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G T Nagami

J Clin Invest. 1992;**89**(3):925-931. <https://doi.org/10.1172/JCI115673>.

Research Article

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Effect of Angiotensin II on Ammonia Production and Secretion by Mouse Proximal Tubules Perfused In Vitro

Glenn T. Nagami

With the technical assistance of Evelyn M. Warech

Medical and Research Services, Veterans Affairs Medical Center West Los Angeles, Los Angeles 90073; and Department of Medicine, University of California at Los Angeles School of Medicine, Los Angeles, California 90024

Abstract

The effects of angiotensin II on total ammonia (tNH₃) production and net secretion were investigated using in vitro microperfused mouse S₂ proximal tubule segments incubated in Krebs-Ringer bicarbonate buffer containing 0.5 mM L-glutamine. Basolateral exposure of mouse S₂ segments to 10⁻¹¹, 10⁻¹⁰, and 10⁻⁹ M angiotensin II stimulated tNH₃ production rates by 23, 52, and 49%, respectively. Addition of 10⁻⁶ M angiotensin II inhibited the tNH₃ production rate by 34%. 10⁻¹⁰ M angiotensin II inhibited net luminal secretion of tNH₃ in the presence of enhanced luminal acidification and in the absence of altered luminal tNH₃ efflux rates. Measurements of intracellular pH (pH_i) and intracellular calcium concentration ([Ca²⁺]_i) suggested that the effects of angiotensin II on tNH₃ production were not mediated by changes in pH_i but by the stimulatory effect of angiotensin II correlated with increased [Ca²⁺]_i. Inhibition of the calcium-calmodulin-dependent pathway with W-7 blocked the stimulatory effect of 10⁻¹⁰ M angiotensin II on tNH₃ production and luminal acidification. These results indicate that angiotensin II has concentration-dependent effects on tNH₃ production; that its action to stimulate tNH₃ production may be mediated by rises in [Ca²⁺]_i and the calcium-calmodulin pathway; and that angiotensin II, at concentrations that stimulate tNH₃ production, inhibits net luminal ammonia secretion by a mechanism that is not mediated by diminished luminal acidification or by changes in luminal ammonia efflux rates. (*J. Clin. Invest.* 1992; 89:925-931.) Key words: ammoniogenesis • ammonia transport • intracellular calcium • intracellular pH

Introduction

Angiotensin II is a potent regulator of proximal tubule functions, including fluid and bicarbonate transport (1-4). Because total ammonia (tNH₃ = NH₃ + NH₄⁺)¹ production and transport are also important functions of the proximal tubule in that they account for the major portion of tNH₃ that appears in the final urine (5-9), the effects of angiotensin II on tNH₃ produc-

tion and transport by the proximal tubule should be explored. Recent studies by Chobanian and Julin (10) have demonstrated a stimulatory effect of 10⁻⁸ M angiotensin II on tNH₃ production by isolated dog proximal tubule fragments. The effects of angiotensin II on tNH₃ transport in the proximal tubule are not known.

In previous studies, we have used the isolated perfused mouse proximal tubule segment to examine the effects of acute changes in luminal flow rates, pH, and potassium concentration in vitro on ammonia production and transport (11-13). The main advantages of the isolated perfused tubule model are that the origin of the examined segment is known; that the tubule lumen is perfused, so that tNH₃ production and luminal secretory rates may be measured; and that during the isolation procedure no proteolytic enzymes are used that could alter the expression of receptor or transporter functions.

The purpose of the present study was to determine the effect of angiotensin II on tNH₃ production and secretion by proximal tubules perfused in vitro. In the present studies, we examined the effects of angiotensin II on tNH₃ production and secretion by isolated perfused mouse proximal tubule segments. The results of this study demonstrate that angiotensin II has concentration-dependent effects on tNH₃ production by the mouse proximal tubule. By contrast, at a concentration that stimulates tNH₃ production rates, angiotensin II inhibits net tNH₃ secretion by the proximal tubule, despite stimulation of amiloride-sensitive luminal fluid acidification, suggesting a dissociation between luminal tNH₃ secretion and Na⁺-H⁺ exchanger activity.

Methods

Isolation and perfusion of mouse proximal tubules. The isolation and perfusion of mouse proximal tubule segments has been previously described in detail (11, 12, 14). As in previous studies, male Swiss Webster mice weighing 25-35 g were maintained on a standard Purina Rodent Chow (Ralston Purina, St. Louis, MO) diet and were given water ad lib. Proximal tubule segments were dissected from the renal cortex under microscopic guidance using needle-tipped forceps. The mid-portion of the proximal tubule ("S₂") consisted of the late convoluted and early straight portions. The mean length of S₂ segments was 0.8±0.1 mm, and ranged from 0.6 to 1.0 mm. A dissected segment was placed in a modified microperfusion chamber in which the volume was reduced to ~ 200 μl. In experiments in which ammonia production rates were to be made, one end of the segment was cannulated and perfused with Krebs-Ringer bicarbonate (KRB) buffer (see Solutions below), and the distal end of the segment remained open to the bath medium. The tubule was washed by exchanging the bath solution several times with the buffer to be used in the incubation. The standard buffer used in the bath solution was KRB buffer containing 0.5 mM L-glutamine, pH 7.4, pre-equilibrated with 95% O₂:5% CO₂ at 37°C. The volume of the incubation buffer was reduced to ~ 150-200 μl and the bath medium and perfused tubule were covered with mineral oil pre-gassed with 95%

Address correspondence to Glenn T. Nagami, M. D., Building 114, Room 317, Veterans Affairs Medical Center West Los Angeles, Wilshire and Sawtelle Boulevards, Los Angeles, CA 90073.

