

Angiotensin II Directly Stimulates Sodium Transport In Rabbit Proximal Convolted Tubules

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Abstract. Numerous previous studies have proposed a role for angiotensin II (AII) in the renal regulation of salt balance. At least one nephron site, the proximal convoluted segment, has been implicated in this role. We used in vitro microperfusion of rabbit proximal convoluted tubules to further examine this question. To insure use of appropriate in vivo concentrations as well as potency of the hormone in vitro, we measured plasma AII levels by radioimmunoassay in normal, sodium-depleted, and adrenalectomized rabbits, and measured AII activity by bioassay after incubation in various microperfusion baths. Plasma levels ranged from $\sim 2 \times 10^{-11}$ to 5×10^{-11} M. AII activity was stable in Ringer's solution plus albumin, but not in rabbit serum or Ringer's solution plus fetal calf serum.

In Ringer's solution plus albumin, physiologic concentrations of AII stimulated volume reabsorption (J_v). 10^{-11} M AII increased J_v by 16% ($P < 0.01$). 10^{-10} M AII produced a lesser increase, 7.5% ($P < 0.05$). At a frequently studied, but probably pharmacologic dose, 10^{-7} M AII inhibited J_v by 24% ($P < 0.001$). AII at 10^{-11} M did not stimulate J_v in the presence of 10^{-7} M saralasin. Though previous studies have suggested agonistic effects of saralasin alone in epithelia, we found no significant effect of 10^{-7} M saralasin on J_v . None of the AII doses measurably changed transepithelial voltage.

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We conclude that AII in physiologic doses directly stimulates J_v in proximal convoluted tubules and this effect is probably receptor mediated and, within the limits of detection, electroneutral.

Introduction

The role of angiotensin II (AII)¹ in salt and water homeostasis has been increasingly well described in recent years. In addition to its role in aldosterone synthesis, AII alters the slope of osmolar antidiuretic hormone release (1), produces a central dipsogenic response and salt appetite (1), and enhances salt and water transport by the small intestine (2, 3) and colon (2, 4, 5).

Since the demonstration by Hughes-Jones et al. in 1949 (6) that systemic infusions of a crude renin preparation produced natriuresis, numerous investigators have examined the role of the renin-angiotensin system, and particularly AII, in renal salt and water transport. Clearance studies in several species demonstrated that, in general, systemic infusion of AII at rates < 100 ng \cdot kg⁻¹ \cdot min⁻¹ produced antinatriuresis, whereas higher infusion rates produced natriuresis (7). When measured, the filtration fraction rose at all AII infusion rates. Since an increased filtration fraction favors proximal tubular volume reabsorption via peritubular mechanisms, a direct effect of AII on proximal function could not be ascertained by these studies.

Though several micropuncture studies utilizing systemic AII administration found no effect on proximal volume reabsorption (8-10), other studies applying AII directly to the peritubular surface found a biphasic dose response. Steven (11) found inhibition of proximal volume reabsorption when 2×10^{-8} M AII was infused into superficial peritubular capillaries. Spinelli and Walther (12) applied AII to the decapsulated cortical surface and found stimulation of proximal reabsorption at $\sim 10^{-10}$ M, but inhibition at $\sim 10^{-5}$ M. Harris and Young (13), using in vivo microperfusion of both lumen and capillaries, showed maximal stimulation of sodium efflux from the lumen at peritubular concentrations of 10^{-11} M, but inhibition at 10^{-6} M.

While suggesting a direct effect of AII, these studies leave several questions unanswered. First, only superficial convolutions

1. Abbreviations used in this paper: AII, angiotensin II; CVP, constant volume pipette; J_v , net volume flux; V_T , transepithelial voltage.

were examined. Since deep nephrons differ from superficial ones in a number of ways, including lower single-nephron renin activities (14) and higher volume reabsorptive rates (15), the response of deep nephrons to AII is of interest. Second, renal nerves were presumably intact in the studies cited. Since AII is known to enhance norepinephrine release from sympathetic nerve endings, and since catecholamines per se can influence proximal reabsorption (16), a direct effect of AII on the epithelium cannot be concluded. Third, AII may be vasoconstrictive, particularly at high doses, and thus resistance changes in peritubular capillaries induced during AII infusion have been suggested (17).

The isolated, perfused tubule appears to be an ideal technique by which to examine AII's effect. Deep convolutions are easily accessible, dissected tubules are free of sympathetic nerve endings by electron microscopy (Tisher, C. Craig, personal communication), and transport mechanisms are relatively easily defined. Although one early isolated tubule study found no effect of 10^{-6} M bath AII on proximal volume reabsorption (18), we have reexamined AII effects using this technique and have found a direct, biphasic dose response of AII on proximal volume reabsorption.

Methods

AII radioimmunoassay (RIA) and plasma levels. AII concentration was measured in plasma using an RIA developed by Arant and Stephenson (19). This assay has a lower limit sensitivity of ~ 3 pg/ml and a cross-reactivity with AII of $\sim 60\%$. Plasma levels were measured in three groups of rabbits: (a) normal rabbits on standard laboratory chow (Wayne Feeds; Chicago, IL) and tap water, who gained an average of 0.26 ± 0.03 kg/wk, (b) normal rabbits given furosemide, 2.0 mg/kg s.c. body weight, followed by 7 d of a zero-sodium diet (ICN Nutritional Biochemicals, Cleveland, OH) and distilled water, during which time the mean weight loss was 0.11 ± 0.06 kg, and (c) rabbits 7–14 d postadrenalectomy (20) fed normal rabbit chow and 0.9% saline for drinking water. At decapitation, free-flowing mixed arteriovenous blood was collected in chilled heparin-containing beakers, centrifuged, and the plasma immediately placed in tubes containing 250 mM 1,10 ortho-phenanthroline and 125 mM EDTA for RIA.

AII bioassay and stability in tubule baths. To determine stability of synthetic AII in various standard isolated tubule baths, a bioassay for AII was used (21). Briefly, rat stomach and colon strips were mounted on tension transducers and superfused with oxygenated Ringer's solution. Spontaneous muscular activity was abolished with a variety of neuromuscular blockers (21), and contractile responses to 1 and 10 ng 5-ile-AII (Beckman Instruments, Inc., Palo Alto, CA) were recorded. 5-ile-AII was incubated in commercial rabbit serum (Irvine Scientific; Santa Ana, CA) or Ringer's bicarbonate plus 5% vol/vol fetal calf serum (Pel-freez Biologicals; Rogers, AR) at 37°C , or in Ringer's bicarbonate plus 6 g/100 ml bovine serum albumin (CRG-7, Armour Pharmaceutical Co., Kankakee, IL) at room temperature and at 37°C .

