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_A receptors are clear, the contribution from endothelial and vascular smooth muscle ET_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_A and ET_B receptor subtypes in human blood vessels and determine the mechanism underlying the vasodilatation to the ET_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_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_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_B receptor antagonism either alone or on a background of ET_A antagonism causes local vasoconstriction, indicating that ET_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_A and the ET_B receptor, which have been characterized and cloned and are pharmacologically distinct. The ET_A receptor has a higher affinity for ET-1 (ET-1>>ET-3), whereas the ET_B receptor is nonisopeptide selective (ET-1=ET-3). ET_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 and systemic vasodilatation in response to inhibitors of the endothelin system. An important role for the ET_A receptor in mediating this response is suggested by the substantial forearm vasodilatation to local administration of the selective ET_A receptor antagonist BQ-123 in healthy subjects.

Initially, it was thought that ET_B receptors were present only on endothelial cells, where they cause vasodilatation through release of endothelium-derived vasodilators, including nitric oxide (NO) and prostacyclin. However, it is now recognized that ET_B receptors are also present on the smooth muscle of human arteries and can mediate vasoconstriction, although their contribution to ET-1–mediated constriction in humans remains to be defined. Therefore, although ET_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_B receptors results predominantly in a vasodilator or constrictor tone.

In addition to mediating vasodilator effects of endothelial ET_B receptor activation, endothelium-derived dilators can in turn modulate the production and actions of ET-1. In the short term NO inhibits production of ET-1 whereas chronic...
exposure causes upregulation of ET\textsubscript{A} receptors.\textsuperscript{16} In addition, endothelin receptor antagonists attenuate the pressor response to NO inhibition,\textsuperscript{7,14} 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\textsuperscript{19} and growth-promoting properties,\textsuperscript{20} ET-1 has also been implicated in the pathophysiology of diseases such as hypertension, heart failure, and renal failure.\textsuperscript{21} 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.\textsuperscript{21,22} We are now in a position where it would be valuable to explore the contribution of ETA and ETB receptor subtypes and their possible interactions with the response to the selective ET\textsubscript{B} receptor antagonist BQ-123 during local inhibition of NO synthase and during systemic inhibition of prostaglandin generation with the response to BQ-123 alone. In the second part of the study, we were able to investigate the role of the ET\textsubscript{A} receptor in the BQ-123–induced vasodilatation, we examined the effects of simultaneous ET\textsubscript{A} and ET\textsubscript{B} receptor blockade compared with ET\textsubscript{A} or ET\textsubscript{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 vasodilating 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 foods, 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 anaesthesia (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\textsubscript{A} receptor antagonist (study 1: American Peptide Co; study 2: ClnaAG). We have demonstrated previously local forearm vasodilatation to intra-arterial infusion of BQ-123 (100 nmol/min).\textsuperscript{3} 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.\textsuperscript{23} BQ-788 (1 nmol/min) was used as a selective ET\textsubscript{B} receptor antagonist\textsuperscript{24} (American Peptide Co). This dose has been shown to completely inhibit venoconstriction to the selective ET\textsubscript{B} receptor agonist sarafotoxin S6c.\textsuperscript{25}

The endogenous NO system in the forearm was inhibited by use of an “NO clamp,” as described previously.\textsuperscript{26} The NO synthase inhibitor L-\textsuperscript{N} monomethyl-arginine (L-NMMA; Institut fur Pharmazie, 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.\textsuperscript{27–29} Sodium nitroprusside (SNP), an exogenous NO donor (Merck) was then infused at titrated doses (12 to 30 ng/min). After 8 minutes of L-NMMA infusion, when steady state forearm blood flow was obtained, SNP was infused in incremental doses and titrated until baseline forearm blood flow had been restored. L-NMMA and SNP were then titrated, 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; Carbalsatrum 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.\textsuperscript{30}

**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.\textsuperscript{27,31} 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,\textsuperscript{31} whereas in study 2, voltage output was transferred to a Macintosh personal computer (Classic 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 semiautomated noninvasive oscillometric method in the noninfused arm (study 2).\textsuperscript{32} 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 stilled. 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>
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<tbody>
<tr>
<td></td>
<td>BQ-123 (100 nmol/min)</td>
<td>BQ-123+ NO-Clamp</td>
</tr>
<tr>
<td>Forearm blood flow (mL/100 mL per minute)</td>
<td>3.7±0.4</td>
<td>3.7±0.3</td>
</tr>
<tr>
<td>Infused arm</td>
<td>4.6±0.5</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>Control arm</td>
<td>78±2</td>
<td>82±3</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>78±2</td>
<td>83±5</td>
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<td></td>
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<td>n=8</td>
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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 SNP, and L-NMMA; BQ-123; BQ-123; BQ-123 and BQ-788; and BQ-788. There were no significant differences in baseline forearm blood flow or mean arterial pressure between the phases of each study.

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=.5). 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).
by NO clamping. However, on the basis of vasodilatation to NO generation. Inhibition of endogenous prostanoid generation by oral administration of aspirin has no effect on basal vasoconstriction. In this situation, enhanced endogenous NO generation may be responsible for the lesser degree of vasodilatation to the combined ETA/ETB blockade in physiological and 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 ETA 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 vasodilatation, 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

This work was supported by grants from The Dutch Heart Foundation, The Wellcome Trust, and The British Heart Foundation. Dr Rabelink was supported by a fellowship of the Royal Dutch Academy of Sciences (KNAW), Dr Newby was supported by a British Heart Foundation Junior Research Fellowship (FS/95009), and F. Strachan was supported by a Wellcome Trust project grant (PG-048560).

References

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_Circulation_. 1998;97:752-756
doi: 10.1161/01.CIR.97.8.752

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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