Coronary Vasodilation and Improvement in Endothelial Dysfunction With Endothelin \(\text{ET}_A\) Receptor Blockade

Julian P.J. Halcox, Khaled R.A. Nour, Gloria Zalos, Arshed A. Quyyumi

Abstract—The endothelium-derived peptide endothelin-1 (ET-1) causes vasoconstriction predominantly via smooth muscle \(\text{ET}_A\) receptor activation. We hypothesized that \(\text{ET}_A\) receptor inhibition would improve human coronary vascular function. We studied unobstructed coronary arteries of 44 patients with atherosclerosis or its risk factors. Epicardial diameter \((D)\) and Doppler flow velocity were measured, and coronary vascular resistance \((\text{CVR})\) was calculated during intracoronary infusions of acetylcholine \((\text{ACH})\) and sodium nitroprusside \((\text{SNP})\), and during cold pressor testing, before and after a 60-minute intracoronary infusion of the \(\text{ET}_A\) receptor antagonist BQ-123. BQ-123 dilated the coronary circulation; \(D\) increased by 5.6±1.0% \((P<0.0001)\), and \(\text{CVR}\) fell by 12±3% \((P<0.01)\). The \(D\) response to \(\text{ACH}\), corrected for the SNP response, improved in segments that constricted with \(\text{ACH}\) at baseline \((P=0.03)\), whereas segments that initially dilated with \(\text{ACH}\) did not change with BQ-123 \((P=\text{NS})\). Improvement in \(D\) and \(\text{CVR}\) responses to \(\text{ACH}\) with BQ-123 inversely correlated with baseline \(\text{ACH}\) responses \((r=-0.44\ [P=0.006]\) and \(r=-0.78\ [P=0.001]\), respectively), indicating greater improvement in those with endothelial dysfunction. Similarly, cold pressor testing–mediated epicardial vasoconstriction \((-2.0±1.1\%)\) was reversed after BQ-123 \((+1.0±0.7\%)\), especially in dysfunctional segments \((\text{from }-5.6±0.9\% \text{ to }+2.2±0.9\%, P<0.001)\). There was no correlation between any risk factor and the response to BQ-123. An arteriovenous difference in ET-1 levels developed after BQ-123, which was consistent with enhanced cardiac clearance of ET-1, probably via ET\(_B\) receptors. Thus, ET-1 acting via the \(\text{ET}_A\) receptor contributes to basal human coronary vasoconstrictor tone and endothelial dysfunction. This suggests that \(\text{ET}_A\) receptor antagonism may have therapeutic potential in the treatment of endothelial dysfunction and atherosclerosis.

Key Words: endothelin • coronary circulation • endothelial function • atherosclerosis

Endothelin (ET), a powerful vasoconstrictor peptide, exists in 3 isoforms, ET-1, ET-2, and ET-3.\(^1\)\(^-\)\(^3\) ET-1, the predominant isopeptide released from endothelial cells, is likely to be physiologically the most important in regulating vascular function via its action on 2 distinct receptor subtypes, \(\text{ET}_A\) and \(\text{ET}_B\).\(^3\) The \(\text{ET}_A\) receptor has a high affinity for ET-1, is selectively expressed on vascular smooth muscle cells, and is the predominant ET receptor in the heart.\(^4\)\(^-\)\(^5\) whereas the \(\text{ET}_B\) receptor has equal affinity for all 3 isoforms of ET and is present on both endothelial and vascular smooth muscle cells.\(^6\) ET-1, via stimulation of \(\text{ET}_A\) receptors on vascular smooth muscle cells, activates phospholipase \(C\), resulting in generation of inositol triphosphate, intracellular calcium accumulation, and vasoconstriction.\(^4\)\(^-\)\(^7\)\(^-\)\(^8\) ET-1 levels are elevated in conditions associated with vascular endothelial dysfunction such as hyperlipidemia, hypertension, smoking, heart failure, and atherosclerosis, suggesting a possible pathophysiological role of ET-1 in these conditions.\(^9\)\(^-\)\(^14\) However, as ET-1 is preferentially secreted abuminally by the endothelial cells, circulating levels appear not to accurately reflect the vascular activity of ET-1, thus necessitating the use of selective antagonists to characterize its physiological effects.\(^15\)

\(\text{ET}_A\) receptor antagonism vasodilates the forearm microcirculation of healthy volunteers and subjects with hypertension, heart failure, and hyperlipidemia, suggesting that endogenous ET-1 regulates resting peripheral vascular tone in humans.\(^16\)\(^-\)\(^21\) Endogenous ET-1 acting via the \(\text{ET}_A\) receptor contributes toward the maintenance of coronary vasoconstrictor tone.\(^22\)\(^-\)\(^24\) Although chronic \(\text{ET}_A\) receptor blockade attenuated the progression of endothelial vasomotor dysfunction in animal models,\(^25\)\(^,\)\(^26\) whether endogenous ET-1 contributes toward endothelial dysfunction in the human coronary circulation in vivo is unknown.

