Selective Defect in Nitric Oxide Synthesis May Explain the Impaired Endothelium-Dependent Vasodilation in Patients With Essential Hypertension

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Background—Patients with essential hypertension have impaired endothelial NO activity, but the mechanism underlying this abnormality is unknown.

Methods and Results—To investigate whether the endothelial dysfunction of hypertensive patients is related to a selective defect in NO synthesis, we studied the forearm blood flow responses to intra-arterial infusion of acetylcholine (7.5 to 30 \( \mu \)g/min), a \( \beta \)-adrenoceptor agonist that stimulates NO production by increasing intracellular cAMP, in 12 normotensive subjects and 12 hypertensive patients. The infusion of isoproterenol was repeated during the concurrent blockade of NO synthesis by N\(^{\text{G}}\)-monomethyl-L-arginine (L-NMMA; 4 \( \mu \)mol/min). The vasodilator response to acetylcholine was significantly reduced in hypertensives compared with normotensives (maximum blood flow: 10.4±4.6 versus 14.4±3.7 mL \cdot min\(^{-1}\) \cdot dL\(^{-1}\); \( P = .008 \)). However, the vasodilator effect of isoproterenol was similar in normotensives and hypertensives (maximum blood flow: 14.4±5.4 versus 13.5±5 mL \cdot min\(^{-1}\) \cdot dL\(^{-1}\); \( P = .56 \)) and was significantly (both \( P < .01 \)) and equally blunted by L-NMMA in both groups (maximum blood flow: 11±3 mL \cdot min\(^{-1}\) \cdot dL\(^{-1}\) in normotensives versus 10.8±3.9 mL \cdot min\(^{-1}\) \cdot dL\(^{-1}\) in hypertensives; \( P = .77 \)). The vasodilator response to sodium nitroprusside (0.8 to 3.2 \( \mu \)g/min), an exogenous NO donor, was similar in both groups and was not modified by L-NMMA.

Conclusions—Hypertensive patients have impaired endothelium-dependent vasodilation in response to acetylcholine but preserved NO activity in response to \( \beta \)-adrenergic stimulation. These findings suggest that the endothelial dysfunction in essential hypertension is due to a selective abnormality of NO synthesis, probably related to a defect in the phosphatidylinositol/Ca\(^{2+}\) signaling pathway. (Circulation. 1998;97:851-856.)

Key Words: receptors, adrenergic, beta \( \cdot \) signal transduction \( \cdot \) acetylcholine \( \cdot \) nitric oxide \( \cdot \) hypertension
hypertension. Therefore, in the present study we compared the endothelium-dependent vasodilator responses to these substances in control subjects and hypertensive patients to determine whether the impaired endothelial vasodilator function in essential hypertension is related to a selective defect in NO synthesis or rather reflects a more generalized abnormality of the vascular endothelium.

### Methods

#### Study Population

The most relevant characteristics of the hypertensive patients and control subjects are reported in the Table. Twelve patients with a well-documented history of chronically elevated blood pressure (≥145/95 mm Hg) without any apparent underlying cause who were monitored at the outpatient clinic of the National Heart, Lung, and Blood Institute (NHLBI) were recruited for this study. Each patient had been treated with one or more antihypertensive agents for ≥3 years. In all patients, resistance of hypertension to changes in lifestyle (including diet adjustments) had been demonstrated before initiation of antihypertensive therapy, and causes of secondary hypertension had been ruled out by use of conventional clinical and laboratory criteria. Patients were asked to discontinue all antihypertensive medications 2 weeks before the day of the study; during this period, patients were closely monitored for any evidence of accelerated or malignant hypertension. Patients in whom the withdrawal of antihypertensive therapy was considered hazardous, mostly because of severely elevated blood pressure despite medications, were excluded from the study. None of the patients had a history of diabetes, hyperlipidemia (total plasma cholesterol <240 mg/dL), peripheral vascular disease, coagulopathy, or any other systemic condition, and none were taking medications at the time of the study.

Twelve normal volunteers matched with the patients for approximate age were selected as a control group. Each subject was screened by clinical history, physical examination, ECG, chest roentgenogram, and routine chemical analyses. None had evidence of present or past hypertension, hyperlipidemia, cardiovascular disease, or any other systemic condition, and none were taking medications at the time of the study.

All participants gave written informed consent, and the study protocol was approved by the NHLBI Investigational Review Board.

