Role of Endothelin-1 in the Active Constriction of Human Atherosclerotic Coronary Arteries

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Background—Atherosclerotic coronary arteries are prone to constriction but the underlying causes are incompletely understood. We tested the hypothesis that endothelin-1 (ET-1), a potent vasoconstrictor, contributes to the heightened tone of atherosclerotic human coronary arteries.

Methods and Results—In 8 patients with coronary artery disease (CAD) and 8 patients with angiographically smooth coronary arteries (normal), we infused BQ-123, an antagonist of the ET_A receptor, into a major coronary artery (infused artery) at 40 nmol/min for 60 minutes. The infused artery in the CAD patients contained a >50% stenosis. Using quantitative angiography, we compared the dilation of the infused artery with another, noninfused coronary artery. To estimate the magnitude of the contribution of ET-1 to coronary tone, we compared the dilation to BQ-123 with that elicited by intracoronary nitroglycerin (200 μg). BQ-123 induced significant dilation in the normal arteries (7.3% at 60 minutes, \( P < 0.001 \) versus noninfused arteries) and a greater dilation in the CAD arteries (16.3% at 60 minutes, \( P < 0.001 \) versus infused normal arteries). The dilation at stenoses was particularly pronounced (21.6% at 60 minutes, \( P < 0.001 \) versus infused CAD arteries). Compared with the dilation from nitroglycerin, ET-1 contributed to 39% of the coronary tone in normal arteries, 74% of tone in CAD arteries, and 106% of tone at stenoses (\( P < 0.001 \)).

Conclusions—ET-1 accounts for nearly all the resting tone in atherosclerotic coronary arteries, especially at stenoses. Inhibitors of ET-1, by relieving constriction, may significantly lessen the hemodynamic significance of coronary stenoses and thereby reduce myocardial ischemia. (Circulation. 2001;104:1114-1118.)

Key Words: endothelin ■ endothelium ■ vasoconstriction ■ atherosclerosis

Atherosclerotic coronary arteries are prone to inappropriate constriction that exacerbates the severity of coronary stenoses and thereby contributes to the pathogenesis of myocardial ischemia.\(^1–5\) Increased vasmomotor tone is partly due to the reduced bioavailability of the endogenous vasodilator, endothelium-derived nitric oxide.\(^6,7\) However, the vessel wall also produces vasoconstrictors that may contribute to the heightened coronary vasmomotor tone in patients with atherosclerosis. One of the most potent vasoconstrictor factors produced in the arterial wall is endothelin-1 (ET-1), a 21-amino acid peptide. Although only scant immunoreactive ET-1 is present in the endothelium of normal human coronary arteries, abundant ET-1 is present throughout the thickened intima of atherosclerotic human coronary arteries.\(^8–10\) Whether this immunoreactive ET-1 contributes to the exaggerated constriction of atherosclerotic human coronary arteries is unknown.

The recent availability of ET receptor antagonists has permitted the investigation of the role of ET-1 in the vasmomotor control of the human circulation in vivo.\(^11–14\) In the forearm, ET-1 binds to 2 specific receptors, termed ET\(_A\) and ET\(_B\). ET\(_A\) receptors are located on vascular smooth muscle and mediate vasoconstriction.\(^15\) ET\(_B\) receptors are located on both endothelial cells, where they mediate dilation by releasing nitric oxide, and on smooth muscle cells, where they contribute to constriction.\(^15\) On the basis of ligand-binding studies, ET\(_A\) receptors are the dominant receptors in the human coronary arteries, accounting for >85% of ET receptors.\(^16\) Hence, ET-1–induced constriction of human coronary arteries would most likely be mediated by ET\(_A\) receptors.

In the present study, we tested the hypothesis that ET-1, acting through the ET\(_A\) receptor, contributes to the exaggerated constriction of atheroslerotic human coronary arteries and, to a lesser degree, to the physiological tone of normal coronary arteries. Accordingly, we evaluated the vasmomotor responses of atherosclerotic and angiographically normal coronary arteries to the intracoronary administration of the selective ET\(_A\) receptor antagonist BQ-123 in patients undergoing cardiac catheterization.

