JCI The Journal of Clinical Investigation

Angiotensin II stimulation of hydrogen ion secretion in the rat early proximal tubule. Modes of action, mechanism, and kinetics.

F Y Liu, M G Cogan

J Clin Invest. 1988;82(2):601-607. https://doi.org/10.1172/JCI113638.

Research Article

Physiologic concentrations of angiotensin II stimulate sodium transport by intestinal and renal early (S1) and late (S2) proximal tubule epithelial cells. We recently found that hydrogen ion secretion, which effects sodium bicarbonate absorption, was a transport function preferentially and potently increased by angiotensin II in S1 cells. S1 cells are normally responsible for half of the total renal hydrogen ion secretion. The mechanism by which angiotensin II regulates intestinal sodium transport is by potentiating sympathetic nerve activity and norepinephrine release. Direct control of hydrogen ion secretion by angiotensin II via receptors on epithelial cells has not been previously demonstrated. We now report that stimulation of in vivo hydrogen ion secretion in the rat early proximal tubule by angiotensin II was not mediated via change in nerve activity. Rather, enhanced hydrogen ion secretion by angiotensin II correlated with increased angiotensin II receptor density on epithelial cells in the early compared to late microdissected proximal tubule. Basolateral as well as luminal angiotensin II stimulated bicarbonate absorption. Angiotensin II reduced bicarbonate permeability and caused alteration in the apparent substrate affinity, but not maximal capacity, of the proximal hydrogen ion secretory system involving the Na+/H+ antiporter.

Angiotensin II Stimulation of Hydrogen Ion Secretion in the Rat Early Proximal Tubule

Modes of Action, Mechanism, and Kinetics

Fu-Ying Liu and Martin G. Cogan
Department of Medicine and Cardiovascular Research Institute, University of California, San Francisco, San Francisco, California 94143

Abstract

Physiologic concentrations of angiotensin II stimulate sodium transport by intestinal and renal early (S_1) and late (S_2) proximal tubule epithelial cells. We recently found that hydrogen ion secretion, which effects sodium bicarbonate absorption, was a transport function preferentially and potently increased by angiotensin II in S_1 cells. S_1 cells are normally responsible for half of the total renal hydrogen ion secretion. The mechanism by which angiotensin II regulates intestinal sodium transport is by potentiating sympathetic nerve activity and norepinephrine release. Direct control of hydrogen ion secretion by angiotensin II via receptors on epithelial cells has not been previously demonstrated. We now report that stimulation of in vivo hydrogen ion secretion in the rat early proximal tubule by angiotensin II was not mediated via change in nerve activity. Rather, enhanced hydrogen ion secretion by angiotensin II correlated with increased angiotensin II receptor density on epithelial cells in the early compared to late microdissected proximal tubule. Basolateral as well as luminal angiotensin II stimulated bicarbonate absorption. Angiotensin II reduced bicarbonate permeability and caused alteration in the apparent substrate affinity, but not maximal capacity, of the proximal hydrogen ion secretory system involving the Na^+/H^+ antiporter.

Introduction

Angiotensin II plays an important role in cardiovascular and extracellular volume homeostasis. The diverse physiologic actions of angiotensin II include control of solute transport in epithelial cells of the jejunum (1) and of the renal late proximal tubule (2–6). Recently, we found angiotensin II had a far greater impact on transport in the early (1st mm) compared to late (2nd–5th mm) rat proximal convoluted tubule (PCT)[1] in vivo (7). Morphologically different epithelial cell types reside in the early and late PCT, termed S_1 and S_2, respectively (8). Sodium bicarbonate reabsorption, which is effected by luminal hydrogen ion secretion, was the transport function in S_1 cells preferentially stimulated by angiotensin II (7).

Address reprint requests to Dr. Cogan, Division of Nephrology, Box 0532, University of California, San Francisco, CA 94143.

Received for publication 23 November 1987 and in revised form 9 February 1988.

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

J. Clin. Invest.

0021-9738/88/08/0601/07 $2.00
Volume 82, August 1988, 601–607

These preliminary observations did not establish the mechanism by which angiotensin II regulated hydrogen ion secretion in S_1 cells. In the jejunum, angiotensin II increases solute transport indirectly: angiotensin II potentiates sympathetic nerve activity and release of norepinephrine, which then changes epithelial cell transport via α_1 receptors (1). Proximal tubule cells are richly innervated (6, 9, 10), but they also have angiotensin II receptors (5, 11–14). However, receptor-mediated stimulation of epithelial cell hydrogen ion secretion by angiotensin II has not been previously described. Direct hormonal control of S_1 cell function could potentially have great physiologic importance because these cells are normally responsible for fully half of renal bicarbonate reabsorption (15).

In the present studies, we first examined whether angiotensin II affects hydrogen ion secretion via the renal sympathetic nerves or via receptors on the epithelial cells themselves. After having established the predominance of the latter mechanism, in the next experiments we examined whether a nonuniform receptor density as a function of tubule length might explain the greater change in hydrogen ion secretion induced by angiotensin II in the early compared to the late PCT and whether basolateral and luminal receptors were both capable of transducing this angiotensin II effect. Finally, we examined the contribution of the Na^+/H^+ antiporter and kinetic aspects of the enhanced acidification induced by angiotensin II.

Methods

Preparation of animals

Male Munich-Wistar rats (weighing 200–220 g) were used in these studies and prepared for microperfusion as previously reported from this laboratory (7, 16, 17). For studies examining the role of renal nerves in the action of angiotensin II, denervation was performed by standard mechanicochemical techniques, as previously described (10).

