Angiotensin-(1-7) Does Not Affect Vasodilator or TPA Responses to Bradykinin in Human Forearm

Terry Wilsdorf, James V. Gainer, Laine J. Murphey, Douglas E. Vaughan, Nancy J. Brown

Abstract—Studies in isolated vessels and rat models of hypertension suggest that angiotensin (Ang)-(1-7) potentiates the vasodilator effect of bradykinin, possibly through ACE inhibition. We therefore tested the hypothesis that Ang-(1-7) potentiates the vasodilator or tissue plasminogen activator (TPA) response to bradykinin in the human forearm vasculature. Graded doses of Ang-(1-7) (10, 100, and 300 pmol/min), bradykinin (47, 94, and 189 pmol/min), and Ang I (1, 10, and 30 pmol/min) were administered through the brachial artery to 8 normotensive subjects in random order. Thirty minutes after initiation of a constant infusion of Ang-(1-7) (100 pmol/min), bradykinin and Ang I infusions were repeated. There were no systemic hemodynamic effects of the agonists. Bradykinin significantly increased forearm blood flow \(P<0.001\), from \(3.8\pm0.5\) to \(13.9\pm3.1\) mL/min per 100 mL at \(189\) pmol/min) and net TPA release \(P=0.007\), from \(1.1\pm1.0\) to \(23.6\pm6.2\) ng/min per 100 mL at \(189\) pmol/min), whereas Ang I caused vasoconstriction \(P=0.003\), from \(3.3\pm0.4\) to \(2.5\pm0.3\) mL/min per 100 mL at \(30\) pmol/min dose). There was no effect of Ang-(1-7) on either forearm blood flow \(P=0.62, 3.3\pm0.4\) to \(3.5\pm0.4\) mL/min per 100 mL at \(300\) pmol/min) or TPA release \(P=0.52, \) from \(0.7\pm0.8\) to \(1.0\pm0.7\) ng/min/100 mL at \(300\) pmol/min). Moreover, there was no effect of \(100\) pmol/min Ang-(1-7) on the vasodilator \(P=0.46\) for Ang-(1-7) effect) or TPA \(P=0.82\) for Ang-(1-7) effect) response to bradykinin or the vasoconstrictor response to Ang I \(P=0.62\) for Ang-(1-7) effect). These data do not support a role of Ang-(1-7), given at supraphysiological doses, in the regulation of human peripheral vascular resistance or fibrinolysis. (Hypertension. 2001;37:1136-1140.)

Key Words: angiotensin ■ angiotensin-converting enzyme ■ bradykinin ■ endothelium ■ plasminogen ■ vasodilator agents

Angiotensin (Ang)-(1-7) is formed from Ang I by the action of the endopeptidase neprilysin (EC 3.4.24.11) and degraded to Ang-(1-5) by ACE.1 Ang-(1-7) concentrations may be increased during ACE inhibition.2-5 In vitro and in animal models, Ang-(1-7) acts as a vasodilator and exhibits antiproliferative effects.4,5,7,8 For example, in isolated canine, porcine, and human arteries, Ang-(1-7) causes nitric oxide–dependent vasodilation.4,5,7,8 Significantly, the vasodilator action of the endopeptidase neprilysin (EC 3.4.24.11) studied extensively in humans. Davie and McMurray15 recently reported that intrabrachial administration of Ang-(1-7) did not cause vasodilation and did not potentiate the vasodilator response to BK in patients with heart failure treated with an ACE inhibitor. However, in this study, pretreatment with an ACE inhibitor may have obscured any BK-potentiating effect of Ang-(1-7). In addition, no studies have reported whether Ang-(1-7) potentiates the effect of BK-stimulated tissue plasminogen activator (TPA) release. BK is known to stimulate endothelial TPA release through its B2 receptor.16,17 We therefore investigated the effect of intrabrachial administration of Ang-(1-7) on forearm blood flow (FBF) and endothelial TPA release when given alone and in combination with BK.

