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Specificity of Angiotensin Antagonists in the Mesenteric Vasculature of Dogs (40977)[1,2]

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Abstract. Experiments were conducted to assess whether the octapeptide antagonist Sar^1–Ile^8-angiotensin II or the heptapeptide antagonist Ile^7-angiotensin III demonstrates specificity for antagonizing the mesenteric vasoconstrictor properties of angiotensin II or angiotensin III. Experiments were performed on 12 dogs anesthetized with sodium pentobarbital. In all dogs, the effects of five graded doses (10, 20, 40, 80, and 160 pmole, bolus injections) of angiotensin II and angiotensin III on superior mesenteric artery blood flow were tested. At all doses tested, angiotensin II was more potent than angiotensin III. Administration of the middle dose (40 pmole) of angiotensin II and angiotensin III was repeated during the infusion of either Ile^7-angiotensin III ($N = 6$) or Sar^1–Ile^8-angiotensin II ($N = 6$) at five different dosages into the superior mesenteric artery. Sar^1–Ile^8-angiotensin II antagonized equally the mesenteric vasoconstrictor effects of both angiotensin II and angiotensin III. Ile^7-angiotensin III was not absolutely specific as an antagonist of the mesenteric vasoconstrictor effects of angiotensin III.

Present evidence indicates that angiotensin II is an important regulator of systemic arterial blood pressure (1) and sodium and potassium balance (2). The availability of angiotensin competitive antagonist analogs has permitted qualitative and quantitative assessment of the role of angiotensin II (3, 4). Recent work, however, brings to our attention the possibility that the heptapeptide angiotensin III (des-Asp^1-angiotensin II) may also be of physiologic importance (5–8). In support of a role in cardiovascular function, our laboratory has demonstrated that angiotensin III is 50% as potent as angiotensin II as a vasoconstrictor in the mesenteric circulation (9) and that pathways are available for the local formation of angiotensin III, via the action of kininase II, from the nonapeptide precrusor des-Asp^1-angiotensin I in the mesenteric (10) and renal (8) vasculatures.

Peptide analogs that demonstrate specificity for antagonizing the effects of either angiotensin II or angiotensin III would be useful tools in defining the possibility of a physiologic role for angiotensin III. The present study was designed to assess whether the octapeptide antagonist Sar^1–Ile^8-angiotensin II or the heptapeptide antagonist Ile^7-angiotensin III demonstrates specificity for antagonizing the mesenteric vasoconstrictor properties of angiotensin II or angiotensin III.

Methods. Experiments were performed on 12 mongrel dogs (12 to 16 kg and of either sex), anesthetized with sodium pentobarbital (25 mg/kg, intravenously). All dogs were maintained on a normal diet of dog chow (Nutrena) and water *ad libitum*. They were fasted for 15 hr before each experiment. A femoral vein was cannulated for the administration of additional anesthetic agent. 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

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selected by reference to the nomogram of Kleinman and Radford (11). Rectal temperature was monitored and maintained at 37 ± 1°C with 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 to avoid traumatizing 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-sec 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 was connected via a low-volume Y tube to two separate polyethylene catheters. One catheter delivered either 0.85% sodium chloride or antagonist compounds at a constant flow rate of 0.1 ml/min. The other catheter was used for the bolus injection of agonist compounds.

After the completion of the operation, 30 min was allowed for stabilization of the preparation. Zero-flow baseline was established at the beginning of each experiment by occluding mechanically the artery distal to the flow probe. Zero flow was checked at least once during the experiment and at the 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 by diverting the flow into a graduated cylinder for 30-sec intervals. At all flow levels, the relationship between the output of the flow probe and the directly measured mesenteric blood flow was linear.

