Effects of Angiotensin-(1-7) on Forearm Circulation in Normotensive Subjects and Patients With Essential Hypertension

Shota Sasaki, Yukihito Higashi, Keigo Nakagawa, Hideo Matsuura, Goro Kajiyama, Tetsuya Oshima

Abstract—Previous animal studies have shown that angiotensin (Ang)-(1-7) is a biologically active component of the renin-angiotensin system, acting as a vasoactive agent, and may play a role in the blood pressure regulation. There is little information, however, on the effect of Ang-(1-7) on human circulation or the mechanism of its action. To investigate the effect of Ang-(1-7) on forearm circulation and to determine whether this effect is altered in patients with essential hypertension, we measured change in forearm blood flow using venous occlusion plethysmography in response to intra-arterial infusion of Ang-(1-7) (10^-10, 10^-9, and 10^-8 mol/min; for 5 minutes) in normotensive control subjects (n=8) and patients with essential hypertension (n=8). Infusion of Ang-(1-7) significantly increased the forearm blood flow response in a dose-dependent manner in both normotensive control subjects (28.7±9.7%, at 10^-8 mol/min; P<0.05) and hypertensive patients (31.8±15.2%, at 10^-8 mol/min; P<0.05). The vasodilatory effect of Ang-(1-7) was similar in the two groups. Intra-arterial infusion of N\textsuperscript{G}-monomethyl-L-arginine, a nitric oxide synthesis inhibitor, did not alter the forearm blood flow response to Ang-(1-7) in either group. These findings suggest that Ang-(1-7) causes vasodilation in forearm circulation of normotensive subjects and patients with essential hypertension through a pathway that is independent of nitric oxide synthesis. (Hypertension. 2001;38:90-94.)

Key Words: angiotensin-(1-7) ■ forearm blood flow ■ nitric oxide ■ hypertension, essential

Recent studies have shown that angiotensin (Ang)-(1-7) is a vasoactive component of the renin-angiotensin system. Although it has been reported that Ang-(1-7) has vasodepressor, vasodilator, and antihypertensive actions and it counteracts the action of Ang II, the mechanism responsible for the effects of Ang-(1-7) is not fully understood. Several investigators have reported that Ang-(1-7) opposes the action of Ang II either directly or by stimulation of prostaglandin or NO. However, these conclusions were based mainly on animal studies, and it is not clear whether they apply in humans.

The important role of NO in endothelium-dependent vasodilation for regulation of cardiovascular homeostasis has been established. NO is responsible for an inhibition of smooth muscle cell proliferation and neointima formation, a reduction in platelet aggregation, regulation of urinary sodium excretion, and a decrease in the accumulation of intracellular calcium in vascular smooth muscle cells. Impaired endothelium-dependent vasodilation has been shown in the forearm, coronary, and renal vasculature of patients with essential hypertension and may be related to the development of atherosclerosis in these patients. Recently, many investigators have described the link between the renin-angiotensin system and NO. On the basis of this link, one could hypothesize that endothelial dysfunction results from alterations in an Ang-(1-7) mediator.

In this study, we compared the effects of Ang-(1-7) in patients with essential hypertension versus normotensive subjects to evaluate the role of Ang-(1-7) on human forearm circulation in relation to NO synthesis and to determine whether Ang-(1-7) is involved in the pathogenesis of essential hypertension.

Methods

Subjects

Eight normotensive Japanese control subjects (7 men and 1 woman; mean age, 49.7±11.2 years) and 8 patients with mild to moderate essential hypertension (6 men and 2 women; mean age, 54.6±9.6 years) were studied. Normotensive control subjects were recruited from healthy members of medical staff and people who were undergoing annual medical checkups. Normal blood pressure was defined as systolic blood pressure <140 mm Hg and diastolic blood pressure <80 mm Hg. The normotensive control subjects exhibited normal findings on physical and routine laboratory examinations.

