Renal nerve stimulation augments effect of intraluminal angiotensin II on proximal tubule transport

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Quan, Albert, and Michel Baum. Renal nerve stimulation augments effect of intraluminal angiotensin II on proximal tubule transport. Am J Physiol Renal Physiol 282: F1043–F1048, 2002. First published December 18, 2001; 10.1152/ajprenal.00279.2001.—The proximal tubule synthesizes and secretes angiotensin II into the lumen, where it regulates transport. Renal denervation abolishes the effect of angiotensin II on proximal tubule transport. Using in vivo microperfusion, we examined whether renal nerve stimulation modulates the effect of angiotensin II on transport. The effect of angiotensin II was assessed by measuring the decrease in volume reabsorption with the addition of 10−4 M luminal enalaprilat. Luminal enalaprilat did not alter volume reabsorption (2.80 ± 0.18 vs. 2.34 ± 0.14 nl·mm−1·min−1). However, with renal nerve stimulation, enalaprilat decreased volume reabsorption (3.45 ± 0.22 vs. 1.67 ± 0.20 nl·mm−1·min−1, P < 0.0005). The absolute and percent decrements in volume reabsorption with luminal enalaprilat were higher with renal nerve stimulation than with native innervation (1.78 ± 0.19 vs. 0.46 ± 0.23 nl·mm−1·min−1, P < 0.02, and 51.8 ± 5.0 vs. 14.6 ± 7.4%, P < 0.05, respectively). Renal nerve stimulation did not alter the glomerular filtration rate or renal blood flow. Renal nerve stimulation augments the stimulatory effect of intraluminal angiotensin II. The sympathetic renal nerves modulate the proximal tubule renin-angiotensin system and thereby regulate proximal tubule transport.

Inhibition of intraluminal angiotensin II production decreases proximal tubule volume reabsorption in hydropenic rats (2, 27, 28). These studies are consistent with the proximal tubule renin-angiotensin system modulating proximal tubule transport independently of circulating angiotensin II.

The effect of angiotensin II on proximal tubule transport is regulated by acute changes in extracellular volume (29). Proximal tubule volume reabsorption is augmented by intraluminal angiotensin II to a greater degree during volume contraction than during volume expansion (29). However, the mechanism by which acute changes in extracellular volume are “translated” into changes in the proximal tubule volume reabsorptive rate, as mediated by intraluminal angiotensin II, is unknown.

We have recently demonstrated that the sympathetic renal nerves modulate the effect of intraluminal angiotensin II on proximal tubule transport. In studies in which the renal nerves were disrupted in vivo, inhibition of proximal tubule angiotensin II production failed to decrease proximal tubule transport in hydropenic animals (30). However, similar inhibition of angiotensin II production in the normally innervated kidney decreases volume transport (27, 30). These studies support a role for renal nerves in modulating the effect of angiotensin II on proximal tubule transport. To further examine whether renal nerves play a role in modulating the proximal tubule renin-angiotensin system, we examined whether renal nerve stimulation modulates the effect of the renin-angiotensin system on proximal tubule transport.

MATERIALS AND METHODS

Preparation of animals. Male Sprague-Dawley rats, 190–250 g, were used for this study. Rat surgical preparation and in vivo microperfusion were performed as described previously (27, 29, 30). After tracheostomy and jugular vein cannulation, 5% bovine serum albumin containing 140 mM NaCl and 4 mM KCl was infused as previously described to maintain extracellular volume (16, 29). This fluid infusion protocol replaces loss of plasma volume incurred during animal surgery, maintains euvolemia, and minimizes native sympathetic renal nerve activity (11, 16, 22). The left kidney was removed to permit bilateral microperfusion. The sympathetic renal nerves innervate the proximal tubule and participate in the regulation of proximal tubule sodium reabsorption (1, 9, 11, 21, 24). Renal nerve stimulation augments proximal tubule transport, whereas renal denervation decreases proximal tubule transport (3–5, 9, 12, 13). The renal nerves directly modulate proximal tubule transport independently of changes in the glomerular filtration rate (GFR), renal blood flow, and peritubular Starling forces (3–5, 9, 12, 13).

