Angiotensin-Converting Enzyme Inhibition, But Not Calcium Antagonism, Improves a Response of the Renal Vasculature to L-Arginine in Patients With Essential Hypertension

Yukihito Higashi, Tetsuya Oshima, Shota Sasaki, Yukiko Nakano, Masayuki Kambe, Hideo Matsuura, Goro Kajiyama

Abstract—Endothelial function has been shown to be impaired in patients with essential hypertension. The purpose of the present study was to determine whether antihypertensive drug therapy improves impaired endothelium-dependent renal vasorelaxation in essential hypertensive patients without atherosclerosis. We evaluated the effects of intravenous infusion of L-arginine (500 mg/kg given over 30 minutes) on systemic and renal hemodynamics in 27 patients with mild to moderate essential hypertension who were randomly assigned to treatment with either the angiotensin-converting enzyme inhibitor imidapril or the calcium antagonist amlodipine for 12 weeks in a double-blind fashion. After the 12 weeks, the decrease in blood pressure was similar in the imidapril (n=14) and amlodipine (n=13) groups. The increase in renal plasma flow was also similar in both groups. L-Arginine–induced renovascular relaxation was increased by imidapril (renal plasma flow, 9.6±5.1% to 14.4±7.4%; renal vascular resistance, −10.4±8.1% to −16.7±9.2%, P<0.05, respectively) but not by amlodipine. Urinary excretion of nitrite/nitrate in response to L-arginine was significantly increased by imidapril (90±29% to 134±63%, P<0.05) but remained unchanged by amlodipine. These findings suggest that angiotensin-converting enzyme inhibition improves the impaired endothelium-dependent renovascular relaxation in patients with essential hypertension due to the increase in nitric oxide production and that the reduction in blood pressure with a calcium antagonist does not play a major role in the potentiation of L-arginine/nitric oxide–mediated effects. (Hypertension. 1998;32:16-24.)

Key Words: angiotensin-converting enzyme inhibitor ■ calcium antagonist ■ nitric oxide ■ endothelium ■ kidney ■ hypertension, essential

Nitric oxide plays an important role in the regulation of systemic and renal hemodynamics.1-3 In hypertensive patients, endothelium-dependent vascular relaxation in coronary4 and forearm arteries5-7 was impaired, and endothelial dysfunction being involved in the development of atherosclerosis increases the risk of cardiovascular and cerebrovascular diseases. We also demonstrated that endothelium-dependent renovascular relaxation was impaired in essential hypertensive patients compared with normotensive subjects.8-10

It is important to determine whether reduction in blood pressure improves endothelial dysfunction in hypertensive subjects. It is unclear whether endothelial dysfunction is a cause or consequence of hypertension. Many investigators have reported an improvement of endothelial dysfunction in forearm circulation of patients with essential hypertension by antihypertensive therapy with ACE inhibitors.11-13 Several lines of evidence in experimental hypertensive models support these findings.14-17 In contrast, others have shown that clinically effective antihypertensive therapy, including ACE inhibitors, did not restore impaired endothelium-dependent vascular relaxation in the forearm.18,19 In addition, although the relationship between the kidney and NO in the development and maintenance of hypertension has been demonstrated,20,21 there is little information regarding the effects of antihypertensive drugs on renal endothelial function in essential hypertensives.

We conducted a 12-week randomized, double-blind, parallel trial to evaluate the effects on renal endothelial function of the ACE inhibitor imidapril compared with the calcium antagonist amlodipine in patients with mild to moderate essential hypertension without atherosclerosis. For this purpose, we measured RVR and the concentration of NOx in response to L-arginine at the beginning and at the end of the 12-week treatment period in the 2 groups.
Methods

Subjects

We recruited 29 Japanese patients with mild to moderate essential hypertension, 27 (16 men and 11 women; mean age, 56±15 years) of whom completed the study. Hypertension was defined as systolic blood pressure of >160 mm Hg and/or diastolic blood pressure of >95 mm Hg, with the subject in a sitting position, on at least 3 different occasions. Measurements were obtained in the outpatient clinic of Hiroshima University School of Medicine. Patients with secondary forms of hypertension were excluded on the basis of complete history; physical examination; radiological and ultrasound examinations; urinalysis; PRA; plasma aldosterone and norepinephrine concentrations; serum creatinine, potassium, calcium, and free thyroxine concentrations; and the 24-hour urinary excretion of 17-hydroxy cortisol, 17-ketogenic steroids, and vanillylmandelic acid. No patients had a history of cardiovascular or cerebrovascular disease, diabetes mellitus, hypercholesterolemia, liver disease, or renal disease. The study protocol was approved by the ethics committee of the First Department of Internal Medicine of Hiroshima University. Informed consent for participation was obtained from all subjects.

