Angiotensin-(1-7) Attenuates Vasoconstriction Evoked by Angiotensin II but Not by Noradrenaline in Man

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Abstract—Angiotensin-(1-7) has been suggested to be a novel vasodilating peptide. We investigated the direct vascular effect of angiotensin-(1-7) in human forearm resistant vessels, particularly with regard to the interaction with angiotensin II, in healthy normotensive men by strain-gauge venous occlusion plethysmography with intra-arterial infusions of peptides. Intravenous infusions of angiotensin-(1-7) at 0.1 to 1000 pmol/min did not cause vasodilatation but rather reduced forearm blood flow by ≈10% at the highest dose. A placebo-controlled study showed that angiotensin-(1-7) at 0.5 to 40 nmol/min caused weak but significant vasoconstriction (P<0.0016 by ANOVA). Angiotensin-(1-7) at 100 pmol/min, but not at 10 pmol/min, significantly shifted the angiotensin II dose-response curve toward the right (mean±SD of percent changes in forearm blood flow: −19±17%, −33±22%, −55±12%, −63±10%, and −68±5% at 5, 10, 25, 50, and 100 pmol/min of angiotensin II, respectively, with saline; 5±13%, 0.9±18%, −40±16%, −54±9%, and −61±6% with angiotensin-(1-7), P=0.0021 by ANOVA). Angiotensin-(1-7) did not affect the dose-response curve of noradrenaline [3±12%, 5±16%, −20±22%, −31±18%, and −40±12% at 25, 50, 100, 300, and 600 pmol/min of noradrenaline, respectively, with saline; −4±15%, −2±23%, −29±22%, −34±16%, and −42±9% with angiotensin-(1-7)]. Our results suggest that angiotensin-(1-7) antagonizes vasoconstriction by angiotensin II in human resistant vessels and might act as an endogenous angiotensin II antagonist. (Hypertension. 2000;35:998-1001.)

Key Words: angiotensin ■ angiotensin II ■ noradrenaline ■ blood flow velocity

It has been suggested that angiotensin-(1-7) [Ang-(1-7)], which is one of bioactive components of the renin-angiotensin system and reportedly formed from angiotensin I (Ang I) and angiotensin II (Ang II), is a novel vasodilating peptide. Release of nitric oxide, production of prostaglandins, and potentiation of effect of bradykinin by Ang-(1-7) have been postulated as possible mechanisms for vasodilatation. Ang-(1-7) is also thought to contribute to a reduction in blood pressure during intravenous Ang-(1-7) infusion at a comparable dose as that in the animal studies that showed a hypotensive effect. This is not surprising because Ang-(1-7) has been known to have a weak agonistic effect. In fact, a short-term pressor effect has been demonstrated in pithed rats. However, interspecies differences in the response to Ang-(1-7) may also exist. We investigated the direct vascular effect of Ang-(1-7), particularly with regard to the interaction with Ang II, in human resistant vessels in vivo.

Methods

Subjects

Twenty-two healthy normotensive men (8 for protocol 1, 6 for protocol 2, and 8 for protocol 3) were recruited for the present study. All subjects underwent normal routine physical and laboratory examinations. Written informed consent was obtained from each subject after a full explanation of the study. All study protocols were approved by the Yokohama City University Hospital Ethics Committee.

Forearm Blood Flow Measurement

All experiments were performed in a quiet, temperature-controlled room. After an overnight fast, all subjects attended our Clinical Research Unit on the study day. Subjects abstained from cigarettes, alcohol, and caffeine-containing drinks for ≥12 hours before the study. Forearm blood flow was measured by bilateral strain-gauge venous occlusion plethysmography. The details of the method and
the reproducibility of the measurement were described elsewhere.\textsuperscript{17,18} Briefly, pediatric cuffs placed around wrists were inflated to 200 mm Hg during each measurement. Collecting cuffs placed around the upper arms were inflated to 40 mm Hg and deflated every 15 minutes. Strain gauges were placed around the forearms 5 cm distal to the olecranon. All drugs were infused into the catheter inserted into the brachial artery of the nondominant arm under local anesthesia with 1% lidocaine. The infusion rate was maintained at 1 mL/min throughout the study unless otherwise indicated.