Microperfusion—general experimental features. Normal rabbits on regular laboratory chow were used in all microperfusion experiments. The general methods of Burg and Orloff (18) and our modifications (15) of in vitro tubule perfusion have been previously described. Female New Zealand white rabbits between 1.5 and 2.5 kg were killed by de-

capitation. The left kidney was quickly removed and decapsulated. Coronal slices 1–2 mm thick were cut and placed into Ringer's bicarbonate plus 6 g/100 ml albumin at 4°C . Dissection was performed in the same chilled solution with the aid of a dissecting microscope. Proximal convoluted tubules were dissected from the deeper one-half of the cortex, usually from areas adjacent to an arcuate artery. Though the distinction between true surface convolutions and juxtamedullary convolutions is clear (15), that between midcortical and juxtamedullary convolutions is less so. Therefore, since not every tubule was from the deepest cortex, we have used the term "nonsurface" for our tubule population. Likewise, although about one-third of our tubules had a glomerulus attached by a neck region, no attempt was made to specifically dissect S_1 vs. S_2 segments.

After dissection, the tubules were transferred to a thermostatically controlled chamber of $\cong 1$ cm³ volume containing ports for constant infusion and removal of bath fluid, as well as for placement of Ringer's bicarbonate agarose bridges. The two ends of the tubule were sucked into glass holding-pipettes containing elastomeric silicone resin (Sylgard 184, Dow Corning Corp.; Midland, MI) at the tips, providing a water and electrical seal. The perfusion pipette, previously centered in one of the holding pipettes, was advanced into the tubule lumen, 50–100 μm . Perfusion rate was controlled hydrostatically. Transepithelial voltage (V_T) was measured continuously at the perfusion pipette via a Ringer's bicarbonate agarose bridge connected to a calomel electrode; a similar agarose bridge in the perfusion chamber, also connected to a calomel electrode, completed the circuit. V_T was measured with a battery-operated Keithley electrometer (model 602, Keithley Instruments Inc., Cleveland, OH) and recorded on a strip-chart recorder (Rikadenki model B261, Soltec Corp.; Sun Valley, CA).

Timed collections of fluid from the tubule were made into a constant-volume pipette (CVP) under water-equilibrated mineral oil. Calibration of this pipette was performed daily as follows. At 37°C , the perfusion pipette was advanced into a pipette containing a short (~ 200 μm) column of Sylgard at the tip and water-equilibrated mineral oil above the Sylgard. A bolus of perfusate was injected above the Sylgard into the oil, and the CVP was filled in triplicate from this perfusate.

Microperfusion—solutions. Solutions were synthetic ones designed to simulate rabbit serum and glomerular ultrafiltrate. Bath solution contained in millimolar: Na, 145; K, 5; Cl, 112; HCO_3^- , 25; Ca, 2.4; PO_4 , 2.3; Mg, 1.0; SO_4 , 1.0; acetate, 10; glucose, 8; alanine, 5. This solution also contained 6 g/100 ml defatted bovine serum albumin (CRG-7). The perfusate was an ultrafiltrate of the bath, prepared by pressure dialysis through Amincon PM-30 membranes (American Instrument Co., Silver Springs, MD). Baths were bubbled at 37°C with 95% O_2 /5% CO_2 to pH 7.40, drawn anaerobically into syringes, and pumped through the perfusion chamber at ≥ 0.6 cm³/min. We have previously shown by on-line bath pH electrodes that this method produces extremely stable bath pH (22). Perfusate was equilibrated with 95% O_2 /5% CO_2 at 37°C , and ^3H -inulin was added (see below). Osmolality of bath and perfusate were matched at 295 mosM with small additions of water or NaCl.

Microperfusion—net volume flux (J_v) measurements. J_v was measured using perfusate methoxy- ^3H -inulin (New England Nuclear, Boston, MA) exhaustively dialyzed by the method of Schafer et al. (23). J_v was calculated from the equation: $J_v = V_i - V_o/L$, where V_i is the perfusion rate in nanoliters per minute, V_o is the collection rate in nanoliters per minute, and L is the tubule length in millimeters. V_o was measured directly, and V_i was calculated from $V_o = \text{cpm}_o/\text{cpm}_i$, where cpm_o and cpm_i are ^3H counts per minute per nanoliter of collected and perfused fluid,

respectively. L was measured directly by eyepiece micrometer. Care was taken to maximize sensitivity and precision of J_v measurements.²

Microperfusion—experimental protocol. The general protocol was the same in all experiments. After being hooked up to the pipettes, the tubule was warmed to 37°C and allowed to equilibrate for 30 min. During this and all subsequent periods the bath contained 1% vol/vol AII vehicle: Tris, 50 mM; glycine, 100 mM, BSA, 0.2 g/100 ml, pH 5.0. This vehicle did not measurably change the bath pH or osmolality. After the equilibration period, control period collections were made (see below).

The continuously flowing bath was then changed to contain 5-ile-AII, the 5-position moiety thought to be normally circulating in the rabbit (24), and/or saralasin. Synthetic 5-ile-AII or saralasin (Beckman Instruments, Inc.) was dissolved in the vehicle described above, aliquoted, and frozen until just before serial dilution and addition to the bath. Fresh stock solution was made frequently. 30 min after exposure of the tubule to AII a second group of collections was made. In some experiments done with AII at 10^{-11} M, bath remaining in the infusion syringe at the end of the experimental period was assayed for AII concentration by RIA as described above. The concentration at 10^{-11} M should be 10.4 pg/ml. Measured values were 11.78 ± 0.62 (SEM) pg/ml ($n = 5$), demonstrating presence of AII in the bath at the desired concentration. All experiments except those with AII plus saralasin contained a third (recovery) period in which the bath again contained only vehicle. 30 min after beginning this period, a final set of collections was made. Five collections were made per period.

