Dietary K⁺ restriction upregulates total and Sch-28080-sensitive bicarbonate absorption in rat tIMCD

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Wall, Susan M., Pramod Mehta, and Thomas D. DuBose, J. R. Dietary K⁺ restriction upregulates total and Sch-28080-sensitive bicarbonate absorption in rat tIMCD. Am. J. Physiol. 275 (Renal Physiol. 44): F543–F549, 1998.—In tubules from the terminal segment of the inner medullary collecting duct (tIMCD) from rats with chronic metabolic acidosis, our laboratory has shown that bicarbonate absorption (J ic o2) is inhibited by removal of K⁺ from the luminal fluid or by the addition of Sch-28080 to the perfusate. The present study asked whether total and/or Sch-28080-sensitive J ic o2 are regulated by changes in systemic K⁺ homeostasis. Rat tIMCD tubules were perfused in vitro in symmetrical, HCO3⁻/CO2-buffered solutions containing 10 mM KCl + 6 mM NH₄Cl. Total and Sch-28080-sensitive J ic o2 were measured in rats with varying K⁺ intake. In K⁺-repleter rats, baseline J ic o2 was 2.1 ± 0.3 pmol·mm⁻¹·min⁻¹ (n = 6). In rats fed a K⁺-deficient diet for 3 days, J ic o2 was 5.4 ± 0.7 pmol·mm⁻¹·min⁻¹ (n = 16, P < 0.05). To determine the mechanism for the increase in HCO₃⁻ absorption observed with K⁺ restriction, the Sch-28080-sensitive component of J ic o2 was measured in each treatment group. Following the addition of Sch-28080 (10 µM) to the perfusate, a 40% reduction in J ic o2 was observed in K⁺-restricted rats. J ic o2 was not reduced following the addition of Sch-28080 in rats with normal K⁺ intake. Because Sch-28080-sensitive J ic o2 was increased in K⁺-restricted rats, Sch-28080-sensitive J ic o2 was studied further in tIMCD tubules from rats in this treatment group. In K⁺-restricted rats, J ic o2 decreased by 20% following the addition of 5 mM ouabain to the perfusate. This ouabain-induced decline in J ic o2 was observed both in the presence and in the absence of Sch-28080. We conclude that total and Sch-28080-sensitive net acid secretion is increased with dietary K⁺ restriction. However, since ~50% of J ic o2 is insensitive to both Sch-28080 and ouabain, future studies will be necessary to define other mechanisms of luminal acidification in the rat tIMCD.

collecting duct; hypokalemia; proton-potassium-adenosine triphosphatase; HKα₁; HKα₂; ouabain; Sch-28080

IT IS WIDELY ACCEPTED that renal net acid excretion is enhanced by hypokalemia (29). In part, this increase in net acid excretion can be attributed to enhanced ammonium production in the proximal tubule (29). Ammonium is produced through conversion of glutamine to glutamate, a reaction catalyzed by phosphate-dependent glutaminase. Ammoniagenesis is regulated in vivo by dietary K⁺ restriction (24, 29) and in vitro by changes in extracellular K⁺ concentration (23, 27). With hypokalemia, distal delivery of NH₄⁺ is increased, whereas the distal delivery of K⁺ is reduced. This combination augments NH₄⁺ uptake by the Na⁺-K⁺-2Cl⁻ cotransporter (BSC-1, NKCC2) on the apical membrane of the thick ascending limb (14), which presumably increases NH₄⁺ concentration in the medullary interstitium. These results suggest that ammonium secretion by the collecting duct is regulated with changes in K⁺ homeostasis.