#### Protocol

All studies were performed in the morning in a quiet room with a temperature of ~22°C. Participants 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 forearm vasculature by means of strain-gauge venous occlusion plethysmography. All drugs used in this study were approved for human use by the Food and Drug Administration in the form of Investigational New Drugs and were prepared by the Pharmaceutical Development Service of the National Institutes of Health according to specific procedures to ensure accurate bioavailability and sterility of the solutions.

While the participants were supine, a 20-gauge polytetrafluoroethylene catheter (Arrow Inc) was inserted into the brachial artery of the nondominant arm (in most cases, the left arm). This arm was slightly elevated above the level of the right atrium, and a mercury-filled silicone elastomer strain gauge was placed in the widest part of the forearm. The strain gauge was connected to a plethysmograph (model EC-4, D.E. Hokanson) that was calibrated to measure the percent change in volume and connected in turn to a chart recorder to record the flow measurements. For each measurement, a cuff placed around 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 ~7 seconds every 15 seconds; seven readings were obtained for each mean value.

Basal measurements were obtained after a 15-minute infusion of saline at 1 mL/min. Forearm blood flow was then measured after the infusion of acetylcholine, isoproterenol, and sodium nitroprusside. Acetylcholine induces vasodilation by stimulating the release of relaxing factors from the vascular endothelium. Isoproterenol was used as a β-adrenoceptor agonist whose vasodilator effect is, at least in part, mediated by NO. Sodium nitroprusside was used as an endothelium-independent vasodilator because its vasodilator effect is largely due to its direct action on smooth muscle cells.

Acetylcholine chloride (Sigma Chemical Co) was infused at 7.5, 15, and 30 μg/min; isoproterenol (Sanofi Winthrop) was infused at 50, 100, and 200 ng/min; and sodium nitroprusside was infused at 0.8, 1.6, and 3.2 μg/min (the infusion rates were 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. A 30-minute rest period was allowed, and another basal measurement was obtained between the infusion of the two drugs. Then, N′-monomethyl-L-arginine (L-NMMA; Sigma), a blocker of NO synthesis, was infused at 4 μmol/min (infusion rate of 1 mL/min) for 15 minutes, and baseline flow measurements were obtained. This dose of L-NMMA has been previously shown to effectively blunt in vivo the synthesis of NO and thereby reduce the vasodilator effect of acetylcholine in the human forearm. Subsequently, cumulative dose-response curves for isoproterenol and sodium nitroprusside were repeated during the concomitant infusion of L-NMMA with the same doses, infusion rates, and resting interval reported above. The infusion of L-NMMA was continued during the resting period. The sequence of infusion of acetylcholine, isoproterenol, and sodium nitroprusside, both before and after the infusion of L-NMMA, was randomized to avoid any bias related to the order of drug infusion.

Because L-NMMA infusion induces a vasoconstrictor response by inhibiting basal release of NO from endothelial cells, the change in baseline flow during L-NMMA administration could nonspecifically affect the vasodilator response to isoproterenol, given that baseline vascular tone is an important determinant of the response to vasodilator stimuli and that intravascular concentrations of isoproterenol are proportionally higher in a vasoconstricted state. Moreover, inhibition of basal NO release by L-NMMA could reduce cGMP content in the underlying smooth muscle cells, thus leading to enhanced activity of cGMP-inhibited phosphodiesterase III, with a consequent increase in cAMP breakdown and blunted vasodilator response to β-adrenoceptor stimulation. To rule out these possibilities, an additional series of experiments was performed in five of the normotensive subjects on a separate occasion. In these experiments, the effect of L-NMMA on the vasodilator response to isoproterenol was assessed with similar time schedule and drug dosages of isoproterenol and L-NMMA, but L-NMMA infusion was accompanied by a low intra-arterial dose of the NO donor sodium nitroprusside. Sodium nitroprusside was infused at 0.2 μg/min because previous studies have shown that this dose is appropriate to counteract the vasocostricter response to L-NMMA.

During the studies, participants were unaware of the drug being infused. All blood pressures were recorded directly from the intra-
Figure 1. Graphs showing forearm blood flow (top) and vascular resistance (bottom) responses to acetylcholine in normotensive subjects and hypertensive patients. Values represent mean±SEM. The probability values refer to the comparison of blood flow and vascular resistance at the three doses of acetylcholine between the two curves.