Treatment Protocol

No patient had a history of antihypertensive treatment before the study. Patients were randomly assigned to treatment either with ACE inhibitor (imidapril) or calcium antagonist (amlodipine) in a double-blind fashion. A 4-week run-in period with a placebo was followed by a 12-week treatment period. During the initial 4 weeks of the active treatment period, patients were treated with single daily doses of imidapril (5 mg) or amlodipine (5 mg) in the morning. When diastolic blood pressure was found to be >90 mm Hg or a decrease of <15 mm Hg was seen at the end of the first 4 weeks, dosages were increased to 10 mg for imidapril and 10 mg for amlodipine during the following 4-week period. If blood pressure was not controlled with this regimen, the daily dosages were increased to 15 mg for imidapril and 15 mg for amlodipine during the last 4 weeks, at which time patients received the same daily dose of each drug.

24-Hour Ambulatory Blood Pressure Monitoring

To confirm the basal blood pressure profile and the effects of antihypertensives, 24-hour ambulatory blood pressure monitoring was also performed at the beginning and end of the treatment period using a TM2420 (AND Co) device, a noninvasive ambulatory blood pressure monitor that is attached to the upper left arm. Blood pressure was measured using the Korotkoff microphone method during stepwise deflations (3.0±1.0 mm Hg per step) of the cuff. The within-run precision of blood pressure and heart rate measurements were ±4.0 mm Hg and ±5.0%, respectively. Blood pressure and heart rate measurements were obtained at 30-minute intervals from daytime (6 AM to 9 PM) and nighttime hours (9 PM to 6 AM).

1-Arginine Infusion Study

The vasodilatory response to L-arginine was evaluated at the beginning and end of the 12-week treatment period. Before and after the 12-week antihypertensive treatment, the L-arginine infusion study began at 8:30 AM. Subjects fasted the previous night for at least 12 hours. They were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 22°C to 25°C) throughout the study. A 19-gauge polyethylene catheter (Terumo Co) was inserted into the right antecubital vein for the infusion of PAH, insulin, and L-arginine. A second catheter was inserted into the left antecubital vein to obtain blood samples. After a 30-minute rest period, an initial dose of PAH (8.0 mg/kg) and insulin (16 mg/kg) was infused as a bolus. PAH and insulin were subsequently infused at constant rates of 12 and 20 mg/min, respectively, by a syringe pump (Terufusion; Terumo Co) throughout the study.26 Sixty minutes after beginning the infusions, we initiated the infusion of L-arginine (500 mg/kg) given over 30 minutes using an infusion pump (PEI-1000; Pal Medical Co). The end of the L-arginine infusion was followed by a 30-minute recovery period. Blood pressure and heart rate were determined every minute by a TM2420 monitor attached to the upper part of the left arm. Mean blood pressure was calculated as the diastolic pressure plus one third of the pulse pressure. Blood samples were obtained to determine serum PAH, insulin, and plasma cGMP, NOx, norepinephrine, and h-ANP at 0 minutes and at 15, 30, and 60 minutes after the start of L-arginine administration. Baseline fasting serum concentrations of total cholesterol, creatinine, insulin, glucose, electrolytes, and ACE activity and PRA, Ang II concentration, and PAC were obtained at 0 minutes. The urinary excretions of cGMP, NOx, creatinine, and electrolytes were obtained during 1 hour before and after the start of L-arginine infusion.

Mean daily dietary intake of NOx in the Japanese population is about 80 mg/d. In the preliminary study, we examined the effect of oral intake of NOx on plasma concentration of NOx in 5 normoten- sive male subjects (mean age, 30±4 years; age range, 26 to 43 years). After the subjects ingested 30 mg NOx, plasma concentration of NOx was measured at 0, 1, and 12 hours. Plasma concentration of NOx was increased from 37±10 to 43±11 μmol/L after 1 hour (P<0.05) and returned to baseline levels after 12 hours in all subjects, suggesting that a 12-hour fast may avoid the effect of dietary intake of NOx on plasma concentration of NOx. Furthermore, the day-to-day variation in plasma concentration of NOx in the fasting concentration in the same individual is small (coefficient of variation, 5.1%).

