Effects of L-Arginine on Forearm Vessels and Responses to Acetylcholine

Tsutomu Imaizumi, Yoshitaka Hirooka, Hiroyuki Masaki, Seiki Harada, Michiko Momohara, Tatsuya Tagawa, and Akira Takeshita

This study was designed to investigate the effects of L-arginine (the substrate of endothelium-derived nitric oxide) in human forearm vessels. We examined whether intra-arterial infusion of L-arginine dilated forearm vessels and augmented vasodilatory responses to acetylcholine in young, healthy humans. The left brachial artery was cannulated for drug infusions and direct measurement of arterial pressure. Forearm blood flow was measured by a strain gauge plethysmograph. Intra-arterial infusions of L-arginine at 10, 20, 40, and 60 mg/min increased forearm blood flow from 4.7±0.6 to 4.9±0.5, 5.7±0.5, 7.2±0.8, and 8.2±0.9 ml·min⁻¹·100 ml⁻¹, respectively (n=8, p<0.01), whereas D-arginine at the same doses did not alter forearm blood flow (n=7). Intra-arterial infusions of acetylcholine (n=7) (4, 8, 16, and 24 μg/min) and sodium nitroprusside (n=5) (0.2, 0.4, 0.8, and 1.2 μg/ml) increased forearm blood flow dose dependently (p<0.01 for both). Arterial pressure was not altered with infusions of these drugs. Responses to acetylcholine were augmented with simultaneous intra-arterial infusion of L-arginine at 10 mg/ml (p<0.01) but not with D-arginine. Responses to sodium nitroprusside were not altered by L-arginine. These results in human forearm resistance vessels support the notion that vasodilation induced by acetylcholine is a result of the conversion from L-arginine to endothelium-derived nitric oxide. Our results suggest that intra-arterial infusion of L-arginine may facilitate production of nitric oxide in the forearm and that L-arginine contributes to the modulation of vascular smooth muscle tone in healthy humans. (Hypertension 1992;20:511-517)

KEY WORDS • endothelium • endothelium-derived relaxing factor • human experimentation • vascular resistance • nitroprusside • plethysmography • arginine • acetylcholine • forearm

Nitric oxide is produced from the conversion of the semiessential amino acid L-arginine into L-citrulline by nitric oxide synthase in endothelial cells.1-4 The biosynthesis of nitric oxide by the endothelium accounts for the vasorelaxation, and nitric oxide is now considered one of the endothelium-derived relaxing factors (EDRF).5-7 Nitric oxide is a potent and direct activator of soluble guanylate cyclase and increases the production of cyclic guanosine 3',5'-monophosphate (cGMP).5-7 It is thought that acetylcholine dilates vessels by releasing nitric oxide from the endothelium.8,9

In in vitro experiments with normal vessels, it has been shown that L-arginine does not dilate vessels or augment acetylcholine-induced vasorelaxation.2-10-15 It also has been shown that intravenous infusion of L-arginine does not augment acetylcholine-induced vasorelaxation in the hindquarter resistance vessels of normal rabbits.16 In humans, it is not known whether L-arginine augments acetylcholine-induced vasodilation and whether L-arginine itself dilates blood vessels. In the present study, by measuring forearm blood flow with a plethysmograph, we examined whether intra-arterial infusion of L-arginine dilated forearm blood vessels and whether L-arginine augmented acetylcholine-induced vasodilation in the forearm of healthy humans.

Methods

General Procedures

Subjects were all young, healthy male students (age, 20-23 years) at our university who volunteered for the study. The protocol was explained, and written informed consent was obtained from each subject. The study was done with subjects in a supine position and in a postabsorptive state in an air-conditioned room with room temperature at 25-26°C. Under local anesthesia with 2% procaine, the left brachial artery was cannulated with a 20-gauge intravenous over-the-needle poly(tetrafluoroethylene) catheter (Quick-Cath, Travenol Laboratories, Inc., Baxter Healthcare Corp., Deerfield, Ill.) for drug infusion, and the catheter was connected by a three-way stopcock to a pressure transducer (Viggo-Spectramed, Oxnard, Calif.) for direct measurement of arterial pressure. The arterial line was kept open by infusion of heparinized saline (0.1 ml/min) while no drug was being infused. In some subjects, a vein in the antecubital region of the ipsilateral, contralateral, or both, arm was cannulated with the same...
cannula as used for the artery to obtain blood samples for measuring plasma L-arginine and cholesterol levels. Heart rate was obtained by counting the pulse rate for a few minutes on arterial pressure recordings.

