Role of Endothelin in the Increased Vascular Tone of Patients With Essential Hypertension

Carmine Cardillo, Crescence M. Kilcoyne, Myron Waclawiw, Richard O. Cannon III, Julio A. Panza

Abstract—We investigated the possible role of endothelin in the increased vasoconstrictor tone of hypertensive patients using antagonists of endothelin receptors. Forearm blood flow (FBF) responses (strain-gauge plethysmography) to intraarterial infusion of blockers of endothelin-A (ET$_A$) (BQ-123) and endothelin-B (ET$_B$) (BQ-788) receptors, separately and in combination, were measured in hypertensive patients and normotensive control subjects. In healthy subjects, BQ-123 alone or in combination with BQ-788 did not significantly modify FBF ($P$=0.78 and $P$=0.63, respectively). In hypertensive patients, in contrast, BQ-123 increased FBF by 33±7% ($P$<0.001 versus baseline), and the combination of BQ-123 and BQ-788 resulted in a greater vasodilator response (63±12%; $P$=0.006 versus BQ-123 alone in the same subjects). BQ-788 produced a divergent vasoactive effect in the two groups, with a decrease of FBF (17±5%; $P$=0.004 versus baseline) in control subjects and transient vasodilation (15±7% after 20 minutes) in hypertensive patients ($P$<0.001, hypertensives versus controls). The vasoconstrictor response to endothelin-1 was slightly higher ($P$=0.04) in hypertensive patients (46±4%) than in control subjects (32±4%). Our data indicate that patients with essential hypertension have increased vascular endothelin activity, which may be of pathophysiologic relevance to their increased vascular tone. In these patients, nonselective ET$_A$ and ET$_B$ blockade seems to produce a greater vasodilator effect than selective ET$_A$ blockade. (Hypertension. 1999;33:753-758.)

Key Words: endothelin  ■  hypertension, essential  ■  vascular tone  ■  receptors, endothelin  ■  blood flow

Endothelin is the most potent vasoconstrictor substance produced by the cardiovascular system, and therefore, a pathophysiologic role for this peptide has been proposed in those conditions, such as arterial hypertension, characterized by increased vascular tone.1 However, the involvement of endothelin in the development or maintenance of human hypertension remains unclear. Although some studies have reported modest increases in plasma levels of endothelin-1 in hypertensive patients,2-4 other investigations have failed to demonstrate higher circulating levels of the peptide in these patients.5-7 The pathophysiologic significance of elevated plasma endothelin levels is, in itself, questionable, because the peptide acts predominantly in an autocrine and paracrine manner and its secretion by endothelial cells is polarized toward the underlying vascular smooth muscle.8 Consequently, plasma endothelin levels are largely the result of variable spillover into the bloodstream, and therefore, the circulating peptide may not necessarily reflect endothelial cell production or its biological effect on smooth muscle cells.

The vasoactive properties of endothelin are mediated by two subtypes of specific receptors: endothelin-A (ET$_A$) and endothelin-B (ET$_B$).9,10 In vascular smooth muscle cells, both receptors seem to mediate vasoconstriction,11,12 whereas ET$_B$ receptors on endothelial cells cause vasodilation through the release of nitric oxide and prostacyclin.13 Pharmacological agents that selectively and nonselectively block ET$_A$ and ET$_B$ receptors have recently been developed. The use of these substances provides an important means for the in vivo assessment of the role of endothelin in cardiovascular homeostasis. We designed the current study to determine the possible involvement of endothelin in the increased vascular tone of patients with essential hypertension by comparing vascular responses to endothelin receptor antagonists in hypertensive patients and normotensive subjects.

Methods

Study Subjects

All participants in the various protocols of this study were taken from an initial population of 19 hypertensive patients and 18 normotensive control subjects who took part in protocol 1. Hypertensive patients who had a well-documented history of chronically elevated blood pressure ($\geq$140/90 mm Hg) without any apparent underlying cause and who were followed at the outpatient clinic of the National Heart, Lung, and Blood Institute (NHLBI) were recruited for this study. Healthy volunteers matched in each protocol with the patients for approximate race, sex, and age were selected as a control group. The selection and exclusion criteria for patients and controls, as well as the screening process they underwent before enrollment have been

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reported elsewhere. The study protocol was approved by the NHLBI Investigational Review Board, and all participants gave written informed consent.

