Effect of luminal angiotensin II on rabbit proximal convoluted tubule bicarbonate absorption

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Baum, Michel, Raymond Quigley, and Albert Quan. Effect of luminal angiotensin II on rabbit proximal convoluted tubule bicarbonate absorption. Am. J. Physiol. 273 (Renal Physiol. 42): F595–F600, 1997.—The present in vitro microperfusion study examined the effect of luminal angiotensin II on proximal convoluted tubule (PCT) volume absorption and bicarbonate transport. Neither 10−11 M, 10−10 M, nor 2×10−8 M luminal angiotensin II significantly affected PCT transport. When tubules were first perfused with enalaprilat to inhibit endogenous angiotensin II production, addition of 10−8 M luminal angiotensin II increased volume absorption (0.72 ± 0.08 vs. 0.86 ± 0.07 nl·mm−2·min−1, P < 0.01) and bicarbonate transport (52.3 ± 3.7 vs. 67.9 ± 4.2 pmol·mm−2·min−1, P < 0.03). Addition of 10−6 M losartan, an AT1 inhibitor, to the luminal perfusate inhibited volume absorption (0.95 ± 0.14 vs. 0.72 ± 0.11 nl·mm−2·min−1, P < 0.05) and bicarbonate transport (65.0 ± 7.3 vs. 54.7 ± 9.2 pmol·mm−2·min−1, P < 0.05). Addition of 10−4 M luminal PD-123319, an AT2 inhibitor, was without effect. In tubules perfused with 10−4 M luminal enalaprilat and 10−4 M luminal PD-123319, addition of 10−10 M luminal angiotensin II in the experimental period resulted in a stimulation in volume absorption (0.61 ± 0.08 vs. 0.81 ± 0.10 nl·mm−2·min−1, P < 0.01) and bicarbonate transport (49.9 ± 6.3 vs. 77.4 ± 14.3 pmol·mm−2·min−1, P < 0.01). In tubules perfused with 10−6 M losartan and 10−4 M enalaprilat, addition of luminal 10−6 M angiotensin II resulted in no change in transport. These data are consistent with endogenous angiotensin II affecting PCT bicarbonate transport in vitro via luminal AT1 receptors.

METHODS

Midcortical and juxtamedullary rabbit PCT were dissected and perfused as previously described (1). Briefly, kidneys from adult female New Zealand White rabbits weighing 2–3 kg were cut in coronal slices. Individual tubules were dissected in cooled (4°C) Hanks’ solution containing (in mM) 137 NaCl, 5 KCl, 0.8 MgSO4, 0.33 Na2HPO4, 0.44 KH2PO4, 1.0 MgCl2, 2 tris(hydroxymethyl)aminomethane hydrochloride, 0.25 CaCl2, 2 glutamine, and 2 l-lactate. Hanks’ solution was bubbled with 100% O2 and had a pH of 7.4.

Tubules were perfused with an ultrafiltrate-like solution containing (in mM) 115 NaCl, 25 NaHCO3, 2.3 Na2HPO4, 10 sodium acetate, 1.8 CaCl2, 1 MgSO4, 5 KCl, 8.3 glucose, and 5 alanine and bathed in a similar solution containing 6 g/dl albumin. The perfusate and bath solutions were bubbled with 95% O2-5% CO2 and had a pH of 7.4. The osmolalities of the bath and perfusate were adjusted to 300 mosmol/kg H2O by the addition of either H2O or NaCl. To maintain the pH and bath osmolality constant, bath fluid was continuously changed at a rate of at least 0.5 ml/min. All tubules were perfused at ~10 nl/min at 39–39°C in a 1.2 ml temperature-controlled bath. The first period began after an equilibration time of 30–60 min. Subsequent periods were separated by an equilibration time of at least 15 min.

Net volume absorption (JV, nl·ml−1·min−1) was measured as the difference between the perfusion (JV0) and collection (JV) rates (nl/min) normalized per millimeter of tubule length (L). Exhaustively dialyzed [methoxy-3H]inulin was added to the perfusate at a concentration of 50–75 µCi/ml, so that the perfusion rate could be calculated. The collection rate was measured with a 60-nl constant-volume pipette. The average tubule length, measured using an eyepiece micrometer, was 1.4 ± 0.1 mm.

Total CO2 (TCO2) measurements were performed using microcalorimetry (Picoptherm, model GVI; World Precision Instruments, New Haven, CT). Net total CO2 flux (J CO2, pmol·mm−2·min−1) was calculated according to the equation: J CO2 = (V̇CO2 - V̇CO2) L, where V̇CO2 and V̇CO2 represent the concentration of TCO2 in the perfused and collected fluid, respectively.