Methods

Patient Population

We approached patients aged 18 to 75 years who were undergoing diagnostic cardiac catheterization for the investigation of coronary artery disease (CAD). Patients were divided into 2 groups on the basis of clinical syndrome and angiographic findings. The first group

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ET A receptor in the circulation of the human forearm. The arteries, and an angiogram taken to assess the maximum dilator through the guiding catheter into both the infused and control
infused with BQ-123 were analyzed offline by quantitative coronary angiography.

Figure 1. Study protocol. The coronary artery was engaged with a guiding catheter, and an infusion catheter was directed into the proximal artery. BQ-123 was infused selectively into one artery (infused artery), and the percent dilation in 3 artery segments was averaged. The dilation in a noninfused artery served as a control. In the CAD group, the dilation of a stenosis (>50%) in the infused artery was also measured. QCA indicates quantitative coronary angiography.

had typical anginal chest pain and at least one 50% stenosis in a major coronary artery (CAD group). The second group had atypical chest pain, angiographically smooth coronary arteries suitable for study, and (at most) minor luminal irregularities in the arteries that were not studied (normal group).

We excluded patients with left ventricular dysfunction (ejection fraction <40%) and severe renal or hepatic abnormalities. Patients taking long-acting nitrates or calcium channel blockers omitted these medications for at least 24 hours before the study.

Coronary risk factors were assessed from a review of the medical records and by patient interview. Hypertension was defined by the use of antihypertensive therapy; current smoking as at least one cigarette per day in the last 30 days; and diabetes mellitus by treatment with diet, oral hypoglycemics, or insulin. Informed consent was obtained from all patients according to the standards of the Human Research Committee of the Brigham and Women’s Hospital.

Study Protocol
After the diagnostic catheterization, one major coronary artery was identified for drug infusion (infused artery). This infused artery was angiographically smooth in normal patients and contained at least one stenosis (>50% by visual inspection) in the CAD patients. Another noninfused artery served as a time control in both groups.

Intravenous heparin (5000 to 10 000 U) was given, and a 0.014-inch guidewire was advanced through a 6F guiding catheter into the infused artery. A 2.5F catheter (Target Therapeutics) was advanced over the guidewire for selective drug infusion. Dextrose 5% was infused through the catheter at 0.8 cc/min for 5 to 10 minutes to establish baseline conditions. After a baseline angiogram, BQ-123 was infused into the artery at 40 nmol/min (0.8 cc/min) for 60 minutes, and angiograms were taken every 15 minutes. This dose of BQ-123 yielded an estimated local blood concentration of 0.5 mM (60-minute duration) which completely inhibited the ET(1) receptor in the circulation of the human forearm. The 60-minute duration of infusion was chosen as the time required for BQ-123 to reach a plateau effect in prior investigations in the human forearm.

After BQ-123 infusion, nitroglycerin (200 μg) was given by bolus through the guiding catheter into both the infused and control arteries, and an angiogram taken to assess the maximum dilator capacity of epicardial coronary arteries.

Epicardial Response
Three segments (proximal, mid, and distal) from the infused coronary artery and 3 segments from the coronary artery that was not infused with BQ-123 were analyzed offline by quantitative coronary angiography. In the CAD group, an additional stenotic segment of the infused artery was also analyzed (Figure 1).

Quantitative Coronary Angiography
End-diastolic cineframes were digitized, and the luminal diameters of 3 segments of the infused artery and 3 segments of the control artery were measured by computerized quantitative angiography (CMS-QCA, MEDIS), as previously described. The percentage change in epicardial diameter from baseline in each of the 3 segments was averaged. In the CAD subjects, the change of an additional stenotic segment (>50% stenosis) in the infused artery was also measured.

Coronary Blood Flow
In the normal patients, changes in coronary microvascular tone over the 60-minute infusion of BQ-123 were assessed by measuring coronary blood flow velocity with a 0.014-inch Doppler Flowwire (Endosonics). The Flowwire was used instead of the guidewire and was maintained in a stable position throughout the study.

