Endogenous angiotensin II modulates rat proximal tubule transport with acute changes in extracellular volume

ALBERT QUAN1 AND MICHEL BAUM1,2
1Departments of Pediatrics and 2Internal Medicine, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235-9063

Quan, Albert, and Michel Baum. Endogenous angiotensin II modulates rat proximal tubule transport with acute changes in extracellular volume. Am. J. Physiol. 275 (Renal Physiol. 44): F74–F78, 1998.—In the present study, we examined whether the effect of endogenously produced angiotensin II on proximal tubule transport in the male Sprague-Dawley rat is regulated by acute changes in extracellular volume. We measured the magnitude of endogenous angiotensin II–mediated stimulation of transport by sequentially perfusing proximal tubules in vivo, first with an ultrafiltrate-like solution, then by perfusion of the same tubule with an ultrafiltrate-like solution containing 10–8 M losartan (angiotensin II receptor antagonist). During volume contraction, 10–7 M losartan decreased volume reabsorption from 4.20 ± 0.50 to 1.70 ± 0.30 nl·mm–1·min–1 (P < 0.05), a decrease of 58.0 ± 7.0%. In contrast, after acute volume expansion, 10–8 M losartan decreased volume reabsorption from 1.84 ± 0.20 to 1.31 ± 0.20 nl·mm–1·min–1 (P < 0.05), a decrease of 29.6 ± 9.0%. In hydropenic rats, addition of exogenous luminal angiotensin II had no effect on transport. However, in volume-expanded rats, addition of 10–8 M angiotensin II increased volume reabsorption from 2.10 ± 0.34 to 4.38 ± 0.59 nl·mm–1·min–1 (P < 0.005). These data are consistent with endogenously produced angiotensin II augmenting proximal tubule transport to a greater degree during volume contraction than after volume expansion.

autocrine; losartan; microperfusion; kidney

THE RENAL PROXIMAL TUBE can synthesize and secrete angiotensin II into the lumen (4, 5, 25, 27). Components of the renin-angiotensin system found within the proximal tubule include angiotensinogen and its mRNA, renin activity in lysates of proximal tubule cells in primary culture, and renin mRNA detected in proximal tubule cells in primary culture by reverse transcription and polymerase chain reaction (13, 20, 21, 24, 29). Angiotensin-converting enzyme activity has been localized to the luminal brush-border membrane, and receptors for angiotensin II have been found on both the basolateral and luminal membranes (6, 7, 9, 10, 29). Angiotensin II has been detected in native tubular fluid and in rat proximal tubules perfused with an artificial tubular fluid at concentrations ranging from 100–1,000-fold higher than in plasma (4, 5, 27).

Using in vivo microperfusion, we have recently shown that endogenously produced and luminally secreted angiotensin II modulates proximal tubule transport in the hydropenic rat (23). Luminal perfusion of 10–8 M losartan (angiotensin II receptor antagonist) or 10–4 M enalaprilat (angiotensin-converting enzyme inhibitor) decreases the rate of proximal tubule transport by 35–40% (23). The decrease in transport observed with enalaprilat was completely reversed with addition of exogenous 10–11 or 10–8 M luminal angiotensin II (23). These observations are consistent with endogenous angiotensin II stimulating proximal tubule transport in an autocrine or paracrine fashion.

The aim of this study was to examine whether the effect of endogenously produced and luminally secreted angiotensin II on proximal tubule volume reabsorption is regulated by acute changes in extracellular volume. The magnitude of the reduction in proximal tubule transport observed after addition of luminal losartan was used as a measure of the magnitude of the effect of angiotensin II on proximal tubule transport.