We hypothesized that ET-1 activity may contribute to both the resting coronary vasomotor tone and the endothelial dysfunction observed in the coronary circulation of patients with atherosclerosis. Therefore, in this study, we investigated the effect of \(\text{ET}_A\) receptor antagonism on coronary vasomotor function and on transcardiac ET-1 uptake in patients with and without coronary atherosclerosis.
TABLE 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>Total Cohort (n=44)</th>
<th>Protocol 1 (n=21)</th>
<th>Protocol 2 (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD, n (%)</td>
<td>34 (77)</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Mean age, years</td>
<td>60±2</td>
<td>62±2</td>
<td>57±2</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>192±6</td>
<td>188±10</td>
<td>195±7</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>117±5</td>
<td>112±9</td>
<td>121±5</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>44±2</td>
<td>41±2</td>
<td>46±3</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>29 (66)</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>9 (20)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>26 (59)</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>9 (20)</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease. There were no significant differences in patient characteristics between the two study protocols.

Materials and Methods

Patients
We studied 44 patients with coronary atherosclerosis (coronary artery disease [CAD]) or normal coronary arteries and risk factors for atherosclerosis undergoing diagnostic cardiac catheterization for investigation of chest pain or abnormal noninvasive cardiac investigations (Table 1). CAD was defined as angiographic evidence of coronary stenosis or plaquing and normal coronary arteries if coronary arteries appeared angiographically smooth. Risk factors analyzed included age, male gender, current tobacco smoking (within past year), diabetes (fasting blood glucose level >7.8 mmol/L), hypertension (systolic blood pressure ≥140 mm Hg and/or diastolic pressure ≥90 mm Hg on at least 3 occasions), and fasting serum cholesterol level. Patients with an unstable coronary syndrome within the previous month or significant valvular heart disease and women of childbearing potential were excluded. Aspirin and angiotensin-converting enzyme therapy were withheld for at least 1 week, and all cardiac medications were discontinued for at least 5 half-lives before the study. The study was approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute and informed, written consent was obtained from all patients.

Protocol
The protocol was initiated after completion of diagnostic coronary angiography. A 7F guide catheter was introduced into a coronary artery with insignificant stenosis (<20%). In 9 patients, the study was performed in a patient saphenous vein graft that inserted into a distally unobstructed major epicardial coronary artery. Blood flow velocity was measured using a 0.014-inch wire equipped with a Doppler crystal at its tip (FloWire, Cardiometrics, EndoSonics Corp, Rancho Cordova, Calif). Medications were infused into the study vessel using a 3F infusion catheter, which was advanced over the Doppler wire.

Infusion Protocols

Protocol 1
Baseline coronary blood flow velocity and coronary angiography were performed after a 10-minute infusion of 5% dextrose at 2 mL/min. Endothelium-dependent coronary vasodilation was estimated in 21 patients by administering incremental 2-minute infusions of acetylcholine (ACH) at 1.5 and 15 g/min (to achieve estimated intracoronary concentrations of 10<sup>-7</sup> and 10<sup>-6</sup>mol/L, respectively). BQ-123 (Bachem), a selective ET<sub>A</sub> receptor antagonist, was then infused for 1 hour at a rate of 200 nmol/min. This dose produces an intravascular concentration ∼10-fold higher than the pA<sub>2</sub> (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<sub>A</sub> receptor, and counteracts the vasoconstrictor effect of ET-1 infusion in the human forearm.† To record the maximal response, measurements were made over 1 hour on the basis of previous observations in the forearm circulation. ACH infusions were then repeated during coadministration of BQ-123.