In the preliminary study, to examine whether the effects of L-arginine on renal hemodynamics are due to its contribution to the release of NO, we administered d-arginine, the enantiomer of L-arginine, as a control for L-arginine. The effects of L-arginine and d-arginine on renal hemodynamics and urinary excretion of NOx in 7 normoten- sive male subjects (mean age, 26±5 years; age range, 23 to 35 years) were measured. These studies were carried out in a double-blind, randomized fashion on a separate day. d-Arginine infusion was performed in a protocol identical to that described for L-arginine. d-Arginine caused a small but significant increase in RPF and a decrease in RVR. The responses of RPF and RVR to L-arginine were significantly greater than those to d-arginine (RPF, 20.4±7.1% versus 7.0±4.2%; P<0.05; and RVR, −28.1±7.6% versus −10.2±5.0%, P<0.05, respectively). The infusion of d-arginine did not significantly alter the GFR. The urinary excretion of NOx markedly increased after L-arginine infusion (from 121.2±47.8 to 286.4±101.1 μmol/mmol creatinine, P<0.001) but not after d-arginine infusion (from 119.5±47.1 to 129.7±67.2 μmol/mmol creatinine). Renal vasorelaxation and urinary NOx responses were much greater after L-arginine than after d-arginine.

Drugs

The ACE inhibitor used was imidapril hydrochloride (Tanabe Pharmaceutical Co). The calcium antagonist was amlodipine (Pfizer Pharmaceutical Co). The L-arginine used for intravenous administration was L-arginine hydrochloride (Morishita-Rusel Pharmaceutical Co). d-Arginine was d-arginine hydrochloride (Sigma Chemical

Selected Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
Ang = angiotensin
FF = filtration fraction
GFR = glomerular filtration rate
h-ANP = human atrial natriuretic peptide
NO = nitric oxide
NOx = nitrite/nitrate
PAC = plasma aldosterone concentration
PAH = para-aminohippurate
PRA = plasma renin activity
RPF = renal plasma flow
RVR = renal vascular resistance

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Co). The administered inulin was Inutest (Laevosan-Gesellschaft Co), whereas the PAH was from Daiichi Pharmaceutical Co.

Analytic Methods

Samples of venous blood were placed in tubes containing EDTA-Na (1 mg/mL) and in polystyrene tubes. The EDTA-containing tubes were promptly chilled in an ice bath. Plasma was immediately separated by centrifugation at 3100×g at 4°C for 10 minutes, and serum at 1000g (at room temperature) for 10 minutes. Samples were stored at −80°C until assayed. Routine chemical methods were used to determine serum concentrations of total cholesterol, creatinine, glucose and electrolytes, and urinary electrolytes. PRA (Gamma Coat PRA, Baxter Travenol Co), plasma Ang II (antiangiotensin II antibody, SRL Co), and PAC (SPAC-S, Aldosterone kits, Daiichi Radio Laboratory Co) were assayed by radioimmunoassay; the intra- and interassay coefficients of variation were 6.2% and 7.6% for PRA, 8.9% and 9.4% for Ang II, and 7.1% and 8.8% for PAC, respectively. Determination of ACE activity was based on the colorimetry of the quininoneimine dye as previously described35; the intra- and interassay coefficients of variation were 2.1% and 3.3%, respectively. The plasma concentration of norepinephrine was measured by high-performance liquid chromatography (HPLC); the intra- and interassay coefficients of variation were 2.4% and 2.1%, respectively. The plasma concentration of h-ANP was assayed by radioimmunoassay (Amersham Co); the intra- and interassay coefficients of variation were 3.8% and 5.3%, respectively. Plasma and urine concentrations of CGMP were measured by radioimmunoassay using a cGMP kit (Yamasu Shoyu Co); the intra- and interassay coefficients of variation were 4.8% and 5.2%, respectively. Plasma and urine concentrations of NOx were assayed by colorimetric methods using NOx assay kits (Cayman Chemical Co). Briefly, nitrate in the sample is converted to nitrite utilizing nitrate reductase. The second step is addition of Griess reagents24 (1% sulfonamide, 0.1% N-1-naphthylethenediamide, and 5% HCl), which convert nitrite into a deep purple azo compound. The absorbance of this azo dye at 540 nm was measured by a microplate reader (M-Tmax; Wako Co). Urine samples were diluted with HPLC-grade water (1:100) because urine contained relatively high levels of nitrate. Urinary excretions of cGMP and NOx were corrected by creatinine excretion. The intra- and interassay coefficients of variation were 0.8% and 1.2%, respectively. The least detectable levels of PRA, Ang II concentration, PAC, ACE activity, norepinephrine, h-ANP, CGMP, and NOx were 0.1 ng/mL per hour, 2 pg/mL, 15 pmol/mL, 0.1 IU/L, 2 pg/mL, 0.5 pmol/L, 0.15 pmol/mL, and 1 pmol/L, respectively.

