Interactions Between Nitric Oxide and Endothelin in the Regulation of Vascular Tone of Human Resistance Vessels In Vivo

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Abstract—Endothelial release of nitric oxide (NO) contributes to the regulation of vascular tone by inducing vascular relaxation. In addition, NO may inhibit the synthesis and hemodynamic effects of endothelin-1 (ET-1), a powerful endothelium-derived vasoconstrictor peptide that may stimulate NO production. However, whether NO and ET-1 physiologically interact to regulate vascular tone in humans has not been defined. In this study, the interactions between the L-arginine NO pathway and the ET-1 system in the regulation of vascular tone in human forearm resistance vessels were examined in vivo. Vasomotor response to the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA, 4 μmol/min for 30 minutes) was measured during either saline infusion or blockade of ET-1 receptors. Endothelin-A (ET_A) and endothelin-B (ET_B) receptor blockade was achieved by infusion of BQ-123 (100 nmol/min) and BQ-788 (50 nmol/min), respectively, separately and in combination. Drugs were infused into the brachial artery, and the forearm blood flow (FBF) response was measured by strain-gauge plethysmography. During saline infusion, L-NMMA administration significantly decreased FBF (25%, $P<0.01$ versus baseline). This effect was significantly blunted during nonselective blockade of ET-1 receptors (7% decrease in FBF, $P=0.02$ versus the effect of L-NMMA during saline infusion). Selective ET_A blockade did not modify the vasoconstrictor response to L-NMMA (26% decrease in FBF, $P=0.66$ versus the effect of L-NMMA during saline infusion), but selective ET_B receptor antagonism caused significant diminution of the hemodynamic response to NO inhibition (8% decrease in FBF, $P=0.04$ versus the effect of L-NMMA during saline infusion). Thus ET-1 contributes to the regulation of vascular tone by stimulating NO activity. This effect is mediated through endothelial ET_B receptors and may be relevant in conditions associated with endothelial dysfunction. **(*Hypertension*. 2000;35:1237-1241.)**

Key Words: endothelin ■ nitric oxide ■ vascular tone ■ receptors, endothelin ■ blood flow

Endothelial synthesis and release of vasoactive mediators has been widely recognized as one of the main mechanisms involved in the regulation of vascular tone.[1] Among several players involved in this process, the L-arginine nitric oxide (NO) pathway and the endothelin (ET-1) system have generated much attention, not only in relation to their vasoactive properties but also because of their role in the overall modulation of vascular homeostasis. Thus, NO is not only a potent vasodilator, but it also inhibits platelet aggregation, monocyte adhesion, and smooth muscle proliferation. ET-1, in addition to its effects on vasomotion mediated by 2 receptors subtypes (endothelin-A [ET_A] and endothelin-B [ET_B]), is an important mediator of mitogenesis and contributes to vascular remodeling by enhancing smooth muscle cell proliferation.[2] Previous observations from in vitro and other experimental models have indicated the complex interactions between NO and ET-1. Thus, NO released from endothelial cells permanently inhibits the synthesis[3,4] and the vasoconstrictor effects[5–8] of ET-1, which in turn may stimulate NO production by means of autocrine interactions with endothelial ET_B receptors.[9,10]

In humans, the role of NO in the maintenance of vascular tone in human resistance vessels has been well established by the use of L-arginine analogues.[11,12] However, there is little information about possible interactions between NO and ET-1 because there have been no appropriate tools with which to investigate ET-1 activity in vivo. Although plasma ET-1 levels have been frequently used in previous studies, they may not necessarily reflect the vascular activity of the peptide because ET-1 acts primarily as a local mediator and is secreted by endothelial cells toward the smooth muscle.[13] Recently, selective and nonselective blockers of ET-1 receptors have become available for clinical studies. This has provided a more suitable tool to assess the role of ET-1 in vascular homeostasis.

This study was designed to investigate the interactions between NO and ET-1 in the regulation of vascular tone in the forearm circulation of healthy subjects. To accomplish this, we analyzed forearm blood flow (FBF) responses to NO synthesis inhibition in the absence or presence of selective or nonselective blockade of ET_A or ET_B receptors.

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Methods

Study Population

Healthy volunteers with no family history of diabetes or hypertension were included in this study. The results of each subject's clinical history, physical examination, routine chemical analyses, and electrocardiographic and chest radiographic studies were used as screening criteria. Those with a history of or evidence of present or past arterial hypertension, hypercholesterolemia (total cholesterol >200 mg/dL), diabetes mellitus, cardiac disease, peripheral vascular disease, coagulopathic conditions, or other disease predisposing them to vasculitis or Raynaud's phenomenon were excluded from the study. None of the volunteers was taking medication or vitamin supplements. The study protocol was approved by the National Heart, Lung, and Blood Institute Investigational Review Board. Participants gave written informed consent for all procedures.

