REGULATION OF INTESTINAL FLUID TRANSPORT BY ANGIOTENSIN II: MECHANISMS AND PHYSIOLOGICAL SIGNIFICANCE*,†

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Angiotensin II, the octapeptide product of the renin-angiotensin hormonal cascade, is physiologically important in sodium and fluid homeostasis. Angiotensin II maintains extracellular fluid volume by stimulating thirst and salt appetite (1, 2), aldosterone secretion (3), vasopressin release (4) and transport of sodium and water across epithelial surfaces (5–7).

The present review is concerned with the effects of angiotensin II on small intestinal fluid transport. Past studies have demonstrated that the actions of angiotensin II on transporting epithelia are concentration-dependent. In the kidney and small intestine, low physiological concentrations of angiotensin II have been shown to stimulate net mucosal to serosal transfer of sodium and water, while high concentrations of the hormone inhibit absorption or stimulate secretion or both (5–7). In vivo, the effects of angiotensin II on transport are not mediated by changes in intestinal blood flow (7). The fact that angiotensin II is effective in the intestinal tract in vitro suggests a direct effect on transporting epithelial cells (7).

After a reduction in extracellular fluid volume, the absorption of sodium and water is increased from all regions of the intestine (8–10). The absorption of sodium and water from the colon in sodium deficient states is mediated by aldosterone (11). In contrast, the factors responsible for the increase in small intestinal absorption under conditions of reduction in extracellular fluid volume are unknown.

The following studies, performed in our laboratory during the past 3 years, were designed to elucidate the mechanism of action and physiologic relevance of angiotensin II as a mediator of small intestinal fluid trans-

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port. The results of these studies indicate that angiotensin II stimulated intestinal fluid absorption occuring at low doses of the peptide is secondary to release of norepinephrine from sympathetic nerve endings in the jejunum. In high doses, angiotensin II inhibits absorption and/or stimulates secretion by a prostaglandin-dependent mechanism. Further, during extracellular fluid volume depletion, the generated angiotensin II increases small intestinal fluid absorption by facilitating norepinephrine release from enteric sympathetic nerves. Thus, angiotensin II is a physiologically important mediator of intestinal fluid absorption.

Mechanism of Angiotensin II Action in the Small Intestine

Jejunal fluid absorption was measured in isolated jejunal sacs of anesthetized male albino Wistar rats by a method described in detail elsewhere (12). In brief, inulin was used as a nonabsorbable marker, so that after an increase in absorption of fluid from the sac, there was an increase in inulin concentration. From the volume of the samples removed from the sac before and after experimental manipulation, fluid absorption from the jejunal segment during 2 consecutive 15 min periods was calculated. Thus, each animal served as its own control. Fluid absorption is defined as loss of fluid from the intestinal sac and is expressed as ml of water transport per g wet weight of intestinal segment per 30 min.

Initial experiments were performed to determine the effect of angiotensin II on jejunal water transport in peripherally sympathectomized rats. In these experiments, the cardiac norepinephrine concentrations of sympathectomized rats were 22% of controls. Infusion of angiotensin II to normal animals at a subpressor dose of 0.7 ng/kg per min significantly stimulated net fluid absorption from the sac, but a pressor dose of 700 ng/kg/min of angiotension II inhibited water transport significantly. In contrast, sympathectomy completely abolished the response to low dose angiotensin II, while the inhibitory response to high dose angiotensin II was potentiated. These results suggested that the angiotension-dependent absorptive response is mediated by the sympathetic nervous system, but that the inhibition of absorption with higher doses of the peptide is not related to sympathetic stimulation.

