Acute Endothelin-Receptor Inhibition Does Not Attenuate Acetylcholine-Induced Coronary Vasoconstriction in Experimental Hypercholesterolemia

David Hasdai, Patricia J.M. Best, Charles R. Cannan, Verghese Mathew, Robert S. Schwartz, David R. Holmes, Jr, Amir Lerman

Abstract—Endothelin (ET) may mediate the enhanced coronary vasoconstriction associated with hypercholesterolemia. We hypothesized that short-term inhibition of ET receptors attenuates the coronary epicardial vasoconstrictor response to acetylcholine in experimental hypercholesterolemia. ET-1 (group I, n=5; 5 ng ⋅ kg⁻¹ ⋅ min⁻¹) and acetylcholine (group III, n=7; 10⁻⁶ to 10⁻⁴ mol/L) were given by intracoronary infusion in pigs. ET-1 and acetylcholine were also infused with the specific ETA-receptor blocker FR-139317 (5 μg ⋅ kg⁻¹ ⋅ min⁻¹; group II, n=6; group IV, n=6). Acetylcholine was also infused with the combined ET-receptor blocker, bosentan (0.5 mg/kg plus 1 mg ⋅ kg⁻¹ ⋅ h⁻¹, group V, n=5). The ETB-receptor agonist sarafotoxin 6c (5 ng ⋅ kg⁻¹ ⋅ min⁻¹; n=4) was also infused. The percentage change in coronary artery diameter (%ΔCAD) to the infusions was measured at baseline and after 10 weeks of high-cholesterol diet in all animals. Sarafotoxin 6c mildly reduced %ΔCAD at baseline and 10 weeks (−10±2% and −12±3%, respectively). FR-139317 did not attenuate the epicardial vasoconstrictor response to ET-1 at baseline (%ΔCAD = 18±8% for group I versus −12±6% for group II; P=NS) but did at 10 weeks (%ΔCAD = 77±14% for group I versus −14±6% for group II; P<.05). FR-139317 did not affect the response to acetylcholine at baseline (%ΔCAD = 5±2% for group III versus 7±3% for group IV, P=NS) or at 10 weeks (%ΔCAD = 23±12% for group III versus −19±7% for group IV; P=NS). Bosentan did not affect the response to acetylcholine at baseline or 10 weeks. Short-term ET-receptor inhibition in experimental hypercholesterolemia attenuated the enhanced coronary epicardial vasoconstrictor effects of ET-1 but not acetylcholine-induced coronary epicardial vasoconstriction, suggesting that acetylcholine-induced coronary epicardial vasoconstriction may not be mediated by ET receptors. (Arterioscler Thromb Vasc Biol. 1998;18:108-113.)

Key Words: pig ■ hypercholesterolemia ■ acetylcholine ■ endothelin ■ endothelin receptor

Endothelins (ETs) are 21–amino acid peptides that act as modulators of coronary vasomotor tone.¹ There are two distinct receptors for ET, ETA and ETB, each encoded by a different gene.²,³ The ETA receptor is expressed in vascular smooth muscle, whereas the ETB receptor is localized to endothelial and smooth muscle cells.⁴ Coronary vasoconstriction is mediated by both receptors.¹ We recently reported that intracoronary infusion of ET-1 at pathophysiological concentrations results in coronary epicardial vasoconstriction in dogs, which was presumed to be mediated predominantly via the ETA receptor.

Hypercholesterolemia is a pathophysiological state characterized by impaired coronary endothelium-dependent arterial relaxation to the endothelium-dependent vasodilator acetylcholine.⁶,⁷ In a porcine model of diet-induced hypercholesterolemia, plasma concentrations of ET are elevated, and coronary tissue ET immunoreactivity is enhanced.⁸ Moreover, intracoronary infusion of acetylcholine causes vasoconstriction and further increases plasma ET concentrations.⁸ Similarly, humans with coronary endothelial dysfunction have enhanced ET immunoreactivity in the coronary circulation, with an additional rise in coronary ET levels during intracoronary infusion of acetylcholine.⁹

These prior studies implicate ET as a potential mediator of endothelial dysfunction in hypercholesterolemia, possibly through stimulation of the ET receptor. The present study was designed to examine the hypothesis that selective inhibition of the ET receptor attenuates the coronary epicardial vasoconstrictor response to acetylcholine in experimental hypercholesterolemia.

