Clinical Investigation

Role of Endothelium-Derived Nitric Oxide in the Abnormal Endothelium-Dependent Vascular Relaxation of Patients With Essential Hypertension

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Background. Patients with essential hypertension have abnormal endothelium-dependent vasodilation. Because the endothelium exerts its action on the vascular smooth muscle through the release of several substances, it is important to identify which of these factors is involved in the abnormal response of hypertensive arteries.

Methods and Results. To investigate the role of endothelium-derived nitric oxide in this abnormality, we studied the vascular effect of the arginine analogue N\textsuperscript{G}-monomethyl-L-arginine, an inhibitor of the endothelial synthesis of nitric oxide, under baseline conditions and during infusion of acetylcholine, an endothelium-dependent vasodilator, and sodium nitroprusside, a direct smooth muscle dilator. The study included 11 hypertensive patients (seven men; age, 46.5±9 years) and 10 normal control subjects (seven men; age, 45.7±7 years). Drugs were infused into the brachial artery, and the response of the forearm vasculature was measured by strain-gauge plethysmography. Basal blood flow was similar in normal control subjects and hypertensive patients (2.97±0.7 versus 2.86±1.1 mL·min\textsuperscript{-1}·100 mL\textsuperscript{-1}, respectively). N\textsuperscript{G}-monomethyl-L-arginine produced a significantly greater decrease in blood flow in control subjects than in patients (1.08±0.6 versus 0.32±0.4 mL·min\textsuperscript{-1}·100 mL\textsuperscript{-1}; p<0.004). The vasodilator response to acetylcholine was reduced in patients compared with control subjects (maximum flow, 8.2±4 versus 16.4±8 mL·min\textsuperscript{-1}·100 mL\textsuperscript{-1}; p<0.001). N\textsuperscript{G}-monomethyl-L-arginine blunted the vasodilator response to acetylcholine in control subjects (maximum flow decreased from 16.4±8 to 7.01±3 mL·min\textsuperscript{-1}·100 mL\textsuperscript{-1}; p<0.004); however, the arginine analogue did not significantly alter the response to acetylcholine in hypertensive patients (maximum flow, 8.2±4 versus 8.01±5 mL·min\textsuperscript{-1}·100 mL\textsuperscript{-1}). N\textsuperscript{G}-monomethyl-L-arginine did not modify the vasodilator response to sodium nitroprusside in either control subjects or patients.

Conclusions. These findings indicate that patients with essential hypertension have a defect in the endothelium-derived nitric oxide system that may at least partly account for both the increased vascular resistance under basal conditions and the impaired response to endothelium-dependent vasodilators. (Circulation 1993;87:1468-1474)

Key Words • hypertension • endothelium • endothelium-derived relaxing factor • nitric oxide • acetylcholine • nitroprusside

It is now recognized that the endothelium importantly determines vascular tone through the release of different relaxing and constricting factors that modulate the contractile activity of the underlying smooth muscle.\textsuperscript{1-5} The critical nature of such endothelial function is further emphasized by the recent discovery of abnormal endothelium-dependent vascular responses in certain specific cardiovascular conditions. In this regard, previous studies from our and other laboratories have demonstrated that patients with essential hypertension have impaired endothelium-dependent vasodilatation.\textsuperscript{6,7} These findings suggest the possibility that an endothelial defect may play a pathophysiological role in the functional abnormalities of resistance arteries characteristic of hypertensive patients.\textsuperscript{8}

Because the endothelium releases several factors that act on the vascular smooth muscle, it is important to identify which of these substances is involved in the abnormal endothelial function of hypertensive arteries. One of the agents that mediate the effect of the endothelium on vascular tone is endothelium-derived relaxing factor, or EDRF.\textsuperscript{1,2,9} Although there might be more than one EDRF, at least one such factor has recently been identified as nitric oxide,\textsuperscript{10} which is synthesized by endothelial cells using the nonessential amino acid L-arginine as a precursor.\textsuperscript{11} Importantly, this process can be inhibited by arginine analogues that competitively antagonize the synthesis of nitric oxide from L-arginine\textsuperscript{12} and thus provide a useful tool to

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investigate the rate of production of nitric oxide by the vascular endothelium.\textsuperscript{13}

The present investigation, therefore, was undertaken with the following objectives: 1) to determine whether a decreased synthesis or release of endothelium-derived nitric oxide can at least partly account for the elevated basal vascular resistance of patients with essential hypertension and 2) to define the role of nitric oxide in the abnormal endothelium-dependent vascular responses observed in these patients.

