Regulation of jejunal sodium and water absorption by angiotensin subtype receptors

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The octapeptide ANG II is a physiologically important sodium-retaining hormone, contributing to the maintenance of extracellular fluid volume by stimulating thirst (16), aldosterone secretion (10), vasopressin release (32), and transport of sodium and water across epithelial tissues (7, 19, 33). These and other effects of ANG II are mediated by binding to specific ANG II receptors (14). At present, the subtype-1 (AT1) ANG II receptor is thought to be the major cellular effector mediating virtually all of the known effects of ANG II (13, 14). Although the subtype-2 (AT2) ANG II receptor has been cloned and sequenced (22, 34), the AT2 receptor has a low degree of expression compared with that of the AT1 receptor, and the physiological effects of ANG II that are mediated by an action at the AT2 receptor are largely unknown (11, 13, 14, 35, 39, 41).

In the gastrointestinal tract, ANG II has been shown to mediate epithelial sodium and water absorption in the jejunum, ileum, and distal colon (25). In the jejunum, the effect of ANG II on sodium and water transport is dose dependent (2, 27, 28). At low doses, ANG II physiologically interacts with a high-affinity ANG II receptor to stimulate net ion and water absorption, whereas at high doses the peptide interacts with low-affinity receptors to inhibit absorption and/or stimulate secretion (2, 27, 28). The receptor subtypes and mechanisms mediating these effects of ANG II on sodium and water transport in the jejunum are unknown (14). The present study was conducted to elucidate which ANG II receptor subtype mediates each of these responses and to determine the mechanisms by which these responses occur.

METHODS

Animals. Experiments were conducted in male Wistar rats weighing 200–250 g obtained from Harlan (Indianapolis, IN). Rats were maintained on a normal diet (containing 0.28% sodium) or a low-sodium diet (containing <0.05% sodium) for 5 days. Both groups of rats were allowed free access to water and were housed in a room with 12:12-h light-dark cycles. Sodium restriction was validated by measurement of sodium in 24-h urine samples collected with the rats in metabolic cages. The animals were fasted overnight before study.

Operative procedure. Rats (n = 6 in each group) were anesthetized with 70 mg/kg pentobarbital sodium (ip), and the trachea and left jugular vein were cannulated. A venous cannula (PE-50) was connected to a Harvard 975 infusion pump (Harvard Apparatus, Millie, MA) through which lactated Ringer solution (vehicle), ANG II (Peninsula Laboratories, Belmont, CA), and/or ANG II receptor subtype agonists and/or antagonists were infused at the rate of 20 µl·kg⁻¹·min⁻¹.

Measurement of jejunal fluid absorption. A ventral midline celiotomy was performed, and the proximal end of the jejunum (the duodenal-jejunal flexure or 30 cm below the pylorus) was loosely ligated. A second ligature was positioned loosely tied 15 cm distal to the first. The resulting 15-cm intestinal segment was washed thoroughly with lactated Ringer solution and gently emptied, forming a closed jejunal loop. After a 15-min rest period, the jejunal loop was filled with lactated Ringer solution and gently emptied, forming a closed jejunal loop. After a 15-min rest period, the jejunal loop was filled with lactated Ringer solution and gently emptied, forming a closed jejunal loop. After a 15-min rest period, the jejunal loop was filled with lactated Ringer solution and gently emptied, forming a closed jejunal loop.
with 1) 3 ml Krebs-Ringer-bicarbonate solution or 2) 3 ml lactated Ringer solution, both containing [14C]inulin (15,000 dpm/ml; 2.2 μCi/mg specific activity; New England Nuclear, Boston, MA), and the jejunal loops were gently agitated to ensure complete mixing. Then a 0.15-ml sample of fluid was removed at time 0 (first sample). The loop was returned to the abdominal cavity, and an intravenous infusion of isotonic saline was initiated (20 μl/min) for 15 min, after which the jejunal loop was exposed and a second 0.15-ml sample was removed. The loop then was returned to the abdomen, and the saline infusion either was continued (control animals, n = 6) or was replaced by an infusion of ANG II, CGP-42112A (CGP; Ciba-Geigy, Basel, Switzerland), a selective AT2 receptor antagonist (30), losartan (Los; Dupont-Merck Pharmaceutical, Wilmington, DE), a specific long-acting nonpeptide AT1 receptor antagonist (6, 46, 47), at 10 mg/kg iv; guanethidine (Ciba-Geigy; 20 mg/kg ip) was administered immediately before measurement of water transport. Drug administration was performed at 10 min, 30 min, and 60 min after the first sample removed at 15 min, and V2 is volume of second sample removed at 30 min (after 15 min of administration of pharmacological agent). Volume of fluid transported in the first period (X) is represented by

\[
(V_1 - V_2) - \left(\frac{dpm \ 0 \ min}{dpm \ 15 \ min}\right) = X
\]