In vivo microperfusion

Only one proximal nephron 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, as previously described (7, 16–18). The entire PCT was initially mapped by injecting a small oil droplet into Bowman's space, as previously described. First studied was the late portion of the PCT, the S_2 subsegment, defined as being 2.5–5 mm from the glomerulus (8). A thermally insulated microperfusion pump (Wolfgang Hampel, Berlin, Federal Republic of Germany) was utilized for orthograde perfusion, at 30 nl/min unless otherwise specified. Timed collections of 3–4 min were performed. The same procedures for perfusion and collection were then performed in the early portion of the same nephron, the S_1 subsegment, defined as the initial millimeter from the glomerulus (8).

The perfusion solution was the standard glomerular ultrafiltrate-like solution (in millimolar): 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, 16–18). The solution was gassed with 93% O_2/7% CO_2 and contained 0.1% FD & C green dye No. 3 and exhaustively dialyzed [*methoxy*-^{3}H]inulin. In

indicated protocols, 4.3 mM amiloride (Sigma Chemical Co., St. Louis, MO) or 1×10^{-12} M or 1×10^{-11} M angiotensin II (Asn^1, Val^5 AII, Sigma Chemical Co.) was added to the perfusate. For determining bicarbonate permeability, the previously described bicarbonate-free, acetazolamide-containing perfusate was used (16–18).

When intravenous angiotensin II (20 ng/kg · min) was used, the protocol was the same as previously described (7). This dose of angiotensin II has minimal effects on systemic or glomerular hemodynamics or the peritubular Starling forces (19).

After completing all collections, the entire tubule was injected with liquid microfil, the kidney was dissolved in acid, and the microfil casts were dissected and photographed for measurement of perfused length. The volume of collected sample was measured and aliquots removed for determination of total CO_2 concentration by microcalorimetry and of chloride concentration by microtitration and for radioactivity counting (7, 16–18).

Calculations were performed as previously described (7, 16–18). Total CO_2 was assumed to principally represent bicarbonate. Perfusion rate (V_0) was calculated in vivo for each collection.

Net bicarbonate flux ($J^{NET}_{HCO_3}$) was calculated as the difference in the absolute amount of bicarbonate perfused and collected: $J^{NET}_{HCO_3} = (C_0V_0 - C_LV_L)/L$, where C_L and C_0 represent the collected and perfused bicarbonate concentrations, V_0 the perfusion rate, and L the perfused length.

To calculate bicarbonate permeability (P_{HCO_3}), bicarbonate influx was divided by the log mean chemical driving force: $P_{HCO_3} = (J^{NET}_{HCO_3}/(C_L/\ln[C_p/(C_p - C_L)])$, where C_p is the plasma bicarbonate concentration, uncorrected for Donnan distribution. As before (16–18), the transepithelial potential difference was not measured or included in this calculation. Spontaneous potential differences in the early PCT are small (≤ -2 mV) and the absence of organic solutes and presence of acetazolamide should minimize the transport-generated potential difference (20, 21).

For the studies investigating the effect of angiotensin II on the kinetics of PCT acidification, net bicarbonate absorption was assumed to be the sum of two components, passive bicarbonate diffusion ($J^{PASS}_{HCO_3}$) plus proton secretion (J_{H^+}): $J^{NET}_{HCO_3} = J^{PASS}_{HCO_3} + J_{H^+}$, where $J^{PASS}_{HCO_3}$ was the product of the P_{HCO_3} at that flow rate and the measured arithmetic mean bicarbonate concentration gradient: $J^{PASS}_{HCO_3} = P_{HCO_3} \times ([(C_0 + C_L)/2] - C_p)$.

There were five microperfusion protocols:

Denervation: effect of angiotensin II. To examine whether angiotensin II-induced changes in early and late PCT transport were mediated via the renal nerves, the effect on transport by intravenous angiotensin II was measured after renal denervation ($n = 5$) and compared to the innervated state (7).

Polarity of angiotensin transport effects. These studies assessed the relative physiologic importance of basolateral versus luminal effects. The magnitude of transport stimulation by intravenous angiotensin II (which raises systemic angiotensin II level from about 1–5 to 5–10 $\times 10^{-12}$ M [22, 23]) with an angiotensin II-free perfusate was compared to that elicited by luminal perfusion with either 1×10^{-12} M ($n = 5$) or 1×10^{-11} M ($n = 5$) angiotensin II under conditions of a constant basolateral environment.

Role of Na^+/H^+ exchanger in stimulation by angiotensin II of proximal acidification. The effect of angiotensin II on PCT acidification was assessed in the presence of 4.3 mM luminal amiloride ($n = 5$), which has been previously shown to potently inhibit the Na^+/H^+ exchanger in vivo (24). In preliminary studies, we confirmed the specificity and potency of amiloride for inhibiting PCT acidification. Amiloride markedly inhibited bicarbonate absorption (−50 to −68%) with only a small effect on [^{14}C]glucose absorption (−16 to −26%).

Effect of angiotensin II on proximal bicarbonate permeability. These studies assessed the possible contribution of depressed paracellular bicarbonate back-leak to the increase in net bicarbonate absorption induced by angiotensin II. A bicarbonate-free perfusate was used at luminal perfusion rates of 15, 30, and 45 nl/min ($n = 5$) and bicarbonate permeabilities were then calculated from rates of luminal bicarbonate appearance during angiotensin II infusion (20 ng/kg · min) to compare with permeabilities previously measured without angiotensin infusion (16–18).

Kinetics of angiotensin II stimulation of proximal acidification. Although angiotensin II can stimulate bicarbonate absorption when bicarbonate transport is initially submaximal, during perfusion at 30 nl/min (7), we investigated whether transport stimulation could still occur if a maximal hydrogen ion secretory rate was initially present. Perfusion was therefore performed at 45 nl/min ($n = 5$), which induces a maximal rate of proton secretion (18). Hydrogen ion secretion was calculated from net bicarbonate absorptive rates by correcting for rates of passive bicarbonate back-leak (calculated from measured bicarbonate permeabilities in the previous protocol, as described above).