Serum PAH concentration was analyzed by spectrophotometry. GFR was measured by the clearance of inulin.33 Serum inulin concentration was calculated by the anthrone method.34 Renal blood flow was calculated from PAH clearance and hematocrit level. RVR was calculated as the mean blood pressure divided by renal blood flow, and FF was calculated as GFR divided by RPF. RPF, GFR, and RVR were normalized to body surface area divided by 1.48 m² (1.48 m² being the average body surface area of the Japanese population).

Statistical Analysis

Results are presented as mean±SD. Values of P<0.05 were considered significant. The Mann-Whitney U test was used to evaluate differences between the imidapril and amlodipine groups concerning the parameters at baseline before treatment. Comparisons between treatment groups with respect to changes in parameters were performed with adjusted means by ANCOVA using baseline data as the covariates. Comparisons of time-course curves of parameters during l-arginine infusion were analyzed by 2-way ANOVA for repeated measures. The data were processed using either StatView IV (Brainpower) or Super ANOVA (Abacus Concepts) software packages.

Results

Clinical Characteristics and Drug Dosages

With a double-blind, randomized, and parallel method, 29 patients were divided into either the imidapril group (n=15) or the amlodipine group (n=14). One of 15 patients in the imidapril group withdrew because of adverse effects resulting in a cough and rash, and 1 of 14 patients in the amlodipine group withdrew because of a change of address. Of the 27 patients completing the study, 14 were randomized to receive imidapril (9 men and 5 women; mean age, 57±11 years) and 13 to receive amlodipine (7 men and 6 women; mean age, 55±11 years). To achieve blood pressure control, 14 patients received an imidapril dose of 8.8±3.7 mg/d and 13 patients received an amlodipine dose of 6.3±2.1 mg/d. There were no significant differences in age and gender in the 2 groups. The baseline values for parameters in the imidapril and amlodipine groups at the beginning of the treatment period were similar in both groups, as shown in Table 1.

Effects of Imidapril and Amlodipine on Baseline Clinical Characteristics

The effects of imidapril and amlodipine on the baseline values of parameters are shown in Table 1. After 12 weeks, the decrease in casual blood pressure was similar in the imidapril and amlodipine groups. The 24-hour ambulatory blood pressure was significantly decreased: with imidapril, systolic fell from 152.0±13.7 to 132.2±12.7 mm Hg, diastolic from 91.6±10.7 to 81.4±10.2 mm Hg, and mean from 111.4±12.8 to 96.2±12.1 mm Hg (all P<0.01); with amlodipine, systolic fell from 153.3±13.8 to 130.2±11.9 mm Hg, diastolic from 92.8±10.9 to 80.1±10.1 mm Hg, and mean from 113.1±13.8 to 95.7±11.5 mm Hg (all P<0.01). There was no significant difference in decline in 24-hour mean ambulatory blood pressure between the 2 groups. Mean daytime and nighttime ambulatory blood pressures were also similar before and after treatment with imidapril and amlodipine (data not shown). ACE activity and Ang II were significantly decreased (69±15% and 78±19%, respectively; P<0.01) by imidapril but remained unchanged by amlodipine. In the imidapril group, PRA tended to increase and PAC tended to be depressed but not significantly. Both treatments increased RPF significantly and decreased RVR significantly. The increase in RPF was similar in both the imidapril and amlodipine groups. Thus, drug-induced decline in RVR was also similar in both groups. Other parameters such as lipid and glucose metabolism remained unchanged by both antihypertensive treatments.

Effects of L-Arginine on Systemic and Renal Hemodynamics at Beginning and End of 12-Week Treatment Period

Figure 1 shows the effects of L-arginine infusion on mean blood pressure and heart rate. After L-arginine administration began, mean blood pressure promptly decreased and plateaued after 20 minutes. A prompt return to the baseline level occurred after the end of l-arginine infusion. Changes in systolic and diastolic blood pressures were exactly paralleled by the change in mean blood pressure (imidapril group: before treatment, systolic −10.2±2.1% and diastolic −9.8±1.8%; after treatment, systolic −9.9±2.1% and diastolic −9.1±1.9%; amlodipine group: before treatment, systolic −10.3±2.2% and diastolic −10.1±1.8%; after treatment, systolic −9.7±2.0% and diastolic −9.4±1.8%). The
time course of percent changes in blood pressures was similar
in the 2 groups and was not changed by either treatment.
Conversely, heart rate gradually increased during l-arginine
infusion and gradually returned to the baseline during the
recovery period. The percent changes in heart rate were
similar in the 2 groups before and after treatment.