Statistics. For any given tubule the means of four to five collections per period were used as values for that tubule. The group means for the control, experimental, and recovery periods were then computed using each tubule mean. In addition, to avoid loss of statistical power due to variability of control J_v values, the experimental and recovery tubule means were also calculated as percent changes from the control

2. For a given experimental perturbation, the minimum increment or decrement of J_v that can be detected is a function of the precision with which the CVP is filled, and the counting accuracy, a square-root function of the total counts. In turn, total counts are determined by perfusate counts per nanoliter, tubule length, J_v , perfusion rate, CVP volume, and total time the collected fluid is counted. With regard to precision of filling the CVP, triplicate calibrations performed daily had a total range around the mean of $\pm 0.5\%$. During an experiment, collections for any given experimental period were made in quintuplicate to minimize sampling error. With regard to counting accuracy, the following mean values apply to these experiments: tubule length, 1.28 mm; perfusate ^3H counts, 61.9 cpm/nl; CVP, 160 nl; J_v , 0.747 nl/mm per min; V_i , 12.1 nl/min. All samples were counted for 10 min in a Tracor model 6892 liquid scintillation counter (Tracor Analytic; Atlanta, GA). Thus, under these circumstances, the total mean counts for one collection are $108,570 \pm 329$, or $\pm 0.30\%$. Using the formula above for J_v and the mean values given, a 0.30% variation in collected counts would appear as a variation in J_v of ± 0.026 nl/mm per min, i.e., the mean detectable change in J_v above counting "noise" is 0.026 nl/mm per min, assuming 0% variability in filling of the CVP. Even by assuming worst-case additive random errors, i.e., scintillation counting at the upper end of the variation range plus overfilling the CVP by 0.5%, with both occurring only during the experimental period, the mean minimal detectable change in J_v rises to 0.069 nl/mm per min, still smaller than the smallest mean change seen with AII in these experiments.

tubule means. Student's two-tailed t test for paired samples was used for mean values derived from the same tubule; the t test for unpaired samples was used for mean values from different tubules. Differences were considered significant if $P < 0.05$. Other values such as tubule length, V_T , and perfusion rate are expressed as mean \pm SEM.

Results

Circulating plasma AII levels. Plasma AII concentration was measured by RIA in the rabbit as a rough approximation of renal cortical values. As shown in Fig. 1, the mixed arteriovenous plasma AII concentration of normal rabbits was 18.5 ± 4 pg/ml. This value rises with maneuvers stimulating renal sodium retention, i.e., diuretic- and diet-induced sodium depletion and adrenalectomy, to 30.4 ± 4 pg/ml and 43.7 ± 10 pg/ml, respectively ($P < 0.05$ normal vs. adrenalectomy). These values translate into molarities of $\sim 2 \times 10^{-11}$ M for normals and $\leq 5 \times 10^{-11}$ M for adrenalectomized rabbits.

Stability of AII in various isolated tubule baths. To insure the presence of AII at desired concentrations in the bath throughout the experiment, the stability of synthetic AII was examined by bioassay following incubation in three standard microperfusion baths. As shown in Fig. 2, rapid loss of bioactivity occurred in commercial rabbit serum, with a $t_{1/2}$ of ~ 10 min. Likewise, the addition of 5% vol/vol fetal calf serum to Ringer's solution led to a loss of $\sim 50\%$ of initial activity. On the contrary, incubation in Ringer's solution plus 6 g/100 ml albumin led to a very slight loss of activity at 37°C, the temperature in the perfusion chamber, and to no loss of activity at 23°C, the temperature of the infusion syringe containing bath plus hormone. Ringer's bicarbonate plus albumin was therefore used in all microperfusion experiments.

Response of J_v to physiological doses of AII. In all microperfusion experiments, AII was added to the bath only, since this is the most likely site for a control function of AII on the proximal tubule (25), and because luminal AII has been shown

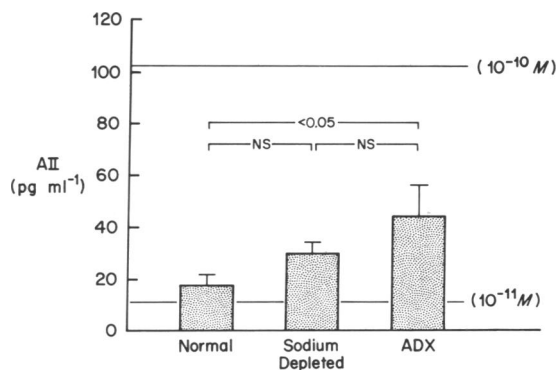


Figure 1. Mixed arteriovenous plasma AII levels measured by RIA in normal rabbits, rabbits given furosemide and a low-sodium diet, and adrenalectomized (ADX) rabbits. $n = 6$ for each group.

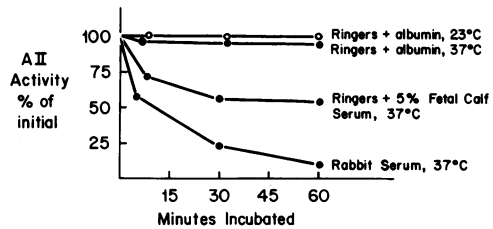


Figure 2. Stability of AII in three standard microperfusion baths. Each point represents the mean of two determinations, which varied by <math><6\%</math>. The open circles represent the incubation conditions which predominated during the microperfusion experiments, i.e., AII remained in a syringe in Ringer's plus albumin at 23°C until the moment it entered the perfusion chamber, when it was warmed to 37°C.

to be rapidly hydrolyzed to small peptide fragments in this segment (26). As shown in Fig. 3, 10^{-11} M AII added to the bath in eight tubules increased J_v in each case. The mean J_v for control, AII, and recovery periods was 0.666 ± 0.07 , 0.767 ± 0.08 , and 0.695 ± 0.08 nl/mm per min. This rise in J_v was significant at $P < 0.01$. When expressed as a percentage of the control period value, J_v rose by a mean of 16% with 10^{-11} M bath AII. Although the AII period J_v was not significantly different from that of the recovery period, it was significantly different ($P < 0.05$) from the combined control and recovery period J_v 's. Mean length of tubules was 1.20 ± 0.07 mm, and mean control period V_T (lumen negative) was -4.6 ± 0.5 mV. Perfusion rates for three consecutive periods were comparable at 12.01 ± 0.25 , 12.78 ± 0.42 , and 11.93 ± 0.38 nl/min, respectively.