Proximal tubule transport is also regulated by the renin-angiotensin system (8, 14, 28). The proximal tubule contains an autonomous renin-angiotensin system in which high levels of angiotensin II are synthesized and luminally secreted (7, 17, 23, 25, 31–33).
exposed with a flank incision, immobilized in a Lucite cup, and bathed with 37°C water-equilibrated mineral oil that had been previously bubbled with 95% O2-5% CO2. The ureter was cannulated with polyethylene tubing (PE-50) to ensure free flow of urine.

In vivo microperfusion. Proximal tubule segments were identified, and a wax block was inserted into the lumen of an early proximal tubule loop with a hydraulic Microdrive (Trent Wells, Coulterville, CA). The wax block prevented native glomerular ultrafiltrate from flowing into the tubule segments distal to the block. Subsequently, a microperfusion pipette was inserted into the lumen immediately distal to the wax block, and an ultrafiltrate-like solution was perfused at 30 nl/min by using a microperfusion pump system (K. Effenberger; Vestavia Scientific, Birmingham, AL). Tubules were perfused with an ultrafiltrate-like solution containing exhaustively dialyzed [methoxy-3H]inulin, as previously described (27, 29, 30). A collection pipette was inserted into the lumen immediately distal to the perfusion pipette, and fluid was collected after an oil block was placed distally. Fluid collections were made over a 2- to 3-min period, and the volume was measured by using a constant-bore pipette. Only perfused tubules with an inulin recovery rate of between 90 and 110% were included.

To examine the effect of \(10^{-4}\) M luminal enalaprilat (angiotensin-converting enzyme inhibitor; Merck, West Point, PA) on proximal tubule transport, each proximal tubule was sequentially perfused twice, with the control ultrafiltrate-like solution and then by a second perfusion with the same ultrafiltrate-like solution containing \(10^{-4}\) M enalaprilat. The initial and subsequent tubule perfusions and fluid collections were made from the same tubule puncture sites, thus allowing paired study data to be obtained. We have previously demonstrated that such “double microperfusion” of the same proximal tubule does not affect the volume reabsorptive rate (29). The difference in volume reabsorptive rates between these two perfusions represents the effect of intraluminal angiotensin II on transport. This double microperfusion technique was performed in rats with normal baseline innervation and with external renal nerve stimulation.

After all collections were performed, the entire tubule was injected with liquid microfil (Flow Tech, Carver, MA), which was allowed to harden, and later placed in 6 N HCl at 37°C for 1 h. The microfil tubule casts were then dissected and photographed, and the tubular lengths between the perfusion and collection sites were measured.

The volume of collected tubular fluid was measured with constant-bore capillary tubing and a micrometer (Mitutoyo, City of Industry, CA). The rate of volume reabsorption was calculated as the difference between perfused and collected volumes divided by the time of collection divided by the tubule length (in mm). One tubule per rat was used for micropuncture. Thus \(n\) represents the number of tubules and rats.

Renal nerve stimulation. The renal nerve plexus near the aortocaval ganglia was identified under a dissecting microscope (surgical microscope model 903093, Leitz), as described previously (5, 20). These nerve fibers were carefully dissected free of surrounding tissues and placed on bipolar hooked electrodes made from 0.008-in.-diameter insulated platinum-iridium wire. The bipolar hooked electrodes were connected to a square-wave electrical nerve stimulator (model S44G, Grass Instrument, Quincy, MA) through a stimulus isolation unit (model SIU5, Grass Instrument). The electrical nerve stimulator delivers a square-wave electrical pulse of a preset frequency (Hz) and voltage (V) for 0.5 ms. To show that the proper renal nerve fibers were hooked to the electrodes, the nerves were stimulated for 5–7 s with a stimulation of 10 Hz and 10 V for 0.5 ms, which caused temporary blanching of the surface of the kidney. The renal nerve was then secured to the electrodes with biocompatible polymerizing vinyl silicone gel (Bisico S4i, Munich, Germany), and the electrical stimulation unit was set to deliver electrical pulses at 1 Hz and 5 V for 0.5 ms. The nerve fibers proximal to the nerve-electrode connection were crushed and cut. These electrical stimulation parameters have previously been shown not to alter glomerular hemodynamics, renal blood flow, or GFR (5, 20). Renal nerve stimulation was carried out continuously throughout the experiment. In vivo microperfusion was started 45 min after initiation of renal nerve stimulation.