The transepithelial potential difference (in mV) was measured using the perfusion pipette as the bridge into the tubular lumen. The perfusion and bath solutions were connected to the recording and reference calomel half-cells, respectively, via a bridge containing an ultrafiltrate of the bathing solution in series with a 3.6 M KCl/0.9 M KNO3 agarose bridge. The recording and reference calomel half-

THE PROXIMAL TUBULE contains all the synthetic machinery to produce angiotensin II (3, 4, 14, 20, 23, 30). Angiotensinogen mRNA and protein are produced by the proximal tubule (14). Renin mRNA has been detected in primary cultures of rabbit proximal tubule cells using reverse transcription and polymerase chain reaction and in dissected proximal tubules from rabbits which received enalapril, an angiotensin converting enzyme inhibitor (23). Renin has also been found in cell lysates from rabbit proximal tubules in culture (23). Angiotensin converting enzyme activity is present on the brush border of the proximal tubule (20). Direct evidence for the production of angiotensin II by the proximal tubule has come from rat in vivo micropuncture and microperfusion studies, which have found luminal concentrations of angiotensin II ~100-fold higher than that in the plasma (3, 4, 30).

We have recently demonstrated that this endogenously produced angiotensin II modulates volume absorption in surface proximal convoluted tubules (PCT) of hydropenic rats (27). Thus, in the volume-depleted rat, angiotensin II acts in an autocrine or paracrine fashion to modulate proximal tubular transport. In this in vitro microperfusion study, we examined whether there was evidence that endogenously produced angiotensin II can affect PCT volume absorption and bicarbonate transport in nonsurface PCT perfused in vitro from euvoletic rabbits. In addition, we examined whether the endogenously produced angiotensin II effect was mediated via AT1 or AT2 receptors.
cells were connected to the high- and low-impedance side, respectively, of an electrometer (model 601; Keithley Instruments, Cleveland, OH).

There were at least four measurements of volume absorption and three of bicarbonate transport in a given period for each tubule. The mean values for individual periods in a given tubule were used to calculate the mean value for that period. Data are expressed as means ± SE; the t-test for paired data was used to determine statistical significance.

RESULTS

Effect of luminal angiotensin II in absence and presence of luminal enalaprilat on PCT volume absorption and bicarbonate transport. The first series of experiments was designed to examine whether luminal angiotensin II affected PCT volume absorption or bicarbonate transport. The results are shown in Table 1. In paired experiments, addition of either 10⁻¹⁰ M or 10⁻¹¹ M angiotensin II to the luminal perfusate, concentrations of angiotensin II that have been previously shown to stimulate PCT volume absorption when added to the bath (15, 29), had no effect on PCT volume absorption. Similarly, addition of 2 × 10⁻⁸ M angiotensin II to the lumen, a concentration measured in the rat PCT luminal fluid (4, 30), also had no effect on the rate of volume absorption. There was a small tendency of 10⁻¹⁰ M and 2 × 10⁻⁸ M luminal angiotensin II to increase the rate of bicarbonate absorption (0.05 < P < 0.10).

To determine whether the lack of an effect of exogenous angiotensin II on PCT transport was due to endogenous angiotensin II production, we examined the effect of 10⁻⁴ M luminal enalaprilat. As shown in Table 2, addition of luminal enalaprilat, a converting enzyme inhibitor, resulted in a small but not significant decrease in the rate of volume absorption. However, addition of 10⁻⁴ luminal enalaprilat significantly inhibited the rate of PCT bicarbonate absorption. These data are consistent with endogenously produced angiotensin II affecting PCT bicarbonate absorption.

In the next series of experiments we examined whether luminal 10⁻¹⁰ M angiotensin II would affect the rate of PCT transport in the presence of 10⁻⁴ M enalaprilat. In these experiments, tubules were perfused with an ultrafiltrate-like solution containing enalaprilat in the control period for ~30 min prior to measurement of volume absorption and bicarbonate transport. As shown in Fig. 1, addition of 10⁻⁴ M luminal angiotensin II increased both the rate of volume production and bicarbonate absorption when the endogenous production of angiotensin II was blocked with enalaprilat.

Effect of losartan on PCT volume absorption and bicarbonate transport. In the next series of experiments we examined the effect of luminal 10⁻⁶ M losartan, an AT₁ antagonist, on proximal tubule transport. As shown in Table 2 and Fig. 2, addition of 10⁻⁶ M luminal losartan resulted in an inhibition in both volume absorption and bicarbonate transport with no effect on the transepithelial potential difference. In this series of experiments there was a large variation in the control rate of volume absorption and bicarbonate transport. However, the reductions in volume absorption and bicarbonate transport were statistically significant when analyzed in a paired fashion. These data are consistent with endogenously produced angiotensin II modulating PCT transport via a luminal AT₁ receptor.

