Local upregulation of colonic angiotensin II receptors enhances potassium excretion in chronic renal failure

MARGUERITE HATCH, ROBERT W. FREEL, AND N. D. VAZIRI
Department of Medicine, Division of Nephrology, University of California at Irvine, Irvine, California 92697

Hatch, Marguerite, Robert W. Freel, and N. D. Vaziri. Local upregulation of colonic angiotensin II receptors enhances potassium excretion in chronic renal failure. Am. J. Physiol. 274 (Renal Physiol. 43): F275–F282, 1998.—The role of angiotensin II (ANG II) in colonic secretion of K\textsuperscript{+} was examined in rats with chronic renal failure (CRF). The basal net secretory flux of \( ^{86}\text{Rb}^+ \) (as a tracer for K\textsuperscript{+}) across the CRF distal colon (\(-0.20 \pm 0.04 \mu\text{eq cm}^{-2}\text{ h}^{-1}\)) was reversed to an absorptive flux (\(0.35 \pm 0.05 \mu\text{eq cm}^{-2}\text{ h}^{-1}\)) by injecting the rats with the AT\textsubscript{1} receptor antagonist, losartan. A similar result was observed when losartan was added to the CRF colonic tissue in vitro. In contrast, an AT\textsubscript{2} receptor antagonist, PD-123319, did not reverse the CRF-induced alterations in Rb\textsuperscript{+} transport across the short-circuited colonic tissue. Plasma concentrations of ANG II, aldosterone, and K\textsuperscript{+}, as well as the ANG II content of colonic tissues from CRF and normal rats, were similar. However, specific \( ^{125}\text{I} \)-labeled ANG II binding sites in rat distal colon increased twofold in CRF [maximal specific binding (\(B_{\text{max}}\)] = 28.6 ± 1.6 fmol/mg protein] compared with normal (\(B_{\text{max}}\) = 15.2 ± 0.4 fmol/mg protein). These studies suggest that CRF-induced secretion of K\textsuperscript{+} by the colon is mediated by an upregulation of AT\textsubscript{1} receptors present in CRF.

Losartan; EXP-3174; PD-123319; jejunum; ileum; absorption; secretion; AT\textsubscript{1} receptor; AT\textsubscript{2} receptor

POTASSIUM HOMEOSTASIS is maintained by both renal and extrarenal mechanisms (15). Normally, the renal distal tubule secretes up to 90\% of the dietary K\textsuperscript{+} load, and the remainder is eliminated via the large intestine (15). When renal function is compromised, such as in chronic renal failure (CRF), plasma K\textsuperscript{+} has been shown to remain stable (23). This plasma K\textsuperscript{+} homeostasis appears to be a consequence of an increased rate of K\textsuperscript{+} secretion by remaining functional nephrons in addition to an increase in colonic K\textsuperscript{+} secretion (2, 23, 24). Both renal and colonic K\textsuperscript{+} transport mechanisms can be regulated by aldosterone; however, investigations of aldosterone involvement in this CRF-adaptive response have yielded inconsistent results in patient studies (4, 23, 24). For example, spironolactone antagonism of colonic K\textsuperscript{+} secretion was not demonstrated in nephrectomized rats, and elevated aldosterone titers have not been reported in this animal model of CRF (2).

In experimental CRF in rats, we have observed a net colonic secretion of anions including chloride, urate, and oxalate, compared with a basal absorptive flux in normal controls (9–11). During the course of these previous investigations, we found that losartan, a specific angiotensin II (ANG II) receptor antagonist, reversed the CRF-induced anion secretion to absorption across CRF rat colon (9). These results implicated the involvement of ANG II in mediating the generalized secretory nature of colonic mucosa in CRF and prompted the present study, which further investigates the possible role of angiotensin in the regulation of colonic K\textsuperscript{+} secretion in CRF.

In this report, we provide new information regarding large intestinal control of K\textsuperscript{+} homeostasis in CRF. The present study suggests that ANG II has an integral role in mediating colonic excretion of K\textsuperscript{+} in CRF by a local upregulation of ANG II binding sites.

MATERIALS AND METHODS

Reagents. We received both AT\textsubscript{1} receptor antagonists, losartan and its metabolite EXP-3174, as gifts from Merck (Rahway, N.J.). The AT\textsubscript{2} receptor antagonist, PD-123319, was a product of Research Biochemicals International (Natick, MA), and [\( ^{38}\text{Asp}^1, {\text{Val}}^5\)ANG II was obtained from Peninsula Laboratories (Belmont, CA). Bestatin, bacitracin, phosphoramidon, leupeptin, Pepstatin, and neomycin were obtained from Calbiochem (La Jolla, CA), and all other reagents were purchased from Sigma Chemical (St. Louis, MO).