**Measurements of Forearm Blood Flow**

Forearm blood flow was measured by using a mercury-in-Silastic strain gauge plethysmograph with a venous occlusion technique. The strain gauge was placed approximately 5 cm below the antecubital crease. Forearm blood flow (milliliters per minute per 100 milliliters forearm blood flow) was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating the cuff on the upper arm. The pressure in the venous occlusion or constricting cuff on the upper arm was 40 mm Hg. Circulation to the hand was arrested by a cuff inflated around the wrist. The wrist cuff was inflated before the determination of forearm blood flow and continuously throughout the measurements. Forearm vascular resistance was calculated by dividing the mean arterial pressure (diastolic pressure plus one third of the pulse pressure in millimeters of mercury) by the forearm vascular resistance was calculated by dividing the mean arterial pressure (diastolic pressure plus one third of the pulse pressure in millimeters of mercury) by the forearm vascular resistance was calculated by dividing the mean arterial pressure (diastolic pressure plus one third of the pulse pressure in millimeters of mercury) by the forearm vascular resistance.

**Measurements of Plasma L-Arginine and Cholesterol Levels**

In four subjects, 5 ml blood was drawn from the antecubital vein of the ipsilateral forearm for measurement of plasma L-arginine and cholesterol levels. Blood was centrifuged immediately, stored in a freezer, and measured at a commercial laboratory (SRL, Tokyo).

**Preparation of Drugs**

Because acetylcholine is unstable in solution, 100 mg acetylcholine (Daichi Pharmaceutical, Tokyo) was lyophilized and stored in a vial (0.4 mg acetylcholine per vial). It was dissolved in physiological saline (10 ml) immediately before use. Sodium nitroprusside (Wakou Junyaku Kogyo, Osaka, Japan) was dissolved in physiological saline at a concentration of 2,000 ng/ml. Special care was taken not to expose sodium nitroprusside to light. For the infusion of L-arginine, commercially available L-arginine solution (0.1 g L-arginine per milliliter, and direct effects and effects on the acetylcholine-induced vasodilation were examined. In this group, effects of intra-arterially infused L-arginine at 20 and 60 mg/min for 5 minutes each on the contralateral forearm blood flow were examined for the last protocol. The volumes of infusion were 0.1, 0.2, 0.4, and 0.6 ml/min for infusion of acetylcholine, sodium nitroprusside, or L- or D-arginine at four different doses. We had previously confirmed that the difference in the volume of infusion by itself did not alter forearm blood flow. Saline at 0.1 ml/min was infused as a control for infusion of L-arginine or D-arginine at 10 mg/min. The last 1-minute measurements of forearm blood flow during infusion of each dose of drugs were used for later analysis.

**Forearm Vascular Responses to Drugs**

After the placement of cannulas and a strain gauge plethysmograph, at least 15 minutes was allowed for subjects to become accustomed to the study conditions before the experiments were begun.

We examined vasodilator responses to intra-arterial infusion of L-arginine (n=8), acetylcholine (n=7), and sodium nitroprusside (n=5) at graded doses. Similarly, we examined effects of intra-arterial infusion of L-arginine (10 mg/min) on forearm responses to simultaneous infusions of acetylcholine (n=7) or sodium nitroprusside (n=5) at graded doses. First, we examined forearm vasodilator responses to intra-arterial infusions of acetylcholine (4, 8, 16, and 24 μg/min) or sodium nitroprusside (0.2, 0.4, 0.8, and 1.2 μg/min) for 2 minutes at each dose. Forearm blood flow reached the steady state by 1 minute after starting infusion of acetylcholine and sodium nitroprusside. After 10 minutes, when forearm blood flow had returned to the baseline values, L-arginine (10, 20, 40, and 60 mg/min) was infused intra-arterially for 5 minutes at each dose, and forearm blood flow was obtained for the last 2 minutes at each dose. After 15 minutes, when blood flow had returned to the baseline values, L-arginine was infused at 10 mg/min for 5 minutes, and then acetylcholine or sodium nitroprusside was infused in the same way as before L-arginine while L-arginine (10 mg/min) was infused continuously and simultaneously. A total of 11 subjects received either single or combined infusion of drugs as follows. In eight subjects, infusions of graded doses of L-arginine were given; in seven subjects, infusions of graded doses of acetylcholine before and during L-arginine (10 mg/min) were given; and in five subjects, infusions of graded doses of sodium nitroprusside before and during L-arginine (10 mg/min) were given. In another group of subjects (n=7), D-arginine (isomer of L-arginine) at the same doses as L-arginine was infused intra-arterially.
Morishita Pharmaceutical, Osaka) was used. d-Arginine was obtained from Sigma Chemical Co., St. Louis, Mo.