Volume of fluid transported in the second period (Y) is represented by

\[
(V_1 - V_2) - (X + V_3)
\]

Because ANG peptides do not affect the water content of the jejunum (27), the volumes of fluid transported in each period (X and Y) were divided by the wet weight of the sac removed from the animal to control for differences in absorptive surface area between experimental animals.

Measurement of cAMP, cGMP, and PGE2 in the jejunal loop.

In separate groups of animals (n = 6 in each group), jejunal loops were prepared and animals were instrumented as previously indicated. However, instead of measuring jejunal fluid absorption, loop concentrations of cAMP, cGMP, and PGE2 were measured in the 0.15-ml samples of fluid recovered at 0, 5, 15, and 30 min in response to infusion of vehicle or ANG II. Drug groups were ANG II, ANG II + Los, ANG II + PD, ANG II + PD + PGE2, or ANG II + Los + PD + or ANG II + prazosin. The production rates of jejunal cAMP, cGMP, and PGE2 were corrected for loop volume changes in response to these pharmacological agents.

Measurement of jejunal interstitial fluid cAMP, cGMP, and PGE2.

For the determination of jejunal interstitial fluid cGMP and PGE2, we constructed a microdialysis probe as previously described for the kidney (37). Recoveries for these substances observed with a perfusion rate of 3 μl/min and were 70% for cAMP, 70% for cGMP, and 63% for PGE2 (37). In separate groups of rats (n = 6 in each group) instrumented as above, but without closed jejunal loops, the serosal surface of the jejunum was penetrated with a 31-gauge needle that was tunneled in the jejunum ~1 mm from the outer serosal surface before it exited by penetrating the serosal surface again. The tip of the needle was inserted into one end of the dialysis probe, and the needle was pulled together with the dialysis tube until the dialysis fiber was situated in the jejunal serosa. The inflow and outflow tubes of the dialysis probes were tunneled subcutaneously through a bevel-tipped stainless steel tube and exteriorized. For collection of jejunal interstitial fluid, the inflow tube was connected to a gas-tight syringe filled with lactated Ringer solution and perfused at 3 μl/min. The effluent was collected from the outflow tube for 30-min sample periods in nonheparinized plastic tubes and stored at −80°C until assayed for cGMP or PGE2. Because limited amounts of jejunal interstitial fluid were available, each experiment was repeated three times and cGMP or PGE2 was measured during each experiment. Experiments were conducted as stated above for measurement of jejunal loop concentrations of cAMP, cGMP, and PGE2 except that interstitial fluid samples were collected over a 30-min period in response to infusion of vehicle or ANG II or CGP or ANG II + PD or ANG II + los or ANG II + PD + Los.

Analytic methods.

Jjejunal interstitial and loop fluid cAMP, cGMP, and/or PGE2 levels in dialysate samples were measured by enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). The sensitivities and specificities of this method for cAMP were 0.10 pmol/ml and 100%, for cGMP 0.11 pmol/ml and 90%, and for PGE2 0.05 pmol/ml and 90%.
RESULTS

Effects of ANG II, PD, and Los on jejunal fluid transport. Figure 1 shows that the rate of fluid absorption from the dosed jejunal loop remained constant over the two consecutive 15-min periods at 0.17 ± 0.01 ml·g wet tissue⁻¹·15 min⁻¹ when the isotonic saline vehicle (control) was infused throughout the experiment. Responses of jejunal fluid transport to infusion of vehicle (control) was infused throughout the experiment from the closed jejunal loop remained constant over the two consecutive 15-min periods at 0.17 ± 0.01 ml·g wet tissue⁻¹·15 min⁻¹. The stimulation of fluid absorption by ANG II was blocked completely by coinfusion of the AT₂ receptor antagonist PD, but was not affected by the administration of the AT₁ receptor antagonist Los. In the absence of exogenous ANG II, neither PD nor Los alone affected baseline jejunal water transport (data not shown). The increase in net jejunal absorption obtained with low-dose exogenous ANG II was blocked completely when PD and Los were combined (data not shown).