When 10^{-10} M AII was added to the bath, a smaller increase in J_v was seen. As shown in Fig. 4, AII caused J_v to rise in seven tubules and to fall slightly in two tubules. Mean J_v 's were

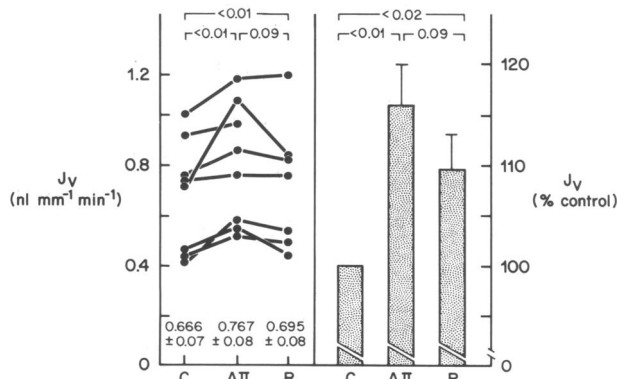


Figure 3. Effect of 10^{-11} M bath AII on proximal convoluted tubule J_v . (Left) Values for individual tubules during control (C), AII, and recovery (R) periods. Mean $J_v \pm \text{SEM}$ for each period is shown. (Right) Mean ($\pm \text{SEM}$) percentage changes, calculated from each tubule's percent change with AII and during recovery (R) compared with its own control value.

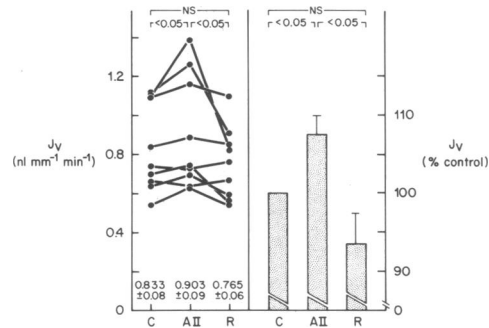


Figure 4. Effect of 10^{-10} M bath AII on proximal convoluted tubule J_v . See Fig. 3 for symbols and format.

0.833 ± 0.08 , 0.903 ± 0.09 , and 0.765 ± 0.06 nl/mm per min for the control, AII, and recovery periods, respectively. Both the J_v increase with AII and the recovery to control values were significant ($P < .05$). Expressed as a percentage of control J_v , AII at 10^{-10} M caused a 7.5% increase in J_v . For this group, mean tubule length was 1.38 ± 0.17 mm, mean V_T was -4.7 ± 0.3 mV; and mean perfusion rates for control, AII, and recovery periods were 11.49 ± 0.44 , 11.63 ± 0.33 , and 11.98 ± 0.35 nl/min.

A comparison of the effect of 10^{-11} vs. 10^{-10} M shows no significant difference when the absolute increments in J_v 's by each dose are compared ($+0.101 \pm 0.03$ vs. $+0.070 \pm 0.03$ nl/mm per min, NS). However, when the percentage increases are examined the two groups are statistically different ($+16\% \pm 4\%$ vs. $+7.5\% \pm 2.5\%$, $P < 0.05$).

Effect of high-dose AII on J_v . Although the AII doses of 10^{-11} to 10^{-10} M are probably close to the physiologic peritubular concentration in vivo, previous investigators have demonstrated an inhibitory effect on proximal tubule sodium transport at doses of $\sim 10^{-7}$ – 10^{-6} M. To confirm these possibly pharmacologic effects, 10^{-7} M AII was examined. Fig. 5 shows that this dose inhibited J_v by 24% ($P < 0.05$ from AII period), with complete recovery. J_v values for control, AII, and recovery periods were 0.877 ± 0.17 , 0.674 ± 0.17 , and 0.797 ± 0.19 nl/mm per

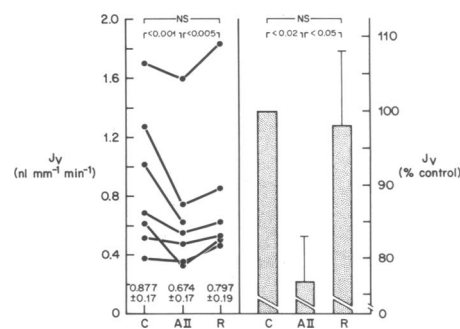


Figure 5. Effect of 10^{-7} M bath AII on proximal convoluted tubule J_v . See Fig. 3 for symbols and format.

min. Mean tubule length was 1.26 ± 0.21 mm, and mean control period V_T was -4.6 ± 0.4 mV. Perfusion rates for the three periods were 11.38 ± 0.28 , 11.65 ± 0.36 , and 11.55 ± 0.43 nl/min.

Time controls. To insure that either increases or decreases in J_v during the middle phase of these lengthy (6 h) experiments were not due to a natural variation with time, five tubules were handled in an identical fashion to the previous experimental tubules, except that no AII was added during the second period. J_v did not change significantly during any period of these studies (0.683 ± 0.14 , 0.684 ± 0.14 , and 0.661 ± 0.12 nl/mm per min, all NS). Perfusion rates for these three periods were 12.75 ± 0.37 , 12.88 ± 0.38 , and 12.67 ± 0.40 nl/min). Thus, the increases in J_v with 10^{-11} and 10^{-10} M, and the decrease with 10^{-7} M AII, cannot be attributed to spontaneous variation.

Effects of saralasin on J_v . A number of micropuncture studies have utilized the AII blocker saralasin to examine the role of AII in proximal tubule reabsorption (27–29). These have been predicated on the concept that, as in vascular beds, saralasin acts as a nearly pure antagonist with little, if any, intrinsic agonistic action. However, in superficial proximal convoluted tubules, some studies have suggested that saralasin is agonistic at 10^{-8} M (30) and at 10^{-7} M (12). In the jejunum, Levens et al. (31) found saralasin agonistic at both low and high doses. Saralasin was therefore examined for intrinsic agonistic activity in rabbit nonsurface proximal convoluted tubules.

