Endothelin-A Receptor Antagonist–Mediated Vasodilatation Is Attenuated by Inhibition of Nitric Oxide Synthesis and by Endothelin-B Receptor Blockade

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Background—The role of endothelin (ET)-1 in maintenance of basal vascular tone has been demonstrated by local and systemic vasodilatation to endothelin receptor antagonists in humans. Although the constrictor effects mediated by the vascular smooth muscle ET\textsubscript{A} receptors are clear, the contribution from endothelial and vascular smooth muscle ET\textsubscript{B} receptors remains to be defined. The present study, in human forearm resistance vessels in vivo, was designed to further investigate the physiological function of ET\textsubscript{A} and ET\textsubscript{B} receptor subtypes in human blood vessels and determine the mechanism underlying the vasodilatation to the ET\textsubscript{A}-selective receptor antagonist BQ-123.

Methods and Results—Two studies were performed, each in groups of eight healthy subjects. Brachial artery infusion of BQ-123 caused significant forearm vasodilatation in both studies. This vasodilatation was reduced by 95% (P<.006) with inhibition of the endogenous generation of nitric oxide and by 38% (P<.001) with coinfusion of the ET\textsubscript{B} receptor antagonist BQ-788. In contrast, inhibition of prostanoid generation did not affect the response to BQ-123. Infusion of BQ-788 alone produced a 20% reduction in forearm blood flow (P<.001).

Conclusions—Selective ET\textsubscript{A} receptor antagonism causes vasodilatation of human forearm resistance vessels in vivo. This response appears to result in major part from an increase in nitric oxide generation. ET\textsubscript{B} receptor antagonism either alone or on a background of ET\textsubscript{A} antagonism causes local vasoconstriction, indicating that ET\textsubscript{B} receptors in blood vessels respond to ET-1 predominantly by causing vasodilatation. (Circulation. 1998;97:752-756.)

Key Words: endothelin • nitric oxide • flow • receptors • prostanoids

The endothelin (ET) family of peptides (ET-1, ET-2, ET-3) are generated in a variety of tissues and act primarily as paracrine and autocrine factors. The major isoform in the cardiovascular system, ET-1, is generated in the endothelium from a precursor, big ET-1, through cleavage by a specific endothelin-converting enzyme (ECE). Its actions are mediated by two receptors, the ET\textsubscript{A} and the ET\textsubscript{B} receptor, which have been characterized and cloned\textsuperscript{1,2} and are pharmacologically distinct. The ET\textsubscript{A} receptor has a higher affinity for ET-1 (ET-1 >> ET-3), whereas the ET\textsubscript{B} receptor is nonisopeptide selective (ET-1 ≈ ET-3). ET\textsubscript{A} receptors are expressed on vascular smooth muscle cells, and their activation by ET-1 leads to vasoconstriction. The physiological importance of endogenous ET-1 in the maintenance of basal vascular tone and blood pressure in humans has been demonstrated by local\textsuperscript{3,4} and systemic\textsuperscript{5} vasodilatation in response to inhibitors of the endothelin system. An important role for the ET\textsubscript{A} receptor in mediating this response is suggested by the substantial forearm vasodilatation to local administration of the selective ET\textsubscript{A} receptor antagonist BQ-123\textsuperscript{5} in healthy subjects.

Initially, it was thought that ET\textsubscript{B} receptors were present only on endothelial cells, where they cause vasodilatation through release of endothelium-derived vasodilators, including nitric oxide (NO) and prostacyclin.\textsuperscript{6,7} However, it is now recognized that ET\textsubscript{B} receptors are also present on the smooth muscle of human arteries\textsuperscript{8} and can mediate vasoconstriction.\textsuperscript{9-11} although their contribution to ET-1–mediated constriction in humans remains to be defined.\textsuperscript{12} Therefore, although ET\textsubscript{A} receptor–mediated vasoconstriction is undisputed, it is unclear whether the balance of the effects of endogenous ET-1 on the endothelial and vascular smooth muscle ET\textsubscript{B} receptors results predominantly in a vasodilator or constrictor tone.