Animals. Male Sprague-Dawley rats (285–325 g) were used in the following studies. The rats had free access to drinking water and Purina Rat Chow 5001. Food intake was determined, over a 48-h period, on a weekly basis in the normal and experimental groups beginning on the second week following surgery through the sixth week.

To produce CRF, a partial nephrectomy was performed on each animal designated to the CRF group. General anesthesia was induced with an intraperitoneal injection of pentobarbital, and the surgical procedure of a right nephrectomy, followed by a left two-thirds nephrectomy 4 days later, was performed extraperitoneally under aseptic conditions. Several series of experiments were conducted using intestinal tissues removed from normal rats and CRF rats that were euthanized by an intraperitoneal injection of pentobarbital sodium 6 wk after surgery. Blood was collected from the rats at this time for the measurement of K\textsuperscript{+} (atomic absorption spectrometry, Perkin-Elmer, Norwalk, CT), aldosterone [solid phase radioimmunoassay (RIA), Coat-A-Count; Diagnostic Products, Los Angeles, CA), ANG II (RIA; Nichols Instruments, San Juan Capistrano, CA), and creatinine (kit 555A; Sigma Chemical). The blood, collected by cardiac puncture from unconscious rats, was divided into several tubes for the various measurements. Within 15 s of opening the body cavity, blood (2 ml) was collected for ANG II determination into a syringe containing the following inhibitor cocktail (75 \( \mu\text{l ml blood) to prevent generation or degradation of the peptide: 0.025 M phenylthionline, 0.125 M EDTA, 2 mM neomycin, 10^{-3} \text{ M enalaprilat, 10^{-5} M pepstatin, and 2% ethanol (14). An internal control provided with the RIA kit for ANG II determination and an immunonuassay tri-level control, CON6 (Diagnostic Products), which was assayed as an unknown along with the plasma samples in the aldosterone RIA, consistently yielded values within the ranges specified. Creatinine was also determined in 24-h urine specimens collected immediately before the animals were euthanized. Creatinine clearance was calculated for each rat, according to

\[ \text{Creatinine clearance} = \frac{(\text{Creatinine in urine} \times \text{Urine volume}) \times \text{Body weight}}{(\text{Creatinine in blood} \times \text{Blood volume}) \times \text{Body weight}} \]

The rats had free access to drinking water and Purina Rat Chow 5001. Food intake was determined, over a 48-h period, on a weekly basis in the normal and experimental groups beginning on the second week following surgery through the sixth week.
the standard formula, and this was used as an indicator of renal function.

In one experimental series, CRF rats were divided into two groups. Half of the rats received intraperitoneal injections of losartan (10 mg/kg), on a daily basis for 7 days, beginning on the fifth week after surgery. This dosage and schedule was chosen, because it was found to achieve the maximal antihypertensive effect in rats (30). At the same time, the remaining half of the group received a placebo injection of saline (150 mM NaCl). The intestinal segments (primarily distal colon, but jejunum and ileum were included in one series) were removed from both normal and CRF rats as previously described (9), rinsed with the standard saline solution (see below), and partially stripped of the serosal muscularis. Flat sheets of tissue were mounted in modified Ussing chambers with an exposed tissue area of 0.64 cm² and bathed on both sides by 10 mL of the standard saline solution (9).

Solutions. The standard saline contained the following solutes (in mmol/l): 134.9 Na⁺, 5.4 K⁺, 1.2 Mg²⁺, 123.2 Cl⁻, 21.0 HCO₃⁻, 1.2 Ca²⁺, 0.6 H₂PO₄⁻, 2.4 HPO₄²⁻, and 10 glucose. Whenever ⁸⁶Rb⁺ was used in the flux experiments, K⁺ was replaced by equimolar Rb⁺. ANG II at 10⁻⁴ M and losartan at 10⁻⁵ M were added to the serosal bathing solution to ensure an effective dose at the receptor location. In the Ussing chambers, these concentrations are necessary to overcome potential time-dependent hydrolysis of the peptide and receptor antagonists (9). To inhibit peptide degradation in the flux experiments involving ANG II addition, the standard saline also contained 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 mM leupeptin, 0.1 mM pep- tide, the standard saline also contained 0.5 mM phenylmeth-