Methods

Subjects

Eight healthy subjects were studied. The study was approved by the Vanderbilt University Institutional Review Board and conducted according to the Declaration of Helsinki. Each subject underwent a
Experimental Protocol

Studies were performed in the morning, in a temperature-controlled room. Subjects were studied in the supine position and in the fasting state. After subdermal administration of 1% lidocaine, a 20-gauge polythene catheter (Cook Inc) was inserted into the brachial artery of the nondominant arm, allowing for intra-arterial administration of drugs and arterial sampling. A catheter was inserted in the antecubital bival of the same arm for venous sampling. Before the infusion of vasoactive drugs, arterial catheter patency was maintained by infusion of 5% dextrose in water at a rate of 1 mL/min. After placement of the intravenous and intra-arterial catheters, subjects were allowed to rest 30 minutes before baseline measurements were made. Blood pressure was monitored in the contralateral arm with an automated blood pressure cuff throughout the study. After measurement of basal FBF and blood sampling, graded doses of Ang-(1-7) (Clinalfa AG), Ang I (Clinalfa AG), or BK (Calbiochem; sterilized and tested for pyrogenicity by the Vanderbilt Investigational Drug Pharmacy) were infused in random order. Ang-(1-7) was infused at 10, 100, and 300 pmol/min; Ang I was infused at 1, 10, and 30 pmol/min; and BK was infused at 50, 100, and 200 ng/min (47, 94, and 189 pmol/min). Each dose was infused for 5 minutes, and FBF was measured during the last 2 minutes. Before infusion of each drug, the 30-minute rest period and basal measurements were repeated. Ang I and BK were each infused twice, in the presence and absence (order randomized) of a 100-pmol/min continuous infusion of Ang-(1-7). Continuous Ang-(1-7) infusion was initiated 30 minutes before concurrent administration of either BK or Ang I. The shortest interval between BK and Ang-(1-7) infusion in any subject was 75 minutes. We have previously determined that tachyphylaxis to BK does not occur over a time interval as short as 1 hour. Throughout the study, drug concentrations in the infusate were adjusted to maintain infusion volumes at 1 mL/min. The doses of Ang I and BK were chosen on the basis of previously published studies from this laboratory and others. The doses of Ang-(1-7) were chosen to be 10-fold higher than the pharmacologically active doses of Ang I.

Forearm Perfusion Measurements

FBF was measured by mercury-in-silastic strain-gauge plethysmography. The wrist was supported in a sling to raise the forearm to above the level of the atrium, and the strain gauge was placed at the widest part of the forearm. The strain gauge was connected to a plethysmograph (model EC-5, D.E. Hokanson), calibrated to measure the percent change in volume, and connected to a chart recorder to record the flow measurements. For each measurement, a cuff placed around the upper arm was inflated to 40 mm Hg with a rapid cuff inflator (model E-10, Hokanson) to occlude venous outflow from the extremity. The hand was excluded from the measurement of blood flow by inflation of a pediatric sphygmomanometer cuff around the wrist to 200 mm Hg before and during measurement of FBF. Flow measurements were recorded for ~7 of 15 seconds, and the slope was derived from the first 3 to 4 pulses; 5 to 7 such readings were obtained for each mean value.

Blood Sampling and Biochemical Assays

After measurement of FBF, simultaneous arterial and venous samples were obtained from the infused arm before and after each dose of study drug. Infusion of drug was interrupted during arterial sampling. All samples were obtained after the first 3 mL of blood was discarded. Blood samples were collected on ice and centrifuged immediately, and plasma was stored at -70°C until the time of assay. Blood for measurement of plasminogen activator inhibitor-1 and TPA was collected in tubes containing 0.105 mol/L acidified sodium citrate, and antigen levels were determined with a 2-site enzyme-linked immunosorbert assay (Biopool AB), as previously described. Because increases in TPA activity parallel increases in TPA antigen in response to BK, TPA activity was not measured separately. Arteriovenous concentration gradients were calculated by subtracting the plasma level measured in simultaneously collected venous and arterial blood. Forearm plasma flow was calculated from the FBF and arterial hematocrit corrected for 1% trapped plasma. Thus, individual net release or uptake rates at each time point were calculated by the formula

Net release = (Cv - Ca) x (FBF x [100 - hematocrit])/100

where Cc and Cs represent the concentration of TPA in the brachial vein and artery, respectively.

Statistics

Data are presented as mean±SEM. Because there was no effect of Ang-(1-7), Ang I, or BK on systemic blood pressure, data are presented in terms of FBF. The effect of Ang-(1-7) on the response to agonist was assessed by ANOVA with repeated measures in which the within-subject variables were the presence and absence of Ang-(1-7) and the dose of agonist. Post hoc comparisons were made with the paired t test or Wilcoxon signed rank test, as appropriate. A 2-tailed probability value of <0.05 was considered statistically significant.