Statistical Analysis

The hemodynamic values during infusions of acetylcholine, sodium nitroprusside, d-arginine, and L-arginine at each dose were compared by one-way analysis of variance (ANOVA) for repeated measures to test treatment effects. The hemodynamic responses to acetylcholine or sodium nitroprusside before and during the infusion of L-arginine at 10 mg/min were compared by two-way ANOVA. Similarly, the hemodynamic responses to acetylcholine before and during infusion of d-arginine at 10 mg/min were compared by two-way ANOVA. All values are expressed as mean±SEM, and a value of p<0.05 was considered to be statistically significant.

Results

Responses to Intra-arterial Infusion of Arginine

Direct intra-arterial infusion of L-arginine (n=8) at doses of 10, 20, 40, and 60 mg/min increased ipsilateral forearm blood flow (p<0.01) and decreased forearm vascular resistance (p<0.05) dose dependently without changes in blood pressure. Results are shown in Figure 1 and Table 1. In contrast to L-arginine, d-arginine did not alter forearm blood flow or forearm vascular resistance (Figure 1 and Table 2). L-Arginine at 20 and 60 mg/min did not alter contralateral forearm blood flow or forearm vascular resistance. Values are mean±SEM. n=8.

Plasma Cholesterol and L-Arginine Levels

The plasma cholesterol level was 141±9 mg/dl (n=4). Infusions of L-arginine at 20 and 60 mg/min increased the plasma L-arginine level to 265±105 µg/l (n=2) and 1,169±298 µg/l (n=4) in the ipsilateral arm, respectively, from 17±2 µg/l at control (n=4). The systemic concentration of L-arginine at 60 mg/min was 35±3 µg/l (n=5).

Discussion

The major findings of this study were that intra-arterial infusion of L-arginine augmented vasodilator responses to acetylcholine but not to sodium nitroprusside in young, healthy subjects and that intra-arterial infusion of L-arginine dilated forearm blood vessels. Intra-arterial infusion of d-arginine (isomer of L-arginine) did not dilate forearm vessels or augment vasodilator responses to acetylcholine. Our results support the notion that acetylcholine dilates blood vessels by converting L-arginine to nitric oxide in human forearm vessels. Our results suggest that infusion of L-arginine may facilitate production of nitric oxide and that L-arginine may contribute to the modulation of vascular tone in the forearm of healthy humans.

Acetylcholine-Induced Vasodilation

Acetylcholine is a vasodilator substance released from cholinergic nerves. Until recently, it was as-

<table>
<thead>
<tr>
<th>TABLE 1. Responses to Intra-arterial Infusion of L-Arginine</th>
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<tbody>
<tr>
<td>Forearm hemodynamics</td>
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<tr>
<td>MBP (mm Hg)</td>
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<tr>
<td>FBF (ml min⁻¹ 100 ml⁻¹)</td>
</tr>
<tr>
<td>FVR (units)</td>
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</tbody>
</table>

ANOVA, analysis of variance; MBP, mean blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance. Values are mean±SEM. n=8.

<table>
<thead>
<tr>
<th>TABLE 2. Responses to Intra-arterial Infusion of D-Arginine</th>
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<tbody>
<tr>
<td>Forearm hemodynamics</td>
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<tr>
<td>MBP (mm Hg)</td>
</tr>
<tr>
<td>FBF (ml min⁻¹ 100 ml⁻¹)</td>
</tr>
<tr>
<td>FVR (units)</td>
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</tbody>
</table>

ANOVA, analysis of variance; MBP, mean blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance. Values are mean±SEM. n=7.
TABLE 3. Responses to Intra-arterial Infusion of Acetylcholine Before and During Simultaneous Intra-arterial Infusion of L-Arginine