In all dogs, the effects of five graded doses (10, 20, 40, 80, and 160 pmole) of angiotensin II [Beckman, 0.92 μmole peptide/mg (≥96% angiotensin II)] and angiotensin III [Beckman, 1.04 μmole peptide/mg (≥96% angiotensin III)] on superior mesenteric blood flow were tested. Also tested were three doses (160, 320, and 640 pmole) of norepinephrine (Winthrop). All 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 sec) into the superior mesenteric artery with 1.0 ml of saline. The injection schedule with respect to dose and agonist was randomized, and sufficient time (8 min) was allowed between injections so that tachyphylaxis did not develop.

In six of the dogs, administration of the middle dose of each agonist (angiotensin II, 40 pmole; angiotensin III, 40 pmole; norepinephrine, 320 pmole) was repeated during infusion of the heptapeptide antagonist Ile^7-angiotensin III at five different dosages (20, 40, 80, 160, and 320 pmole/min/kg). Each dose of the antagonist was delivered directly into the superior mesenteric artery for 15 min before the agonists were tested. Agonists were administered, as in the control situation, every 8 min in random order. Delivery of the antagonist continued during the injection of the test dose of the agonists. Ile^7-angiotensin III was administered in incremental doses beginning with the lowest dosage (20 pmole/min/kg). Ten minutes intervened between discontinuing the delivery of one dose of the antagonist and commencing the administration of the next higher dose.

In the other six dogs, the effects of the octapeptide antagonist Sar^1-Ile^8-angiotensin II on the responses to angiotensin II, angiotensin III, and norepinephrine were examined with use of the same methods as described above for the heptapeptide antagonist. The dosages of Sar^1-Ile^8-angiotensin II used were 2.5, 5, 10, 20, and 40 pmole/min/kg.

Changes in superior mesenteric artery blood flow consequent to bolus injections of angiotensin II, angiotensin III, or norepinephrine were examined by measuring two characteristics of the blood-flow response (Fig. 1): (1) maximal changes in the amplitude of the blood-flow response, as measured from its preinjection level, and (2) graphic integration (area) of the flow response as a function of the maximal possible flow change that could have occurred

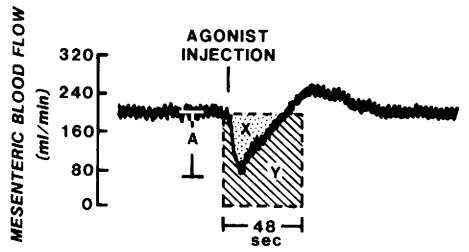

FIG. 1. Changes in mesenteric blood flow consequent to agonist administration were examined by evaluating two variables: (1) amplitude (*A*) of vasoconstrictor response and (2) graphic integration (area) of flow-change response. Integrated responses are expressed as percentage of maximal possible flow change (area *x*/area *x* + area *y*) that could have occurred during the 48 sec after injection of an agonist.

during the 48 sec after the injection of an agonist. Integration for 48 sec was chosen because this duration represents approximately the longest response time achieved with our highest dose of angiotensin II administered. 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 sec 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 was then traced on this piece of graph paper, cut out, and weighed. The percentage 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 sec (areas *x* + *y*) and multiplying the quotient by 100.

The statistical significance of differences between the responses to angiotensin II and angiotensin III was evaluated by Student's paired *t* test (12); *P* values of <0.05 were considered significant for differences. Values are reported as means ± 1 SE.

Results. Injections of angiotensin II, angiotensin III, or norepinephrine directly into the superior mesenteric artery caused dose-dependent decreases in mesenteric blood flow when the responses were analyzed on the basis of amplitude or area (Fig. 2). Amplitude analysis revealed no significant difference in potency between angiotensin II and angiotensin III. Area analysis, however, showed that at each dose tested angiotensin II was more potent than angiotensin III. The dose–response curves for angiotensin II and angiotensin III were parallel on the basis of both area and amplitude. On the average, the analysis by