degradation in the flux experiments involving ANG II addi-

Fig. 1. Correlation between simultaneously measured ⁴²K⁺ and ⁸⁶Rb⁺ unidirectional fluxes (J K⁺ and J Rb⁺, respectively) across rat distal colon. Each point represents the mean ± SE for 3 animals. Open symbols, fluxes measured during 4 intervals in period I (control, unstimulated); filled symbols, fluxes determined during 3 intervals in period II subsequent to addition of 0.5 mM dibutyryl-cAMP (dBCAMP) to the serosal compartment. Line through the variates is the least squares regression as given by the equation. Intercept is not significantly different from zero. Each flux sample was counted twice by gamma-spectrometry, immediately to evaluate activity measured in the second counting was decay corrected to the first counting and represents ⁸⁶Rb activity in the sample. Potassium activity in the sample is the difference between total initial activity and the ⁸⁶Rb activity. S-M, serosal-to-mucosal flux; M-S, mucosal-to-
serosal flux.

equilibration period preceded period II, following the addition of a drug to the tissue.

Determination of tissue ANG II content. Distal colonic segments removed from both normal and CRF rats were rinsed thoroughly with 0.9% saline containing the same inhibitor cocktail that was used for blood collection (see above). The tissues were stripped of the serosal muscularis in the same way as described for the Ussing chamber preparation and dropped into ice-cold methanol (10% wt/vol) containing 100 µl of 8 M urea and 0.1% Triton X-100 (16). The tissue was homogenized with four 10-s pulses using a Brinkmann Polytron (Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged for 10 min at 13,000 g, and the supernatant was removed and dried under a stream of N₂ gas. The dried extract was reconstituted into a tris(hydroxy-
methyl)aminomethane (Tris) buffer, pH 7.4, and stored at −20°C for less than 1 wk before the RIA was conducted. In a pilot series, it was determined that this procedure yielded 81.06 ± 2.8% (n = 5 tissues) recovery of the tracer ¹²⁵I-ANG II that was added to the tissue sample prior to homogenization. Two further extractions of the recovered pellet in methanol, as described above, yielded tracer recoveries of 11.74 ± 22.2% and 5.33 ± 0.8%, respectively, and independent RIA determination of the three dried supernatant extracts gave comparable values. On the basis of these results, the tissue extraction procedure was standardized to one homogenization only. Both tissue and plasma results were corrected for losses during extraction.

Preparation of membrane fragments for receptor binding assay. Distal colonic segments were removed from both normal and CRF rats. The tissues were handled exactly as described in the previous section except these stripped tissues were snap frozen in liquid nitrogen and stored at −70°C. The mucosa was homogenized with four 10-s pulses, using the Brinkmann Polytron, in 20 vol of 250 mM sucrose (pH 7.6)
containing the following: 10 mM triethanolamine HCl, 0.1 mM PMSF, 0.1 mM bacitracin, 50 µM phenanthroline, 10 µM phosphoramidon, 130 µM bestatin, and 1 µM leupeptin, pepstatin, and captopril. The homogenate was centrifuged for 10 min at 50,000 g (model L5–75B, Beckman). The pellet was recovered, rehomogenized, and centrifuged once again. The final pellet was resuspended in an appropriate volume of 10 mM triethanolamine, containing 0.1 mM PMSF at pH 7.6 to give a solution containing -1 mg/ml protein. Protein was determined using the Bradford method (Bio-Rad Protein Kit; Bio-Rad, Richmond, CA).

Receptor binding assay. The assay buffer (pH 7.4) contained the following: 120 mM NaCl, 20 mM Tris·HCl, 5 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 0.1 mM PMSF, 0.2% bovine serum albumin (BSA, heat treated at 56°C for 30 min), 100 µM bacitracin, 50 µM phenanthroline, 10 µM phosphoramidon, 130 µM bestatin, and 1 µM leupeptin, pepstatin, and captopril. The homogenate was centrifuged for 10 min at 50,000 g (model L5–75B, Beckman). The pellet was recovered, rehomogenized, and centrifuged once again. The final pellet was resuspended in an appropriate volume of 10 mM triethanolamine, containing 0.1 mM PMSF at pH 7.6 to give a solution containing ~1 mg/ml protein. Protein was determined using the Bradford method (Bio-Rad Protein Kit; Bio-Rad, Richmond, CA).