Results

Local infusions of Ang-(1-7), BK, and Ang I did not affect MAP or heart rate. For example, MAP was 83.3±3.1 mm Hg during 300 pmol/min Ang-(1-7) versus 83.6±2.5 mm Hg at baseline. MAP was 83.6±3.6 at 189 pmol/min BK versus 87.5±3.1 baseline and 86.6±2.7 at 30 pmol/min Ang I versus 85.1±2.5 mm Hg at baseline.

Forearm Blood Flow

Figure 1 shows the effect of Ang-(1-7), Ang I, and BK on FBF. There was no effect of Ang-(1-7) on FBF at doses up to 300 pmol/min (P=0.62, Figure 1A). BK significantly increased FBF from 3.8±0.5 mL/min per 100 mL at baseline to 39.3±3.1 mL/min per 100 mL at the 189-pmol/min dose (P<0.001, Figure 1B). There was no effect of concurrent Ang-(1-7) administration on the vasodilator response to BK [P=0.46 for Ang-(1-7) effect]. Ang I caused a significant decrease in FBF from 3.3±0.4 mL/min per 100 mL at baseline to 2.5±0.3 mL/min per 100 mL at the 30 pmol/min dose (P=0.003, Figure 1C). There was no effect of concurrent Ang-(1-7) administration on the vasoconstrictor response to Ang I [P=0.62 for Ang-(1-7) effect].

Net TPA Release

Figure 2 shows net TPA release across the forearm during Ang-(1-7) and BK infusion. There was no effect of Ang-(1-7) on net TPA release (P=0.52). BK caused a significant increase in net TPA release from 1.1±1.0 ng/min per 100 mL at baseline to 23.6±6.2 ng/min per 100 mL at the 189-pmol/min dose (P=0.007) without a concomitant increase in plasminogen activator inhibitor-1 antigen (P=0.44). There was no effect of Ang-(1-7) on the TPA response to BK [P=0.82 for effect of Ang-(1-7)].
Discussion

In this study, we set out to test the hypothesis that Ang-(1-7) causes vasodilation in the peripheral human vasculature, either through direct or indirect effects. We examined the effect of Ang-(1-7) on both the vasodilator and the TPA responses to BK because previous studies in humans have demonstrated that BK acts as a potent stimulus to TPA release from the human endothelium through effects at its B2 receptor. In addition, we examined the effect of Ang-(1-7) on the vasoconstrictor response to Ang I. We hypothesized that if Ang-(1-7) acts through ACE inhibition, then it would both potentiate responses to BK and attenuate the vasoconstrictor response to Ang I. In contrast, if Ang-(1-7) acts by altering BK receptor sensitivity, we hypothesized that it would potentiate the responses to BK but not the response to Ang I. Finally, if Ang-(1-7) acts as an AT1 receptor antagonist, we hypothesized that it would attenuate the vasoconstrictor effect of Ang I without potentiating the responses to BK. The results suggest that Ang-(1-7), given at supraphysiological doses, does not cause vasodilation of the human peripheral vasculature. Moreover, the study results do not support an effect of Ang-(1-7) on either ACE activity or BK receptor function in the human peripheral vasculature.

Data from this study are consistent with those from 3 prior studies in humans. In 1986, Kono et al reported that Ang-(1-7) acted as a weak pressor when administered systemically to humans, with a potency 0.028% of that of Ang II. Because Ang-(1-7) was administered systemically, however, it has been proposed that a compensatory baroreflex response may have masked any vasodepressor effect of Ang-(1-7). In the present study, Ang-(1-7) was administered directly into the brachial artery, and there were no systemic effects of any of the agonists administered. More recently, Ueda et al reported no vasodilator response to intra-arterial Ang-(1-7) at doses up to 2000 pmol/min, although these investigators observed a vasoconstrictor response to intra-arterial Ang-(1-7) at higher doses. In contrast, Davie and McMurray reported no significant vasoactive effect of intra-arterial Ang-(1-7) administered at doses up to 50 000 pmol/min in the forearm vasculature of patients with congestive heart failure. The authors also reported that Ang-(1-7) did not potentiate the vasodilator response to BK in the presence of ACE inhibition. However, this study could not exclude the possibility that Ang-(1-7) potentiates the effects of BK by inhibiting ACE. This study, performed in the absence of ACE inhibitor therapy, does not support this possibility.