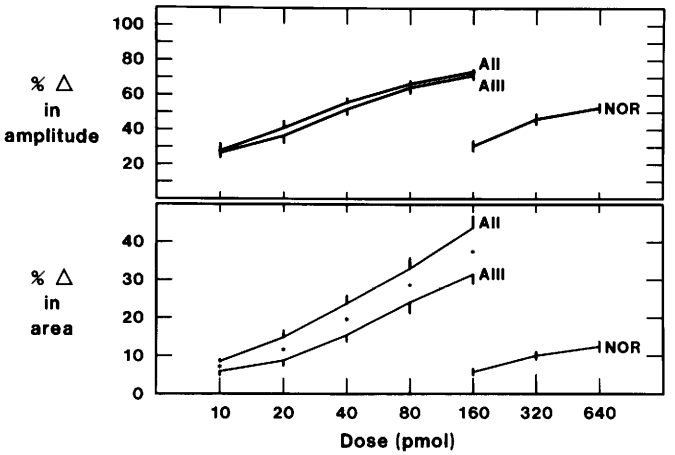

FIG. 2. Dose–response curves for changes in two variables of mesenteric blood flow (see Fig. 1) caused by angiotensin II, angiotensin III, and norepinephrine. Each point represents the mean response of 12 dogs; vertical lines represent ±1 SE. Agonist dose is shown on a log scale. (*$P < 0.05$ for angiotensin II compared with angiotensin III responses.)

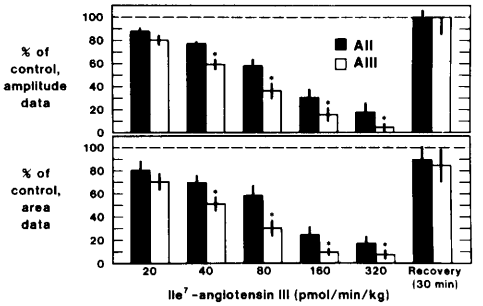

FIG. 3. Effects of administration of repeated doses (40 pmole each) of angiotensin II and angiotensin III on mesenteric blood flow during the administration of increasing doses of the heptapeptide antagonist Ile^7-angiotensin III. 100% represents the responses to angiotensin agonists before administration of Ile^7-angiotensin III. Responses are shown as mean ± 1 SE ($N = 6$). (*$P < 0.05$ for percentage of control response with angiotensin II compared with angiotensin III at each level of antagonist administered.)

area showed that angiotensin II was 49% more potent than angiotensin III as a mesenteric vasoconstrictor. Responses to either angiotensin agonist were rapid in onset (2 to 3 sec), attained maximal levels within 6 to 8 sec, and returned to control levels during the following 1 to 2 min. Injections of the saline vehicle produced no alteration in mesenteric blood flow.

Analysis by both amplitude and area showed that Ile^7-angiotensin III caused dose-dependent decreases in the response to a fixed dose of angiotensin II or angiotensin III (Fig. 3). Furthermore, this heptapeptide analog attenuated the responses to angiotensin III to a greater extent than those to angiotensin II. Thirty minutes after cessation of administration of the last (highest) dose of Ile^7-angiotensin III, the responses to both angiotensin II and angiotensin III had returned to values that were similar to those found before the onset of administration of the antagonist.

We chose dose ranges of Ile^7-angiotensin III and Sar^1–Ile^8-angiotensin II that produced approximately equal antagonistic effects on the mesenteric blood flow responses to angiotensin II. Sar^1–Ile^8-angiotensin II was approximately seven to eight times more potent than Ile^7-angiotensin III as an angiotensin II antagonist (Figs. 3 and 4).

Administration of Sar^1–Ile^8-angiotensin II inhibited equally the responses to angiotensin II and angiotensin III (Fig. 4) on the basis of both area and amplitude. Thirty minutes after cessation of the administration of this octapeptide antagonist, the responses to both agonists had recovered to only 40 to 50% of the preantagonist value.