Study Protocol

All studies were performed in the morning in a quiet room with a temperature of approximately 22°C. Participants were asked to refrain from drinking alcohol or beverages containing caffeine and from smoking for at least 24 hours before the studies. While each participant was supine, a 20-gauge Teflon catheter (Arrow Inc) was inserted into the brachial artery of the left arm for drug infusion.

The infused 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.[14] The strain gauge was connected to a plethysmograph (model EC-4, D.E. Hokanson), calibrated to measure the percent change in volume, and was connected to a chart recorder to record the flow measurements. To obtain 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. One minute before each measurement, a wrist cuff was inflated to suprasystolic pressures to exclude the hand circulation.[15] Flow measurements were recorded for ≈7 seconds every 15 seconds, and 7 readings were obtained for each mean value. During the studies, blood pressure was recorded directly from the intra-arterial catheter immediately after each flow measurement, and heart rate was continuously recorded by ECG.

Effects of Nonselective ET-1 Blockade on NO Synthesis Inhibition

To investigate the effects of nonselective blockade of ET-1 receptors on vascular responses to NO synthesis inhibition, 12 subjects (8 men and 4 women; age 49±2 years) underwent assessment of the hemodynamic response to N^G-monomethyl-L-arginine (L-NMMA) in the absence and presence of ET-1 receptor antagonism. Because of the prolonged time required to assess the hemodynamic action of the infused drugs and their relatively long-lasting effects, studies were performed on separate days at least 1 week apart and in random sequence. Volumes infused throughout the studies were matched by the administration of saline in variable amounts.

On one study day, after having undergone forearm preparation, subjects received intra-arterial infusion of saline for 15 minutes at 1 mL/min, and baseline blood flow was measured. Then, intra-arterial infusion of L-NMMA (Calbiochem; 4 μmol/mL solution) was started at 4 μmol/min (infusion rate 1 mL/min), and blood flow was measured 30 minutes later. L-NMMA is an arginine analogue that competitively antagonizes the synthesis of NO from L-arginine,[16] and prior studies[11,12] have indicated that the dose of 4 μmol/min effectively inhibits endogenous NO activity.

On a different day, subjects underwent forearm preparation, after which baseline measurements were taken. The study participants then received a combined intra-arterial infusion of BQ-123 and BQ-788. BQ-123 (Bachem) is a synthetic peptide with high potency of antagonism for the ET_A receptor.[17] BQ-788 (Peninsula) is a synthetic and highly selective antagonist of the ET_B receptor.[18] BQ-123 (100 nmol/mL solution) was infused at 100 nmol/min (infusion rate, 1 mL/min), and BQ-788 (50 nmol/mL solution) was given at 50 nmol/min (infusion rate, 1 mL/min). Doses of BQ-123 and BQ-788 were similar to those used in other studies[19–21] and were originally chosen to allow an intravascular concentration ≈10-fold higher than the pA_2 (a 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[17] and the ETB receptors,[18] respectively. BQ-123 and BQ-788 were infused for 1 hour; thereafter, an intra-arterial infusion of L-NMMA was superimposed in each subject at 4 μmol/min, and FBF measurements were obtained 30 minutes later.

To determine the specificity of the effect of ET-1 receptor blockade on L-NMMA–induced vasoconstriction, on a different day we investigated the effect of BQ-123 and BQ-788 on vasoconstriction induced by norepinephrine. After having undergone forearm preparation, subjects received an intra-arterial infusion of saline at 1 mL/min for 15 minutes. Subsequent baseline blood flow measurements were obtained, after which an intra-arterial infusion of norepinephrine (Sanofi Winthrop; 240 pmol/mL solution) was administered at 60, 120, and 240 pmol/min (infusion rates 0.25, 0.5, and 1.0 mL/min, respectively). Each dose was given for 5 minutes, and FBF was measured during the last 2 minutes. After a 60-minute resting period, another blood flow measurement was obtained, after which an intra-arterial infusion of BQ-123 and BQ-788 was administered at the same doses and for the same time indicated above. The infusion of norepinephrine described previously was repeated during the concurrent blockade of ET-1 receptors.

