A Comparison of Angiotensin II and Angiotensin III as Vasoconstrictors in the Mesenteric Circulation of Dogs

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SUMMARY Angiotensin II is more potent as a vasopressor than angiotensin III when given intravenously. We tested the hypothesis that differential changes in mesenteric blood flow contribute to this difference in potency. The effects of angiotensin II and angiotensin III on mesenteric blood flow were compared in 31 dogs anesthetized with pentobarbital. These agonists were administered either as bolus injections (10-160 pmol) or as constant infusions (30 pmol/min per kg) directly into the vasculature supplied by the superior mesenteric artery. Approximately equipressor doses of angiotensin II (40 pmol/min per kg) and angiotensin III (80 pmol/min per kg) also were given intravenously. On the basis of duration and graphic integration of the response in mesenteric blood flow, but not on the basis of absolute change in amplitude, angiotensin II was consistently more potent than angiotensin III as a mesenteric vasoconstrictor. The intra-arterial and intravenous constant infusion doses were repeated after the administration of mepolafenamate (4 mg/kg, iv). Mepolafenamate did not alter any of the responses to angiotensin II or angiotensin III. We conclude that the differential constrictor properties of these compounds in the mesenteric vasculature contribute to the greater potency of intravenously administered angiotensin II on arterial pressure. The results provide no evidence for an interaction between prostaglandins and the vasoconstrictor properties of angiotensin II or III in the intact mesenteric vasculature of the anesthetized dog. Circ Res 46: 146-151, 1980

RECENT studies have raised the possibility that angiotensin III is an important intermediate of the renin-angiotensin system (Goodfriend and Peach, 1975; Freeman et al., 1976; Peach, 1977; Carey et al., 1978). Because the pressor activity of intravenously administered angiotensin III is approximately 20-30% of the activity of angiotensin II (Carey et al., 1978), angiotensin III may be expected to be a less potent vasoconstrictor in the major peripheral vasculatures. Angiotensin II and angiotensin III, however, have been reported to have equal vasoconstrictor effects in the renal (Freeman et al., 1975; Taub et al., 1977) and in the mesenteric and femoral vasculatures (Caldicott et al., 1977). This finding indicates that mechanisms associated with differential responses in mesenteric blood flow to angiotensin II and angiotensin III are not involved in the contrasting responses in arterial pressure elicited by these compounds. Preliminary observations in our laboratory suggested that angiotensin II and angiotensin III, when administered as bolus injections directly into the vasculature supplied by the superior mesenteric artery, were equipotent as vasoconstrictors when the primary response evaluated was the maximal change in amplitude of the flow. When the responses were analyzed by duration and graphic integration, however, angiotensin II was considerably more potent than angiotensin III. Results presented in this study show that angiotensin II is a more potent mesenteric vasoconstrictor than angiotensin III when these compounds are delivered either by bolus injections or by constant infusions. These findings demonstrate that a portion of the difference in arterial pressor activity between angiotensin II and angiotensin III is attributable to their differences in potencies as vasoconstrictors in the mesenteric circulation.

Blumberg et al. (1977a) have shown that angiotensin III is appreciably more potent than angiotensin II in inducing the release of a prostaglandin E-like substance from the mesenteric vasculature. Prostaglandin E has been demonstrated to be a vasodilator in the mesenteric circulation (Shehadeh et al., 1969). Therefore, we also tested the hypothesis that the difference in mesenteric vasoconstrictor potency between angiotensin II and angiotensin III could be accounted for by the selective release of prostaglandins. We present evidence that blockade of prostaglandin synthesis with mepolafenamate did not alter the mesenteric vasoconstrictor properties of either angiotensin II or angiotensin III.
Methods

Experiments were performed on 31 mongrel dogs (8-13 kg and of either sex), anesthetized with sodium pentobarbital (25 mg/kg, iv). All dogs were maintained on a normal diet of dog chow (Nutrena) and water ad libitum. They were fasted for 15 hours before each experiment. A femoral vein was cannulated for the administration of drugs and additional anesthetic agents. Femoral artery blood pressure was measured with a pressure transducer (Statham P23Db) and recorded on a polygraph (Grass). All dogs were ventilated mechanically with a respirator (Harvard), and the minute volume ventilation was selected by reference to the nomogram of Kleinman and Radford (1964). Rectal temperature was monitored and maintained at 37 ± 1°C by a heating pad and heat lamp.