Received for publication 4 June 1991 and in revised form 22 October 1991.

1. Abbreviations used in this paper: BCECF-AM, 2-bis(carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester; [Ca²⁺]_i, intracellular calcium concentration; tNH₃, total ammonia.

The Journal of Clinical Investigation, Inc.
Volume 89, March 1992, 925-931

O₂:5% CO₂. The tubule was incubated for 20–30 min at 37°C. During the incubation period, the mineral oil directly above the tubule was bubbled with a gas jet of 95% O₂:5% CO₂, which served to mix the incubation medium. At the end of the incubation period, an aliquot of the bath medium was taken and assayed for tNH₃, as described by us previously (11, 14), using enzymatic reactions coupled to the bacterial luciferase bioluminescence reaction. The volume of the incubation medium was determined from the dilution of added amounts of trypan blue dye.

In studies examining net tNH₃ secretion, the fluid leaving the distal end of the perfused segment was collected under mineral oil as timed fluid samples. The rate at which tNH₃ left the distal end of the perfused segment represented the net luminal secretion of tNH₃.

Total ammonia assay. The ultramicroassay used to determine the concentration of tNH₃ in the incubation bath was based on a series of enzymatic reactions coupled to the bacterial luciferase bioluminescence reaction (11). The tNH₃ present in the sample or standard reacts with 2-oxoglutarate and NADH to form glutamate and NAD⁺. The NAD⁺ thus formed was equal to the amount of tNH₃ present in the original sample and was measured using a modification of the bacterial luciferase method of Brolin et al. (15). The rate of light emission was measured using a photometer (model 20; Turner Designs, Mountain View, CA). The steady-state light emission rate was proportional to the amount of tNH₃ present in the sample.

The tNH₃ production rate (pmol/min per mm) is bath volume (μl) × tNH₃ concentration (μM)/incubation time (min) × tubule length (mm).

Net luminal secretion of tNH₃ was calculated from the rate at which tNH₃ left the distal end of the perfused segment in timed luminal fluid collections, so that net tNH₃ secretion (pmol/min per mm) is total content (pmol)/collection time (min) × tubule length (mm).

Measurement of intraluminal pH. The pH of the fluid leaving the distal end of the perfused segment was measured using a pH-sensitive liquid ion exchanger microelectrode as described by us before (14). In these studies, the microelectrode was introduced into the collection pipette so that the tip was placed adjacent to the distal end of the perfused segment. The tip of the electrode was designed to be thin enough to be held next to the luminal opening without impeding flow. The electrode was connected via a silver–silver chloride wire to a high input impedance electrometer (Duo 773; World Precision Instruments, Sarasota, FL).

Measurement of intracellular pH (pH_i). Changes in pH_i were monitored in perfused mouse S₂ segments using the cell permeant, pH-sensitive fluorescent probe 2-bis(carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester (BCECF-AM). The dissected tubule segment was held, cannulated, and microperfused using concentric pipettes and was bathed in a modified, temperature-controlled perfusion chamber with an inlet port that permitted constant perfusion of the tubule and an exit drain port. The perfusion of the bath solution and perfusion of the lumen ensured the removal of any dye that may have leaked into the extracellular compartments. The fluid entering the chamber could be changed rapidly by switching the source of fluid from one reservoir to another. The reservoir and glass tubing leading to the chamber were water-jacketed to maintain a constant temperature so that the fluid entering the chamber was 37°C. The solutions in the reservoirs were gassed with 95% O₂:5% CO₂. A minimum amount of plastic tubing was used to connect sections of glass tubing to avoid excessive loss of gas. The pH of the fluid entering the chamber was 7.44±0.01 (*n* = 20) and was monitored continuously using a small pH probe (Lazar Research Laboratories, Los Angeles, CA).

