Effect of Increased Availability of Endothelium-Derived Nitric Oxide Precursor on Endothelium-Dependent Vascular Relaxation in Normal Subjects and in Patients With Essential Hypertension

Julio A. Panza, MD; Philip R. Casino, MD; Diane M. Badar, RN; and Arshed A. Quyyumi, MD

Background. Patients with essential hypertension have a deficit in the endothelium-derived nitric oxide system that results in impaired endothelium-dependent vascular relaxation. The objective of this study was to determine whether this abnormality is caused by a deficiency of substrate for nitric oxide synthesis.

Methods and Results. The vascular responses to acetylcholine (an endothelium-dependent vasodilator infused at 7.5, 15, and 30 μg/min) and sodium nitroprusside (a direct smooth muscle dilator infused at 0.8, 1.6, and 3.2 μg/min) were studied during combined administration of dextrose 5% or l-arginine (substrate for nitric oxide synthesis infused at 40 μmol/min) in 12 normal control subjects (seven men and five women; age, 49.3±7 years) and 14 hypertensive patients (nine men and five women; age, 48.4±7 years). In addition, the effect of d-arginine (stereoisomer of arginine that is not a precursor of nitric oxide) on the vascular responses to acetylcholine was studied in eight normal control subjects and seven hypertensive patients.

Drugs were infused into the brachial artery, and the response of the forearm vasculature was measured by strain gauge plethysmography. The vasodilator response to acetylcholine was significantly blunted in hypertensive patients compared with normal control subjects (maximum flow, 8.9±5 versus 15.7±6 mL·min⁻¹·100 mL⁻¹, respectively; p<0.007); however, no difference was observed in the response to sodium nitroprusside (11.4±6 and 11.7±6 mL·min⁻¹·100 mL⁻¹, respectively). L-Arginine did not significantly change basal blood flow or vascular resistance in either group. In normal control subjects, the infusion of l-arginine significantly augmented the vasodilator response to acetylcholine (maximum flow, 15.7±6 versus 21.4±8 mL·min⁻¹·100 mL⁻¹ before and after l-arginine, respectively; p<0.001). In contrast, in hypertensive patients, the infusion of l-arginine did not alter the response to acetylcholine (maximum flow, 8.9±5 and 8.4±4 mL·min⁻¹·100 mL⁻¹ before and after l-arginine, respectively). The administration of l-arginine did not modify the response to sodium nitroprusside in either group. Similarly, the infusion of d-arginine did not alter the response to acetylcholine in either group.

Conclusions. In normal humans, availability of substrate for production of nitric oxide is a rate-limiting step for endothelium-dependent vascular relaxation. In contrast, increased availability of nitric oxide precursor does not modify endothelium-mediated vasodilation in hypertensive patients. These findings provide further evidence of a defect in the endothelium-derived nitric oxide system in hypertension and indicate that this abnormality is not related to decreased availability of substrate for nitric oxide production. (Circulation 1993;87:1475–1481)

Key Words • endothelium-derived relaxing factor • nitric oxide • arginine • hypertension

The endothelium importantly regulates vascular tone through the release of several factors that modulate the contractile activity of the underlying smooth muscle.1–5 This regulatory function of the endothelium is abnormal in certain cardiovascular conditions,6–9 including essential hypertension, as demonstrated by previous studies from our and other laboratories.8,9

One of the factors released by the endothelium that is a major determinant of both basal vascular tone and endothelium-dependent vascular relaxation is endothelium-derived relaxing factor (EDRF).1,2 Although endothelial cells may release various EDRFs, at least one of them is endothelium-derived nitric oxide,10,11 which is synthesized by the endothelium using the nonessential amino acid l-arginine as a substrate,12 and it plays a major role in the physiological regulation of basal vascular tone and in the response to endothelium-dependent agents.13,14

We recently have shown that patients with essential hypertension have a deficit in the endothelium-derived nitric oxide system of the forearm vasculature, both under basal conditions and during stimulation with the...
endothelium-mediated vasodilator acetylcholine, confirming previous studies in animal models of hypertension. These findings raised the possibility that decreased availability of the natural precursor for nitric oxide formation might be responsible for the abnormal endothelial function of hypertensive patients. However, whether the availability of substrate for endothelial production of nitric oxide is an important limiting factor in the vascular responses mediated by the endothelium has not been definitely established.