The dogs were placed in a metal frame that held them in a position approximating their normal standing posture. The superior mesenteric artery was approached through a left flank incision. At its origin from the aorta, a short segment of the artery was dissected free from surrounding connective tissue, with care being taken not to traumatize periarterial nerves or the nearby adrenal gland. Superior mesenteric artery blood flow was measured by placing a noncannulating electromagnetic flow probe (Carolina Electronics Co.) around the artery. Mean flow was recorded through a low-pass filter with a 0.6-second time constant. Distal to the flow probe, a curved 23-gauge needle attached to polyethylene tubing (PE 50) was inserted into the artery for the administration of drugs. This arterial needle delivered either 0.85% sodium chloride or agonists.

After completion of surgery, an interval of 30 minutes was allowed for stabilization of the preparation. Zero-flow baseline was established at the beginning of each experiment by mechanically occluding the artery distal to the flow probe. Zero flow was checked at least once during the experiment and at its termination. The flow probe was calibrated at the end of the experiment by cannulating the superior mesenteric artery distal to the position of the flow probe and diverting flow into a graduated cylinder for 30-second intervals. At all flows, the relationship between the output of the flow probe and the directly measured mesenteric blood flow was linear.

Dose-Response Relationships

In 26 dogs, we tested the effects of five graded doses (10, 20, 40, 80, and 160 pmol) of angiotensin II [Beckman, 0.92 μmol peptide/mg (>96% angiotensin II)] and angiotensin III [Beckman, 1.04 μmol peptide/mg (>96% angiotensin III)] on superior mesenteric blood flow. Both agonists were dissolved in saline and injected in equivalent volumes (0.2 ml) into the injection catheter (0.25-ml dead space), after which they were flushed rapidly (5 seconds) into the superior mesenteric artery blood flow with 1.0 ml of saline. The injection schedule with respect to dose and agonist was randomized, and sufficient time (8 minutes) was allowed between injections so that tachyphylaxis did not develop.

In five additional dogs, the effects of angiotensin II or angiotensin III given as a constant infusion intravenously or directly into the superior mesenteric artery for a duration of 10 minutes were examined. Equimolar doses (30 pmol/min per kg) of angiotensin II and angiotensin III were delivered into the superior mesenteric artery at a rate of 0.1 ml/min. Approximately equipressor doses of angiotensin II (40 pmol/min per kg) and of angiotensin III (80 pmol/min per kg) also were administered intravenously at a rate of 1 ml/min. These intravenous and intra-arterial constant infusions were repeated after the blockade of prostaglandin synthesis with meclofenamate (4 mg/kg, iv). Blockade of prostaglandin synthesis was evaluated by injecting a bolus test dose of arachidonic acid (250 μg, Nu-Chek Prep, Inc.) into the superior mesenteric artery before and 40 minutes after the administration of meclofenamate (Warner-Lambert/Parke-Davis). Disappearance of the arachidonic acid-induced vasodilation was interpreted as an indication of adequate blockade of prostaglandin synthesis.