Protocol 2
In a further 23 patients, in addition to the 2 doses of ACH, 3-minute intracoronary infusions of sodium nitroprusside (SNP) at 10 and 20 g/min were administered to assess endothelium-independent coronary vasodilation, before and after BQ-123. Thirteen of these patients were also subjected to cold pressor testing (CPT) by immersion of one of their hands in ice-cold water for 90 to 120 seconds.

Measurement of Coronary Blood Flow and Diameter
Coronary blood flow was derived from the Doppler flow velocity and diameter (D) measurements using the formula (π×average peak velocity×0.125×D<sup>2</sup>). Coronary vascular resistance (CVR) was calculated as mean arterial pressure/coronary blood flow. For calculating flow, D was measured beyond the tip of the flow wire using quantitative angiography by a blinded observer using the CAAS II software package (PIE Medical, Inc). After each intervention, additional D measurements were made in the middle and distal native coronary arteries in segments free of plaquing in 41 patients with a single measurement in 3 patients. Additionally, in 10 subjects with CAD, minimum lumen D at the site of lesions (<20% stenosis) was measured before at baseline and after BQ-123.

Reproducibility
Reproducibility of the responses to ACH and SNP were evaluated in 8 patients. Basal D+CVR values were similar before and after 1 hour (2.02±0.12 versus 2.04±0.10 mm Hg·mL<sup>-1</sup>·min<sup>-1</sup> [r=0.98]) and 3.57±0.37 versus 3.46±0.36 mm Hg·mL<sup>-1</sup>·min<sup>-1</sup> [r=0.9], respectively. The percentage change in CVR with 15 μg/min ACH (−31±8% and
TABLE 2. Effect of BQ-123 on Hemodynamic Parameters

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>60 Minutes</th>
<th>Percentage Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, min⁻¹</td>
<td>75±2</td>
<td>79±2 *</td>
<td>+5±2</td>
</tr>
<tr>
<td>Mean BP, mm Hg</td>
<td>111±3</td>
<td>103±2 †</td>
<td>−6±1</td>
</tr>
<tr>
<td>SVR, units</td>
<td>23.9±1.8</td>
<td>21.3±1 †</td>
<td>−10±2</td>
</tr>
<tr>
<td>Mean PAP, mm Hg</td>
<td>17.2±0.9</td>
<td>15.3±0.8*</td>
<td>−15±3</td>
</tr>
<tr>
<td>Mean</td>
<td>10.7±0.9</td>
<td>8.1±0.6†</td>
<td>−21±4</td>
</tr>
<tr>
<td>PWP, mm Hg</td>
<td>1.61±0.16</td>
<td>1.48±0.16*</td>
<td>−15±6</td>
</tr>
<tr>
<td>PVR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO, L/min</td>
<td>5.0±0.3</td>
<td>5.3±0.2</td>
<td>+7±4</td>
</tr>
</tbody>
</table>

HR indicates heart rate; BP, mean systemic arterial pressure; SVR, systemic vascular resistance; PAP, pulmonary artery pressure; PWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; and CO, cardiac output. Data represent mean±SEM.

*P<0.01, †P<0.001 vs baseline value.

−31±9% [r=0.98)] and with 20 μg/min SNP (−41±17% and −39±7% [r=0.9]) and percentage change in D with ACH (−1.4±1.4% and −0.7±1.5% [r=0.9]) and SNP (19±2% and 20±2% [r=0.8]) were similar during repeat testing.

Measurement of Plasma BigET-1 and ET-1
Blood was drawn in EDTA tubes and immediately chilled on ice and centrifuged at 4°C at 2500 rpm for 10 minutes, and plasma was frozen at −70°C. Plasma levels of ET-1 and BigET-1 were measured using radioimmunoassay techniques.¹⁴,²⁹

Statistical Analysis
Data are mean±SEM. Differences between means were compared by paired or unpaired Student t test as appropriate. Dose-response curves were compared by ANOVA (SAS version 6.12; SAS Institute, Cary, NC). If the F value was significant, a Bonferroni multiple-comparison test was performed. Univariate correlations were performed using the Pearson correlation coefficient for continuous variables, and the Pearson χ² test for categorical variables. Multiple stepwise regression analysis was performed to test whether the magnitude of change with BQ-123 was related to the age, sex, atherosclerosis, hypertension, diabetes, cigarette use, or total LDL and HDL cholesterol levels (SAS, version 6.12). All probability values are 2-tailed, and a P value of <0.05 was considered of statistical significance.