METHODS

Preparation of animals. Male Sprague-Dawley rats weighing between 180 and 240 g were used for this study. Rat preparation and the in vivo microperfusion procedure described below have been previously described in detail (16, 17, 23). Briefly, all animals were allowed free access to food and water before anesthesia with intraperitoneal Inactin (100 mg/kg). Rats were placed on a servo-controlled heated table set to maintain body temperature at 37°C. The jugular vein was cannulated for infusion of normal saline at 2.8 ml/h. A flank incision was used to expose the left kidney, which was then immobilized in a Lucite cup. The kidney was bathed with water-equilibrated light mineral oil heated to 37°C that was previously bubbled with 95% O2-5% CO2. The ureter was cannulated with polyethylene tubing to ensure free flow of urine. After microperfusion experiments during the hydropenic period were completed, the rat was volume expanded with normal saline and a normal saline solution containing 5% bovine serum albumin (1, 12). Normal saline was infused at a volume equal to 10% body weight over 1 h (~20 ml/h), followed by 3% body wt/h (~6 ml/h) (1). The 5% bovine serum albumin solution contained 140 mM NaCl and 4 mM KCl and was infused at 1 ml/100 g weight over 45 min (~2 ml/45 min), followed by 0.15 ml·100 g·wt–1·h–1 (~0.3 ml/h) (12). Serial hemocrits were obtained at the time of vessel cannulation (prior to further surgery), 1 h after all surgery had been completed, and 1 h after volume expansion had been completed. Micropuncture surgery has previously been shown to reduce plasma volume and raise serial hemocrits (18, 23). As seen in Table 1, hemocrits and plasma angiotensin II levels rose with volume contraction and fell to presurgical levels following volume expansion (P < 0.001). Conversely, urine flow rate and urinary sodium excretion were low during volume contraction and rose significantly with volume expansion. Using a very similar volume expansion protocol, Braam et al. (5) demonstrated a similar fall in hematocrit and a rise in plasma angiotensin II levels, mean arterial pressure, urinary flow rate, and urinary sodium excretion, as well as fractional excretion of sodium.

In vivo microperfusion. Proximal tubule segments on the surface of the kidney were initially mapped with an injection of a small droplet of oil, and early and late loops were identified. A wax block was inserted into the lumen of an early loop by a hydraulic Microdrive (Trent Wells, Coulterville,
Table 1. Comparison of data in presurgical, volume-contracted, and volume-expanded rats

<table>
<thead>
<tr>
<th></th>
<th>Presurgery</th>
<th>Volume Contraction</th>
<th>Volume Expansion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>96.7 ± 3.0</td>
<td>106.7 ± 2.1P</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>45.1 ± 0.7</td>
<td>51.3 ± 0.8P</td>
<td>43.0 ± 0.7P</td>
</tr>
<tr>
<td>Plasma angiotensin II, pM</td>
<td>115.8 ± 34.5</td>
<td>198.4 ± 57.5P</td>
<td>85.6 ± 23.1P</td>
</tr>
<tr>
<td>Urine flow, μl/min</td>
<td>1.2 ± 0.2</td>
<td>19.7 ± 5.3P</td>
<td>P &lt; 0.0005</td>
</tr>
<tr>
<td>Urine Na excretion, nmol/min</td>
<td>99.6 ± 36.2</td>
<td>2.994.5 ± 998.2P</td>
<td>P &lt; 0.002</td>
</tr>
</tbody>
</table>

P < 0.001, volume contraction vs. presurgery and volume expansion vs. volume contraction; P < 0.05, volume contraction vs. presurgery and volume expansion vs. volume contraction; P > 0.05, compared with volume contraction; P < 0.0005, compared with volume contraction; P < 0.02, compared with volume contraction.

We next examined the effect of luminal angiotensin II on proximal tubule transport during volume contraction and volume expansion. These studies were performed to investigate the effect of losartan, an angiotensin II receptor antagonist, on the proximal tubule volume reabsorptive rate during volume contraction and volume expansion. As seen in Fig. 1, administration of luminal 10⁻⁸ M losartan significantly inhibited the rate of volume reabsorption by the proximal tubule during volume contraction (4.2 ± 0.50 vs. 1.70 ± 0.30 nl·mm⁻¹·min⁻¹, P < 0.05).
reabsorption to a far lesser degree (1.84 ± 0.20 vs. 1.31 ± 0.20 nl·mm⁻¹·min⁻¹, P < 0.05). Time controls performed in the volume-expanded rats demonstrated no change in volume reabsorption rates between the first and second perfusions (2.22 ± 0.78 vs. 2.13 ± 0.81 nl·mm⁻¹·min⁻¹). As seen in Fig. 2, the absolute and percent decrement in proximal tubule transport resulting from the administration of luminal 10⁻⁸ M losartan was significantly greater during volume contraction (58 ± 7.0%) than after volume expansion (29.6 ± 9.0%, P < 0.05).

