PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **181**, 125–130 (1986)

Evaluation of Angiotensins II and III as Vasoconstrictors in the Hepatic and Hindlimb Vasculatures of Dogs (42233)

STEVEN L. BRITTON, THEODORE F. RONAU, AND PATRICIA J. METTING

Cardiovascular Laboratory, Department of Physiology, Medical College of Ohio, Toledo, Ohio 43699

Abstract. Previous work has demonstrated that intravenously administered angiotensin II is more potent than angiotensin III as a systemic vasopressor agent. We tested the hypothesis that this difference in potency is caused at least partially by angiotensin II being more potent than angiotensin III as a vasoconstrictor in the hindlimb and hepatic vasculatures. The effects of angiotensins II and III on hindlimb and hepatic blood flow were evaluated in 14 dogs anesthetized with pentobarbital. Blood flows were measured electromagnetically. Graded doses of angiotensins II and III were administered as bolus injections directly into the arterial supply of the hindlimb and liver. On the basis of duration and graphic integration of the flow responses, but not on the basis of absolute changes in amplitude, angiotensin II was significantly more potent than angiotensin III as a vasoconstrictor in the hindlimb vasculature. In the hepatic circulation the flow changes produced by angiotensin II and angiotensin III were not significantly different on the basis of duration, graphic integration, or amplitude. We conclude that (i) differential vasoconstrictor responses of the hindlimb, but not the hepatic circulation, to angiotensins II and III contribute to the difference in systemic vasopressor potency between these two peptides, and (ii) because flow responses are an integral event with duration and constantly varying amplitude, evaluation of vasoconstrictor potency based only upon amplitude of the flow changes can be misleading.

It is well documented that angiotensin II is an important regulator of systemic arterial blood pressure (1) and sodium and potassium balance (2). More recent work has suggested that angiotensin III (des-Asp[1]-angiotensin II) is also an important agonist peptide. Angiotensin III has been identified in the blood of man (3) and animals (4–6), and pathways for the formation of angiotensin III have been evaluated (7–11). The pressor activity of intravenously administered angiotensin III is approximately 20–40% of the activity of angiotensin II (12–14). Thus, angiotensin II may be expected to be more potent than angiotensin III as a vasoconstrictor in the major peripheral vascular beds. Other investigators have concluded, however, that angiotensins II and III are equal as vasoconstrictors in the renal (15, 16) and in the mesenteric and femoral vasculatures (17). These findings suggest that the greater vasopressor potency of angiotensin II relative to angiotensin III is not due to angiotensin II being a more potent vasoconstrictor in most major vasculatures. In subsequent studies, however, we concluded that the difference in arterial pressor activity between angiotensins II and III is at least partially attributable to their differences in potencies as vasoconstrictors in the mesenteric (18) and renal (19) circulations.

The purpose of the present study was to evaluate the potencies of angiotensins II and III as vasoconstrictors in the hepatic and hindlimb vasculatures of dogs. Our data demonstrate that these peptides are equipotent in the hepatic arterial circulation but that angiotensin II is more potent than angiotensin III in the hindlimb vasculature.

Methods. Experiments were performed on 14 mongrel dogs (12–18 kg) of either sex that were maintained on a normal diet of dog chow and water *ad libitum*. They were fasted for 15 hr before each experiment and then anesthetized with sodium pentobarbital (30 mg/kg, intravenously). The right femoral vein was cannulated for administration of additional anesthetic agent. Femoral artery blood pressure was measured with a pressure transducer (Statham P23Db) and recorded on a Beckman R611 polygraph. Heart rate was measured with a Beckman cardiotachometer. All dogs were ventilated mechanically with a Harvard respirator and the minute-volume ventilation was selected by reference to the nomogram of

0037-9727/86 $1.50

Kleinman and Radford (20). Esophageal temperature was monitored and maintained at 37 ± 1°C with a heat lamp.