An attempt was made to mimic in vitro the peritubular saralasin concentrations that would prevail in vivo in the micropuncture studies cited above (27–29). Pharmacokinetic data on saralasin plasma levels at various systemic infusion rates were taken from a study by Pettinger et al. (32) and plotted as a linear regression in Fig. 6. The systemic saralasin infusion rates used in references 27 and 29 are shown to result in a predicted plasma saralasin concentration of $\sim 10^{-7}$ M. Therefore, since there is no intrarenal generation of saralasin, this value was used as the likely peritubular concentration. Shown in Fig.

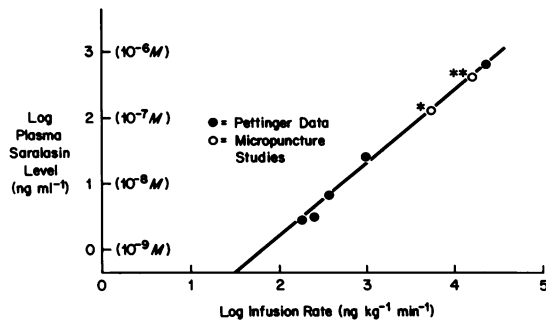


Figure 6. Plasma saralasin concentrations as a function of infusion rate. Closed circles and the regression line are from data of reference 32. Two micropuncture studies using saralasin infusion (open circles, *, reference 27; **, reference 29) result in predicted plasma saralasin concentrations of $\sim 10^{-7}$ M. $y = 1.05x - 1.8$. $r = 0.988$. $P < 0.01$.

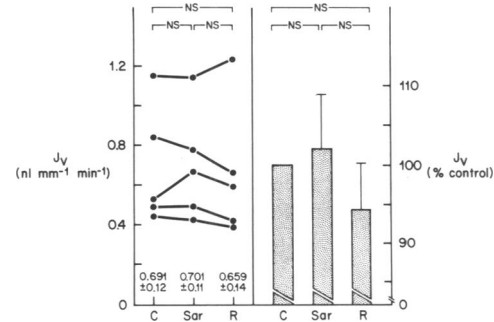


Figure 7. Effect of 10^{-7} M bath saralasin on proximal convoluted tubule J_v . See Fig. 3 for format. Sar, saralasin, R, recovery.

7 is the effect of 10^{-7} M bath saralasin on J_v in five tubules. Mean J_v 's for control, saralasin, and recovery periods were 0.691 ± 0.12 , 0.701 ± 0.11 , and 0.659 ± 0.14 nl/mm per min, respectively (NS control vs. experimental vs. recovery). For this group of tubules, mean length was 1.28 ± 0.12 mm and mean V_T was -6.0 ± 0.28 mV. Perfusion rates for the three periods were 13.41 ± 0.62 , 13.28 ± 0.70 , and 13.33 ± 0.54 nl/min. Thus, at peritubular concentrations likely to prevail during infusion studies, saralasin has no direct intrinsic agonistic effect on J_v .

Effects of saralasin plus AII. In five tubules, both saralasin at 10^{-7} M plus AII at 10^{-11} M were added in concert. This in vitro experiment thus approximated the in vivo saralasin studies previously cited. As shown in Fig. 8, saralasin, at this peritubular concentration, completely blocked the stimulatory effect of 10^{-11} M AII. The J_v 's for control and saralasin-plus-AII periods were 0.65 ± 0.05 and 0.61 ± 0.10 nl/mm per min (NS). The perfusion rates were 10.19 ± 0.67 and 10.41 ± 0.62 nl/mm per min, respectively.

AII effect on V_T . In our laboratory, proximal tubules meeting morphological criteria for experimentation tend to have an increase in lumen-negative V_T throughout the experiment. This increase in V_T is relatively rapid for ~ 1 – 2 h and then appears

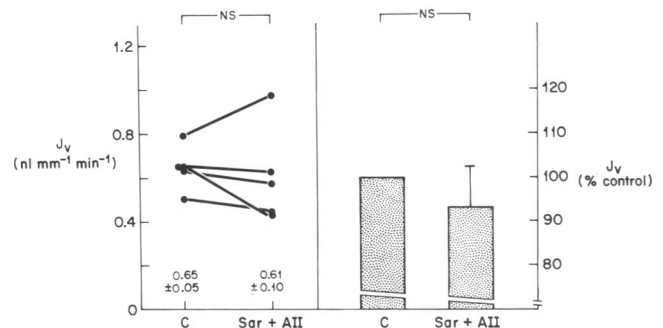


Figure 8. Effect of 10^{-7} M bath saralasin plus 10^{-11} M AII on proximal convoluted tubule J_v . See Fig. 3 for format.

to approach an asymptote. Fig. 9 shows V_T vs. time in two representative AII experiments. As shown, neither high-dose (10^{-7} M) nor low-dose (10^{-10} M) AII, which had opposite effects on J_v , noticeably altered V_T . Likewise, the rate of rise of V_T , i.e., the slope of this curve, did not change upon addition of AII, nor were the slopes different in the time controls compared with AII experiments (data not shown). Therefore, both stimulatory and inhibitory effects of AII on J_v appeared to be electroneutral.

Discussion

These studies are important in that they: (a) demonstrate a direct effect of AII on the proximal convoluted tubule in vitro under open-lumen transport conditions, (b) show AII effects on nonsurface proximal tubules, (c) directly measure transepithelial voltage in the proximal tubule during AII administration, demonstrating that the transport effect of AII is not associated with a measurable change in transepithelial voltage, and (d) measure plasma AII levels in rabbits by RIA. Our data also confirm the biphasic dose-response pattern seen in other studies.

Three major aspects of our studies deserve particular attention: the directness, magnitude, and mechanism of the AII effect.