Angiotensin II receptor binding

The methods of Mujais et al. were followed (11). Briefly, the left kidney of an anesthetized Munich-Wistar rat was perfused with a cold collagenase-containing solution. Thin slices were incubated for 7 min at 35°C in the same medium and then rinsed with cold microdissection solution. An entire PCT ($n = 14$) was dissected under stereomicroscopic observation. The attached glomerulus was removed and the tubule divided into three parts: early (0–1 mm), middle (1–3 mm), and late (3–5 mm) portions. Each segment was photographed to determine length and placed in a well with 2 μl of cold incubation medium at 25°C, pH 7.4, for 30 min without agitation followed by addition of stop solution. Total ^{125}I-angiotensin II (New England Nuclear, Boston, MA; specific activity 1,880 μCi/ug) binding was determined in duplicate at several different ligand concentrations as was nonspecific binding (^{125}I-angiotensin II plus 1,000-fold excess unlabelled angiotensin II). Counts per minute of total binding were 105–765 times and 26–230 times background in the early and mid-to-late PCT, respectively.

Statistical analysis

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

Results

Mode of action of angiotensin II on early proximal acidification

Role of renal sympathetic nerves. Whether renal sympathetic nerves mediate the changes in transport induced by angiotensin II was first examined. In vivo microperfusion was performed in the early and late PCT of normally innervated kidneys, as previously reported (7), or of denervated kidneys. In each case, transport rates were measured during a control period and following 20 ng/kg · min angiotensin II i.v. Denervation itself reduced transport rates by 10 to 50% depending on the solute, in roughly similar proportions in the early and late PCT (Table I). As illustrated in Fig. 1, the angiotensin II-induced increments in chloride and volume absorption were markedly attenuated by renal denervation, indicating neural mediation of these transport effects. In contrast, denervation did not prevent stimulation of bicarbonate absorption by angiotensin II in either early (169±25 vs. 190±10 peq/mm · min) or late (27±2 vs. 60±6 peq/mm · min) PCT. Thus, the ability for angiotensin II to stimulate bicarbonate absorption was independent of renal nerve activity.

Angiotensin II receptors on PCT epithelial cells. Greater angiotensin II-induced transport stimulation occurred in the early compared to late PCT independent of the renal nerves (Fig. 1). Increased angiotensin II receptor density in the early

Table I. Effects of Denervation on Angiotensin II Stimulation of Water, Bicarbonate, and Chloride Transport in the Early and Late Proximal Tubule

Location	Treatment	Perfusion rate	Perfused length	$[HCO_3^-]$ Perfused	$[HCO_3^-]$ Collected	$[Cl^-]$ Perfused	$[Cl^-]$ Collected	J_v	J_{Cl^-}	$J_{HCO_3^-}$
		nl/min	*mm*	*mM*	*mM*	*meq/liter*	*meq/liter*	*nl/mm · min*	*peq/mm · min*	*peq/mm · min*
Early PCT	Control*	29.4±0.1	0.73±0.06	23.9±0.3	17.4±1.2	119.3±0.2	130.0±1.0	5.0±0.3	291±14	345±9
	AII*	29.6±0.1			13.8±1.3		136.3±0.9	7.5±0.4	313±15	514±22
	P‡				<0.001		<0.001	<0.001	<0.001	<0.001
	Denervation	29.8±0.05	0.74±0.04	23.9±0.4	20.7±0.6§	119.4±0.1	130.7±0.7	4.6±0.3	140±2‖	216±11‖
	Denervation + AII	29.7±0.2			15.6±0.5		131.5±0.6‖	5.0±0.3‖	161±4‖	406±6‖
	P‡				<0.001		<0.001	<0.001	<0.001	<0.001
Late PCT	Control*	29.7±0.2	2.25±0.02	23.9±0.3	10.7±0.6	119.3±0.2	129.0±0.8	2.7±0.1	225±1	202±3
	AII*	29.7±0.2			9.1±0.8		134.2±1.2	3.7±0.2	292±10	229±5
	P‡				<0.001		<0.001	<0.001	<0.001	<0.001
	Denervation	29.7±0.1	2.27±0.05	23.9±0.4	16.4±0.4‖	119.4±0.1	131.8±0.4§	2.1±0.05‖	112±4‖	132±5‖
	Denervation + AII	29.7±0.1			11.4±0.3§		132.8±0.5	2.4±0.06‖	142±6‖	192±3‖
	P‡				<0.001		<0.005	<0.001	<0.001	<0.001

n = 5 in each group. Abbreviations: AII, angiotensin II; $J_{HCO_3^-}$, net bicarbonate absorption; J_v, net water absorption; J_{Cl^-}, net chloride absorption. * Data from Liu and Cogan (7). ‡ P values of paired comparison to control and denervation groups, respectively; § $P < 0.05$, ‖ $P < 0.005$, unpaired comparison to values of control group or control + AII group.

compared to late PCT could reasonably account for this axial heterogeneity of transport response. However, Mujais et al. found no change in angiotensin II receptor density (300 amol · cm^{-1}) with PCT length (11). This apparent discrepancy might be explained if these workers had failed to examine the very early (S_1) tubule. Accordingly, we measured angiotensin II binding in paired segments of the early (S_1, 0–1 mm of tubule length), middle (S_2, 1–3 mm), and late (S_2, 3–5 mm) microdissected PCTs. As shown in Fig. 2, nonspecific binding was linear and was 10–15% (early) or 25–30% (mid-to-late) of total binding. Average tubule lengths in the three subsegments were 0.9±0.04, 1.8±0.1, and 1.8±0.1 mm.

Fig. 3 shows that maximal specific ^{125}I-angiotensin II binding in the S_1 segment (~ 4000 amol · cm^{-1}) was 10-fold higher than that in the mid-to-late S_2 segments (300–500 amol · cm^{-1}) and than reported by Mujais et al. (11). The angiotensin II concentration associated with half-maximal binding was similar, 5–6 nM, in all segments. Thus, this in vitro biochemical evidence showing markedly higher angiotensin II receptor density in the early compared to late PCT (Figs. 2 and 3) correlated well with the physiologic in vivo results showing greater angiotensin II stimulation of hydrogen ion secretion in the early PCT (Fig. 1).