To examine whether endogenous angiotensin II affected the AT₁ receptors on the basolateral membrane, 10⁻⁶ M losartan was added to the bathing solution. As shown in Table 2, bath losartan had no effect on PCT transport. These data also indicate that losartan is not having a nonspecific toxic effect on the tubule. In the final period, we added 10⁻¹¹ M angiotensin II to the bathing solution in the presence of 10⁻⁶ M bath losartan. The rates of volume absorption and bicarbonate transport were 1.12 ± 0.14 nl·mm⁻¹·min⁻¹ and 83.3 ± 9.6 pmol·mm⁻¹·min⁻¹, respectively, in the presence of

Table 1. Effect of luminal ANG II on PCT potential difference, Jᵥ, and JᵥCO₂

<table>
<thead>
<tr>
<th>Protocol</th>
<th>n</th>
<th>Control (PD, mV)</th>
<th>Expt (PD, mV)</th>
<th>Control (Jᵥ, nl·mm⁻¹·min⁻¹)</th>
<th>Expt (Jᵥ, nl·mm⁻¹·min⁻¹)</th>
<th>Control (JᵥCO₂, pmol·mm⁻¹·min⁻¹)</th>
<th>Expt (JᵥCO₂, pmol·mm⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time control</td>
<td>4</td>
<td>-5.2 ± 0.9</td>
<td>-4.5 ± 0.6</td>
<td>0.83 ± 0.08</td>
<td>0.86 ± 0.07</td>
<td>61.6 ± 5.6</td>
<td>62.8 ± 7.2</td>
</tr>
<tr>
<td>10⁻¹² M Luminal ANG II</td>
<td>5</td>
<td>-4.2 ± 0.8</td>
<td>-3.1 ± 0.1</td>
<td>0.69 ± 0.11</td>
<td>0.65 ± 0.10</td>
<td>61.5 ± 6.2</td>
<td>60.0 ± 6.1</td>
</tr>
<tr>
<td>10⁻¹¹ M Luminal ANG II</td>
<td>6</td>
<td>-3.6 ± 0.8</td>
<td>-3.8 ± 0.4</td>
<td>0.71 ± 0.12</td>
<td>0.75 ± 0.10</td>
<td>48.8 ± 8.8</td>
<td>53.6 ± 8.3</td>
</tr>
<tr>
<td>2 × 10⁻⁸ M Luminal ANG II</td>
<td>6</td>
<td>-4.3 ± 0.7</td>
<td>-4.3 ± 0.5</td>
<td>0.74 ± 0.07</td>
<td>0.80 ± 0.08</td>
<td>53.8 ± 7.3</td>
<td>62.4 ± 9.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of experiments. PD, potential difference; ANG II, angiotensin II; Jᵥ, volume absorption; JᵥCO₂, bicarbonate transport; PCT, proximal convoluted tubules.

Table 2. Effect of luminal enalaprilat, luminal and bath losartan and luminal PD-123319 on PCT transport

<table>
<thead>
<tr>
<th>Protocol</th>
<th>n</th>
<th>Control (PD, mV)</th>
<th>Expt (PD, mV)</th>
<th>Control (Jᵥ, nl·mm⁻¹·min⁻¹)</th>
<th>Expt (Jᵥ, nl·mm⁻¹·min⁻¹)</th>
<th>Control (JᵥCO₂, pmol·mm⁻¹·min⁻¹)</th>
<th>Expt (JᵥCO₂, pmol·mm⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻⁴ M Luminal enalaprilat</td>
<td>6</td>
<td>-4.6 ± 1.1</td>
<td>-3.6 ± 0.9</td>
<td>0.78 ± 0.07</td>
<td>0.74 ± 0.09</td>
<td>60.9 ± 7.8</td>
<td>50.2 ± 8.6</td>
</tr>
<tr>
<td>10⁻⁶ M Luminal losartan</td>
<td>6</td>
<td>-4.6 ± 0.6</td>
<td>-4.4 ± 0.9</td>
<td>0.95 ± 0.14</td>
<td>0.72 ± 0.11*</td>
<td>65.0 ± 7.3</td>
<td>54.7 ± 9.2*</td>
</tr>
<tr>
<td>10⁻⁶ M Bath losartan</td>
<td>5</td>
<td>-4.5 ± 1.1</td>
<td>-4.9 ± 1.1*</td>
<td>1.08 ± 0.20</td>
<td>1.12 ± 0.14</td>
<td>77.2 ± 9.2</td>
<td>83.3 ± 9.6</td>
</tr>
<tr>
<td>10⁻⁴ M Luminal PD-123319</td>
<td>5</td>
<td>-3.8 ± 1.1</td>
<td>-3.5 ± 1.0</td>
<td>0.96 ± 0.16</td>
<td>0.94 ± 0.15</td>
<td>84.5 ± 9.0</td>
<td>85.8 ± 4.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of experiments. *P < 0.05 vs. control.
10-6 M losartan and were 1.06 ± 0.14 nl·mm⁻¹·min⁻¹ and 81.4 pmol·mm⁻¹·min⁻¹ when 10⁻¹¹ M angiotensin II was added to the bathing solution. Thus the previously well-described stimulation in PCT volume absorption by angiotensin II was blocked by 10⁻⁶ M losartan (12, 15, 29).

