorption, and negative numbers denote net secretion. Total CO_2 content was measured by a picapnotherm (GVH-1, World Precision Instruments, Inc.) and displayed a linear response throughout the range used. The slope of all curves exhibited a sensitivity $> 120 \text{ U/meq/liter}$ and could detect reliably differences in TCO_2 of 1.0 mmol/liter. This value is less than one-fourth the mean difference in perfused and collected fluid TCO_2 under basal conditions. $[\text{K}]$ and $[\text{Na}]$ in the perfused and collected fluid were measured by flameless atomic absorption spectrophotometry (13). This method can detect reliably differences in the perfusate $[\text{K}]$ of 0.6 meq/liter. This is less than one-half the mean difference in perfused and collected $[\text{K}]$ under basal conditions. This method can detect reliably differences in the perfusate $[\text{Na}]$ of 4.2 meq/liter. The mean paired difference in basal perfused and collected fluid $[\text{Na}]$ was $5.3 \pm 1.2 \text{ mmol/liter}$. Because this value was close to the sensitivity of the assay, we examined the paired differences between the perfused and collected $[\text{Na}]$. By paired analysis in 16 tubules the collected fluid $[\text{Na}]$ was always less than the perfusate $[\text{Na}]$, and this difference was highly significant ($P < 0.0001$). Were the difference between the perfused and collected fluid $[\text{Na}]$ beyond the limit of resolution of the assay, the difference in the perfused and collected fluid $[\text{Na}]$ should have been randomly distributed about zero.

Studies examining proton secretion were conducted in two separate series of experiments. In both series, after perfusion for at least 30 min at 37°C , at least six collections were obtained for TCO_2 determination during the basal period, and then the perfusate was exchanged for either vehicle or omeprazole (0.1 mM). After 20 min at least six additional collections were obtained for TCO_2 determination. Six different samples of the perfusate were analyzed in an identical manner.

Studies examining the effect of omeprazole on net Na and K flux followed a protocol similar to the bicarbonate flux studies except that three collections were obtained during the basal period for $[\text{Na}]$ and $[\text{K}]$. The perfusate was then exchanged for either vehicle or omeprazole (0.1 mM). After 20 min three additional collections were obtained. Six different samples of the perfusate were analyzed identically. In addition, each separate sample including the three basal samples (collections), three vehicle or omeprazole samples, and six perfusate samples were analyzed at least in quadruplicate for $[\text{Na}]$ and $[\text{K}]$ (13). Thus, each period represents an average of at least 12 analyses for $[\text{Na}]$ and $[\text{K}]$ in the collected fluid and 24 analyses for $[\text{Na}]$ and $[\text{K}]$ in the perfusate.

Omeprazole was dissolved in pure polyethylene glycol (PEG-400, Fisher Scientific Co., Pittsburgh, PA) to make a stock solution of 10

mM. The experimental perfusate was prepared by adding 20 μl of the stock solution to 2 ml of the perfusate. The vehicle-treated perfusate was prepared in an identical manner by adding 20 μl of PEG-400 to 2 ml of perfusate. The passive paracellular K flux (J_{K}^p) was calculated according to the Goldman flux equation:

$$J_{\text{K}}^p = \frac{zF}{RT} \cdot V_T \cdot P_K \frac{[\text{K}]_b - [\bar{\text{K}}]_i \exp[V_T zF/(RT)]}{1 - \exp[V_T zF/(RT)]}$$

where P_K is the paracellular K permeability, $[\text{K}]_b$ is the bath $[\text{K}]$, $[\bar{\text{K}}]_i$ the mean luminal $[\text{K}]$, and z , F , R , and T have their usual meaning.

Statistics. Analysis of variance or paired t test were performed as appropriate, and the null hypothesis was rejected at the 0.05 level of significance. Means are reported \pm the standard error of the mean. Paired differences in the perfused and collected $[\text{TCO}_2]$, $[\text{Na}]$, or $[\text{K}]$ were computed for each set of experiments. All the statistically significant results for net flux were matched by statistically significant results for the corresponding concentration differences.