Coronary blood flow velocity was calculated as follows: APV/2 (APV indicates average peak velocity). Coronary blood flow volume was calculated as follows: (diameter measured 5 mm distal to the Flowire tip using quantitative coronary angiography).

Blood flow was not measured in the CAD patients due to the presence of a >50% stenosis in the epicardial artery, which would have invalidated this assessment of microvascular tone.

Statistical Analysis
The primary end point of the study was the change in epicardial diameter of the infused artery to BQ-123, as measured by quantitative coronary angiography. The responses to BQ-123 and to nitroglycerin were normally distributed. To analyze the responses over the 60 minutes of BQ-123 infusion, we performed 3 analyses. First, the percent changes in epicardial diameter of the CAD and normal infused arteries were compared with their respective noninfused arteries. The significance of these comparisons was assessed by group-vessel-time interaction terms from randomized effects models with generalized least squares estimator. Second, we similarly compared the dilation from BQ-123 of the CAD infused arteries to the dilation of the normal infused arteries and the dilation of the stenoses to the dilation of the CAD infused arteries. Finally, the ratio of the dilation to BQ-123 at 60 minutes to the dilation elicited by nitroglycerin was used to assess the percentage of resting vasmotor tone attributable to ET-1 in the normal and CAD infused arteries and the stenoses. A multivariate randomized-effects model was used to assess the effect of BQ-123 after adjusting for baseline variables that differed between the groups. Coronary blood flow changes were compared with baseline using similar models. Differences were assessed with 95% confidence intervals (95% CI) and t tests. All data were analyzed using STATA (Statacorp).

Results
We enrolled 8 patients with CAD and 8 patients with angiographically normal coronary arteries for study. Their coronary risk factors are shown in Table 1. As expected, HDL cholesterol was lower, statin use higher, and the proportion of men higher in the CAD group. In the CAD group, 3 subjects (38%) had one-vessel disease (>50% stenosis by visual inspection), 4 (50%) had 2-vessel disease, and 1 (12%) had 3-vessel disease.

There was a slight decrease in the mean blood pressure over the 60-minute infusion of BQ-123. This change in mean blood pressure was similar in magnitude in the CAD group (from 90±7 to 86±9 mm Hg at 60 minutes) and normal group (from 95±12 to 86±13 mm Hg at 60 minutes, P=NS). The average heart rate did not change significantly in either
Epicardial Responses to BQ-123

There were no significant differences in the baseline diameters between the normal and CAD groups (Table 2). Figure 2 displays the vasodilation of the epicardial arteries as a percentage change from baseline during the 60-minute infusion of BQ-123. There was no detectable change in the diameter of the noninfused arteries of the normal or CAD group over the 60 minutes. There was significant vasodilation of the BQ-123–infused arteries of the normal group (7.3±1.9%) at 60 minutes (P<0.001 versus noninfused normal arteries) and an even greater dilation of the BQ-123–infused artery of the CAD group (16.3±4.3%) at 60 minutes (P<0.001 versus noninfused arteries of the CAD group, P<0.001 versus infused arteries of the normal; Figure 1). In the CAD group, the dilation at the stenoses was even greater than that observed in the averaged segments of the CAD infused artery (21.6±11.7%) at 60 minutes (P<0.001; Figure 1).

Contribution of ET to Epicardial Vasomotor Tone

The relative contribution of ET-1 to vasomotor tone was assessed by comparing the vasodilation achieved by BQ-123 to that elicited by intracoronary nitroglycerin, a maximal dilator of epicardial arteries. The dilation to nitroglycerin was 21±6% in the normal infused artery, 24±9% in the CAD infused artery, and 22±16% in the stenosis (P=NS for all comparisons). ET-1 contributed to 39% (95% CI, 24%, 55%) of total vasomotor tone in the normal vessels, 74% (95% CI, 56%, 92%) of total vasomotor tone in the CAD vessels, and 106% (95% CI, 88%, 124%) of the total vasomotor tone at the stenoses (Figure 3; P<0.01 for all comparisons).

