Angiotensin II: A Potent Regulator of Acidification in the Rat Early Proximal Convoluted Tubule

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Abstract

The early proximal convoluted tubule (PCT) is the site of 50% of bicarbonate reabsorption in the nephron, but its control by angiotensin II has not been previously studied. In vivo microperfusion was used in both the early and late PCT in Munich-Wistar rats. Systemic angiotensin II administration (20 ng/kg · min) or inhibition of endogenous angiotensin II activity with saralasin (1 μg/kg · min) caused profound changes in bicarbonate absorption in the early PCT (169±25 and −187±15 peq/mm · min, respectively). Because the bicarbonate absorptive capacity of the early PCT under free-flow conditions is 500 peq/mm · min, angiotensin II administration or inhibition affected > 60% of proton secretion in this segment. Both agents less markedly affected bicarbonate absorption in the late PCT (±28 peq/mm · min) or chloride absorption (±68–99 peq/mm · min) in both the early and late PCT. Because of its potential for controlling the majority of bicarbonate absorption in the early PCT (hence ≥ 30% of bicarbonate absorption in the entire nephron), angiotensin II may be a powerful physiologic regulator of renal acidification.

Introduction

The proximal convoluted tubule (PCT)[1] is a target for the action of angiotensin II. There are high-affinity receptors on the PCT (1) and, with physiologic low doses of angiotensin II, sodium transport in the PCT is increased in vitro (2) as well as in vivo (3–7). Previous studies have examined the effect of angiotensin II only in the late PCT, and the change in sodium transport has generally been relatively modest (2–7).

Unexplored to date is whether transport in the early (S_1 subsegment) PCT is affected by angiotensin II. The impact of angiotensin II on overall nephron transport might be accentuated if the early PCT were affected, because this tubule subsegment has substantially higher sodium, bicarbonate, and chloride transport rates than the late (S_2 subsegment) PCT (8). It is also unknown which cellular transport system(s) is modified by angiotensin II. The transport effect might be selective, affecting primarily sodium bicarbonate or sodium chloride reabsorption, or generalized, affecting both processes.

The purposes of the present study were to investigate whether angiotensin II differentially affected solute and water absorption in the early versus late PCT and whether sodium bicarbonate and/or sodium chloride transport was altered.

Methods

Preparation of animals. 15 male Munich-Wistar rats, weighing 215±3 g, were used in these studies.

Preparation of rats for microperfusion has been previously reported from this laboratory (9). Briefly, each rat was allowed free access to food and water before being anesthetized with Inactin (100–110 mg/kg i.p.). The rat was placed on a heated table to maintain body temperature at 37°C. The left femoral artery was catheterized for monitoring blood pressure and obtaining samples for bicarbonate measurement. Both jugular veins were catheterized for lissamine green injection and for infusion of bicarbonate Ringer's solution during surgical preparation (2.5 ml for 1 h, then 1.5 ml/h). A flank incision was used to expose the left kidney, which was immobilized in a lucite holder. The surface of the kidney was bathed with mineral oil that was water-equilibrated, bubbled with 95% O_2/5% CO_2, and heated to 37°C. The ureter was cannulated to ensure free drainage of urine. 20 μl of 10% lissamine green was injected intravenously to ensure that PCT transit time was 8–10 s. Plasma volume losses induced by micropuncture surgery were not replaced (10) to allow relatively high levels of endogenous angiotensin.

In vivo microperfusion. Only one proximal nephron (glomerulus with several early and late PCT surface loops) was used for each kidney. Microperfusion was performed in both the early and late portions of the same PCT in both a control and an experimental period. An intravenous saline infusion of 2 ml/h was maintained during both periods.