<table>
<thead>
<tr>
<th>Forearm hemodynamics</th>
<th>Control</th>
<th>ACh (μg/min)</th>
<th></th>
<th></th>
<th>ANOVA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arginine</td>
<td>82±1</td>
<td>79±2</td>
<td>79±2</td>
<td>78±2</td>
<td>77±2</td>
</tr>
<tr>
<td>FBF (ml • min⁻¹ • 100 ml⁻¹)</td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>5.4±0.4</td>
<td>170±22</td>
<td>196±12</td>
<td>274±24</td>
<td>328±31</td>
</tr>
<tr>
<td>FVR (units)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arginine</td>
<td>15.7±1.1</td>
<td>5.3±0.8</td>
<td>4.2±0.2</td>
<td>3.1±0.3</td>
<td>2.7±0.3</td>
</tr>
</tbody>
</table>

ACh, acetylcholine; ANOVA, analysis of variance; MBP, mean blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance. Values are mean±SEM. n=7.

Assumed that acetylcholine causes vasodilation via prejunctional inhibition of adrenergic neurotransmission. Acetylcholine also releases prostacyclin from the blood vessels. Since Furchgott and Zawadzki first demonstrated that endothelial cells release an EDRF after stimulation with acetylcholine, it has been shown in many in vitro experiments that acetylcholine releases EDRF from the endothelium; EDRF is now considered to be nitric oxide, and it relaxes vascular smooth muscles. Thus, three possible mechanisms could mediate the vasodilator actions of acetylcholine in vivo: an inhibitory effect of norepinephrine from sympathetic nerve endings, stimulation of the production of prostacyclin from the blood vessels, and release of EDRF from the endothelium. In in vitro experiments using aortic strips of animals, it has been shown that the vasodilation by acetylcholine is endothelium dependent, because the vasodilation by acetylcholine was abolished after endothelium denudation. Furthermore, it has been shown that acetylcholine releases nitric oxide derived from the metabolites of L-arginine. However, the mechanisms of acetylcholine-induced vasodilation in human resistance vessels are not well known. Linder et al examined the mechanisms of the vasodilator action of acetylcholine in human forearms. They demonstrated that the inhibitory effect on the norepinephrine release from the nerve endings was not likely, because the vasodilator action of acetylcholine was similar before and after phentolamine. The contribution of vascular prostacyclin to the vasodilator actions of acetylcholine was excluded, because intravenous infusion of acetylsalicylic acid did not affect the vasodilator actions of acetylcholine. From the above indirect evidence, they argued that acetylcholine releases EDRF and dilates resistance vessels of the human forearm in vivo. In contrast to acetylcholine, sodium nitroprusside is a spontaneous releaser of nitric oxide and does not involve the enzymatic conversion from L-arginine to nitric oxide. The finding in the present study that intra-arterial infusion of L-arginine increased vasodilator responses of the forearm blood vessels to acetylcholine but not to sodium nitroprusside strongly supports the above notion that acetylcholine dilates forearm resistance vessels by releasing nitric oxide converted from L-arginine. This mechanism was also suggested by the finding that the forearm vasodilation by acetylcholine was inhibited by NG-nitro-L-arginine (L-NMMA), a specific inhibitor of the synthesis of nitric oxide.

Direct Vasodilation and Augmented Responses to Acetylcholine by L-Arginine

It is well established that L-arginine is a substrate of nitric oxide. In normal vessels, there appears to be sufficient endogenous L-arginine to saturate the nitric oxide-forming enzyme, because the addition of L-arginine did not cause vasorelaxation or enhance acetylcholine-induced vasorelaxation in in vitro preparations of large vessels and in vivo resistance vessels. In the present study, however, intra-arterial infusions of...
The vasodilating effects and effects on acetylcholine-induced vasodilation might have been a result of nonspecific effects of L-arginine. Recently, Calver et al. infused L- and D-arginine at doses of 2.1, 8.4, and 33.6 mg/min and demonstrated that the highest doses of L- and D-arginine caused forearm vasodilation in healthy humans. They suggested that the actions of arginine supplementation are not caused by activation of an L-arginine/nitric oxide pathway through the provision of excess substrate. The findings in the present study are different from those reported by Calver et al. In our study, L-arginine not only dilated forearm blood vessels but also augmented vasodilator responses to acetylcholine, and there were no such effects for D-arginine. Thus, we believe that effects of L-arginine on forearm vessels are not nonspecific but rather are related to activation of the pathway from L-arginine to nitric oxide.

