Improved Endothelium-Dependent Vasodilation After Blockade of Endothelin Receptors in Patients With Essential Hypertension

Carmine Cardillo, MD; Umberto Campia, MD; Crescence M. Kilcoyne, RN, MS; Melissa B. Bryant, RN; Julio A. Panza, MD

Background—Hypertensive patients have both impaired endothelium-dependent vasodilation and increased activity of the endothelin (ET-1) system, which participate in their increased vascular tone and may predispose them to atherosclerosis. This study investigated the contribution of increased ET-1 activity to the impaired endothelium-dependent vasodilator function of hypertensive patients.

Methods and Results—Forearm blood flow (FBF) responses to intraarterial infusion of acetylcholine (ACh; 7.5, 15, and 30 μg/min) and sodium nitroprusside (SNP; 0.8, 1.6, and 3.2 μg/min) were assessed by strain-gauge plethysmography before and after nonselective blockade of ETₐ and ETᵦ receptors by combined infusion of BQ-123 (ETₐ blocker; 100 nmol/min) and BQ-788 (ETᵦ blocker; 50 nmol/min). During saline administration, the vasodilator response to ACh was significantly blunted in hypertensive patients compared with controls (P<0.001), whereas the vasodilator effect of SNP was not different between groups (P=0.74). Blockade of ET-1 receptors resulted in a significant increase in FBF from baseline in hypertensive patients (P<0.008) but not in controls (P=0.15). In hypertensive patients, a combined ETₐ/ᵦ blockade resulted in a significant potentiation of the vasodilator response to ACh compared with saline (P=0.01), whereas the response to SNP was unchanged (P=0.44). In contrast, the response to ACh was not significantly modified by ET-1 receptor antagonism in healthy subjects (P=0.14 compared with saline).

Conclusions—These findings indicate that blockade of ET-1 receptors improves endothelium-dependent vasodilator function in hypertensive patients, thereby suggesting that an increased ET-1 activity may play a role in the pathophysiology of this abnormality. (Circulation. 2002;105:452-456.)

Key Words: endothelin  ■  endothelium  ■  vasodilation  ■  hypertension

Several studies from our and other laboratories¹–⁴ have shown that patients with essential hypertension have impaired endothelium-dependent vasodilation, largely because of decreased activity of endothelial nitric oxide (NO).⁵,⁶ Given the potent antiatherogenic effects of NO,⁷,⁸ this form of endothelial dysfunction has been associated with the increased predisposition toward the development of atherosclerosis and other vascular complications observed in hypertensive patients. The precise mechanisms leading to impaired endothelial function in hypertensive vessels, however, have not been fully elucidated.

Accumulating evidence indicates that endothelial control of vasomotor function results from a complex balance between endothelium-derived relaxing and contracting factors. Therefore, a disruption of the critical equilibrium between these opposing forces may predispose the vascular smooth muscle to increased tone and decreased vasomotion.⁹ Principal among the substances produced by vascular endothelial cells is endothelin (ET-1), a powerful vasoconstrictor peptide that exerts its vasoactive effects through interaction with 2 subtypes of specific receptors, ETₐ and ETᵦ.⁹,¹⁰ Over the last few years, a growing interest in the role of ET-1 as a factor involved in the vascular abnormalities of human hypertension has been sparked by the availability of antagonists of ET-1 receptors. In a recent study, we showed that nonselective antagonism of ETₐᵦ receptors results in a significant vasodilator response in hypertensive patients, but not in healthy controls,¹¹ thereby suggesting that an increased activity of the ET-1 system may indeed contribute to the increased vascular tone of patients with hypertension. Whether this abnormality also plays a role in the blunted endothelium-dependent vasorelaxation of hypertensive patients has not been investigated. Therefore, this study was designed to evaluate the potential involvement of ET-1 in the development of endothelial dysfunction in hypertensive resistance vessels in vivo. To this end, we examined the effects of ET-1 receptor antagonism on endothelium-dependent and endothelium-independent vasodilation in a group of hypertensive patients...
Clinical Characteristics of the Study Subjects

<table>
<thead>
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<th>Normal Controls</th>
<th>Hypertensive Patients</th>
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<td>111 ± 4</td>
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<tr>
<td>Triglycerides, mg/dL</td>
<td>104 ± 19</td>
<td>107 ± 18</td>
<td>0.92</td>
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</tbody>
</table>

Data are expressed as mean ± SEM. MAP indicates mean arterial pressure.

and compared the results to those obtained in a control group of healthy subjects.

Methods

Study Subjects

Eighteen patients (Table 1) with a well-documented history of hypertension (blood pressure > 140/90 mm Hg) who were followed at the outpatient clinic of the National Heart, Lung and Blood Institute (NHLBI) were recruited for this study. None of the patients had a history of diabetes, hypercholesterolemia, peripheral vascular disease, coagulopathy, or any disease predisposing them to vasculitis or Raynaud’s phenomenon.