Numerous studies have elucidated an important role for the H⁺-K⁺-ATPases in K⁺ and acid-base homeostasis. There are at least two and possibly three α isoforms of the H⁺-K⁺-ATPase that localize to the collecting duct (19). In the terminal portion of the rat inner medullary collecting duct (tIMCD), both HKα₁ (the "gastric" H⁺-K⁺-ATPase) and HKα₂ (the "colonic" H⁺-K⁺-ATPase) have been identified (1, 2). In the kidney, expression of both HKα₂ mRNA and protein abundance increase during dietary K⁺ restriction (2, 7, 12, 26). HKα₂, in contrast to HKα₁, is sensitive to ouabain at high concentrations (24). However, the role of HKα₂ in luminal acidification along the collecting duct has not been fully defined. Moreover, it is not known whether HKα₂-mediated transport is modulated in the kidney in vivo in response to changes in K⁺ homeostasis.

Although HKα₁ mRNA has been detected throughout the collecting duct (1), the regulatory response of HKα₁ mRNA, upon changes in K⁺ homeostasis, is apparently confined to the renal cortex (3). Since HKα₁ is fully inhibited by Sch-28080 at concentrations below 10 µM, the Sch-28080-sensitive component of J ic o2 has been taken as an index of H⁺ secretion mediated by the α₁ isoform of the H⁺-K⁺-ATPase (33). However, in the rabbit outer medullary collecting duct (OMCD), total and Sch-28080-sensitive bicarbonate absorption (J ic o2) are not enhanced with dietary K⁺ restriction (34).

Our laboratory has shown that bicarbonate absorption, J ic o2, is inhibited by removal of K⁺ from the luminal fluid or by the addition of Sch-28080 to the perfusate (32) in tIMCD tubules from rats with chronic metabolic acidosis. Nevertheless, the specific α-H⁺-K⁺-ATPase that mediates Sch-28080-sensitive J ic o2 in vivo, as well as the conditions in vivo that alter its activity, are not well understood. The purpose of the present study was to determine whether changes in K⁺ homeostasis alter J ic o2 in the rat tIMCD and to determine whether these changes are mediated through changes in activity of H⁺-K⁺-ATPases previously detected in this segment.

METHODS

Animal conditioning. Pathogen-free male Sprague-Dawley rats weighing 65–120 g (Rm. 205G; Harlan, Indianapolis, IN) were employed and housed in microisolator cages. Unless otherwise specified, animals were fed a rat chow containing 7.8 g K⁺/kg food, 0.664 g Na⁺/kg food (Zeigler Brothers,
Flow rate was determined as described previously (31). Total were collected under oil in calibrated constriction pipettes.

**RESULTS**

Effect of changes in K⁺ homeostasis on JtCO₂. Rats ingested a K⁺-restricted diet (0.0041 g K⁺/kg food, 1.03 g Na⁺/kg food, group B) or a normal K⁻ diet (2.3 g K⁺/kg food, group A) for 3 days prior to death. This time period was selected because maximal renal K⁺ conservation following dietary K⁺ restriction is observed at 3 days (21). Moreover, we have observed that if the animals ingest this K⁺-restricted diet for more than 3 days, then tMCMD tubules became very difficult to dissect. Serum and urine electrolytes and urinary pH were measured in both treatment groups (Table 1). As shown, serum K⁺ was lower in the K⁺-restricted group than in controls. Moreover, K⁺-restricted rats developed a mild metabolic alkalosis, increased urinary pH.

**Table 1. Serum and urine electrolytes**

<table>
<thead>
<tr>
<th></th>
<th>Controls (Group A)</th>
<th>K⁺ Restricted (Group B)</th>
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<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺, mM</td>
<td>146.0 ± 0.4</td>
<td>145.2 ± 0.5</td>
</tr>
<tr>
<td>K⁺, mM</td>
<td>4.6 ± 0.2</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>Cl⁻, mM</td>
<td>109.3 ± 0.6</td>
<td>101.8 ± 0.3</td>
</tr>
<tr>
<td>CO₂, mM</td>
<td>26.9 ± 0.3</td>
<td>31.7 ± 0.8</td>
</tr>
<tr>
<td>Osmolality, mosmol/kgH2O</td>
<td>275 ± 1</td>
<td>275 ± 3</td>
</tr>
<tr>
<td>Urine osmolality, mosmol/kgH2O</td>
<td>650 ± 97</td>
<td>195 ± 16</td>
</tr>
<tr>
<td>pH</td>
<td>6.15 ± 0.05</td>
<td>7.40 ± 0.12</td>
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Values are means ± SE; n = no. of experiments.
RESTRICTION INCREASES J\textsubscript{tCO2} IN RAT tIMCD