**Protocols**
Each study consisted of infusion of drugs into the brachial artery and measurement of the response of the forearm vasculature by means of strain-gauge venous-occlusion plethysmography, according to a methodology reported elsewhere. All drugs used in this study were approved for human use by the Food and Drug Administration in the form of Investigational New Drug (IND) 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. Forearm blood flow (FBF) tracings were analyzed by an experienced observer (C.M.K.) who, at the time of the analysis, was unaware of the subjects’ diagnoses and of the responses of the overall subject population. The data were grouped according to diagnosis (hypertensive or normotensive) after analysis of all the individual results was completed.

Because of the prolonged infusion time required to assess the hemodynamic effect of the different substances and their relatively long-lasting effects, in subjects and patients participating in more than one protocol, studies were performed on separate days at least 1 week apart. Normotensive subjects and hypertensive patients were studied approximately on alternate days throughout the study period.

**Protocol 1: Assessment of Vascular Responses to ET\textsubscript{A} Receptor Blockade**
Basal measurements were obtained after a 15-minute infusion of saline at 1 mL/min. Then, 18 normotensive subjects and 19 hypertensive patients (whose clinical characteristics are reported in the Table) received concurrent intraarterial infusion of BQ-123 (100 nmol/min) and BQ-788. BQ-788 (Peninsula Laboratories; 50 nmol/mL solution) is a synthetic and highly selective antagonist of ET\textsubscript{B} receptors and 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 more than 10-fold higher than the pA\textsubscript{2} at the ET\textsubscript{B} receptor. The combination of BQ-123 and BQ-788 was infused for 60 minutes, and FBF was measured every 10 minutes.

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Data are mean±SEM. NT indicates normotensive subjects; HT, hypertensive patients; MAP, mean arterial pressure; and HDL, high-density lipoprotein.

**Protocol 2: Assessment of Vascular Responses to Nonselective ET\textsubscript{A} and ET\textsubscript{B} Blockade**
After basal measurements were obtained, 10 normotensive subjects and 10 hypertensive patients (Table) received concurrent intraarterial infusion of BQ-123 (100 nmol/min) and BQ-788. BQ-788 (Peninsula Laboratories; 50 nmol/mL solution) is a synthetic and highly selective antagonist of ET\textsubscript{B} receptors and 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 more than 10-fold higher than the pA\textsubscript{2} at the ET\textsubscript{B} receptor. The combination of BQ-123 and BQ-788 was infused for 60 minutes, and FBF was measured every 10 minutes.

**Protocol 3: Assessment of Vascular Responses to Selective ET\textsubscript{B} Blockade**
After basal measurements were obtained, 10 normotensive subjects and 11 hypertensive patients (Table) received intraarterial infusion of BQ-788 (50 nmol/min) for 60 minutes, and FBF was measured every 10 minutes.

**Protocol 4: Assessment of Vascular Responses to Norepinephrine and Endothelin-1**
After basal measurements were obtained, 12 normotensive subjects and 12 hypertensive patients (Table) received intraarterial infusion of norepinephrine and endothelin-1. Because the vascular hypertrophy commonly present in hypertensive patients may be responsible for a nonspecifically enhanced responsiveness to vasoconstrictor substances, norepinephrine was used to rule out the possibility that a difference between the two groups in the vasoconstrictor response to endothelin-1 could be attributable to structural changes leading to nonspecific responses of the hypertensive vasculature. Norepinephrine (Sanofi Winthrop; 240 pmol/mL solution) was infused at 60, 120, and 240 pmol/min (infusion rates were 0.25, 0.5, and 1 mL/min, respectively). Each dose was given for 5 minutes, and FBF was measured during the last 2 minutes.

After a 30-minute resting period, another FBF measurement was obtained to ascertain the return to baseline values, and endothelin-1 infusion was started. Endothelin-1 (Bachem Inc; 5 pmol/mL solution) was given at 5 pmol/min (1 mL/min infusion rate) for 60 minutes. FBF was measured after 5 minutes and then at 10-minute intervals up to 30 minutes after the end of infusion; one last blood flow measurement was obtained 60 minutes after the end of endothelin-1 infusion.