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From the First Department of Internal Medicine (S.S., Y.H., K.N., H.M., G.K.) and the Department of Clinical Laboratory Medicine (T.O.), Hiroshima University School of Medicine, Japan.


Correspondence to Shota Sasaki, MD, Hiroshima University School of Medicine, First Department of Internal Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. E-mail sshota@mcai.med.hiroshima-u.ac.jp

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Hypertension was diagnosed as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg or both in the sitting position on at least 3 different occasions in the outpatient clinic of the Hiroshima University School of Medicine. Patients who had cardiovascular or cerebrovascular diseases, renal failure, diabetes mellitus, or hypercholesterolemia were excluded. Five patients were receiving no treatment, and 3 patients were taking anti-hypertensive drugs, including calcium antagonists or ACE inhibitors. Thus, anti-hypertensive agents were withdrawn 2 weeks before the study. The Ethics Committee of the First Department of Internal Medicine, Hiroshima University School of Medicine, approved the study protocol. Informed consent for participation was obtained from each subject.

Measurements of Forearm Blood Flow

Forearm blood flow (FBF) was measured with a mercury-filled Silastic strain-gauge plethysmograph (EC-5R, D.E. Hokanson Inc) as previously described. Briefly, a strain gauge was attached to the upper part of the left arm, connected to a plethysmography device, and supported above the level of the right atrium. A wrist cuff was inflated to a pressure of 50 mm Hg above the systolic blood pressure to exclude the hand circulation during the measurement of FBF. The upper arm-congesting cuff was inflated to 40 mm Hg for 7 seconds in each 15-second cycle to occlude venous outflow from the arm with a period cuff inflator (EC-20, D.E. Hokanson Inc). The FBF output signal was transmitted to a recorder (U-228, Advance Co).

FBF was expressed as milliliters per minute per 100 milliliters of forearm tissue volume.

In a preliminary study, we examined the effect of saline infusion as a control for Ang-(1-7) in 5 normotensive subjects (3 men and 2 women; mean age, 52.3±9.6 years) and 6 essential hypertensive patients (4 men and 2 women; mean age, 58.6±11.6 years). Forearm blood flow was not altered by saline infusion throughout the study. The average of 4 plethysmographic measurements was used for analysis of FBF at baseline and during administration of drugs. The intraobserver coefficient of variation was 3.0±1.8%.

Study Protocol

The study began at 8:30 AM. Subjects fasted over the previous night for at least 12 hours. They were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature 22°C to 25°C) throughout the study. A 23-gauge polyethylene catheter (Hakkow Co) was inserted into the left brachial artery for the infusion of Ang-(1-7) and Nω-monomethyl-L-arginine (L-NMMA) and for the recording of arterial pressure with an AP-641G pressure transducer (Nihon Kohden Co) under local anesthesia (1% lidocaine). A 20-gauge catheter was inserted into the left deep antecubital vein to obtain blood samples after Ang-(1-7) infusion at a maximal dose.

After the patient had been in the supine position for 30 minutes, we measured basal FBF and arterial blood pressure. Next, the effects of Ang-(1-7) on forearm hemodynamics were measured. Ang-(1-7) (10⁻⁸, 10⁻⁷, or 10⁻⁶ mol/min) was infused intra-arterially for 5 minutes at each dose by a constant-rate infusion pump (Terfusion STG-523, Termo Co). FBF was measured during the last 2 minutes of the infusion. In the preliminary study, after the infusion of a maximal dose of Ang-(1-7) (10⁻⁶ mol/min), FBF returned to baseline within 30 minutes. Thus, the end of the response to Ang-(1-7) was followed by a 30-minute recovery period. After a 30-minute rest period, L-NMMA, an inhibitor of NO synthesis, was infused intra-arterially at a dose of 8 μmol/min for 5 minutes while the basal FBF and arterial blood pressure were recorded. The FBF was measured during the last 2 minutes of the drug infusion. In a preliminary study, we examined the effects of 4 dosages of L-NMMA (1, 4, 8, and 16 μmol/min) on forearm hemodynamics (n=4). Although L-NMMA at doses of 1, 4, and 8 μmol/min significantly attenuated basal FBF (5.6±1.9%, 30.2±9.4%, and 47.1±12.0%, respectively) and acetylcholine-induced increases in FBF without altering arterial blood pressure, 16 μmol/min L-NMMA significantly elevated mean arterial blood pressure from 108.7±7.8 to 114.5±8.5 mm Hg (P<0.05). Therefore, we decided to use 8 μmol/min of L-NMMA in the final study.