Ile^7-angiotensin III produced a potentiation of the responses to norepinephrine at the dosage of 160 pmole/min/kg on the basis of change in area (Fig. 5). In contrast,

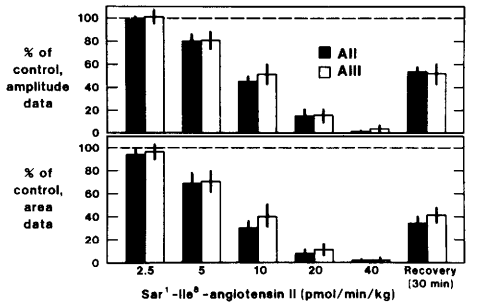

FIG. 4. Effects of administration of repeated doses (40 pmole each) of angiotensin II and angiotensin III on mesenteric blood flow during the administration of increasing doses of the octapeptide antagonist Sar^1–Ile^8-angiotensin II. 100% represents the responses to angiotensin agonist before administration of Sar^1–Ile^8-angiotensin II. Responses are shown as mean ± 1 SE ($N = 6$).

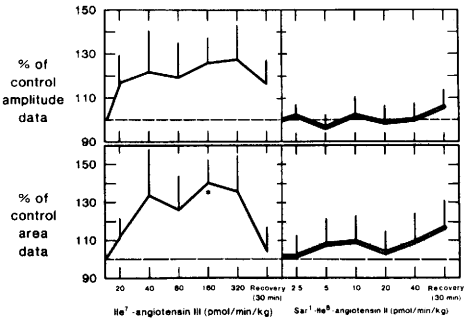

FIG. 5. Effects of administration of a repeated dose (320 pmole) of norepinephrine on mesenteric blood flow during the administration of increasing doses of Ile^7-angiotensin III (left panel) or Sar^1–Ile^8-angiotensin II (right panel). Responses are shown as mean ± 1 SE ($N = 6$). (*$P < 0.05$ as compared with control.)

Sar^1-Ile^8-angiotensin II did not change the responses to norepinephrine at any dose.

The average superior mesenteric blood flow was 243 ± 32 ml/min at the beginning of the experiment and 224 ± 24 ml/min at the end. Corresponding values for mean systemic arterial blood pressure were 123 ± 5 mm Hg and 118 ± 5 mm Hg, respectively. Neither Ile^7-angiotensin III nor Sar^1-Ile^8-angiotensin II caused a change in baseline systemic arterial blood pressure or mesenteric blood flow.

Discussion. Integration of the response in mesenteric blood flow elicited by bolus injections of agonists was chosen as the variable best representative of the effects of the angiotensin compounds on mesenteric blood flow. Because any physiologic flow response has a duration and a varying amplitude during that duration, conclusions regarding the potency of an agonist or antagonist based on only amplitude data can be misleading. Our finding that angiotensin II was significantly more potent than angiotensin III when compared on the basis of change in area of the response, but not on the basis of change in amplitude, demonstrates this point and corroborates the findings of a previous report (9).

Norepinephrine was administered originally to serve as a nonangiotensin receptor-mediated index of responsiveness of mesenteric vascular smooth muscle. Because angiotensin II has been shown to potentiate the effects of exogenously administered norepinephrine in the mesenteric vasculature (13), we anticipated that blockade of angiotensin receptors should have either no discernible effect or an attenuating effect on the responses to norepinephrine (14). We observed, however, that although Ile^7-angiotensin III antagonized the effects of angiotensin II and angiotensin III, it also potentiated the mesenteric vasoconstrictor responses to norepinephrine. These unusual properties of the heptapeptide antagonist were not displayed by the octapeptide antagonist Sar^1-Ile^8-angiotensin II. Campbell and Jackson (15) have also observed that Ile^7-angiotensin III potentiates the response to exogenously administered norepinephrine in the isolated mesenteric vasculature of the rat perfused with Krebs' solution. The mechanism of this potentiation is unknown but may be related to partial agonistic properties of Ile^7-angiotensin III.

Ten minutes intervened between discontinuing the delivery of one dose of the antagonists and commencing the administration of the next higher dose. Because 10 min is insufficient time for recovery of inhibitory action produced by either Sar^1-Ile^8-angiotensin II or Ile^7-angiotensin III, the antagonist effects of these peptides were likely accumulative. Such an accumulative effect, however, does not preclude our ability to compare the specificity of these antagonists because the vasoconstrictor effects of angiotensin II and angiotensin III were always compared at the same level of antagonist action. We chose doses of antagonists that produced effects ranging from minimal antagonist action to almost complete inhibition of the responses to angiotensin II and angiotensin III.