Figures 2 and 3 show the effects of l-arginine infusion on
renal hemodynamics such as RPF, RVR, GFR, and FF in the

Table 1. Clinical Characteristics of Patients Before and After Treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Imidapril (n=14)</th>
<th>Amlodipine (n=13)</th>
</tr>
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<tbody>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.3±3.3</td>
<td>22.3±3.3</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>154.2±12.2</td>
<td>135.5±11.2†</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>92.9±7.1</td>
<td>83.4±6.7†</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>63.9±5.9</td>
<td>63.1±5.2</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>4.5±1.1</td>
<td>4.4±1.1</td>
</tr>
<tr>
<td>Serum glucose, mmol/L</td>
<td>4.8±0.4</td>
<td>4.7±0.4</td>
</tr>
<tr>
<td>Serum creatinine, µmol/L</td>
<td>86.9±27.0</td>
<td>85.3±26.2</td>
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<tr>
<td>Renin-angiotensin system</td>
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<td></td>
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<tr>
<td>PRA, ng/mL per hour</td>
<td>1.59±1.20</td>
<td>2.08±1.50</td>
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<td>PAC, pg/mL</td>
<td>86.5±45.5</td>
<td>78.2±35.1</td>
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<tr>
<td>Ang II, pg/mL</td>
<td>16.6±8.1</td>
<td>3.7±1.1†</td>
</tr>
<tr>
<td>Serum ACE activity, IU/L</td>
<td>12.9±3.3</td>
<td>4.0±1.8†</td>
</tr>
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<td>Plasma norepinephrine, nmol/L</td>
<td>1.10±1.10</td>
<td>1.15±1.22</td>
</tr>
<tr>
<td>Plasma h-ANP, pmol/L</td>
<td>6.8±4.4</td>
<td>6.5±4.8</td>
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<tr>
<td>Plasma NOx, µmol/L</td>
<td>44.6±38.5</td>
<td>41.8±36.3</td>
</tr>
<tr>
<td>Plasma cGMP, pmol/mL</td>
<td>2.4±1.9</td>
<td>2.1±2.2</td>
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<tr>
<td>Urine NOx, µmol/mmol creatinine</td>
<td>132.1±59.9</td>
<td>126.8±58.1</td>
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<tr>
<td>Urine cGMP, mmol/mmol creatinine</td>
<td>44.3±16.7</td>
<td>42.1±15.1</td>
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<td>Renal hemodynamics</td>
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<td>RPF, mL/min/1.48 m²</td>
<td>608±111</td>
<td>647±104*</td>
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<td>GFR, mL/min/1.48 m²</td>
<td>90±11.0</td>
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<td>RVR, mm Hg/mL/min/1.48 m²</td>
<td>0.117±0.026</td>
<td>0.092±0.022†</td>
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<tr>
<td>FF</td>
<td>0.148±0.018</td>
<td>0.142±0.015*</td>
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</table>

All results are presented as mean±SD.

*P<0.05, †P<0.01 vs before treatment; ‡P<0.05 vs amlodipine (after treatment).

Figure 1. Effects of l-arginine infusion on mean blood pressure and heart rate before and after treatment in the imidapril and amlodipine groups. The responses of mean blood pressure and heart rate to l-arginine were similar in the 2 groups. Results are presented as mean±SD. Probability value refers to the comparison of time-course curves using ANOVA for repeated measurements.
imidapril and amlodipine groups at the beginning and at the end of the antihypertensive treatment period. L-Arginine infusion caused RPF to increase significantly and RVR and FF to decrease significantly and did not produce a significant change in GFR in either group before or after treatment. RPF, RVR, and FF returned to baseline level at 30 minutes after the end of L-arginine infusion. Before treatment, the degree of L-arginine–induced renovascular relaxation was similar in the imidapril and amlodipine groups. After 12 weeks, the responses of RPF and RVR to L-arginine were augmented (9.6±5.1% to 14.4±7.4% and −10.4±8.1% to −16.7±9.3%, respectively; P<0.001) by imidapril, whereas L-arginine–induced changes in the parameters were not altered by amlodipine.