Finally, we examined whether the stimulation in volume absorption seen with 10⁻¹⁰ M luminal angiotensin II in the presence of 10⁻⁴ M enalaprilat was affected by 10⁻⁶ M luminal losartan. In these experiments, tubules were perfused with 10⁻⁴ M enalaprilat and 10⁻⁶ M losartan in the control period. In the experimental period, 10⁻¹⁰ M angiotensin II was added to the luminal perfusate. As shown in Fig. 3, 10⁻⁶ M losartan blocked the increase in transport previously found with angiotensin II in the presence of enalaprilat. These data are consistent with luminal angiotensin II mediating an increase in PCT transport via the AT₁₂ receptor.

Effect of luminal PD-123319 on PCT volume absorption and bicarbonate transport. In the next series of experiments, we examined the effect of luminal 10⁻⁴ M PD-123319 on PCT volume absorption and bicarbonate transport. As shown in Table 2, addition of 10⁻⁴ M luminal PD-123319 had no effect on PCT volume absorption, potential difference, or rate of bicarbonate transport. Finally, we examined the effect of 10⁻¹⁰ M angiotensin II in the presence of 10⁻⁴ M PD-123319 and 10⁻⁴ M enalaprilat. As shown in Fig. 4, 10⁻⁴ M PD-123319 did not inhibit the luminal angiotensin II-mediated increase in bicarbonate absorption on volume absorption in the presence of enalaprilat. These data are consistent with the absence of a significant role for
the AT$_2$ receptor in mediating the effect of luminal angiotensin II.

**DISCUSSION**

Angiotensin II plays an important role in modulating proximal tubule transport (2, 8, 11–13, 15, 19, 21, 22, 27–29, 31, 32). Systemic infusion of angiotensin II at doses that do not affect glomerular hemodynamics or blood pressure results in a significant increase in proximal tubule transport (17, 18). A reduction in angiotensin II levels by the systemic infusion of an angiotensin-converting enzyme inhibitor or administration of angiotensin II receptor antagonist produces a reduction in proximal tubule transport (8, 13, 18, 31, 32). Both in vivo and in vitro microperfusion studies demonstrate that physiological concentrations of peritubular angiotensin II increase proximal tubule transport (12, 15, 29).

A significant role for luminal angiotensin II to modulate proximal tubule transport seemed unlikely. Most studies found that luminal perfusion with angiotensin II produced either no effect or a trivial increase in proximal tubule transport (12, 16, 17, 27). Any filtered angiotensin II would be quickly hydrolyzed by enzymes on the brush-border membrane (25, 26). However, recent studies provide evidence for the local production and luminal secretion of angiotensin II by the PCT, which maintains a luminal concentration higher than

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**Fig. 3.** Effect of luminal $10^{-10}$ M luminal angiotensin II on PCT volume absorption (A) and bicarbonate transport (B) in presence of $10^{-4}$ M enalaprilat and $10^{-6}$ M losartan. Tubules ($n = 5$) were perfused with an ultrafiltrate-like solution containing $10^{-4}$ M enalaprilat to inhibit endogenous angiotensin II production and AT$_1$ receptor antagonist losartan. During experimental period, $10^{-10}$ M angiotensin II was added to luminal perfusate.

**Fig. 4.** Effect of $10^{-10}$ M luminal angiotensin II on PCT volume absorption (A) and bicarbonate transport (B) in presence of $10^{-4}$ M luminal enalaprilat and $10^{-4}$ M PD-123319. Tubules ($n = 5$) were perfused with an ultrafiltrate-like solution containing $10^{-4}$ M enalaprilat to inhibit endogenous angiotensin II production and AT$_2$ receptor antagonist PD-123319. During experimental period $10^{-10}$ M angiotensin II was added to luminal perfusate. *$P < 0.01$ vs. control.
that in the systemic circulation along the proximal tubule (3, 4, 30).