**Protocol 1: Effect of Ang-(1-7) on Forearm Blood Flow**

Eight subjects (body mass index (BMI) 22.1±2.7 kg/m\(^2\), mean±SD, age 20 to 25) received Ang-(1-7) (ClinAlfa AG) intra-arterially at 0.1, 1.0, 5.0, 10, 50, 100, 250, 500, 1000, and 2000 pmol/min, for 10 minutes per dose, on a single study day. Forearm blood flow was measured after each dose of Ang-(1-7).

**Protocol 2: Effect of Relatively High Dose of Ang-(1-7) on Forearm Blood Flow**

Six subjects (BMI 19.7±2.4 kg/m\(^2\), age 20 to 25) received placebo (0.9% saline) and Ang-(1-7) at 0.1, 0.5, 1, 2.5, 5, 10, 20, and 40 nmol/min for 10 minutes per dose in a double-blind, crossover fashion. There was a washout period for 30 minutes between Ang II and noradrenaline. The order of these 2 vasoconstrictors was randomized but kept the same in the same subject.

**Protocol 3: Effect of Ang-(1-7) on Vasoconstriction Induced by Ang II and Noradrenaline**

Eight subjects (BMI 22.0±2.7 kg/m\(^2\), age 20 to 25) received noradrenaline (25, 50, 100, 300, and 600 pmol/min, at 10 minutes per dose) (Sankyo) and Ang II (5, 10, 25, 50, and 100 pmol/min, at 10 minutes per dose) (Yamanouchi) intra-arterially with concomitant intra-arterial infusions of Ang-(1-7) at 10 pmol/min, 100 pmol/min, or placebo (isotonic saline) on 3 study days in a double-blind, crossover fashion. There was a washout period for 30 minutes between Ang II and noradrenaline. The order of these 2 vasoconstrictors was randomized but kept the same in the same subject.

**Analysis of Forearm Blood Flow Data**

All forearm blood flow data were obtained via a Mac Laboratory 4 chart recorder (AD instruments). The percentage change in ratio of forearm blood flow from baseline was calculated as \( \frac{F(i)D / F(ni)D - F(i)B / F(ni)B}{F(i)B / F(ni)B} \times 100\% \). \( F(i) \) and \( F(ni) \) represent forearm blood flow during infusions of Ang-(1-7) and saline, respectively.

**Statistical Analysis**

Data are shown as mean±SD unless otherwise indicated. Comparison between changes in forearm blood flow with infusions of Ang-(1-7) and saline (protocol 2) was made by repeated measure of ANOVA. Comparison between changes in forearm blood flow during Ang II and noradrenaline infusion with concomitant infusions of Ang-(1-7) or saline was made by repeated measure of ANOVA, ie, interaction between treatment [Ang-(1-7) or placebo] and the doses of agonists.

**Results**

**Protocol 1: Effect of Ang-(1-7) on Forearm Blood Flow**

Figure 1 shows forearm blood flow during the infusion of Ang-(1-7) at various doses. There were no significant changes in forearm blood flow at 0.5 to 250 pmol/min of Ang-(1-7). Forearm blood flow during infusions of Ang-(1-7) at 500 to 2000 pmol/min tended to be reduced but the changes did not reach statistical significance.

**Protocol 2: Effect of Ang-(1-7) on Forearm Blood Flow, Double-Blind, Crossover Study**

Figure 2 shows changes in forearm blood flow during infusions of Ang-(1-7) and placebo. Reduction of forearm blood flow during Ang-(1-7) infusion was significantly greater than that during placebo infusion \((P=0.0016, \text{by ANOVA})\).