Responses of jejunal fluid transport to infusion of a high dose of ANG II (700 pmol·kg⁻¹·min⁻¹) during the second 15-min period are depicted in Fig. 1B. High-dose ANG II infusion resulted in a significant inhibition of water absorption below control levels. Los blocked completely this effect of high-dose ANG II, which also was blocked to an equivalent degree by the combination of Los and PD (data not shown). The effect of high-dose ANG II to inhibit absorption was significantly augmented when ANG II was infused in the presence of PD (P < 0.05). Los or PD alone in the absence of ANG II did not affect baseline jejunal absorption (data not shown).

ANG II at the intermediate infusion rate of 70 pmol·kg⁻¹·min⁻¹ (data not shown) had no significant effect on water transport. However, in the presence of Los, this dose of ANG II increased water absorption from baseline values, whereas in the presence of PD, ANG II inhibited absorption. In these experiments, Los or PD alone or combined together with ANG II (Los + PD + ANG II) had no effect on water transport.

Figure 2 shows the dose-dependent relationship between exogenous ANG II and net fluid absorption in the isolated jejunal segments. No significant change in net absorption was observed at ANG II infusion rates below 0.7 pmol·kg⁻¹·min⁻¹. The maximum increase in net absorption occurred at 0.7 pmol·kg⁻¹·min⁻¹ of ANG II. With escalating infusion rates of ANG II above 10 pmol·kg⁻¹·min⁻¹, a decrease in net absorption was observed until, at 700 pmol·kg⁻¹·min⁻¹, absorption fell below control levels. Figure 2 shows that in the presence of AT₂ receptor blockade with PD, ANG II did not cause any increase in net absorption at any infusion rate and that above an ANG II infusion rate of 30 pmol·kg⁻¹·min⁻¹, ANG II inhibited absorption below control levels when coadministered with PD. Figure 2 also shows that in the presence of AT₂ receptor blockade with Los, ANG II at low infusion rates (0.7–10 pmol·kg⁻¹·min⁻¹) was able to mediate a full absorptive response, but that at infusion rates above 10 pmol·kg⁻¹·min⁻¹ the effect of ANG II to inhibit absorption was impaired (P < 0.01 for infusion rates of 30, 70,
and 700 pmol·kg\(^{-1}\)·min\(^{-1}\) compared with the same infusion rate of ANG II alone). At an ANG II infusion rate of 700 pmol·kg\(^{-1}\)·min\(^{-1}\) in the presence of Los, the inhibition of absorption below control values by ANG II was abolished.

Effects of CGP, PD, and Los on jejunal water absorption. Figure 3 depicts the effect of CGP, a selective AT\(_2\) receptor agonist, on jejunal fluid transport. CGP induced an inverse dose-dependent effect on water absorption in the jejunum. At the low infusion rate of 0.1 µg·kg\(^{-1}\)·min\(^{-1}\) CGP increased absorption maximally (5-fold from baseline values). At an infusion rate above that level (0.1 µg·kg\(^{-1}\)·min\(^{-1}\) and above) CGP did not induce a significant increase in absorption. With increasing infusion rates of CGP, above 0.1 µg·kg\(^{-1}\)·min\(^{-1}\), a stepwise inhibitory response was observed. The action of CGP (0.1 µg·kg\(^{-1}\)·min\(^{-1}\)) to increase water absorption was blocked completely by AT\(_2\) receptor blockade with PD but was not affected by AT\(_1\) receptor blockade with Los.

Effect of sodium restriction on jejunal water transport. Dietary sodium restriction decreased urinary sodium excretion from 8.1 ± 2.5 to 0.01 ± 0.08 meq/24 h (P < 0.0001) after 5 days. Dietary sodium restriction increased baseline values of net fluid absorption (0.29 ± 0.06 ml·g wet tissue\(^{-1}\)·15 min\(^{-1}\)) compared with non-sodium-restricted animals (0.19 ± 0.02 ml·g wet tissue\(^{-1}\)·15 min\(^{-1}\); P < 0.05). In a separate group of sodium-restricted animals, Los administration increased jejunal absorption and PD administration inhibited absorption (Fig. 4). When Los and PD were combined, there was no net effect on fluid absorption in the jejunum (data not shown).