**Statistical Analysis**
Within-group analyses were performed by paired t test and one-way and two-way analysis of variance for repeated measures. Group
comparisons were performed using unpaired Student’s t test and two-way analysis of variance, as appropriate. Factors potentially affecting responses to selective ET\textsubscript{A} blockade in normotensive subjects and hypertensive patients were identified by linear regression analysis. The covariates considered were mean arterial pressure, plasma cholesterol, age, weight, sex, and race. All covariates were examined as predictors of FBF response to endothelin receptor blockers as a group in a multivariate model and in a stepwise regression model. All calculated probability values are two-tailed, and a \( P < 0.05 \) was considered to indicate statistical significance. Group data are reported as mean \( \pm \) 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. Baseline FBF was similar in hypertensive patients and control subjects at all times (all \( P > 0.05 \)).

Vascular Responses to ET\textsubscript{A} Receptor Blockade
Changes in FBF in the two groups after selective ET\textsubscript{A} antagonism are shown in Figure 1. In control subjects, infusion of BQ-123 did not significantly modify FBF from baseline (\( P = 0.78 \)). In contrast, in hypertensive patients, BQ-123 administration resulted in a significant vasodilator response (\( P < 0.001 \) versus baseline). Analysis of the FBF response to BQ-123 in the 16 hypertensive patients who were nonsmokers demonstrated that the vasodilator response to ET\textsubscript{A} receptor blockade (maximum increase in FBF from baseline: 33 \( \pm \) 7\%) was similar to that of the overall hypertensive population (\( P = 0.91 \)).

The results of multivariate analysis performed to identify potential predictors of the response to BQ-123 in normotensive subjects showed that male sex was the only positive independent predictor in the stepwise approach (\( r = 0.52, P = 0.03 \) and tended to be a significant predictor (\( P = 0.07 \)) in the full model. No significant predictor of the response to ET\textsubscript{A} receptor blockade was found in hypertensive patients.

Vascular Responses to Nonselective ET\textsubscript{A} and ET\textsubscript{B} Blockade
Changes in FBF in the two groups after nonselective endothelin antagonism are shown in Figure 2. In control subjects, infusion of BQ-123 and BQ-788 did not significantly modify FBF from baseline (\( P = 0.63 \)). In contrast, in hypertensive patients, the combination of BQ-123 and BQ-788 resulted in a significant vasodilator response (\( P < 0.001 \) versus baseline). Importantly, the vasodilator effect of nonselective ET\textsubscript{A} and ET\textsubscript{B} blockade in hypertensive patients was significantly greater than that produced in the same patients by selective ET\textsubscript{A} antagonism (Figure 3).

As expected, baseline forearm vascular resistance was significantly higher in hypertensive patients than in controls (\( P < 0.016 \)). In hypertensive patients, the combination of BQ-123 and BQ-788 for 60 minutes reduced forearm vascular resistance by 37\% (from 33 \( \pm \) 2.4 mm Hg/mL min \(^{-1} \) dL \(^{-1} \); \( P < 0.001 \)). In contrast, no significant change in forearm vascular resistance from baseline (22.8 \( \pm \) 2.3 mm Hg/mL min \(^{-1} \) dL \(^{-1} \)) was determined by nonselective endothelin blockade (22.6 \( \pm \) 3.2 mm Hg/mL min \(^{-1} \) dL \(^{-1} \)) in control subjects (\( P = 0.91 \)). As a result of this differential effect in normotensive subjects and hypertensive patients, no

Figure 1. FBF responses to intraarterial infusion of BQ-123 (100 nmol/min) in 18 control subjects and 19 hypertensive patients. Values are mean \( \pm \) SEM. \( P \) value refers to comparison between the two groups in blood flow changes from baseline during selective ET\textsubscript{A} blockade by two-way ANOVA.

Figure 2. FBF responses to intraarterial infusion of BQ-123 (100 nmol/min) in combination with BQ-788 (50 nmol/min) in 10 control subjects and 10 hypertensive patients. Values are mean \( \pm \) SEM. \( P \) value refers to comparison between the two groups in blood flow changes from baseline during selective ET\textsubscript{A} blockade by two-way ANOVA.