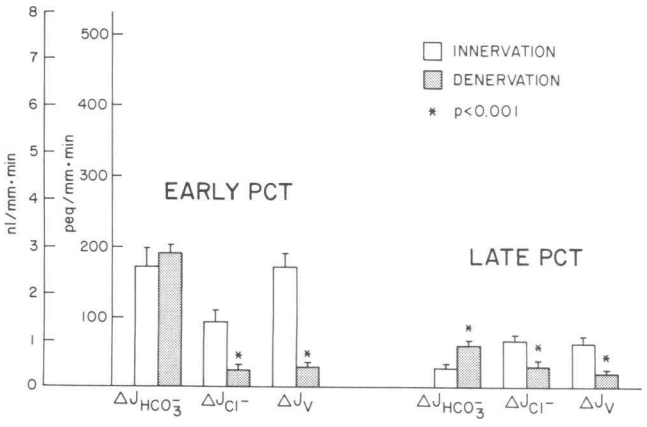

Figure 1. The increment in bicarbonate, chloride, and water absorption in the early and late PCT in response to angiotensin II (20 ng/kg · min i.v.) is depicted in control, innervated (*open bars*) kidneys, as previously reported (7), and denervated (*closed bars*) kidneys. Mean±SEM is shown (n = 5 in each group). Asterisk (*) indicates significant difference ($P < 0.001$) from innervated value.

Basolateral vs. luminal angiotensin II signaling. In the above perfusion experiments in which lumens were perfused with an angiotensin II-free solution, the augmented hydrogen ion secretion after intravenous angiotensin II was signaled via the basolateral route (12, 14). The systemic angiotensin II level under normal conditions in the rat is ~ 1–5 × 10^{-12} M and rises to ~ 5–10 × 10^{-12} M after low-dose intravenous angiotensin II administration (22, 23). Since membrane receptors facing the tubular lumen also exist (13, 14), occupancy of luminal receptors by comparable doses of angiotensin II might also affect transport.

As shown in Fig. 4 *A*, increments in bicarbonate absorption after luminal perfusion with physiologic concentrations of angiotensin II of 10^{-12} or 10^{-11} M were 94±6 and 53±5 peq/mm · min, in the early PCT. The changes in the late PCT were 8±1 and 13±2 peq/mm · min, respectively. All values were, however, substantially less than after intravenous angiotensin II infusion. Thus, angiotensin II can stimulate proximal hydrogen ion secretion via both luminal and basolateral mechanisms.

Mechanism and kinetics of angiotensin II-stimulated acidification

Participation of Na^+/H^+ antiporter. Most, but not all, proximal acidification is effected by the Na^+/H^+ antiporter. To deter-

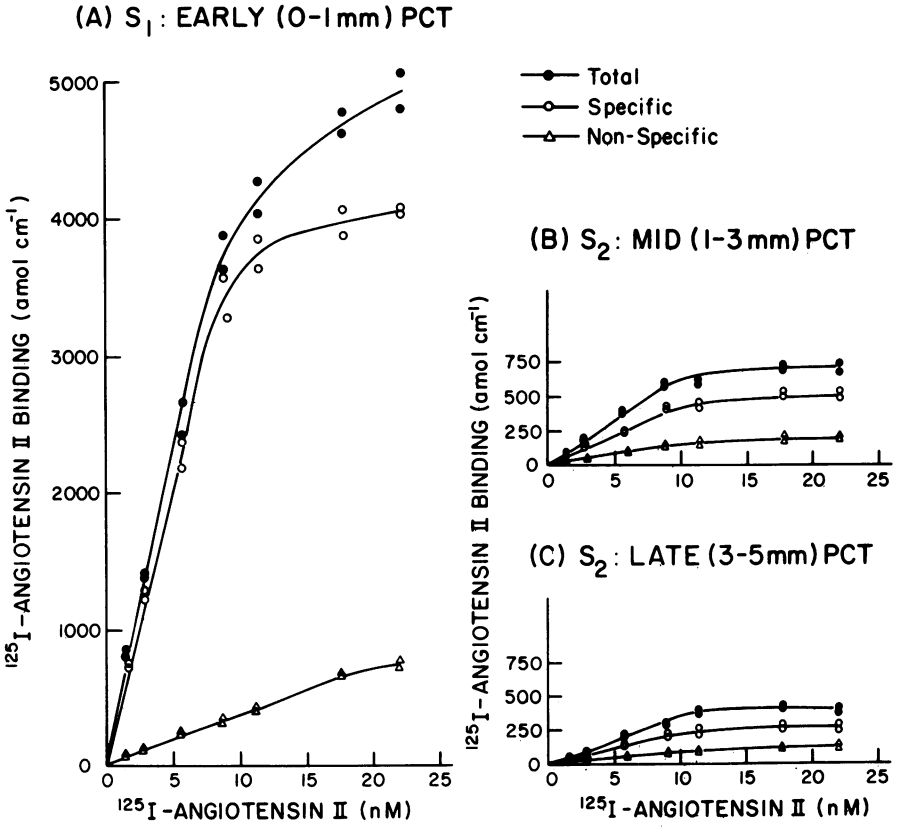

Figure 2. Total, specific, and nonspecific ^{125}I-angiotensin II binding as a function of ^{125}I-angiotensin II concentration in three microdissected segments of the Munich-Wistar PCT (n = 14): (*A*) S_1: early (0–1 mm); (*B*) S_2 mid (1–3 mm); and (*C*) S_2 late (3–5 mm). The protocol of Mujais et al. (11) was used. Specific binding was the difference in total and nonspecific (containing 1,000-fold excess angiotensin) binding.

mine whether angiotensin II-stimulation of proximal hydrogen ion secretion involved by the Na^+/H^+ antiporter, we perfused the lumen with 4.3 mM amiloride, a concentration which potently and selectively inhibits the PCT Na^+/H^+ antiporter in vivo (24). Luminal amiloride reduced basal rates of bicarbonate aborption by 58% in the early and by 65% in the late PCT (Table II). The increment in bicarbonate transport by angiotensin II was substantially reduced by 83%, to 29 peq/mm · min in the early PCT (Fig. 4 *B*) and by 52%, to 13 peq/mm · min in the late PCT. Thus, Na^+/H^+ activity is required for the expression of the change in acidification in response to angiotensin II.