After background fluorescent measurements were taken, the proximal tubule segment was loaded with 1 μM BCECF-AM for 20 min. After the excess dye was washed away, the fluorescence emission at 535 nm was measured in response to alternating excitation wavelengths of 440 and 495 nm using the UMANS data acquisition and analysis system (Chester Regen, Urbana, IL). The microfluorometer consisted of an inverted epifluorescent microscope (Diaphot; Nikon Inc., Melville, NY) equipped with a photon counter and with an impeller driven by a

computer-controlled stepper motor for alternating filters in front of the xenon lamp light source.

pH_i was calculated by comparing ratios of emission fluorescence intensities at 535 nm obtained at excitation wavelengths of 495 and 440 nm with ratios obtained in the same tubule using the nigericin–high potassium pH clamping technique of Thomas et al. (16). In preliminary studies, ammonium chloride loading led to acute alkalization of pH_i and removal led to acute acidification in a way consistent with results obtained by others in the proximal tubule (17).

Measurement of intracellular calcium concentration ([Ca²⁺]_i). Changes in [Ca²⁺]_i were followed using the calcium-sensitive, cell-permeant fluorescent dye, fura 2-AM (18). The tubule segments were perfused in the same way as that described above for the measurement of pH_i. The cells of the perfused segment were loaded with 2–5 μM fura 2-AM for 30 min after an initial check of the background fluorescence of the tubule. With this system, ratios of emission intensities minus background at 510 nm in response to excitation at 340 and 380 nm could be made at 0.5-s intervals. Because of photobleaching of the dye, prolonged continuous observation of [Ca²⁺]_i was not possible so that intermittent fluorescence measurements were obtained to determine calcium measurements over a 20-min period. With these precautions, emission fluorescence fell by < 10% and the ratios of emission intensities remained stable in this flow-through system in the absence of changes in the extracellular environment.

[Ca²⁺]_i was calculated from the ratio of fluorescent emission intensities minus background at 340 and 380 nm excitation wavelength (*R*), from the maximum ratio of fluorescent emission intensities (*R*_{max}), and from the minimum ratio of fluorescent emission intensities (*R*_{min}) using the formula of Grynkiewicz et al. (18):

$$[Ca^{2+}]_i = K_d \times \frac{(R - R_{min})}{(R_{max} - R)} \times (S_f/S_b),$$

where *K_d* applies to the binding of calcium to fura 2 (220 nM), *S_b* is the fluorescence of the calcium-bound dye, and *S_f* is the fluorescence of the calcium-free dye at 380 nm excitation light. *R*_{max} was obtained in the presence of 5 μM ionomycin and 1 mM extracellular calcium, whereas *R*_{min} was obtained in the presence of 5 μM ionomycin, 0 mM extracellular calcium, and 5 mM EGTA. In preliminary studies, addition of 0.2 or 1 μM ionomycin in the presence of extracellular calcium caused a marked rise in intracellular calcium.

Solutions. KRB buffer contained the following electrolytes (in mM): 140 NaCl, 25 NaHCO₃, 5 KCl, 1 MgCl, 1 NaH₂PO₄, and 1 CaCl. Angiotensin II and [Sar¹, Val⁵, Ala⁸]-angiotensin II (saralasin; Sigma Chemical Co., St. Louis, MO) were added in concentrations specified in the results. BCECF-AM, fura 2-AM, and W-7 (Molecular Probes, Eugene, OR) were prepared in 10,000X stock solutions of DMSO and added to achieve the final concentrations specified in results.

Statistical analysis. All data are presented as means±SE. Comparisons between two groups were made using Student's *t* test and comparisons of means among greater than two groups were made using analysis of variance with multiple comparisons (method of Scheffe) (19).

Results

The rates of tNH₃ production and net luminal secretion of tNH₃ were stable for ≥ 45 min at 37°C under the conditions studied. All samples used in this study were obtained within 45 min of initiating the incubation period. All tubules excluded trypan blue, introduced at the conclusion of the incubation period.

Effects of angiotensin II on tNH₃ production rates in S₂ proximal tubule segments. tNH₃ production rates were measured in S₂ segments of the mouse proximal tubule (*n* = 5 in each group) in the presence of various concentrations of angiotensin II in the basolateral bath solution (Fig. 1). The mean luminal perfusion flow rate was 20.8±0.9 nl/min, with no sig-

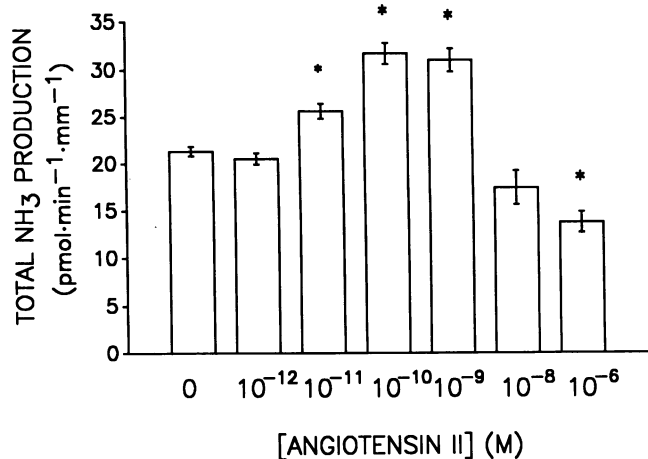


Figure 1. Angiotensin II stimulates the tNH₃ production rate at 10⁻¹¹–10⁻⁹ M but inhibits it at 10⁻⁶ M. **P* < 0.05 vs. 0 M angiotensin II.

