The Physiological Role of Endogenous Endothelin in the Regulation of Human Coronary Vasomotor Tone

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Published by Elsevier Science Inc. PII S0735-1097(00)01042-1

© 2001 by the American College of Cardiology ISSN 0735-1097/01/$20.00
Journal of the American College of Cardiology Vol. 37, No. 1, 2001

OBJECTIVES The study was done to investigate the physiological role of endogenous endothelin-1 in the human coronary circulation by studying the effect of an intracoronary infusion of the specific endothelin receptor subtype A (ET\textsubscript{A}) receptor antagonist BQ123 on coronary vasomotor tone.

BACKGROUND Endothelin-1 contributes to the maintenance of peripheral vascular tone in humans. However, its physiological role in the human coronary vasculature is unknown.

METHODS We studied 12 patients (mean age 54.7 ± 2.5 years, 3 men) undergoing cardiac catheterization for investigation of atypical chest pain, with angiographically normal coronary arteries. Coronary artery cross-sectional area was measured with digital quantitative coronary angiography, and coronary blood flow was assessed with an intracoronary Doppler flow wire. Flow-mediated (adenosine, 18 μg) and agonist-mediated (substance P, 20 pmol/min for 2 min) endothelial responses were measured prior to study. BQ123 (40 nmol/min for 15 min and monitored for a further 15 min) was infused into the left coronary artery.

RESULTS The BQ123 caused significant dilation of the proximal (artery cross-sectional area: 8.08 ± 0.9 to 8.88 ± 0.9 mm\textsuperscript{2}; p < 0.05), mid (5.32 ± 0.8 to 6.49 ± 0.8 mm\textsuperscript{2}; p < 0.001) and distal study vessel (2.11 ± 0.2 to 2.50 ± 0.2 mm\textsuperscript{2}; p < 0.05). There was an increase in coronary blood flow (26.8 ± 2.8 to 32.8 ± 3.4 ml/min; p < 0.001) but no change in systemic hemodynamics. Baseline flow- or substance P-induced epicardial vasodilation did not correlate with the degree of vasodilation induced by BQ123.

CONCLUSIONS These data uncover a role of endogenous endothelin-1 in the maintenance of basal vasomotor tone in patients with angiographically normal coronary arteries. (J Am Coll Cardiol 2001;37:137–43) © 2001 by the American College of Cardiology

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Received February 2, 2000; revised manuscript received June 12, 2000; accepted September 7, 2000.

METHODOLOGY

The study conformed to the principles outlined in the Declaration of Helsinki and was conducted with the approval of the Institutional Research and Ethics Committee. All subjects provided written informed consent.
We studied 12 patients (3 men, mean age 54.7 ± 2.5 years) undergoing diagnostic cardiac catheterization for the investigation of atypical chest pain. None had diabetes or clinical/electrocardiographic (ECG) features of coronary spasm or syndrome X. Patients with valvular heart disease, left ventricular hypertrophy and left ventricular dysfunction were excluded by echocardiography prior to study. All medications were withheld and all subjects abstained from alcohol, caffeine-containing drinks, and cigarettes for at least 24 h, and from food for at least 6 h before cardiac catheterization. Patient characteristics are shown in Table 1.

Cardiac catheterization was performed via the right femoral approach in a quiet, temperature-controlled cardiac catheterization laboratory, using biplane digital cineangiography (Philips Optimus 2000). After diagnostic coronary angiography, 10,000 iU of heparin was given, and a 7F Judkins guiding catheter was introduced into the left main coronary artery. A 3F infusion catheter (Boston Scientific) was advanced over a 0.014-in. (0.0356-cm) Doppler flow-guide wire (Cardiometrics, California) and positioned in a nonbranching segment of the mid-left anterior descending coronary artery (LAD, n = 11) or circumflex coronary artery (Cx, n = 1). The Doppler wire was then manipulated ~1 cm distal to the tip of the infusion catheter into a position that gave a stable velocity signal. The Doppler wire was interfaced with a real-time spectral analysis system (FlowMap, Cardiometrics, California) to enable recording of the average peak (Doppler) velocity (APV) of blood flow. Coronary blood flow was calculated by multiplying one-half of this value by the coronary artery area at the tip of the Doppler flow wire on the corresponding angiogram. The technique was identical to that used in a previous study (14), and the accuracy of this system has been verified in a separate publication (15). Adenosine was administered selectively into the coronary artery via the infusion catheter, but all other drugs were administered through the guiding catheter.