Next, we examined the effect of guanethidine on angiotensin II stimulated jejunal fluid transfer. As shown in Figure 1, in the absence of guanethidine, angiotensin II demonstrated a dose-dependent dual action on jejunal absorption. Low doses of the octapeptide (less than 70 ng/kg/min) resulted in stimulation of fluid transport, while a high dose (700 ng/kg/min) was associated with inhibition of absorption. Guanethidine

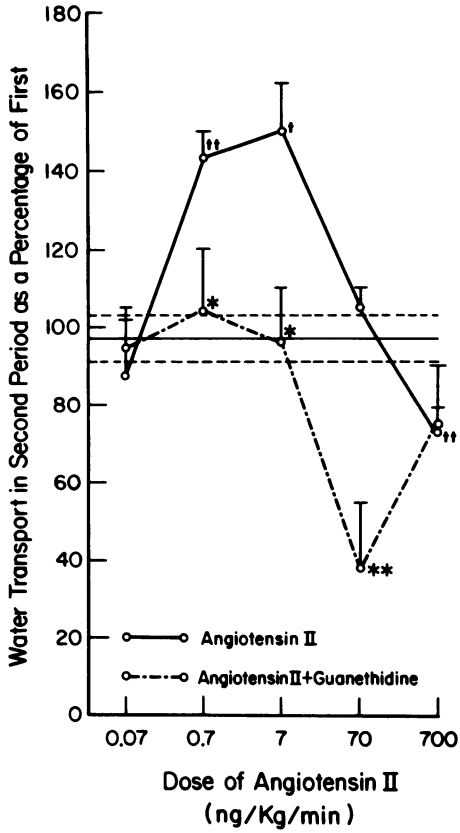

FIG. 1. Effect of angiotensin II on jejunal water transport in the presence and absence of guanethidine. Results are expressed as percent change of water transport in the experimental animals in the second period when angiotensin was infused above that in the first period when saline was infused. The horizontal line and accompanying broken lines represent the mean ± 1 SE of change occuring when saline was infused over both periods in the absence of guanethidine (control animals). Guanethidine did not affect the percent change in water transfer occuring in control animals. Each point represents the mean ± 1 SE, ↑, *, $p < 0.05$, ↑↑, **, $p < 0.01$. Statistics are relative to the percent change in fluid transport induced by angiotensin in the presence and absence of guanethidine (*) or are relative to the changes in water absorption produced by angiotensin II above control animals (↑). Guanethidine (20 mg/kg) was injected 48 and 24h before measurement of water transport.

completely abolished the effect of low dose angiotensin II on stimulation of absorption, but had no significant effect on the inhibitory response at high dose of peptide. Since angiotensin II failed to stimulate jejunal transport at any dose in sympathectomized or guanethidine-treated animals, increased peripheral sympathetic neuronal activity must be respon-

sible for stimulation of jejunal fluid transport after administration of low doses of angiotensin II. In addition, catecholamines are not responsible for the inhibitory action of high doses of the peptide.

Since angiotensin II did not stimulate jejunal fluid transport in sympathectomized animals, it was likely that angiotensin II-induced transport was not mediated by release of catecholamines from the adrenal medulla because the adrenal medulla was still intact (13). In other experiments, we demonstrated that bilateral adrenalectomy did not block the angiotensin II stimulatory response. Infusion of low dose angiotensin II (7 ng/kg/min) significantly stimulated jejunal fluid transport to the same extent in normal and adrenalectomized rats. Thus, angiotensin induced adrenal catecholamine release was not involved in the regulation of fluid absorption. Antiotensin II at 700 ng/kg/min inhibited fluid transport significantly in both intact and adrenalectomized rats, but the inhibitory response was potentiated by adrenalectomy (73 ± 6%, compared to 22 ± 18% in controls, $P < 0.05$).

Analogs of angiotensin II with aliphatic amino acid substitutions for the carboxy terminal phenylalanine possess low agonist activity and are potent competitive inhibitors of the parent peptide (14). However, the substituted analog, [Sar^1,Leu^8]-angiotensin II has even greater potency than the parent peptide in stimulating jejunal fluid absorption. [Sar^1,Leu^8]-angiotensin II stimulates jejunal fluid absorption in normal animals. Since angiotensin II antagonists have significant agonist activity in facilitation of sympathetic neurotransmission (15, 16), it was likely that [Sar^1,Leu^8]-angiotensin II stimulated jejunal fluid transport by means of an agonist activity at peripheral sympathetic neurons. However, it was possible that stimulation of jejunal transport in response to [Sar^1,Leu^8]-angiotensin II may have been due at least in part to increased endogenous angiotensin II production mediated by an enhancement of renin release. Treatment with the angiotensin converting enzyme inhibitor, captopril, at doses which reduced plasma angiotensin converting enzyme activity to undetectable levels and totally abolished angiotensin I-induced fluid transport had no significant effect on angiotensin II or [Sar^1,Leu^8]-angiotensin II mediated responses. Further, in separate experiments reduction of plasma renin activity to undetectable levels by means of acute bilateral nephrectomy had no significant effect on the ability of [Sar^1,Leu^8]-angiotensin II to stimulate jejunal fluid absorption. Therefore, stimulation of jejunal transport during administration of [Sar^1,Leu^8]-angiotension II could not have been due to enhanced endogenous angiotensin II production as a result of renal renin release.