Therefore, the present investigation was undertaken with these objectives: 1) to determine whether increased availability of substrate for endothelium-derived nitric oxide can modify endothelium-dependent vascular relaxation in normal humans and 2) to further define the mechanisms responsible for the defect in the endothelium-derived nitric oxide system of hypertensive patients.

Methods

Study Population

Fourteen patients with a well-documented history of chronically elevated blood pressure (≥145/95 mm Hg) without any apparent underlying cause who were followed at the outpatient department of the National Heart, Lung, and Blood Institute were recruited for the study. There were nine men and five women. Mean age was 48.4 ± 7 years. Each patient had been treated for at least 5 years with one or more antihypertensive agents. Patients were asked to discontinue all antihypertensive medications 2 weeks before the day of the study; during that period, they were closely monitored for any evidence of accelerated or malignant hypertension. Patients in whom the withdrawal of antihypertensive agents was considered hazardous (mostly because of severely elevated blood pressure despite medication) were not included in the study. None of the patients had a history of diabetes, hyperlipidemia, peripheral vascular disease, coagulopathy, or any disease predisposing them to vasculitis or Raynaud’s phenomenon.

A population of 12 normal volunteers (seven men and five women) matched with the patients for approximate age and sex was selected as a control group. Mean age was 49.3 ± 7 years. Each of these subjects was screened by clinical history, physical examination, ECG, chest radiograph, and routine chemical analyses and had no evidence of present or past hypertension, hyperlipidemia, cardiovascular disease, or any other systemic condition. None of the control subjects were taking medications at the time of the study.

All participants gave written informed consent for all procedures. This study was approved by the National Institutes of Health Investigational Review Board.

Protocol

All studies were performed in the morning in a quiet room with a temperature of approximately 22°C. Participants were asked to refrain from drinking alcohol or beverages containing caffeine and from smoking for at least 24 hours before studies.

Each study consisted of the infusion of drugs into the brachial artery and the measurement of the response of the forearm vasculature by forearm plethysmography. While the participants were supine, a needle was inserted into the brachial artery of the nondominant arm (left, in most cases). This arm was slightly elevated above the level of the right atrium, and a mercury-filled Silastic strain gauge was placed on the widest part of the forearm. The strain gauge was connected to a plethysmograph (model EC-4, D.E. Hokanson, Issaquah, Wash.) calibrated to measure the percent change in volume; in turn, the plethysmograph was connected to a chart recorder to record the forearm flow measurements. For each measurement, a cuff placed on the upper arm was inflated to 40 mm Hg with a rapid cuff inflator (model E-10, Hokanson) to occlude venous outflow from the extremity. A wrist cuff was inflated to suprasystolic pressures 1 minute before each measurement to exclude the hand circulation. Flow measurements were recorded for approximately 7 seconds every 15 seconds; seven readings were obtained for each mean value.

Basal measurements were obtained after a 3-minute infusion of 5% dextrose solution at 1 mL/min. Forearm flows were then measured after the infusion of sodium nitroprusside and acetylcholine. Sodium nitroprusside was used as an endothelium-independent substance because its vasodilator effect is largely due to its direct action on smooth muscle cells. In contrast, acetylcholine induces vasodilation by stimulating the release of relaxing factors from the vascular endothelium.

Sodium nitroprusside was infused at 0.8, 1.6, and 3.2 μg/min, and acetylcholine chloride (Sigma Chemical, St. Louis, Mo.) was infused at 7.5, 15, and 30 μg/min (infusion rates, 0.25, 0.5, and 1 mL/min, respectively, for each drug). Each dose was infused for 5 minutes, and forearm flow was measured during the last 2 minutes of the infusion. A 30-minute rest period was allowed, and another basal measurement was obtained between the infusion of the two drugs.