nificant differences among the groups studied. The rate of tNH₃ production was significantly higher (*P* < 0.05) in the groups exposed to 10⁻¹¹, 10⁻¹⁰, and 10⁻⁹ M angiotensin II (25.6 ± 0.8, 31.7 ± 1.1, and 31.0 ± 1.2 pmol/min per mm, respectively) than in tubules exposed to 0 or 10⁻¹² M angiotensin II. Exposure to 10⁻⁸ M angiotensin II had no significant effect on the rate of tNH₃ production (17.4 ± 1.8 pmol/min per mm). At a higher concentration of 10⁻⁶ M, the rate was significantly lower than control rates (13.7 ± 1.1, *P* < 0.05). Thus, angiotensin II had a biphasic effect on tNH₃ production by the mouse S₂ segment such that tNH₃ production rates were higher in S₂ segments exposed to 10⁻¹¹–10⁻⁹ M angiotensin II but were lower in segments exposed to 10⁻⁶ M angiotensin II.

Effect of saralasin on tNH₃ production. Saralasin, a direct angiotensin II antagonist, was added at 10⁻⁷ M to the bath solution surrounding the perfused proximal tubule segments in the presence or absence of 10⁻¹⁰ M angiotensin II. As shown in Fig. 2, the tNH₃ production rates with the addition of saralasin by itself or with angiotensin II did not significantly differ from control rates (20.8 ± 0.5 for saralasin + angiotensin II group; *n* = 5 in each group). Thus, saralasin blocked the effect of 10⁻¹⁰ M angiotensin II to stimulate the tNH₃ production rate by

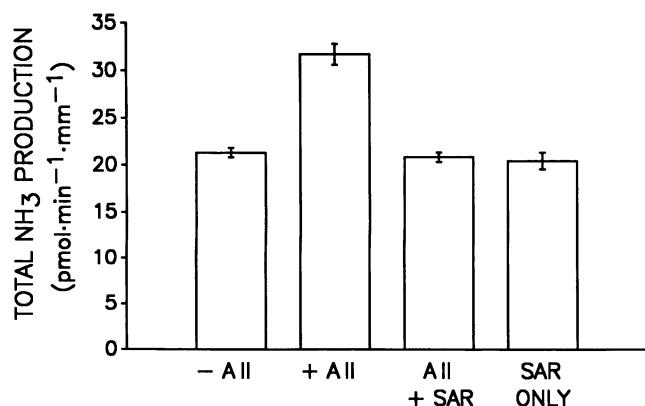


Figure 2. 10⁻⁷ M saralasin (SAR) inhibits the stimulatory effect of 10⁻¹⁰ M angiotensin II (A II) on the tNH₃ production rate in mouse S₂ segments.

mouse proximal tubules without exerting a significant effect on tNH₃ production rates by itself.

Effect of angiotensin II on net tNH₃ secretion and luminal fluid acidification. The effect of the addition of 10⁻¹⁰ M angiotensin II to the bath solution on net tNH₃ secretion was examined (Fig. 3). The net luminal tNH₃ secretory rate was significantly (*P* < 0.05) lower in tubules bathed in KRB buffer containing angiotensin II (7.9 ± 0.4 pmol/min per mm) than in tubules bathed without angiotensin II (11.0 ± 0.7). Thus, at a concentration of angiotensin II that stimulated the tNH₃ production rate, angiotensin II inhibited net tNH₃ secretion such that the percentage of the tNH₃ produced that was secreted into the lumen was reduced from 55% in control proximal tubule segments to 30% in angiotensin II-treated segments.

Na⁺-H⁺ exchanger activity in the proximal tubule is stimulated by angiotensin II (20). Stimulation of the Na⁺-H⁺ exchange would be expected to enhance net tNH₃ secretion by increasing the luminal fluid acidity and by increasing Na⁺-NH₄⁺ exchange (14). To ascertain whether luminal acidification was stimulated by angiotensin II in isolated perfused mouse proximal tubule segments, angiotensin II (10⁻¹⁰ M) was added to the peritubular bath solution and luminal pH was monitored using a pH-sensitive microelectrode placed at the opening of the distal end of the perfused segment. The luminal fluid pH fell significantly in response to angiotensin II in a reversible fashion (Fig. 4). Addition of 1 mM amiloride to the luminal perfusion solution completely blocked luminal acidification in the presence of peritubular angiotensin II (*n* = 5 in each group; luminal fluid pH = 7.44 ± 0.01 in angiotensin II + amiloride vs. 7.29 ± 0.01 in angiotensin II alone; *P* < 0.01). Thus, angiotensin II enhanced amiloride-sensitive luminal fluid acidification in mouse proximal tubule segments.