Figure 3. FBF responses to intraarterial infusion of BQ-123 (100 nmol/min), alone or in combination with BQ-788 (50 nmol/min) in 10 hypertensive patients. Values are mean \( \pm \) SEM. \( P \) value refers to comparison between the two curves by two-way ANOVA for repeated measures.
The infusion of increasing doses of norepinephrine induced a progressive vasoconstrictor response in hypertensive patients and controls. At the highest doses of norepinephrine, FBF was reduced by 23% (from 2.7±0.2 to 2.1±0.2 mL min⁻¹ · dL⁻¹; P<0.001 versus baseline) in hypertensive patients and 24% (from 2.9±0.2 to 2.2±0.2 mL min⁻¹ · dL⁻¹; P<0.001 versus baseline) in controls, without a significant difference between the two groups (P=0.59).

Discussion

The present study demonstrates that selective ET₄ and nonselective blockade of endothelin receptors determines a vasodilator effect in patients with essential hypertension but not in normotensive controls. These findings indicate that the vasoconstrictor activity of endogenous endothelin is enhanced in patients with essential hypertension. The increase in vascular resistance that is characteristic of these patients was normalized by nonselective endothelin receptor blockade, suggesting that endothelin may play a role in the pathophysiology of the hypertensive process.

Our results are in keeping with those of a previous study in experimental models of hypertension demonstrating that nonselective antagonism of ET₄ and ET₆ receptors markedly lowers blood pressure in hypertensive animals but has only minor effect in healthy controls, thereby suggesting that the pressor activity of endothelin is upregulated in hypertension. In contrast, under physiological conditions, the peptide does not seem to modulate vasoconstriction. In fact, it may even act as a mild vasodilator, as suggested by the results of endothelin-1 gene targeting studies in experimental animals. Mice in which endothelin-1 production is decreased by heterozygous knockout of the gene have higher blood pressure than normal controls, indicating that the peptide physiologically acts as a depressor rather than a pressor agent. However, other studies have reported that systemic administration of endothelin receptor blockers decreases peripheral vascular resistance and blood pressure in normotensive humans as well. Moreover, selective ET₆ and nonselective endothelin antagonism result in a slight vasodilator response in the human skin microcirculation. Also, other investigators have reported that in the forearm circulation, ET₆ receptor blockade with BQ-123 induces a vasodilator response in normal subjects. The discrepancy between our results and those of previous studies with BQ-123 cannot be accounted for by differences in the study protocols, because we used doses and infusion times of BQ-123 similar to those used in previous studies. It is possible that interindividual variability in the hemodynamic responsiveness to blockade of ET₆ receptors could be responsible for the discrepancies between different studies, but the causes of this phenomenon remain to be elucidated.

To investigate whether selective ET₆ or nonselective blockade of endothelin receptors provides a greater vasodilator effect in hypertensive patients, we compared the responses to BQ-123 alone and to the combination of BQ-123 and BQ-788. Our findings demonstrated that combined blockade of ET₆ and ET₄ receptors produces a higher degree of vasodilation compared with selective ET₆ blockade. This finding is in keeping with the results of a recent study.

Figure 4. FBF responses to intraarterial infusion of BQ-788 (50 nmol/min) in 10 control subjects and 11 hypertensive patients. Values are mean±SEM. P value refers to comparison between the two groups after the combined infusion of BQ-123 and BQ-788 (P=0.65).

Figure 5. FBF responses to endothelin-1 (ET-1, 5 pmol/min) in control subjects and hypertensive patients. Values are mean±SEM. P value refers to difference between the two groups in FBF response to endothelin-1 infusion by two-way ANOVA.

significant difference in forearm vascular resistance was observed between the two groups after the infusion of BQ-123 and BQ-788 (P=0.65).

Vascular Responses to ET₆ Receptor Blockade

In control subjects, infusion of BQ-788 resulted in a decrease in FBF from baseline (P=0.004). In hypertensive patients, however, BQ-788 administration resulted in a short-lasting vasodilator effect, followed by the return of FBF to levels similar to baseline (P=0.32). As a result of the divergent hemodynamic effect in normotensive subjects and hypertensive patients, FBF during BQ-788 infusion was significantly higher in hypertensive patients than in controls (Figure 4).