Eighteen normal volunteers matched with the patients for approximate race, sex, and age were selected as a control group (Table). Each subject was screened by clinical history, physical examination, ECG, chest x-ray, and routine chemical analyses. None had evidence of present or past hypertension, hyperlipidemia, diabetes, cardiovascular disease, or any other systemic condition, and none of them was taking medications at the time of the study. The study protocol was approved by NHLBI Institutional Review Board, and all participants gave written informed consent.

Protocols

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 and beverages containing caffeine and from smoking for at least 24 hours before the studies. Each study consisted of an infusion of drugs into the brachial artery and the measurement of the response of the forearm vasculature by means of strain-gauge plethysmography. All drugs used in this study were approved for investigational use in humans by the Food and Drug Administration and were prepared by the Pharmaceutical Development Service of the National Institutes of Health following specific procedures to ensure accurate bioavailability and sterility of the solutions.

While the participants were supine, a 20-gauge Teflon catheter (Arrow Inc) 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 in the widest part of the forearm. The strain gauge was connected to a plethysmograph (model EC-4, D.E. Hokanson), calibrated to measure the percent change in volume, and connected to a chart recorder. 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; 7 readings were obtained for each mean value. Blood pressure was recorded directly from the intraarterial catheter after each flow measurement. Heart rate was recorded from an electrocardiographic lead. Throughout all studies, volumes infused were matched by administration of variable amounts of saline.

Assessment of Vascular Responses to Acetylcholine and Sodium Nitroprusside

Basal measurements were obtained after a 20-minute infusion of saline. Forearm blood flow was then measured after the infusion of acetylcholine (ACH) and sodium nitroprusside (SNP). ACh induces vasodilation by stimulating the release of relaxing factors from the vascular endothelium. SNP was used as an endothelium-independent vasodilator because its effect is largely due to its direct action on smooth muscle cells. ACh chloride (Sigma Chemical Co.) was infused at 7.5, 15, and 30 μg/min, and SNP 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 blood flow was measured during the last 2 minutes. A 30-minute rest period was allowed, and another basal measurement was obtained between the infusions of the 2 drugs. The sequence of ACh and SNP was randomized to avoid bias related to the order of these procedures.

Assessment of Vascular Responses to ET-1 Receptor Blockade

After infusion of ACh and SNP, a 30-minute period of saline infusion was observed to return the forearm blood flow to baseline, and combined infusion of BQ-123 and BQ-788 was then started. Results of a previous study from our laboratory showed that, in hypertensive patients, there is greater vasodilator responsiveness to nonselective ET-A than to a selective ET-A or ET-B blockade. Therefore, we postulated that complete removal of ET-1–dependent vasoconstrictor tone would result in maximal improvement of endothelial function in these patients. For this reason, we chose to use the combination of both BQ-123 and BQ-788 for the purpose of this study.

BQ-123 (Peninsula Laboratories), a synthetic peptide with high potency of antagonism for the ET-A receptor, was infused at 100 nmol/min (100 nmol/mL solution). This dose results in an intravascular concentration ~10-fold higher than the pA2 (negative logarithm of the molar concentration of antagonist that causes a 2-fold parallel shift to the right of the concentration-response curve) at the ET-A receptor, and has been previously shown to effectively counteract the vasoconstrictor effect of endothelin-1 infusion in the human forearm. BQ-788 (Peninsula Laboratories; 50 nmol/mL solution), a synthetic and highly selective antagonist of ET-B receptors, was given at 50 nmol/min (1 mL/min infusion rate). The dose of BQ-788 was selected to achieve a local concentration in the forearm greater than 10-fold higher than the pA2 at the ET-B receptor. BQ-123 and BQ-788 were given for 60 minutes (each at 1 mL/min infusion rate), and forearm blood flow was measured every 10 minutes.

Assessment of Vascular Responses to ACh and SNP During ET-1 Blockade

Infusion of BQ-123 and BQ-788 was then continued at the same doses as before, and forearm blood flow was measured in both groups during administration of ACh at the same doses as before. In hypertensive patients, vascular response to SNP, given at the same doses as during saline infusion, was also assessed during ET-1 receptor antagonism.

Statistical Analysis

Intragroup analyses were performed by paired t tests, 1-way ANOVA, and 2-way ANOVA for repeated measures, as appropriate. Group comparisons were performed using unpaired t test and 2-way ANOVA, as appropriate. All calculated probability values are 2-tailed, and a P value < 0.05 was considered statistically significant. All group data are reported as mean ± SEM.
Results

Mean arterial pressure and heart rate did not change significantly after infusion of any of the drugs used in the study, thus indicating that the drug effects were limited to the infused forearm and did not extend to the systemic circulation. Baseline forearm blood flow was similar in hypertensive patients and healthy controls (Table).