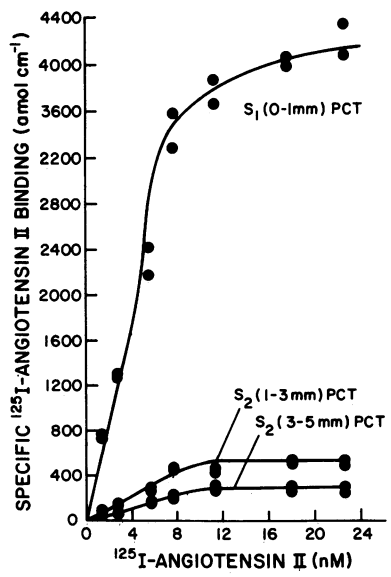

Figure 3. Specific ^{125}I-angiotensin II binding in microdissected early (S_1) compared to mid-to-late (S_2) PCT segments (n = 14) of Munich-Wistar rats using the protocol of Mujais et al. (11). Specific binding was the difference in total and nonspecific (containing 1,000-fold excess angiotensin) binding.

Bicarbonate permeability. Although the precise intracellular mechanism by which angiotensin II regulates epithelial hydrogen ion secretion is not currently known, we performed the following studies to gain some insight into kinetic aspects of the process. First, it is possible that angiotensin II altered net bicarbonate absorption at least in part by changing the rate of bicarbonate back-leak. Bicarbonate permeability was therefore measured using standard techniques and, compared to normal (16–18), was found to be reduced by about 40% (19–44%) at the flow rates tested (Table III).

Effect on V_{max}. To further examine the transport kinetic changes induced by angiotensin II, we compared the response to angiotensin II when the basal rate of proximal bicarbonate absorption was submaximal (using a 30-nl/min microperfusion rate as described above) to that when the rate was initially at a maximal level (15, 17, 18), achieved by increasing the microperfusion rate to 45 nl/min (18). As shown in Table IV, when perfusion rate was 45 nl/min and bicarbonate absorption was maximal, angiotensin II caused little further change in net bicarbonate absorption in the early PCT (from 548±9 to 557±18 peq/mm · min, NS) (Fig. 4 *C*) or late PCT (177±5 to 188±4 peq/mm · min, $P < 0.005$).

Net bicarbonate absorption is the sum of active hydrogen ion secretion and passive bicarbonate back-leak. When corrected for altered bicarbonate permeability (see above) and hence bicarbonate back-leak, there was no change in the rate of hydrogen ion secretion by angiotensin II: 627±17 to 608±24 and 202±9 to 205±6 peq/mm · min, respectively (Table IV). Thus, angiotensin II did not affect the maximal rate (V_{max}) of hydrogen ion secretion.

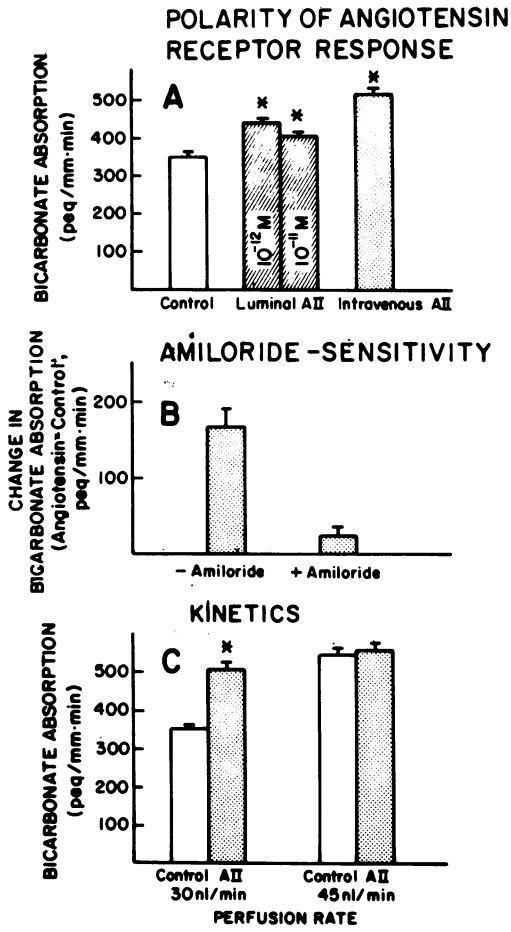

Figure 4. Polarity, mechanism, and kinetics of angiotensin II effect on hydrogen ion secretion in the early (S_1) PCT. (*A*) The early proximal (S_1) bicarbonate absorptive response is shown when neither luminal nor intravenous angiotensin was used (control), when 10^{-12} or 10^{-11} M angiotensin was added to the luminal perfusion solution, or when intravenous angiotensin (20 ng/kg · min) was administered with no luminal angiotensin ($n = 5$ in each group). Means±SEM are shown. Asterisk (*) indicates significant difference ($P < 0.001$) from control. (*B*) Change in early proximal (S_1) bicarbonate absorption in response to angiotensin II (20 ng/kg · min i.v.) with or without 4 mM amiloride in the luminal perfusate to inhibit Na^+/H^+ antiporter activity (22). There were five tubules in each group. Means±SEM are shown and there was a significant difference ($P < 0.001$) between groups. (*C*) Early proximal (S_1) bicarbonate absorption with or without intravenous angiotensin administration (20 ng/kg · min) at two microperfusion rates of 30 or 45 nl/min. 45 nl/min perfusion elicits maximal bicarbonate transport rates (18). There were five tubules in each group. Mean±SEM is shown and asterisk (*) indicates significant difference ($P < 0.001$) from control.

