Endogenous Production of Angiotensin II Modulates Rat Proximal Tubule Transport

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Abstract

There is evidence that angiotensin II is synthesized by the proximal tubule and secreted into the tubular lumen. This study examined the functional significance of endogenously produced angiotensin II on proximal tubule transport in male Sprague-Dawley rats. Addition of 10^{-11}, 10^{-8}, and 10^{-6} M angiotensin II to the lumen of proximal convoluted tubules perfused in vivo had no effect on the rate of fluid reabsorption. The absence of an effect of exogenous luminal angiotensin II could be due to its endogenous production and luminal secretion. Luminal 10^{-8} M Dup 753 (an angiotensin II receptor antagonist) resulted in a 35% decrease in proximal tubule fluid reabsorption when compared to control (J_v = 1.64±0.12 nl/mm · min vs. 2.55±0.32 nl/mm · min, $P < 0.05$). Similarly, luminal 10^{-4} M enalaprilat, an angiotensin converting enzyme inhibitor, decreased fluid reabsorption by 40% (J_v = 1.53±0.23 nl/mm · min vs. 2.55± 0.32 nl/mm · min, $P < 0.05$). When 10^{-11} or 10^{-8} M exogenous angiotensin II was added to enalaprilat (10^{-4} M) in the luminal perfusate, fluid reabsorption returned to its baseline rate (J_v = 2.78±0.35 nl/mm · min). Thus, addition of exogenous angiotensin II stimulates proximal tubule transport when endogenous production is inhibited. These experiments show that endogenously produced angiotensin II modulates fluid transport in the proximal tubule independent of systemic angiotensin II. (*J. Clin. Invest.* 1996. 97: 2878–2882.) Key words: kidney • renin • autocrine • enalaprilat • microperfusion

Introduction

Recent findings support an autocrine/paracrine role for angiotensin II within the proximal tubule. All the components of the renin–angiotensin system exist within the proximal tubule (1–10). Angiotensinogen and its mRNA are present in the proximal tubule (1, 2) and renin activity along with renin mRNA are present in lysates of proximal tubule cells in primary culture (8). Angiotensin converting enzyme activity has been localized on the luminal brush border membrane (3–7) and receptors for angiotensin II have been found on both the basolateral and luminal membranes (11–13). Angiotensin II has been measured in both endogenous luminal fluid and in the lumen of proximal convoluted tubules perfused with an artificial tubular fluid at concentrations 100–200-fold higher than in plasma (9, 10). These results indicate that angiotensin II is synthesized and secreted into the lumen of the proximal tubule. Despite the evidence for endogenous intraluminal secretion of angiotensin II, proof of proximal tubule transport regulation by such endogenous angiotensin II has previously been elusive. The purpose of this study is to investigate the role of endogenous angiotensin II in modulating proximal tubule transport.

Methods

Preparation of animals. Male Sprague-Dawley rats weighing between 180 and 230 grams were used for this study. Rat preparation and the microperfusion procedure described below has been previously described (14, 15). 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 mineral oil heated to 37°C that was previously bubbled with 95%O_2/5%CO_2. The ureter was cannulated with polyethylene tubing to ensure free flow of urine.[1]

In vivo microperfusion. Proximal tubule segments on the surface of the kidney were initially mapped with injection of a small droplet of oil and early and late loops identified. A wax block was inserted into the lumen of an early loop by a hydraulic Microdrive (Trent Wells, Coulterville, CA) which prevented any 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 perfused at 30 nl/min. Perfusion was accomplished by using a microperfusion pump system (K. Effengerger, Vestavia Scientific, Birmingham, AL). In a late proximal tubule loop distal to the perfusion pipette, a collection pipette was inserted and the perfused ultrafiltrate-like solution collected after an oil block was placed distally. Fluid collections were made over 2–3 min. The length of the tubule between the perfusion and collection sites was estimated to be 1.5–3.0

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Received for publication 4 January 1996 and accepted in revised form 4 April 1996.

1. Serial hematocrits were performed at the time of femoral artery catheterization (0 time), 2 and 4 h later (International Microcapillary Centrifuge, Needham Heights, MA). Micropuncture surgery has previously been shown to reduce plasma volume and raise serial hematocrits (29). In our rats, the initial hematocrit (0 time) was 45.7±1.5% and rose to 49.6±2.0% at 2 h and 51±3.0% at 4 h ($P < 0.001$).