The infusion of increasing doses of isoproterenol progressively raised forearm blood flow and reduced forearm vascular resistance in both groups (Fig 2). In contrast to the comparison of the vasodilator effect of acetylcholine, both the increase in forearm blood flow and the decrease in forearm vascular resistance induced by isoproterenol were similar in the two groups (Fig 2).

During administration of sodium nitroprusside at the highest dose (3.2 µg/min), forearm blood flow increased to 11.4±3.3 mL·min⁻¹·dL⁻¹ in control subjects and to 11±1.8 mL·min⁻¹·dL⁻¹ in hypertensives; forearm vascular resistance fell to 33±13% of baseline in control subjects and to 27±6% in hypertensives. Both the increase in forearm blood flow and the decrease in forearm vascular resistance induced by sodium nitroprusside were not significantly different in the two groups (P=.48 for forearm blood flow and P=.07 for forearm vascular resistance, respectively).

Effect of L-NMMA on the Vascular Responses to Isoproterenol and Sodium Nitroprusside
No significant change in systemic blood pressure or heart rate was observed with infusion of L-NMMA in either control subjects or hypertensives.

L-NMMA administration significantly blunted the vasodilator effect of isoproterenol in both normotensives and hypertensives compared with saline (Fig 3), with no significant difference between the two groups (P=.77); similarly, forearm vascular resistance fell to 24±5% of baseline in control subjects (P=.005 versus saline), and to 25±9% in hypertensives (P=.001 versus saline), with no significant difference between the two groups (P=.61).

In the additional series of experiments in five of the normotensive subjects, the vasoconstrictive effect of L-NMMA was counteracted by the concomitant infusion of a small dose (0.2 µg/min) of sodium nitroprusside. Therefore, basal forearm blood...
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The main finding of the present study is that compared with control subjects, patients with essential hypertension have a blunted vasodilator response to acetylcholine but a preserved vasorelaxation to isoproterenol. Because isoproterenol-mediated vasodilation is the result of combined effects on both vascular endothelial and smooth muscle cells, we used L-NMMA to determine whether the endothelial NO-dependent component of isoproterenol-induced vasodilation could be defective in hypertensives. We observed that the response to isoproterenol during NO synthesis inhibition by L-NMMA was equally blunted in control subjects and hypertensives, indicating that NO activity during isoproterenol administration was not different between the two groups. L-NMMA attenuation of isoproterenol-induced vasodilation could not be attributed to either change in basal vascular tone or reduction in basal cGMP content in smooth muscle because restoration of baseline conditions by coinfusion of a small dose of sodium nitroprusside did not change the results. This observation is in keeping with the results of a recent study showing that L-NMMA reduces forearm vasorelaxing response to β-adrenergic agonists but not to prostacyclin, a vasodilator that increases cAMP content in vascular smooth muscle, or to verapamil, an endothelium-independent vasodilator that does not use the NO pathway, and supports the view that β-adrenergic stimulation is indeed able to induce endothelial synthesis of NO in the human forearm. In the present study, the response to sodium nitroprusside was similar in control subjects and hypertensives and was not significantly modified by L-NMMA in either group. These findings confirm that the blunted response to acetylcholine observed in hypertensive patients was not related to a nonspecific defect in the responsiveness of vascular smooth muscle cells to nitrovasodilators and demonstrate that the influence of L-NMMA on the response to isoproterenol was specifically related to its inhibition of endogenous production of NO.

Potential Mechanism of Endothelial Dysfunction in Essential Hypertension

We have previously shown that the decreased vasodilator response to acetylcholine observed in patients with essential hypertension...
hypertension is largely related to reduced activity of NO. In a series of other studies,7,8 we have demonstrated that the defect of endothelial NO vasodilator function in hypertensive patients is not related to an isolated abnormality of the muscarinic receptor and is unlikely to be mediated by a selective loss in function of a single G protein because NO-dependent vasodilator responses to other agents, such as substance P and bradykinin, are decreased as well. The results of the present study expand those previous observations by demonstrating that the release of NO after β-adrenoceptor stimulation is preserved, thus suggesting that the defect is not related to a generalized abnormality of endothelial synthesis of NO.