A previous in vitro rabbit microperfusion study examined the effect of luminal angiotensin II on volume absorption (15). Li et al. (15) found that addition of 10^{-11} M, but not 10^{-12} or 10^{-10} M, angiotensin II stimulated volume absorption. They also found that addition of 10^{-8} M angiotensin II to the luminal perfusate inhibited volume absorption to a rate not different from zero. Our results are at significant variance from these. We found that in the absence of enalaprilat, addition of luminal angiotensin II at 10^{-11} M, 10^{-10} M, and 2 \times 10^{-8} M had no significant effect on the rate of volume absorption. The higher concentrations produced a small increase in the rate of bicarbonate absorption that did not reach statistical significance (0.05 < P < 0.10). The cause for the discrepancy between these results is unclear. The absence of an effect of addition of exogenous luminal angiotensin II in vitro could be due to endogenous production of angiotensin II as has been found in the rat (3, 4, 30). To examine the effect of inhibition of angiotensin II production on proximal tubule transport we used enalaprilat. Luminal enalaprilat at 10^{-8} M resulted in an inhibition in PCT bicarbonate reabsorption; the small decrease in volume absorption did not reach significance. The reason why enalaprilat did not inhibit volume absorption is not clear. Angiotensin-converting enzyme inhibitors may be affecting proximal tubule transport by a secondary mechanism in addition to decreasing angiotensin II production. A significant decrease in both volume absorption and bicarbonate transport were observed with blockade of the AT_1 receptor by the addition of luminal losartan. Furthermore, addition of 10^{-10} M angiotensin II to the luminal perfusate in the presence of enalaprilat resulted in a significant increase in both volume absorption and bicarbonate transport. The volume absorption with luminal losartan and 20\% increase in volume absorption with luminal angiotensin II in the presence of enalaprilat are consistent with comparable regulation of proximal tubule transport by luminal and peritubular angiotensin II in the rabbit (29). Taken together, our data are consistent with endogenous production of angiotensin II by the proximal tubule. The lack of an effect of exogenous luminal angiotensin II is consistent with ongoing in vitro production of angiotensin II in the proximal tubule. Angiotensin II binding sites are abundant along the nephron, with the highest density in the PCT (24). The proximal tubule has angiotensin II receptors on the apical and basolateral membrane (5, 7, 9, 10). Whether AT_1 or AT_2 receptors would mediate any luminal effect of angiotensin II was unclear. Dulin et al. (10) described angiotensin II receptors on both the apical and basolateral membrane. They found no evidence for apical membrane AT_1 receptors in the rabbit. [^{125}I]-labeled saralasin was displaced with high concentrations of AT_2 receptor antagonists (10). On the other hand, Burns et al. (7) found that 10^{-8} M losartan displaced over 90\% of labeled [^{125}I]-angiotensin II from brush-border membrane vesicles and found no evidence for apical membrane AT_2 receptors. Our data is consistent with the presence of AT_1 receptors mediating the luminal effect of angiotensin II on proximal tubule transport. We found that addition of losartan inhibited PCT bicarbonate transport and volume absorption. The increase in PCT transport in the presence of luminal enalaprilat by 10^{-10} M luminal angiotensin II was not seen when losartan was in the luminal perfusate. On the other hand, the AT_2 inhibitor PD-123319 had no effect on volume absorption and bicarbonate transport when added to the luminal perfusate. In addition, the angiotensin II-mediated increase in PCT transport in the presence of enalaprilat was observed in the presence of the AT_2 antagonist PD-123319.

Several studies have shown that angiotensin II stimulates the Na^+/-H^+ antiporter (2, 11, 28). This may be mediated by G protein activation of phospholipase A_2 (21). Our data are consistent with the activation of the Na^+/-H^+ antiporter by endogenously produced angiotensin II. We found that addition of enalaprilat and losartan inhibited the rate of bicarbonate reabsorption in the absence of a change in the transepithelial potential difference. Furthermore, addition of luminal angiotensin II in the presence of enalaprilat resulted in an electroneutral stimulation in bicarbonate reabsorption. Thus luminal angiotensin II is likely an important regulator of proximal tubule bicarbonate reabsorption.

In summary, our data are consistent with the presence of endogenously produced angiotensin II affecting PCT bicarbonate absorption from euvolemic rabbits. This effect is mediated entirely via the AT_1 receptor.

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