To ensure that renal nerve stimulation (1 Hz and 5 V for 0.5 ms) did not alter glomerular hemodynamics, renal blood flow and GFR were measured. After the renal nerve fibers were placed on the hooked platinum-iridium electrodes as described immediately above, a 2-mm Silastic cuff fitted with a Doppler crystal flow probe (Crystal Biotech, Holliston, MA) was placed around the renal pedicle such that the crystal was opposite the renal artery. The Doppler crystal flow probe was connected to a VF-1 pulsed Doppler flow system (Crystal Biotech), which was connected to a standard Linseis chart recorder. Doppler wave signals from the crystal probe that indicated renal blood flow were measured in kilohertz and converted into a waveform to assess blood flow velocity (33). Renal blood flow (kHz) was then measured during renal nerve stimulation at 10 Hz and 10 V for 0.5 ms, which caused the surface of the kidney to blanch, and at 1 Hz and 5 V for 0.5 ms (Fig. 1 and Table 1).

Measurement of GFR. Sprague-Dawley rats were allowed free access to food and water until the beginning of the study. Rats were anesthetized with intraperitoneal Inactin and placed on a servo-controlled heated table that was set to maintain a constant body temperature of 37°C, as described in Preparation of animals. A tracheostomy was performed, and catheters were placed in the internal jugular vein and femoral artery. Intravenous fluids and the total infusion rate were given as described in Preparation of animals. [Methoxy-3H]inulin clearances were used to determine the GFR, as described previously (10, 26). Briefly, a prime of 6 µCi of [methoxy-3H]inulin was infused followed by a maintenance

Fig. 1. Renal arterial blood flow as measured by a Doppler crystal (in kHz) during renal nerve stimulation (RNS; see Table 1). With electrical stimulation at 10 Hz and 10 V for 0.5 ms, the kidney surface blanched and renal blood flow was reduced. With electrical stimulation at 1 Hz and 5 V for 0.5 ms, renal blood flow was unchanged from baseline.
Table 1. Comparison of glomerular filtration rate and renal blood flow during renal nerve stimulation

<table>
<thead>
<tr>
<th>Renal Nerve Stimulation</th>
<th>GFR, ml·min⁻¹·g⁻¹ kidney⁻¹</th>
<th>RBF, kHz</th>
</tr>
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<tr>
<td>Baseline 10 Hz, 10 V, 5 ms</td>
<td>0.90 ± 0.07</td>
<td>1.65 ± 0.15</td>
</tr>
<tr>
<td>1 Hz, 5 V, 0.5 ms</td>
<td>1.0 ± 0.07</td>
<td>0.95 ± 0.10⁶</td>
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GFR, glomerular filtration rate; RBF, renal blood flow. There was no change in GFR between baseline and renal nerve stimulation at 1 Hz and 5 V for 0.5 ms. *P < 0.05 vs. baseline and renal nerve stimulation at 1 Hz and 5 V for 0.5 ms.

infusion of 16 μCi of inulin/h. After a 1-h equilibration period, three to four 30-min urine and midpoint blood samples were collected for analysis of [methoxy-³H]inulin by liquid scintillation counting. The GFR was calculated by using the following formula:

\[ \text{GFR} = \frac{UV}{P} \]

where \( U \) is the measured radioactivity of the [methoxy-³H]inulin in the urine, \( V \) is the urine volume in milliliters divided by the 30-min collection period, and \( P \) is the measured radioactivity of the [methoxy-³H]inulin in the serum obtained from the blood collection. The final GFR was divided by the kidney weight (ml·min⁻¹·g⁻¹; Table 1).