Fig. 1. Effect of dietary K\textsuperscript{+} restriction on bicarbonate absorption (J\textsubscript{tCO2}). In rats eating a normal K\textsuperscript{+} diet (control), J\textsubscript{tCO2} was 2.1 ± 0.3 pmol·mm\textsuperscript{-1}·min\textsuperscript{-1} (n = 6). J\textsubscript{tCO2} increased to 5.4 ± 0.7 pmol·mm\textsuperscript{-1}·min\textsuperscript{-1} in rats ingesting a K\textsuperscript{+}-restricted diet (n = 16, P < 0.05).

Changes in K\textsuperscript{+} homeostasis, Sch-28080, and because H\textsuperscript{+}-K\textsuperscript{+}-ATPase activity with activity of the HK\textsubscript{α3} isoform of the H\textsuperscript{+}-K\textsuperscript{+}-ATPase. Results of these experiments are shown in Fig. 2A (Table 2). In K\textsuperscript{+}-restricted rats (group B), baseline J\textsubscript{tCO2} was 6.0 ± 1.3 pmol·mm\textsuperscript{-1}·min\textsuperscript{-1} but decreased to 3.6 ± 0.7 pmol·mm\textsuperscript{-1}·min\textsuperscript{-1} upon the addition of Sch-28080 (10 μM) to the perfusate (n = 8, P < 0.05; series 1). To determine whether the Sch-28080-induced reduction in J\textsubscript{tCO2} is a time-dependent phenomenon, time-control experiments were performed (Fig. 2B; Table 2, series 2). To do so, tIMCD tubules from K\textsuperscript{+}-restricted rats were perfused under the same conditions as above in series 1. Baseline J\textsubscript{tCO2} was measured (period 1). In period 2, a mock perfusate exchange was performed, and Sch-28080 vehicle (ethanol) was introduced into the perfusate, without the addition of Sch-28080. As shown, the decline in J\textsubscript{tCO2} observed upon the addition of Sch-28080 to the perfusate was not observed in time controls (Fig. 2B).

To determine whether the Sch-28080-sensitive component of J\textsubscript{tCO2} is regulated through perturbations in K\textsuperscript{+} homeostasis, Sch-28080-sensitive J\textsubscript{tCO2} was measured in rats eating a K\textsuperscript{+}-replete diet (Fig. 3; Table 2, series 3). In rats ingesting a normal K\textsuperscript{+} diet (group A, 2.3 g K\textsuperscript{+}/kg food), no change in J\textsubscript{tCO2} was detected upon the addition of Sch-28080 to the perfusate. Thus, with dietary K\textsuperscript{+} restriction, both total and Sch-28080-sensitive J\textsubscript{tCO2} are increased.

Mechanism of Sch-28080-sensitive J\textsubscript{tCO2}. Since total and Sch-28080-sensitive J\textsubscript{tCO2} were increased in rats placed on dietary K\textsuperscript{+} restriction, the mechanism of Sch-28080-sensitive net acid secretion was studied further in rat tIMCD tubules from this treatment group. HK\textsubscript{α3} mRNA and protein have been detected in the tIMCD (2, 7). Both H-K\textsuperscript{+}\textsubscript{-ATPase mRNA and protein abundance are increased in rat medulla with dietary K\textsuperscript{+} restriction (2, 7, 12, 26). When expressed in Xenopus laevis oocytes and measured in the presence of 1 mM ouabain to the extracellular fluid (8). Therefore, since HK\textsubscript{α3} is sensitive to high concentrations of ouabain, we asked whether J\textsubscript{tCO2} is sensitive to luminal ouabain at high concentrations. Results of these experiments are shown in Fig. 4A (Table 2, series 4). In K\textsuperscript{+}-restricted rats (group B), baseline J\textsubscript{tCO2} was 5.2 ± 1.1 pmol·mm\textsuperscript{-1}·min\textsuperscript{-1} but fell to 4.3 ± 1.0 pmol·mm\textsuperscript{-1}·min\textsuperscript{-1} with the addition of 5 mM ouabain to the luminal fluid (n = 4, P < 0.05). Thus J\textsubscript{tCO2} is sensitive to high concentrations of luminal ouabain.