Results

Systemic and Pulmonary Hemodynamic Changes With BQ-123
Mild systemic vasodilation was observed with intracoronary BQ-123; after 1 hour, mean arterial pressure fell by 8 mm Hg (P<0.001), heart rate increased by 4 bpm (P=0.003), and cardiac output was unchanged (Table 2). Mean pulmonary artery pressure and pulmonary artery wedge pressure also fell by 1.9 mm Hg (P<0.01) and 2.6 mm Hg (P<0.001), respectively (Table 2).

Coronary Vascular Effects of BQ-123
Epicaerdi coronary vasodilation was significant after 30 minutes and reached a maximum of 5.6±1.0% after 1 hour of BQ-123 (P<0.001; Figure 1). Middle and distal segments of the coronary arteries dilated by a similar amount (6.3±1.6% and 5.1±1.3% respectively, P=0.57). Significant vasodilation was also observed at the site of mild atherosclerotic plaquing (minimum lumen D increased by 12%, from 1.55±0.12 to 1.77±0.20 mm after BQ-123).

There was progressive microvascular dilation with BQ-123; coronary blood flow increased by 9.1±3.5% (P<0.02), and CVR fell by −12.1±3.1% (P<0.02) after 1 hour (Figure 2). There was no correlation between the magnitude of epicaerdi or microvascular vasodilation observed with BQ-123 and the presence of CAD or its risk factors.

Effect of BQ-123 on the Coronary Vascular Responses to ACH and SNP
ACH infusion at baseline produced progressive microvascular dilation, measured as a reduction in CVR (−32.2±5.5%, P<0.005) and epicaerdi coronary artery constriction

![Figure 1](image-url)

Figure 1. Epicardial diameter (A) and CVR (B) responses to 60-minute intracoronary infusion of BQ-123. Data are mean±SEM. *P<0.02 compared with baseline (one-way ANOVA).
(−1.9±1.4%, \(P<0.02\)) at 15 \(\mu\)g/min ACH. After BQ-123, CVR was lower and \(D\) was greater at each dose of ACH (Figure 2).

To exclude the contribution of the baseline coronary vasodilation due to BQ-123 on the ACH response, we measured the percentage changes in CVR and \(D\) with ACH, before and after BQ-123. Whereas ACH caused significant epicardial vasoconstriction at baseline, repeat administration of ACH after BQ-123 nonsignificantly attenuated the constrictor response (\(P=0.076\) by ANOVA; Figure 2). The increase in the coronary blood flow with ACH (from 84±15% to 100±14%, \(P=0.25\), at 15 \(\mu\)g/min) and the further fall in CVR with ACH after BQ-123 also did not reach statistical significance (Figure 2).

Similar findings were noted with SNP. With each dose of SNP, \(D\) was greater; CVR was lower after BQ-123 (Figure 3). To exclude the contribution of the baseline coronary vasodilation by BQ-123, the percentage changes in \(D\), flow, and CVR were compared from the baselines before and after BQ-123 (Figure 3). There was no significant percentage change in \(D\) (\(P=NS\)) or coronary blood flow (from 69±13% to 86±13%, \(P=0.2\), at 20 \(\mu\)g/min); however, the percentage change in CVR with SNP was enhanced by BQ-123 (\(P=0.04\) by ANOVA; Figure 3).

**Effect of BQ-123 on Endothelial Dysfunction**

To assess endothelial function with ACH independent of the nonspecific dilator effect of BQ-123 and of SNP responses, we calculated the ratio of ACH:SNP responses in the epicardial coronary arteries before and after BQ-123 in the 23 patients. There was a significant improvement in the epicardial ACH:SNP ratio after BQ-123 (\(P=0.029\) by ANOVA). We also determined whether the response to ACH after ET\(_A\) receptor blockade varied according to the severity of the underlying endothelial dysfunction. There was a significant inverse correlation between the baseline response to ACH and the magnitude of improvement in this response after BQ-123 (\(r=−0.44\), \(P=0.006\)), indicating that ET\(_A\) receptor antagonism improved epicardial endothelial function most in patients with the greatest degree of baseline constriction with ACH and vice versa. Thus, when the response to BQ-123 was separately assessed in epicardial segments that initially constricted in response to ACH (denoting endothelial dysfunction), epicardial diameter with ACH, corrected for the SNP response (ACH:SNP ratio), was significantly improved after BQ-123. In contrast, in segments with more normal endothelial function that dilated with ACH, the ACH:SNP ratio was similar before and after BQ-123 (Figure 4).