Arterial blood flows to the liver and hindlimb were measured with noncannulating electromagnetic flow probes. Access to blood vessels was made through an abdominal midline and left subcostal incision. For measurement of hepatic flow a probe was positioned around the celiac artery at its origin from the aorta. The left gastric artery was ligated and cannulated in the retrograde direction with a small polyethylene catheter (PE 50). This catheter was maneuvered until its tip was positioned in the hepatic artery and then advanced orthogradely into the hepatic artery for approximately 1 cm. This catheter was used to deliver drugs. The splenic artery was then ligated at its origin from the celiac. Ligation of the splenic and left gastric arteries caused all blood flow through the celiac artery to be delivered to the hepatic artery. A hydraulic occluder was positioned around the hepatic artery for determination of zero blood flow. For measurement of hindlimb blood flow a noncannulating probe was positioned around the left external iliac artery. An occluder was positioned distal to the probe. Distal to the occluder, a curved 23-gauge needle attached to polyethylene tubing (PE 50) was inserted into the artery for the administration of drugs. After completion of the operation, 30 min was allowed for stabilization of the preparation before beginning drug injections.

Zero-flow baselines were established at the beginning of each experiment and checked periodically by inflation of the hydraulic occluding cuffs with 10 lb/in.^{2} of air pressure. The flow probes were calibrated *in situ* at the end of each experiment by cannulating the hepatic and external iliac arteries distal to the position of the flow probes and diverting flow into a graduated cylinder for 30-sec intervals. At all flow levels, the relationship between the output of the flow probes and the directly measured blood flows was linear.

In each dog, the effects of graded doses of angiotensin II [>0.917 mole of peptide/mg (>97% angiotensin II)] and angiotensin III [>1.032 mole of peptide/mg (>96% angiotensin III)] upon hepatic and hindlimb blood flows were evaluated. All of the angiotensin peptides were purchased from Beckman (Palo Alto, Calif.). All agonists were dissolved in saline and each dose (vol = 0.2 ml) was administered as a bolus injection directly into the arterial catheter of the hepatic and hindlimb vasculatures. A constant infusion of saline (1.0 ml/min) to each vasculature delivered the bolus injections of agonists through the injection catheters into the arterial blood; this method allowed each dose to be delivered to each vascular bed in a standardized fashion. The injection schedule with respect to agonist and dose was randomized, and sufficient time (8 min) as determined in preliminary studies elapsed between injections so that tachyphylaxis did not develop.

Changes in hepatic and hindlimb blood flow consequent to bolus injections of angiotensins II or III were examined by measuring three characteristics of the blood flow response (Fig. 1): (i) maximal change in the amplitude of the blood flow response as measured from its preinjection value; (ii) duration of the response as measured from the time of agonist injection until the flow returned to the preinjection value; and (iii) graphic integration (area) of the flow response as a function of the maximal possible flow change that could have occurred during the 400 sec after injection of an agonist. Integration for 400 sec was chosen because flow in both vascular beds had returned to preinjection levels, or within 10% of preinjection levels, in all of the experiments within that time. Integration of flow responses was performed with a compensating polar planimeter (Lasico, Model l-10). The percentage change in area was calculated as the area of change in flow (area x) divided by the area

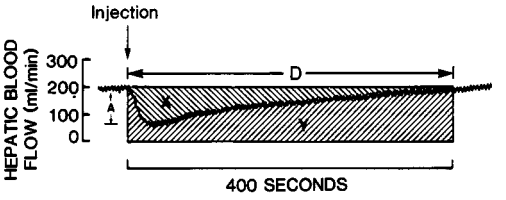

FIG. 1. Changes in hindlimb and hepatic blood flow produced by the agonist peptides were evaluated by measuring three variables: (1) duration (D) of the flow decrease; (2) amplitude (A) of the flow decrease; and (3) graphic integration (area) of flow change response. Integrated responses are expressed as percentage of maximal possible flow change (area x/area x + area y) that could have occurred during the 400 sec after injection of the peptides.