Effects of Selective ET_A or ET_B Blockade on NO Synthesis Inhibition

To investigate the mechanism involved in the effect of ET-1 receptor blockade on the hemodynamic response to NO inhibition, we assessed the effect of selective blockade of either ET_A or ET_B blockade on the vasomotor response to L-NMMA.

To determine the specific contribution of ET_A receptors, 8 subjects (4 men and 4 women; age 46±2 years) underwent forearm preparation, and baseline measurements were taken. Each study participant then received the selective ET_A receptor antagonist BQ-123 at 100 nmol/min (infusion rate 1 mL/min) for 60 minutes, after which measurements of FBF were taken. After 1 hour of ET_A receptor blockade, an intra-arterial infusion of L-NMMA was superimposed in each subject at 4 μmol/min, and FBF measurements were obtained 30 minutes later.

To determine the specific contribution of ET_B receptors, the effect of BQ-788 on the vascular response to L-NMMA was studied in a different group of 8 subjects (5 men and 3 women; age 49±2 years). After each participant underwent forearm preparation, baseline measurements were taken and the selective ET_B receptor antagonist BQ-788 was infused at 50 nmol/min (infusion rate 1 mL/min) for 60 minutes, after which FBF measurements were obtained. After 1 hour of ET_B receptor blockade, an intra-arterial infusion of L-NMMA was superimposed in each subject at 4 μmol/min, and FBF measurements were obtained 30 minutes later.

Statistical Analysis

All comparisons were performed by paired or unpaired Student's *t* test and by 1-way ANOVA for repeated measures, as appropriate. All calculated probability values are 2-tailed, and $P<0.05$ indicates statistical significance. 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 substances used in the different studies, which indicates that the hemodynamic effects of the study drugs were limited to the infused forearm.

Effects of Nonselective ET-1 Blockade on NO Synthesis Inhibition

During saline infusion, NO synthesis inhibition resulted in a significant vasoconstrictor response: Forearm blood flow

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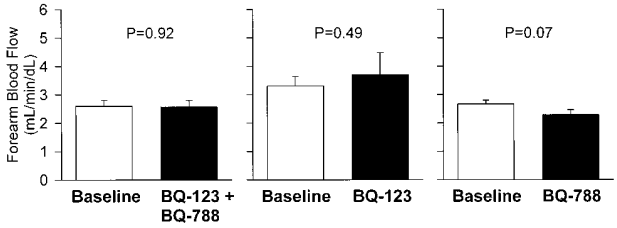


Figure 1. FBF values at baseline (open bars) and during 60 minutes of intra-arterial infusion (solid bars) of BQ-123 (100 nmol/min) in combination with BQ-788 (50 nmol/min; left), BQ-123 alone (100 nmol/min; center), and BQ-788 alone (50 nmol/min; right). Data are mean±SEM.

decreased from 2.4±0.2 mL · min^{-1} · dL^{-1} at baseline to 1.7±0.1 mL · min^{-1} · dL^{-1} after 30 minutes of L-NMMA administration (25% decrease, $P<0.01$). Nonselective blockade of ET_A and ET_B receptors did not result in any significant change in FBF from baseline. Thus FBF was 2.6±0.2 mL · min^{-1} · dL^{-1} at baseline and 2.6±0.2 mL · min^{-1} · dL^{-1} after 60 minutes of coinfusion of BQ-123 and BQ-788 (Figure 1, left). In contrast to the results observed during concomitant saline administration, L-NMMA produced only a slight and nonsignificant decrease in FBF during nonselective blockade of ET-1 receptors: 2.5±0.2 mL · min^{-1} · dL^{-1} before and 2.3±0.2 mL · min^{-1} · dL^{-1} after L-NMMA (7% change, $P=0.10$). Thus the vasoconstrictor effect of L-NMMA was significantly higher in the absence than in the presence of nonselective ET-1 receptor blockade (Figure 2).

Infusion of norepinephrine resulted in a significant vasoconstrictor response during the infusion of either saline (FBF 2.9±0.2 mL · min^{-1} · dL^{-1} before and 2.2±0.2 mL · min^{-1} · dL^{-1} after norepinephrine; 25% change, $P<0.01$) or ET-1 receptor blockers (FBF 4.3±0.5 mL · min^{-1} · dL^{-1} before and 3.3±0.3 mL · min^{-1} · dL^{-1} after norepinephrine; 24% change, $P=0.02$). Thus, in contrast to the results obtained with L-NMMA, the vasoconstrictor effect of norepinephrine was not modified by nonselective ET-1 receptor antagonism (Figure 2).