Effect of guanethidine and prazosin on jejunal fluid transport. Figure 5 depicts responses of jejunal fluid absorption to high and low infusion rates of ANG II in the presence of the sympathetic nervous system inhibitor guanethidine and the \(\alpha_1\) adrenergic receptor antagonist prazosin. The decrease in absorption engendered by high-dose ANG II was unaffected by either agent. However, the increase in fluid absorption stimulated by low-dose ANG II was inhibited by both guanethidine (P < 0.05) and prazosin (P < 0.01).

Effect of ANG II, PD, and Los on jejunal loop cAMP, cGMP, and PGE\(_2\). cAMP in the jejunal loop fluid is shown in Fig. 6. A high infusion rate of ANG II (700 pmol·kg\(^{-1}\)·min\(^{-1}\)) caused a time-dependent decrease in cAMP, whereas a low infusion rate (0.7 pmol·kg\(^{-1}\)·min\(^{-1}\)) produced no significant change (data not shown).
The decrease in cAMP in response to high-dose ANG II infusion for 30 min (Fig. 6) was blocked to control values by Los and by the combination of Los and PD (data not shown) but not by PD alone. Low-dose ANG II alone or in the presence of Los and/or PD did not alter cAMP significantly.

Figure 7 shows jejunal loop fluid cGMP. Low-dose ANG II produced a time-dependent increase in cGMP, whereas a high dose of the peptide caused no significant change (data not shown). Low-dose ANG II infusion for 30 min (Fig. 7) caused an approximate eightfold rise in cGMP, which was blocked to control levels by PD and by PD and Los combined (data not shown), and by prazosin, but not by Los alone. High-dose ANG II alone or in the presence of Los and/or PD did not cause a significant change in cGMP.

PGE2 in the jejunal loop is depicted in Fig. 8. High-dose ANG II caused a time-dependent stimulation of jejunal PGE2, whereas low-dose ANG II did not alter PGE2 significantly (data not shown). Los blocked the ~60-fold increase in PGE2 stimulated by high-dose ANG II infusion for 30 min (Fig. 8). PD did not alter the increase in PGE2 elicited by high-dose ANG II, but the combination of Los and PD blocked PGE2 to control levels (data not shown). In contrast to high-dose ANG II, low-dose ANG II alone, or combined with PD or Los, did not alter jejunal PGE2.

Effect of ANG II, CGP, PD, and Los on jejunal interstitial fluid cGMP and PGE2. Jejunal interstitial fluid levels of cGMP and PGE2 are shown in Fig. 9, A and B, respectively. As shown in Fig. 9A, both 0.1 µg·kg⁻¹·min⁻¹ CGP and 0.7 pmol·kg⁻¹·min⁻¹ ANG II increased interstitial fluid cGMP. The increase in interstitial fluid cGMP was blocked completely by PD but not by Los (data not shown). As shown in Fig. 9B, high-dose ANG II increased interstitial fluid PGE2, and this response was blocked by Los but not by PD (data not shown).
Effect of 8-BrcGMP on jejunal fluid transport. Figure 10 shows that administration of 8-BrcGMP (0.6 µmol/rat) into loop caused an inhibition of fluid absorption. However, mesenteric artery administration of the same quantity of 8-BrcGMP caused an increase in fluid absorption from jejunal loop. Administration of 8-BrcGMP into the jejunal interstitial space also caused an increase in fluid absorption (P < 0.001).

DISCUSSION

Our data show that jejunal fluid absorption is regulated by the AT2 receptor. ANG II at low infusion rates (0.7 pmol·kg\(^{-1}·\min^{-1}\)) stimulated jejunal fluid absorption. This response was blocked completely by the selective AT2 receptor antagonist PD but not by the AT1 receptor antagonist Los. The combination of PD and Los resulted in the same degree of blockade of ANG II-stimulated jejunal absorption as with PD alone, suggesting that the entire increase in absorption was mediated by AT2 receptors. These findings were confirmed by the marked increase in absorption in response to the AT2 receptor agonist CGP at a low dose specific for AT2 receptors (29, 30). Although CGP is a highly selective ligand at the AT2 receptor (IC\(_{50}\) 5 × 10\(^{-10}\) and 2 × 10\(^{-8}\) M for AT2 and AT1 receptors, respectively), at high concentrations (>1 µM) CGP occupies both AT1 and AT2 receptors (29, 30). At the doses employed in the present study, CGP is specific for the AT2 receptor. CGP was demonstrated to blunt pressure-induced natriuresis in the rat kidney at a dose 100-fold higher than that which increased absorption maximally in the present study (30). Instead, the inverse dose-response relationship of CGP on intestinal absorption observed in the present study is typical of AT2 receptor responses to this agonist in other systems (30). The maximal absorptive response to CGP (at an infusion rate of 0.1 µg·kg\(^{-1}·\min^{-1}\)) was blocked completely by PD, but not by Los, indicating that the effect of CGP on jejunal fluid absorption was mediated by AT2 receptors. Taken together, these data strongly support the thesis that jejunal fluid absorption is mediated by ANG II through an action at the AT2 receptor.