Vascular Responses to Endothelin-1

Endothelin-1 caused a slow-onset, long-lasting vasoconstrictor response in both hypertensive patients (P<0.001) and control subjects (P<0.001 versus baseline), but this effect was higher in hypertensive patients than in normotensive subjects (Figure 5).
showing that bosentan, a nonselective antagonist of endothelin receptors, significantly lowers systemic blood pressure in patients with essential hypertension and suggests that vasoconstriction mediated through smooth muscle ET<sub>A</sub> receptors may contribute to the increased vascular tone of hypertensive patients. This view is also supported by the results of our experiments of selective blockade of ET<sub>A</sub> receptors. Thus, in normotensive subjects, infusion of BQ-788 alone resulted in vasoconstriction, in keeping with the results reported by other investigators using similar methodology. This indicates that under physiological conditions, release of vasodilator substances from vascular endothelium due to stimulation of ET<sub>B</sub> receptors participates in endothelin-mediated regulation of vascular tone. In hypertensive patients, in contrast, selective ET<sub>B</sub> blockade led to transient vasodilation, followed by the return of FBF to baseline values. This suggests an impairment of ET<sub>B</sub>-mediated vasodilation in these patients, probably in relation to the endothelial dysfunction found in essential hypertension. The finding of the vasodilator response to BQ-788 in hypertensive patients fading over time could be related to increased availability of endothelin to produce ET<sub>B</sub>-mediated vasoconstriction due to displacement of the peptide from the ET<sub>B</sub> clearance receptors.

To ascertain whether the increased endothelin-mediated vasoconstrictor tone observed in our group of hypertensive patients could be attributable to an increased vasoconstrictor effect of the peptide, we compared the vasoactive effect of exogenous endothelin in hypertensive patients and control subjects. We observed that the vasoconstrictor response to endothelin is higher in hypertensive than in normotensive individuals. This effect is unlikely to be explained by non-specific enhancement of vascular reactivity to vasoconstrictor stimuli induced by structural changes of the hypertensive vessels because the response to norepinephrine was similar in the two groups. Moreover, an enhanced response to endothelin-1 infusion in hypertensive patients has previously been observed also in the peripheral veins, which do not undergo vascular hypertrophy as a consequence of high blood pressure. The increased vasoconstrictor effect of endothelin in hypertensive patients may be related to enhanced sensitivity of vascular smooth muscle (upregulation of endothelin receptors or postreceptor sensitization) or, alternatively, to decreased production of vasodilator substances in response to endothelial ET<sub>B</sub> receptor stimulation. An augmented production of the peptide may also play a part in the enhanced endothelin-dependent vasoconstrictor tone of hypertensive patients, as supported by previous findings of Schiffrin et al showing enhanced expression of the endothelin-1 gene in resistance arteries of hypertensive individuals.

The findings of the present study may have important pathophysiological and therapeutic implications. First, endothelin is able to induce proliferation of vascular smooth muscle and hypertrophy of cardiac myocytes. Thus, the enhanced activity of endothelin in the cardiovascular system of hypertensive patients not only may result in increased vascular tone but also may be involved in the structural changes of the heart and blood vessels associated with hypertension. In fact, a protective effect against cardiac remodeling, with improvement in survival, has recently been shown in animals with chronic heart failure treated with BQ-123. Second, the present demonstration that endothelin activity is increased in hypertensive patients and plays a role in their increased vascular tone suggests that endothelin receptor antagonists may be an attractive therapeutic alternative in essential hypertension.

In conclusion, patients with essential hypertension have increased vascular endothelin activity that may be of pathophysiological relevance to their increased vascular tone. This phenomenon seems predominantly related to increased production of the peptide and/or to dysfunctional ET<sub>A</sub>-mediated vasodilation. In hypertensive patients, nonselective ET<sub>A</sub> and ET<sub>B</sub> blockade seems to produce a greater vasodilator effect than selective ET<sub>A</sub> blockade.

References


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