Discussion

Control by angiotensin II of epithelial transport may be mediated by the nervous system via central and/or presynaptic peripheral nerve receptors (1, 6, 25), or by receptors on the cells themselves (5, 11–14). In previous studies, the magnitude of the hormonal response was relatively small and the solute specificity (whether angiotensin II affected sodium bicarbonate and/or sodium chloride transport systems) was not elucidated (1–6). We recently reported that angiotensin II had a profound impact on sodium bicarbonate absorption in S_1 cells of the early PCT (7), and now provide evidence that this transport regulation correlated with a high receptor density on these cells (Figs. 2 and 3) and did not require the presence of renal sympathetic nerves (Fig. 1). Direct receptor-mediated stimulation of epithelial cell hydrogen ion secretion by angiotensin II has not been previously described.

In contrast to the marked change in bicarbonate absorption in the early PCT, the stimulation by angiotensin II of chloride and volume absorption was relatively small and required intact renal innervation. The present studies are the first, in fact, to demonstrate any functional renal nerve effects on transport in S_1 cells of the PCT (Table I). The renal nerves mediate and/or condition the proximal sodium chloride transport response to angiotensin II, similar to the mechanism by which angiotensin II alters intestinal transport (1). Under free-flow conditions, control of sodium chloride reabsorption by angiotensin II in the innervated PCT may have great importance in regulating renal sodium chloride excretion (26). Thus, angiotensin II has two modes of action for regulating PCT transport: a non-neural, direct, cell receptor-mediated effect on sodium bicarbonate absorption, and an effect on sodium chloride absorption requiring intact renal innervation (Fig. 1).

The far greater stimulation of bicarbonate absorption in the S_1 cells of the early PCT compared to S_2 cells in the mid-to-late PCT correlated well with the axial heterogeneity of epithelial cell receptor density (Figs. 2 and 3). The amount of specific angiotensin II binding was 10-fold higher in the early compared to late PCT, without change in the concentration effecting half-maximal binding. Both luminal and basolateral receptors exist (12–14) and angiotensin II occupancy of both affected bicarbonate absorption (Figs. 1 and 4 *A*). The increase in bicarbonate absorption induced by luminal 10^{-11} M angiotensin II was no higher than by 10^{-12} M, and less than the increase elicited by raising systemic angiotensin II concentration (Fig. 4 *A*). However, it is difficult to rigorously compare the relative physiologic importance of these two receptor systems without further kinetic characterization and without knowledge of in vivo intrarenal angiotensin levels and their indirect, nonepithelial cell receptor effects.

The physiologic mechanism by which angiotensin II receptor occupancy is transduced such that bicarbonate absorption is stimulated could involve (*a*) reduced bicarbonate permeability and hence reduced passive back-leak, (*b*) increased luminal acidification involving the Na^+/H^+ antiporter; and/or (*c*) increased luminal proton pump activity.

In absolute terms, proximal bicarbonate permeability is normally small, although relatively higher in the early PCT, and is flow-dependent (16–18). Angiotensin II reduced PCT bicarbonate permeability (Table III), but this had only a minor impact on net bicarbonate absorption because the baseline back-leak rate was low relative to the proton secretory rate (Table IV). Nevertheless, it is intriguing that a paracellular property, bicarbonate permeability, was altered by a hormone acting on cellular function. This phenomenon is reminiscent of, although opposite in orientation to, the increase by cAMP of nonelectrolyte permeability noted by Jacobson (27). The two observations are conceivably related since angiotensin II has been reported to reduce cellular cAMP (14). In any case, the interesting possibility is raised that altered cell morphology, which changes paracellular permeation properties, also may be involved in and/or mediate the change in active transport.

The studies with luminal amiloride (Table II and Fig. 4 *B*)

Table II. Effect of Amiloride on Angiotensin II Stimulation of Water, Bicarbonate, and Chloride Transport in the Early and Late Proximal Convoluted Tubule

Location	Treatment	Perfusion Rate	Perfused Length	$[HCO_3^-]$		$[Cl^-]$		J_v	J_{Cl^-}	$J_{HCO_3^-}$
				Perfused	Collected	Perfused	Collected			
		nl/min	*mm*	*mM*	*mM*	*meq/liter*	*meq/liter*	*nl/mm·min*	*peq/mm·min*	*peq/mm·min*
Early PCT	Amiloride	29.8±0.07	0.84±0.03	24.7±0.2	21.1±0.4	122.0±0.1	123.1±0.3	0.9±0.1	70±2	145±9
	Amiloride + AII	29.7±0.07			20.4±0.4		123.5±0.5	1.1±0.01	93±3	174±10
	*P**				<0.05			<0.02	<0.001	<0.01
Late PCT	Amiloride	29.8±0.07	2.12±0.07	24.7±0.02	20.9±0.2	122.0±0.1	124.5±0.3	0.8±0.05	66±3	70±2
	Amiloride + AII	29.8±0.08			20.1±0.5		124.4±0.4	0.9±0.04	82±2	83±6
	*P**				<0.05			<0.005	<0.001	<0.02

$n = 4$ in each group. Abbreviations: AII, angiotensin II; $J_{HCO_3^-}$, net bicarbonate absorption; J_v, net water absorption; J_{Cl^-}, net chloride absorption. * *P* Values of paired comparison to respective control groups in first (amiloride) period.

suggest the Na^+/H^+ antiporter participates in the angiotensin II enhancement of hydrogen ion secretion. Caution should be exercised in the interpretion of these results in that amiloride is not a completely specific inhibitor of the Na^+/H^+ antiporter. Amiloride can affect other signal transduction systems (28) and other sodium-dependent transport process, though only a small effect on proximal glucose transport was observed in our preliminary studies (see Methods). It is equally plausible that angiotensin II changes a component of the bicarbonate absorptive process in series with the Na^+/H^+ antiporter, such as the $Na(HCO_3)_3$ exit step. Altered luminal proton-translocating ATPase number and/or activity is not excluded but a major role for this mechanism in mediating the acidification effects induced by angiotensin II is not indicated.