The CRF rat model. A comparison of plasma creatinine concentration and creatinine clearance between the normal group of rats and the CRF group confirmed a significant reduction in renal function in CRF rats 6 wk after five-sixths nephrectomy. Plasma creatinine increased significantly from 0.047 ± 0.001 mM in the normal group (n = 18) to 0.096 ± 0.008 mM in the CRF group (n = 18). Urinary clearance of creatinine was reduced ~50% in the CRF group compared with the normal group (normal, 1.95 ± 0.09, n = 18; CRF, 1.0 ± 0.1 ml/min, n = 18). Weight gain in the normal group was greater (Δ = 73 ± 3 g) than in the CRF group (Δ = 59 ± 3 g) over the 6-wk time period. However, during this period there was no significant difference in mean

![Fig. 2. Basal 42K⁺ fluxes with associated electrical characteristics across jejunum, ileum, and distal colon of normal and chronic renal failure (CRF) rats. Tissue conductance (Gₜ) is presented in mS/cm², and short-circuit current (Iₛ𝐜) is given in µeq·cm⁻²·h⁻¹. Jₛₑₘ, serosal-to-mucosal flux; Jₑₘₛ, mucosal-to-serosal flux; Jₑₙₑₜ, net flux. Error bars are ± SE above or below the mean; n = 9 tissue pairs for each segment within each group. *Significant difference (P ≤ 0.05) between CRF and normal animals.](http://ajprenal.physiology.org/)

![Fig. 3. Basal ⁸⁶Rb transport with associated electrical parameters of distal colonic tissues removed from CRF rats injected with placebo (open bars) or losartan (hatched bars) for 7 days before study. Gₜ is given in mS/cm², and Iₛ𝐜 is given in µeq·cm⁻²·h⁻¹. Control fluxes were determined at 10-min intervals over two 30-min periods (Per I and Per II). Error bars are ± SE about the mean; n = 10 tissue pairs, from 7 rats in each group. *Significant difference (P ≤ 0.05) between losartan treatment and placebo treatment within the given time period.](http://ajprenal.physiology.org/)
marked alterations in the direction and magnitude of Rb⁺ fluxes across the colonic tissues compared with colonic Rb⁺ fluxes in CRF rats injected with placebo (Fig. 3). It was apparent that chronic losartan administration abolished the characteristic net secretory flux of Rb⁺ across CRF colon by way of coordinated changes in both unidirectional fluxes. The results presented in Fig. 3 also show that there were no significant time-dependent changes in the fluxes or the associated electrical parameters in either series over the duration of two flux periods (i.e., periods I and II). These results suggest that chronic antagonism of ANG II receptors in vivo inhibits net potassium (Rb⁺) secretion in this CRF model.

The possibility of acute, in vitro antagonism of CRF-induced secretion was also evaluated. In a separate experimental series, when losartan was added to the serosal solution bathing the CRF tissue preparation in vitro, similar significant alterations in Rb⁺ transport were observed, as depicted in Fig. 4. Furthermore, the addition of EXP-3174, a metabolite of losartan also known to have ANG II receptor antagonist activity (30), produced similar results. In another experimental series (n = 6), Jₘₛ increased significantly from 0.26 ± 0.05 to 0.36 ± 0.06 µeq cm⁻² h⁻¹, and Jₐₛ was significantly reduced from 0.47 ± 0.07 to 0.28 ± 0.06 µeq cm⁻² h⁻¹ following serosal EXP-3174 addition between periods I and II. These changes in both unidirectional fluxes resulted in a reversal of Jₐₛ from 0.21 ± 0.02 to +0.08 ± 0.01 µeq cm⁻² h⁻¹. Similar to the effects of losartan on the electrical parameters of this tissue, EXP-3174 significantly decreased Jₘₛ from 5.39 ± 0.27 to 2.63 ± 0.77 µeq cm⁻² h⁻¹ in period II.

Food intake between the two groups (normal, 21.0 ± 0.5; CRF, 22.7 ± 0.7 g·rat⁻¹·day⁻¹).

Intestinal potassium transport in CRF rats. Unidirectional fluxes of ⁴ᴷ⁺ were measured and compared across segments of colon, ileum, and jejunum, removed from control rats and CRF rats, 6 wk after five-sixths nephrectomy. The colonic segments from the normal rats supported a net absorptive flux of K⁺, which was reversed to a significant net secretory flux in CRF (Fig. 2). This change occurred via alterations in both unidirectional fluxes in this segment. The significant increase in Iₛ across the CRF colonic tissues confirms our previous observations of a concomitant electroneutral chloride secretion (9–11). In the small intestine of the normal rats, there was no significant net flux of K⁺ in either direction. However, in CRF ileum, the absorptive component of the transepithelial flux of K⁺ was reduced; consequently, the CRF ileum supported a small but significant net secretion. There was no net secretion of K⁺ across the CRF jejunum, and unidirectional K⁺ fluxes in this segment were not different from normal.