Vascular Responses to Acetylcholine and Sodium Nitroprusside

During the concurrent infusion of saline, infusion of increasing doses of ACh resulted in a progressive increase in forearm blood flow from baseline both in normotensive and hypertensive subjects. The vasodilator response to ACh, however, was significantly blunted in hypertensive patients compared with controls (Figure 1, left). Administration of SNP induced a dose-dependent vasodilator response in both normotensive and hypertensive subjects. In contrast to the ACh results, the vasodilator response to SNP was not significantly different between hypertensive patients and controls (Figure 1, right).

Vascular Responses to ET-1 Receptor Blockade

Changes in forearm blood flow in the 2 groups after ET-1 receptor blockade are presented in Figure 2. In healthy subjects, the combined infusion of BQ-123 and BQ-788 did not significantly modify forearm blood flow from baseline. In contrast, the combination of BQ-123 and BQ-788 resulted in a significant vasodilator response in hypertensive patients. As a result, forearm blood flow values after nonselective ETA/ETB blockade were significantly higher in hypertensive patients than in healthy controls.

Effects of ET-1 Receptor Blockade on Vascular Responses to ACh and SNP

In normal subjects, combined administration of BQ-123 and BQ-788 did not result in any significant change in the vasodilator effect of ACh compared with saline (Figure 3, left). In contrast, nonselective blockade of ETa and ETA receptors resulted in a significant increase in the vasodilator response to ACh compared with saline in hypertensive patients (Figure 3, right). As a result, the vasodilator effect of ACh during ET-1 receptor blockade was not significantly different between healthy subjects and hypertensive patients (P=0.15).

In hypertensive patients, ET-1 receptor blockade was not associated with significant changes in the vasodilator response to SNP compared with saline (Figure 4). Because in hypertensive patients basal forearm blood flow was higher during ET-1 receptor blockade than during saline, both before ACh (P=0.002) and before SNP (P=0.008), comparisons of vascular responses to ACh and SNP between saline and the combination of BQ-123/BQ-788 were also analyzed in terms of forearm blood flow increases from baseline. The average increase in forearm blood flow from baseline during the 3 doses of ACh was significantly higher during blockade of ET-1 receptors (6.2±0.7 mL/min/dl) compared with saline (3.5±0.6 mL·min⁻¹·dL⁻¹; P<0.001). As a result, the average increase in forearm blood flow response to ACh during BQ-123/BQ788 administration was not different between healthy subjects and hypertensive patients (7.3±0.8 and 6.2±0.7 mL·min⁻¹·dL⁻¹, respectively; P=0.20). In contrast to the ACh results, the average increase in forearm blood flow from baseline during the 3 doses of SNP in...
hypertensive patients was similar during ET-1 antagonism (4.9±0.6 mL·min⁻¹·dL⁻¹) and during saline infusion (4.6±0.5 mL·min⁻¹·dL⁻¹; P=0.42).

Discussion

The main new finding of this study is that, in patients with essential hypertension, the blunted endothelium-dependent vasodilator responsiveness to ACh is reversed after blockade of ET-1 receptors. In contrast, endothelium-independent vasorelaxation to SNP is not significantly modified by ET-1 receptor blockade. It has been recently demonstrated that an increased vasoconstrictor activity of ET-1 may contribute to the defective endothelial vasomotor dysfunction in an experimental model of hypertension.

In this study, in the absence of ET_A/B antagonism, hypertensive patients had impaired vasodilator response to ACh, whereas the vasorelaxing effect of SNP was not different from that observed in normal controls. Blockade of ET-1 receptors resulted in a significant vasodilator response in hypertensive patients, but not in normotensive controls, suggesting an increased ET-1 vasoconstrictor tone in hypertensive vessels. Therefore, our group of hypertensive patients was characterized by both impaired endothelium-dependent vasodilation and increased vascular activity of ET-1.

Although previous studies have suggested that the impaired endothelium-dependent vasorelaxation in hypertensive patients is predominantly related to decreased availability of NO, other vasoactive factors released by the endothelium may also play a part in the vasomotor abnormalities characteristic of the hypertensive process. To our knowledge, this study is the first to demonstrate that an increased vasoconstrictor activity of ET-1 may contribute to the defective endothelium-dependent vasodilator function of hypertensive patients. Thus, after infusion of BQ-123 and BQ-788, the vasodilator response to ACh was significantly enhanced in hypertensive patients, resulting in the abolition of the difference in endothelium-dependent vasodilation between patients and controls observed during saline infusion. In contrast, the vasodilator effect of an exogenous NO donor was not significantly modified by ET-1 receptor blockade in hypertensive patients, indicating that endothelium-independent vasodilation is not affected. Taken together, these findings underscore the importance of increased vascular activity of the ET-1 system in the pathophysiology of the defective endothelium-dependent vasorelaxation of patients with hypertension.