We conclude from our present results that the heptapeptide Ile^7-angiotensin III is a more potent antagonist of the mesenteric vasoconstrictor effects of angiotensin III than of angiotensin II. The octapeptide antagonist Sar^1-Ile^8-angiotensin II, however, did not display specificity; it blocked the mesenteric vasoconstrictor effects of angiotensin II and angiotensin III equally. Taub *et al.* (16) found that Ile^7-angiotensin III produced identical blockade of renal vasoconstrictor responses to angiotensin II and angiotensin III. Previous results from our laboratory support this finding (8). Thus, Ile^7-angiotensin III appears to have no specificity for angiotensin III in the renal vasculature and only relative specificity in the mesenteric vasculature. Nevertheless, Hall *et al.* (17) purport to have examined the intrarenal role of angiotensin III in sodium-depleted dogs. They tested the efficacy of Ile^7-angiotensin III blockade by administering exogenous angiotensin III. They did not demonstrate, however, that responses to angiotensin II were still intact in the presence of Ile^7-angiotensin III. Before an antagonist can be used to examine physiologic mechanisms, it must display substantial specificity against a particular endogenous agonist. The present study

demonstrates that Ile^7-angiotensin III is not absolutely specific as an antagonist of the mesenteric vasoconstrictor effects of exogenous angiotensin III.

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1. Peach, M. J., Physiol. Rev. **57**, 313 (1977).
2. Laragh, J. H., Baer, L., Brunner, H. R., Buhler, F. R., Sealey, J. E., and Vaughan, E. D., Jr., Amer. J. Med. **52**, 633 (1972).
3. Peach, M. J., and Ackerly, J. A., Fed. Proc. **35**, 2502 (1976).
4. Bumpus, F. M., Fed. Proc. **36**, 2128 (1977).
5. Goodfriend, T. L., and Peach, M. J., Circ. Res. (Suppl. 1), **36**, 38 (1975).
6. Freeman, R. H., Davis, J. O., Lohmeier, T. E., and Spielman, W. S., Circ. Res. (Suppl. 2), **38**, 99 (1976).
7. Carey, R. M., Vaughan, E. D., Jr., Peach, M. J., and Ayers, C. R., J. Clin. Invest. **61**, 20 (1978).
8. Britton, S. L., Beierwaltes, W. H., Fiksen-Olsen, M. J., and Romero, J. C., Circ. Res. **44**, 666 (1979).
9. Britton, S. L., Sexton, J. M., Fiksen-Olsen, M. J., Werness, P. G., and Romero, J. C., Circ. Res. **46**, 146 (1980).
10. Sexton, J. M., Britton, S. L., Beierwaltes, W. H., Fiksen-Olsen, M. J., and Romero, J. C., Amer. J. Physiol. **237**, H218 (1979).
11. Kleinman, L. I., and Radford, E. P., Jr., J. Appl. Physiol. **19**, 360 (1964).
12. Zar, J. H., "Biostatistical Analysis." Prentice–Hall, Englewood Cliffs, N.J. (1974).
13. Malik, K. U., and Nasjletti, A., Circ. Res. **38**, 26 (1976).
14. Zimmerman, B. G., J. Pharmacol. Exp. Ther. **185**, 486 (1973).
15. Campbell, W. B., and Jackson, E. K., Amer. J. Physiol. **236**, H211 (1979).
16. Taub, K. J., Caldicott, W. J. H., and Hollenberg, N. K., J. Clin. Invest. **59**, 528 (1977).
17. Hall, J. E., Coleman, T. G., Guyton, A. C., Balfe, J. W., and Salgado, H. C., Amer. J. Physiol. **236**, F252 (1979).

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