Data Analysis

Changes in superior mesenteric blood flow consequent to bolus injections of angiotensin II or angiotensin III were examined by measuring three characteristics of the blood flow response (Fig. 1): (1) duration of the response, as measured from the time of agonist injection until the flow returned to baseline; (2) amplitude of the response; and (3) graphic integration (area) of change in blood flow. Integrated responses are expressed as percentage of maximal possible flow change (area x/area x + area y) that could have occurred during 48 seconds after injection of an agonist.
the preinjection value; (2) maximal changes in the amplitude of the blood flow response, as measured from its preinjection level; and (3) graphic integration (area) of the flow response as a function of the maximal possible flow change that could have occurred during the 48 seconds after the injection of an agonist. Integration for 48 seconds was chosen because this duration represents approximately the longest response time achieved with our highest dose of angiotensin II. The maximal possible response was determined by examining the original flow trace recorded with the polygraph and cutting a rectangular piece of graph paper such that its height represented the absolute blood flow (ml/min) occurring just before agonist injection and its width represented 48 seconds of the flow recording. The resultant piece of graph paper was weighed on an analytical balance (Mettler H10 T). The area of the flow response then was traced on this piece of graph paper, cut out, and weighed. The percent change in flow, on an area basis, was calculated by dividing the weight of that proportion of the graph paper representing the change in flow (area x) by the weight of the graph paper representing the maximal possible response occurring in 48 seconds (areas x + y) and multiplying the quotient by 100.

The response to constant infusions of the agonists also was determined by graphic integration of the flow record. The integral flow value was determined every minute for the duration of administration of the constant infusion.

The statistical significance of differences between the responses to angiotensin II and angiotensin III was evaluated by Student's paired t-test (Zar, 1974); P values of <0.05 were considered significant for differences. Values are reported as means ± 1 SE.

Results

Superior mesenteric blood flow decreased in a dose-dependent fashion in response to direct intra-arterial bolus injections of angiotensin II and angiotensin III (Fig. 2). The log-dose response relationships for each agonist were parallel, at the midrange of doses tested, for each of the characteristics of the response examined (see Fig. 1). There was no significant difference in the responses elicited by either agonist, at any dose level, when the response was evaluated on the basis of percent change in amplitude. However, angiotensin II was significantly more potent than angiotensin III when examined on the basis of (1) percent of maximal possible change in area and (2) duration of the response. On the average for the five doses tested, the response to angiotensin II was 39.0% longer in duration than the response to an equimolar dose of angiotensin III. On the basis of area, angiotensin II averaged 43.6% more potent than angiotensin III.

The qualitative characteristics of the responses in mesenteric blood flow to bolus injections of angiotensin II and angiotensin III were similar. After injection of agonist, blood flow decreased within 2 or 3 seconds, reached a minimal level in 5–8 seconds, and returned to baseline level within 30–45 seconds (Fig. 1). The initial vasoconstriction usually was followed by a transient increase in flow, lasting 20–60 seconds before returning to baseline. Systemic arterial pressure did not change in response to the lower angiotensin doses; however, it increased slightly (2–4 mm Hg) consequent to the injection of the higher doses of angiotensin agonists.

Equimolar quantities (30 pmol/min per kg) of both angiotensin II and angiotensin III produced decreases in superior mesenteric blood flow when delivered as a constant infusion intra-arterially for 10 minutes (Fig. 3). The maximal decrease in flow for both agonists occurred after 2 minutes of infu-
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**Figure 3** Responses in mesenteric blood flow, as determined by graphic integration, to 10-minute constant infusion of angiotensin II (30 pmol/min per kg) or angiotensin III (30 pmol/min per kg) administered directly into superior mesenteric artery. Each point represents the mean response of five dogs; vertical lines represent ± 1 SE. (*P < 0.05 for angiotensin II compared with angiotensin III responses."

Angiotensin II, however, was more potent than angiotensin III as a mesenteric vasoconstrictor for most of the duration of the infusion. Intra-arterial infusion of these doses of angiotensin agonists produced no alteration in systemic arterial pressure.

The response in systemic arterial pressure to angiotensin II (40 pmol/min per kg) was not significantly different from the response to angiotensin III (80 pmol/min per kg) at any time during the 10 minutes of intravenous administration (Fig. 4). The responses in superior mesenteric blood flow to intravenously administered angiotensin II reached their maximum after 2 minutes of infusion. Angiotensin II produced a significantly greater decrease than angiotensin III in mesenteric flow for the duration of the infusion.