The entire PCT was initially mapped by injecting a small oil droplet into Bowman's space, as previously described (8). First studied in the control period was the late portion of the PCT, the S_2 subsegment, defined as being 2.5–5 mm from the glomerulus (11). The length to be perfused was estimated at 2.0–2.5 mm. The microperfusion pipette was placed in the lumen at the beginning of this segment for orthograde perfusion. A thermally insulated microperfusion pump (Wolfgang Hampel, Berlin, FRG) set at 30 nl/min was utilized. This perfusion rate was used for two reasons. First, it represents the lower limit of luminal flow rate in the early PCT and the upper limit of flow in the late PCT observed under physiologic free-flow conditions (8). Second, a moderately high but submaximal rate (~ 70–75% of the highest possible transport rate) of bicarbonate absorption occurs in both the early and late PCT at a perfusion rate of 30 nl/min during control conditions (8), so that both transport stimulation and inhibition potentially could occur during the experimental period. The glomerular ultrafiltratelike perfusion solution contained (in mM): NaCl, 120; $NaHCO_3$, 25; KCl, 5; $MgSO_4$, 1; $CaCl_2$, 1.8; Na_2PO_4, 1; glucose, 5; alanine, 5; and urea, 5 (7). The perfusion solution was gassed with 93% O_2/7% CO_2, and contained 0.1% FD and

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1. *Abbreviation used in this paper:* PCT, proximal convoluted tubule.

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C green dye No. 3 and exhaustively dialyzed [methoxy-^{3}H]inulin. A red-stained oil block was then placed proximal to the perfusion pipette, and a hole was left for endogenous tubular fluid to leak out. The collection pipette was placed in the end-proximal loop, a stained, Hepes/10% CO_2 equilibrated paraffin oil block was inserted and maintained distally, and a timed (3–4 min) collection was made.

The early PCT of the same nephron was then studied. Since the S_1 segment is shorter (1–2 mm) (11), the length perfused for early PCT measurements was less (about 1 mm or 1–2 surface loops) than for the late PCT. In general, the same procedures for perfusion and collection were used as in the late PCT. To ensure easy escape of glomerular ultrafiltrate, two holes were made in Bowman's space before perfusion. After the perfusion pipette was inserted into the lumen ~ 0.1–0.2 mm from Bowman's space, the oil block was inserted at the junction of Bowman's space and the beginning of the PCT. The collection pipette was then placed about 1 mm downstream, a block inserted, and the collection performed (23 min).

In the experimental period, there were three different groups of five rats each. Either angiotensin II (Asn^1, Val^5 AII, Sigma, 20 ng/kg/min) or saralasin (Sar^1, Ala^8 AII, Sigma, 1 μg/kg/min) was added to the intravenous saline infusion, or the saline vehicle was continued as a time control. These doses of angiotensin II and saralasin were chosen because they have been previously shown to have minimal systemic or glomerular hemodynamic effects and to not alter the peritubular Starling forces (12, 13). A 1-h equilibration period elapsed before the second period of late and early PCT collections were performed by repuncture of the same nephron segments as described above.

After all collections were completed, the entire tubule was injected with liquid microfil, and a drawing of the tubule was made to document the perfusion and collection sites in the early and late PCT. The kidney was later placed in 6 N HCl at 37°C for 40 min. The microfil casts were then dissected and the two segments between the sites of perfusion and collection were photographed for measurement of perfused length.

Analysis. Collected samples were transferred into constant-bore tubing for measurement of volume. Aliquots were removed for determination of total CO_2 concentration by microcalorimetry and for chloride concentration by the microtitrimetric method, and the remaining fluid was mixed with scintillation fluid for radioactivity counting (8, 9).

Calculations. Calculations were performed as previously described (9). Collected total CO_2 was assumed to represent bicarbonate (9). Perfusion rate was calculated in vivo for each collection and the anion and volume absorptive rates were calculated as the difference in perfused and collected amounts of anion or volume.

Data are presented as mean±SEM. Significance was assessed using the paired *t* test for results obtained in the same tubule.

Results

Angiotensin II or saralasin at the doses utilized minimally changed blood pressure (9±1 and −7±1 mmHg, respectively), as previously reported (12, 13).