Previous studies from our laboratory have indicated that ANG II stimulates sodium and water absorption via a high-affinity ANG II receptor located on sympathetic nerve terminals in close proximity to intestinal epithelial cells (27, 28). ANG II has been shown to stimulate norepinephrine release from presynaptic sympathetic nerve terminals (28). Evidence for this mode of ANG II action in the jejunum includes prevention of the ANG II-mediated increase in absorption by α- but not β-adrenergic receptor blockade, chemical sympathectomy, or treatment with 6-hydroxydopamine or guanethidine (27, 28). Because neither chemical sympathectomy nor 6-hydroxydopamine or guanethidine influenced norepinephrine release from the adrenal medulla or central nervous system, it is generally accepted that ANG II increases absorption by an action at enteric sympathetic neurons (1, 24, 40). To date, most studies have shown that the presynaptic ANG II receptor mediating norepinephrine release is the AT1 receptor in the vasculature and kidney of the rat (9, 21, 45). However, a recent study indicated that in the rat carotid artery and vas deferens, both AT1 and AT2 receptors stimulate norepinephrine release (9). In the present study, we redocumented that the action of ANG II at low infusion rates to increase absorption was blocked by both guanethidine and prazosin. Because low infusion rates of ANG II stimulate AT2 but not AT1 receptors, these results indicate that in the jejunum AT2 receptor stimulation releases norepinephrine, which acts at postsynaptic α2-adrenergic receptors to increase absorption. Clearly, more work will be required to clarify the precise manner in which jejunal AT2 receptors mediating fluid and sodium absorption act to facilitate sympathetic neurotransmission. However, the data of the present study strongly suggest that this presynaptic receptor is an ANG II receptor of the AT2 subtype.

In the present study, we demonstrated that low-dose ANG II and CGP stimulated cGMP release into the jejunal interstitial and/or luminal (loop) fluid. The increase in cGMP induced by ANG II was blocked by PD and by prazosin but not by Los. These data suggest that ANG II increases fluid absorption via the AT2 receptor by a cGMP-dependent mechanism. Previous studies have shown that luminal cGMP mediates net secretion in the intestine, the putative mechanism by which the heat-stable toxin (STa) of Escherichia coli causes secretion and diarrhea (20, 42). In our experiments, however, an increase in cGMP was associated with an increase in absorption. We clarified this difference by administering the cGMP analog 8-BrcGMP into the jejunal loop and directly into the mesenteric vascular or the jejunal interstitial compartment. We demonstrated that, similar to the literature, 8-BrcGMP inhibited absorption when administered into the loop. In marked contradistinction, 8-BrcGMP administered into the mesenteric vascular or jejunal interstitial space caused a highly significant absorptive response. Thus cGMP has
different effects on jejunal transport, depending on the compartment into which it is introduced. In the kidney, cGMP has been shown to be released into the extracellular environment (5), and we have recently demonstrated that cGMP is released into renal interstitial fluid by an action of ANG II at the AT2 receptor (37). Extruded cGMP may mediate sodium and water transport across the renal tubule (4). Although the mechanism of cGMP formation and extrusion in the jejunum in response to ANG II is uncertain, nitric oxide is a likely candidate, as we have recently demonstrated in the kidney (38). This interpretation is consistent with the recent report of Schirgi-Degen and Beubler (36), who found that intravenous Nω-nitro-L-arginine methyl ester caused net fluid secretion in the rat jejunum and concluded that nitric oxide enhances absorption in the intestine.