Effects of ANG II receptor antagonists on ⁸⁶ᴿᵇ fluxes across colonic tissues. Seven days of losartan in vivo administration (by injection) to CRF rats resulted in...
without any alterations in \( G_T \) (8.59 ± 0.75 in period I and 8.59 ± 0.53 mS/cm\(^2\) in period II).

The sensitivity of CRF tissues to exogenous ANG II was examined by adding the peptide to the serosal solution bathing CRF colonic tissue. The addition of ANG II at \( 10^{-4} \) M produced significant changes in the secretory component of rubidium flux (\( \Delta J_{sc}^{Rb} = 0.18 ± 0.01 \) μeq·cm\(^{-2}\)·h\(^{-1}\)) and \( I_{sc} \) (\( \Delta I_{sc} = 0.85 ± 0.27 \) μeq·cm\(^{-2}\)·h\(^{-1}\)) between period I and period II in five tissues from four CRF rats. The time course of these responses is illustrated in Fig. 5 and compared with CRF control tissues that were not treated with ANG II. When ANG II at \( 10^{-5} \) M was added to CRF tissues, the increase in serosal-to-mucosal flux was not significant (\( \Delta J_{sc}^{Rb} = 0.05 ± 0.02 \) μeq·cm\(^{-2}\)·h\(^{-1}\), \( n = 5 \)). A small, transient increase in \( I_{sc} \) (\( \Delta I_{sc} = 0.07 ± 0.06 \) μeq·cm\(^{-2}\)·h\(^{-1}\), \( n = 5 \)) was observed following the addition of ANG II at \( 10^{-6} \) M, which peaked over one 10-min flux period, but was not sustained through period II. Changes in \( J_{sc}^{Rb} \) could not be resolved during period II with ANG II addition at \( 10^{-6} \) M. Presumably, an effective dose of ANG II does not reach the receptor location at these lower concentrations because of the local degradation of the octapeptide.\(^1\)

Losartan inhibition of CRF-induced ion secretion across the distal colonic segment in vivo and in vitro (Fig. 3 and 4, respectively) suggests antagonism of the AT\(_2\) receptor subtype. The next question addressed was whether an AT\(_2\) receptor antagonist would similarly affect the CRF-induced \( \text{Rb}^{+} \) secretion. As shown in Fig. 6, unidirectional and net fluxes of \( \text{Rb}^{+} \) and the associated electrical parameters across CRF colon were not affected by the addition of the AT\(_2\) receptor antagonist, PD-123319, to colonic segments from CRF rats.

Plasma ANG II, aldosterone, and potassium. Since the foregoing results strongly suggest a role for ANG II in the mediation of colonic electrolyte secretion in CRF, the plasma ANG II concentration was compared in CRF and normal rats. In addition, since changes in colonic \( \text{K}^{+} \) transport can be mediated via aldosterone (3, 29), plasma \( \text{K}^{+} \) and aldosterone were also determined in both groups. The results of these studies confirmed no difference in either plasma ANG II or aldosterone concentrations in CRF rats compared with normal rats (Table 1), and despite CRF, plasma \( \text{K}^{+} \) was also comparable in both groups. The constancy of plasma ANG II levels in CRF does not preclude a change in tissue ANG II content (1). Yet we were unable to detect a significant difference between the ANG II content in tissues from CRF (698 ± 148 pmol/kg tissue wet wt, \( n = 7 \)) and normal rats (907 ± 220 pmol/kg tissue wet wt, \( n = 7 \)). Since there were no differences in either the circulating or the local tissue concentrations of ANG II, the possibility of a CRF-induced upregulation of ANG II receptors within the large intestinal segment was considered (1, 6). From the results of saturation binding studies, we determined that there was an increase in the number of specific \(^{125}\text{I}-\text{ANG II} \) binding sites in crude homogenates of colonic mucosa from CRF rats with the addition of CRF tissues to exogenous ANG II.