Effects of L-Arginine on cGMP and NOx at Beginning and End of 12-Week Treatment Period
L-Arginine infusion significantly increased the urinary excretions of NOx and cGMP and plasma concentrations of cGMP and h-ANP but did not change plasma concentrations of NOx and norepinephrine (Figure 4 and Tables 2 and 3). The increment in urinary excretion of NOx in response to L-arginine infusion was significantly increased from 90±29% to 134±63% (P<0.05) by imidapril treatment but was unchanged by amlodipine treatment (from 88±32% to 102±58%) (Figure 4, top). The increment in urinary excretion of cGMP in response to L-arginine was also significantly increased from 119±37% to 187±97% (P<0.05) by imidapril treatment but was unchanged by amlodipine treatment.
discussing the effects of chronic treatment of the calcium antagonist amlodipine on the responses of renal hemodynamics and urinary NOx excretion to the different dosages of L-arginine (250, 500, and 1000 mg/kg, respectively) in 5 of 13 patients. The duration of amlodipine treatment was 37 to 49 weeks. The response of RPF to each dose of L-arginine was similar before and after amlodipine treatment (3.9±3.3% to 3.6±3.0%, 9.2±5.3% to 10.1±5.6%, and 13.4±6.2% to 14.1±6.5%, respectively). The increment in urinary excretion of NOx in response to each dose of L-arginine was also similar before and after amlodipine treatment (42±22% to 44±30%, 122±53% to 133±56%, and 164±62% to 171±65%, respectively).

**Discussion**

In the present study, we demonstrated that ACE inhibition with imidapril improved impaired endothelium-dependent renovascular relaxation in patients with essential hypertension without atherosclerosis. Urinary excretion of NOx, as an index of renal NO release, in response to L-arginine was significantly augmented by the imidapril treatment, suggesting that improvement of renal endothelial dysfunction by ACE inhibitors may be due to the increase in NO production. Reduction in blood pressure per se did not play a major role in this potentiation of L-arginine/NO/cGMP–mediated effects because there was no alteration in the renal endothelial function in the amlodipine-treated group despite a hypotensive action and renal vasodilation similar to those of the imidapril group.

Because several lines of evidence have suggested that endothelial function is impaired as blood pressure increases, and that the degree of endothelial dysfunction is related to the severity of blood pressure elevation, the hypothesis has been that improvement of renal endothelial dysfunction by ACE inhibitors may be due to the increase in NO production. Reduction in blood pressure per se did not play a major role in this potentiation of L-arginine/NO/cGMP–mediated effects because there was no alteration in the renal endothelial function in the amlodipine-treated group despite a hypotensive action and renal vasodilation similar to those of the imidapril group.

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<td>cGMP, pmol/mL</td>
<td>Before</td>
<td>2.4±1.9</td>
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<td>After</td>
<td>2.1±2.2</td>
<td>2.6±2.6</td>
<td>3.2±2.9*</td>
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<td>NOx, μmol/L</td>
<td>Before</td>
<td>44.6±38.5</td>
<td>43.2±36.6</td>
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<td>After</td>
<td>41.8±36.3</td>
<td>40.9±37.4</td>
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<td>42.0±37.7</td>
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<td>Norepinephrine, nmol/L</td>
<td>Before</td>
<td>1.10±1.10</td>
<td>1.14±1.20</td>
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<td>After</td>
<td>1.12±1.10</td>
<td>1.15±1.20</td>
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<td>h-ANP, pmol/L</td>
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<td>After</td>
<td>6.5±4.8</td>
<td>6.9±5.9</td>
<td>8.1±7.8*</td>
<td>8.4±8.1*</td>
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All results are presented as mean±SD.

*P<0.05 vs 0 minutes.