**Study protocol.** We assessed endothelial function (both epicardial dilation following an increase in coronary flow induced by adenosine and agonist-mediated vasodilation) in all patients prior to BQ123 infusion. An 18 μg bolus of adenosine was given via the infusion catheter, and angiograms were taken 30 s after the resultant peak APV. This protocol for assessing flow-mediated dilation is similar to that used in a previous study (14), and adenosine in a bolus dose of 18 μg has been shown to achieve adequate coronary vasodilation for this purpose (16). Substance P (20 pmol/min for 2 min; dissolved in 0.9% saline) was used to assess agonist-mediated vasodilation. This agent appears to be more selective than acetylcholine in modulating vascular tone by the release of NO; furthermore, it does not directly affect smooth muscle tone (17,18). The dose–response relationship for substance P-induced in vivo epicardial coronary vasodilation in humans has been well characterized (19). Endothelium–independent dilation was also assessed as the response to intracoronary glyceryl trinitrate (GTN) (250 μg bolus) at the end of the study protocol.

The BQ123 (American Peptide, Sunnyvale, California) was infused into the left coronary artery at a dose of 40 nmol/min (flow rate 2 ml/min; dissolved in 0.9% saline) for 15 min, using an IVAC P4000 anesthesia syringe pump (Welmed, Hampshire, United Kingdom). Previous studies by Verhaar et al. (4) have shown that an infusion of 10 nmol/min BQ123 in the human forearm induced maximal vasodilatation, yet remained locally active (i.e., blood flow was unchanged in the noninfused forearm). We chose a dose of 40 nmol/min to achieve a roughly equivalent local concentration (~0.4 μmol/liter, assuming a maximum total

**Table 1. Clinical Characteristics of the Patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Gender</th>
<th>Vessel</th>
<th>Total Cholesterol (mmol/liter)</th>
<th>Triglycerides (mmol/liter)</th>
<th>Hypertension</th>
<th>Smoker</th>
<th>FHx of Vascular Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td>M</td>
<td>LAD</td>
<td>4.2</td>
<td>2.2</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>M</td>
<td>LAD</td>
<td>6</td>
<td>1.8</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>49</td>
<td>F</td>
<td>Cx</td>
<td>5.8</td>
<td>1.1</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>F</td>
<td>LAD</td>
<td>7.1</td>
<td>1.2</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>F</td>
<td>LAD</td>
<td>9.2</td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>56</td>
<td>F</td>
<td>LAD</td>
<td>6.2</td>
<td>1.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>57</td>
<td>F</td>
<td>LAD</td>
<td>8.6</td>
<td>3</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>58</td>
<td>F</td>
<td>LAD</td>
<td>6.6</td>
<td>1.6</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>74</td>
<td>F</td>
<td>LAD</td>
<td>8</td>
<td>2.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>M</td>
<td>LAD</td>
<td>5.2</td>
<td>3.2</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>50</td>
<td>F</td>
<td>LAD</td>
<td>5.5</td>
<td>2.7</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>45</td>
<td>F</td>
<td>LAD</td>
<td>9.0</td>
<td>3.1</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

Cx = circumflex coronary artery; FHx = family history; LAD = left anterior descending coronary artery.
coronary flow rate of approximately 100 ml/min). This concentration is 10- to 100-fold higher than the reported affinity of binding sites for BQ123 in human coronary vascular smooth muscle (Kd 0.85 nmol/liter) (13), yet still selective for the ET
A receptor (13,20,21). The study protocol is shown in Figure 1.