Norepinephrine combines with alpha-adrenergic receptors at postsynaptic membranes and also exerts negative feedback inhibition of catecholamine release from adrenergic nerve endings by combining with

$alpha_2$ receptors on presynaptic neurons (17). The alpha-adrenergic antagonist, phentolamine, inhibits sympathetic function by combining with both $alpha_1$ and $alpha_2$ receptors (17). Thus, it is not possible with phentolamine to determine on which side of the synapse released norepinephrine affects intestinal fluid transport. The alpha-adrenergic antagonist, prazosin, has high affinity and specificity for postsynaptic $alpha_1$ receptors (18). Both prazosin and phentolamine inhibit angiotensin II-induced fluid transport. Therefore, angiotensin II binds to receptors located on postsynaptic sympathetic nerve terminals in close proximity to the transporting epithelial cells of the small intestine, resulting in facilitation of norepinephrine release. The liberated norepinephrine subsequently binds to $alpha_1$ receptors leading to an increase in fluid transport.

Stimulation of jejunal fluid transport after administration of [Sar^1,Leu^8]-angiotensin II also was abolished by phentolamine. This indicates that the analog stimulates jejunal water transport by facilitating norepinephrine release from sympathetic nerve endings.

Both absorptive and secretory processes have been shown to occur simultaneously within the intestinal tract. Under normal physiological conditions, absorption usually is greater than secretion, resulting in a net uptake of fluid from the intestinal lumen. Stimulation of intestinal alpha adrenergic receptors increases absorption and inhibits opposing secretion (19). In contrast, prostaglandins E and F inhibit absorption and stimulate secretion (19). Angiotensin II also appears to stimulate absorptive and secretory processes simultaneously within the intestine. At low doses, the stimulatory (sympathetic) response predominates. At progressively higher doses the peptide induces dose-dependent inhibition of absorption (secretion). As shown in Figure 2, the cyclooxygenase inhibitors, meclofenamate and idomethacin, abolish the inhibition of intestinal absorption (secretion) produced by angiotensin II. Thus, at high doses, the hormone acts by stimulating intestinal prostaglandin production.

The inhibition of transport induced by high doses of angiotensin II was significantly potentiated in sympathectomized animals. Since angiotensin II stimulates absorption and secretion simultaneously, it was possible that only the secretory response to angiotensin II would be elicited in sympathectomized animals with high doses of the hormone. Thus, inhibition of sympathetic nervous system activity would lead to relatively greater inhibition of absorption. This hypothesis was supported by the findings that angiotension II inhibited fluid transport was potentiated in the presence of phentolamine or prazosin. Also, in animals treated acutely with guanethidine, a potentiated inhibition of transport was expressed with high doses of angiotensin II (Figure 1). However, at