Studies examining a possible direct effect of AII on epithelial transport have been complicated by interactions with the sympathetic nervous system. Angiotensin's ability to enhance norepinephrine release from sympathetic nerve endings is well known (16). Indeed, the stimulation of jejunal volume reabsorption by low doses of AII appears due solely to the release of norepinephrine (33), whereas the effects on colonic transport persist after denervation (2). In the kidney, the initial clearance

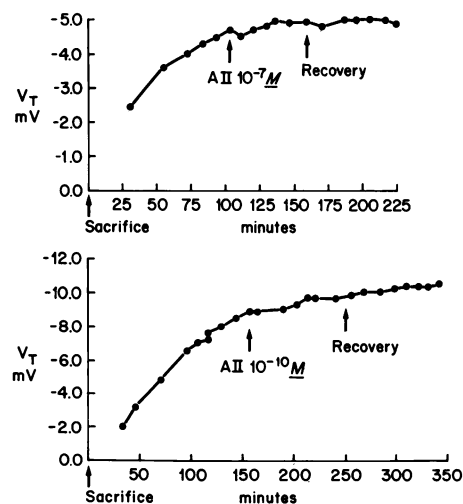


Figure 9. V_T (lumen negative) vs. time in two representative experiments. Addition of both AII at 10^{-7} M (top) or 10^{-10} M (bottom) had no discernible effect on V_T .

studies by McGiff (34) suggested that blockade of the sympathetic nervous system ablated the antinatriuretic effect of low-dose AII infusion. However, subsequent clearance studies by Waugh (35) and proximal tubule micropuncture studies by Pelayo and Blantz (36) have suggested an antinatriuretic effect of exogenous or endogenous AII that is independent of catecholamine release. Our studies, in which adrenergic nerve endings on tubular basement membranes have been removed during the dissection process, show a direct effect of peritubular AII on proximal tubule J_v . Such a direct effect is most likely mediated by hormone-receptor binding.

Receptors for AII have been shown on both brush border and basolateral membranes of (primarily proximal) tubule suspensions (30, 37). Although the precise function of each set of receptors remains to be determined, current evidence suggests that luminal AII undergoes rapid hydrolysis with tubular uptake of fragments (26), whereas basolateral AII produces changes in epithelial transport (12, 13). Presumably, basolateral AII receptors mediate this latter effect. The dissociation constants for these basolateral receptors are consistent with the transport responses at hormonal concentrations in the 10^{-11} – 10^{-10} M range which we have demonstrated. Moreover, this concentration range is probably physiologic, since our measured plasma AII values range from 2×10^{-11} in sodium-replete rabbits to $\sim 5 \times 10^{-11}$ M in adrenalectomized rabbits.

The magnitude of J_v changes demonstrated here compare well with the findings of others: Spinelli and Walther (12) found stimulation of J_v by 17% at 10^{-10} M peritubular AII, comparable to our 16% at 10^{-11} M. Also, our 24% inhibition at 10^{-7} M agrees with Steven's 23% at 2×10^{-8} M (13). Our results in nonsurface convolutions are thus similar to those of rat superficial convolutions. The physiological significance to the organism of the high-dose inhibition is uncertain, as there is little evidence that peritubular AII concentrations reach these levels in vivo. Moreover, inhibition of proximal reabsorption is often not reflected in the final urine, owing to compensatory reabsorption by the loop of Henle and distal nephron segments. On the other hand, even small increases in proximal reabsorption are reflected in the final urine and in overall volume status. For example, in the human with a glomerular filtration rate of 180 liters/24 h and a fractional proximal reabsorption of 60%, a sustained 16% increase in J_v would result in the addition of more than 17 liters of reabsorbate per 24 h to the extracellular space in the absence of other compensatory changes. Moreover, it is likely that the magnitude of stimulation demonstrated here represents a minimal effect of AII. Studies using AII blockade with saralasin or angiotensin converting enzyme inhibition have shown larger effects of endogenous AII on proximal reabsorption in states associated with high circulating AII levels compared with control states (27, 38). It is not clear if this change in AII effect represents a change in tubule responsiveness to AII or some other intrarenal action of AII. Our normal rabbits are on a relatively high-sodium diet. We therefore attempted to examine the effects of AII on tubules harvested from sodium-depleted

rabbits. However, these proximal convoluted tubules were extremely friable upon dissection, and no tubules of sufficient viability for these experiments were obtained. No attempts were made at perfusing convolutions from adrenalectomized rabbits.

We examined the competitive AII antagonist saralasin. Some data have suggested that the action of saralasin in relationship to AII is different in epithelial as opposed to vascular tissues. In the rat jejunum, saralasin is agonistic at all doses. It does not antagonize the stimulatory effects of low-dose AII, and it blocks the inhibitory effect of high-dose AII, allowing the latter to be stimulatory (31). In renal tubules, saralasin alone has been reported to be stimulatory at 10^{-7} M (12) and at 10^{-9} M (30). We addressed this issue by first examining saralasin alone at a dose (10^{-7} M) which probably prevailed in micropuncture experiments using saralasin infusions, and found no significant agonistic activity. In addition, this dose prevented the rise in J_v seen with 10^{-11} M AII. These experiments are thus consistent with an action of saralasin on the proximal tubule as an AII receptor antagonist only. It is possible, however, that other saralasin-AII dose combinations could yield other results.

The action of AII on the proximal convolution appears to involve an electroneutral transport process(es), at least as assessed by V_T . This is characteristic of AII's action in other epithelia, i.e., colon, (4), jejunum (3), and gallbladder (39). In the proximal tubule, Harris and Young (13) indirectly assessed V_T by measuring chloride distribution across the epithelium. Although they found no effect of AII on V_T , their method is based on the assumption that all chloride movement is passive and paracellular. However, recent evidence has suggested that at least a portion of chloride movement may be transcellular and coupled to sodium entry (40), therefore challenging the validity of this method. Our studies directly measured V_T at the lumen pipette and found no measurable change with AII at doses that either stimulated or inhibited J_v . It is, of course, possible that the relatively small changes in J_v (0.100 nl/mm per min) induced by AII were associated with proportionately small changes in V_T which went undetected. The relationship between J_v driven by electrogenic processes (i.e., coupled organic solute co-transport) and the contribution of these processes to V_T have not been systematically examined. Extrapolation from several available studies (15, 41, 42) suggests that electrogenic processes produce changes in V_T of 0.3–1.2 mV per 0.100 nl/mm per min change in J_v . Our system should be able to detect changes of this magnitude. The larger changes in J_v produced by high-dose AII probably should have produced detectable changes in V_T . We did not observe such changes. However, the transport process(es) inhibited by high-dose AII may not be the same one(s) stimulated by physiologic concentrations. In any event, further experiments with AII involving omission of luminal organics are required to be confident that the AII effect is electroneutral.