Methods

Animals and Instrumentation

The following studies were performed after approval by the Mayo Clinic Institutional Animal Care and Use Committee. All experiments were conducted using juvenile domestic crossbred pigs, each weighing 25 to 37 kg. In phase I, we verified that intracoronary infusion of FR-139317, a selective ETA receptor antagonist,¹⁰ inhibits the coronary vasoconstrictor response elicited by ET-1 after 10 weeks of high-cholesterol diet, in light of previous in vitro studies that have alluded to an increased vasoconstrictor response to ET in pathophys-

Received June 19, 1997; revision accepted September 26, 1997.
From the Division of Internal Medicine and Cardiovascular Diseases, Mayo Clinic and Foundation, Rochester, Minn.
Correspondence to Amir Lerman, MD, Division of Cardiovascular Diseases, Mayo Clinic, 200 First St SW, Rochester, MN 55905.
E-mail lerman.amir@mayo.edu
© 1998 American Heart Association, Inc.
ological states, such as hypercholesterolemia and atherosclerosis. In phase II, we examined the effect of intracoronary infusion of FR-139317 before intracoronary acetylcholine on coronary artery diameter in the steady state and after 10 weeks of diet-induced hypercholesterolemia. In phase III, we examined the effect of intracoronary infusion of the nonselective ET-receptor antagonist bosentan before intracoronary acetylcholine on coronary artery diameter in the steady state and after 10 weeks of diet-induced hypercholesterolemia. In phase IV, we further examined the role of the ETB receptor in the coronary epicardial vasoconstrictor to ETs at baseline and after 10 weeks of high-cholesterol diet.

Each animal was fasted overnight before the day of the study but allowed ad libitum access to tap water. Animals were initially anesthetized with ketamine 30 mg/kg IM and xylazine 5 mg/kg IM, and additional anesthesia was given using a tritrate intravenous infusion of a solution containing ketamine (5 g/L) and xylazine (7.5 mg/L) to maintain a constant level of anesthesia. The external or internal jugular vein was exposed by cutdown and cannulated with a 17F venous sheath. A flow-directed thermolodion catheter was advanced through the venous sheath for the measurement of PAP, PCWP, and CO. The external carotid artery was also exposed by cutdown and cannulated with an 8F arterial sheath. After injecting 10 000 U of heparin intravenously (followed by intravenous infusion of 1000 U of heparin per hour), an 8F Judkins left coronary guiding catheter was used to engage the left main coronary artery. A 2.2F coronary-infusion catheter (Ultrasound, SciMed Life System) was advanced over a guidewire and positioned into the proximal portion of the left anterior descending coronary artery. All infusions (see below) were delivered at a rate of 1 mL/min through the coronary infusion catheter. MAP and HR were continuously monitored throughout the procedure. Arterial blood samples for lipid profile and circulating ET-1 were obtained before each procedure.

After the baseline study, all animals were placed on a diet of 2% cholesterol and 15% lard by weight (TD 93296, Harlan Teklad) for 10 weeks. The respective studies were repeated in their entirety at 10 weeks.

**Study Groups**

**Phase I**

The animals were divided into two groups: ET-1 infusion (group I, n=5) and FR-139317 infusion followed by ET-1 infusion (group II, n=6). In pilot studies designed to determine the optimal dosage of ET-1, we found that intracoronary infusion of ET-1 (Peninsula Laboratories) at a rate of 2.5 ng · kg⁻¹ · min⁻¹ did not cause significant coronary epicardial vasoconstriction, whereas at a rate of 10 ng · kg⁻¹ · min⁻¹, there was severe vasoconstriction. When ET-1 was administered at a rate of 5 ng · kg⁻¹ · min⁻¹, moderate coronary epicardial vasoconstriction was achieved. In group I, normal saline was given by intracoronary infusion for 20 minutes, followed by infusion with ET-1 for 30 minutes. In group II, FR-139317 (Abbott Laboratories) was infused at 5 ng · kg⁻¹ · min⁻¹ for 20 minutes before infusion with ET-1. This dosage for FR-139317 was derived from previous studies performed in vivo in dogs.