**Effect of luminal angiotensin II on proximal tubule transport during volume expansion.** These studies were conducted to investigate the effect of 10⁻⁶, 10⁻⁸, and 10⁻¹⁰ M luminal angiotensin II on proximal tubule volume reabsorption in the acutely volume-expanded rat. We and others have previously shown that administration of 10⁻⁶, 10⁻⁸, and 10⁻¹¹ M luminal angiotensin II in the volume-contracted rat had little or no effect on proximal tubule transport (11, 17, 23). As shown in Fig. 3, 10⁻⁸ M luminal angiotensin II increased proximal tubule volume reabsorption in the volume-expanded rat from 2.10 ± 0.34 to 4.38 ± 0.59 nl·mm⁻¹·min⁻¹ (P < 0.005). This rate of proximal tubule volume absorption was comparable to that observed in volume-contracted rats. In contrast, the rates of proximal tubule volume reabsorption observed with 10⁻⁶ and 10⁻¹⁰ M luminal angiotensin II (2.22 ± 0.29 and 1.97 ± 0.20 nl·mm⁻¹·min⁻¹, respectively) were unchanged, compared with that observed in the volume-expanded control (2.10 ± 0.34 nl·mm⁻¹·min⁻¹).

**DISCUSSION**

Angiotensin II has long been known to directly affect proximal tubule transport independent of changes in the glomerular filtration rate (3, 11, 14, 16, 17, 26). Physiological doses of angiotensin II stimulate proximal tubule transport in the absence of changes in glomerular hemodynamics or blood pressure (3, 11, 14, 16, 17). Using in vitro and in vivo microperfusion, physiological doses of peritubular or systemic angiotensin II stimulate proximal tubule transport (15–17).
Systemic administration of the angiotensin II antagonist saralasin in whole animal studies decreased proximal tubule fractional and absolute volume reabsorption (22, 28).

Recently, the proximal tubule has been found to synthesize and luminally secrete angiotensin II at concentrations ranging from 100- to 1,000-fold higher than that in plasma (4, 5, 27). This robust endogenous production of angiotensin II within the proximal tubule is consistent with an autocrine/paracrine role for endogenous angiotensin II to regulate proximal tubule transport independent of systemic angiotensin II. We recently demonstrated that blockade of the production or action of endogenous angiotensin II in vivo microperfused proximal tubules by administration of luminal \(10^{-8}\) M losartan or \(10^{-4}\) M enalaprilat inhibited the proximal tubule volume reabsorptive rate by 35–40% (23).

Our current data is consistent with an autocrine/paracrine role for angiotensin II in the regulation of proximal tubule transport by demonstration that the effect of luminal angiotensin II is regulated by acute changes in extracellular volume. The decrease in proximal tubule transport caused by luminal losartan is a measure of angiotensin II’s contribution to regulation of proximal tubule transport. The proportionately greater decrease in transport observed with luminal losartan during volume contraction (58.0 \(\pm\) 7.0 vs. 29.6 \(\pm\) 9.0%, \(P < 0.05\)) indicates greater stimulation of net proximal tubule transport by luminal angiotensin II levels during acute volume contraction than during acute volume expansion.

The proposed autocrine or paracrine role for angiotensin II in the regulation of proximal tubule transport does not preclude the role of other systemic and peritubular physical factors that also concomitantly affect proximal tubule transport. Alterations in extracellular volume affect the systemic renin-angiotensin system, the permeability properties of the paracellular pathway, peritubular physical forces, renal nerve activity, and systemic catecholamine levels, which were not examined in this study (19). The net change in proximal tubule transport occurring with alterations in extracellular volume likely result from the combined effect of these systemic and peritubular factors, as well as endogenous proximal tubule angiotensin II.