**TABLE 2. Effects of L-Arginine on Plasma cGMP, NOx, Norepinephrine, and h-ANP in Imidapril Group**

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<td>After</td>
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<td>2.2±2.5</td>
<td>3.1±2.2*</td>
<td>3.5±2.5*</td>
</tr>
<tr>
<td>NOx, μmol/L</td>
<td>Before</td>
<td>35.8±15.8</td>
<td>34.6±16.6</td>
<td>36.2±17.3</td>
<td>36.0±16.2</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>31.3±14.4</td>
<td>30.2±15.8</td>
<td>32.2±16.2</td>
<td>32.1±15.1</td>
</tr>
<tr>
<td>Norepinephrine, nmol/L</td>
<td>Before</td>
<td>1.02±1.20</td>
<td>1.13±1.31</td>
<td>1.18±1.41</td>
<td>1.14±1.31</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>1.16±1.31</td>
<td>1.18±1.32</td>
<td>1.21±1.41</td>
<td>1.19±1.31</td>
</tr>
<tr>
<td>h-ANP, pmol/L</td>
<td>Before</td>
<td>7.1±5.1</td>
<td>7.3±5.6</td>
<td>8.6±8.3*</td>
<td>8.8±8.6*</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>6.9±5.4</td>
<td>7.3±5.8</td>
<td>8.6±8.6*</td>
<td>8.9±9.1*</td>
</tr>
</tbody>
</table>

All results are presented as mean±SD.

*P<0.05 vs 0 minutes.
proposed that endothelial dysfunction is a consequence of hypertension. If this hypothesis is correct, endothelial dysfunction should be improved with the normalization of blood pressure by antihypertensive drug treatment. Thus, in the present study, we evaluated the effects of the reduction in blood pressure by the ACE inhibitor imidapril or the calcium antagonist amiodipine on renal endothelial function. After a 12-week study, the responses of RPF and RVR to \( \text{L-arginine} \) were augmented in the imidapril group, whereas \( \text{L-arginine} \)-induced changes within those parameters remained unchanged in the amiodipine group, indicating that only imidapril produced a significant improvement of renal vasorelaxation in response to \( \text{L-arginine} \), although the decrease in blood pressure and increase in RPF were similar in both groups. Our findings are consistent with previous studies in that there was no relationship between the improvement of endothelial dysfunction and the decrease in blood pressure in the brachial artery and small arteries of essential hypertensives.\(^{11,13,27}\) These findings suggest that endothelial dysfunction may not be a consequence of hypertension.

Many investigators have reported an improvement of endothelial dysfunction with ACE inhibitors in patients with essential hypertension.\(^{11,13,27}\) Results of ACE inhibitor treatment in several experimental models of hypertension support these findings.\(^{14–17,28}\) On the other hand, Creager and Roddy\(^{19}\) reported that antihypertensive therapy for up to 7 to 8 weeks with the ACE inhibitors captopril or enalapril did not improve endothelium-dependent vasodilatation in the brachial artery of patients with essential hypertension, regardless of whether a sulfahydryl group is present. Although the precise reason for this discrepancy is unknown, it may be due to the differences in patients or treatment periods.

Some possible mechanisms by which the ACE inhibitor imidapril augments endothelium-dependent renovascular relaxation have been postulated. First, ACE inhibitors decrease the production of Ang II through the inhibition of circulating ACE activity and renal tissue ACE. Ang II causes vasoconstriction of the renal artery and a decrease in renal blood flow.\(^{29,30}\) Sumners and Myers\(^{31}\) showed that reduced generation of Ang II may alter the activity of the NO/cGMP pathway. It is postulated that Ang II increases superoxide anions through the stimulation of NADH and NADPH oxidase activity in the smooth muscle cells, leading to an inactivation of NO.\(^{32}\) Sigmon et al\(^{33}\) reported that local interaction of Ang II and NO may be an important factor in selective regulation of renal blood flow. Several lines of evidence show that NO synthesis inhibition with \( N^\text{2}-\text{nitro-L-arginine methyl ester} \) elevates blood pressure and RVR, and decreases RPF in control rats, but has little effect on renal hemodynamics in rats treated with an ACE inhibitor, suggesting that renovascular constriction evoked by NO synthesis inhibition is mainly due to Ang II.\(^{35,36}\) In the present study, after 12 weeks, imidapril inhibited basal circulating ACE activity by 69% and basal circulating Ang II level by 78%. On the basis of animal experiments, we speculate that the local level of Ang II is also diminished. Thus, during \( \text{L-arginine} \) infusion, in the imidapril group the influence of Ang II may be somewhat removed.