Statistical analyses. Analysis of variance and Student’s t-tests (paired and unpaired) were used to determine statistical significance. The post hoc test used after analysis of variance included the Tukey-Kramer multiple comparisons tests. All data are expressed as means ± SE. Statistically significant data were determined with a \( P \) value of < 0.05.

RESULTS

Renal nerve stimulation and renal blood flow. Renal blood flow, measured in kilohertz with a Doppler crystal flow probe, is shown in Fig. 1 (a sample) and Table 1. Renal nerve stimulation at 10 Hz and 10 V for 0.5 ms caused the surface of the kidney to blanch and reduced the renal blood flow below baseline (1.65 ± 0.15 vs. 0.95 ± 0.10 kHz, \( P < 0.05 \)). This reduction in renal blood flow ensures that the appropriate renal nerve fibers have been hooked to the electrodes and are capable of electrical stimulation. When the renal nerve was stimulated at 1 Hz and 5 V for 0.5 ms, renal blood flow was unchanged from baseline (1.58 ± 0.16 vs. 1.65 ± 0.15 kHz). These last renal nerve stimulation parameters (1 Hz and 5 V for 0.5 ms) were used for the remainder of the studies discussed herein. Thus renal blood flow was unchanged from control in all renal nerve stimulation studies.

Renal nerve stimulation and GFR. The GFRs of control and renal nerve stimulated kidneys are shown in Table 1. As seen, the GFR did not change with renal nerve stimulation at 1 Hz and 5 V for 0.5 ms. Thus, in all renal nerve stimulation studies, both GFR and renal blood flow were unchanged.

Effect of luminal enalaprilat on proximal tubule volume reabsorption during renal nerve stimulation. The effect of renal nerve stimulation on the regulation of proximal tubule transport by intraluminal angiotensin II is summarized in Fig. 2. In these studies, the regulation of transport by intraluminal angiotensin II was measured by sequentially perfusing proximal tubules in vivo in a "paired" fashion, first with a control ultrafiltrate-like solution and then by reperfusion of the same tubule with a ultrafiltrate-like solution with \( 10^{-4} \) M enalaprilat. The observed difference in volume reabsorptive rates represents the effect of intraluminal angiotensin II on transport. These studies were performed under euvolemic conditions to minimize the native renal nerve activity (11). Under euvolemic conditions with no external renal nerve stimulation, luminal administration of \( 10^{-4} \) M enalaprilat resulted in no decrease in proximal tubule volume reabsorption (2.80 ± 0.18 vs. 2.34 ± 0.14 nl·mm⁻¹·min⁻¹). However, with renal nerve stimulation, administration of luminal \( 10^{-4} \) M enalaprilat resulted in a large and significant decrease in proximal tubule volume reabsorption (3.45 ± 0.22 vs. 1.67 ± 0.20 nl·mm⁻¹·min⁻¹, \( P < 0.0005 \)). Thus inhibition of intraluminally produced angiotensin II in the proximal tubule resulted in a greater decrement of volume reabsorption when the renal nerves were stimulated. As demonstrated in Fig. 2, renal nerve stimulation increases the proximal tubule volume reabsorptive rate, which is consistent with its stimulatory role in proximal tubule transport (3.45 ± 0.22 vs. 2.80 ± 0.18 nl·mm⁻¹·min⁻¹, \( P < 0.05 \)) (5, 13).