There was a significant correlation between baseline microvascular endothelial function, measured as percentage change in CVR with ACH, and the observed improvement in this response after BQ-123 (\(r=−0.78\), \(P<0.001\)), indicating that patients with the greatest impairment of microvascular endothelial function at baseline derived the greatest improvement with ET\(_A\) receptor antagonism and vice versa (Figure 5).

**Effect of BQ-123 on CPT**

During CPT, heart rate and blood pressure increased by 10% and 18% before BQ-123 and by 11% and 18% after BQ-123, respectively (both \(P=NS\) before versus after BQ-123).

There was significant reduction in \(D\) (−2.0±1.1%, \(P=0.05\)) with CPT at baseline, but after BQ-123 epicardial dilation occurred (+1.0±0.7%, \(P=0.056\) before versus after; Figure 6). \(D\) changes with CPT were heterogeneous; after

**Figure 2.** Effect of ACH (1.5 to 15 \(\mu\)g/min) on epicardial diameter (A), percentage change in epicardial diameter (B), CVR (C), and percentage change in CVR (D) before and after BQ-123. Data are mean±SEM; probability values are results of two-way ANOVA.
BQ-123, significant improvement was observed in segments that initially constricted with CPT (from $-5.6\pm0.9\%$ to $+2.2\pm0.9\%$, $P<0.0001$), whereas dilating segments remained unchanged.

CVR during CPT was unaltered after BQ-123 (percentage CVR from $+2.4\pm4.6\%$ versus $+0.4\pm2.4\%$ [$P=NS$]; Figure 6). As in the epicardial circulation, the microvascular responses were heterogeneous; after BQ-123, significant improvement was observed only in patients with constriction to CPT at baseline (percentage CVR from $+17.4\pm5.4\%$ to $-0.8\pm3.4\%$ after BQ-123 [$P<0.05$]).

**Figure 3.** Effect of SNP (10 to 20 $\mu$g/min) on epicardial diameter (A), percentage change in epicardial diameter (B), CVR (C), and percentage change in CVR (D) before and after BQ-123. Data are mean±SEM; probability values are results of two-way ANOVA.

**Figure 4.** Ratio of ACH:SNP responses in the epicardial circulation before and after BQ-123 in segments constricting with ACH (A) at baseline and in segments with baseline vasodilation with ACH (B). Data are mean±SEM. *$P<0.01$.

**Effect of BQ-123 on Plasma Levels of BigET-1 and ET-1 in Coronary Circulation**

No correlation was observed between baseline levels of ET-1 or BigET-1 and the coronary vascular responses to ACH, BQ-123, or the magnitude of improvement of ACH response with BQ-123.

Arterial levels of BigET-1 and ET-1 were unchanged after 60 minutes of BQ-123 infusion. Coronary sinus BigET-1 levels were lower than in arterial blood before and after BQ-123 (arteriovenous difference $=1.99\pm0.62$ pg/mL [$P=0.006$] and $2.70\pm1.06$ pg/mL [$P=0.02$] before and after
BQ-123, respectively). ET-1 levels were similar in arterial and coronary sinus blood at baseline. However, after BQ-123 infusion, coronary sinus ET-1 levels were significantly lower than arterial levels (arteriovenous difference = 1.37 ± 1.29 pg/mL [P = 0.31] and 3.45 ± 1.49 pg/mL [P = 0.035] before and after BQ-123 respectively), indicating enhanced extraction of ET-1 in the coronary circulation.

**Discussion**

We demonstrate that ET-1 modulates basal coronary vascular tone in patients with CAD and those with risk factors for atherosclerosis. Blockade of ET_A receptors vasodilates coronary conductance and resistance vessels, confirming the tonic vasoconstrictor effect of endogenous ET-1 via activation of ET_A receptors in the human coronary circulation in vivo.

Additionally, we demonstrate that ET_A receptor blockade improves abnormal coronary vascular reactivity to endothelium-dependent physiological and pharmacological stressors, providing the first evidence that endogenous ET-1, via ET_A receptor activation, contributes to human coronary vascular endothelial dysfunction in vivo.