Results

Bicarbonate flux. Measurements of J_{TCO_2} were made in 11 tubules. There was no significant difference by paired analysis in the mean perfusion rate ($5.4 \pm 0.6 \text{ nl/min}$) or tubule length ($1.5 \pm 0.2 \text{ mm}$) between the five vehicle-treated tubules and the six omeprazole-treated tubules ($4.6 \pm 0.2 \text{ nl/min}$ and $1.4 \pm 0.1 \text{ mm}$). Moreover, by paired analysis there was no significant difference in the perfusion rate before ($4.7 \pm 0.2 \text{ nl/min}$) and after ($4.6 \pm 0.2 \text{ nl/min}$) addition of vehicle, nor was there any significant difference in the mean perfusion rate for tubules perfused in the absence ($5.5 \pm 0.9 \text{ nl/min}$) and presence ($5.3 \pm 0.8 \text{ nl/min}$) of omeprazole. Administration of vehicle did not significantly change V_T ($5.7 \pm 2.6 \text{ mV}$, basal period vs. $8.1 \pm 3.3 \text{ mV}$, vehicle period). Likewise, omeprazole had no significant effect on V_T ($4.9 \pm 1.6 \text{ mV}$, basal period vs. $5.1 \pm 1.9 \text{ mV}$, omeprazole period). Moreover, the voltages for both groups were not significantly different by analysis of variance for repeated measures.

There was no time-dependent change in bicarbonate absorption (vehicle-treated tubules). J_{TCO_2} was $15.9 \pm 4.3 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$ during the basal period and 20.6 ± 5.5

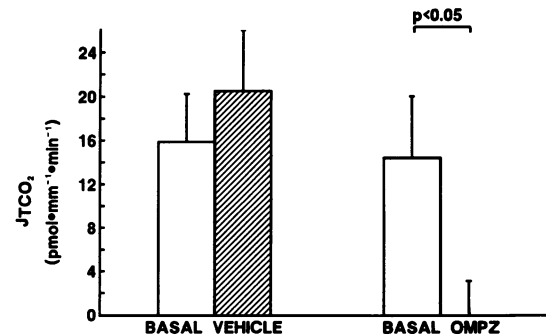


Figure 1. The effect of vehicle and omeprazole (OMPZ), 0.1 mM, on J_{TCO_2} . In five vehicle-treated tubules, shown on the left, J_{TCO_2} did not change significantly between the first period (basal period, open bar) and second period (vehicle period, hatched bar). In six omeprazole-treated tubules, shown on the right, J_{TCO_2} decreased significantly from the first period (basal period, open bar) to the second period (omeprazole period, hatched bar).

3. At physiologic pH (7.40), the vast majority of total CO_2 content reflects bicarbonate ion concentration. The terms net bicarbonate absorption and total CO_2 absorption will be used interchangeably.

$\text{pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$ during the vehicle period (Fig. 1). In contrast, omeprazole abolished net bicarbonate absorption ($14.5 \pm 5.6 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$, basal period vs. $-0.1 \pm 3.1 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$, omeprazole period [$P < 0.05$]).

Sodium and potassium flux. To examine the effects of omeprazole on Na and K transport, 16 tubules were perfused in a similar manner as for the bicarbonate flux studies. Eight tubules (mean length $1.1 \pm 0.1 \text{ mm}$ and mean perfusion rate $4.6 \pm 0.1 \text{ nl/min}$) received vehicle during the second period, and eight tubules (mean length $1.2 \pm 0.1 \text{ mm}$ and mean perfusion rate $4.9 \pm 0.2 \text{ nl/min}$) received 0.1 mM omeprazole during the second period. There was no significant difference in the mean tubule length or perfusion rate between the vehicle-treated and omeprazole-treated groups. Perfusion rates were identical before ($4.6 \pm 0.1 \text{ nl/min}$) and after ($4.6 \pm 0.1 \text{ nl/min}$) luminal addition of vehicle. Likewise, perfusion rates were the same before ($4.8 \pm 0.2 \text{ nl/min}$) and after ($4.9 \pm 0.3 \text{ nl/min}$) luminal addition of omeprazole.

Neither vehicle nor omeprazole significantly altered V_T , consistent with the previous observations. In the vehicle group, V_T was $2.6 \pm 0.6 \text{ mV}$ (basal period) vs. $3.0 \pm 1.3 \text{ mV}$ (vehicle period); in the omeprazole group V_T was $7.3 \pm 2.8 \text{ mV}$ (basal period) vs. $5.2 \pm 1.6 \text{ mV}$ (omeprazole period). The voltages were not significantly different between the vehicle-treated and omeprazole-treated tubules by analysis of variance for repeated measures.

Effect of omeprazole on sodium absorption. Neither vehicle nor omeprazole significantly affected J_{Na} . In vehicle-treated tubules, basal Na absorption was $17.4 \pm 5.4 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$ and was not significantly different after addition of vehicle ($9.5 \pm 3.2 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$). In omeprazole-treated tubules, basal Na absorption was $22.8 \pm 6.3 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$ and was unchanged after the addition of omeprazole ($19.2 \pm 8.7 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$).