Although we infused L-arginine intra-arterially, systemic effects of L-arginine should be considered. Systemic effects of L-arginine were unlikely, however, because neither contralateral forearm blood flow nor blood pressure was altered during intra-arterial infusion of L-arginine at 20 and 60 mg/min. Furthermore, the systemic level of L-arginine was 35 ± 3 µg/l at 60 mg/min of infusion, which was almost in the normal range. Although we did not measure contralateral forearm blood flow or systemic concentration of L-arginine dur-

### Table 4. Responses to Intra-arterial Infusion of Acetylcholine Before and During Simultaneous Intra-arterial Infusion of D-Arginine

<table>
<thead>
<tr>
<th>Forearm hemodynamics</th>
<th>Control</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>24</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP (mm Hg)</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Saline</td>
<td>91±2</td>
<td>89±2</td>
<td>91±3</td>
<td>91±3</td>
<td>90±2</td>
<td>NS</td>
</tr>
<tr>
<td>D-Arginine</td>
<td>94±3</td>
<td>91±2</td>
<td>93±2</td>
<td>92±2</td>
<td>91±2</td>
<td></td>
</tr>
<tr>
<td>FBF (ml·min⁻¹·100 ml⁻¹)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>4.9±1.1</td>
<td>13.6±3.5</td>
<td>12.6±2.6</td>
<td>22.5±7.0</td>
<td>32.6±6.4</td>
<td>NS</td>
</tr>
<tr>
<td>D-Arginine</td>
<td>4.6±1.0</td>
<td>10.7±3.1</td>
<td>11.1±2.7</td>
<td>18.2±4.3</td>
<td>29.2±6.9</td>
<td></td>
</tr>
<tr>
<td>FVR (units)</td>
<td></td>
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<tr>
<td>Saline</td>
<td>23.8±4.8</td>
<td>12.2±4.6</td>
<td>10.8±3.7</td>
<td>7.1±2.2</td>
<td>3.8±1.1</td>
<td>NS</td>
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<tr>
<td>D-Arginine</td>
<td>24.7±4.0</td>
<td>13.9±4.6</td>
<td>12.2±3.4</td>
<td>8.9±3.4</td>
<td>4.5±1.1</td>
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</tbody>
</table>

ACh, acetycholine; ANOVA, analysis of variance; MBP, mean blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance. Values are mean±SEM. n=7.

L-arginine dilated forearm blood vessels dose dependently and augmented vasodilating responses to acetylcholine. Therefore, our findings are different from those reported previously in vitro and in vivo experiments in animals.¹¹⁻¹³,¹⁶ There are several possibilities to account for the differences. Recently, Gold et al. showed that L-arginine caused endothelium-dependent relaxation and cGMP formation in L-arginine-depleted rings of the bovine pulmonary artery. Similar results were reported by Schini and Vanhoutte, who showed that L-arginine evoked concentration- and time-dependent relaxations in rat aortic rings incubated for a long time. Therefore, it is possible that L-arginine was depleted in the forearm vessels of our subjects. However, this possibility is unlikely. The baseline plasma arginine level in our subjects was 17±3 µg/l (normal range, 8.3–29.1 µg/l); thus, L-arginine was not depleted in our subjects. We also measured other essential amino acids, which were all within normal ranges.

Recently, it has been shown that impaired acetylcholine-induced relaxation was normalized in the hypercholesterolemic rabbit by exposure to L-arginine.¹⁴⁻¹⁶ Thus, if our subjects had had hypercholesterolemia, they might have had improved responses to L-arginine. This possibility was unlikely, however, because our subjects were young and healthy and had normal cholesterol levels.
beneficial effects on the endothelium even in healthy humans. It is possible that sufficient doses of L-arginine may have beneficial effects on the endothelium even in healthy humans.

In summary, we demonstrated that direct intra-arterial infusion of L-arginine dilated forearm vessels and augmented acetylcholine-induced vasodilation in young, healthy humans. Our results may suggest that the conversion from L-arginine to nitric oxide is not maximal at rest and that infusion of L-arginine facilitates production of nitric oxide in forearm vessels of healthy humans.

Acknowledgment

We greatly appreciate Dr. Makoto Kawashige for preparing the drugs used in this study.

References


FIGURE 3. Line graph shows sodium nitroprusside (SNP) caused dose-dependent increases in forearm blood flow (FBF) during simultaneous infusions of saline (○) and L-arginine (●) (p<0.01). Increases in FBF to SNP were similar during infusion of saline and infusion of L-arginine (NS) (n=5).
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