**ET-1–Mediated Coronary Vasoconstrictor Tone**

We demonstrate that ET-1–mediated ET_A receptor activation contributes to resting coronary vasoconstrictor tone in patients with atherosclerosis or its risk factors, confirming findings from previous smaller studies. In one study, BQ-123 was only administered for 15 minutes, which likely underestimated its full effect. We noted that ET_A receptor blockade dilated epicardial arteries to \( \sim 40\% \) of the extent

**Figure 5.** Difference in percentage change in CVR (\( \Delta \% \text{CVR} \)) in response to ACH (15 \( \mu \text{g/min} \)) after administration of BQ-123 (y-axis) vs percentage change in CVR (\%CVR) in response to ACH at baseline (x-axis).

**Figure 6.** Effect of cold pressor test on epicardial diameter (A), percentage change in epicardial diameter (B), CVR (C), and percentage change in CVR (D) before and after BQ-123. Data are mean±SEM.
observed with the nitrovasodilator SNP. Similarly, despite the tight autoregulatory control, ET_A receptor blockade produced a modest vasodilation of the coronary microcirculation. In contrast, antagonists of other endogenous autocrine and paracrine vasoactive substances known to modulate coronary vasoconstrictor tone in vitro, including angiotensin II, constrictor prostaglandins, and norepinephrine, do not dilate the coronary microvessels to the extent observed with ET-1 receptor blockade in this study.30–32 Bosentan, a combined ET_A and ET_B receptor antagonist, produced epicardial but not microvascular coronary vasodilation. However, ET-1 levels rose after bosentan, whereas they were reduced in the coronary circulation after BQ-123.33 Thus, it is likely that the differences observed between these studies could be attributed to the incremental ET_A receptor blockade from bosentan and potentially to additional reflex effects from systemic administration of bosentan in that study. Similar to our observations, another recent study with BQ-123 demonstrated greater vasodilation at the site of significant stenoses than in nonstenosed segments of the same vessels.34

Increased ET_A receptor–mediated vasoconstrictor tone in hypertensive and hypercholesterolemic patients compared with healthy subjects has been observed in the forearm microcirculation, suggesting that ET-1 activity is lower in healthy individuals.18,19,21 In our study, the vasodilator effect with BQ-123 on coronary vascular tone was unrelated to any of the conventional risk factors for atherosclerosis or to baseline endothelial function, indicating that none of these factors was the sole determinant of coronary vascular ET-1 activity.

We observed an arteriovenous difference in plasma BigET-1 levels across the coronary circulation at baseline that was unchanged after BQ-123. This is consistent with either extraction of BigET-1 or, more likely, conversion of BigET-1 to ET-1 in the heart, a process that is not significantly affected by ET_A receptor antagonism. The observed development of an arteriovenous difference in ET-1 levels after BQ-123 infusion is consistent with increased transcardiac extraction of ET-1, which is probably accounted for by enhanced ET_B receptor–mediated clearance of circulating ET-1 during selective blockade of ET_A receptors. This may further contribute to BQ-123–mediated vasodilation. Of note, basal levels of BigET-1 and ET-1 did not correlate with the coronary vascular responses to BQ-123, suggesting that they are poor predictors of tissue ET-1 activity.

Whether the observed vasodilator effect of BQ-123 was entirely due to inhibition of ET-1–mediated constriction or by additional attenuation of angiotensin II and norepinephrine–mediated pressor effects could not be determined from our study.35–37

Systemic and Pulmonary Effects of ET_A Receptor Blockade

When given intravenously, the 1.2-g total dose of BQ-123 administered intracoronarily in this study effectively counteracts the forearm constrictor effect of ET-1.27 The dose and duration of administration of BQ-123 were based on previous experience in the forearm circulation in which the peak effect occurred after 1 hour at the intravascular concentrations achieved in our study.18 The significant systemic vasodilation observed is compatible with previous studies and demonstrates that endogenous ET-1, acting via the ET_A receptors, is an important determinant of peripheral vasoconstrictor tone in these patients. Additionally, the modest fall in pulmonary vascular resistance observed with BQ-123 is consistent with findings in subjects with chronic heart failure.38

Effect of ET_A Receptor Blockade on Endothelium-Dependent and -Independent Vasomotion

Epicardial arteries were more dilated and CVR was further lowered when both ACH and SNP were administered after ET_A receptor blockade with BQ-123. However, this finding, which implies that there was a generalized improvement in vasodilator capacity of the coronary vasculature, is complicated by the baseline vasodilation observed with BQ-123. To overcome this, we analyzed the effects of ACH and SNP as percentage change from the new baseline after BQ-123. This analysis revealed a strong trend toward a selective improvement in endothelium-dependent responses after BQ-123. Because our study population exhibited a wide range of endothelial responses, we analyzed our data according to the baseline response to ACH. Epicardial segments that constricted with ACH improved significantly with BQ-123, whereas segments that dilated at baseline did not change significantly. Similarly in the microcirculation, those with the greatest impairment of endothelial function derived the greatest improvement after ET_A blockade.