As shown in Table I and Fig. 1 (*solid squares*), angiotensin II caused a marked stimulation of bicarbonate absorption in the early PCT by 169±25 peq/mm · min (from 345±9 to 514±22 peq/mm · min, $P < 0.001$). Saralasin (*solid hexagons*) had an equal but opposite effect, causing early PCT bicarbonate absorption to change by −187±15 peq/mm · min (from 382±9 to 195±20 peq/mm · min, $P < 0.001$). Because the normal capacity of S_1 PCT bicarbonate absorption under free-flow conditions is ~ 500 peq/mm · min (8), pharmacologic stimulation or inhibition of angiotensin II activity was capable of controlling ~ 60% of the normal proton secretory rate in the early PCT.

Effects on bicarbonate absorption by angiotensin II and saralasin were less marked in the late PCT, accounting for changes of 27±2 and −28±5 peq/mm · min, respectively (Fig. 1, *open squares and hexagons*). Even given the lower baseline values, the changes in bicarbonate absorption were qualitatively less impressive in the late compared with the early PCT (±13% vs. ±49%).

Table I. Effect of Angiotensin II and Saralasin on Bicarbonate, Chloride, and Water Absorption in Early (S_1) Versus Late (S_2) PCT

	Perfused				Collected			Absorption		
	Length	$[HCO_3^-]$	[Cl]	Rate	$[HCO_3^-]$	[Cl]	Rate	$J_{HCO_3^-}$	J_{Cl}	J_v
	mm	*meq/liter*	*meq/liter*	*nl/min*	*meq/liter*	*meq/liter*	*nl/min*	*peq/mm · min*	*peq/mm · min*	*nl/mm · min*
Early PCT										
Control	0.73±0.06	23.9±0.3	119.3±0.2	29.4±0.1	17.4±1.2	130.0±1.0	25.8±0.3	345±9	219±14	5.0±0.3
Angiotensin II				29.6±0.1	13.8±1.3*	136.3±0.9*	24.2±0.3*	514±22*	313±15*	7.5±0.4*
Control	0.81±0.04	24.2±0.3	119.3±0.2	29.5±0.2	16.1±0.8	132.3±1.0	25.2±0.3	382±9	223±13	5.3±0.4
Saralasin				29.6±0.2	20.8±0.8*	127.8±0.4*	26.9±0.1*	195±20*	125±3*	3.4±0.3*
Control	0.77±0.04	24.3±0.5	119.1±0.2	29.1±0.4	16.6±0.8	129.4±1.2	25.5±0.2	365±23	213±17	4.8±0.6
Control				29.5±0.2	17.1±1.0	129.2±1.6	25.9±0.2	359±19	224±18	4.8±0.7
Late PCT										
Control	2.25±0.08	23.9±0.3	119.3±0.3	29.6±0.1	10.7±0.6	129.0±0.8	23.5±0.2	202±3	225±11	2.7±0.1
Angiotensin II				29.7±0.2	9.1±0.8*	134.2±1.2*	21.5±0.1*	229±5*	292±10*	3.7±0.2*
Control	2.45±0.05	24.2±0.3	119.3±0.3	29.7±0.2	10.1±0.1	127.5±0.2	23.8±0.1	198±5	206±10	2.4±0.1
Saralasin				29.5±0.2*	11.7±0.3*	122.9±0.7*	25.9±0.3*	170±3*	135±7*	1.4±0.1*
Control	2.25±0.02	24.3±0.5	119.1±0.2	29.7±0.2	10.9±0.5	128.4±0.8	23.9±0.3	208±10	214±20	2.5±0.3
Control				29.6±0.1	10.8±0.3	128.2±0.5	23.7±0.3	209±13	224±29	2.6±0.2

$J_{HCO_3^-}$, bicarbonate absorption; J_{Cl}, chloride absorption; J_v, water absorption. * Significance at $P < 0.005$ for paired comparison to comparable control value.

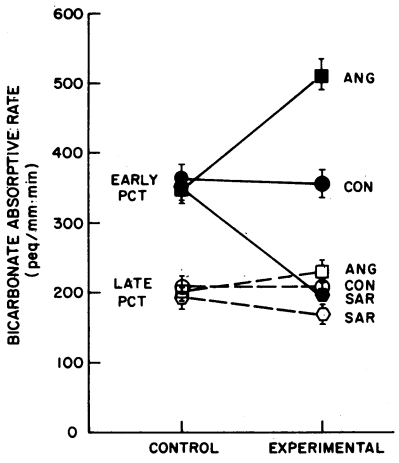

Figure 1. Bicarbonate absorptive rate in the early (*solid symbols*) and late (*open symbols*) PCT in a control period and after systemic angiotensin II (*ANG, squares*), saralasin (*SAR, hexagons*), or vehicle (*CON, circles*) administration. Mean±SEM are shown for paired measurements in each group.