One possible mechanism by which angiotensin II could reduce net luminal tNH₃ secretion despite enhanced Na⁺-H⁺ exchanger activity would be through an increase in backflux of tNH₃ out of the lumen. To examine whether angiotensin II affected luminal efflux of tNH₃, we added known amounts of tNH₃ (10 mM) to the luminal perfusion solution and determined the rate of efflux of tNH₃ out of the lumen by measuring the tNH₃ leaving the distal end of the perfused segment. As illustrated in Table I, there was no significant difference between the rate of efflux of tNH₃ in tubules bathed in control KRB buffer without angiotensin II and tubules bathed in KRB

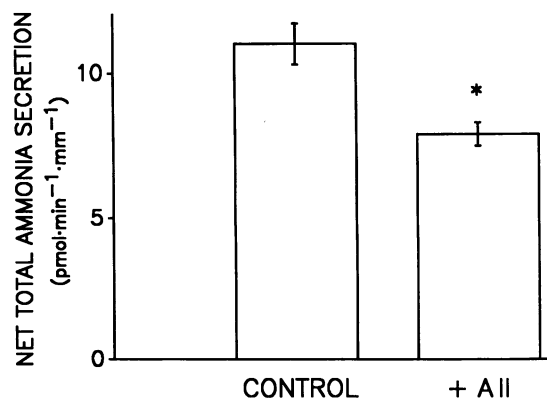


Figure 3. Angiotensin II (A II) at 10⁻¹⁰ M inhibited net tNH₃ secretion. **P* < 0.05 vs. control (0 M angiotensin II).

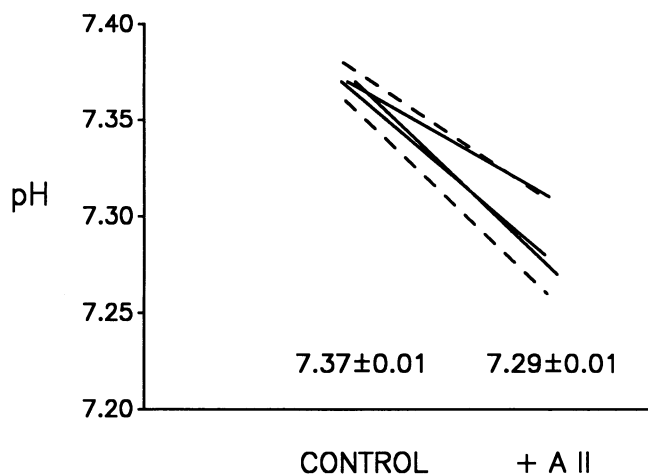


Figure 4. Angiotensin II (A II) at 10^{-10} M significantly stimulated luminal fluid acidification. Dashed lines represent experiments in which luminal pH was measured in S_2 segments exposed to A II and then A II was removed, whereas the solid lines represent segments initially exposed to no A II and then A II was added. * $P < 0.01$ A II vs. control.

buffer containing 10^{-10} M angiotensin II. Thus, angiotensin II had no measurable effect on tNH_3 efflux out of the lumen.

Mediation of the stimulatory effect of angiotensin II on ammonia production and luminal acidification. pH_i may mediate the stimulatory effect of systemic acidosis on tNH_3 production and luminal acidification. Angiotensin II could stimulate both processes by primarily reducing the pH_i . As shown in Fig. 5, 10^{-10} M angiotensin II, which stimulated luminal acidification, had no net effect on pH_i (control 7.01 ± 0.02 vs. angiotensin II 7.03 ± 0.03 ; $n = 8$ in each group). Similarly, 10^{-6} M angiotensin II, which inhibited tNH_3 production rates, also had no significant effect on pH_i (control 7.02 ± 0.03 vs. angiotensin II 7.02 ± 0.02 ; $n = 7$ in each group). Thus, no measurable changes in pH_i were involved in mediating the effects of angiotensin II on tNH_3 production and luminal acidification. Furthermore, since luminal acidification involves H^+ exit from the cell to luminal fluid, the results suggest that exposure to 10^{-10} M angiotensin II stimulates acid secretion and base removal at equal rates. These results suggest that, when stimulated by angiotensin II, luminal acidification (cellular acid extrusion) was balanced by equal rates of base removal or acid entry, leading to stable pH_i measurements.

Chobanian and Julin (10) recently demonstrated that inhibitors of calcium-calmodulin-dependent pathway (W-7, trifluoperazine) inhibit the stimulatory effect of angiotensin II on tNH_3 production in isolated dog proximal tubule segments.

Table I. Total Ammonia Efflux

Angiotensin II concentration	Tubule length	Flow rate	tNH_3 efflux rate
M	mm	nl/min	pmol/min per mm
0	0.6 ± 0.1	20.9 ± 0.9	220 ± 7
10^{-10}	0.6 ± 0.1	21.4 ± 1.0	230 ± 6

$n = 6$ in each group. No statistically significant differences between groups.