Basal potassium absorption. During the basal period for all tubules the mean transepithelial voltage was $5.1 \pm 1.6 \text{ mV}$. This lumen-positive V_T could drive some K absorption. Thus, we calculated the rate of K absorption from the Goldman equation that could be ascribed to passive diffusion via the paracellular pathway, assuming a very large paracellular K permeability ($P_K = 1.0 \times 10^{-5} \text{ cm/s}$). This value is larger than the apparent K permeability of the OMCD_i that has been directly measured by Stokes (14) or by this laboratory (5). Using this generous estimate of the paracellular K permeability,⁴ the rate of paracellular K absorption (J_K^P) was $1.0 \pm 0.4 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$. This represents less than one-fifth the observed rate of net K absorption of $5.0 \pm 1.0 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$. This difference between J_K^P and J_K was highly significant ($P < 0.01$) and indicates that K absorption was largely active.⁵

4. The following analysis assumes that passive net K transport proceeds via a paracellular pathway. Although passive tracer K flux may also include transcellular K movement, in most transporting epithelia net cellular K entry is energetically unfavorable and against the electrochemical gradient for passive net K movement (15). Thus, it appears plausible to consider passive net K transport to be confined to a paracellular route.

5. The K tracer rate coefficient (K_K) approximates an apparent K permeability for the OMCD_i during K repletion (14). Although K_K is $< 1.0 \times 10^{-5} \text{ cm/s}$ during K repletion, it has been demonstrated that

Effect of omeprazole on potassium absorption. Despite the lack of an effect of omeprazole on transepithelial voltage and Na transport, there was a dramatic effect of omeprazole on K absorption (Fig. 2). K absorption was unchanged by vehicle ($4.8 \pm 1.7 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$, basal period vs. $4.8 \pm 2.9 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$, vehicle period). In contrast, addition of 0.1 mM luminal omeprazole abolished net K absorption ($5.1 \pm 1.0 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$, basal period vs. $0.1 \pm 2.5 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$, omeprazole period, $P < 0.05$) (Fig. 2).

Discussion

The present studies demonstrate that omeprazole abolishes both proton secretion and K absorption in the OMCD_i of rabbits conditioned to a low-K diet. These changes occurred without significant changes in V_T or J_{Na} . These observations are consistent with the hypothesis that omeprazole inhibits a primary active pump that energizes the active exchange of protons for K ions across the apical membrane. This model is similar to that developed to explain proton secretion by the gastric mucosa in which omeprazole inhibits active proton secretion and active K absorption (16). Indeed, the CD of both the rabbit and the rat has K-ATPase activity that exhibits inhibition characteristics similar to the gastric proton pump (i.e., insensitive to ouabain but inhibited by vanadate and omeprazole (6–8)). Furthermore, this enzyme activity is stimulated by K depletion (7). Morphologic studies have demonstrated consistently that K depletion induces hypertrophy of the intercalated cells of the OMCD_i (1–4). The morphologic similarity of the intercalated cell of the CD and the parietal cell of the gastric mucosa makes the intercalated cell a plausible cell type that may be responsible for K absorption and H secretion.

Omeprazole fully inhibited proton secretion in the OMCD_i of these K-restricted rabbits. This might suggest that omeprazole also inhibited an electrogenic proton ATPase (H-ATPase), which has been proposed to be the major enzyme responsible for luminal acidification in this segment (17). However, the H-ATPase is electrogenic and, therefore, omeprazole should have inhibited V_T , whereas our results show clearly that V_T was in fact maintained. J_{TCO_2} was indistinguishable from zero after omeprazole. This implies that either the H-ATPase was not functional under the conditions of these experiments or that significant bidirectional proton flux is present in this segment. Because removal of luminal Cl enhances acidification in the OMCD_i, the latter possibility is not excluded (18). However, it should be stressed that K-ATPase activity is present in rabbits on a normal K diet (7, 8). Furthermore, in the colon aldosterone stimulates a putative H-K-ATPase (19) similar to the stimulation of acidification in the OMCD_i. Taken together, these facts suggest that both H-ATPase and H-K-

K_K increases dramatically after K restriction (5). It is instructive, therefore, to examine the rate of passive K transport predicted by the Goldman flux equation using a value of $P_K = 2.0 \times 10^{-5} \text{ cm/s}$. This value is comparable to the value for the rate coefficient that was measured during K restriction. Using this value for P_K the rate of passive K flux is less than half the observed rate of K absorption ($J_K^P = 1.9 \pm 0.8 \text{ pmol} \cdot \text{mm}^{-1} \cdot \text{min}^{-1}$) and the difference between J_K^P and J_K is highly significant ($F = 8.0, P < 0.01$).