Although previous investigators have examined the effect of Ang-(1-7) on BK-mediated vasodilation, this study is unique in measuring the effect of Ang-(1-7) on the TPA response to BK. TPA is stored in small dense granules in endothelial cells. Recent studies indicate that BK stimulates the release of TPA through a B2-dependent but nitric oxide synthase–independent, cyclooxygenase-independent, pathway. The observation that coadministration of Ang-(1-7) does not potentiate BK-stimulated TPA release provides additional and complementary evidence that Ang-(1-7) does not alter B2-mediated responses, whether by affecting BK degradation or B2-receptor sensitivity.

The finding that Ang-(1-7) does not potentiate the vasodilator effects of BK in the human forearm conflicts with data from many in vitro studies as well as studies in normotensive and hypertensive animal models. Several possible factors may account for the discrepancy between the vasodilator effect of Ang-(1-7) observed in vitro and in animal studies and the lack of such an effect on BK-mediated
vasodilation in this and other human studies. First, there may be interspecies differences in the effects of Ang-(1-7). Second, Ang-(1-7) may contribute to vascular tone in hypertensive but not in normotensive animals or humans. In this regard, Benter et al. have reported differential susceptibility to the effects of Ang-(1-7) in hypertensive versus normotensive rat models.

Differences in dose of Ang-(1-7) used may account to a large extent for the discrepancy between data from human and prior in vitro and animal studies. The EC50 for the vasodilator effect of Ang-(1-7) has been reported to be 2.2 ± 0.4 μmol/L in porcine coronary rings and 2.73 ± 0.58 μmol/L in canine coronary arteries, although the EC50 appears to be lower in rat aorta. Roks et al. have reported an IC50 for inhibition of ACE activity in human plasma in the 10−8 mol/L range. In contrast, the concentration of Ang-(1-7) in human plasma appears to be in the range of 10−1−10−5 mol/L.

The maximal dose of Ang-(1-7) infused in this study was calculated to give local concentrations of Ang-(1-7) ≈ 10−8 mol/L or 1000-fold higher than physiologic concentrations. In addition, the constant dose of Ang-(1-7) infused was 10-fold higher than the minimal vasoconstrictor dose of Ang I and 30-fold higher than the minimal vasodilator dose of BK. By comparison, the molar ratio of Ang-(1-7) to Ang I in human plasma is <1. Thus, the study does not support the hypothesis that Ang-(1-7) contributes to BK-mediated peripheral vasodilation in humans at physiologically relevant concentrations. On the other hand, the study does not address the possibility that Ang-(1-7) affects BK-mediated peripheral vasodilation when given at the micromolar concentrations used in many in vitro studies. Similarly, the study does not address the possibility that Ang-(1-7) may act as an autacoid in microenvironments where concentrations may be greatest; studies with specific inhibitors will be necessary to address the role of endogenous Ang-(1-7) in humans.

The finding that Ang-(1-7) did not attenuate the forearm vasoconstrictor effect of Ang I contrasts with data from Roks et al., who demonstrated that Ang-(1-7) (10−5 mol/L) antagonized the vasoconstrictor effects of Ang I and Ang II in human internal mammary arteries. Our findings also diverge from those of Ueda et al., who reported that Ang-(1-7), at the same concentration used in this study, attenuated the forearm vasoconstrictor response to Ang II in 8 healthy volunteers. It is possible that the use of Ang I, which must be converted to Ang II, rather than Ang II in the present study may have obscured an effect of Ang-(1-7) at the level of the AT1 receptor. Indeed, the vasoconstrictor response to Ang I measured in the present study was markedly less than that reported by Ueda et al in response to similar concentrations of Ang II.

The finding that Ang-(1-7) does not cause peripheral vasodilation or potentiate the effects of BK in normotensive subjects also does not exclude a role for Ang-(1-7) in the regulation of blood pressure in humans through other mechanisms. For example, Ang-(1-7) could contribute to blood pressure regulation through its central nervous system effects. In the kidney, Ang-(1-7) causes natriuresis independent of changes in renal blood flow or glomerular filtration. Urinary Ang-(1-7) concentrations appear to be decreased in animal models of hypertension and in patients with essential hypertension. Thus, it is possible that Ang-(1-7) plays a role in the regulation of blood pressure through local paracrine effects in the brain or kidney, without causing peripheral vasodilation.

**Summary**

We examined the interactive effect of Ang-(1-7) on BK-stimulated vasodilation and vascular TPA release, two B2 receptor–mediated responses, in the forearm of healthy humans. The results do not support the hypothesis that Ang-(1-7), given at supraphysiological concentrations, potentiates the peripheral effects of BK in humans.

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