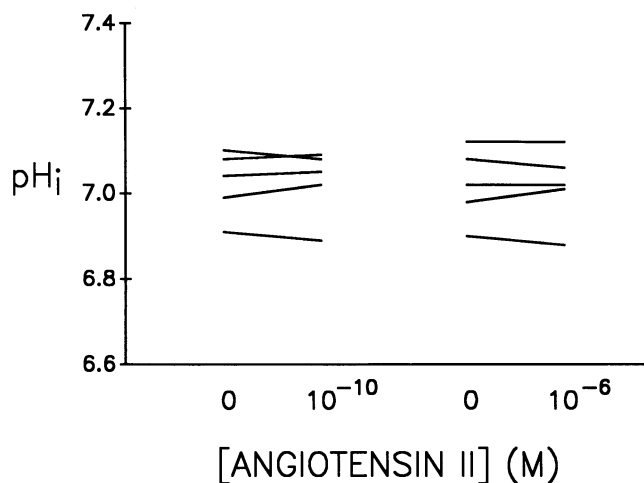


Figure 5. Absence of an effect of addition of 10^{-10} or 10^{-6} M angiotensin II on intracellular pH.

We examined the effect of addition of bath (basolateral) $10 \mu M$ W-7 on the effects of 10^{-10} M angiotensin II on luminal acidification and tNH_3 production rates. Although W-7 by itself had no significant effect on luminal acidification (pH 7.37 ± 0.01 with and 7.36 ± 0.01 without W-7, $n = 5$), it blocked the stimulatory effect of angiotensin II on luminal acidification such that luminal pH increased from 7.28 ± 0.01 for angiotensin II to 7.39 ± 0.01 for angiotensin II + W-7 ($n = 5$). In addition, W-7 inhibited the stimulatory effect of 10^{-10} M angiotensin II on tNH_3 production rate (Table II). Thus, both the effects of 10^{-10} M angiotensin II to stimulate tNH_3 production and luminal acidification may be mediated through a calcium-calmodulin-dependent mechanism.

To explore further the role of $[Ca^{2+}]_i$ as a mediator of the actions of angiotensin II, $[Ca^{2+}]_i$ was determined in response to different concentrations of angiotensin II (Fig. 6). Control $[Ca^{2+}]_i$ without angiotensin II ($n = 5$) was 100 ± 5 nM. Exposure of cells to angiotensin II at 10^{-10} M resulted in a significant sustained increase in $[Ca^{2+}]_i$, to 165 ± 5 nM, whereas 10^{-6} M angiotensin II had no significant effect on $[Ca^{2+}]_i$ (103 ± 7 nM). Such results are consistent with a role of an increased $[Ca^{2+}]_i$ in the stimulation of tNH_3 production in the mouse S_2 segment since the concentrations of angiotensin II that increased $[Ca^{2+}]_i$ are the same as those that increased tNH_3 production rates (Fig. 1). On the other hand, the inhibitory effect of 10^{-6} M angiotensin II on tNH_3 production occurred in the absence of changes in $[Ca^{2+}]_i$.

The effect of 10^{-10} M angiotensin II on tNH_3 production was also examined with the removal of extracellular calcium in

Table II. Effects of Angiotensin II and W-7 on tNH_3 Production

Conditions	tNH_3 production rates
	pmol/min per mm
10 mM W-7	18.6 ± 1.0
10^{-10} angiotensin II	$30.7 \pm 1.0^*$
10 mM W-7 + 10^{-10} angiotensin II	19.9 ± 0.8

$n = 5$ in each group. * $P < 0.01$ vs other groups.

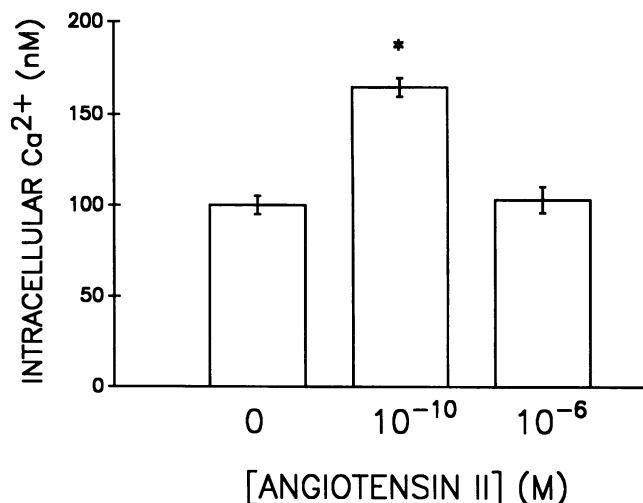


Figure 6. Angiotensin II increases $[Ca^{2+}]_i$ in proximal tubule cells at 10^{-10} M but not at 10^{-6} M. * $P < 0.01$ vs. 0 M angiotensin II.