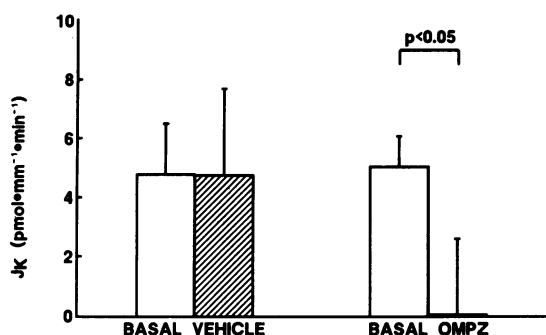


Figure 2. The effect of vehicle and omeprazole (OMPZ), 0.1 mM, on J_K . In eight vehicle-treated tubules, shown on the left, J_K did not change significantly between the first period (basal period, open bar) and the second period (vehicle period, hatched bar). In eight omeprazole-treated tubules, shown on the right, K absorption (J_K) decreased significantly from the first period (basal period, open bar) to the second period (omeprazole period, hatched bar).

ATPase activity are present in the OMCD_i normally and that both may be stimulated by aldosterone. Potassium restriction may act as an additional factor that stimulates the H-K-pump (6). Because the diet used in this study profoundly suppressed plasma aldosterone concentration (5), electrogenic acidification may be small or absent during K restriction as supported by the present data.⁶ Finally, the lack of any effect of omeprazole on V_T and J_{Na} demonstrates that the profound action of omeprazole on both J_{TCO_2} and J_K cannot be attributed to non-specific or toxic effects.

An important finding of the present study is that the CD is capable of active K absorption. Thus, renal K excretion is determined not only by active K secretion localized largely to the renal cortex but also by active K absorption localized at least in part to the OMCD.

The presence of a functional H-K-ATPase necessary for K conservation may be relevant to chloride-resistant metabolic alkalosis. This form of metabolic alkalosis is usually secondary to mineralocorticoid excess and requires K depletion to produce maximal alkalinization of the extracellular fluid (21–23). Chronic hyperaldosteronism or exogenous mineralocorticoid therapy results in a three- to fourfold increase in K secretion in the cortical CD and initial collecting tubule (24–26), although effects on urinary K excretion are relatively minor (24). If net K secretion in the cortical CD and initial collecting tubule exceeds K intake, profound K depletion would ensue unless there were a compensatory increase in K-absorptive flux in more terminal nephron segments. Activation of a K conservation mechanism in the OMCD_i, which is coupled to proton secretion, would generate an extracellular metabolic alkalosis. These findings are entirely consistent with the known effects of

6. The precise origin of the lumen positive voltage is not clear. Whereas Lombard et al. (20) have reported that acetazolamide (0.1 mM) abolishes the lumen positive voltage in the OMCD_i from K-replete rabbits, acetazolamide (0.1 mM) clearly does not abolish this voltage in the OMCD_i from rabbits on a K-restricted diet (unpublished observations).

mineralocorticoids to enhance renal net acid excretion and the marked augmentation of this effect in the presence of K deprivation (21, 27).