After another 30-minute rest period, flow measurements were obtained to corroborate return to basal values. Then, L-arginine was infused at 40 μmol/min (infusion rate, 1 mL/min) for five minutes and forearm blood flow was measured during the last two minutes of the infusion. Subsequently, cumulative dose–response curves for acetylcholine and sodium nitroprusside were repeated using the same doses, infusion rates, and resting interval mentioned above. The infusion of L-arginine was discontinued during the rest period but reinstated before obtaining the second of these dose–response curves. The sequence of administration of acetylcholine and sodium nitroprusside, both before and after infusion of L-arginine, was randomized to avoid any bias related to the order of drug infusion.

In seven hypertensive patients and eight normal control subjects, D-arginine was infused into the brachial artery at 40 μmol/min (infusion rate, 1 mL/min), and subsequently another cumulative dose response for acetylcholine was obtained using the doses mentioned above. D-Arginine is an optically different form of arginine that is not incorporated into proteins and is not a substrate for endothelial synthesis of nitric oxide. Therefore, D-arginine was used to determine whether the effect of L-arginine on basal vascular tone and in the response to acetylcholine was due to the latter’s property of being the natural nitric oxide precursor.

In addition, the effect of administration of increasing concentrations of nitric oxide precursor on endotheli-
um-dependent vascular relaxation was studied in seven normal control subjects and seven hypertensive patients on a separate day. In these subjects, a submaximal dose of acetylcholine (15 µg/min) was infused intra-arterially for 5 minutes, and blood flow measurements were obtained during the last 2 minutes of the infusion. Subsequently and during the continuous administration of acetylcholine, L-arginine was infused at 10, 20, 40, 80, and 160 µmol/min (infusion rates, 0.25, 0.5, 1, 2, and 4 mL/min, respectively). Each dose of L-arginine was infused for 5 minutes, and blood flow was measured during the last 2 minutes of each infusion. After a 30-minute resting period, the experiment was repeated using D-arginine at the same doses and infusion rates used for L-arginine.

During the studies, the participants did not know which drug was being infused. All blood pressures were recorded directly from the intra-arterial catheter before each measurement. Forearm vascular resistance was calculated as the mean arterial pressure divided by the forearm blood flow.

**Statistical Analysis**

Differences between two means were compared by paired or unpaired Student’s t test, as appropriate. The responses to sodium nitroprusside and acetylcholine were compared by ANOVA for repeated measures. Because basal forearm blood flow was similar in patients and control subjects, absolute values were used for all comparisons. However, because the basal resistance was significantly different between the two groups, changes in vascular resistance were expressed as the percentage of the baseline value for all comparisons between the two groups. All calculated p values are two tailed. All values of p<0.05 were considered to indicate significance. All group data are reported as mean±SD unless otherwise indicated.

**Results**

**Vascular Responses to Acetylcholine and Sodium Nitroprusside**

Similar to the findings of previous studies, the increase in blood flow and decrease in vascular resistance with acetylcholine were significantly reduced in hypertensive patients compared with normal control subjects (Figure 1). At the highest dose (30 µg/min), forearm blood flow was 15.7±6 mL·min⁻¹·100 mL⁻¹ in the control subjects and 8.9±5 mL·min⁻¹·100 mL⁻¹ in the patients (p<0.007).

However, no significant differences were found between the two groups in the forearm blood flow and vascular resistance response to sodium nitroprusside (Figure 2). At the highest dose (3.2 µg/min), forearm blood flow was 11.7±4 mL·min⁻¹·100 mL⁻¹ in the control subjects and 11.4±6 mL·min⁻¹·100 mL⁻¹ in the hypertensive patients.

**Effect of L-Arginine and D-Arginine on Basal Blood Flow and Vascular Resistance**

The basal forearm blood flow, measured at the beginning of the study, was similar in hypertensive patients and normal control subjects (2.95±1.4 versus 3.07±0.8 mL·min⁻¹·100 mL⁻¹, respectively). As expected, the basal vascular resistance was significantly elevated in patients compared with control subjects (50.2±24 versus 29.5±5 mm Hg·mL⁻¹·min⁻¹·100 mL⁻¹, p<0.01).