Effects of Selective ET_A or ET_B Blockade on NO Synthesis Inhibition

Selective blockade of ET_A receptors did not result in a change in FBF: 3.3±0.3 mL · min^{-1} · dL^{-1} before and 3.7±0.8 mL · min^{-1} · dL^{-1} after BQ-123 infusion (Figure 1, center), but selective blockade of ET_B receptors was associated with a 15% decrease in FBF from 2.7±0.1 mL · min^{-1} · dL^{-1} at baseline to 2.3±0.2 mL · min^{-1} · dL^{-1} after 60 minutes of BQ-788 infusion, although this change did not achieve statistical significance (Figure 1, right).

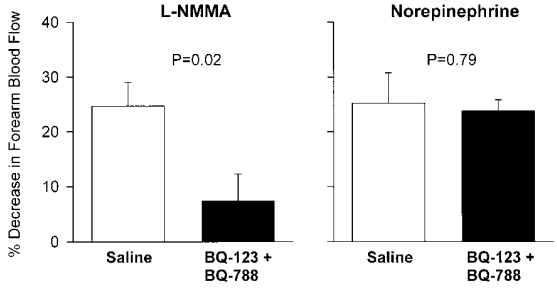


Figure 2. Changes in FBF from baseline after intra-arterial administration of L-NMMA (4 μmol/min; left) or norepinephrine (240 pmol/min; right) during infusion of saline (open bars) or nonselective blockade of ET-1 receptors by BQ-123 (100 nmol/min) in combination with BQ-788 (50 nmol/min; solid bars). Data are mean±SEM.

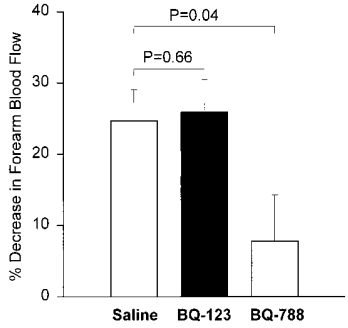


Figure 3. Changes in FBF from baseline after intra-arterial administration of L-NMMA (4 μmol/min) during infusion of saline (open bar) and after selective ET_A receptor blockade by BQ-123 (100 nmol/min; solid bar) or ET_B receptor blockade by BQ-788 (50 nmol/min; hatched bar). Data are mean±SEM.

During BQ-123 infusion, NO synthesis inhibition resulted in a significant decrease in FBF (from 3.6±0.6 mL · min^{-1} · dL^{-1} before to 2.5±0.1 mL · min^{-1} · dL^{-1} after L-NMMA; 26% change, $P=0.01$). The magnitude of this vasoconstrictor response was not significantly different from that observed during saline administration (Figure 3). In contrast, during selective ET_B blockade there was only a slight decrease in FBF from baseline after L-NMMA administration (from 2.3±0.2 to 2.1±0.2 mL · min^{-1} · dL^{-1} after L-NMMA; 8% change, $P=0.14$). Thus the vasoconstrictor effect of L-NMMA was significantly lower during BQ-788 administration than during saline infusion (Figure 3).

Discussion

The results of this study demonstrate that nonselective blockade of ET-1 receptors significantly diminishes the vasoconstictor response to NO synthesis inhibition. This observation indicates that, in the forearm circulation of healthy humans, NO and ET-1 continuously released from endothelial cells reciprocally interact to maintain basal vascular tone.

Nonspecific inhibition of the vasoconstrictor capacity after the removal of ET-1–mediated smooth muscle contraction could account for the blunted response to L-NMMA during ET-1 receptor blockade. To rule out that possibility, we analyzed the effect of ET-1 receptor blockade on the vascular response to another vasoconstrictor agent, the adrenergic agonist norepinephrine. In contrast to the findings observed with NO inhibition, the norepinephrine-induced vasoconstriction was not affected by blockade of ET-1 receptors. This indicates that the blunted response to L-NMMA during ET-1 receptor blockade was specifically related to an interaction between ET-1 and NO.

Another potential mechanism that could explain the decreased response to NO synthesis inhibition after ET-1 receptor antagonism is the removal of ET-1–mediated vasoconstriction. Thus, because NO synthesis inhibition modifies

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the balance between vasoconstrictor and vasodilator forces within the vessel wall, the ensuing vasoconstriction may be due to unopposed ET-1–related smooth muscle contraction mediated primarily by the stimulation of smooth muscle ET_A receptors.[22] Although ET_B receptors that mediate vasoconstriction have been identified in human arteries,[23,24] their functional role is questionable. Studies in animal models suggest that our results may be explained by the removal of ET-1–mediated vasoconstriction. Thus, administration of bosentan, a nonselective blocker of ET-1 receptors, reduced the pressor response to systemic administration of the NO synthase inhibitor L-NAME in rats.[25] Similar results were obtained with selective ET_A antagonism by BQ-123[25] or BQ-610,[26] which suggests that in those animals, NO synthesis inhibition may unmask a tonic pressor influence exerted by ET-1 through its ET_A receptor subtype.