Previous studies have demonstrated little or no effect of exogenous luminal angiotensin II on proximal tubule transport in the hydronephatic rat (16, 23). To examine whether this was the result of elevated endogenous production and luminal levels of angiotensin II during volume contraction, we perfused angiotensin II into the lumen of proximal tubules after acute volume expansion. The twofold increase in volume reabsorption with addition of \(10^{-8}\) M luminal angiotensin II during volume expansion is consistent with endogenous physiological concentrations of angiotensin II having a greater stimulatory effect on volume absorption during volume contraction. Pharmacological or subphysiological concentrations of exogenous luminal angiotensin II (\(10^{-6}\) and \(10^{-10}\) M) had neither an inhibitory nor stimulatory effect on proximal tubule volume reabsorption.

The above data (Figs. 2 and 3) underscore the important and central role played by luminal angiotensin II levels. These data are supported by the results of two other recent studies that have examined the effect of changes in extracellular volume on luminal angiotensin II levels (4, 5). Braam et al. (5) found that, although not statistically significant, the luminal angiotensin II concentration in native tubular fluid fell from 14 nM during volume contraction to 8 nM after acute volume expansion. Conversely, Boer et al. (4) found that proximal tubule angiotensin II rose 3.5-fold when renal perfusion pressure was decreased 20%. This rise in angiotensin II with renal hypoperfusion and potential fall in angiotensin II with volume expansion are consistent with regulation of endogenously produced and luminally secreted angiotensin II by acute changes in extracellular volume. The absence of a statistically significant change in luminal angiotensin II levels induced by acute changes in extracellular volume in both studies might result from the lack of adequate sensitivity and precision in detecting small differences of luminal angiotensin II during different volume states. The small tubular fluid sample volumes along with sample dilution during the assay all contribute to variation in obtaining the final angiotensin II level. Such “background variation or noise” could potentially obscure any differences in luminal angiotensin II levels occurring between different volume states.

Alternatively, the decrease in luminal angiotensin II levels might represent dilution by the greater volume of tubular fluid rather than an actual decrease in production and secretion of angiotensin II. The absence of a statistically significant change in angiotensin II levels induced by acute changes in extracellular volume in both studies might result from the lack of adequate sensitivity and precision in detecting small differences of luminal angiotensin II during different volume states. The small tubular fluid sample volumes along with sample dilution during the assay all contribute to variation in obtaining the final angiotensin II level. Such “background variation or noise” could potentially obscure any differences in luminal angiotensin II levels occurring between different volume states.

Another equally plausible hypothesis to explain regulation of proximal tubule transport by angiotensin II involves modulation of angiotensin II receptor density or modulation of the sensitivity of the signal transduction mechanism for angiotensin II. After changes in extracellular volume, the effect of luminal angiotensin II would “translate” into greater or lesser stimulation of proximal tubule transport via alterations in receptor density or sensitivity of signal transduction. Cheng et al. (8) recently found that angiotensin II upregulates expression of angiotensin II receptors in the proximal...
tubule. Thus the stimulation of proximal tubule transport by angiotensin II could be significantly amplified via upregulation of its own receptor. Our observations regarding heightened stimulation of proximal tubule transport by endogenous angiotensin II during volume contraction may, in part, result from an increase in angiotensin II receptor expression caused by angiotensin II itself. Alternatively, changes in extracellular volume might also alter the affinity of the angiotensin II receptor for angiotensin II. Extracellular volume expansion may decrease the angiotensin II receptor affinity, whereas extracellular volume contraction may increase the angiotensin II receptor affinity. This notion would reconcile the currently observed stimulation of proximal tubule transport with $10^{-8}$ M angiotensin II in volume-expanded but not volume-contracted rats with the previous observation by Harris and Young (11) of stimulation of proximal tubule transport with $10^{-9}$ M angiotensin II in volume-contracted rats (11, 23). Although speculative, these possibilities provide an intriguing alternative to the mechanism of action of endogenous angiotensin II.

We are grateful for the able secretarial assistance of Patricia Wilson and Janelle McQuinn and the technical assistance of Lisa K. Worrall.

This work was supported by a grant from the American Heart Association, Texas Affiliate (to A. Quan), National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-41612 (to M. Baum), and by grants from the National Kidney Foundation of Texas (to A. Quan and M. Baum).

Address for reprint requests: A. Quan, Dept. of Pediatrics, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9063.

Received 22 July 1997; accepted in final form 6 March 1998.

REFERENCES