The superior mesenteric artery infusions and intravenous infusions of angiotensin II and angiotensin III (Figs. 3 and 4) were repeated 40 minutes after the blockade of prostaglandin synthesis with meclofenamate. The administration of meclofenamate did not result in a significant change in baseline systemic arterial pressure or baseline superior mesenteric flow. Furthermore, the presence of meclofenamate did not alter the flow or pressure responses to intravenous or intra-arterial infusions of angiotensin II or angiotensin III. The intravenous administration of meclofenamate (4 mg/kg) completely inhibited the mesenteric vasodilator response to arachidonic acid (Fig. 5).

Baseline systemic arterial pressure and mesenteric blood flow remained stable for the duration of the experiment. The average superior mesenteric blood flow was 268 ± 20 ml/min at the beginning of the experiment and 257 ± 18 ml/min at the end. Corresponding values for mean systemic arterial pressure were 125 ± 4 mm Hg and 120 ± 5 mm Hg, respectively.

**Discussion**

We conclude from these results that angiotensin II is a more potent mesenteric vasoconstrictor than angiotensin III. This conclusion is based on the following observations: (1) when delivered directly into the superior mesenteric artery as bolus injections, angiotensin II at all doses produced a greater vasoconstrictor response in terms of duration of effect and graphic integration than did angiotensin III; (2) when administered as constant infusions

**Figure 4** Responses in mesenteric blood flow, as determined by graphic integration, to 10-minute constant intravenous infusion of angiotensin II (40 pmol/min per kg) or angiotensin III (80 pmol/min per kg). Each point represents the mean response of five dogs; vertical lines represent ± 1 SE. (*P < 0.05 for angiotensin II compared with angiotensin III responses."

**Figure 5** Representative tracing of response in mesenteric blood flow to arachidonic acid (intra-arterial) before and after intravenous administration of meclofenamate. Arrows represent time of injection of arachidonic acid.
directly into the superior mesenteric artery, angiotensin II was a more effective vasoconstrictor than angiotensin III; (3) in response to approximately equipressor doses given as constant infusions intravenously, angiotensin II (40 pmol/min per kg) caused a greater decrease in mesenteric blood flow than did angiotensin III (80 pmol/min per kg). The observations that baseline mesenteric blood flow and systemic arterial pressure were not significantly altered throughout the experimental period suggest that the preparation maintained adequate stability and responsiveness.

Integration of the response in mesenteric blood flow elicited by bolus injections of agonists was chosen as the variable best representative of the effect of the angiotensin compounds on mesenteric blood flow. Because any physiological flow response has a duration and a varying amplitude during that duration, conclusions regarding the potency of a drug based only on amplitude or duration data can be misleading. We chose to integrate graphically the flow response for a duration of 48 seconds because results of preliminary experiments showed that this was approximately the average duration obtained with our highest dose of angiotensin II administered.

The responses elicited by the injections of angiotensins into the superior mesenteric artery could have been due partially to neural reflex interactions or to the direct release of catecholamines from the adrenal medulla (Feldberg and Lewis, 1964; Peach, 1971). Some observations from the present study show that the responses were attributable totally to the effects of angiotensin acting locally on mesenteric vasculature smooth muscle. First, the mesenteric vasoconstrictor responses were rapid in onset after injection of angiotensin (2-3 seconds) and occurred in the absence of significant changes in systemic arterial pressure. Second, as found previously (Sexton et al., 1979), high doses of angiotensin II or angiotensin III, when injected directly into the superior mesenteric artery, caused no alterations in flow to the renal vasculature.

Blumberg et al. (1977b) have demonstrated, in the isolated Kreb's perfused mesenteric vasculature of the rabbit, that angiotensin III is considerably more potent than angiotensin II in eliciting the release of prostaglandin E-like substances. Because prostaglandin E is a vasodilator in the mesenteric circulation (Shehadeh et al., 1969), the differential vasoconstrictor properties of angiotensin II and angiotensin III may be related to their ability to release prostaglandin E. In accord with this hypothesis, Blumberg et al. (1977b) have observed that the administration of indomethacin enhances the vasoconstrictor response to angiotensin II in the isolated Kreb's perfused mesenteric vasculature of the rabbit.