An infusion of normal saline at 2 ml/min was substituted between drug administrations. Recordings of a biplane angiogram, Doppler APV, aortic pressure and ECG were obtained after each drug administration.

Quantitative coronary angiography. Serial angiograms with nonionic contrast medium (Omnipaque) were performed in each patient using a biplanar radiographic system with the study artery positioned near the isocenter and avoiding vessel overlap. The angle of projection was set at the beginning of the protocol and was not altered thereafter. Digital analysis was performed off-line with an edge-detection system (EasyVision, Philips) using a previously described and validated method (14,22,23). For each angiogram, diameter measurements were made (using geometric edge detection) from three end-diastolic frames and averaged. In each frame, mean vessel diameter was measured in the proximal, mid- and distal study vessel in both views of the biplanar angiogram, using side branches, the infusion catheter, and Doppler flow wire tip as anatomic landmarks. Cross-sectional arterial area was calculated on the assumption of an elliptical lumen.

Statistics. Data are expressed as mean ± SEM. Values for coronary artery cross-sectional area and coronary blood flow before and after adenosine, substance P and GTN were compared with paired \( t \)-tests. Similar values before, immediately after BQ123 infusion and 15 min after discontinuation of BQ123 were compared with one-way analysis of variance (ANOVA) for repeated measures with Tukey post hoc testing, using a statistical software package (Statview™ version 4.5). All analyses were two-tailed, and a value of \( p < 0.05 \) was considered to indicate statistical significance.

RESULTS

There were no complications in any of the patients studied, and no adverse effects to BQ123 were observed. No significant change occurred in heart rate, ECG or aortic pressure in any patient after any of the drugs were administered.

Changes in arterial cross-sectional area. Infusion of BQ123 led to significant vasodilation in all segments of the study vessel (i.e., proximal, mid and distal) at the end of the infusion (Fig. 2). This change was most marked in the mid study vessel, in which the artery cross-sectional area increased by 24.3 ± 4.9% (mean 5.32 ± 0.8 mm² to 6.49 ± 0.8 mm², \( p < 0.001 \)). The cross-sectional area in the distal vessel increased by 22.3 ± 8.6% (mean 2.11 ± 0.2 mm² to 2.50 ± 0.2 mm², \( p < 0.05 \)); in the proximal vessel, the increase was 10.7 ± 3.9% (mean 8.08 ± 0.9 mm² to 8.88 ± 0.9 mm², \( p < 0.05 \)). This vasodilation had diminished by 15 min post-BQ123 infusion in the mid- and distal vessel (to 5.95 ± 0.9 mm² and 2.38 ± 0.2 mm², respectively; \( p = \text{NS} \)), but remained significantly greater than baseline in the proximal vessel (9.10 ± 0.9 mm²; \( p < 0.05 \)).

Changes in coronary flow. Following BQ123 infusion, coronary flow increased significantly (Fig. 3) by 24.3 ± 5.1% (mean 26.8 ± 2.8 ml/min to 32.8 ± 3.4 ml/min; \( p < 0.001 \)). Coronary flow had returned to 30.0 ± 3.3 ml/min by 15 min post-BQ123 infusion, which was not significantly greater than at baseline.

Endothelial function. Of the 12 patients studied, 11 had vasodilatory responses to flow- and agonist-mediated stimuli. All 12 responded to the endothelium-independent
stimulus of GTN (mean data shown in Table 2). One patient (Patient 5) failed to exhibit epicardial vasodilation in response either to an increase in coronary flow induced by adenosine or to substance P, but nevertheless did show a vasodilatory response to BQ123. If this patient had been excluded from analysis, the effect of BQ123 on mid study vessel cross-sectional area and coronary flow remained highly significant. No correlation was found between the magnitude of epicardial vasodilator response to flow (adenosine) or agonist (substance P) and the epicardial vasodilatory response to BQ123 in individual patients ($r = 0.54$ and $-0.01$, respectively; both $p = NS$). There was no correlation between changes in coronary flow induced by substance P and those induced by BQ123 ($r = -0.30$, $p = NS$), nor was there a correlation between serum cholesterol and vasodilator response to BQ123 ($r = -0.02$; $p = NS$).