Effect of ET_A Receptor Blockade on CPT

We examined coronary vasomotor changes during CPT to determine whether the improvement in vascular function observed with ET_A receptor blockade translated into improvement in physiological coronary vasomotion. We found that CPT-induced epicardial constriction was abrogated by BQ-123. However, only those with dysfunctional, constrictor responses to CPT and not those with more normal, dilator responses improved after ET_A antagonism, further supporting the notion that acute ET_A antagonism differentially improves endothelial function in dysfunctional vessels. Alternatively, it is possible that the effects observed in our study could be primarily attributed to ET_A receptor blockade, because CPT can stimulate ET-1 release.39

Potential Mechanisms

Our physiological study was not designed to provide a biochemical explanation for the observed improvement. It is known that oxidant stress reduces NO bioavailability, contributing to endothelial dysfunction in patients with atherosclerosis and its risk factors.40,41 ET-1–stimulated production of oxygen free radicals by neutrophils in vitro is inhibited by ET_A receptor blockade.42,43 and postischemic coronary endothelial dysfunction can be attenuated by pretreatment with BQ-123.44 Thus, reduced oxidative consumption of endothelium-derived NO provides a compelling explanation for our findings. Additionally, because activation of endothelial ET_B receptors stimulates NO synthesis, it is possible that enhanced endothelial ET_B receptor activation after selective ET_A recept-
tor blockade may have contributed toward the improvement of endothelial function.

Our findings in humans with CAD and risk factors for atherosclerosis differ from observations in pigs, in which acute ET\textsubscript{A} antagonism with FR-139317 did not improve the response to ACH.\textsuperscript{45} These discrepancies may be due to interspecies differences and the shorter duration of ET\textsubscript{A} receptor blockade used in that study. In contrast, longer-term ET\textsubscript{A} antagonism attenuates the progressive deterioration in endothelial function in the porcine model.\textsuperscript{25}

**Limitations**

We did not perform intravascular ultrasound of the coronary arteries, so it is possible that some intimal thickening may have been missed in patients classified as having normal coronary arteries. Additionally, we have not studied healthy subjects with normal coronary arteries without any risk factors for atherosclerosis or those with severe stenoses and therefore cannot extend our observations to these populations or to patients with congestive heart failure.

Our study cohort was heterogeneous because of the ethical considerations underlying recruitment of subjects for such studies. We attempted to minimize this variable by recruiting 44 subjects, and although we did not find that CAD or any of its risk factors were determinants of the response to BQ-123 by univariate or multivariate analysis, it is possible that much larger groups of patients with individual risk factors may reveal such a relationship.

The peripheral vasodilation observed with BQ-123 may have activated reflex coronary constriction. Despite this, we observed coronary vasodilation, indicating that ET\textsubscript{A} receptor blockade is a powerful vasodilator stimulus. Also, the magnitude of coronary dilation with BQ-123 was similar in patients with and without hypertension at baseline despite the differential effect of BQ-123 on systemic blood pressure in these subjects.

**Conclusions and Implications**

Tonic basal ET\textsubscript{A} receptor–mediated constrictor effects of ET-1 are demonstrated in the epicardial and microvascular coronary circulation of patients with risk factors for atherosclerosis and those with mild atherosclerosis. ET\textsubscript{A} receptor blockade selectively improved endothelial dysfunction and the abnormal vasomotion during physiological stress. Transcardiac clearance of circulating ET-1 also became apparent after ET\textsubscript{A} receptor blockade. Our finding that acute ET\textsubscript{A} receptor antagonism can improve coronary tone at rest and during stress, in addition to lowering blood pressure, may have important implications for the treatment of myocardial ischemia and hypertension. ET\textsubscript{A} receptor antagonist–mediated improvement of coronary endothelial dysfunction may favorably impact on the consequences of atherosclerosis; however, this requires further study with novel orally available agents.

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