To determine whether the ouabain-sensitive component of J\textsubscript{tCO2} is distinct from the Sch-28080-sensitive portion of J\textsubscript{tCO2}, ouabain-sensitive J\textsubscript{tCO2} was measured in K\textsuperscript{+}-restricted rats (group B) in the presence of luminal Sch-28080 (Fig. 4B; Table 2, series 5). With Sch-28080 present in the luminal perfusate, J\textsubscript{tCO2} was 4.6 ± 1.4 but declined to 3.4 ± 1.1 when both ouabain and Sch-28080 were added to the perfusate (n = 6, P < 0.05).2 Thus J\textsubscript{tCO2} is a \textit{gastric} isofrom of the H\textsuperscript{+}-K\textsuperscript{+}-ATPase (33). Therefore, J\textsubscript{tCO2} was measured before and following the application of Sch-28080 to the luminal perfusate. The difference between these two values, or the Sch-28080-sensitive component of J\textsubscript{tCO2}, was taken to be consistent with activity of the HK\textsubscript{α3} isoform of the H\textsuperscript{+}-K\textsuperscript{+}-ATPase.

1 Taken from period 1 of series 3 (Table 2) plus one other tubule.
2 Taken from period 1 of series 1, 2, and 4 plus one other tubule.
Therefore, \( J_{\text{ICO}_2} \) was reduced by \( \sim 1.0 \) pmol·mm⁻¹·min⁻¹ upon the addition of ouabain to the luminal fluid, both in the presence and in the absence of Sch-28080. Thus the Sch-28080- and the ouabain-sensitive components of \( J_{\text{ICO}_2} \) are additive, which implies that the mechanisms of action of these inhibitors on \( J_{\text{ICO}_2} \) are not identical.

\( J_{\text{ICO}_2} \) fell by only \( \sim 20\% \) of the baseline value upon the addition of ouabain to the perfusate. Therefore, the effect of ouabain on \( J_{\text{ICO}_2} \) is small. However, high levels of HK\( \alpha_2 \) activity might not be detected under the conditions of the experiment given above. For example, if HK\( \alpha_2 \) were only partially inhibited by ouabain at a concentration of 5 mM, then the ouabain-sensitive component of \( J_{\text{ICO}_2} \) might be small, despite significant activity of this transporter. Therefore, we asked whether luminal ouabain at a 5 mM concentration only partially inhibits HK\( \alpha_2 \). We have shown previously that removal of K⁺ from the perfusate reduces \( J_{\text{ICO}_2} \). Since the H⁺-K⁺-ATPases utilize K⁺ as a substrate, we asked whether luminal K⁺ removal further reduces \( J_{\text{ICO}_2} \) when measured in the presence of both ouabain and Sch-28080 in the luminal fluid. Tubules from K⁺-restricted rats were perfused with both Sch-28080 and ouabain present in perfusate containing K⁺-restricted rats (n = 5).

**DISCUSSION**

It is well recognized that dietary K⁺ depletion increases urinary pH and ammonium excretion (29). The increase in urinary pH has been attributed to enhanced ammoniagenesis, which presumably increases medullary interstitial NH₃ concentration (29). With increased NH₃ in the medullary interstitium, NH₃ secretion into the medullary collecting duct is augmented. Thus luminal buffering is increased, which augments net acid excretion (13, 15). The present study demonstrates that
the increase in net acid secretion observed with K+ restriction in vivo occurs, in part, as a result of increased apical proton secretion. Moreover, since this study was performed in isolated perfused tIMCDs, it demonstrates a "memory" for the increase in H+ secretion observed in response to dietary K+ restriction. HK1 in the collecting duct, including the tIMCD, has been the subject of much interest over the past decade. HK1 has demonstrated a "memory" for the increase in H+ secretion observed with K+ depletion (2, 3, 7, 10, 17, 26).