Multivariate Analysis

Using multivariate analysis, we assessed the effect of the baseline characteristics that differed between the CAD and normal groups, which were HDL cholesterol, sex, and statin use. There was no independent effect of these variables on the response of the infused arteries to BQ-123.

Coronary Microvascular Response in Normal Patients

Coronary blood flow was measured in 7 normal patients (in one other patient, a stable Doppler wire position could not be obtained). In the 7 normal patients studied, there was no significant change in coronary blood flow velocity over the 60-minute infusion of BQ-123 (6±18% at 60 minutes, P=0.7). Compared with baseline, coronary blood flow volume increased modestly in the 7 normal subjects (19±6% at

### TABLE 1. Clinical Characteristics of the Study Group

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=8)</th>
<th>Coronary Disease (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>52±9</td>
<td>54±8</td>
</tr>
<tr>
<td>Mean, n (%)</td>
<td>2 (25)</td>
<td>6 (75)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>3 (38)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Current smoking status, n (%)</td>
<td>2 (25)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>0 (0)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Statin use, n (%)</td>
<td>1 (13)</td>
<td>6 (75)*</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>190±59</td>
<td>170±50</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>101±43</td>
<td>103±37</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>62±22</td>
<td>37±11*</td>
</tr>
</tbody>
</table>

Values are mean±SD or n (%).

*P<0.05.

### TABLE 2. Coronary Artery Diameters Before and After the 60-Minute Infusion of BQ-123 in Each of the Segments Studied

<table>
<thead>
<tr>
<th>Coronary Artery Diameters, mm</th>
<th>Baseline</th>
<th>60 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.78±0.51</td>
<td>1.76±0.49</td>
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<tr>
<td>BQ-123 infused artery</td>
<td>1.79±0.53</td>
<td>1.92±0.58*</td>
</tr>
<tr>
<td>CAD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.19±0.71</td>
<td>2.19±0.72</td>
</tr>
<tr>
<td>BQ-123 infused artery</td>
<td>1.83±0.38</td>
<td>2.11±0.42*</td>
</tr>
<tr>
<td>BQ-123 infused stenosis</td>
<td>1.40±0.44</td>
<td>1.68±0.41*</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P<0.01 compared with change in corresponding control vessel.
60 minutes, \(P=0.001\). This change was entirely related to the increase in epicardial cross-sectional area.

**Discussion**

This study demonstrates the pivotal role that ET-1, acting via the ET\(_A\) receptor, plays in the active constriction of human atherosclerotic coronary arteries. At sites of stenoses, it accounts essentially for all of the resting vasomotor tone. To a lesser degree, ET-1 also contributes to the resting tone of angiographically normal epicardial arteries and resistance vessels.

**Use of Selective ET\(_A\) Antagonist BQ-123**

Actions of ET-1 are mediated by ET\(_A\) and ET\(_B\) receptors. ET\(_A\) is the principal constrictor receptor, whereas ET\(_B\) has mixed actions with direct constriction and nitric oxide–dependent dilation. ET\(_A\) receptors are the predominant receptors in the coronary arteries, accounting for \(>85\%\) of all ET receptors.\(^{16}\) Therefore, we used BQ-123, a potent and highly specific ET\(_A\) receptor antagonist to probe the effect of ET-1 in human coronary arteries. The dose of BQ-123 in the present study was chosen to yield a blood concentration that is 10 to 100-fold higher than the reported half-maximum inhibitory concentration of BQ-123 in ligand-binding studies yet one that is still highly selective for the ET\(_A\) receptor.\(^{19}\) In the human forearm vasculature, a similar concentration of BQ-123 effectively blocked the constriction in response to exogenously infused ET-1.\(^{20}\)

**Source of ET-1 in Atherosclerotic Human Coronary Arteries**

Although ET-1 was initially discovered as a product of endothelial cells, it is also released from activated macrophages and smooth muscle cells, which are abundant in atherosclerotic arteries.\(^{8,10,21}\) Oxidized LDL and various cytokines and growth factors that participate in atherogenesis are capable of stimulating the production of ET-1 by macrophages and vascular smooth muscle cells.\(^{22–26}\) Indeed, immunoreactive ET-1 is dramatically increased in the intima of human atherosclerotic coronary arteries, where it preferentially localizes to areas rich in macrophages and activated smooth muscle cells.\(^{8,10,21}\) Accordingly, we hypothesized that ET-1 would play a greater role in the constriction of atherosclerotic coronary arteries than normal coronary arteries.

