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^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 vasodilator, vasopressor, and antihypertensive actions and it counteracts the action of Ang II,1–4 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 prostaglandin5 or NO.6 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 aggregability, regulation of urinary sodium excretion, and a decrease in the accumulation of intracellular calcium in vascular smooth muscle cells.7–11 Impaired endothelium-dependent vasodilation has been shown in the forearm,12 coronary,13 and renal vasculature14 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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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 antihypertensive drugs, including calcium antagonists or ACE inhibitors. Thus, antihypertensive 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.

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 N\textsuperscript{3}-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\textsuperscript{-10}, 10\textsuperscript{-9}, or 10\textsuperscript{-8} 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\textsuperscript{-8} 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 \mu mol/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 \mu mol/min) on forearm hemodynamics (n=4). Although L-NMMA at doses of 1, 4, and 8 \mu mol/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 \mu mol/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 \mu mol/min of L-NMMA in the final study.

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, NO\x3csub>x\x3c/sup>, 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 significant at doses ≥10\textsuperscript{-9} mol/min above basal concentra-
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 not address this issue, our data are consistent with these observations.

Sample Table

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensives</th>
<th>Hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>125.1±4.7</td>
<td>167.1±12.3</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>68.3±7.1</td>
<td>92.5±10.7</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>65.0±8.2</td>
<td>68.8±9.2</td>
</tr>
<tr>
<td>Plasma insulin, mmol/L</td>
<td>76.8±9.8</td>
<td>104.5±12.5</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.88±0.9</td>
<td>5.13±0.6</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.37±0.5</td>
<td>1.21±0.4</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.87±0.6</td>
<td>3.26±0.5</td>
</tr>
<tr>
<td>Plasma renin activity, ng/mL per hour</td>
<td>2.6±1.7</td>
<td>3.0±2.6</td>
</tr>
<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, μmol/L</td>
<td>33.1±13.9</td>
<td>53.4±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>
</tbody>
</table>

Results are presented as mean±SD. *P<0.05 vs normotensive control subjects.
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 McMurray 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 publication 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, the 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 arterial 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. 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 angiotensin.

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 concentrations 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 demonstrated in genetically hypertensive rats and dogs with renovascular hypertension. Benter et al 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 al 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 pathogenesis of essential hypertension. However, exogenous Ang-(1-7) evoked similar vasodilating effects in both normotensive 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. Administration 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 independent of the release of NO. Exogenous administration of Ang-(1-7) may increase blood flow and decrease blood pressure in patients with essential hypertension. Supplementation 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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