The intracellular mechanism of J_v stimulation at low doses and inhibition at high doses remains speculative. Many peptide hormones appear to alter transport via effects on cyclic AMP.

Available evidence, however, suggests that AII does not raise, and may even lower, cyclic AMP levels in renal cortex (43–46). Since cyclic AMP inhibits J_v in the proximal tubule (47), a fall in cyclic AMP might stimulate J_v . On the other hand, changes in cytosolic calcium activity, a mediator of AII action in many tissues, might produce the effects seen here. Although some studies have shown inhibition of J_v in the proximal tubule with maneuvers thought to increase cytosolic calcium (48), others have shown enhanced J_v (49). Moreover, sodium entry into cultured epithelial cells is enhanced when cytosolic calcium is moderately raised. However, when calcium loading is extended still further, stimulation of sodium entry falls below the peak value (50). It is therefore possible that progressively larger increases in cytosolic calcium, due to AII, might produce the biphasic dose response. The stimulation of transport might be mediated through cyclic AMP-independent protein phosphorylation, as has been shown to occur in the hepatocyte (1). The inhibitory effect might be a direct action of calcium per se on sodium transport (48), or could be due to stimulation of prostaglandin synthesis by calcium, though the evidence for a significant role of prostaglandins in the proximal tubule is poor (51). The role of cytosolic calcium in the action of any hormone on renal tubules, and of AII in particular, remains poorly understood.

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References

1. Peach, M. J. 1981. Molecular actions of angiotensin. *Biochem. Pharmacol.* 30(20):2745–2751.
2. Davies, N. T., K. A. Munday, and B. J. Parsons. 1970. The effect of angiotensin on rat intestinal fluid transfer. *J. Endocrinol.* 48:39–46.
3. Bolton, J. E., K. A. Munday, B. J. Parsons, and B. G. York. 1975. Effects of angiotensin II on fluid transport, transmural potential difference and blood flow by rat jejunum in vivo. *J. Physiol. (Lond.)* 253:411–428.
4. Levens, N. R., M. J. Peach, R. M. Carey, J. A. Poat, and K. A. Munday. 1981. Changes in an electroneutral transport process mediated by angiotensin II in the rat distal colon in-vivo. *Endocrinology.* 108(4):1497–1504.
5. Hornych, A., P. Meyer, and P. Milliez. 1973. Angiotensin, vasopressin, and cyclic AMP: Effects on sodium and water fluxes in rat colon. *Am. J. Physiol.* 224(5):1223–1229.
6. Hughes-Jones, N. C., G. W. Pickering, P. H. Sanderson, H. Scarborough, and J. Vandenbrouckey. 1949. The nature of the action of renin and hypertensin on renal function in the rabbit. *J. Physiol. (Lond.)* 109:288–307.