The role of the H+-K+-ATPases in acid secretion by the collecting duct, including the tIMCD, has been the subject of much interest over the past decade. HKα1 is exquisitely sensitive to Sch-28080 (4). Therefore, Sch-28080-sensitive J\textsubscript{tCO2} has been taken as a representation of the gastric H+-K+-ATPase-mediated, or HKα1-mediated, transport (33). In cultured rat and mouse tIMCD cells, Sch-28080 inhibits ATP hydrolysis (18) and K+-dependent intracellular pH (pHi) recovery following NH\textsubscript{4}Cl addition and then withdrawal (25). In tIMCD tubules from rats with metabolic acidosis, J\textsubscript{tCO2} is reduced by 50% with either the application of Sch-28080 (10 µM) to the luminal fluid or with removal of luminal K+ (32). Collectively, these studies demonstrate that Sch-28080-sensitive transport in the tIMCD is an active process which mediates K+-dependent apical proton secretion. HKα1 mRNA has been detected in the tIMCD in some (1), but not in all (6), reports. These previous studies are consistent with the hypothesis that Sch-28080-mediated K+/OH-/H+/HCO\textsubscript{3} flux is mediated by HKα1.

However, with in vivo conditioning, the response of HKα1 mRNA and protein differ from Sch-28080-sensitive transport. In the present study, we demonstrate that in the rat tIMCD, the Sch-28080-sensitive component of J\textsubscript{tCO2} is increased with dietary K+ restriction. In tIMCD tubules from K+-restricted rats, ~40% of proton secretion is Sch-28080 sensitive. In contrast, in K+ replete controls, Sch-28080-sensitive J\textsubscript{tCO2} was not detectable. Thus Sch-28080-sensitive J\textsubscript{tCO2} is upregulated in the tIMCD with hypokalemia. Studies in rat tIMCD cells in suspension and cultured mouse OMCD cells have also demonstrated a role of K+ homeostasis in the regulation of Sch-28080-sensitive H+/OH-/HCO\textsubscript{3} transport (10, 17). In permeabilized rat tIMCD cells, Sch-28080-sensitive ATP hydrolysis was increased with dietary K+ restriction in vivo (10). Similarly, cultured mouse OMCD cells, grown in medium with a reduced K+ concentration, show increased Sch-28080-sensitive pHi recovery following NH\textsubscript{4}Cl addition and withdrawal relative to cells grown in medium with a higher K+ concentration (17).

Nevertheless, following K+ restriction, it is not clear whether HKα1 is the sole transporter responsible for
the observed increase in Sch-28080-sensitive J\(^{14}CO_2\), ATP hydrolysis, and pH\(_1\) recovery following an acid load. In the medulla, HK\(_\alpha_1\) mRNA and protein abundance are not increased by dietary K\(^+\) restriction (3, 7, 12). Enhanced HK\(_\alpha_1\) activity could occur through posttranslational regulation, or through trafficking of HK\(_\alpha_1\) between the plasma membrane and the cytoplasm. In the stomach, regulation of HK\(_\alpha_3\)-mediated H\(^+\)-secretion occurs through trafficking of HK\(_\alpha_3\) protein between the plasma membrane and the intracellular vesicles (9). In the kidney, it is possible that the increase in H\(^+\)-K\(^+\)-ATPase activity observed following dietary K\(^+\) restriction occurs through increased insertion of HK\(_\alpha_1\) into the apical membrane. Alternatively, the Sch-28080-sensitive component of J\(^{14}CO_2\) could represent activity of a transporter other than HK\(_\alpha_1\).