\textbf{Methods}

\textit{Study Population}

Eleven 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 seven men and four women. Mean age was 46.5±9 years. Each patient had been treated for at least 5 years with one or more antihypertensive agents. Medications included calcium channel blockers in five patients (verapamil in three and nifedipine in two), diuretics in five, angiotensin converting enzyme inhibitors in five (enalapril in four and captopril in one), \( \beta \)-blockers in two (atenolol in both), and prazosin in one. Six patients were receiving one antihypertensive agent, three patients were on two medications, and two patients were being treated with three antihypertensive drugs. 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 10 normal volunteers (seven men and three women), matched with the patients for sex and approximate age, was selected as a control group. Mean age was 45.7±7 years. Each of these subjects was screened by clinical history, physical examination, ECG, chest x-ray film, 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.

The most relevant clinical characteristics of hypertensive patients and normal control subjects are summarized in Table 1. Except for systemic blood pressure (measured at the time of the study), no significant differences were observed between the two groups.

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

\textit{Protocol}

All studies were performed in the morning in a quiet room with a temperature of approximately 22°C (72°F). Participants were instructed to continue with their regular diet and were asked to refrain from drinking alcohol or beverages containing caffeine and from smoking for at least 24 hours before the studies.

Each study consisted of the infusion of drugs into the brachial artery and the measurement of the response of the vasculature (changes in regional blood flow) by means of forearm plethysmography. While the participants were supine, a needle was inserted into the brachial artery of the nondominant arm (the left arm, 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.\textsuperscript{14,15} The strain gauge was connected to a plethysmograph (model EC-4, D.E. Hokanson, Issaquah, Wash.)\textsuperscript{16} calibrated to measure the percent change in volume; the plethysmograph in turn 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.\textsuperscript{17} 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, since its vasodilator effect is largely caused by its direct action on smooth muscle cells.\textsuperscript{16,19} Acetylcholine, in contrast, induces vasodilation by stimulating the release of relaxing factors from the vascular endothelium.\textsuperscript{1,2,7,13} Sodium nitroprusside was infused at 0.8, 1.6, and 3.2 \( \mu \)g/min and acetylcholine chloride (Sigma Chemical Co., St. Louis, Mo.) at 7.5, 15, and 30 \( \mu \)g/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 infusions of the two drugs.

After another 30-minute rest period, flow measurements were obtained to corroborate return to basal

\begin{table}[h]
\centering
\caption{Characteristics of the Study Population}
\begin{tabular}{lccc}
\hline
 & Normal control subjects & Hypertensive patients & \( p \) \\
\hline
Age (years, mean±SD) & 45.7±7 & 46.5±9 & NS \\
Sex (M/F) & 7/3 & 7/4 & NS \\
Mean blood pressure (mm Hg) & 76.5±8 & 111.2±8 & <0.001 \\
Height (cm) & 170.1±9 & 167.2±9 & NS \\
Weight (kg) & 75.9±12 & 79.5±18 & NS \\
Serum potassium (mEq/L) & 4.1±0.2 & 4.0±0.3 & NS \\
Serum sodium (mEq/L) & 138.1±3 & 138.0±2 & NS \\
Creatinine (mg/L) & 1.09±0.2 & 1.11±0.3 & NS \\
Cholesterol (mg/dl) & 170.1±34 & 177.0±46 & NS \\
\hline
\end{tabular}
\end{table}
values. Then, the arginine analogue N⁰-monomethyl-L-arginine was infused at 4 μmol/min (infusion rate, 1 mL/min) for 5 minutes, and forearm blood flow was measured during the last 2 minutes of the infusion. Subsequently, cumulative dose–response curves for acetylcholine and sodium nitroprusside were repeated with the same doses, infusion rates, and resting interval as mentioned above. The infusion of N⁰-monomethyl-L-arginine was discontinued during the rest period but reinstituted before the second of these dose–response curves was obtained.