We evaluated the issue of peptide hydrolysis in the CRF colonic preparation by determining the concentration of ANG II in extracellular tissue space (ECS) of tissues incubated in the presence of ANG II. Briefly, tissue segments, stripped of serosal muscularis (150 mg wet wt), were incubated in buffer solution (see flux studies in MATERIALS AND METHODS) containing 10 \( \mu \)M ANG II or \( [14\text{C}] \)inulin. The tissues and incubation buffers that were used were rinsed thoroughly in buffer without any alterations in ECS. The tissues from the [14C]inulin designated for ANG II measurements were handled and assayed as described in MATERIALS AND METHODS. The tissues from the [14C]inulin-containing buffer were further subdivided into two pieces; one was set for water content determination, and the other pieces were each digested in 1 ml of Beckman Tissue Solubilizer. Tracer activity was determined in the digestate and bath samples at constant quench by liquid scintillation spectrometry. Mean ECS in tissues removed from five CRF rats was 0.17 ± 0.04 ml extracellular water/g wet tissue wt, and the calculated concentration of ANG II in that volume of ECS was 2.70 ± 0.18 × 10\(^{-6}\) M. Since the measured concentration of ANG II added to the buffers was 1.02 ± 0.02 × 10\(^{-6}\) M (\( n = 5 \)), the difference in concentration of ANG II between the bath and the tissue is approximately two orders of magnitude.

**Table 1.** Plasma concentrations of \( \text{K}^{+} \), aldosterone, and ANG II in normal rats and rats with CRF

<table>
<thead>
<tr>
<th>Plasma Solute</th>
<th>Normal Rat</th>
<th>CRF Rat</th>
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<tbody>
<tr>
<td>Potassium, mM (( n = 10 ))</td>
<td>5.2 ± 0.2</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>Aldosterone, pM (( n = 10 ))</td>
<td>216 ± 27</td>
<td>219 ± 26</td>
</tr>
<tr>
<td>ANG II, pM (( n = 9 ))</td>
<td>89 ± 15</td>
<td>66 ± 8</td>
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Values are means ± SE from \( n \) animals. CRF, chronic renal failure.
compared with normal rats (Fig. 7). Specific binding of
$^{125}$I-ANG II to this preparation was saturable at \( \sim 5 \) nM. Maximal specific binding ($B_{\text{max}}$) of $^{125}$I-ANG II in
colonic homogenates from CRF rats (28.6 \pm 1.6 fmol/mg protein) was
significantly greater than that from normal animals (15.22 \pm 0.04 fmol/mg protein). Ligand
concentration at half-maximal binding was the same in both preparations (3.6 \pm 0.06 in normal vs. 3.3 \pm 0.13
nM in CRF).

**DISCUSSION**

Previous studies conducted in this laboratory demon-
strated that transepithelial fluxes of Cl$^-$ and organic
anions (urate and oxalate) are markedly altered in the
distal colon of rats with CRF compared with normal
rats (9–11). These anions, which are normally absorbed
by the rat distal colon, are secreted in response to CRF.
On the basis of the losartan sensitivity of CRF-induced
secretory pathways, we proposed that anion secretion
across this intestinal segment in CRF involves ANG II
secretory pathways, we proposed that anion secretion
across this intestinal segment in CRF involves ANG II
involves ANG II (9). In the present study, we have more completely
addressed the possible role of ANG II in mediating
enteric elimination of K$^+$ in CRF.

Extrarenal K$^+$ elimination by colonic secretion of K$^+$
is known to occur in patients with renal insufficiency
and in animals with CRF (2, 18, 21, 23, 24). We have
confirmed this phenomenon in the present study by
directly measuring colonic fluxes of K$^+$ across short-
circuited tissues that were removed from both normal
and rats and rats with CRF (five-sixths nephrectomized).
We also confirm that the primary site for K$^+$ adapta-
tion, within the intestinal tract, is the large intestine,
where coordinated alteration in both the absorptive
and the secretory components of transepithelial K$^+$
transport are shown to occur. CRF had a limited affect
on small intestinal K$^+$ transport, which was confined to
a reduction in the absorptive component of K$^+$ trans-
port across the ileum; in contrast, K$^+$ fluxes across the
jejunal segment were not altered.

Mechanisms of K$^+$ absorption and secretion. In the
rat distal colon, active K$^+$ absorption is an electroneu-
tral, sodium-independent, and partly chloride-indepen-
dent process (26). An apical uptake mechanism involv-
ing an H$^+$-K$^+$-ATPase has been suggested, and a
basolateral exit mechanism via a barium-sensitive conductive process has been proposed (26). The me-
chanisms explaining transepithelial active K$^+$ secretion
involve uptake at the basolateral membrane via a ouabain-sensitive Na$^+$-K$^+$-2Cl$^-$ cotransporter (3). Exit
across the apical membrane is conductive through barium-sensitive channels (3, 26). Modulation of co-
lonic K$^+$ transport has been demonstrated in response
to changes in dietary K$^+$ (2, 8), and for example, K$^+$
secretion can be induced in animals fed a high-K$^+$ diet
(22). The mechanism of K$^+$ adaptation in renal insuffi-
ciency appears to be somewhat similar to the response
following an oral K$^+$ load (22). Since aldosterone concen-
trations are elevated in response to a high dietary K$^+$
(19) and because the role of aldosterone in enhancing
renal excretion of K$^+$ is well established, a central role
for aldosterone in mediating enteric elimination of K$^+$
has been generally assumed (13).