Drugs

The following drugs were used: Ang-(1-7) and L-NMMA (both from Sigma Chemical Co). All drugs were dissolved in saline (0.9% NaCl; Ohtsuka Pharmaceutical Co) immediately before use.

Analytical Methods

Samples of venous blood were placed in polystyrene tubes containing EDTA-Na (1 mg/mL). The EDTA-containing tubes were chilled promptly in an ice bath. Plasma was immediately separated by centrifugation at 3100g at 4°C for 10 minutes; serum, at 1000g at room temperature for 10 minutes. Samples were stored at −80°C until assay. Serum concentrations of total cholesterol, triglycerides, HDL cholesterol, glucose, and electrolytes were determined by routine chemical methods. Plasma norepinephrine was measured by high-performance liquid chromatography. Serum levels of LDL were estimated by Friedewald’s method. Plasma renin activity (Gamma Coat PRA, SRL Co) and the concentration of Ang II were determined by radioimmunoassay. Nitrite/nitrate (NOx) levels were measured by a colorimetric assay based on the Griess reaction.

Statistical Methods

Values are expressed as mean±SD. The Mann-Whitney U test was used to evaluate differences between patients with essential hypertension and normotensive subjects with regard to baseline parameters. A 2-tailed Student’s paired r test was used to evaluate differences between parameters before and after Ang-(1-7) infusion. The response of FBF to Ang-(1-7) was examined by 2-way ANOVA for repeated measures followed by Scheffe’s F test. Results were considered significant at P<0.05.

Results

Baseline Clinical Characteristics of Normotensive Control Subjects and Hypertensive Patients

The clinical characteristics of normotensive control subjects and hypertensive patients are summarized in Table 1. Patients with essential hypertension had significantly higher systolic and diastolic blood pressures. Other parameters, however, including the concentrations of plasma insulin, NOx, plasma renin activity, Ang II, norepinephrine, and the lipid profiles, were similar in normotensive control subjects and patients with essential hypertension. Basal FBF was also similar in the two groups.

Clinical Characteristics Before and After Ang-(1-7) Infusion in Normotensive Control Subjects and Hypertensive Patients

The clinical characteristics before and after Ang-(1-7) infusion are summarized in Table 2. Ang-(1-7) did not change systemic hemodynamics, including blood pressure and heart rate. In addition, neither plasma renin activity nor the concentrations of plasma Ang II or norepinephrine were altered after Ang-(1-7) infusion in either group.

FBF to Ang-(1-7) Infusion in Normotensive Control Subjects and Hypertensive Patients

The FBF response to Ang-(1-7) is shown in Figure 1. Ang-(1-7) increased the FBF response in a dose-dependent manner in normotensive control subjects and hypertensive patients. The increase in FBF response was statistically significantly at doses ≥10⁻⁶ mol/min above basal concentra-
tions in both groups. There was no difference in the response of FBF to Ang-(1-7) between the two groups.