ANG II at high doses has been shown to inhibit fluid absorption and/or stimulate secretion in the jejunum (27, 28). The present study indicates that at infusion rates above 10 pmol·kg−1·min−1 ANG II inhibited absorption. This inhibition of absorption in response to ANG II was blocked completely by Los, indicating that this response was mediated by the AT1 receptor. The combination of Los and PD also blocked ANG II-mediated inhibition of absorption to the same degree as Los alone, indicating that the inhibition of absorption in response to ANG II was mediated by AT1 receptors. Interestingly, however, AT2 receptor blockade with PD alone enhanced significantly the inhibition of absorption in response to high-dose ANG II. The ability of AT2 receptor blockade to enhance the inhibitory response to high-dose ANG II may be due to receptor “cross-talk,” in which blockade of one receptor subtype leads to an enhanced response to the exogenous agonist via the other receptor subtype. This effect can be mediated by increased production of the agonist if negative feedback suppression of the hormone (agonist) secretion is interrupted by specific subtype receptor blockade (26, 37). However, we recently demonstrated that AT2 receptor blockade with PD augmented AT1-receptor-mediated renal PGE2 production in the rat in the absence of a change in plasma renin activity (37). Thus blockade of the AT2 receptor may augment ANG II action at the AT1 receptor even in the absence of increased agonist production.

It is generally acknowledged that absorptive and secretory processes occur simultaneously in the intestine. Under normal physiological conditions, absorption is generally greater than secretion, leading to a net uptake of ions and water from the intestinal lumen. In our model, none of the experimental manipulations resulted in a decrease in net absorption below zero, so we could not clearly document a secretory event. Therefore, we refer to a decrease in absorption below control values as inhibition of absorption even though we recognize that simultaneous secretion may be taking place.

In contrast to the effects of PD or Los to block absorption or to block inhibition of absorption, respectively, stimulated by exogenous ANG II, neither subtype ANG II receptor antagonist affected basal absorption. However, we were interested to determine whether changes in jejunal absorption could be mediated physiologically by endogenous ANG II. Past studies from our laboratory have shown that jejunal sodium and water absorption can be enhanced in response to extracellular fluid volume depletion due to profound sodium restriction with peritoneal dialysis or dehydration (26). In the present study, dietary sodium restriction also increased basal jejunal fluid absorption. During dietary sodium restriction, AT1 receptor blockade with Los increased absorption, indicating that endogenous ANG II may have a tonic physiological effect to inhibit absorption via an action at the AT1 receptor and/or that AT1 receptor blockade increased absorption by unmasking a tonic effect of ANG II at the AT2 receptor. In contrast, AT2 receptor blockade in the presence of sodium restriction inhibited baseline absorption, suggesting that endogenous ANG II may stimulate absorption physiologically and that AT2 receptor blockade may unmask ANG II-inhibited absorption through the AT1 receptor. The similarity of responses to specific receptor subtype blockade in the presence of sodium depletion and during infusion of the intermediate dose of ANG II (70 pmol·kg−1·min−1) suggests the possibility that the level of circulating ANG II achieved during exogenous ANG II infusion at 70 pmol·kg−1·min−1 approximated that engendered by dietary sodium depletion. Evidence that low sodium diet brings out significant subtype receptor effects by increasing endogenous ANG II has been provided by the observation that the jejunal response to sodium restriction can be prevented by inhibition of the renin-angiotensin system (2). Therefore, it is highly likely that endogenous ANG II increases the functional activity of the subtype receptor responses to the peptide. These findings support the concept of physiological cross-talk between the AT1 and AT2 receptors. When both receptors were blocked simultaneously, absorption was unaltered from basal values, suggesting that other non-AT1 or -AT2 receptors are unlikely to mediate transport processes physiologically.

At progressively higher doses of ANG II than 10 pmol·kg−1·min−1, the peptide is thought to interact with a lower-affinity receptor on epithelial cells to stimulate prostaglandin release (27, 28). In past studies, our laboratory has shown that blockade of prostaglandin synthase with medofenamate or indomethacin prevented this effect of ANG II (27, 28). The present study strongly supports the concept that the jejunal response to high-dose ANG II is accompanied by an increase in PGE2, as ANG II administration resulted in a marked increase of both jejunal interstitial and loop fluid PGE2. Furthermore, the ANG II-induced increase in PGE2 was blocked with Los, but not by PD, indicating that the receptor mediating this action of ANG II is the AT1 receptor. The reduction of cAMP levels associated with AT1 receptor-stimulated PGE2 and reduced absorption is of interest. Indeed, the effects of cholera toxin on secretion have been associated with increases in prostaglandin and platelet-activating factor, and indomethacin and platelet-activating factor antago-
nists have been shown to block cholera toxin-induced secretion without altering cAMP levels (17, 23, 43).