The infusion of L-arginine did not produce any significant change in blood flow or vascular resistance in either group. In hypertensive patients, blood flow was 3.39±1.3 and 3.16±1.4 mL·min⁻¹·100 mL⁻¹ (p=NS) and vascular resistance was 41.2±17 and 45.3±17 mm Hg·mL⁻¹·min⁻¹·100 mL⁻¹ (p=NS) immediately before and after L-arginine infusion, respectively. In normal control subjects, blood flow was 3.44±1.3 and 3.59±1.4 mL·min⁻¹·100 mL⁻¹ (p=NS), and vascular resistance was 30.1±11 and 29.4±12 mm Hg·mL⁻¹·min⁻¹·100 mL⁻¹ (p=NS) immediately before and after L-arginine infusion, respectively.

Similarly, no changes in blood flow or vascular resistance were observed in either group after the infusion of D-arginine. In the seven hypertensive patients studied, blood flow was 2.77±0.7 and 2.67±0.7 mL·min⁻¹·100 mL⁻¹ (p=NS) and vascular resistance was 47.6±16 and 49.6±15 mm Hg·mL⁻¹·min⁻¹·100 mL⁻¹ (p=NS) immediately before and after D-arginine infusion, respectively.

In the eight normal control subjects studied, blood flow was 2.88±0.6 and 2.90±0.7 mL·min⁻¹·100 mL⁻¹ (p=NS) and vascular resistance was 31.8±6 and 33.0±11 mm Hg·mL⁻¹·min⁻¹·100 mL⁻¹ (p=NS).
Effect of L-Arginine on the Vascular Responses to Acetylcholine and Sodium Nitroprusside

In normal control subjects, the vasodilator response to acetylcholine was significantly potentiated after infusion of L-arginine (Figure 3). At the highest dose of acetylcholine (30 μg/min), blood flow was 15.7±6 mL·min⁻¹·100 mL⁻¹ before and 21.4±8 mL·min⁻¹·100 mL⁻¹ after infusion of L-arginine (p<0.001). An augmentation of the maximum flow with acetylcholine of ≥20% in response to the combined administration of L-arginine was observed in 10 of the 12 subjects. The infusion of L-arginine, however, did not modify the vasodilator response to sodium nitroprusside in normal control subjects (maximum blood flow, 11.7±4 versus 12.8±6 mL·min⁻¹·100 mL⁻¹ before and after L-arginine infusion, respectively).

In contrast, in hypertensive patients, the response to acetylcholine was not significantly altered by the infusion of L-arginine (Figure 4). At the maximum dose of acetylcholine, blood flow was 8.9±5 mL·min⁻¹·100 mL⁻¹ before and 8.4±4 mL·min⁻¹·100 mL⁻¹ after infusion of L-arginine (p=NS). Similarly, the infusion of L-arginine did not produce any significant difference in the response to sodium nitroprusside in hypertensive patients (maximum blood flow, 11.3±6 versus 11.0±5 mL·min⁻¹·100 mL⁻¹ before and after L-arginine infusion, respectively).

In the separate studies using a submaximal dose of acetylcholine and subsequent infusion of increasing doses of L-arginine (Figure 5), the single dose of acetylcholine produced significantly greater vasodilation in normal control subjects than in hypertensive patients (blood flow, 7.9±4 versus 3.9±2 mL·min⁻¹·100 mL⁻¹ of forearm volume, respectively; p<0.05). In normal control subjects, the subsequent infusion of L-arginine produced a dose-dependent augmentation of the response to acetylcholine starting at the 20-μmol/min dose (Figure 5). In hypertensive patients, however, no significant change in the response to acetylcholine was observed with L-arginine until the maximum dose of 160 μmol/min was reached (Figure 5).