In addition to mediating vasodilator effects of endothelial ET\textsubscript{B} receptor activation, endothelium-derived dilators can in turn modulate the production and actions of ET-1.\textsuperscript{6,13-15} In the short term NO inhibits production of ET-1\textsuperscript{13} whereas chronic
exposure causes upregulation of ET_A receptors. In addition, endothelin receptor antagonists attenuate the pressor response to NO inhibition, suggesting that this response may not simply be due to loss of basal NO-mediated dilator tone. These interactions indicate the existence of a complex relationship between the endothelin and NO systems.

As a consequence of its potent vasoconstrictor and growth-promoting properties, ET-1 has also been implicated in the pathophysiology of diseases such as hypertension, heart failure, and renal failure. The recognition of the endothelin system as a new therapeutic target in the treatment of cardiovascular disease has lead to the rapid development of pharmacological agents that inhibit either the production of ET-1 or its actions. Recently, potent intravenous and orally active endothelin receptor antagonists with different pharmacological profiles have become available for clinical studies. We are now in a position where it would be valuable to explore the contribution of the ET_B receptor to the vascular effects of ET-1.

The present study, in human forearm resistance vessels in vivo, was designed to further investigate the physiological role of ET_A and ET_B receptor subtypes and their possible interactions in mediating the vasodilator response to selective ET_A receptor antagonism. The first part of the study aimed to investigate whether increased release of the endothelium-dependent relaxant factors NO and prostacyclin contributes to the vasodilator response to selective ET_A receptor antagonism. We therefore compared the response to the selective ET_B receptor antagonist BQ-123 during local inhibition of NO synthase and during systemic inhibition of prostanooid generation with the response to BQ-123 alone. In the second part of the study, to investigate the role of the ET_B receptor in BQ-123–induced vasodilatation, we examined the effects of simultaneous ET_A and ET_B receptor blockade compared with ET_A or ET_B receptor blockade alone.

Methods

Subjects

Twenty-two healthy subjects (1 woman) ranging in age from 20 to 43 years participated in two studies that were performed in the University Hospital Utrecht (study 1) and the University Department of Medicine, Western General Hospital, Edinburgh (study 2), with the approval of the local research ethics committees of each hospital and the written informed consent of each subject. The investigations conformed with the principles outlined in the Declaration of Helsinki. No subjects had received vasoactive medication or nonsteroidal anti-inflammatory drugs within the week before each phase of a study, and all subjects abstained from alcohol for 24 hours and from food, caffeine-containing drinks, and tobacco for at least 4 hours before any measurements were made. All studies were performed in a quiet room maintained at a controlled temperature between 22°C and 24.5°C.

Drug Administration

The brachial artery of the nondominant arm was cannulated with a 22 (study 1) or 27 SWG cannula (study 2) under lidocaine local anesthesia (lidocaine 2%; Astra Pharmaceuticals Ltd). Drugs, with the exception of aspirin, were dissolved in physiological saline (0.9%; Baxter Healthcare Ltd) and infused intra-arterially at locally active doses. The infusion rate was kept constant at 80 mL/h (study 1) or 60 mL/h (study 2). All solutions were prepared aseptically from sterile stock solutions or ampules on the day of the study.

Drugs

BQ-123 (100 nmol/min, study 1; 10 nmol/min, study 2), was used as a selective ET_A receptor antagonist (study 1: American Peptide Co; study 2: Clara AG). We have demonstrated previously local forearm vasodilatation to intra-arterial infusion of BQ-123 (100 nmol/min). In study 2, we used a 10-fold lower dose of BQ-123 (10 nmol/min) because more recent studies have shown that this causes vasodilatation of equal magnitude to that seen with the higher dose. BQ-788 (1 nmol/min) was used as a selective ET_B receptor antagonist (American Peptide Co). This dose has been shown to completely inhibit vasoconstriction to the selective ET_B receptor agonist sarafotoxin S6c.