In contrast to the selective modulation of acidification in the early PCT, the effects of angiotensin II and saralasin on chloride transport were quantitatively similar in the early and late PCT (Fig. 2 *A, solid and open squares and hexagons*). Angiotensin II stimulated chloride absorption by 94±14 and 68±5 peq/mm · min in the early and late PCT, respectively, whereas saralasin inhibited chloride absorption by −99±15 and −72±15 peq/mm · min, respectively. The effect on water absorption by angiotensin II and saralasin (Fig. 2 *B, solid and open squares and hexagons*) was twofold higher in the early compared with late PCT (±2 vs. ±1 nl/mm · min, respectively), predominantly because of the marked changes in bicarbonate absorption in the early segment.

Time controls with saline vehicle infusion were associated with no significant changes in anion or volume absorption (Table I and Figs. 1 and 2, *closed and open circles*).

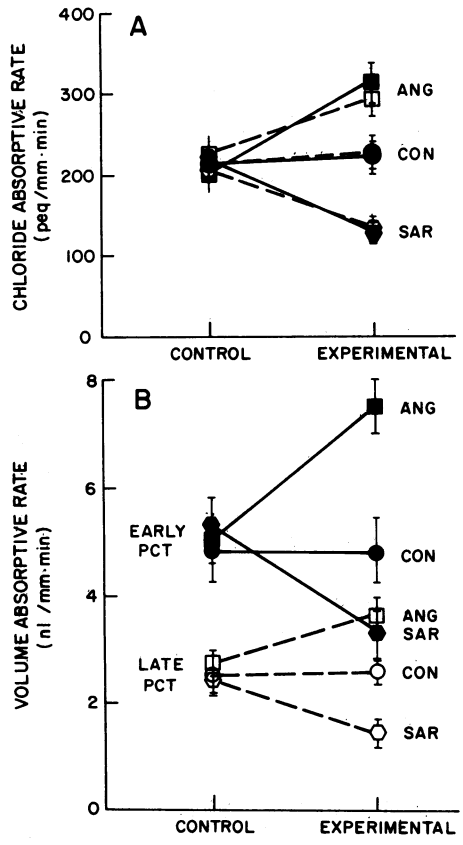

Figure 2. Chloride (*A*) and volume (*B*) absorptive rates in the early (*closed symbols*) and late (*open symbols*) PCT in a control period and after systemic angiotensin II (*ANG, squares*), saralasin (*SAR, hexagons*), or vehicle (*CON, circles*) administration. Mean±SEM are shown for paired measurements in each group.

Discussion

In addition to the well-established hemodynamic and endocrine actions of angiotensin II for regulating renal function, direct effects on tubular transport systems may also occur (3). Direct transport effects reported to date, however, have been relatively modest in magnitude, but only the late (S_2) PCT had been examined (2–7). We have now shown that modulation of angiotensin II activity can profoundly alter renal solute transport, specifically bicarbonate absorption in the early (S_1) PCT.

At least 95% of renal proton secretion is expended in reabsorbing bicarbonate under normal euvolemic conditions. Of this, approximately half (500 peq/min per superficial nephron) occurs in the first millimeter of the PCT, the S_1 subsegment (8). In the present studies, exogenous angiotensin II administration or inhibition of endogenous angiotensin II by saralasin changed the baseline S_1 PCT bicarbonate absorptive rate by 169 and −187 peq/mm · min, respectively (Fig. 1, *solid squares and hexagons*). The combined range of early PCT bicarbonate absorption affected by pharmacologic modulation of angiotensin II activity was therefore > 300 peq/min, or ≥ 60% of the total bicarbonate absorption that normally occurs under freeflow conditions in the first millimeter of the PCT, and hence ≥ 30% of proton secretion by the entire nephron. Thus, angiotensin II may be an important regulator of renal acidification. Though the limits of its control under physiologic circumstances remain to be defined, the magnitude of renal acidification potentially regulated by angiotensin II exceeds that of any other known hormone, such as aldosterone or parathyroid hormone.