The epithelial effects of ANG II appear to be the most sensitive responses described for the hormone (18). Whether the responses in jejunal fluid transport demonstrated in the present study represent direct actions at ANG II receptors on epithelial cells is uncertain. Cox et al. (8) have shown an electrogenic effect for ANG II on transporting epithelia in the jejunum, which was blocked by prostaglandin synthase inhibition, suggesting eicosanoid involvement in a direct effect at the epithelial cell. Although ANG II in concentrations from $10^{-13}$ to $10^{-10}$ M stimulates sodium and water absorption from isolated preparations of jejunal mucosa, this effect of ANG II in vivo is blocked by inhibition of the sympathetic nervous system (27, 28). Certainly, in the proximal renal tubule and the isolated frog skin, ANG II stimulates sodium and water uptake directly by mechanisms that do not involve catecholamines (7, 19, 33). These cells are functional analogs of intestinal epithelial cells. Further clarification will be needed to determine whether ANG II changes absorption physiologically by a direct action at jejunal epithelial cells.

Changes in jejunal blood flow could have accounted for the changes in fluid absorption or secretion observed in the present study (31). However, ANG II stimulates jejunal absorption at doses that do not affect mean arterial pressure or mesenteric blood flow (27). Flow distribution within the jejunal wall also is unaffected by the low doses of the octapeptide, which increased absorption in the present study (27). Also, the increase in small intestinal absorption engendered by sympathetic nerve stimulation is not accompanied by alteration in jejunal blood flow or blood flow distribution. Thus it is highly unlikely that low-dose ANG II stimulated absorption in the present study through changes in enteric hemodynamics. However, it is likely that the high dose of ANG II, which produced inhibition of absorption, constricted jejunal resistance vessels. Clarification of the relative roles of enteric vasoconstriction and prostaglandin formation in the inhibition of absorption and/or stimulation of secretion requires further study.

In addition to vasoconstriction, ANG II stimulates aldosterone and vasopressin secretion, either of which potentially could have influenced jejunal transport (10, 32). However, aldosterone has been shown to have no significant action in the jejunum, and the jejunal response to ANG II is uninfluenced by adrenalectomy (3, 44). Therefore, aldosterone cannot be the mediator of ANG II in the jejunum. Vasopressin inhibits sodium and water absorption from the small intestine (12). It is clear then that ANG II-stimulated absorption cannot be due to vasopressin release. However, inhibition of absorption in response to high concentrations of ANG II may be partially mediated by vasopressin release, and further study will be required to clarify the possible role of vasopressin in ANG II-induced secretion.

The ANG II receptor subtypes have not yet been localized in the gastrointestinal tract to our knowledge. Duggan et al. (15) have determined that ANG II binding sites are present in the jejunum, localized to the muscularis. The same study documented the presence of angiotensin-converting enzyme in the mucosa and muscularis, and the colocalization of angiotensin-converting enzyme with ANG II receptors suggested to these authors the possibility that local generation of ANG II may play a role in intestinal function (15). Further studies to localize the AT$_1$ and AT$_2$ receptor subtypes structurally are indicated.

In summary, we demonstrated the presence of AT$_1$ and AT$_2$ receptors in the rat jejunum. ANG II stimulates jejunal sodium and water absorption by an action at the AT$_2$ receptor mediated by the sympathetic nervous system accompanied by epithelial cell extrusion of cGMP. Although cGMP inhibited absorption when it was administered via the intestinal lumen, cGMP stimulated absorption when introduced into the local arterial vascular or interstitial compartment. ANG II promotes inhibition of sodium and water absorption and/or stimulation of secretion via the AT$_1$ receptor accompanied by inhibition of cAMP and generation of PGE$_2$. During dietary sodium restriction, a reduction in the function of the AT$_2$ receptor may lead to an augmentation of an ANG II action through the AT$_1$ receptor, and inhibition of the AT$_1$ receptor may augment the action of the octapeptide at the AT$_2$ receptor.

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