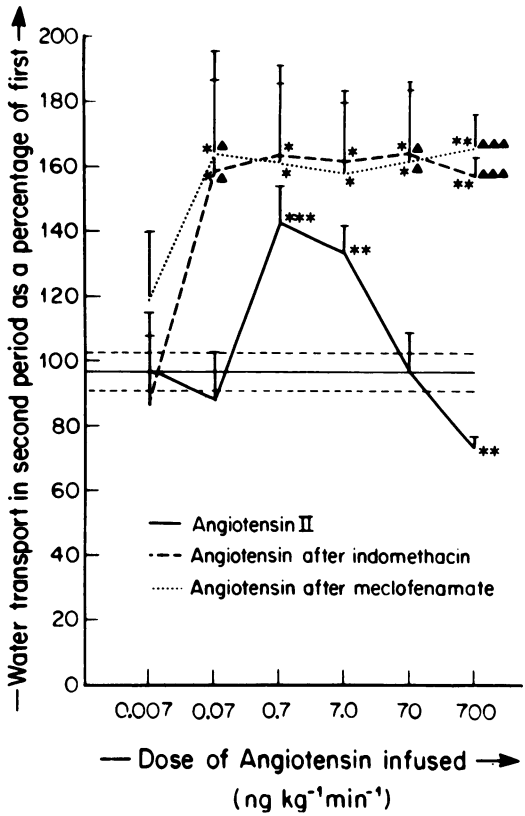

FIG. 2. Effect of angiotensin II on jejunal water transport in the presence and absence of meclofenamate and indomethacin. Results are expressed as percent change of water transport in the second period, when angiotensin was infused (experimental animals). The horizontal line and accompanying broken lines represent mean ± 1 SE of the percent change when saline was infused over both periods in the absence of meclofenamate or indomethacin (control animals). Neither prostaglandin synthetase inhibitor affected the percent change in transfer occuring in control animals. Each point represents mean ± 1 SE, *, Δ, $p < 0.05$; **, $p < 0.01$; ***, ΔΔΔ, $p < 0.001$. Statistics are relative to the percent change in fluid transport in control animals of each experimental treatment (*) or are relative to changes in fluid absorption produced by angiotensin II in the presence or absence of meclofenamate or indomethacin (Δ). Statistics were determined by unpaired Student's "t" test. Percentage potencies of angiotensin (100) in the presence or absence of indomethacin (1,000) and meclofenamate (2,200) were calculated from ED_{50} of each dose-response curve.

the highest dose of angiotensin II infused transport returned to control levels (Figure 1). The lack of potentiated inhibition of absorption at the highest dose of angiotension II might be related to adrenal catecholamine release leading to increased absorption, as angiotensin II-inhibited transport was significantly potentiated in adrenalectomized animals. Further,

a greater percentage of the adrenal medulla may have been destroyed in the sympathectomized animals than in those animals treated acutely with guanethidine. Thus, it is possible that the lack of a potentiated inhibition of absorption at the highest doses of angiotensin II in rats treated acutely with guanethidine may also be due to a relatively greater quantity of medullary norepinephrine release than in sympathectomized animals.

Based on the above observations, we have developed a model to delineate the effects of angiotensin II on jejunal fluid transport (Figure 3). According to this model, at low doses angiotensin II interacts with a high affinity receptor on postganglionic sympathetic nerve endings in close proximity to the transporting epithelial cells. Consequently, norepinephrine is released and subsequently binds to postsynaptic $alpha_1$ receptors on the basolateral membrane of the epithelial cells, leading to increased sodium and water absorption. At higher doses, angiotensin II interacts with a lower affinity receptor on epithelial cells, stimulating production of prostaglandins, which results in decreased absorption or increased secretion or both. The analog [Sar^1,Leu^8]-angiotensin II is a full agonist at stimulating norepinephrine release from sympathetic nerve endings (increased fluid transport), but is an antagonist of angiotensin II induced intestinal prostaglandin production.

PHYSIOLOGIC SIGNIFICANCE OF ANGIOTENSIN II MEDIATED INTESTINAL FLUID ABSORPTION AND SECRETION

In order to determine whether or not the angiotensin II mediated changes in small intestinal fluid transport described above are significant physiologically, fluid absorption was measured in various models of

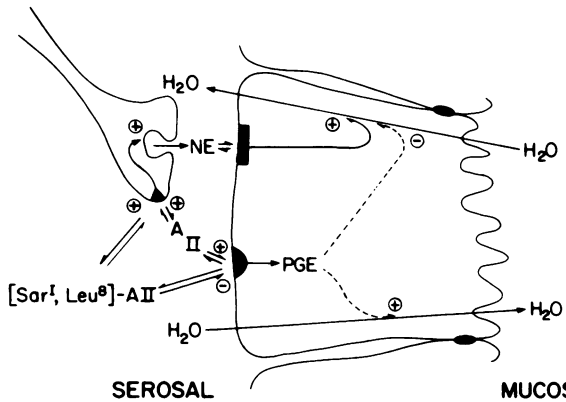

FIG. 3. Theoretical model for effects of angiotensin on jejunal water transport. Water flows are transepithelial.