Results of the present study provide no evidence for an interaction between prostaglandins and the vasoconstrictor properties of angiotensin II or angiotensin III in the intact mesenteric vasculature of the anesthetized dog. Blockade of prostaglandin synthesis with meclofenamate did not alter significantly the baseline systemic arterial pressure, the baseline superior mesenteric blood flow, or the vasoconstrictor responses to angiotensin II or angiotensin III. Blockade of prostaglandin synthesis was confirmed by the absence of vasodilation in response to an intra-arterial bolus injection of arachidonic acid. Such a criterion was adopted in view of several considerations. First, measurement of effluent prostaglandins does not necessarily reflect synthesis because a large proportion of prostaglandins that could be synthesized in the arterial wall in response to vasoactive peptides, such as angiotensin II (Zusman and Keiser, 1977), may be metabolized before passing into the circulation (Samuelsson et al., 1975). In accord with this possibility is the finding of Blumberg et al. (1977b) that the intra-arterial administration of concentrations of arachidonic acid adequate to produce mesenteric vasodilation did not produce detectable prostaglandin E levels in the effluent. Second, prostaglandin I2, which is synthesized in the mesenteric arterial wall, may be an important modulator of vascular responses (Bunting et al., 1976; Armstrong et al., 1978). Finally, local mesenteric prostaglandin synthesis can be induced rapidly by a bolus injection of arachidonic acid, which results in vasodilation (Rose et al., 1974); blockade of this vasodilation with anti-inflammatory drugs, such as meclofenamate, appears to be a good index of blockade of prostaglandin synthesis (Armstrong et al., 1978).

Numerous other factors may contribute to the differential constrictor properties of angiotensin II and angiotensin III. First, there may be a differential enzymatic catabolism of the peptides. Blumberg et al. (1977a) have reported no difference between the percentages of inactivation of the peptides during one transit through the isolated mesenteric vasculature. Nevertheless, this does not preclude the possibility that differential destruction of the peptides occurred proximal to the site of angiotensin-mediated vascular smooth muscle contraction. In contrast to what Blumberg et al. (1977b) found in the mesenteric vasculature, Goodfriend and Peach (1975) reported that, in adrenal glomerulosa cells in vitro, angiotensin III is more susceptible to degradation than is angiotensin II. Second, there may be differential binding of the peptides to specific or nonspecific receptors, which are unrelated to smooth muscle contraction. Third, angiotensin II and angiotensin III may act on the same receptor, and the receptor affinity for each peptide may differ. Furthermore, there may be different receptors for angiotensin II and angiotensin III. In regard to receptor specificity, Blumberg et al. (1977a) has provided evidence suggesting that angiotensin I, angiotensin II, and angiotensin III act on the same receptor in the isolated Kreb's perfused mesenteric vasculature of the rabbit to produce the release of
prostaglandin E. Results of the present study allow no assessment of receptor specificity.

Unpublished observations from our laboratory have shown that angiotensin II and angiotensin III, when administered as either bolus injections or constant infusions, are equipotent as vasoconstrictors in the renal vasculature on the basis of amplitude, duration, and graphic integration. Freeman et al. (1975) also have found that the intrarenal administration of a constant infusion of equal doses of angiotensin II or angiotensin III produces equivalent decreases in renal blood flow. Our findings that angiotensin II and angiotensin III are equipotent as mesenteric vasoconstrictors when evaluated on the basis of change in amplitude of the blood flow response correspond to those of Caldicott et al. (1977). However, graphic integration of the flow response revealed that angiotensin II is significantly more potent than angiotensin III as a mesenteric vasoconstrictor. Therefore, we conclude that the differential constriction properties of these compounds in the mesenteric vasculature contribute to the observed greater potency of intravenously administered angiotensin II on systemic arterial pressure. Our results suggest that prostaglandins are not involved importantly in determining the greater potency of angiotensin II in comparison with angiotensin III in the intact mesenteric vasculature of the anesthetized dog.

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