Effect of D-Arginine on the Vascular Responses to Acetylcholine

In the eight normal control subjects and seven hypertensive patients studied, the vasodilator response to acetylcholine was not significantly altered after infusion of D-arginine.

In normal control subjects, at the highest dose of acetylcholine (30 μg/min), blood flow was 14.6±7 mL·min⁻¹·100 mL⁻¹ before and 14.5±6 mL·min⁻¹·100 mL⁻¹ after infusion of D-arginine (p=NS). In hypertensive
patients, blood flow was $9.7 \pm 5 \text{ mL min}^{-1} \cdot \text{100 mL}^{-1}$ before and $9.6 \pm 7 \text{ mL min}^{-1} \cdot \text{100 mL}^{-1}$ after infusion of D-arginine ($p=\text{NS}$).

In normal control subjects and hypertensive patients, the administration of increasing doses of D-arginine did not augment the vasodilator response to acetylcholine until the maximum dose of 160 $\mu$mol/min was reached (Figure 6). Of note, in normal control subjects, the serial blood flow measurements obtained with increasing doses of L-arginine during continuous infusion of acetylcholine were significantly greater than those obtained with infusion of D-arginine ($p<0.01$ by ANOVA for repeated measures); however, no difference between the measurements obtained with infusion of increasing doses of L- and D-arginine was observed in the hypertensive patients.

**Discussion**

**Effect of L-Arginine in Normal Control Subjects**

The results of the present investigation demonstrate that in normal humans, increased availability of the substrate for endothelial production of nitric oxide does not affect basal vascular tone. It does, however, augment the endothelium-dependent vascular responses to acetylcholine.

Previous studies have shown that L-arginine can produce vasodilation and even hypotension if infused intravenously.$^{23}$ This effect of L-arginine, however, is only observed with high doses of the amino acid, and it most likely is not related to increased endothelial production of nitric oxide because it also can be induced by infusion of high doses of D-arginine, the stereoisomer that is not a substrate for nitric oxide formation.$^{24}$ Based on these previous observations, it is possible that our finding of an augmentation of endothelium-dependent vasodilation by L-arginine was a nonspecific response of the vasculature to the amino acid. However, the infusion of an equimolar dose of D-arginine did not modify the vasodilator response to acetylcholine in our study. Furthermore, the infusion of L-arginine did not alter the vascular response to the direct smooth muscle vasodilator sodium nitroprusside. Both these observations support the concept that the enhanced acetylcholine-induced vasodilation observed with L-arginine truly reflects a potentiation of endothelium-dependent vascular relaxation, most likely as a consequence of an increase in the endothelial production of nitric oxide stimulated by acetylcholine.

Analysis of serial measurements obtained during administration of increasing doses of L- and D-arginine in the presence of a submaximal dose of acetylcholine provides further insight into this issue. Such experiments demonstrated that at doses between 20 and 80 $\mu$mol/min, L-arginine produces a significantly greater augmentation of the vasodilator effect of acetylcholine than D-arginine, most likely as a consequence of in-
creased production of nitric oxide with L-arginine but not with D-arginine. However, at high doses (160 μmol/min), both isomeric forms of the amino acid can augment the vasodilator response to acetylcholine. This latter observation is in agreement with a previous study on the effect of arginine on basal hemodynamics and emphasizes the importance of using D-arginine as a control substance in studies related to the physiology and pathophysiology of the nitric oxide system.

Based on these findings, we conclude that in normal humans, the availability of substrate for endothelial production of nitric oxide does not importantly influence basal vascular tone but becomes rate limiting when the synthesis of nitric oxide is stimulated by endothelium-dependent agents.

Previous investigations that analyzed the effect of L-arginine on coronary vascular responses to acetylcholine, however, have shown lack of a significant change in the vasodilator action of acetylcholine in response to the combined infusion of L-arginine in normal coronary arteries. The discrepancy between those observations and the findings of our study could reflect differences in the behavior of the coronary and peripheral vascular beds. The contrast between our findings and those of other investigators who studied the effect of L-arginine on endothelium-dependent vasodilation of peripheral arteries could be explained by dissimilar intravascular concentra-

tions of L-arginine, because in those studies the amino acid was given intravenously.