J. Clin. Invest.

0021-9738/96/06/2878/05 $2.00
Volume 97, Number 12, June 1996, 2878–2882

mm. The composition of the ultrafiltrate-like solution was (in mM): NaCl, 120; $NaHCO_3$, 25; KCl, 5; $MgSO_4$, 1; $CaCl_2$, 1.8; Na_2PO_4, 1; glucose, 5; alanine, 5; urea, 5; 0.1% and FD&C green dye No. 3. Exhaustively dialyzed methoxy-^{3}H-inulin was added as a volume marker. After all collections were performed, the entire tubule was injected with liquid microfil (Flow-Tek, Boulder, CO) and allowed to harden overnight. The kidney was later placed in 6N HCl at 37°C for 1 h. The microfil tubule casts were then dissected, photographed and the tubular length between the perfusion and collection sites measured. Microfil dissection and calculation of the rate of volume absorption (J_v) was performed without knowledge of the specific experimental protocol (see below).

First, the effect of luminal angiotensin II (Asn^1, Val^5 AII; Sigma Chemical Co., St. Louis, MO) at 10^{-11}, 10^{-8}, and 10^{-6} M on the rate of proximal convoluted tubule volume absorption was examined. Next, we examined the effect of luminal 10^{-8} M Dup 753 (Dupont, Wilmington, DE), an angiotensin II receptor antagonist, on the rate of proximal tubule volume absorption (16–18). Tubules were randomly perfused with either Dup753 or control. We also examined the effect of 10^{-4} M enalaprilat on the rate of proximal tubule volume absorption. Enalaprilat (Merck & Co., West Point, PA), an angiotensin converting enzyme inhibitor, prevents the production of angiotensin II from angiotensin I (19). Similarly, tubules were randomly perfused with either enalaprilat or control to avoid bias. Finally, to examine the effect of exogenous angiotensin II without its endogenous production, angiotensin II (10^{-11} and 10^{-8} M) in conjunction with enalaprilat (10^{-4} M) were added to the luminal perfusion solution and the rate of proximal convoluted tubule volume absorption was measured. To avoid bias, tubules were randomly perfused with either enalaprilat or enalaprilat plus angiotensin II. The ultrafiltrate-like solution was perfused alone in control experiments.

Analysis. All collected tubular fluid was transferred to constant-bore glass tubing for measurement of volume, and then mixed with scintillation fluid for radioactivity counting. The rate of fluid reabsorption was calculated as the difference between perfused and collected volumes divided by the time of collection divided by the tubule length. The average tubule length was 2.0±0.1 mm. Analysis of variance and Student's *t* test were used to determine statistical significance. All data are expressed as the mean±SEM.

Radioimmunoassay. To verify the concentration of angiotensin II in the angiotensin II (10^{-8}, 10^{-11} M) containing ultrafiltrate-like solution, a radioimmunoassay was performed (Peninsula Laboratories, Belmont, CA) with tubular fluid from a perfusion micropipette. Angiotensin II at 10^{-8} M and 10^{-11} M was assayed at $1.2\pm0.18 \times 10^{-8}$ and $2.5\pm1.9 \times 10^{-11}$ M, respectively. Thus, angiotensin II in the ultrafiltrate-like solution is delivered to the perfused tubules at the expected concentrations.

High pressure liquid chromatography. To verify the absence of degradation of angiotensin II in the ultrafiltrate-like solution, HPLC of the angiotensin II containing solution was performed (Dionex, Sunnyvale, CA) on a C_{18} microbore (5 μm) reverse phase Vydac column (150 × 1mm) (Separations Group, Hesperia, CA). A gradient was run over 20 min beginning with 100% 0.02M K_2HPO_4: ACN (80:20) at 0.03 μl/min and ending with 100% 0.5 M K_2HPO_4: ACN (80:20) at 0.05 μl/min. Under these conditions, angiotensin II retention time was 15 min, while angiotensin III, a degradation product, had a retention time of 22 min. HPLC of the angiotensin II containing ultrafiltrate-like solution from the perfusion micropipette resulted in a single peak whose retention time was identical to that for angiotensin II. No angiotensin III nor any other peak was noted. Thus, angiotensin II is not degraded in the ultrafiltrate-like solution perfused into tubules.