To test this hypothesis, we measured the vasoconstrictor response to L-NMMA after selective blockade of ET_A receptors. In our study, the administration of BQ-123 alone was not associated with significant changes in the hemodynamic response to L-NMMA. This suggests that in the forearm circulation of healthy humans, the effects of NO synthesis inhibition are not dependent on a tonic vasoconstrictor influence of ET-1 through its ET_A receptor subtype. The observation that selective ET_A blockade does not result in a significant hemodynamic effect conflicts with the findings from other studies,[19,20,27] which have shown a vasodilator response to BQ-123 in healthy subjects. Because all studies have used similar populations of healthy subjects and similar doses and infusion times of BQ-123, these discrepancies cannot be explained easily. Interindividual variability in the hemodynamic responsiveness to blockade of ET_A receptors could account for the discrepancies among different studies, but the causes of that phenomenon are unknown.

Our findings could also be explained by the stimulatory effect that ET-1 may exert on NO production through stimulation of endothelial ET_B receptors.[9,10] This mechanism would explain the reduced availability of NO and the consequently blunted response to L-NMMA during selective ET_B antagonism. To test this possibility, we analyzed the effects of selective ET_B receptor blockade by BQ-788 on the vasoactive effect of L-NMMA. In contrast with the results obtained with BQ-123, the administration of BQ-788 resulted in a significant reduction in the response to L-NMMA. This observation indicates that NO availability is decreased during blockade of ET_B receptors, which suggests that, in normal humans, endothelial release of ET-1 physiologically exerts autocrine regulation of NO activity through ET_B receptors. In this investigation as well as in previous studies,[21,27] the selective blockade of ET_B receptor blockade was associated with mild local vasoconstriction. A recent report[28] has demonstrated that systemic blockade of ET_B receptors results in increased vascular resistance. These findings suggest that the stimulation of NO activity through ET_B receptors is important in determining the hemodynamic effect of ET-1 in healthy humans, a theory that confirms the results obtained in experiments of ET-1 gene targeting. Thus mice in which ET-1 production is reduced by heterozygous knock-out of the ET-1 gene have higher blood pressure values than those of their normal counterparts,[29] which suggests that the ET-1 system may act physiologically as a dilator rather than as a pressor mechanism.

Certain methodological aspects must be considered in the interpretation of the effect of NO inhibition during ET_B receptor blockade. First, the forearm vascular resistance is higher during selective ET_B blockade than during saline infusion, which could account for the blunted vasoconstrictor effect of L-NMMA during BQ-788 administration, because basal vascular tone affects the response to vasoactive substances. However, a blunted response to L-NMMA was also observed during nonselective $ET_{A/B}$ antagonism. Because nonselective blockade of ET-1 receptors did not modify forearm blood flow, the reduced vasoconstrictor effect of L-NMMA under those conditions must be related to the decreased availability of NO and not to differences in basal vascular tone. Second, because a single dose of L-NMMA was used in our study, we cannot ascertain that maximal inhibition of endogenous NO activity was achieved. Therefore we cannot rule out the possibility that higher doses of L-NMMA could result in a greater vasoconstrictor response in the presence of ET_B receptor antagonism. However, the observation that L-NMMA produced vasoconstriction only when it was infused with saline, and not when it was infused with BQ-788, indicates that ET_B receptor blockade diminishes the basal release of NO, even if the dose of L-NMMA used in this study did not produce maximal inhibition of NO activity.

In conclusion, the results of our study demonstrate that ET-1 is involved in the stimulation of basal release of NO through the activation of ET_B receptors. Because several cardiovascular conditions such as arterial hypertension and atherosclerosis are associated with endothelial dysfunction and reduced NO activity, the vasoconstrictor effect of ET-1 on smooth muscle receptors may remain unopposed. This hypothesis is supported by the results of previous studies[21] indicating that ET-1–mediated vasoconstrictor activity is increased in the vasculature of patients with essential hypertension when compared with that in normal subjects. The prevailing vasoconstrictor and mitogenic effects of ET-1 may contribute to the increased risk of cardiovascular disease, and drugs targeting the ET-1 system may prevent cardiovascular complications in those patients.

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