**Effect of L-Arginine in Hypertensive Patients**

In contrast to the findings in normal subjects, patients with essential hypertension did not show an augmentation of endothelium-dependent vasodilation in response to the increased availability of nitric oxide precursor. Thus, in hypertensive patients, the vasodilatation observed with acetylcholine was diminished in comparison to normal control subjects, a response that was not modified by the administration of L-arginine. In concurrence with the results observed in normal subjects, D-arginine did not alter the vascular responses to acetylcholine in hypertensive patients. Similarly, the response to sodium nitroprusside was not affected by the combined infusion of L-arginine.

The results obtained with increasing doses of L- and D-arginine during continuous infusion of acetylcholine showed that there was no difference between the responses to the two forms of the amino acid in hypertensive patients, a finding that contrasts with the observation in normal control subjects. The effect observed at the maximum dose of arginine used in our study probably is not related to increased production of nitric oxide because it was observed with both isomeric forms of the amino acid and most likely reflects a nonspecific vasodilator action of arginine.

Therefore, the results of the present investigation confirm the findings of previous studies that showed that endothelium-dependent vascular relaxation is impaired in patients with essential hypertension and expand those observations by demonstrating that this abnormality is not related to a decreased availability of the natural precursor for endothelial synthesis of nitric oxide. Moreover, because the normal response to increased availability of nitric oxide precursor is augmentation of endothelium-dependent vascular relaxation, the lack of such a response in hypertensive patients, which appears to be independent of the dose of L-arginine infused, further supports the concept of a deficit in the endothelium-derived nitric oxide system. This concept is consistent with the results of a recent investigation performed in our laboratory to study the role of the nitric oxide system in the endothelium-mediated vasodilatation of hypertensive patients. Such a study showed that the vascular responses to inhibition of the synthesis of nitric oxide with arginine analogues are attenuated in patients with essential hypertension, indicating a reduced activity of the nitric oxide system in these patients.

The findings of the present investigation, however, do not allow us to determine whether this defect exists at the level of the synthesis or the release of nitric oxide by endothelial cells. Similarly, we cannot determine whether the administration of L-arginine in our study patients actually resulted in increased intracellular concentration of the substrate for nitric oxide because it is possible that the defect lies in the capacity of the endothelial cell to uptake the amino acid. These possibilities therefore merit further investigation. Regardless of the exact location of the defect in the nitric oxide system, this abnormality may play a major role in the impaired endothelium-dependent vasodilation of pa-
tients with essential hypertension, thus contributing to the pathophysiology of this condition.

Finally, it also must be noted that vasodilator factors other than nitric oxide, such as endothelium-derived hyperpolarizing factor, 29,30 are released by the endothelium in response to certain stimuli, including administration of acetylcholine. The release of these factors also may be impaired and thus contribute to the abnormal endothelium-mediated vasodilatation of hypertensive patients.

Conclusions

The present investigation demonstrates that increased availability of the precursor for endothelial production of nitric oxide normally results in augmentation of endothelium-dependent vascular relaxation in humans. Such a response, however, is not observed in patients with essential hypertension. The latter finding provides further evidence of an abnormality in the endothelium-derived nitric oxide system in hypertension and indicates that such abnormality is not due to decreased availability of substrate for nitric oxide production. Further investigation therefore is warranted to elucidate the primary mechanism of the deficit in the endothelium-derived nitric oxide system that may play a significant role in the pathophysiology of the hypertensive process.

References

15. Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA: Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. Circulation 1993;87:1468–1474
Effect of increased availability of endothelium-derived nitric oxide precursor on endothelium-dependent vascular relaxation in normal subjects and in patients with essential hypertension.

J A Panza, P R Casino, D M Badar and A A Quyyumi

Circulation. 1993;87:1475-1481
doi: 10.1161/01.CIR.87.5.1475

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circ.ahajournals.org/content/87/5/1475

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at: http://circ.ahajournals.org/subscriptions/