7. Navar, L. G., and H. G. Langford. 1974. Effects of angiotensin on the renal circulation. In *Handbuch der experimentellen Pharmakologie Angiotensin*, Irvine H. Page and F. Meslin Bumpus, editors, Springer-Verlag, New York. 37:455–474.
8. Horster, M., W. Nagel, J. Schnermann, and K. Thurau. 1966. Zur Frage einer direkten Angiotensinwirkung auf die Natrium-resorption im proximalen Tubulus und in der Henleschen Schleife der Rattenniere. *Pflügers Arch.* 292:118–128.
9. Lowitz, H. D., K. O. Stumpe, and B. Ochwaldt. 1969. Micro-puncture study of the action of angiotensin-II on tubular sodium and water reabsorption in the rat. *Nephron.* 6:174–187.
10. Myers, B. D., W. M. Deen, and B. M. Brenner. 1975. Effects of norepinephrine and angiotensin II on the determinants of glomerular ultrafiltration and proximal tubule fluid reabsorption in the rat. *Circ. Res.* 37:101–110.
11. Steven, K. 1974. Effect of peritubular infusion of angiotensin II on rat proximal nephron function. *Kidney Int.* 6:73–80.
12. Spinelli, F., and A. Walther. 1978. Biphasic tubular effect of angiotensin II and Sar¹ Ala⁸ on net water and sodium reabsorption in the proximal tubule of the rat kidney. *Kidney Int.* 13:532. (Abstr.)
13. 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. *Pflügers Arch.* 367:295–297.
14. Flamenbaum, W., and R. J. Hamburger. 1974. Superficial and deep juxtaglomerular apparatus renin activity of the rat kidney. *J. Clin. Invest.* 54:1373–1381.
15. Jacobson, H. R. 1979. Characteristics of volume reabsorption in rabbit superficial and juxtamedullary proximal convoluted tubules. *J. Clin. Invest.* 63(3):410–418.
16. Lifschitz, M. D., and J. H. Stein. 1981. Renal vasoactive hormones. In *The Kidney*. B. M. Brenner and F. C. Rector, editors. Second Ed. W.B. Saunders Co., Philadelphia. 1:650–720.
17. Jensen, P. K., and K. Steven. 1977. Angiotensin II induced reduction of peritubular capillary diameter in the rat kidney. *Pflügers Arch.* 371:245–250.
18. Burg, M. B., and J. Orloff. 1968. Control of fluid absorption in the renal proximal tubule. *J. Clin. Invest.* 47:2016–2024.
19. Arant, B. S., Jr., and W. H. Stephenson. 1982. Developmental changes in cardiac output and peripheral resistance compared with arterial plasma concentrations of prostaglandins and angiotensin II in conscious dogs. *Fed. Proc.* 41:1544. (Abstr.)
20. Wingo, C. S., D. W. Seldin, J. P. Kokko, and H. R. Jacobson. 1982. Dietary modulation of active potassium secretion in the cortical collecting tubule of adrenalectomized rabbits. *J. Clin. Invest.* 70:579–586.
21. Regoli, D., and J. R. Vane. 1964. A sensitive method for the assay of angiotensin. *Br. J. Pharmacol.* 23:351–359.
22. Jacobson, H. R. 1981. Effects of CO₂ and acetazolamide on bicarbonate and fluid transport in rabbit proximal tubules. *Am. J. Physiol.* 240(Renal Fluid Electrolyte Physiol 9):F54–F62.
23. Schafer, J. A., S. L. Troutman, and T. E. Andreoli. 1974. Volume reabsorption, transepithelial potential difference, and ionic permeability properties in mammalian superficial proximal straight tubules. *J. Gen. Physiol.* 64:582–607.
24. Nakajima, T., T. Nakayama, and H. Sokabe. 1971. Examination of angiotensin-like substances from renal and extrarenal sources of mammalian and nonmammalian species. *Gen. Comp. Endocrin.* 17:458–466.
25. Burghardt, W., H. Schweisfurth, and H. Dalheim. 1982. Juxtaglomerular angiotensin II formation. *Kidney Int.* 22:(Suppl. 12):S49–S54.
26. Peterson, D. R., G. Chrabaszca, W. R. Peterson, and S. Oparil. 1979. Mechanism for renal tubular handling of angiotensin. *Am. J. Physiol.* 236(Renal Fluid Electrolyte Physiol 5):F365–F372.
27. Steiner, R. W., B. J. Tucker, and R. C. Blantz. 1979. Glomerular hemodynamics in rats with chronic sodium depletion. *J. Clin. Invest.* 64:503–512.
28. Reineck, H. J., and R. Parma. 1982. Angiotensin II mediates avid proximal reabsorption in volume expanded animals with immediate aortic clamping. *Kidney Int.* 21:285. (Abstr.)
29. Ploth, D. W., and R. N. Roy. 1982. Renal and tubuloglomerular feedback effects of [Sar¹,Ala⁸] angiotensin II in the rat. *Am. J. Physiol.* 242(Renal Fluid Electrolyte Physiol 11):F149–F157.
30. Freeland, A. E., and T. L. Goodfriend. 1977. Angiotensin receptors and sodium transport in renal tubules. *Fed. Proc.* 36:481. (Abstr.)
31. Levens, N. R., M. J. Peach, R. M. Carey, J. A. Poat, and K. A. Munday. 1981. Response of rat jejunum to angiotensin II: role of norepinephrine and prostaglandins. *Am. J. Physiol.* 240(Gastrointest. Liver Physiol. 3):G17–G24.
32. Pettinger, W. A., K. Keeton, and K. Tanaka. 1975. Radioimmunoassay and pharmacokinetics of saralasin in the rat and hypertensive patients. *Clin. Pharmacol. Ther.* 17:146–158.
33. Levens, N. R., M. J. Peach, and R. M. Carey. 1981. Interactions between angiotensin peptides and the sympathetic nervous system mediating intestinal sodium and water absorption in the rat. *J. Clin. Invest.* 67:1197–1207.
34. McGiff, J. C. 1967. Natriuretic effect of angiotensin in dogs revealed after administration of reserpine and guanethidine. *Circ. Res.* 20:664–675.
35. Waugh, W. H. 1972. Angiotensin II. Local renal effects of physiological increments in concentration. *Can. J. Physiol. Pharmacol.* 50:711–716.
36. Pelayo, J. C., and R. C. Blantz. 1982. Interactions of angiotensin II and renal adrenergic nervous activity on glomerular hemodynamics and proximal fluid reabsorption. *Clin. Res.* 30:541A. (Abstr.)
37. Brown, G., and J. Douglas. 1983. Angiotensin II-binding sites in rat and primate isolated renal tubular basolateral membranes. *Endocrinology.* 112:2007–2014.
38. Huang, W. C., D. W. Ploth, and L. G. Navar. 1982. Angiotensin mediated alterations in nephron function in Goldblatt hypertensive rats. *Am. J. Physiol.* 243(Renal Fluid Electrolyte Physiol 12):F553–F560.
39. Leyssac, P. P., L. O. Kristensen, P. Christensen, and O. Fredriksen. 1974. The effect of angiotensin on isosmotic fluid absorption by the rabbit gall-bladder in-vitro. *Acta Physiol. Scand.* 92:508–516.
40. Warnock, D. G., and J. Eveloff. 1982. NaCl entry mechanisms in the luminal membrane of the renal tubule. *Am. J. Physiol.* 242(Renal Fluid Electrolyte Physiol 11):F561–F574.
41. Burg, M., C. Patlak, N. Green, and D. Villey. 1976. Organic solutes in fluid absorption by renal proximal convoluted tubules. *Am. J. Physiol.* 231:627–637.
42. Imai, M., D. W. Seldin, and J. P. Kokko. 1977. Effect of perfusion rate on the fluxes of water, sodium, chloride, and urea across the proximal convoluted tubule. *Kidney Int.* 11:18–27.
43. Munday, K. A., B. J. Parsons, J. A. Poat, G. A. D'Auriac, and P. Meyer. 1976. The role of cyclic 3'-5' adenosine monophosphate in

the responses of the intestine and kidney to angiotensin. *J. Endocrinol.* 69:297-298.

44. Guder, W. G., and A. Repprecht. 1976. Hormonal regulation of gluconeogenesis in isolated rat kidney tubule fragments. *In Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies*, J. M. Tager, H. D. Söling, and J. R. Williamson, editors. North Holland Publishing Company, Amsterdam. 379-389.

45. Torres, V. E., T. E. Northrup, R. M. Edwards, S. V. Shah, and T. P. Dousa. 1978. Modulation of cyclic nucleotides in isolated rat glomeruli. *J. Clin. Invest.* 62:1334-1343.

46. Woodcock, E. A., and C. I. Johnston. 1982. Inhibition of adenylate cyclase by angiotensin II in rat renal cortex. *Endocrinology.* 111(5):1687-1691.

47. 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.

48. Friedman, P. A., J. F. Figueiredo, T. Maack, and E. E. Windhager. 1981. Sodium-calcium interactions in the renal proximal convoluted tubule of the rabbit. *Am. J. Physiol.* 240(Renal Fluid Electrolyte Physiol. 9):F558-F568.

49. McKeown, J. W. 1983. Effects of calcium ionophore A23187 on fluid and phosphate reabsorption in rabbit proximal convoluted tubules. *Kidney Int.* 23(1):106. (Abstr.)

50. Taub, M., and M. H. Saier, Jr. 1979. Regulation of $^{22}\text{Na}^+$ transport by calcium in an established kidney epithelial cell line. *J. Biol. Chem.* 254(22):11440-11444.

51. Kokko, J. P. 1981. Effect of prostaglandins on renal epithelial electrolyte transport. *Kidney Int.* 19:791-796.