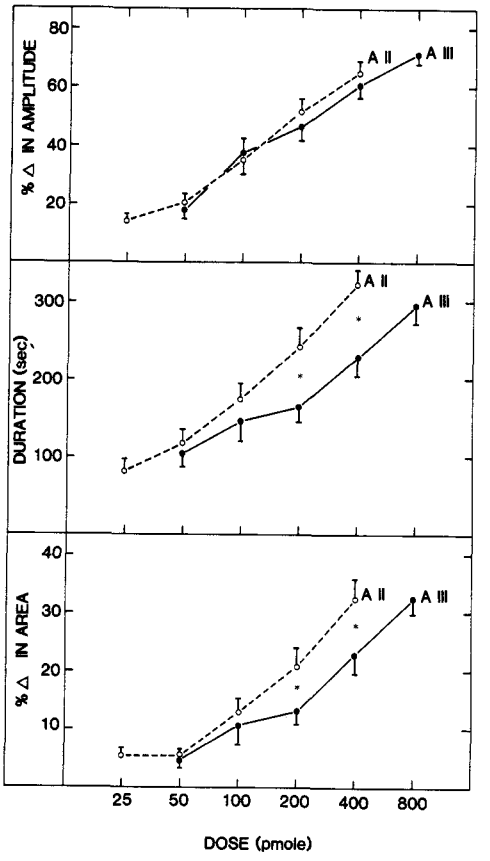

FIG. 2. Dose-response curves for changes in three variables of hindlimb blood flow caused by angiotensin II (AII, unfilled circles) and angiotensin III (AIII, filled circles). Each point represents the average response from 14 dogs; vertical lines represent ±1 SEM. (*<0.05 for angiotensin II compared to angiotensin III responses.)

representing the maximal possible change in flow (area x and y), and this quotient was multiplied by 100.

The statistical significance of differences between the responses to angiotensin II and angiotensin III were evaluated by Student's paired t test (21); P values of <0.05 were considered significant for differences. Values are reported as means ±1 SEM.

Results. *Hindlimb.* Hindlimb blood flow decreased in a dose-dependent fashion in response to direct intraarterial bolus injections of both angiotensins II (25-400 pmole) and III (50–800 pmole) (Fig. 2). There were no significant differences in potency between angiotensins II and III, at any dose tested, when the responses were evaluated on the basis of percentage change in amplitude of the flow response. On the basis of duration of the flow change and percentage change in area of the flow response, however, angiotensin II was significantly more potent than angiotensin III at the higher doses tested. At the dosage of 200 pmole, angiotensin was 45% more potent than angiotensin III on the basis of duration, and 62% more potent on the basis of percentage change in area.

Figure 3 shows the average change in hindlimb blood flow (as % of control value) that occurred in response to 400 pmole of each angiotensin peptide for all 14 animals studied. The qualitative responses to both angiotensin II and angiotensin III were similar. The maximal change in amplitude of the flow response occurred at 40 sec after the injection for both

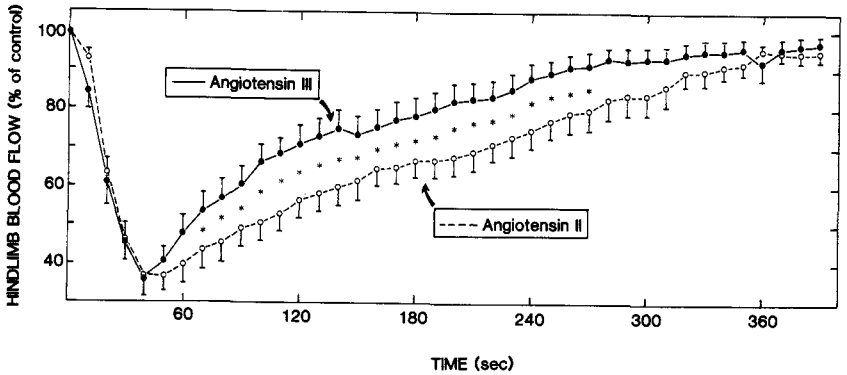

FIG. 3. Average hindlimb blood flow responses to 400 pmole of angiotensins II and III taken each 10 sec after the start of agonist injection. Each point represents the average response from 14 dogs; vertical lines represent ±1 SEM. (*<0.05 for angiotensin II compared to angiotensin III responses).

peptides. Angiotensin II, however, maintained a significantly greater reduction in flow for a longer duration relative to the responses to angiotensin III. Flow responses to angiotensin III had returned to 90% of the baseline value (control) by 260 sec, while similar flow recovery for the angiotensin II responses required 340 sec on the average. These data clearly demonstrate that an assessment of relative potency based only upon measurement of amplitude of flow responses is inadequate.