The absolute and percent decrements in the proximal tubule volume reabsorptive rate observed on administration of luminal \( 10^{-4} \) M enalaprilat with renal nerve stimulation are shown in Fig. 3, A and B, respectively. As can be seen, the absolute decrement in prox-
imal tubule volume reabsorption resulting from administration of luminal enalaprilat was significantly higher after renal nerve stimulation compared with control in euvolemic animals ($1.78 \pm 0.19$ vs. $0.46 \pm 0.23$ nl mm$^{-1}$ min$^{-1}$, $P < 0.02$). Because the proximal tubule volume reabsorptive rates were higher after renal nerve stimulation, comparison of the percent decrement in transport may more accurately reflect the proportional decrease in transport observed with luminal $10^{-4}$ M enalaprilat. As seen in Fig. 3B, the percent decrement in proximal tubule volume reabsorption resulting from luminal $10^{-4}$ M enalaprilat after renal nerve stimulation was more than threefold higher after renal nerve stimulation ($51.8 \pm 5.0$ vs. $14.5 \pm 7.5\%$, $P < 0.01$).

DISCUSSION

Proximal tubule transport is modulated by both the renin-angiotensin system and the sympathetic renal nerves (1, 2, 8, 11, 12, 21, 24, 27, 28). In addition to systemically circulating angiotensin II, the proximal tubule contains an autonomous renin-angiotensin system that synthesizes and secretes high levels of angiotensin II into the lumen (7, 17, 23, 25, 31–33). We have previously examined the role of the proximal tubule renin-angiotensin system by inhibiting its "local" action or intraluminal production using in vivo microperfusion. Our studies have demonstrated that $10^{-8}$, $10^{-10}$, and $10^{-11}$ M luminal angiotensin II had no effect on proximal tubule volume reabsorption in hydropenic animals (27). The absence of an effect of exogenous luminal angiotensin II on volume reabsorption is consistent with existing high levels of luminal angiotensin II (6, 7, 32). With the use of in vivo microperfusion in the rat, luminal administration of losartan (AT$_1$ angiotensin II receptor antagonist) to inhibit the action of intraluminal angiotensin II resulted in a reduction in proximal tubule volume reabsorption of 35% in hydropenic animals (27). A similar reduction in volume reabsorption (40%) was noted with luminal administration of $10^{-4}$ M enalaprilat, an angiotensin-converting enzyme inhibitor (27). Subsequent addition of $10^{-8}$ M angiotensin II to the lumen of proximal tubules containing $10^{-4}$ M enalaprilat resulted in an increase in proximal tubule volume reabsorption rates to control rates (27). These results show that intraluminally synthesized and secreted angiotensin II modulates proximal tubule volume reabsorption independently of systemically circulating angiotensin II. These results were confirmed in studies using rabbit proximal tubules perfused in vitro (2).

The effect of intraluminal angiotensin II on proximal tubule transport is modulated by acute changes in extracellular volume. We have previously demonstrated that intraluminal angiotensin II regulates proximal tubule transport to a greater degree under extracellular volume contraction than under volume expansion (29). These studies are consistent with increased production or action of intraluminal angiotensin II during volume contraction, which augments proximal tubule transport to a greater degree than during volume expansion. However, the mechanism by which acute changes in the extracellular volume result in changes in the proximal tubule renin-angiotensin system is unknown. We hypothesized that the sympathetic renal nerve may help to mediate alterations in the effect of intraluminally produced angiotensin II on proximal tubule transport resulting from changes in the extracellular volume.
The sympathetic renal nerves richly innervate the proximal tubule and have been shown to regulate proximal tubule transport (1, 3–5, 11, 12, 24). Renal nerve stimulation increases proximal tubule transport, whereas renal denervation decreases transport (3–5, 11, 12). We have recently examined whether the sympathetic renal nerve can modulate the effect of intraluminal angiotensin II on transport. With the use of in vivo microperfusion in the denervated kidney, administration of luminal \(10^{-4}\) M enalaprilat to inhibit intraluminal angiotensin II production failed to decrease the volume reabsorptive rate in hydropenic rats (30). In contrast, a 35–40% decrease in volume reabsorption with luminal \(10^{-4}\) M enalaprilat was observed in normally innervated proximal tubules in hydropenic rats (27). The absence of an effect of luminal enalaprilat in the denervated proximal tubule is consistent with the diminished role for intraluminal angiotensin II in the regulation of transport after renal denervation. Addition of \(10^{-8}\) M angiotensin II to the lumen of denervated proximal tubules raised the volume reabsorptive rate to levels observed in innervated tubules (30). In contrast, addition of \(10^{-8}\) M angiotensin II to the lumen of innervated proximal tubules had no effect on volume reabsorption in control rats (27). These data support a role for the sympathetic renal nerves in modulating intraluminal angiotensin II production and thereby regulating proximal tubule transport.