The sequence of administration of acetylcholine and sodium nitroprusside both before and after infusion of the arginine analogue was randomized to avoid any bias related to the order of drug infusion. During the study, the participants did not know which drug was being infused. All blood pressures were recorded directly from the intra-arterial catheter immediately 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. Since 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. Relations between variables were assessed by means of Pearson’s correlation coefficient and linear regression analysis. All calculated probability 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

As in previous studies,⁶,⁷ 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 16.4±8 mL·min⁻¹·100 mL⁻¹ in the control subjects and 8.2±4 mL·min⁻¹·100 mL⁻¹ in the patients (p<0.001).

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 10.6±3 mL·min⁻¹·100 mL⁻¹ in the control subjects and 9.4±4 mL·min⁻¹·100 mL⁻¹ in the hypertensive patients.

Effect of the Arginine Analogue

N⁰-Monomethyl-L-Arginine on Basal Flow and Resistance

The basal forearm blood flow was similar in hypertensive patients and normal control subjects (2.86±1.1 versus 2.97±0.7 mL·min⁻¹·100 mL⁻¹, respectively). As expected, the basal vascular resistance was significantly elevated in patients compared with control subjects (48.6±18 versus 31.3±9 mm Hg·mL⁻¹·min⁻¹·mL⁻¹; p<0.01).

The infusion of N⁰-monomethyl-L-arginine produced significant decrease in blood flow and increase in vascular resistance in both groups. In hypertensive patients, blood flow decreased from 2.86±1.1 to 2.53±1.1 mL·min⁻¹·100 mL⁻¹ (p<0.04), and vascular resistance increased from 48.6±18 to 59.1±31 mm Hg·mL⁻¹·min⁻¹·mL⁻¹ (p<0.05). In normal control subjects, blood flow decreased from 2.97±0.7 to 1.89±0.5 mL·min⁻¹·100 mL⁻¹ (p<0.0003), and vascular resistance increased from 31.3±9 to 47.1±16 mm Hg·mL⁻¹·min⁻¹·mL⁻¹ (p<0.003). Of note, the vascular resistance measured in normal control subjects after infusion of the arginine analogue (47.1±16 mm Hg·mL⁻¹·min⁻¹·mL⁻¹) was similar to the resistance measured in hypertensive patients under baseline conditions (48.6±18 mm Hg·mL⁻¹·min⁻¹·mL⁻¹).

The magnitude of the changes in blood flow and vascular resistance with N⁰-monomethyl-L-arginine was significantly greater in normal control subjects than in hypertensive patients (decrease in blood flow, 1.08±0.6 versus 0.32±0.4 mL·min⁻¹·100 mL⁻¹, respectively [p<0.004], and increase in resistance 51±39% versus 16±26%, respectively [p<0.03]) (Figure 3). Of note, a significant inverse correlation was observed between the systolic blood pressure at the time of the study and the vascular responses to infusion of N⁰-monomethyl-L-arginine.
arginine (Figure 4). Moreover, a significant inverse correlation was also found between the decrease in blood flow with \(N^O\)-monomethyl-L-arginine and the blood flow response to acetylcholine (\(r = -0.45\); \(p < 0.05\)).

No significant changes in systemic blood pressure were observed with the infusion of \(N^O\)-monomethyl-L-arginine in either hypertensive patients or normal control subjects.

**Effect of \(N^O\)-Monomethyl-L-Arginine on the Vascular Responses to Acetylcholine and Sodium Nitroprusside**

In normal control subjects, the vasodilator response to acetylcholine was significantly blunted after infusion of \(N^O\)-monomethyl-L-arginine (Figure 5). At the highest dose of acetylcholine (30 \(\mu\)g/min), blood flow was 16.4 ± 8 mL/min·100 mL⁻¹ before and 7.01 ± 3 mL/min·100 mL⁻¹ after infusion of \(N^O\)-monomethyl-L-arginine (\(p < 0.004\)). The infusion of the arginine analogue, however, did not modify the vasodilator response to sodium nitroprusside in normal control subjects (maximum blood flow, 10.6 ± 3 versus 9.9 ± 3 mL/min·100 mL⁻¹ before and after \(N^O\)-monomethyl-L-arginine infusion, respectively).