Hepatic. Figure 4 shows the effects of angiotensins II and III (25–400 pmole) upon flow in the hepatic circulation. On the basis of percentage change in amplitude and percentage change in area of the flow response, no differences were found between the responses to angiotensin II and angiotensin III in the hepatic vasculature. At the lowest dosage only (25 pmole), however, the average duration of the responses to angiotensin III was 45% longer than the responses to angiotensin II; at all other doses the durations of responses to the peptides were not significantly different. Figure 5 shows the average change in hepatic blood flow (as percentage of control value) that occurred in response to 400 pmole of each peptide for all 14 animals. At all times during the response the flow changes caused by angiotensins II and III were not significantly different.

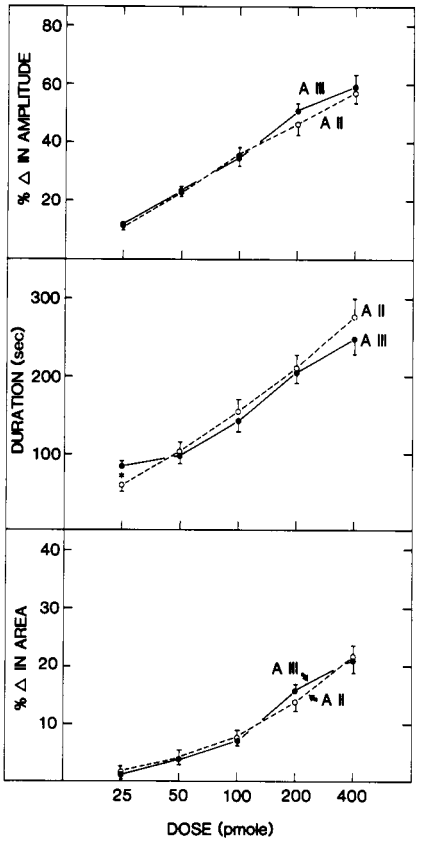

FIG. 4. Dose–response curves for changes in three variables of hepatic blood flow caused by angiotensin II (AII, unfilled circles) and angiotensin III (AIII, filled circles). Each point represents the average response from 14 dogs; vertical lines represent ±1 SEM. (*<0.05 for angiotensin II versus angiotensin III responses).

Baseline systemic arterial blood pressure, iliac blood flow, and hepatic blood flow remained stable throughout the procedure. The average iliac blood flow was 78 ± 10 ml/min at the beginning of the experiment and 78 ± 14 ml/min at the end. Hepatic blood flow averaged 252 ± 28 ml/min at the beginning of the experiment and averaged 284 ± 37 ml/min at the end; corresponding mean arterial blood pressures were 125 ± 4 and 127 ± 4 mm Hg, respectively. Mean systemic arterial blood pressure increased on the average less than 3 mm Hg in response to the intraarterial injections of the two higher doses of both peptides into each vasculature. Although this increase in pressure was small, it occurred consistently, and was thus significant statistically ($P < 0.05$).

Discussion. We conclude from our results that angiotensins II and III are equipotent as vasoconstrictors in the hepatic arterial vascular bed. The hepatic blood flow responses to both of the peptides were similar when analyzed on the basis of amplitude, duration, or area. In the hindlimb vasculature, however, angiotensin II was more potent than angiotensin III on the basis of duration and area of the vasoconstrictor responses. Analysis of hindlimb blood flow responses by measuring amplitude of the responses revealed no significant difference in potency between angiotensins II and III. We think that analysis by integration of the flow responses is the best method for evaluating the relative potency of vasoconstrictor agents. Flow changes in response to agonists are integral events that have duration and varying amplitudes during their duration that are often of complex configuration. The sum of all interacting factors that determine the extent to which a vasoactive compound alters blood