**Protocol 3: Effect of Ang-(1-7) on Vasopressor Responses to Ang II and Noradrenaline**

Figure 3 shows the effect of concomitant infusion of Ang-(1-7) on vasoconstriction evoked by Ang II and noradrenaline. No significant differences existed in baseline forearm blood flow in the infused arm between before Ang II with saline and with Ang-(1-7) (2.78±0.79 versus 2.91±0.69 mL \( \cdot \min^{-1} \cdot \text{dL}^{-1} \)) and between before noradrenaline with saline and with Ang-(1-7) (2.64±0.76 versus 2.58±0.53 mL \( \cdot \min^{-1} \cdot \text{dL}^{-1} \)). The magnitude of reduction of forearm blood flow by Ang II infusion was significantly attenuated by the infusion of Ang-(1-7) at 100 pmol/min [percent changes in forearm blood flow: \(-19±17\%\), \(-33±22\%\), \(-55±12\%\), \(-63±10\%\), and \(-68±5\%\) at 5, 10, 25, 50, and 100 pmol/min of Ang II with saline; 5±13\%, 0.9±18\%, \(-4±16\%\), \(-54±9\%\), and \(-61±6\%\) with Ang-(1-7) at 100 pmol/min, \(P=0.0021\) by ANOVA, placebo versus 100 pmol/min of Ang-(1-7).
Ang-(1-7) but not at 10 pmol/min. Ang-(1-7) infusion at either 10 pmol/min or 100 pmol/min did not affect the noradrenaline dose-response curve.

**Discussion**

**Is Ang-(1-7) a Vasodilating Peptide?**

We demonstrated that the intra-arterial infusion of Ang-(1-7) itself had no direct vasodilating effect, at least at the doses that we used in human forearm resistant vessels. Ang-(1-7) caused weak but significant vasoconstriction at the high doses. The forearm concentration of Ang-(1-7) in our study (protocol 2) was assumed to be 2 to 3 μmol/L at the highest dose (40 mmol/min), which was almost the same as the concentration that caused 50% vasorelaxation in canine coronary arteries (2.7 μmol/L) and in porcine coronary arteries (2.2 μmol/L). This, our results are the antithesis of several previous reports that showed the direct vasodilating effect of Ang-(1-7) in isolated arteries and in vivo hypertensive effect. However, there has not necessarily been universal agreement with the vasodilating property of this peptide. Abbas et al showed that Ang-(1-7) at 20 to 380 nmol/rat had an angiotensin type 1 (AT1) receptor-mediated hypertensive effect unless exogenous bradykinin was administered. This suggests that the vasodilating effect of Ang-(1-7) may require sufficient bradykinin. Kono and colleagues showed the hypertensive effect of Ang-(1-7) at 18 mmol·kg⁻¹·min⁻¹ for 15 minutes, which was comparable with the dose used in the study by Abbas et al, in normotensive healthy subjects. These various vascular and blood pressure responses to Ang-(1-7) among studies may result in part from methodological differences. Different vascular beds (e.g., forearm vessels versus coronary artery) from different species (rat versus human) may have different responses to Ang-(1-7). The duration of infusion of Ang-(1-7) might also affect the result. Indeed, Benter et al demonstrated a biphasic acute blood pressure response to Ang-(1-7), which consisted of an initial pressor component and a subsequent depressor component after a bolus injection of Ang-(1-7) at 300 nmol/rat in rats.