In hypertensive patients, in contrast, the response to acetylcholine was not significantly altered by the infusion of \(N^O\)-monomethyl-L-arginine (Figure 6). At the maximum dose of acetylcholine, blood flow was 8.2 ± 4 mL/min·100 mL⁻¹ before and 8.0 ± 5 mL/min·100 mL⁻¹ after infusion of the arginine analogue. Similarly, the infusion of \(N^O\)-monomethyl-L-arginine did not produce any significant difference in the response to sodium nitroprusside in hypertensive patients (maximum blood flow, 9.4 ± 4 versus 8.5 ± 3 mL/min·100 mL⁻¹ before and after \(N^O\)-monomethyl-L-arginine infusion, respectively).

**Figure 2.** Graphs showing forearm blood flow and vascular resistance responses to sodium nitroprusside in 10 normal control subjects (open circles) and 11 hypertensive patients (closed circles). Values represent mean ± SEM.

**Figure 3.** Bar graphs showing decrease in basal forearm blood flow (top panel) and increase in basal vascular resistance (bottom panel) produced by infusion of \(N^O\)-monomethyl-L-arginine at 4 \(\mu\)mol/min in 10 normal control subjects (solid bars) and 11 hypertensive patients (hatched bars). Values represent mean ± SEM.

**Figure 4.** Scatterplots showing correlation between systolic blood pressure (measured on the day of the study) and decrease in blood flow (top panel) and increase in vascular resistance (bottom panel) with \(N^O\)-monomethyl-L-arginine (L-NMMA) in 10 normal control subjects (open circles) and 11 hypertensive patients (closed circles).
mL\(^{-1}\) before and after \(N^G\)-monomethyl-L-arginine infusion, respectively).

**Discussion**

The endothelium plays a key role in the homeostasis of the cardiovascular system by regulating vascular tone. This endothelial action is exerted through the release of different substances that modulate the contractile activity of the vascular smooth muscle.\(^3\)\(^-\)\(^5\) One of these substances is EDRF.\(^1\)\(^,\)\(^2\)\(^,\)\(^9\) Although the endothelium may release more than one EDRF, at least one has been recently identified as nitric oxide or a molecule containing nitric oxide.\(^10\) The synthesis of nitric oxide by endothelial cells uses the nonessential amino acid L-arginine as the substrate\(^11\) and can be specifically and competitively antagonized by arginine analogues such as \(N^G\)-monomethyl-L-arginine, which binds to the enzymatic pathway in a process that can be reverted by increased availability of L-arginine.\(^12\)\(^,\)\(^13\) This phenomenon permits the study of the role of endothelium-derived nitric oxide in the physiology and pathophysiology of the cardiovascular system.\(^13\)

Previous studies have shown that patients with essential hypertension have a selective impairment in the response to acetylcholine, an endothelium-dependent vasodilator.\(^6\)\(^,\)\(^7\) Because several substances are released by the vascular endothelium, the finding of an impaired response to acetylcholine does not define the mechanisms that mediate this abnormal endothelial response of hypertensive patients.

The results of the present investigation indicate that patients with essential hypertension have a specific deficit in the endothelium-derived nitric oxide system and suggest that this defect may play a role in determining both the increased vascular resistance of these patients and their impaired response to endothelium-dependent agents. Thus, in comparison to normal control subjects, hypertensive patients had a less pronounced decrease in basal blood flow and blood flow response to acetylcholine in response to the arginine analogue \(N^G\)-monomethyl-L-arginine. This difference indicates that the basal and stimulated release of nitric oxide by the endothelium of hypertensive arteries is diminished and therefore is less affected by inhibition of its synthesis.

The finding of a normal vasodilator response to sodium nitroprusside in hypertensive patients both before and after the infusion of \(N^G\)-monomethyl-L-arginine rules out the possibility that the observed abnormality in the nitric oxide system of these patients is caused by a defective response of vascular smooth muscle to nitrovasodilators. However, the results of this study do not allow us to determine whether such abnormality is a consequence of diminished synthesis of endothelium-derived nitric oxide, of normal synthesis but impaired release from the endothelial cells, or even of a defect in the diffusion of this substance between the
endothelium and the vascular smooth muscle. These possibilities merit further investigation.