Results

The first experiments were performed to investigate the effect of exogenous addition of luminal angiotensin II on fluid absorption by the proximal tubule. As seen in Fig. 1, perfusion with a solution containing exogenous angiotensin II at 10^{-11}, 10^{-8} M, and 10^{-6} M concentrations had no significant effect on the rate of fluid transport. In these and all other experiments, the proximal wax block prevents endogenous glomerular ultrafiltrate from entering the tubule segment perfused. These data indicate that addition of exogenous luminal angiotensin II at a wide range of concentrations has no effect on proximal tubule transport.

The second experiments were performed to investigate the effect of Dup 753, an angiotensin II receptor antagonist, on proximal convoluted tubule volume absorption (16–18). As shown in Fig. 2, luminal 10^{-8} M Dup 753 inhibited the rate of fluid absorption rate by 35% (1.64±0.12 nl/min · mm vs.

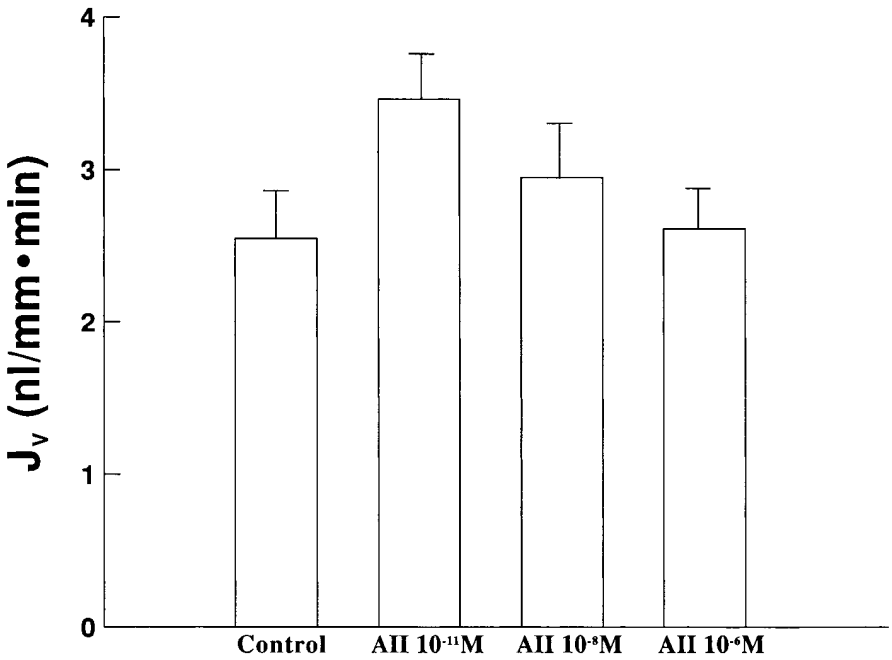

Figure 1. Proximal tubule fluid reabsorption rates J_v (nl/min · mm) with addition of angiotensin II to the luminal perfusate. There was no significant effect of addition of luminal angiotensin II on the rate of fluid reabsorption.