The endogenous NO system in the forearm was inhibited by use of an “NO clamp,” as described previously. The NO synthase inhibitor l-N^monomethyl-arginine (L-NMMA; Institut fur Pharmazeutische, Universitat Leipzig) was continuously infused at a rate of 200 µg/100 mL forearm volume per minute to achieve maximal inhibition of local NO synthase. Sodium nitroprusside (SNP), an exogenous NO donor (Merck) was then confused at titrated doses (12 to 30 µg/min). After 8 minutes of L-NMMA infusion, when steady state forearm blood flow was obtained, SNP was confused in incremental doses and titrated until baseline forearm blood flow had been restored. L-NMMA and SNP were then coinfused, at these rates, for the remainder of the study. This allowed simulation of normal basal NO activity during continuous inhibition of endogenous NO synthesis.

Aspirin (600 mg calcium acetylsalicylic acid, Carbasalatum Calcium, Dagra Pharma BV) was administered orally 30 minutes before measurements in one phase of study 1. Aspirin irreversibly inhibits cyclooxygenase (EC 1.14.99.1), which is responsible for the production of prostaglandins and thromboxanes. When given at a dose of 600 mg, aspirin inhibits bradykinin–stimulated endothelial production of prostacyclin by at least 85% with recovery occurring over the next 6 hours.

Measurements

Forearm Blood Flow

Forearm blood flow was measured simultaneously in both arms by venous occlusion plethysmography using calibrated mercury-in-Silastic strain-gauges applied to the widest part of the forearm. The hands were excluded from the circulation during each measurement period by inflation of a wrist cuff to 220 mm Hg. Upper arm cuffs were intermittently inflated to 40 mm Hg for 10 seconds every 15 seconds to temporarily prevent venous outflow from the forearm and thus obtain plethysmographic recordings. Recordings of forearm blood flow were made over 2.5-minute periods at 5-minute intervals (study 1) and over 3-minute periods at 10-minute intervals (study 2). Venous occlusion plethysmography was performed using a dual-channel strain-gauge plethysmograph (Hokanson), and calibration was achieved using the internal standard of the Hokanson plethysmography unit. In study 1, a microcomputer-based R-wave–triggered system for online semicontinuous monitoring was used, whereas in study 2, voltage output was transmitted to a Macintosh personal computer (Class II; Apple Computer) using a MacLab analog-digital converter and Chart software (version 3.2.8; both from AD Instruments).

Blood Pressure

Blood pressure was monitored during each study using either continuous intra-arterial measurements in the infused arm (study 1) or a semi-automated noninvasive oscillometric method in the noninfused arm (study 2). Blood pressure in study 2 was measured immediately after each forearm blood flow measurement, thereby avoiding any effect of venous congestion caused by this procedure on blood flow.

General Study Design

Subjects rested recumbent throughout each study with both forearms resting slightly above the level of the heart. Strain gauges and arm cuffs were applied, and the left brachial artery cannula was stit. Before the administration of drugs, saline was infused for at least 30 minutes,
Baseline hemodynamic values during saline infusion before infusion of drugs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BQ-123 (100 nmol/min)</td>
<td>BQ-123 + NO-Clamp (100 nmol/min)</td>
</tr>
<tr>
<td>Forearm blood flow (mL/100 mL per minute)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infused arm</td>
<td>3.7±0.4</td>
<td>3.7±0.3</td>
</tr>
<tr>
<td>Control arm</td>
<td>4.6±0.5</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>78±2</td>
<td>82±3</td>
</tr>
</tbody>
</table>

Forearm blood flow in infused and control arm and mean arterial pressure in each phase of studies 1 and 2 at baseline before infusion of, respectively, BQ-123; BQ-123 + NO-clamp; and BQ-788. No significant differences in baseline forearm blood flow or mean arterial pressure between the phases of each study. 

n=8 in each phase of studies 1 and 2.

during which baseline measurements of forearm blood flow were made.

**Study 1: Inhibition of NO Synthase and Prostanoid Generation With ETₐ Receptor Blockade**

Eight subjects were studied on three separate occasions, each separated by at least 1 week. After baseline infusion of saline, BQ-123 was infused for 90 minutes: on one occasion, during saline infusion; on another, after stabilization of the NO-clamp; and on another, after systemic inhibition of prostanoid generation. The effects of the NO-clamp on forearm blood flow were studied during a 2-hour period in 3 subjects (time control NO-clamp).