Stimulation of bicarbonate absorption by angiotensin II occurred when transport was initially submaximal (30 nl/min) but not when maximal (45 nl/min) (Table IV and Fig. 4 *C*). These findings imply that angiotensin II does not alter the total number (V_{max}) of hydrogen ion transporters but rather their apparent substrate affinity. Such a kinetic response in vectorially transporting epithelial cells in vivo is qualitatively similar to the finding of Berk et al. (29) that angiotensin II alters the K_m but not V_{max} of the Na^+/H^+ exchanger in vascular smooth muscle cells cultured in vitro. The intracellular second messenger system that serves to transduce the angiotensin II signal to augment hydrogen ion secretion in epithelial cells is currently unknown. The comparatively larger transport effect at the lower flow rate fits with the well-known physiological observation that angiotensin II is needed and has a greater role in controlling renal transport during conditions of extracellular volume contraction, when luminal flow rate is typically low.

In conclusion, angiotensin II, via direct receptor signaling

Table III. Bicarbonate Permeability in the Early (S_1) and Late (S_2) Proximal Convoluted Tubule in Response to Angiotensin II Administration

Treatment	Location	Perfusion rate	Perfused length	Plasma $[HCO_3^-]$	Collected $[HCO_3^-]$	HCO_3^- Permeability
		nl/min	*mm*	*mM*	*mM*	$\times 10^{-7}$ *cm²/s*
Control*		14.9±0.02	0.75±0.03	24.9±0.2	5.3±0.3	7.3±0.8
	Early PCT	29.9±0.03			6.8±0.4	20.4±2.0
		44.7±0.2			7.8±0.4	36.4±3.0
		14.9±0.02	2.1±0.1	24.3±0.4	3.2±0.3	1.6±0.2
	Late PCT	30.0±0.1			4.3±0.3	4.6±0.4
		45.0±0.04			5.3±0.2	8.3±0.4
AII	Early PCT					
		14.8±0.03	0.86±0.02	24.5±0.6	3.8±0.1	4.5±0.3
	P				<0.005	<0.01
		29.7±0.06			4.8±0.1	11.8±0.9
	P				<0.001	<0.005
		44.8±0.02			5.9±0.08	22.7±1.0
	P				<0.005	<0.005
	Late PCT					
		14.8±0.02	2.3±0.02	24.5±0.6	2.1±0.08	0.9±0.03
	P				<0.005	<0.005
		29.8±0.07			3.1±0.2	2.8±0.2
	P				<0.01	<0.005
		44.8±0.002			4.7±0.2	6.7±0.4
	P				<0.025	<0.025

$n = 5$ at each perfusion rate. Angiotensin II was administered intravenously (20 ng/kg·min). Abbreviation: AII, angiotensin II. *P* values show significance using unpaired *t* test compared to control value. * Data from references 16–18.

Table IV. Effect of Angiotensin II Administration on Maximal Water, Chloride, Bicarbonate, and Hydrogen Ion Transport Rates in the Early and Late Proximal Tubule

Location	Treatment	Perfusion rate	Perfused length	$[HCO_3^-]$		$[Cl^-]$		J_v	J_{Cl^-}	$J^{net}_{HCO_3^-}$	J_{H^+}
				Perfused	Collected	Perfused	Collected				
		nl/min	*mm*	*mM*	*mM*	*meq/liter*	*meq/liter*	*nl/mm · min*	*peq/mm · min*	*peq/mm · min*	*peq/mm · min*
Early PCT	Control	44.6±0.2	0.82±0.03	24.4±0.5	17.6±0.4	118.0±0.5	133.5±1.5	10.2±0.7	532±11	548±9	627±17
	AII	44.7±0.1			17.4±0.6		133.6±1.5	10.3±0.8	522±15	557±18	608±24
Late PCT	Control	44.8±0.06	2.51±0.1	24.4±0.5	16.6±0.6	118.0±0.5	121.4±0.6	2.3±0.2	221±11	177±5	202±9
	AII	44.7±0.06			15.9±0.6*		121.0±1.0	2.2±0.2	212±11	188±4*	205±6

$n = 5$ in each group. Abbreviations: AII, angiotensin II; J_v, water absorption; J_{Cl^-}, chloride absorption; $J^{net}_{HCO_3^-}$, net bicarbonate absorption; J_{H^+}, proton secretion. * $P < 0.005$, compared to value in control group.

in S_1 proximal epithelial cells in vivo, alters the apparent substrate affinity but not maximal capacity of the hydrogen ion secretory system involving the Na^+/H^+ antiporter.

Acknowledgments

These studies were supported in part by grants (DK-37423 and DK-27045) from the National Institute of Diabetes and Digestive and Kidney Diseases and an Established Investigator Award from the American Heart Association.

References

1. Levens, N. R. 1985. Control of intestinal absorption by the renin-angiotensin system. *Am. J. Physiol.* 249 (Gastrointest. Liver Physiol. 12):G3–G15.

2. Harris, P. J., and J. A. Young. 1977. Dose-dependent stimulation and inhibition of proximal tubular sodium reabsorption by angiotensin II in the rat kidney. *Pfluegers Arch. Eur. J. Physiol.* 367:295–297.

3. Harris, P. J., L. G. Navar, and D. W. Ploth. 1984. Evidence for angiotensin-stimulated proximal tubular fluid reabsorption in normotensive and hypertensive rats: effect of acute administration of captopril. *Clin. Sci. (Lond.).* 66:541–544.