The results of the present investigation are in agreement with those of previous studies showing that a blockade of ET-1 receptors is associated with improved endothelium-mediated vasodilation in conditions characterized by both increased ET-1 activity and endothelial vasodilator dysfunction, such as heart failure. Thus, an improvement in flow-mediated vasorelaxation in the brachial artery of patients with congestive heart failure a few weeks after a blocker of ET_A receptors was added to their conventional treatment has recently been reported. Similarly, an improvement in endothelial vasomotor dysfunction has been observed after treatment with ET-1 receptor blockers in rats with heart failure after myocardial infarction. Furthermore, an improvement in endothelial vasodilator function has also been reported after either nonselective ET_A/B, selective ET_A, or selective ET_B blockade in segments of the internal mammary artery obtained from patients with various combinations of cardiovascular risk factors undergoing coronary artery bypass grafting, in whom the ET-1 system may be activated.

Also, the results of this study are in keeping with those of a recent report showing that ET-1 receptor antagonism is able to prevent upregulation of vascular ET-1 and correct endothelial dysfunction in an experimental model of hypertension. In apparent contrast with our findings are those of a previous study showing that, in a group of healthy volunteers, increased ET-1 activity achieved by infusion of exogenous ET-1 does not significantly affect the vasodilator responsiveness to ACh. The reason for this discrepancy, however, may be related to the fact that administration of exogenous ET-1 may not necessarily mimic the complex abnormalities of endothelial regulation of vascular tone observed in hypertension. For example, a differential functional activity of ET_B receptors is present in healthy subjects and hypertensive patients, because in normal subjects ET_B receptors stimulate NO production, a phenomenon that may participate in endothelium-dependent vasorelaxation, whereas in hypertensive patients this mechanism is impaired.

One potential mechanism for the beneficial effects of ET-1 receptor antagonism on endothelial function in hypertensive patients could be the reversal of the imbalance between vasoconstrictor and vasodilator forces within hypertensive vessels after removal of the vasoconstrictor effect of ET-1 on vascular smooth muscle cells. However, the increase in the responsiveness to SNP in hypertensive patients after an ET-1 receptor blockade was modest and did not reach statistical significance. Moreover, a mild, albeit not statistically significant, increase in the responsiveness to ACh after blockade of ET-1 receptors was also observed in healthy controls, in whom ET-1 antagonism was not associated with any vasodilator effect. Therefore, the possibility of nonspecific improvement in the vasodilator function in hypertensive vessels during ET-1 antagonism does not appear to be the only mechanism to explain our study results.

It is reasonable to speculate that other, more specific, mechanisms may contribute to the improvement of endothelial vasodilator function in hypertensive patients after the blockade of ET-1 receptors. It has been recently demonstrated...
in animal models that increased activity of the ET-1 system is associated with augmented vascular release of superoxide anions.22 Further, an imbalance between NO and superoxide anion production has been previously associated with endothelial dysfunction, not only in experimental animals,27 but also in humans.28 In addition to enhanced oxidative stress, other mechanisms involving a cross talk between the ET-1 system and the NO pathway might be involved. For example, ET-1 is known to stimulate leukocyte adhesion, leading to altered vascular homeostasis in states characterized by increased ET-1 activity.29 In addition, angiotensin II stimulates the production of both ET-1 and NO from endothelial cells,30 leading to a complex interaction between these factors by which increased ET-1 release may counteract AT1 receptor-mediated stimulation of NO production. It may therefore be postulated that a reduction in the activity of the ET-1 system after ET-1 receptor blockade may restore NO bioavailability within hypertensive vessels, thus leading to the improvement in endothelium-dependent vasorelaxation observed in hypertensive patients. Although in this study we did not perform experiments during NO inhibition, previous investigations from our6 and other28 laboratories have demonstrated that the impaired endothelium-dependent vasodilator function in hypertensive patients is predominantly related to the decreased bioavailability of NO. Therefore, it seems reasonable to postulate that an augmentation of NO activity is a likely mechanism for the improved endothelium-mediated vasorelaxation observed in hypertensive patients after the blockade of ET-1 receptors.

In conclusion, our results demonstrate that the blockade of ET-1 receptors improves endothelium-dependent vasorelaxation in hypertensive patients. This effect may be of considerable clinical relevance, because endothelial dysfunction is increasingly recognized as a therapeutic target to reduce the atherosclerotic potential in hypertension. In addition to the demonstrated ability of ET-1 antagonists to reduce vascular tone11 and lower blood pressure,31 their capacity to improve endothelial function, demonstrated in this study, may represent an important therapeutic advantage to lower the cardiovascular risk of patients with hypertension.

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References
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