**DISCUSSION**

We have shown that selective blockade of ET$_A$ receptors in humans with angiographically normal coronary arteries causes vasodilation of the epicardial vessels and an increase in coronary blood flow. These data imply for the first time that endogenous ET plays an important physiological role in the regulation of vasomotor tone in human conduit and resistance coronary vessels. Our results demonstrate that the previous findings of a role for endogenous ET in peripheral arteries in humans (5,24) can now be extended to the normal human coronary vasculature.

Previous studies by Haynes et al. (5) showed that brachial artery infusion of BQ123 caused progressive vasodilation, increasing forearm blood flow by 64% at 60 min in normal human subjects, while administration of the ET-converting enzyme inhibitor phosphoramidon had similar effects. In animal studies, systemic BQ123 administration lowered blood pressure, suggesting that this tonic effect of ET is present in vascular beds other than just the forelimb (25). Intracoronary administration of the selective ET$_A$ antagonist Ro-61-1790 in conscious dogs caused epicardial coronary vasodilation (26). The physiological effects of ET in the human coronary circulation, however, have remained unknown. From previous studies it is apparent that responses to ET receptor antagonists vary, depending on species, vascular bed and presence of vascular disease. It cannot therefore be assumed that ET has similar physiological importance in different vascular beds.

In a recent study, Wenzel and colleagues (27) assessed...
coronary vasomotor tone after the systemic IV administration of the ET A/B receptor antagonist bosentan in patients with angiographically documented coronary artery disease. These investigators reported an increase in epicardial coronary artery diameter after bosentan, an effect that was seen particularly in arterial segments in which there was little angiographic evidence of atherosclerotic disease and which correlated inversely with plasma low density lipoprotein (LDL)-cholesterol levels. In contrast to the present study, no change in coronary flow was detected. However, the data are difficult to interpret because bosentan was given systemically (intravenously) and resulted in a decrease in systolic blood pressure and an increase in heart rate. These systemic effects may have secondarily affected coronary vasomotor tone, although such confounding effects would have affected both the patients with normal coronary arteries and those with coronary artery disease. Baseline endothelial function was also not assessed, so that no conclusions could be drawn about the relationship between the effect of ET and the functional integrity of the endothelium. It is important to note that both the generation of and response to endogenous ET may change in the presence of coronary disease and endothelial dysfunction. Under these circumstances, impaired NO production, for example, may attenuate dilator responses of ETB receptors and promote constrictor responses via ETA receptors (28).

In the current study, we selected patients without angiographic evidence of coronary artery disease, 11 out of 12 of whom demonstrated endothelial vasodilator responses. Furthermore, we used a specific ETA-receptor antagonist that was selectively infused into the coronary artery at a dose that did not give rise to systemic effects so as to ensure that observed changes in coronary tone were not confounded by alterations in systemic hemodynamics. Epicardial vasodilation was observed in the proximal, mid and distal study vessel at the end of BQ123 administration, and these effects were largely reversed 15 min after discontinuing the infusion. The vasodilatory effects of BQ123 appeared to be consistently greater in the mid and distal vessel. This differential effect may be explicable by the distribution of ETA receptors in human coronary arteries. In vitro studies have shown that ETA receptors mediate contractile responses in distal, pre-resistant human epicardial coronary arteries, whereas other ET receptor types are involved in constriction of more proximal vessel segments (29).