After intra-arterial infusion of L-NMMA, basal FBF was decreased in both groups (normotensive control subjects, 6.5 ± 1.0 to 4.6 ± 0.7 mL/100 mL per tissue; hypertensive patients, 5.4 ± 1.8 to 4.1 ± 1.3 mL/100 mL per tissue; P < 0.05, respectively). L-NMMA infusion did not alter the increase in FBF induced by Ang-(1-7) in either group (Figure 2). There was no significant correlation between the increased FBF response to Ang-(1-7) and parameters such as blood pressure, plasma insulin, lipid profile, renin activity, Ang II, or norepinephrine.

Discussion
Recent studies describe the diverse enzymatic pathways by which Ang-(1-7) is cleaved from Ang-1 by tissue-specific endopeptidases. It has been suggested that vascular endothelial cells also have the capacity to synthesize Ang-(1-7). In addition, endothelial cells have been reported to contain a unique non-Ang type 1, non-Ang type 2 angiotensin receptor that preferentially binds Ang-(1-7). Thus, it is possible that Ang-(1-7) has vasoactive action through a novel receptor. Although the vasodilator response evoked by Ang-(1-7) has been demonstrated in coronary arteries, mesenteric arteries, and piglet pial vessels in animal studies, the underlying mechanisms for its action are unclear.

Porst et al reported that Ang-(1-7) induced a concentration-dependent dilator response that was markedly attenuated by an NO synthase inhibitor or a bradykinin type 2 receptor antagonist in porcine coronary arteries. Brosnihan et al also described that pretreatment of canine coronaries with an NO synthase inhibitor abolishes the vascular relaxation evoked by Ang-(1-7). These findings suggest that the vasodilatory effect of Ang-(1-7) may be dependent in part on the release of NO and that bradykinin may alter the effect of this peptide. Therefore, this study tested the hypothesis that the elevation of plasma Ang-(1-7) would increase human FBF and determined whether the physiological action of Ang-(1-7) is dependent on the release of NO. Our data document that Ang-(1-7) causes a dose-dependent relaxation of human forearm arteries. This effect of Ang-(1-7) was not altered in the presence of an NO synthase inhibitor. The discrepancy between our findings and those of other authors may be because of the different species or regional vascular beds. In addition, it is possible that the increase in FBF response to Ang-(1-7) was enhanced when expressed as a percent change because the basal blood flow was reduced by the L-NMMA. Therefore, we could not exclude the role of NO completely. It has been suggested that prostaglandins may be involved in Ang-(1-7)–induced vasodilation. Benter et al reported that Ang-(1-7) caused a depressor effect when injected into the circulation of the rat, and this action is blocked completely by indomethacin. Although our study did

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensives</th>
<th>Hypertensives</th>
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<tbody>
<tr>
<td>Blood pressure, mm Hg</td>
<td>125.1 ± 4.7</td>
<td>167.1 ± 12.3</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>68.3 ± 7.1</td>
<td>92.5 ± 10.7</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>65.0 ± 8.2</td>
<td>68.9 ± 9.2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>76.8 ± 9.8</td>
<td>104.5 ± 12.5</td>
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<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.88 ± 0.9</td>
<td>5.13 ± 0.6</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>1.37 ± 0.5</td>
<td>1.21 ± 0.4</td>
</tr>
<tr>
<td>Plasma renin activity, ng/mL per hour</td>
<td>2.87 ± 0.6</td>
<td>3.26 ± 0.5</td>
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<tr>
<td>Plasma Ang II, pg/mL</td>
<td>9.4 ± 2.8</td>
<td>15.5 ± 10.7</td>
</tr>
<tr>
<td>Plasma norepinephrine, pmol/L</td>
<td>234.4 ± 89.9</td>
<td>247.5 ± 142.6</td>
</tr>
<tr>
<td>Plasma NOx, mol/L</td>
<td>33.1 ± 13.9</td>
<td>53 ± 19.5</td>
</tr>
<tr>
<td>FBF, mL/min per dL forearm</td>
<td>6.8 ± 1.6</td>
<td>5.6 ± 1.2</td>
</tr>
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Results are presented as mean ± SD.

*P < 0.05 vs normotensive control subjects.