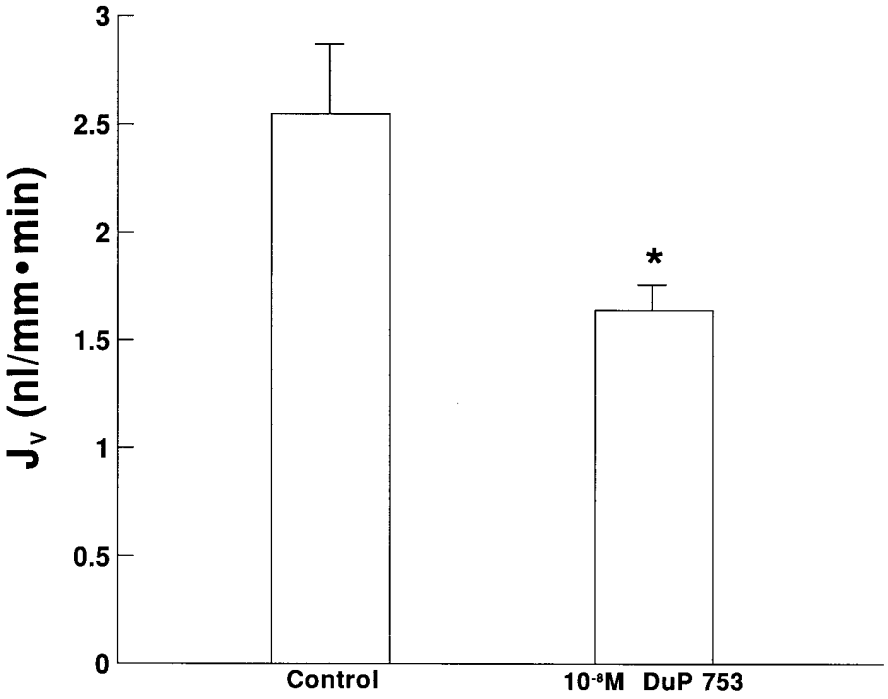

Figure 2. Effect of 10^{-8} M DuP 753, an angiotensin II-receptor antagonist, on the rate of proximal tubule transport in the absence of exogenous angiotensin II. DuP 753 inhibited the rate of proximal tubule transport in the absence of exogenous angiotensin II, consistent with endogenous production of angiotensin II affecting the rate of proximal tubule transport.; $*P < 0.05$ vs. control.

2.55±0.32 nl/min · mm, $P < 0.05$). Next, to examine whether production of endogenous angiotensin II affects fluid reabsorption, 10^{-4} M enalaprilat was added to the perfusion solution. Enalaprilat blocks angiotensin converting enzyme and prevents the formation of angiotensin II from its precursor, angiotensin I (19). As seen in Fig. 3, the fluid absorption rate likewise fell by 40% (1.53±0.23 nl/min · mm vs. 2.55±0.32 nl/min · mm, $P < 0.05$). Thus, both inhibition of the action and production of endogenous angiotensin II results in a similar decrease in the rate of proximal tubule transport. These data are consistent with endogenously produced angiotensin II augmenting proximal tubule transport.

The final experiments were performed to examine whether angiotensin II, without its endogenous production, augments proximal tubule transport. Proximal tubules were perfused with a solution containing both enalaprilat and angiotensin II. As seen in Fig. 3, addition of angiotensin II at 10^{-11} and 10^{-8} M completely reversed the inhibition by 10^{-4} M enalaprilat and restored the rate of fluid absorption to control levels (1.53±0.23 nl/min · mm vs. 2.78±0.35 nl/min · mm, $P < 0.05$).

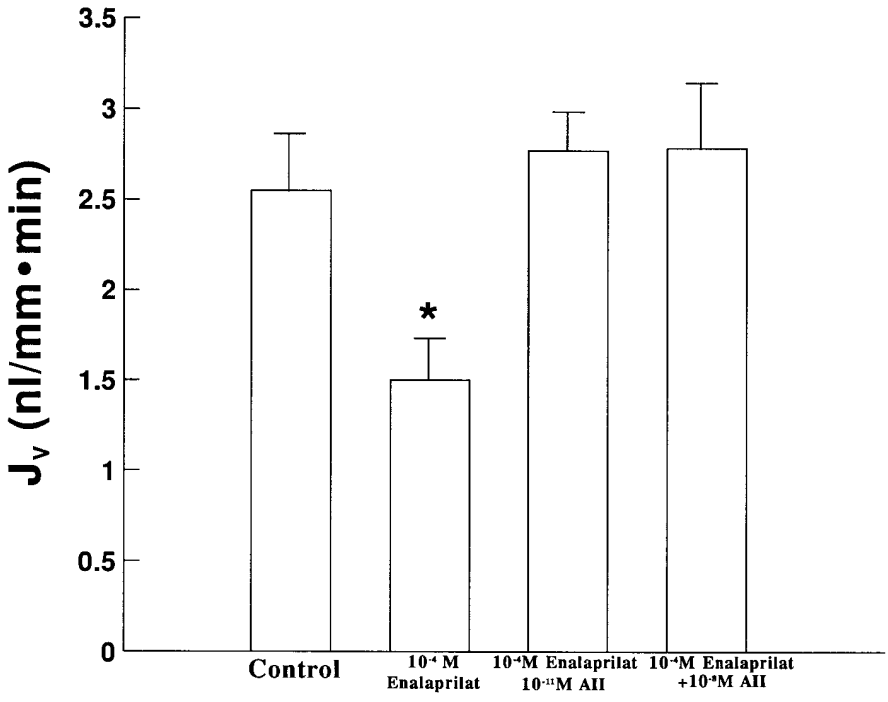

Figure 3. Comparison of the rate of proximal tubule transport in control, those perfused with 10^{-4} M enalaprilat, and those with 10^{-4} M enalaprilat plus angiotensin II at 10^{-11} and 10^{-8} M. Enalaprilat, a converting enzyme inhibitor, reduced the rate of fluid reabsorption ($P < 0.05$). Luminal addition of angiotensin II restored fluid reabsorption rates to control levels in the presence of enalaprilat. $*P < 0.05$ vs. other groups.