The mechanism by which ETA receptor blockade with BQ123 leads to coronary vasodilation is speculative and potentially complex. It is possible that the effects were due to the removal of a tonic vasoconstrictive effect of endoge-

![Figure 3. Mean values for study vessel blood flow at baseline, at the end of the 15-min BQ123 infusion (peak BQ123) and 15-min post-BQ123 infusion. Lower panel shows percentage change from baseline. *p < 0.001 vs. baseline.](image)

**Table 2.** Arterial Cross-Sectional Area and Coronary Flow at Baseline and After Flow-Mediated (Adenosine) and Agonist-Mediated (Substance P) Vasodilation and Endothelium-Independent (GTN) Vasodilation

<table>
<thead>
<tr>
<th>Study Vessel Cross-Sectional Area (mm²)</th>
<th>Flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>Mid</td>
</tr>
<tr>
<td>Baseline</td>
<td>7.99 ± 0.80</td>
</tr>
<tr>
<td>Flow-mediated dilation</td>
<td>9.58 ± 1.02*</td>
</tr>
<tr>
<td>Agonist-mediated dilation</td>
<td>9.54 ± 1.00</td>
</tr>
<tr>
<td>Endothelium-independent dilation</td>
<td>9.98 ± 1.17</td>
</tr>
</tbody>
</table>

All values for flow-mediated, agonist-mediated and endothelin-independent dilation are significantly greater than their respective baseline values (p < 0.05). *Vasodilator effect mediated by an increase in study vessel blood flow; †vasodilator effect mediated by local adenosine administration.
Endothelin-1 has been implicated in the pathophysiology of a wide range of vascular diseases, including hypertension (28,31), coronary artery spasm (8,9), atherosclerosis (both early [10] and advanced [11]), myocardial infarction (7) and heart failure (32). It is known that ET can act as a mitogen, enhancing the growth of vascular smooth muscle cells (33), and it plays a role in neointima formation after arterial injury (34). There is therefore much interest in the potential therapeutic applications of ET receptor antagonists. In a recent clinical study (35), oral bosentan decreased blood pressure in patients with essential hypertension to a similar degree as the angiotensin-converting enzyme (ACE) inhibitor enalapril, while the IV administration of bosentan led to a fall in mean arterial and pulmonary artery pressure in patients with heart failure (36). The results of the present study illustrate the potentially beneficial coronary vasodilatory effects of ETA receptor antagonism, although the importance of these effects in disease states requires further investigation.

Study limitations. A number of limitations of our study merit discussion. First, our data probably underestimate the magnitude of the BQ123 effect, as previous studies in the peripheral vasculature have shown that this antagonist has a progressive action for up to 60 min (4,5). Prolonged invasive monitoring was not feasible in this study, however, for ethical reasons. Significant responses to ET antagonists have been found, however, in other animal and human studies (4–6,37), within 15 min of infusion. Second, we only used a single agent, ETA receptor antagonist, and therefore can make no conclusions about the mechanism of the vasodilator response to BQ123 or the role of ETB receptors. Finally, several of our patients had risk factors for endothelial dysfunction (Table 1) and one patient did not have any flow- or agonist-mediated vasodilator response. It is therefore possible that the effect of intracoronary BQ123 might be different in subjects without any cardiovascular risk factors (38), but such individuals rarely come to cardiac catheterization. However, we found no correlation between the magnitude of epicardial vasodilator response to flow- or agonist-mediated stimuli and the vasodilator response to BQ123, suggesting that the presence of cardiovascular risk factors did not significantly influence the present results.

Summary. In this study, we have demonstrated that the ETA receptor antagonist BQ123 causes significant coronary vasodilation in the absence of angiographic coronary artery disease, implying for the first time that endogenous ET-1 is important in the physiological regulation of basal coronary vasomotor tone. The precise mechanisms of the physiological role of ET-1, the receptors via which it is mediated, and the extent to which these effects are altered in disease states require further investigation.

Acknowledgments
We would like to thank the nursing staff and the radiographers of the Cardiac Catheterization Laboratories, University Hospital of Wales, for their expert assistance throughout this study.

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