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References

- Spargo, B. 1954. Kidney changes in hypokalemic alkalosis in the rat. *J. Lab. Clin. Med.* 43:802–814.
- Ordóñez, M. G., and B. H. Spargo. 1976. The morphologic relationship of light and dark cells of the collecting tubule in potassium-depleted rats. *Am. J. Pathol.* 84:317–322.
- Hansen, G. T., C. C. Tisher, and R. R. Robinson. 1980. Response of the collecting duct to disturbances of acid base and potassium balance. *Kidney Int.* 17:326–337.
- Stetson, D. L., J. B. Wade, and G. Giebisch. 1980. Morphologic alterations in the rat medullary collecting duct following potassium depletion. *Kidney Int.* 17:45–56.
- Wingo, C. S. 1987. Potassium transport by medullary collecting tubule of rabbit: effects of variation in K intake. *Am. J. Physiol.* 253(Renal Fluid Electrolyte Physiol. 22):F1136–F1141.
- Imbert-Teboul, M., A. Doucet, S. Marsy, and S. Siamel-Perez. 1987. Alterations in enzymatic activity along rat cortical tubule in potassium depletion. *Am. J. Physiol.* 253(Renal Fluid Electrolyte Physiol. 22):F408–F417.
- Doucet, A., and S. Marsy. 1987. Characterization of K-ATPase activity in distal nephron: stimulation by potassium depletion. *Am. J. Physiol.* 253(Renal Fluid Electrolyte Physiol. 22):F418–F423.
- Garg, L. C., and N. Narang. 1988. Ouabain-insensitive K-adenosine triphosphatase in distal nephron segments of the rabbit. *J. Clin. Invest.* 81:1204–1208.
- Burg, M., J. Grantham, M. Abramow, and J. Orloff. 1966. Preparation and study of fragments of single rabbit nephrons. *Am. J. Physiol.* 210:1293–1298.
- Wingo, C. S. 1986. Effect of acidosis on chloride transport in the cortical thick ascending limb of Henle perfused in vitro. *J. Clin. Invest.* 78:1324–1330.
- Schmidt-Nielsen, B. 1972. Mechanisms of urea excretion by the vertebrate kidney. In *Nitrogen Metabolism and the Environment*. J. W. Campbell and L. Goldstein, editors. Academic Press, Inc., New York. 79–103.
- Schafer, J. A., S. L. Troutman, and T. E. Andreoli. 1974. Volume reabsorption, transepithelial potential differences, and ionic permeability properties in mammalian superficial proximal straight tubules. *J. Gen. Physiol.* 64:582–607.
- Wingo, C. S., G. B. Bixler, C. H. Park, and S. G. Straub. 1987. Picomole analysis of alkali metals by flameless atomic absorption spectrophotometry. *Kidney Int.* 31:1225–1228.
- Stokes, J. B. 1982. Na and K transport across the cortical and outer medullary collecting tubule of the rabbit: evidence for diffusion across the outer medullary portion. *Am. J. Physiol.* 242(Renal Fluid Electrolyte Physiol. 11):F514–F520.
- Biagi, B., M. Sohtell, and G. Giebisch. 1981. Intracellular potassium activity in the rabbit proximal straight tubule. *Am. J. Physiol.* 241(Renal Fluid Electrolyte Physiol. 10):F677–F686.

16. Reenstra, W. W., J. D. Bettencourt, and J. G. Forte. 1986. Active K⁺ absorption by the gastric mucosa: inhibition by omeprazole. *Am. J. Physiol.* 250(Gastrointest. Liver Physiol. 13):G455–G460.
17. Stone, D. K., D. W. Seldin, J. P. Kokko, and H. R. Jacobson. 1983. Mineralocorticoid modulation of rabbit medullary collecting duct acidification: a sodium-independent effect. *J. Clin. Invest.* 72:77–83.
18. Stone, D. K., D. W. Seldin, J. P. Kokko, and H. R. Jacobson. 1983. Anion dependence of rabbit medullary collecting duct acidification. *J. Clin. Invest.* 71:1505–1508.
19. Perrone, R. D., and D. E. McBride. 1988. Aldosterone and PCO₂ enhance rubidium absorption in rat distal colon. *Am. J. Physiol.* 254(Gastrointest. Liver Physiol. 17):G898–G906.
20. Lombard, W. E., J. P. Kokko, and H. R. Jacobson. 1983. Bicarbonate transport in cortical and outer medullary collecting tubules. *Am. J. Physiol.* 244(Renal Fluid Electrolyte Physiol. 13):F289–F296.
21. Seldin, D. W., L. G. Welt, and J. H. Cort. 1956. The role of sodium salts and adrenal steroids in the production of hypokalemic alkalosis. *Yale J. Biol. Med.* 29:229–247.
22. Kassirer, J. P., A. M. London, D. M. Goldman, and W. B. Schwartz. 1970. On the pathogenesis of metabolic alkalosis in hyperaldosteronism. *Am. J. Med.* 49:306–315.
23. Arruda, J. A. L., and N. A. Kurtzman. 1977. Metabolic and respiratory alkalosis. In *Pathophysiology of the Kidney*. N. A. Kurtzman and M. Martinez-Maldonado, editors. Charles C. Thomas, Springfield, IL. 356–388.
24. Wingo, C. S. 1984. Effect of ouabain on K secretion in cortical collecting tubules from adrenalectomized rabbits. *Am. J. Physiol.* 247(Renal Fluid Electrolyte Physiol. 16):F588–F595.
25. Stanton B., L. Pan, H. Deetjen, V. Guckian, and G. Giebisch. 1987. Independent effects of aldosterone and potassium on induction of potassium adaptation in rat kidney. *J. Clin. Invest.* 79:198–206.
26. Stanton, B. A. 1986. Regulation of adrenal corticosteroids of sodium and potassium transport in loop of Henle and distal tubule of rat kidney. *J. Clin. Invest.* 78:1612–1620.
27. Hutler, H. N., J. F. Sigala, and A. Sebastian. 1978. K⁺ deprivation potentiates the renal alkalosis-producing effect of mineralocorticoid. *Am. J. Physiol.* 235(Renal Fluid Electrolyte Physiol. 4):F298–F309.