Although early observations suggested that hyperal-
dosteronism does not contribute to enteric K$^+$ excretion
in CRF (12), subsequent conflicting reports did not
exclude a role for aldosterone in mediating K$^+$ secretion
by the large intestine (4, 13). Consequently, although
aldosterone clearly affects colonic K$^+$ transport (3), it is
not certain whether the mineralocorticoid has an inte-
gral role in the CRF-induced enteric excretion of K$^+$
(4, 23, 24). We have concluded from the present study that
it is unlikely that primary signal-initiating colonic K$^+$
elimination in CRF is aldosterone. First, although
aldosterone stimulates K$^+$ secretion across the rat
distal colon (3), the mineralocorticoid does not stimu-
late Cl$^-$ secretion across this segment (29). The distal
colon, in the CRF rat model, consistently supports a net
secretion of both Cl$^-$ and other organic anions known
to have an affinity for Cl$^-$ transport systems in large
intestinal epithelia (9–11). Second, dietary K$^+$ intake
and circulating concentrations of aldosterone were not
different in CRF rats compared with normal rats; the
latter finding is in agreement with previous reports
addressing this specific question (23, 24). Third, the
addition of ANG II to short-circuited CRF tissue prepa-
risations resulted in an immediate alteration in electro-
lyte transport as judged by the increases in $I_{sc}$. Since
this response occurred within minutes of adding the
octapeptide, the time frame of this effect on $I_{sc}$ and on
Rb$^+$ fluxes is not consistent with an aldosterone-
mediated effect on colonic transport. The foregoing
arguments do not, however, preclude the possibility
that hyperaldosteronism in the setting of renal insuffi-
ciency may frequently occur and may serve as a supple-
mentary mechanism in further enhancing colonic K$^+$
secretion.
ANG II mediation of colonic secretion in CRF. Initial, convincing evidence for ANG II involvement in CRF-induced colonic anion secretion was provided in a recent report from our laboratory (9). The present study provides further, substantial evidence that ANG II has an integral role in modulating colonic K\(^+\) secretion in CRF. ANG II is known to have a dual action on renal and intestinal epithelia (17). At low concentrations, ANG II stimulates sodium absorption via norepinephrine release from enteric sympathetic nerves (17). At high concentrations, ANG II inhibits absorption by prostaglandin production (17). Interestingly, ANG II-induced Cl\(^-\) secretion by cultured tracheal epithelial cells was found to be sensitive to both the prostaglandin inhibitor indomethacin and to the ANG II receptor antagonist losartan (28). In the present study, we demonstrated the losartan sensitivity of CRF-induced net Rb\(^+\) secretion across the rat colon. We also showed that the basal serosal-to-mucosal flux of Rb\(^+\) across CRF colon further increased with the addition of ANG II. Although the latter experiments indicated that CRF secretory tissues responded to exogenously applied ANG II in a dose-dependent manner, quantifying the relationship between an increase in ANG II receptor density (or ANG II sensitivity) and the rate of ANG II-stimulated Rb\(^+\) secretion in CRF colon would be difficult given this experimental design. First, receptor agonistic activity may be coupled to one or more signal transduction pathways, possibly involving both intercellular and intracellular mediators. Second, to what extent each component of the secretory machinery and signaling pathways is activated and sustained is not easily resolved. Simply stated, a twofold increase in ANG II receptor density may not correlate with a twofold increase in the rate of ANG II-stimulated Rb\(^+\)/K\(^+\) secretion across the isolated CRF colonic tissue that is already primed for secretion. Although the concentrations of ANG II employed here to further stimulate the basal CRF-induced secretion of Rb\(^+\) are high, it was demonstrated\(^3\) that tissue degradation of the peptide is significant. The issue of peptide hydrolysis within intestinal mucosa has also been addressed experimentally by Cox et al. (5) in a study of ANG II receptor binding in the small and large intestine. These investigators examined the susceptibility of ANG II to hydrolysis by endogenous proteases in intestine and determined that after 5 min at 22°C, as well as in the presence of inhibitors and nonspecific proteins, 70% of the free hormone had been hydrolyzed (5). In the present study, the effective dose of ANG II within the tissue is estimated to be two orders of magnitude less than the bath concentration of 10\(^{-8}\) M. If one assumes that this dose-degradation relationship can be linearly extrapolated, then a local tissue concentration of 10\(^{-6}\)–10\(^{-7}\) M ANG II further stimulates K\(^+\) secretion across the CRF distal colon. Although the tissues responded to a lower concentration, as judged by the response in \(I_{sc}\) changes in the secretory flux of Rb\(^+\) could not be resolved.