extracellular fluid volume depletion. The rat models included 1) non-hypotensve hemorrhage of 1% of body weight; 2) sodium depletion by peritoneal dialysis; and 3) dehydration by witholding food and water for 24 h.

As shown in Figure 4, the jejunum responded to extracellular volume depletion induced by dehydration, non-hypotensive hemorrhage and sodium depletion with an increase in fluid absorption. In each case, the increase in jejunal absorption was of similar magnitude, 30–40% above that recorded for control animals in normal sodium and volume balance. Each model of extracellular fluid volume depletion was associated with an increase in circulating angiotensin II from control values, a finding which validates the fact that the animals were indeed volume depleted at the time of study.

The three models of extracellular volume depletion have differing effects on the status of intracellular volume. Thus, non-hypotensive hemorrhage results in only extracellular fluid reduction, whereas sodium depletion and dehydration increase and decrease the volume of the intracellular compartment, respectively. Alterations in enterocyte size as a result of changes in the intracellular compartment could greatly affect intestinal absorption by altering the size and permeability of the tight

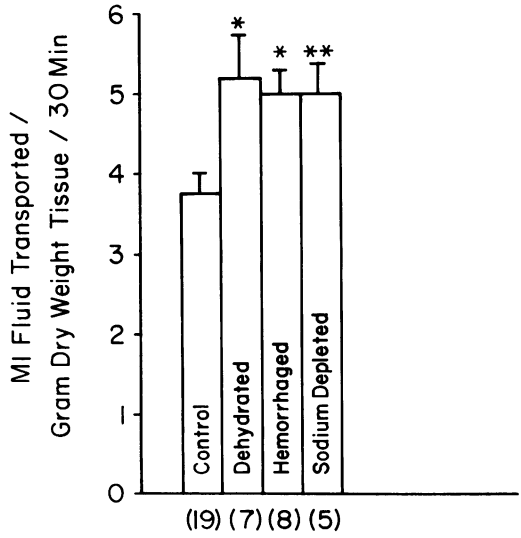

FIG. 4. Effect of extracellular fluid volume depletion on jejunal fluid absorption. Histograms represent the mean ± 1 SE of jejunal fluid transport measured over a single 30 min period in separate animals. *, $p < 0.02$; **, $p < 0.01$. Statistics compare changes produced by dehydration, hemorrhage and sodium depletion to control animals by an unpaired Student's "t" test. Numbers in parenthesis are the number of experiments.

junctions (20). However, all three models manifested a similar increase in jejunal absorption, an observation which demonstrated that extracellular fluid volume depletion per se, not a change in the intracellular compartment, is responsible for the increased fluid absorption from the small intestine.

In previous studies, attempts to delineate the factors responsible for the increase in fluid absorption from the small intestine following extracellular volume depletion have been unsuccessful. The various models of volume depletion are associated with an increase in circulating angiotensin II, which is a potent stimulator of aldosterone and vasopressin secretion. Thus, following extracellular volume depletion the concentrations of all three hormones are elevated in the circulation.

We have shown, however, that neither aldosterone nor vasopressin can account for the increase in jejunal fluid absorption following extracellular volume depletion. Rats which previously had been adrenalectomized responded to extracellular volume depletion with an increase in fluid absorption of similar magnitude to that obtained in intact rats. Vasopressin has been shown either to have no effect or to inhibit fluid absorption in the gut, a response directly opposite that observed in our studies (21–23). Nephrectomized animals did not respond to extracellular volume depletion with an increase in fluid absorption. These observations indicate that a renal factor is responsible for the increase in absorption following extracellular volume contraction.