A potential explanation for a selective impairment in the endothelium-dependent vasodilator responsiveness to acetylcholine but not to isoproterenol in hypertensive patients may be related to the different intracellular signaling pathways used by these two agents to stimulate NO production. Thus, pharmacological evidence in experimental vascular preparations24 as well as in the human forearm25 indicates that the endothelium-dependent relaxation to acetylcholine is predominantly mediated by the M3 muscarinic receptor subtype, which couples to stimulate phospholipase C.24,26 This leads to hydrolysis of plasma membrane phosphatidylinositol-4,5-biphosphate into inositol-1,4,5-triphosphate (IP3) and diacylglycerol, with subsequent binding of IP3 to specific receptors on the sarcoplasmic reticulum and cytoplasmic release of Ca2+.27 The increase in intracellular Ca2+ induced by acetylcholine13,14 leads to activation of eNOS through Ca2+/calmodulin binding.28 In contrast, studies using vascular preparations in vitro have shown that β-adrenoceptor stimulation evokes NO-dependent relaxation using cAMP as a second messenger. Indeed, forskolin, a direct activator of adenylyl cyclase, is able to mimic both the increase in cAMP and the vasorelaxation induced by β-adrenergic agonists in rat thoracic aorta, and both of these effects are abolished or strongly blunted by endothelial removal or preincubation with 1-arginine analogues.29 Moreover, a recent study30 directly comparing the intracellular signal transduction mediating acetylcholine- and isoproterenol-induced NO formation in rat aortic rings has shown that inhibition of adenylyl cyclase attenuates the NO/cGMP-mediated vasorelaxing response to isoproterenol but does not affect the response to acetylcholine; conversely, inhibition of intracellular Ca2+ release abolished acetylcholine-induced vasodilation but did not affect the vasodilator effect of isoproterenol.

Hence, taken in conjunction with the results of previous investigations, the present study findings of impaired responsiveness to acetylcholine but preserved NO-dependent response to isoproterenol in essential hypertensives suggest that a defect in the phosphatidylinositol/Ca2+ signaling pathway may be responsible for their endothelial dysfunction. Our results also argue against other potential mechanisms of decreased NO activity in hypertension, such as reduced expression of eNOS, deficit of cofactors (eg, tetrahydrobiopterin) involved in NO synthesis, and increased breakdown of NO, because none of these defects could explain normal NO activity in response to β-adrenoceptor stimulation.

**Study Limitations**

It must be recognized that the present investigation shares the limitations common to all in vivo studies of the intact human circulation. In this regard, our findings do not provide direct evidence of the specific intracellular pathways activated by acetylcholine and isoproterenol to produce endothelium-dependent vasodilation. It is only in the context of previous experimental observations that we can speculate about the intracellular events that occur during the administration of different endothelial agonists and, in light of our results, advance our understanding of the pathophysiology of endothelial dysfunction in essential hypertension.

Also, the present study included a relatively small number of hypertensive patients who had previously been treated with antihypertensive drugs. Therefore, we cannot rule out the possibility that a different subset of essential hypertensives (eg, patients with either milder or more severe forms of hypertension or patient whose hypertension is sensitive to changes in lifestyle) may have a different response to the endothelial agonists used in the present study. For example, previous studies28,29 have shown that the vasorelaxing response to isoproterenol is blunted in borderline hypertensive subjects. The reason for this discrepancy is probably related to the fact that borderline hypertensives have elevated sympathetic activity.29,30 Because prolonged exposure to adrenoceptor agonists may lead to downregulation,31 it is possible that a catecholamine-induced desensitization of β-adrenoceptors could explain the decreased vasodilator response to isoproterenol previously reported in borderline hypertension but not observed in our group of patients with established hypertension.

Finally, because acetylcholine can release other vasoactive factors from vascular endothelium in addition to NO, such as endothelium-derived hyperpolarizing factor (EDHF) and endothelium-derived contracting factors (EDCFs), a blunted vasodilator response to acetylcholine in hypertensive patients could be potentially related to an abnormality in the vascular activity of these factors.23–26 Because we did not assess the contribution of EDHF and EDCFs to the vasoactive response to acetylcholine in the present investigation, we cannot determine their potential involvement in the blunted vasodilation observed in hypertensive patients.

**Conclusions**

In conclusion, the present study demonstrates that patients with essential hypertension with depressed endothelium-dependent vasodilator response to acetylcholine have preserved NO-dependent vasorelaxation to isoproterenol. In light of the different intracellular signaling pathways likely involved in eNOS activation in response to acetylcholine and isoproterenol, these findings suggest that this form of endothelial dysfunction is due to a selective abnormality in NO synthesis, probably related to a defect in the phosphatidylinositol/Ca2+ signaling pathway.

**References**

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