These results indicate that angiotensin II is responsible for augmenting transport in the proximal convoluted tubule.

Discussion

This study demonstrates that endogenously produced angiotensin II substantially modulates proximal tubule fluid absorption. These data show that the intrarenal renin–angiotensin system described above can act in concert to affect proximal tubule transport in an autocrine/paracrine fashion.

Earlier work by Liu and Cogan demonstrated a profound effect of systemic angiotensin II on proximal tubule transport (14, 15, 20). In their studies, subpressor doses of systemic angiotensin II increased fluid absorption 50% over control in the first millimeter of the proximal tubule and by 37% over control, in the subsequent second through fifth millimeter. These experiments were performed in Munich-Wistar rats, whose surface glomeruli allow the entire length of the proximal tubule accessible for micropuncture. Furthermore, when angiotensin II was perfused into the lumen, the effect on transport of bicarbonate was small and less than the effect of systemic angiotensin II (14, 20). They concluded that systemic angiotensin II exerted powerful control over proximal tubule transport. In our study we found that inhibition of endogenous angiotensin II action or production inhibited volume absorption by the proximal convoluted tubule. These results are consistent with an autocrine/paracrine role for angiotensin II in regulating proximal tubule transport in addition to the systemic effect of angiotensin II.

In support of autocrine/paracrine control of transport, previous work by Seikaly, et al., found that the concentration of angiotensin II in the lumen of the proximal tubule was 10^{-8} M, nearly 1,000-fold higher than in plasma (9). Furthermore, the concentration of angiotensin II along the entire length of the proximal tubule was constant. Since the proximal tubule has angiotensinases (21), the rate of angiotensin II production must equal the rate of its degradation. Braam et al., confirmed the findings of Seikaly et al., and further demonstrated, using in vivo microperfusion, that angiotensin II was secreted into artificial luminal perfusion solution originally free of angiotensin II (10). Thus, angiotensin II was synthesized by the proximal tubule and secreted into the lumen. More recently, angiotensin I as well as angiotensin II has been identified in the lumen of the proximal tubule (22). The findings of Braam, et al. and Seikaly et al., along with our findings, indicate control of proximal tubule transport by endogenously generated luminal angiotensin II.

Previous in vivo rat microperfusion studies on the effect of adding luminal angiotensin II have found either a small increase or no change in transport in the proximal tubule (14, 20, 23). As seen in Fig. 1, we also found that the addition of exogenous angiotensin II to the perfusion solution did not significantly affect fluid reabsorption. Our data are consistent with the absence of an effect of exogenous angiotensin II because of ongoing stimulation of proximal tubule transport in the presence of endogenously produced angiotensin II. However, in isolated perfused rabbit proximal convoluted tubules, addition of 10^{-11} M angiotensin II produced a twofold increase in the rate of volume absorption which was a greater stimulation than that produced by peritubular angiotensin II (24).

It is interesting that the decrement in fluid reabsorption seen with perfusion of Dup 753 or enalaprilat were both nearly identical. Previous investigators have suggested that other proteolytic enzymes not inhibitable by enalaprilat, i.e., cathepsin or kallikrein, could also convert angiotensin I into angiotensin II (25–28). The similarity between Dup 753 and enalaprilat suggest that nearly all angiotensin II formed in the proximal tubule may be derived from angiotensin I via angiotensin converting enzyme.

In conclusion, we have demonstrated that blockade of angiotensin II receptor binding by Dup 753 or inhibition of angiotensin II formation with enalaprilat both decrease the rate of proximal tubule transport. The decrease in transport seen with enalaprilat is completely reversed by addition of exogenous angiotensin II to the lumen. These data show a functional role for luminal angiotensin II in modulating proximal tubule transport and support the existence of an intrarenal renin–angiotensin system.

Acknowledgments

This work is dedicated to the memory of Martin G. Cogan, a good friend to both authors, who made important contributions to our understanding of proximal tubule transport.

This work was supported by grants from the American Heart Association, Texas Affiliate (Albert Quan) and National Institutes of Health grant DK-41612 (Michel Baum).

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