the bath and perfusion solutions by the addition of 1 mM EGTA to 0 mM calcium solutions. Removal of extracellular calcium blocked the stimulatory effect of angiotensin II on tNH_3 production such that tubules incubated with angiotensin II produced tNH_3 at a rate of 13.0 ± 1.3 pmol/min per mm, whereas those incubated with calcium removal only produced tNH_3 at a rate of 15.4 ± 0.8 ($n = 4$ in each group). Similarly, removal of extracellular calcium blocked the sustained rise in $[Ca^{2+}]_i$ observed with exposure to 10^{-10} M angiotensin II. The $[Ca^{2+}]_i$ of four proximal tubule segments was examined in the absence of extracellular calcium and with 10^{-10} M angiotensin II in the absence of extracellular calcium. The baseline $[Ca^{2+}]_i$ of proximal tubule segments in the absence of extracellular calcium was 92 ± 4 nM and remained unchanged after the addition of angiotensin II (93 ± 2 nM). Thus, the effects of 10^{-10} M angiotensin II on tNH_3 production and $[Ca^{2+}]_i$ were blocked by the removal of extracellular calcium.

Discussion

Angiotensin II is a potent regulator of transport by the proximal tubule (1, 2, 4, 21). The results of the present study demonstrate important effects of angiotensin II on tNH_3 production and secretion by mouse proximal tubules. Angiotensin II stimulates tNH_3 production at concentrations ranging from 10^{-11} to 10^{-9} M and inhibits tNH_3 production rates at concentrations $> 10^{-8}$ M. In addition, the effect of angiotensin II at a concentration of 10^{-10} M was completely inhibited by the angiotensin II antagonist saralasin, suggesting a receptor-mediated event.

These data differ in certain ways from the data of Chobanian and Julin (10). First, the stimulatory effect of angiotensin II on tNH_3 production was observed at much higher concentrations of angiotensin II in the dog proximal tubule than in the mouse proximal tubule (10^{-11} M in the mouse vs. 10^{-8} M in the dog). Second, an inhibitory effect of angiotensin II was observed at high concentrations in mouse proximal tubules but not in dog tubules. The reason for the differences in these observations may be related to differences in the species used or to differences in the preparations studied (isolated perfused tubules vs. tubule fragment preparations).

A concentration-dependent effect of angiotensin II on tNH_3 production follows a pattern similar to one observed by others (1, 2) for its effect on net fluid reabsorption by the proximal tubule. Net fluid reabsorption in rabbit proximal convoluted tubules was increased in the presence of 10^{-11} M angiotensin II but was suppressed by 10^{-6} M angiotensin II (2). The explanation for the biphasic nature of the effects of angiotensin II on net fluid reabsorption and on tNH_3 production is not clear but may be related to differences in secondary messenger responses induced by different concentrations of angiotensin II.

The role of $[Ca^{2+}]_i$ as a mediator for the stimulatory effect of angiotensin II on tNH_3 production was suggested by the effect of W-7, an inhibitor of calcium-calmodulin-dependent kinase, to inhibit the action of 10^{-10} M angiotensin II on tNH_3 production. The present results are consistent with the results of Chobanian and Julin (10), who demonstrated that the stimulatory effect of angiotensin II on tNH_3 production by dog proximal tubules was blocked by W-7. In the present studies, direct measurements of $[Ca^{2+}]_i$ suggest a correlation between the abilities of angiotensin II to induce a rise in $[Ca^{2+}]_i$ and to stimulate tNH_3 production. Concentrations of angiotensin II that do not stimulate tNH_3 production do not cause a rise in $[Ca^{2+}]_i$. Thus, a rise in $[Ca^{2+}]_i$ induced by lower concentrations of angiotensin II is associated with an increase in the rate of tNH_3 production, while the mediator for the inhibitory effect of higher concentrations of angiotensin II is unclear and requires additional study.

These data contrast with studies demonstrating that angiotensin II at 10^{-5} M induced a rise in $[Ca^{2+}]_i$ in cultured rabbit proximal tubular cells (22) and in a rat proximal tubule preparation (23). These data also are inconsistent with recent studies by Chatsudthipong and Chan (24), who suggested that the inhibitory effect of angiotensin II on fluid and bicarbonate reabsorption was mediated by a rise in $[Ca^{2+}]_i$. An explanation for the differences in results may be related to the differences in the models used. In the present study, the same isolated perfused tubule model was used to study the effects of angiotensin II on tNH_3 production and on $[Ca^{2+}]_i$. In contrast, Chatsudthipong and Chan used the *in vivo* perfused rat proximal tubule to study the effects of angiotensin II on tubular transport and a different model, namely, a collagenase-treated proximal tubule preparation, to study the effect of angiotensin II on $[Ca^{2+}]_i$. It is possible that during the isolation procedure, the tubules lost some of their sensitivity to angiotensin II so that higher concentrations of angiotensin II were required to observe a rise in $[Ca^{2+}]_i$. Indeed, studies by Mujais (25) on collagenase-treated isolated rat proximal tubule segments demonstrated maximal specific binding at 10 nM angiotensin II, a concentration 100 times higher than that required to achieve maximal stimulation of tNH_3 production in the present study.