Another important finding of our study is that the arginine analogue produced a significant decrease in blood flow and increase in vascular resistance in normal control subjects, in agreement with previous reports.\(^{13}\) Since \(N^5\)-monomethyl-L-arginine does not have an intrinsic effect on vascular smooth muscle,\(^{13}\) this observation indicates that endothelium-derived nitric oxide is a major determinant of normal vascular tone and suggests that a defect in the synthesis or release of nitric oxide may result in abnormally increased vascular resistance. In this regard, it is noteworthy that the vascular resistance of normal subjects after infusion of \(N^5\)-monomethyl-L-arginine was similar to that of hypertensive patients under basal conditions. Furthermore, the importance of nitric oxide in the regulation of vascular tone is emphasized by the observation of a significant inverse correlation between systolic blood pressure and the vascular responses to \(N^G\)-monomethyl-L-arginine. Thus, individuals with a greater impairment in the basal production of nitric oxide (and therefore a reduced response to inhibition of its synthesis with the arginine analogue) are those who have a higher basal systemic blood pressure.

With respect to the comparison of changes in basal blood flow and vascular resistance between the two subject groups, it is important to emphasize that, because resistance arteries of patients with essential hypertension have an increased wall-to-lumen ratio,\(^{20,21}\) hypertensive patients are predisposed to a more pronounced response to any given vasoconstrictor agent.\(^{22,23}\) The decreased response to \(N^G\)-monomethyl-L-arginine that we observed in patients with essential hypertension, therefore, cannot be ascribed to such a response, further supporting the concept that this finding truly reflects a specific deficit of endothelium-derived nitric oxide under basal conditions.

In normal control subjects, \(N^5\)-monomethyl-L-arginine significantly blunted the vasodilator response to acetylcholine but did not alter the response to sodium nitroprusside. This observation, which is in agreement with previous studies,\(^{13}\) indicates that endothelium-derived nitric oxide is an important mediator of the vasodilator effect of acetylcholine. This concept is further supported by the inverse relation observed between the vascular effects of \(N^G\)-monomethyl-L-arginine and acetylcholine. In contrast to the results in normal control subjects, inhibition of the endothelial synthesis of nitric oxide by \(N^G\)-monomethyl-L-arginine did not significantly modify the response to acetylcholine in hypertensive patients, indicating that the release of nitric oxide in response to endothelium-dependent agents is significantly impaired in these patients. Another implication of this latter finding is that, since acetylcholine still produced substantial vasodilation in hypertensive patients even after infusion of the arginine analogue, this effect of acetylcholine must be importantly mediated by other factors released by the endothelium, such as endothelium-derived hyperpolarizing factor,\(^{24}\) which is not affected by arginine analogues.

The observation that inhibition of the synthesis of nitric oxide in normal subjects can cause a rise in vascular resistance to hypertensive levels and the finding of abnormal production of nitric oxide in hypertensive patients suggest that this abnormality may play a primary role in the causative mechanisms of essential hypertension. Alternatively, such a defect may be a consequence and not the cause of the elevated blood pressure. Previous studies from our laboratory have shown that normalization of blood pressure with conventional antihypertensive therapy does not improve the blunted response to endothelium-dependent agents characteristic of patients with essential hypertension,\(^{25}\) suggesting that this defect either is primary or becomes irreversible once the hypertensive process has been established. In either case, this endothelial abnormality may play an important role in the genesis or perpetuation of the hypertensive process and thus contribute to the morbid consequences of the disease.

In conclusion, the present study demonstrates that the abnormal endothelial function of patients with essential hypertension is related to a defect in the endothelium-derived nitric oxide system because of either reduced synthesis, release, or diffusion of nitric oxide to vascular smooth muscle. This finding may account for both the increased vascular resistance under basal conditions and the impaired response to stimulation with endothelium-dependent vasodilators. This abnormality may play an important role in the pathophysiology of the hypertensive process, and its identification may lead to the development of more specific therapies.

References

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