The present findings in this study support a role for the sympathetic renal nerves in modulating the proximal tubule renin-angiotensin system. Here, we studied euolemic animals to minimize the effect of native sympathetic renal nerve activity on the proximal tubule renin-angiotensin system under control conditions (11, 12, 29). The large decrease in proximal tubule volume reabsorption observed with inhibition of intraluminal angiotensin II production when the renal nerves are stimulated is consistent with a greater role for intraluminal angiotensin II in transport during conditions with heightened renal nerve activity. The mechanism by which this occurs is presently unknown. A larger effect of angiotensin II on proximal tubule transport may result from an increase in its production and/or its luminal secretion or a modulation in the effect of angiotensin II on transport. Such a modulation in the effect of angiotensin II may involve an increase in angiotensin II receptor density, or binding affinity, or a change in the sensitivity of the signal transduction mechanism. The present study, however, does not distinguish between these alternative mechanisms. Although extracellular volume expansion is associated with a decrease in the proximal tubule volume reabsorptive rate, a recent micropuncture study demonstrated that volume expansion did not alter luminal angiotensin II levels (6, 29). Thus modulation in the effect of angiotensin II on transport may involve mechanisms other than alteration in its production or luminal secretion, as described immediately above.

Because renal nerve activity is heightened during volume contraction and reduced during volume expansion (11, 12), the present studies and our findings with renal denervation (30) support the hypothesis that modulation of the effect of intraluminal angiotensin II on proximal tubule transport may be mediated by sympathetic renal nerves. Thus sympathetic renal nerves may “translate” changes in extracellular volume into changes in proximal tubule transport, in part, through modulation of the production and/or luminal secretion of angiotensin II. Given the rapidity with which the nervous system can act, the sympathetic renal nerve is a prime candidate in mediating the acute changes in extracellular volume into a rapid and compensatory change in proximal tubule transport.

Regulation of proximal tubule transport by the sympathetic renal nerve may also involve interaction with endocrine or autocrine/paracrine systems other than the proximal tubule renin-angiotensin system. For example, the stimulatory effect of the renal nerves on renal sodium reabsorption requires production of circulating angiotensin II by the systemic renin-angiotensin system (14, 18, 19). The rise in sodium reabsorption accompanying renal nerve stimulation occurs only in the presence of physiological nonpressor levels of circulating angiotensin II (18). Whether circulating angiotensin II levels are lowered with saline loading or captopril, the rise in sodium reabsorption with renal nerve stimulation is markedly blunted (18). Circulating angiotensin II may facilitate adrenergic transmission at the renal nerve-renal epithelial cell junction (14, 18, 19).

In summary, we have demonstrated that inhibition of intraluminal production of angiotensin II in the proximal tubule with renal nerve stimulation decreases volume reabsorption to a greater degree than under control conditions in euolemic rats. From these results, we conclude that renal nerve stimulation augments the effect of intraluminal angiotensin II on proximal tubule volume reabsorption. The present study adds to the results from our previous study, wherein we demonstrated that renal denervation abolished the effect of intraluminal angiotensin II on proximal tubule volume reabsorption (30). Taken together, these results suggest that the renal nerve may mediate the compensatory changes in proximal tubule transport resulting from changes in extracellular volume.

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REFERENCES
F1048 RENAL NERVE AUGMENTS ANGIOTENSIN II-MEDIATED PT TRANSPORT