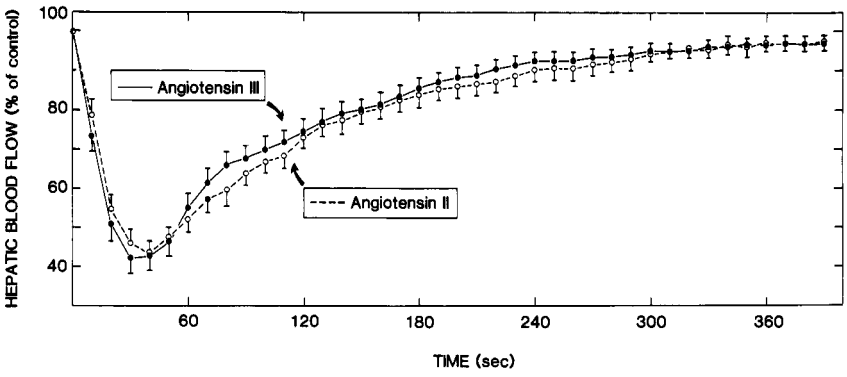

FIG. 5. Average hepatic blood flow responses to 400 pmole of angiotensins II and III taken each 10 sec after the start of agonist injection. Each point represents the average response from 14 dogs; vertical lines represent ±1 SEM.

flow is revealed in integral analysis. Thus, conclusions regarding the potency of a compound based solely on analysis of amplitude of the responses can be misleading. Differences in analysis of data could explain why we conclude (19), in contrast to other reports (15, 17), that angiotensin II is more potent than angiotensin III as a vasoconstrictor in the renal and hindlimb vasculatures.

Our data suggest that differential vasoconstrictor potency between angiotensins II and III in the hindlimb, but not the hepatic circulation, contribute to the observed greater potency of angiotensin II administered intravenously as a constant infusion on systemic arterial blood pressure (12). Differences in vasoconstrictor potency are clearly not the only mechanism by which angiotensin II could possess greater pressor activity than angiotensin III. For example, Stokland *et al.* (22) concluded that about one-half of the rise in arterial blood pressure produced by an infusion of angiotensin II was due to an increase in end-diastolic blood volume that occurred primarily because of a redistribution of blood from the splanchnic circulation. To our knowledge, the effects of angiotensin III upon cardiac preload have not been evaluated. Further, it would be interesting to know if equipressor doses of angiotensins II and III have similar or different effects upon cardiac output and its distribution.

Our data demonstrate that angiotensin II is a more powerful vasoconstrictor than angiotensin III in the hindlimb, but not in the hepatic circulation. Several factors could contribute to the differential vasoconstrictor properties of angiotensins II and III. First, angiotensin peptides, besides acting directly upon vascular smooth muscle, can modulate the release and action of other vasoactive agents such as norepinephrine and prostaglandins (1). Second, angiotensins II and III may act on the same receptor site, but the receptor affinity for each peptide may differ. In contrast, there may be different receptor sites for the two peptides (23). Third, there may be differential binding of the peptides to specific or nonspecific receptor sites, which are unrelated to smooth muscle contraction. Fourth, there may be differential enzymatic catabolism of the peptides. In accord with this last possibility is the observation of Goodfriend and Peach (23) that angiotensin III is more susceptible to degradation than is angiotensin II in adrenal glomerulosa cells. Finally, it is possible that angiotensin II is more potent than angiotensin III in the hindlimb circulation because it is more potent as a potentiator of sympathetic nervous system activity. Numerous studies have demonstrated that angiotensin II potentiates sympathetically mediated neural vasoconstriction by both presynaptic and postsynaptic mechanisms (1). To our knowledge, the efficacy of angiotensins II and III for potentiating sympathetic neural activity in the hindlimb circulation has not been evaluated.

The expert secretarial assistance of Marianne Miller and Cathy Metzker is gratefully acknowledged. This work was supported in part by a research grant from the Northwestern Ohio Affiliate of the American Heart Association.