The following parameters were measured at baseline (P1), after 20 minutes of either normal saline or FR-139317 (P2), and after an additional 30 minutes of coinfusion of either normal saline or FR-139317 with ET-1 (P3): HR, MAP, PAP, PCWP, and CO (average of three measurements). At each time interval, systemic vascular resistance was calculated using the formula Systemic Vascular Resistance = MAP/CO. Also at each time interval, coronary angiography was performed with the use of nonionic contrast medium (Omnipaque, Winthrop Laboratories) after the hemodynamic measurements had been taken. The angles, skew rotation, and table height were kept constant during the procedure and were used again during the study at 10 weeks. CAD was measured by an independent investigator blinded to the drug infused, using a computer-based image-analysis system, as previously described and validated. The left anterior descending coronary artery was divided into three segments: proximal, middle, and distal. For each segment, the measurements were performed in the region where the greatest change had occurred. For each time interval, the diameter refers to the mean of the three segments.

**Phase II**

The animals were divided into two groups: control (group III, n=7) and FR-139317 (group IV, n=6). In group III, normal saline was given by intracoronary infusion for 20 minutes, followed by coinfusion of normal saline and acetylcholine. Group IV received FR-139317 at 5 µg · kg⁻¹ · min⁻¹ for 20 minutes, followed by coinfusion of FR-139317 and acetylcholine. Acetylcholine (Iolab Pharmaceuticals), at concentrations of 10⁻⁶, 10⁻⁵, and 10⁻⁴ mol/L (to achieve estimated final blood concentrations in the coronary bed of 10⁻⁶, 10⁻⁵, and 10⁻⁴ mol/L, assuming coronary blood flow of 80 mL/min), was infused for 3 minutes at each concentration, as previously described. The same measurements described above for phase I were performed at the following time intervals: baseline (P1), after 20 minutes of infusion of either normal saline or FR-139317 (P2), and after coinfusion of either normal saline or FR-139317 with each dose of acetylcholine. Results are presented for each dose of acetylcholine, as well as for the highest dose of acetylcholine administered (P3).

**Phase III**

In this phase, the response to acetylcholine was examined in animals (group V, n=5) receiving bosentan (Ro 47-2023, intracoronary bolus of 0.5 mg/kg followed by an infusion at 1 mg · kg⁻¹ · h⁻¹; Hoffman La Roche) at baseline and after 10 weeks of high-cholesterol diet. The same measurements described above for phase I were performed at the following time intervals: baseline (P1), after 20 minutes of infusion of bosentan (P2), and after coinfusion of bosentan with each dose of acetylcholine. Results are presented for each dose of acetylcholine, as well as for the highest dose of acetylcholine administered (P3). In preliminary experiments, we examined the systemic hemodynamic effects of bosentan at different doses; after an intracoronary bolus of 0.5 mg/kg, bosentan was given by intracoronary infusion at 0.1, 1, and 5 ng · kg⁻¹ · h⁻¹ for 20 minutes at each dose. At 1 mg · kg⁻¹ · h⁻¹, bosentan caused mild systemic hypotension (~10% reduction in MAP), whereas at 5 mg · kg⁻¹ · h⁻¹, it reduced MAP by >20%. We thus elected to infuse bosentan at 1 mg · kg⁻¹ · h⁻¹ in phase III. In addition, bosentan at this dose significantly attenuated the severe vasoconstrictor response to 5 ng · kg⁻¹ · min⁻¹ ET-1 in two animals after 10 weeks of high-cholesterol diet (reduction in CAD of only 4±1%).

**Phase IV**

In this phase, we examined the coronary epicardial vasoconstrictor response to the ETB-receptor agonist S6c in baseline and after 10 weeks of high-cholesterol diet. S6c (Phoenix Pharmaceuticals) was infused at 5 ng · kg⁻¹ · min⁻¹ (ie, same dose as ET-1 infusion in phase I) through the coronary infusion catheter in four pigs at baseline and after 10 weeks of high-cholesterol diet. The same measurements described above for phase I were performed at the following time intervals: baseline (P1), after 20 minutes of normal saline infusion (P2), and after 30 minutes of coinfusion of normal saline and S6c (P3).
ET-Receptor Inhibitors and Endothelial Dysfunction

Statistics
Data from each period are expressed as mean±SEM. Comparison between groups were analyzed by ANOVA and Student’s unpaired t test. Within each group, repeated measurements were analyzed with repeated measures ANOVA and Student’s paired t test. Statistical significance was achieved with a value of P<.05.