The apparent ANG II-mediated effects on electrolyte transport across the CRF colon do not, however, result from either an elevation in circulating levels of ANG II or a change in local ANG II concentrations within colonic tissue. Normal circulating concentrations of ANG II are maintained up to 48 h after bilateral nephrectomy, and the persistence of these normal levels, given the short biological half-life of the octapeptide (1), is presumably due to the remnant kidney and extrarenal generation by other tissues. It was not, therefore, surprising to find a comparable plasma ANG II concentration in the CRF and control groups; however, the finding of no increase in tissue ANG II content was unexpected. It is notable that tissue ANG II concentrations are 10-fold higher than plasma concentrations, suggesting local production of the octapeptide. Although we found no indication that local production of ANG II is increased in CRF tissues, local generation may also partly explain the lack of effect of exogenous ANG II at the lower bath concentrations.

Autoradiographic studies confirming the presence of specific ANG II receptors in the small and large intestine (5, 7) show that the density of ANG II receptors is greatest in the colon (7). Our saturation binding studies confirmed the presence of specific \(^{125}\text{I}-\text{ANG II}\) binding sites in the normal rat colon, and the \(B_{max}\) of 15.22 ± 0.4 fmol/mg protein was comparable to that (11.31 ± 2.66 fmol/mg, \(n = 3\)) found by Cox et al. (5) for the same tissue preparation, under similar assay conditions. On the basis of the pharmacological designation of ANG II receptor subtypes, the inhibition of CRF-induced electrolyte secretion by losartan and the lack of inhibition by PD-123319 together indicate that the predominant receptor subtype in CRF rat distal colon is \(AT_1\). This conclusion is consistent with previous characterizations of the ANG II receptor subtypes within the rat colon (7, 25).

The present study clearly shows that there is a twofold increase in specific \(^{125}\text{I}-\text{ANG II}\) binding in CRF colon and a comparable increase in CRF rat distal colon. Although the concentrations of ANG II employed here to further stimulate the basal CRF-induced secretion of Rb\(^+\) are high, it was demonstrated\(^3\) that tissue degradation of the peptide is significant. The issue of peptide hydrolysis within intestinal mucosa has also been addressed experimentally by Cox et al. (5) in a study of ANG II receptor binding in the small and large intestine. These investigators examined the susceptibility of ANG II to hydrolysis by endogenous proteases in intestine and determined that after 5 min at 22°C, as well as in the presence of inhibitors and nonspecific proteins, 70% of the free hormone had been hydrolyzed (5). In the present study, the effective dose of ANG II within the tissue is estimated to be two orders of magnitude less than the bath concentration of 10\(^{-8}\) M. If one assumes that this dose-degradation relationship can be linearly extrapolated, then a local tissue concentration of 10\(^{-6}\)–10\(^{-7}\) M ANG II further stimulates K\(^+\) secretion across the CRF distal colon. Although the tissues responded to a lower concentration, as judged by the response in \(I_{sc}\) changes in the secretory flux of Rb\(^+\) could not be resolved.

The apparent ANG II-mediated effects on electrolyte transport across the CRF colon do not, however, result...
arterial blood pressure has been shown to be significantly elevated compared with normal; $\Delta^1 = 66$ mmHg, see Ref. 9) is consistent with the notion that an upregulation of AT$_1$ gene expression in either hypertensive rat model may not be confined exclusively to the kidneys (20) or ventricular myocardium (27) or, indeed, colonic epithelium (present study).

In conclusion, ANG II has various agonistic activities in numerous target tissues resulting in a broad range of physiological effects. Although we cannot exclude other neuroimmune/paracrine elements or hemodynamic influences, the results of this study suggest that ANG II is involved in modulating transepithelial electrolyte transport in $K^+$ adaptation in CRF via an upregulation of colonic ANG II receptors.

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Address for reprint requests: M. Hatch, Dept. of Medicine, Division of Nephrology, Univ. of California at Irvine, Medical Sciences 1, Rm. C380, Irvine, CA 92697.

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