Many questions are raised regarding the extent and mechanisms of the control of early PCT acidification exercised by pharmacologic manipulation of angiotensin II activity. Whether even greater stimulation or inhibition of proximal acidification might be effected by different doses of angiotensin II or saralasin, respectively, and whether transport in juxtamedullary nephrons is comparably affected are questions deserving further study. The reason for the attenuated effect on late PCT acidification, perhaps diminished receptor density, also requires elucidation. Because filtered angiotensin II was excluded from luminal contact with the PCT in the present microperfusion experiments, the observed transport changes must have been mediated by peritubular signaling, but luminal angiotensin II effects may also occur (14). Possible participation and/or mediation of the changes in transport induced by angiotensin II and saralasin by renal nerves (7, 15), prostaglandins (16), phosphoinositol turnover and intracellular calcium (17), direct changes in Na/H antiporter kinetics (18), or other factors certainly warrant further investigation.

The physiologic role of changes in the endogenous angiotensin II concentration on S_1 acidification is, of course, a very important question provoked by the present results. Unfortunately, intrarenal tissue angiotensin II levels are not quantifiable. The present data were obtained in the moderate plasma volume contracted state of hydropenia (10), probably associated with a

relatively high endogenous angiotensin II level. How physiologic perturbation of angiotensin II levels (systemic and intrarenal) by acute and chronic alterations in extracellular volume might affect PCT transport is an important future direction for study. That angiotensin and aldosterone coordinately regulate proximal and distal nephron acidification is a provocative hypothesis.

The effect by angiotensin II and saralasin on chloride absorption was quantitatively less impressive than on early PCT bicarbonate absorption, but there was no axial selectivity. Changes in chloride transport were equivalent (±68–99 peq/mm · min) in both the S_1 and S_2 PCT (Fig. 2 *A*). These studies did not elucidate whether chloride transport was affected directly or indirectly, due to concomitant changes in bicarbonate absorption. (In the latter, a relatively greater coupling of chloride flux to change in bicarbonate transport occurred in the late PCT.) Though changes in peritubular colloid osmotic pressure can affect active proximal chloride transport (19), it is important to emphasize that the identical doses and structural preparations of angiotensin II and saralasin have been previously found (12, 13) to minimally alter systemic and glomerular hemodynamics and to not affect the peritubular protein concentration (efferent arteriolar colloid osmotic pressure changed from 28±1 to 29±2 mmHg, NS, with angiotensin II [12], and from 35±1 to 33±2 mmHg, NS, with saralasin [13]).

The ultimate physiologic consequence of the powerful modulation by angiotensin II of acidification in the S_1 PCT should not necessarily be construed as a primary change in acid-base balance under free-flow conditions. Although highly speculative, change in early PCT acidification might be ultimately translated into regulation of sodium chloride homeostasis. For instance, if angiotensin II activity were physiologically reduced (comparable to the effect of saralasin), the increased delivery of bicarbonate out of the S_1 PCT would be reclaimed in large measure by the S_2 PCT, because this latter segment has a significant latent capacity for bicarbonate reabsorption (8, 9) and is relatively spared from modulation by angiotensin II (Fig. 1). However, chloride transport would be diminished along the entire length of the PCT (Fig. 2 *A, open hexagons*), a direct consequence of the reduced angiotensin activity and/or a secondary consequence of the length-averaged depression in the transepithelial chloride concentration gradient required for passive chloride reabsorption.

In conclusion, both angiotensin II administration and inhibition of endogenous angiotensin II profoundly altered bicarbonate absorption in the early PCT. By virtue of the magnitude of this control, representing ≥30% of bicarbonate absorption that normally occurs in the entire nephron, angiotensin II has the potential of being a powerful hormonal regulator of renal acidification under physiologic conditions.

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