As demonstrated earlier, angiotensin II exerts a dose dependent dual action on jejunal fluid transport. At low doses, angiotensin II stimulates net mucosal to serosal transfer of fluid, whereas high concentrations of the hormone inhibit transport or stimulate secretion. The angiotensin II concentration in rat plasma ranges between 10 and 150 pg/ml, depending upon the sodium status of the animal (24, 25). We have demonstrated that low doses of the hormone which double jejunal fluid absorption are associated with only a small increase in circulating angiotensin II, which is well within limits of the physiologic range of the hormone. In contrast, the inhibitory (secretory) effects of angiotensin II occur only at high levels of the hormone (400 pg/ml) which are unlikely to be encountered under normal physiological circumstances. Therefore, stimulation of jejunal fluid absorption is the physiologically important effect of the hormone.

In summary, we have demonstrated that extracellular volume depletion is associated with significantly increased intestinal fluid absorption. The increase in absorption is not influenced by adrenalectomy, but is abolished by nephrectomy. Based upon these and previous observations, we suggest that angiotensin II is responsible for the increase in absorption by the small intestine. The small intestine absorbs the majority of the

ions and water presented to the gastrointestinal tract. Therefore, an action of angiotensin II to increase sodium and water absorption in this region of the intestine would have far greater physiological importance than the effect of aldosterone in the distal colon.

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DISCUSSION

Goldberg (Cincinnati): They were some very interesting observations, Dr. Carey. I wonder if your preparation could exclude or separate out the effects of changes in the circulation. As I understand it, your model is an isolated loop which still has the blood flow intact, so the question is: Are the effects you are observing due to direct effects on the transporting surfaces or membranes, or can they be explained by changes in the circulation?

Carey: Angiotensin II acts in vivo totally independently of intestinal blood flow and further, angiotensin II stimulates fluid absorption in vitro. So, therefore, we feel that this is a direct effect at post-ganglionic sympthetic neurons located in close proximity to the small intestinal cell.

Farrar (Richmond): Two questions, Bob, One, do you know whether this agent affects the villous cells or does it possibly affect the crypt cells. There is some evidence that secretion takes place through crypt cells. Also, do you know whether the effect is on absorption or on secretion or both.

Carey: We have not explored the specific type of cell which is involved in this response. Both absorptive and secretory processes occur simultaneously and one overrides the other under various physiological conditions or in certain pathophysiological states. With angiotensin II, we are probably not only inhibiting absorption but also stimulating secretion, but absorption continues to occur in spite of those actions of the hormone.

James (Birmingham): I've noticed in one of your earlier slides you included the effect of angiotension II in increasing heart rate. Some years ago my colleagues and I became interested in the question of whether this was a direct action on the heart, and if so was it due to local release of catacolamines or not. We found that angiotensin II at no concentration—very small to very high—had any effect on the sinus node directly, leading us to the interpretation that any action it had of the accelerating kind had to be mediated outside the heart, presumably by the low concentration effect of ganglion stimulation, including sympathetic ganglion stimulation, perhaps in the brain as well. This wouldn't be the first example where a hormone or an autocoid would have a different effect in one system, the intestine, than it does in another, in the heart.

Carey: That's an interesting comment. During mild volume depletion, as a homeostatic mechanism, sodium is reabsorbed in the gastrointestinal tract. But when one gets overwhelming volume depletion and the circulating angiotensin levels rise to very high concentrations then we get inhibition of absorption and we get into a vicious cycle in which the gut actually is secreting a net amount of fluid. In that case, I believe that there might be a therapeutic role for an angiotensin analogue to break up that cycle.

Langford (Mississippi): You also, like I do, hold a union card in Endocrinology. The diarrhea of the untreated Addisonian has always been a little physiological embarassment for me. Do you consider your mechanism as a possible cause of the Addisonian diarrhea?

Carey: Not at the moment. We haven't investigated the role of angiotensin in the diarrhea of adrenal insufficiency.

Oates (Nashville): You indicated PGE2 as the prostaglandin involved. Is there any specific evidence for that?

Carey: No, other than the inhibition by blockers of the cyclooxygerose enzyme. We have not specifically measured PGE2 so there could be other prostaglandins involved and we're presently investigating that aspect of it.