Figure 1. Effects of Ang-(1-7) on FBF in normotensive subjects and hypertensive patients. Ang-(1-7) increased FBF in dose-dependent manner in normotensive and hypertensive subjects. Response of FBF to Ang-(1-7) infusion was similar in groups. Results are presented as mean ± SD. Probability value refers to comparison of time course curves by ANOVA for repeated measurements.
Ang-(1-7). A second, more recent publication21 did not see a
trend in their subjects might have obscured the real response to
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with ACE inhibitors; they reported that Ang-(1-7) did not
not address this possibility, these findings might explain the
unchanged FBF response to Ang-(1-7) after L-NMMA
infusion.

Two reports have described the effect of Ang-(1-7) on
human FBF. Davie and McMurray20 examined the actions of
this peptide in patients with heart failure who were treated
with ACE inhibitors; they reported that Ang-(1-7) did not
alter blood flow. It is possible that the particular conditions in
their subjects might have obscured the real response to
Ang-(1-7). A second, more recent publication21 did not see a
direct vasodilative effect of Ang-(1-7) but reported
the antagonist effect of this peptide to Ang II. In their study,
subjects were young volunteer groups, and the dose of
Ang-(1-7) was slightly higher than in our study. Therefore,
different experimental conditions may affect the
discrepancy.

Ang II has been shown to be the main biologically active
product that contributes significantly to the pathogenesis of
gastrointestinal hypertension through its vasoconstrictive effects.
Recently, it has been reported that subtype-selective Ang type
1 or type 2 receptor antagonists do not inhibit its activation,
but a nonselective Ang II receptor antagonist attenuates
Ang-(1-7)–induced vasodilation.18 Although the cloning of
the Ang-(1-7) receptor has not yet been performed, there is a
possibility for the existence of a novel receptor for this
hepatopeptide.

It is well known that Ang II and norepinephrine, an index
of sympathetic nervous activity, are vasoconstrictors. In the present study, Ang-(1-7) infusion did not alter the concentra-
tions of plasma Ang II or norepinephrine in normotensive
subjects or hypertensive patients. The assessments of local
changes in Ang II and norepinephrine would allow us more
specific conclusions concerning the local effects of Ang-(1-7)
on these vasoconstrictors.

Vasodepressor effects of Ang-(1-7) have been demon-
strated in genetically hypertensive rats4 and dogs with reno-
vascular hypertension.22 Benter et al23 reported that the intravenous infusion of Ang-(1-7) decreased systolic blood
pressure in spontaneously hypertensive rats, although the
effect was transient (over several days). Iyer et al24 also
reported that the infusion of a specific Ang-(1-7) antibody
caused acute elevations in arterial pressure in lisinopril- and
losartan-treated rats. They considered that the increase in
plasma Ang-(1-7) concentration may be responsible for the
remarkable depressor effects. Thus, it is likely that Ang-(1-7)
has a role in blood pressure regulation. In the present study,
we examined whether the vasodilative effect of Ang-(1-7)
was decreased in patients with essential hypertension to test
the hypothesis that Ang-(1-7) may be involved in the patho-
genesis of essential hypertension. However, exogenous Ang-
(1-7) evoked similar vasodilating effects in both normoten-
sive subjects and hypertensive patients.

In conclusion, this is the first study to compare the effect of
Ang-(1-7) on human forearm hemodynamics in patients with
essential hypertension versus normotensive subjects. Admin-
istration of L-NMMA did not alter the FBF response to
Ang-(1-7) in normotensive or hypertensive subjects. These
findings suggest that Ang-(1-7) stimulates vasodilation inde-
dependent of the release of NO. Exogenous administration of
Ang-(1-7) may increase blood flow and decrease blood
pressure in patients with essential hypertension. Supplemen-
tation of Ang-(1-7) or an increase in endogenous Ang-(1-7)
may be expected to be beneficial in essential hypertension.

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