In the kidney, Sch-28080 inhibits transporters other than HK\(_\alpha_1\) (5, 16), although Sch-28080 (10 \(\mu M\)) does not inhibit the H\(^+\)-ATPase in the rat tIMCD (32). The present study cannot exclude the possibility that Sch-28080 inhibits K/\(\alpha\)OH/H\(^+\) transporters other than HK\(_\alpha_1\), H\(^+\)-K\(^+\)-ATPases other than HK\(_\alpha_1\) have been defined that also show sensitivity to Sch-28080. ATP1AL1, a gene encoding a polypeptide of P-type K\(^+\)-dependent ATPases, is sensitive to both ouabain and Sch-28080 when expressed in COS cells (16). The mechanism of the Sch-28080- and ouabain-sensitive components of J\(^{14}CO_2\) observed in the present study, however, cannot be attributed to ATP1AL1, since the Sch-28080- and ouabain-sensitive components of J\(^{14}CO_2\) are additive. This additivity cannot be explained by only partial inhibition of the transporter by these agents when administered separately at concentrations employed in the present study. Moreover, ATP1AL1 has not been detected in the collecting duct (16).

It is well established that along the collecting duct, HK\(_\alpha_2\) mRNA and protein abundance is upregulated by chronic hypokalemia (2, 7, 12, 26). Moreover, splice variants of HK\(_\alpha_2\) have been identified (20). Both HK\(_\alpha_{2a}\) and HK\(_\alpha_{2b}\) display a similar tissue distribution, and both are upregulated by dietary K\(^+\) restriction. HK\(_\alpha_{2a}\) expression increases from the more proximal (cortical collecting duct) to the more distal (tIMCD) segments of the collecting duct (2). Thus HK\(_\alpha_{2a}\) is highly expressed in the rat tIMCD. However, participation of either HK\(_\alpha_{2a}\) or the splice variant, HK\(_\alpha_{2b}\), in luminal acidification has not been established unequivocally in the tIMCD. The present study demonstrates that a component of net acid secretion is sensitive to ouabain present in the luminal fluid at high concentrations, consistent with a role for HK\(_\alpha_2\) in mediating net acid secretion (7, 26). Although HK\(_\alpha_2\) mRNA and protein abundance are increased by hypokalemia, it is not known whether changes in extracellular K\(^+\) concentration in vivo or in vitro modulate activity of HK\(_\alpha_2\). The present study demonstrates a small component of J\(^{14}CO_2\) sensitive to ouabain in the luminal fluid. However, since the ouabain-induced change in tCO\(_2\) concentration measured in collected samples approaches the limit of detectability of the fluorometer (28, 30), it was not possible in the present study to determine whether ouabain-sensitive J\(^{14}CO_2\) is reduced in K\(^+\)-replete rats. Whether the component of J\(^{14}CO_2\) sensitive to luminal ouabain is regulated in vivo by K\(^+\) restriction remains to be established, therefore.

Although a component of J\(^{14}CO_2\) sensitive to ouabain was observed, consistent with a contribution to J\(^{14}CO_2\) by HK\(_\alpha_2\), this ouabain-sensitive component of J\(^{14}CO_2\) is quite small. The observation that the ouabain-sensitive component of J\(^{14}CO_2\) is small cannot be explained by only partial inhibition of HK\(_\alpha_2\) by ouabain at a concentration of 5 \(\mu M\).

In conclusion, net acid secretion in the rat tIMCD is upregulated by dietary K\(^+\) restriction. This increase in H\(^+\)-secretion is mediated through transport processes sensitive to both Sch-28080 (10 \(\mu M\)) and ouabain (5 \(\mu M\)). The Sch-28080- and ouabain-sensitive components of J\(^{14}CO_2\) appear to be distinct. These observations could be the result of enhanced bicarbonate absorption mediated through the combined response of both HK\(_\alpha_1\) and HK\(_\alpha_2\) to dietary K\(^+\) restriction.

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