1. Peach MJ. Renin-angiotensin system: Biochemistry and mechanisms of action. Physiol Rev **57**:313–370, 1977.
2. Laragh JH, Baer L, Brunner FR, Sealey JE, Vaughan ED Jr. Renin, angiotensin and aldosterone system in pathogenesis and management of hypertensive vascular disease. Amer J Med **52**:633–652, 1972.
3. Semple PF, Boyd AS, Dawes PM, Morton JJ. Angiotensin II and its heptapeptide (2-8), hexapeptide (3-8), and pentapeptide (4-8) metabolites in arterial and venous blood of man. Circ Res **39**:671–678, 1976.
4. Rio CG del, Smellie SWA, Morton JJ. des-Asp-angiotensin I: Its identification in rat blood and confirmation as a substrate for converting enzyme. Endocrinology **108**(1):406–412, 1981.
5. Fei DTW, Coghlan JP, Fernley FT, Scoggins BA, Tregear GW. Peripheral production of angiotensin II and III in sheep. Circ Res (Suppl I) **46**(6):I135–I137, 1980.
6. Semple PF, Morton JJ. Angiotensin II and angiotensin III in rat blood. Circ Res (Suppl II) **38**:122–126, 1976.
7. Chiu AT, Ryan JW, Stewart JM, Dorer FE. Formation of angiotensin III by angiotensin-converting enzyme. Biochem J **155**:189–192, 1976.
8. Tsai BS, Peach MJ, Khosla MC, Bumpus FM. Synthesis and evaluation of [des-Asp^1] angiotensin II ("angiotensin III"). J Med Chem **18**:1180–1183, 1975.
9. Sexton JM, Britton SL, Beierwaltes WH, Fiksen-Olsen MJ, Romero JC. Formation of angiotensin III from [des-Asp^1] angiotensin I in the mesenteric vasculature. Amer J Physiol **237**:H218–H223, 1979.
10. Larner A, Vaughan ED Jr, Tsia BS, Peach MJ. Role of converting enzyme in the cardiovascular and adrenal corticol responses to [des-Asp^1] angiotensin I. Proc Soc Exp Biol Med **152**:631–634, 1976.
11. Britton SL, Beierwaltes WH, Fiksen-Olsen JM, Romero JC. Intrarenal vascular effects of des-Asp^1-angiotensin III in dogs. Circ Res **44**:666–671, 1979.
12. Carey RM, Vaughan ED Jr, Peach MJ, Ayers CR. Activity of [des-Asp^1]-angiotensin II and angiotensin III in man: Differences in blood pressure and adrenocorticol responses during normal and low sodium intake. J Clin Invest **61**:20–31, 1978.
13. Vaughan ED Jr, Peach MJ, Ackerly JA, Tsai BS, Larner A. Pressor and steroidogenic actions of [des-Asp^1] angiotensin I. Circ Res (Suppl I):I94–I97, 1977.
14. Blair-West JR, Cochlan JP, Denton DA, Fei DTW, Hardy KJ, Scoggins BA, Wright RD. A dose–response comparison of the actions of angiotensin II and angiotensin III in sheep. J Endocrinol **87**:409–417, 1980.
15. Freeman RH, David JO, Lohmeier TE. des-1-Asp-angiotensin II: Possible intrarenal role in homeostasis in the dog. Circ Res **37**:30–34, 1975.
16. Taub KJ, Caldicott WJH, Hollenberg NK. Angiotensin antagonists with increased specificity for the renal vasculature. J Clin Invest **59**:528–535, 1977.
17. Caldicott WHJ, Taub KJ, Hollenberg NK. Identical mesenteric, femoral and renal vascular responses to angiotensins II and III in the dog. Life Sci **20**:517–521, 1977.
18. Britton SL, Sexton JM, Fiksen-Olsen MJ, Wernes PG, Romero JC. A comparison of angiotensin II and angiotensin III as vasoconstrictors in the mesenteric circulation of dogs. Circ Res **46**(1):146–151, 1980.
19. Britton SL, Fiksen-Olsen MJ, Houck PC, Romero JC. Intrarenal vascular effects of angiotensin II and angiotensin III in the dog. J Hypertens **1**(1):95–101, 1983.
20. Kleinman LI, Radford EP Jr. Ventilation standards for small mammals. J Appl Physiol **19**:360–362, 1964.
21. Zar JH. Biostatistical Analysis. Englewood Cliffs, N.J., Prentice–Hall, 1974.
22. Stokland O, Thorvaldson J, Ilebekk A, Kiil F. Mechanism of blood pressure elevation during angiotensin infusion. Acta Physiol Scand **115**:455–465, 1982.
23. Goodfriend TL, Peach MJ. Angiotensin III: (des-aspartic-acid^1)-angiotensin II; evidence and speculation for its role as in important agonist in the renin–angiotensin system. Circ Res **36**(Suppl 1):38–48, 1975.

Received May 29, 1985. P.S.E.B.M. 1986, Vol. 181.
Accepted September 16, 1985.