4. Harris, P. J., and L. G. Navar. 1985. Tubular transport responses to angiotensin II. *Am. J. Physiol.* 248 (Renal Fluid Electrolyte Physiol. 17):F621–F630.

5. Schuster, V. L., J. P. Kokko, and H. R. Jacobson. 1984. Angiotensin II directly stimulates sodium transport in rabbit proximal convoluted tubules. *J. Clin. Invest.* 73:507–515.

6. Pelayo, J. C., and R. C. Blantz. 1984. Analysis of renal denervation in the hydropenic rat: interactions with angiotensin II. *Am. J. Physiol.* 246 (Renal Fluid Electrolyte Physiol. 15):F87–F95.

7. Liu, F.-Y., and M. G. Cogan. 1987. Angiotensin II: a potent regulator of acidification in the rat early proximal convoluted tubule. *J. Clin. Invest.* 80:272–275.

8. Maunsbach, A. B. 1966. Observations on the segmentation of the proximal tubule in the rat kidney. *J. Ultrastruct. Res.* 16:239–258.

9. Barajas, L. 1978. Innervation of the renal cortex. *Fed. Proc.* 37:1192–1201.

10. Cogan, M. G. 1986. Neurogenic regulation of proximal bicarbonate and chloride reabsorption. *Am. J. Physiol.* 250 (Renal Fluid Electrolyte Physiol. 19):F22–F26.

11. Mujais, S. K., S. Kauffman, S., and A. I. Katz. 1986. Angiotensin II binding sites in individual segments of the rat nephron. *J. Clin. Invest.* 77:315–318.

12. Brown, G. P., and J. G. Douglas. 1983. Angiotensin II-binding sites in rat and primate isolated renal tubular basolateral membranes. *Endocrinology.* 112:2007–2014.

13. Brown, G. P., and J. G. Douglas. 1982. Angiotensin II binding sites on isolated rat renal brush border membranes. *Endocrinology.* 111:1830–1836.

14. Douglas, J. G. 1987. Angiotensin receptor subtypes of the kidney cortex. *Am. J. Physiol.* 253 (Renal Fluid Electrolyte Physiol. 22):F1–F7.

15. Liu, F.-Y., and M. G. Cogan. 1986. Axial heterogeneity of bicarbonate, chloride and water transport in the rat proximal convoluted tubule: effects of change in luminal flow rate and of alkalemia. *J. Clin. Invest.* 78:1547–1557.

16. Liu, F.-Y., and M. G. Cogan. 1987. Acidification is inhibited in the late proximal convoluted tubule during chronic metabolic alkalosis. *Am. J. Physiol.* 253 (Renal Fluid Electrolyte Physiol. 22):F89–F94.

17. Liu, F.-Y., and M. G. Cogan. 1987. Kinetics of bicarbonate absorption in the early (S_1) proximal convoluted tubule. *Am. J. Physiol.* 253 (Renal Fluid Electrolyte Physiol. 22):F912–F916.

18. Liu, F.-Y., and M. G. Cogan. 1988. Flow-dependence of bicarbonate transport in the early (S_1) proximal convoluted tubule. *Am. J. Physiol.* 254 (Renal Fluid Electrolyte Physiol. 23):F851–F855.

19. Blantz, R. C., K. S. Konnen, and B. J. Tucker. 1976. Angiotensin II effects upon the glomerular microcirculation and ultrafiltration coefficient of the rat. *J. Clin. Invest.* 57:419–434.

20. Fromter, E., and K. Gessner. 1975. Effect of inhibitors and diuretics on electrical potential differences in rat kidney. *Pfluegers Arch. Eur. J. Physiol.* 357:209–224.

21. Imai, M., E. W. Seldin, and J. P. Kokko. 1977. Effect of perfusion rat on the fluxes of water, sodium, chloride and urea across the proximal convoluted tubule. *Kidney Int.* 11:18–27.

22. Douglas, J. G. 1987. Subpressor infusions of angiotensin II alter glomerular binding, prostaglandin E_2, and cyclic AMP production. *Hypertension.* 9(Suppl. III):III-49–III-56.

23. Aguilera, G., A. Schirar, A. Baukal, and K. J. Catt. 1980. Angiotensin II receptors: Properties and regulation in adrenal glomerulosa cells. *Circ. Res.* 46(Suppl. I):I-118–I-127.

24. Preisig, P. A., H. E. Ives, E. J. Cragoe, Jr., R. A. Alpern, and F. C. Rector, Jr. 1987. Role of the Na^+/H^+ antiporter in rat proximal tubule bicarbonate absorption. *J. Clin. Invest.* 80:970–978.

25. Zimmerman, B. G. 1978. Actions of angiotensin on adrenergic nerve endings. *Fed. Proc.* 37:199–202.

26. Liu, F.-Y., and M. G. Cogan. 1988. Critical role for angiotensin II in proximal glomerulo-tubular balance. *Kidney Int.* 33:421.

27. Jacobson, H. R. 1979. Altered permeability in the proximal tubule response to cyclic AMP. *Am. J. Physiol.* 236 (Renal Fluid Electrolyte Physiol. 5):F71–F79.

28. Howard, M. J., M. D. Mullen, and P. A. Insel. 1987. Amiloride interacts with renal alpha- and beta-adrenergic receptors. *Am. J. Physiol.* 253 (Renal Fluid Electrolyte Physiol. 22):F21–F25.

29. Berk, B. C., M. S. Aronow, T. A. Brock, E. Cragoe, Jr., M. A. Gimbrone, Jr., and R. W. Alexander. 1987. Angiotensin II-stimulated Na^+/H^+ exchange in cultured vascular smooth muscle cells: evidence for protein kinase C-dependent and -independent pathways. *J. Biol. Chem.* 262:5057–5064.