**Interaction With Ang II**

The present study also demonstrated that Ang-(1-7) significantly and dose dependently attenuated vasoconstriction evoked by Ang II but not by noradrenaline. This result raises the possibility that Ang-(1-7) may be an endogenous antagonist of AT1 receptor although the concentration of Ang-(1-7) in the forearm vascular beds (0.01 μmol/L) during the infusion at 100 pmol/min was much lower than IC50 (>1 μmol/L) for AT1 receptor. Interestingly, Mahon et al showed a significant rightward shift of the dose-response curve of Ang II by Ang-(1-7) in rabbit aortic rings and attenuation of the pressor response to Ang II by Ang-(1-7) in rats, whereas the administration of Ang-(1-7) itself had no hypotensive effect. Because this effect was observed specifically with Ang II, but not with other vasoconstrictors, and was reversed by losartan, they speculated that Ang-(1-7) modulated the effect of Ang II via the AT1 receptor. Our results are consistent with their report. There are, however, several other possible mechanisms for Ang-(1-7) mediated attenuation of the vascular effect of Ang II. Evidence suggests that Ang-(1-7) stimulates release of vasodilating prostaglandin and NO and also potentiates the effect of bradykinin. Although this effect has not been confirmed in human vessels, these agents could attenuate the vascular effect of Ang II. This, however, may not be the case for our results, because these vasodepressor agents must affect the effect of noradrenaline as well. Therefore, albeit no experiment with angiotensin receptor antagonists was conducted in our study, we speculate that Ang-(1-7) attenuated the vascular action of Ang II via the AT1 receptor. Experiments to investigate the interaction among Ang-(1-7), and Ang II, AT1 receptor antagonist and inhibitors of prostaglandins, nitric oxide, and bradykinin in man are clearly warranted to confirm this hypothesis. One would claim that Ang-(1-7) should show direct vasodilating effect if it has an AT1 receptor antagonistic effect. However, evidence suggests that Ang II does not contribute to maintaining vascular tone in salt-repleted healthy subjects. Therefore, it is not surprising that Ang-(1-7) alone has no vasodilating effect on our subjects.

Two different mechanisms seem at work for the vascular effect of Ang-(1-7). First through the AT1 receptor directly and second through the production of vasodilating agents that...
might involve non-AT₁ and AT₂ angiotensin receptor(s). In contrast to our study, Benter et al.²⁷ showed nonspecific effect of Ang-(1–7), ie, pressor responses to not only Ang II but also phenylephrine, was attenuated by the chronic administration of Ang-(1–7) in spontaneously hypertensive rats. This non-specific vasodilating effect of Ang-(1–7) may be explained by stimulated production of vasodopressor agents by Ang-(1–7).

**Are These Results Physiologically or Pathophysiologically Significant?**

Our study showed that the vascular effects of Ang II at 2.5 to 5 nmol/L was inhibited by Ang-(1–7) at 10 nmol/L.²⁸ Therefore, although the concentration of Ang-(1–7) in this study was 1000× higher than the physiological concentration in man (10 pmol/L), the ratio of the concentration of Ang II to Ang-(1–7) was 0.25 to 0.5, which could be seen after ACE inhibition.²⁸,²⁹ The hypotensive effect of ACE inhibitors was not necessarily associated only with reduction in plasma Ang II levels.³⁰ Increased Ang-(1–7) after ACE inhibition may, at least in part, contribute to effect of ACE inhibitors by attenuation of the effect of Ang II. Our results also suggest that Ang-(1–7) might work as an endogenous Ang II antagonist in the presence of high Ang II from the activated renin-angiotensin system, whereas Ang-(1–7) appears to be, as shown in protocol 1 and 2, weak Ang II in salt-repleted subjects with normal or inhibited renin-angiotensin system. Ang-(1–7), therefore, can be regarded as a counterregulatory peptide to Ang II in patients with high-renin activity, such as patients with heart failure and renovascular hypertension. Thus, the roles of Ang-(1–7) in these cardiovascular diseases should be investigated.

**Conclusion**

We conclude that Ang-(1–7) itself does not have a vasodilating effect, at least at the dose that we used, but attenuates vasoconstriction evoked only by Ang II in human forearm resistance vessels. Thus, Ang-(1–7) is supposed to act as an endogenous angiotensin receptor antagonist and might be physiologically relevant when the renin-angiotensin system is activated.

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