Second, ACE inhibitors have been shown to inhibit the breakdown of bradykinin through the inhibition of kininase II. The prevention of bradykinin degradation by ACE inhibitors is speculated to induce an augmentation of the production of endothelium-derived relaxing factors such as NO via endothelial bradykinin \( B_2 \) receptors, potentiating the vasodilator.\(^{35–37}\) In addition, the increase in bradykinin per se directly causes the vasodilation.\(^{38}\) In the present study, imidapril solely enhanced \( \text{L-arginine} \)-induced renovascular relaxation and increased the production of NO.

h-ANP induces natriuresis, diuresis, and vasodilation. Both h-ANP and NO cause vascular relaxation by generating cGMP through the activation of the particulate and soluble guanylate cyclases, respectively.\(^{39}\) In the present study, \( \text{L-arginine} \)-induced increase in h-ANP was similar in both the imidapril and amiodipine groups. Thus, differences in the \( \text{L-arginine} \)-induced increase in cGMP between the 2 groups may have been due to differences in the production of NO. Unlike NO, h-ANP may not have played a paracrine role in the increase in \( \text{L-arginine} \)-induced renovascular relaxation in the imidapril group. Hishikawa et al\(^{40}\) demonstrated that h-ANP was increased by both \( \text{L-arginine} \) and a saline vehicle in humans, suggesting that volume load may elevate h-ANP.

In our study, the dihydropyridine calcium antagonist amiodipine did not restore \( \text{L-arginine} \)-induced renovascular relaxation, although it is clinically effective antihypertensive therapy, suggesting that the antihypertensive effect of amiodipine may be accompanied with vasodilation in renal artery independence of the \( \text{L-arginine} \)/NO pathway. Our findings are consistent with many clinical and experimental studies in that calcium antagonists did not improve impaired endothelial function. In renal and forearm arteries, calcium antagonists may not be beneficial in improving endothelial dysfunction with lowered blood pressure in patients with essential hypertension.

In the present study, the subjects did not ingest a constant amount of NOx. Because it is well known that the measurement of NOx is affected by dietary intake of these substances in food and drinking water, one would raise the possibility that the patients ingested more NOx during the imidapril period. However, as shown in the preliminary study, the increase in plasma NOx by food intake is abolished after 12 hours. A 12-hour fast may avoid the effect of dietary intake of NOx. Furthermore, because the day-to-day variation in plasma NOx in the fasting concentration is small, this possibility is unlikely.

In the preliminary study, exogenous \( \text{d-arginine} \), not being a substrate for the NO pathway, had a small effect on renal hemodynamics and did not produce an increase in NO generation. It has been reported that other amino acids, which are not substrates for NO, also produce renal vasodilation.\(^{41}\) Although the precise mechanism is unclear, only one third of the effects of \( \text{L-arginine} \) on RPF can be explained by the effects of these amino acids. Although we consider that \( \text{L-arginine} \)-induced renovascular relaxation is mainly due to activation of an \( \text{L-arginine}/\text{NO}/\text{cGMP} \) pathway, these findings may suggest that exogenous \( \text{L-arginine} \) infusion may cause renal vasorelaxation mostly through the release of NO rather than the nonspecific effect of amino acids.
The use of specific NO synthase inhibitors, such as N\textsuperscript{G}-monomethyl-L-arginine and N\textsuperscript{G}-nitro-L-arginine methyl ester, and agonists to stimulate NO release, such as acetylcholine or bradykinin, would allow us to draw more specific conclusions concerning the role of the basal and stimulated release of NO in the renal circulation. However, because the intravenous infusion of NO synthase inhibitors can increase the blood pressure and vascular resistance, these agents may lead to adverse effects in hypertensive patients. We therefore did not investigate these agents from certain aspects due to ethical considerations.

Plasma concentration of NOx was not altered significantly by L-arginine infusion, whereas urinary NOx excretion markedly increased after L-arginine infusion. These results are consistent with previous observations.\textsuperscript{3,24} Wennewmalm et al\textsuperscript{44} reported that NO produced in the endothelial cells was rapidly oxidized to NOx in blood, and NOx was subsequently rapidly excreted via urine. The measurement of plasma NOx is not adequate to determine NO production in vivo. However, although we measured the urinary NOx levels as one of the indices of renal NO production, we could not directly measure NO produced from endothelial cells under the physiological conditions and stimuli. Such direct measurement would allow us to draw more specific conclusions concerning the role of NO per se.

In conclusion, the present findings suggest that the effects of ACE inhibitor on L-arginine--induced renovascular relaxation may be due in part to changes in endothelial function. ACE inhibition is beneficial not only in lowering blood pressure but also in improving renal endothelial dysfunction in patients with essential hypertension.

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References


Angiotensin-Converting Enzyme Inhibition, But Not Calcium Antagonism, Improves a Response of the Renal Vasculature to l-Arginine in Patients With Essential Hypertension

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