**Study 2: Separate and Combined Blockade of ETₐ and ETₐ Receptors**

On 2 separate study days, in 8 subjects, the ETₐ receptor antagonist BQ-123 was infused for 120 minutes alone or during coinfusion of BQ-788, also for 120 minutes. On a separate occasion, BQ-788 was infused alone for 120 minutes in 8 subjects (2 of whom also participated in the earlier parts of study 2).

**Analysis**

Blood flow in both forearms was obtained from the mean of the last five consecutive recordings of each measurement period. Because wrist cuff inflation results in a transient forearm vasoconstriction, recordings made in the first 60 seconds after wrist cuff inflation were not used for analysis. The ratio of flows in the infused and noninfused arms was calculated for each time point and expressed as percentage change from baseline or, in the NO-clamp experiments, as percentage change from the average of the last four recordings during NO-clamping, before the administration of BQ-123. In both studies, plethysmographic data listings were extracted from data files, and forearm blood flows were calculated for individual venous occlusion cuff inflations using a template spreadsheet (Excel 5.0; Microsoft). All results are expressed as mean±SEM. Data were examined by repeated measures ANOVA (study 1, SigmaStat; Jandel Corp; study 2, Excel 5.0; Microsoft). Statistical significance was taken at the 5% level.

**Results**

There were no significant changes in baseline hemodynamics between phases of each study (Table) and no change in blood pressure or blood flow in the noninfused forearm during the course of the studies.

**Study 1**

Baseline forearm blood flow was restored during the NO-clamp (basal infused forearm blood flow; 3.7±0.3; during basal NO clamp; 3.4±0.2; P=.15) and kept stable for at least 40 minutes before BQ-123 infusion was started. Blood flow in the infused forearm in the time control NO clamp protocol varied by <5% between baseline (pre–NO clamp) and with 120 minutes of NO clamping in 3 subjects.

BQ-123 caused progressive vasodilatation during coinfusion of saline and after inhibition of prostanoid generation (P<.01 for both). The response appeared to plateau at 60 minutes, and no differences were observed in these responses (38±9% versus 42±7% at 90 minutes; P=.95). The vasodilator response to BQ-123 was markedly reduced during NO-clamping (2±5% at 90 minutes, P=.006 versus saline coinfusion) (Fig 1).

**Study 2**

Both BQ-123 alone and coadministration of BQ-123 and BQ-788 caused progressive vasodilatation (P<.001) that appeared to plateau at 60 minutes (Fig 2). The vasodilatation to BQ-123 alone was significantly greater than that during coinfusion with BQ-788 (76±13% versus 47±14% at 120 minutes, P<.001). BQ-788 alone caused a small but consistent reduction in forearm blood flow (20±3% at 120 minutes, P<.001) (Fig 2).

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*Figure 1.* Eight subjects received brachial artery infusion of BQ-123 (100 nmol/min) during coinfusion of saline (○), BQ-123 (100 nmol/min) during inhibition of prostanoid generation (△), or BQ-123 (100 nmol/min) during inhibition of NO generation (□). Slow-onset vasodilatation occurred in response to BQ-123; this response was attenuated during NO clamp but not during inhibition of prostanoid generation.
Discussion

In two centers, we have demonstrated slow-onset forearm vasodilation in response to local arterial infusion of the selective ETA receptor antagonist BQ-123, confirming the importance of endogenous ET-1 in the mediation of vascular tone. From these data, it appears that this vasodilator response is caused in large part by increased generation of NO, which could be mediated by stimulation of the endothelial ETB receptor. Indeed, our observation that the vasodilator response to combined ETA and ETB receptor antagonism was significantly less than that to selective ETA receptor antagonism alone probably reflects the presence of an endogenous ETB-mediated vasodilator tone. This is further supported by the local vasoconstrictor effect of ETB receptor antagonism in the forearm resistance vessels.