Changes in pH_i could also serve as a primary stimulus for tNH_3 production. Yet, angiotensin II at concentrations that stimulated (10^{-10} M) or inhibited (10^{-6} M) tNH_3 production rates did not significantly affect pH_i . Thus, the effect of angiotensin to stimulate tNH_3 production was not mediated by measurable changes in pH_i .

Because the pH_i remained constant during stimulation of luminal acidification (cellular acid exit), an increased rate of base exit must have accompanied the increased rate of acid exit. These data are consistent with the results of Geibel and colleagues (26), who demonstrated that angiotensin II stimulated both luminal Na^+-H^+ exchange and basolateral Na^+

HCO₃⁻ cotransport in the isolated perfused rabbit superficial S₁ segment.

In contrast to the stimulatory effect of 10⁻¹⁰ M angiotensin II on tNH₃ production, 10⁻¹⁰ M angiotensin II inhibited net luminal secretion of tNH₃ so that the percentage of the tNH₃ produced that was secreted was much lower in the presence of angiotensin II than in its absence. The inhibitory effect of angiotensin II on net tNH₃ secretion occurred in the presence of enhanced luminal fluid acidification and in the absence of measurable changes in rates of tNH₃ loss from the luminal fluid. These studies suggested that inhibition of net tNH₃ secretion by angiotensin II resulted from diminished tubular cell secretion into the lumen rather than diminished production or enhanced luminal exit of tNH₃.

The apparent dissociation between net tNH₃ secretion and Na⁺-H⁺ exchanger activity was surprising in view of both our previous study (14), demonstrating a marked dependence of net tNH₃ secretion on Na⁺-H⁺ exchange, and our present results, demonstrating stimulation of luminal fluid amiloride-sensitive acidification. The results of the present studies suggested that angiotensin II did not alter net luminal tNH₃ secretion by altering tNH₃ efflux rates. One possible explanation for the apparent dissociation between angiotensin II-stimulated Na⁺-H⁺ exchange activity and net tNH₃ secretion could be that the affinity of the Na⁺-H⁺ exchanger for H⁺ compared with NH₄⁺ was enhanced, so that H⁺ secretion was enhanced, whereas NH₄⁺ secretion was diminished.

The blocking effect of W-7 on the stimulatory effect of angiotensin II on luminal acidification suggested a role of the calcium-calmodulin pathway. The latter result contrasted with results of Weinman and co-workers (27), who demonstrated that calcium-calmodulin protein kinase II inhibited the activity of the reconstituted renal brush border Na⁺-H⁺ exchanger. The reasons for the differences observed are unclear but may be related to differences in preparations (intact tubule vs. reconstituted Na⁺-H⁺ exchanger).

The overall effect of angiotensin II on tNH₃ delivery out of the proximal tubule depends on the net result of the contrasting effects of angiotensin II on tNH₃ production and net secretion rates. At lower concentrations, angiotensin II stimulates tNH₃ production and inhibits net luminal secretion by mouse proximal tubules such that the overall net effect is a reduction in net tNH₃ secretion by the isolated perfused mouse proximal tubule. The countervailing effects of angiotensin II on production and transport of tNH₃ may be important in limiting the effect of angiotensin II on overall acid-base balance. We have observed similar counteracting effects of luminal and bath potassium concentrations on tNH₃ production and secretion in mouse proximal tubules (13). The opposing effects of angiotensin II on tNH₃ production and secretion rates represent another example of the dissociation between tNH₃ production and transport in the proximal tubule.

The mechanism by which angiotensin II reduces net luminal secretion of tNH₃ is unclear. The inhibitory effect of angiotensin II on net secretion could result from the inhibition of the luminal secretory step, from the stimulation of the basolateral exit pathway, or from the stimulation of the reabsorption of tNH₃ from the lumen. The present data, which demonstrate that the luminal exit rate of tNH₃ is not affected by angiotensin II, suggest that changes in reabsorption of tNH₃ do not occur with angiotensin II. Whether angiotensin II, which stimu-

lates the luminal Na⁺-H⁺ exchanger (20) and basolateral Na⁺-K⁺ ATPase (28), also alters the transport characteristics of these transporters for NH₄⁺ (14, 29-31) is speculative and requires further study.

In conclusion, angiotensin II has a concentration-dependent effect on tNH₃ production; production is stimulated in the lower concentration ranges but inhibited at high concentrations. The stimulatory effect of angiotensin II is paralleled by angiotensin II-induced increases in intracellular calcium and is inhibited by the calmodulin pathway inhibitor, W-7. At a concentration that stimulates tNH₃ production, angiotensin II inhibits net luminal tNH₃ secretion by a mechanism not related to changes in transepithelial permeability to tNH₃ and does not parallel changes in Na⁺-H⁺ exchange.

Acknowledgments

This research was supported by the Department of Veterans Affairs and by a grant from the National Institute of Diabetes and Digestive and Kidney Diseases (DK-38463). G. T. Nagami is a Clinical Investigator of the Department of Veterans Affairs.

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