In the present study, we found that the vasoconstriction induced by endogenous ET-1 was greatest at sites of atherosclerotic stenoses, where activated macrophages and smooth muscle cells tend to be abundant, and least in relatively normal epicardial arteries and normal resistance arterioles. When expressed as a fraction of dilation induced by nitroglycerin, ET-1 accounted for one-third of the resting tone in normal epicardial arteries, two-thirds of the resting tone in atherosclerotic arteries, and virtually all of the resting tone at sites of atherosclerotic stenoses.

**Potential Relationship Between ET-1–Induced Constriction of Human Coronary Arteries and Myocardial Ischemia**

ET-1 exerts a sustained constriction lasting for up to several hours.\(^{13,27}\) In contrast, myocardial ischemia is typically transient. So how may ET-1 contribute to the pathogenesis of ischemia? It is likely that the \(\geq 20\%\) constriction at stenoses by endogenous ET-1 observed in the present investigation markedly increases the hemodynamic severity of such lesions and thereby reduces the threshold to ischemia. We postulate that other short-lived constrictors, not necessarily as potent, that are related to sympathetic activation\(^{28}\) or platelet aggregation\(^{29}\) narrow stenoses further and predispose to brief episodes of ischemia. Increases in myocardial oxygen demand may also account for the intermittency of ischemia.

**Balance of ET-1–Induced Constriction and Nitric Oxide–Mediated Dilation in Atherosclerosis**

Coronary tone is governed by a balance of constrictor and dilator forces. Deficiency of a dilator or excess of a constrictor can “tip the scale” in favor constriction. Reductions in the bioavailability of the endothelium-derived vasodilator nitric oxide in human atherosclerotic arteries have been amply documented from this\(^{4,7,28}\) and other\(^{30–32}\) laboratories, and it is thought to be the principal reason for hypercontractility of atherosclerotic arteries. The unexpected finding in the present study is the large contribution of ET-1 to the abnormal constriction of human atherosclerotic coronary arteries. As noted previously, this ET-1 may not be derived solely from dysfunctional endothelial cells, but rather from inflammatory macrophages and activated smooth muscle cells.\(^{8,10,21}\)

As in the human forearm circulation, BQ-123 reverses the ET-1–mediated coronary constriction slowly, over 60 minutes. A recent study examined the contribution of ET-1 to the constriction of normal human coronary arteries.\(^{33}\) In that study, BQ-123 was administered for only 15 minutes and, thus, an incomplete response may have been observed. The 60-minute intracoronary infusion of BQ-123 in the present study may have induced a biphasic response with early and late dilation. Whether this complex response represents antagonisms of different sources of ET-1 throughout the intima or another mechanism is presently not known.

**Limitations**

We were unable to assess the contribution of ET-1 to blood flow and microvascular resistance in subjects with CAD because interpretation of the flow data are unreliable in the presence of flow-limiting stenoses. This is a limitation of flow wire assessment recognized by other catheterization laboratories that use this technique. Some subjects in our control group had risk factors for atherosclerosis. However, controls without risk factors would only have exaggerated the differences between normal and atherosclerotic arteries.

**Therapeutic Implications**

The present study has potentially important therapeutic implications. Inhibitors of ET-1, by reducing coronary constriction, would be expected to improve myocardial ischemia.

In conclusion, we have found that endogenous ET-1 tonically constricts human coronary arteries, especially those with atherosclerosis, and accounts for nearly all of the resting tone at coronary artery stenoses. These findings implicate ET-1 in the abnormal constriction of atherosclerotic coronary arteries and thereby in the pathophysiology of myocardial ischemia.
ischemia in atherosclerosis. Therapies that reduce ET-1 concentration and inhibit its action should be of interest for reducing myocardial ischemia.

Acknowledgment
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