In the present study, to exclude the influence of the endogenous NO system in mediation or modulation of the effects of ET-1, L-NMMA was infused to inhibit endogenous local generation of NO. SNP was coinfused with L-NMMA to restore baseline blood flow because local inhibition of NO would otherwise result in vasoconstriction. In this situation, endogenous NO is replaced with exogenous NO, in effect applying a clamp to the local endogenous NO system. Using this technique we have shown, for the first time in humans in vivo, that the vasodilatation to BQ-123 is in large part related to NO generation. Inhibition of endogenous prostaglandin generation by oral administration of aspirin has no effect on basal forearm blood flow or systemic hemodynamics and, more importantly, had no effect on the response to BQ-123, indicating that the dilator prostaglandins do not provide an important contribution to the vasodilator response to BQ-123. Almost all of the response to BQ-123 appeared to be blocked by NO clamping. However, on the basis of vasodilatation to the ECE inhibitor phosphoramidon in previous studies, we think it is likely that at least part of the response to BQ-123 is directly due to withdrawal of endogenous ETA-mediated vasoconstriction.

Selective ETA antagonist inhibits the actions of ET-1 at the ETA receptor while allowing its actions at the ETB receptor to be unopposed. ET-1 can stimulate both the endothelial ETB receptor to cause dilatation and the vascular smooth muscle ETB receptor to cause vasoconstriction. Therefore, the overall effect depends on a balance between these two actions. Unfortunately, there are no available pharmacological tools that have been shown clearly to distinguish between the endothelial and vascular smooth muscle ETB receptors. We have shown that coinfusion of the ETB receptor antagonist BQ-788 reduces the vasodilator response to BQ-123, suggesting that the balance of effects of ET-1 favors vasodilatation via the endothelial ETB receptor. This is further supported by the vasoconstriction in these vessels to BQ-788 alone and by the lesser degree of vasodilatation to the combined ETA/ETB endothelin receptor antagonist TAK-044 than to the ETA-selective agent BQ-123. It is possible that the predominant effects of intra-luminal infusion of BQ-788 selectively affect the endothelial ETB receptor because the drug has better access to the endothelial than to the smooth muscle receptors. However, we believe this is unlikely because ET-1 and BQ-123 find ready access to the smooth muscle. The response to BQ-788 may indicate either displacement of ET-1 from, or failure of clearance of ET-1 by, ETB receptors. However, our present results cannot distinguish between these effects.

The observation that selective ETA receptor blockade not only antagonizes direct ETA receptor–mediated constriction but also preserves beneficial ETB receptor–mediated vasodilator tone and enhances endogenous NO generation may have important implications in the use of endothelin antagonists as treatments in cardiovascular disease. For example, the increased NO generation caused by ETA receptor antagonists is potentially beneficial in ischemic heart disease. However, the clinical relevance of our findings in various pathophysiological conditions cannot be fully determined from the present study because endothelin receptors may be modified under these circumstances. Indeed, in ischemic heart disease, there appears to be upregulation of human coronary ETB receptors and this is associated, in heart failure, with enhanced vasoconstrictor responses to sarafotoxin S6c in both the forearm and coronary circulation, whereas the response to BQ-788 appeared similar to that of controls. Clearly, at some stage, it will be necessary to examine the integrated physiology of systemic ETA and ETB blockade in physiological and pathophysiological conditions to fully understand the relative importance of the receptor subtypes.

In summary, we have demonstrated that the local vasodilator response to selective ETA receptor antagonism in human forearm resistance vessels is derived in large part from increased NO-mediated vasodilation, most probably mediated by the endothelial ETB receptor. Although our observations were made in the forearm resistance vessels, these vessels are generally representative of other vascular beds and, importantly, reflect the interaction of these systems in vivo. Our results may indicate new therapeutic uses for ETA receptor antagonists because increased NO synthesis may be a desirable effect in, for example, ischemic heart disease. One could also postulate that enhanced endogenous NO generation may be responsible for the headaches that are a recognized side effect of ET receptor antagonists.
Acknowledgments
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