Results

Lipid Profile
Plasma total cholesterol levels were significantly higher in animals fed a high-cholesterol diet than in pigs that were given a normal diet (376±24 versus 95±4 mg/dL; P<.0001). The increase in total plasma cholesterol levels could be attributed primarily to elevated LDL levels (289±15 versus 45±5 mg/dL; P<.0001), although HDL levels were also higher (79±5 versus 48±2 mg/dL; P<.0001).

ET Levels
Circulating ET levels were significantly higher after 10 weeks of high-cholesterol diet compared with baseline (13.5±1.2 versus 6.2±0.8 pg/mL; P<.05).

Hemodynamics and Coronary Artery Diameter
There were no significant differences in systemic hemodynamic parameters between the six groups at baseline or at 10 weeks before drug infusion.

Phase I
Intracoronary ET-1 infusion at baseline did not affect MAP significantly (94±6 mm Hg at P1 versus 91±6 mm Hg at P3; P=NS). Similarly, at 10 weeks, MAP was preserved (109±4 mm Hg at P1 versus 107±5 mm Hg at P3; P=NS). Hemodynamic parameters measured at baseline and at 10 weeks were not affected differently by infusion of FR-139317 or normal saline before and during intracoronary infusion of ET-1 (data not shown). As shown in the Figure, ET-1 given by intracoronary infusion in the baseline studies resulted in an 18±8% decrease in CAD. FR-139317 did not attenuate the vasoconstrictor response significantly (12±6% reduction in CAD; P=NS). However, after 10 weeks of a high-cholesterol diet, ET-1 caused an enhanced reduction in CAD (77±14%; P<.05 versus the response to ET-1 at baseline). Infusion of the specific ETA-receptor antagonist FR-139317 significantly attenuated the reduction in CAD observed with intracoronary ET-1 infusion (14±6% reduction in CAD with ET-1 and FR-139317 coinfusion versus 77±14% without FR-139317; P<.05).

Phase II
Systemic hemodynamic parameters measured at baseline and at 10 weeks were not affected differently by infusion of FR-139317 or normal saline before and during infusion of acetylcholine (Table 1). At baseline, intracoronary infusion of FR-139317 for 20 minutes resulted in a slight increase in CAD (5±1%), which was comparable to the effect attained with normal saline infusion (3±2%; P=NS). Coinfusion of FR-139317 and acetylcholine resulted in an additional 7±3% increase in CAD, which was not significantly different from the effect achieved with coinfusion of normal saline and acetylcholine (5±2%; P=NS) (Table 2 and Fig 1). At 10 weeks, CAD was affected similarly by a 20-minute infusion of normal saline and FR-139317 (4±3% and 2±1% increase in CAD with normal saline and FR-139317, respectively; P=NS). Likewise, FR-139317 coinfusion did not affect the vasoconstrictor response to acetylcholine infusion (23±12% and 19±7% reduction in CAD with coinfusion of normal saline and FR-139317, respectively; P=NS) (Table 2 and Fig 1).

Phase III
In phase II, we demonstrated the lack of inhibition of acetylcholine-induced coronary epicardial vasoconstriction by FR-139317, a selective ETA-receptor antagonist. In phase III, we aimed to examine whether the intracoronary administration of bosentan, a combined ETA- and ETB-receptor antagonist, at the highest tolerated dose (ie, without untoward hemodynamic effects) would attenuate the vasoconstrictive effects of acetylcholine. Intracoronary infusion of bosentan alone did not affect CAD at baseline (2±5%) and 10 weeks (0±1%). MAP decreased by 11.4±3.6% at baseline and 2.6±3.8% at 10 weeks (P=NS for comparison of baseline versus 10 weeks). HR increased with bosentan infusion at baseline (17.8±5.8%) and 10 weeks (5.0±4.4%), with nonsignificant changes in CO and PAP. The coinfusion of bosentan and acetylcholine did not affect the response to acetylcholine at baseline (increase in CAD of 0±4% with bosentan versus 5±2% with acetylcholine alone, P=NS) or at 10 weeks (decrease in CAD of 30±19% with bosentan versus 23±12% with acetylcholine alone; P=NS) (Table 2).
The results of the present studies demonstrate that experimental hypercholesterolemia in the pig resulted in a coronary epicardial vasoconstrictor response to the endothelium-dependent vasodilator acetylcholine, as well as an enhanced coronary epicardial vasoconstrictor response to ET-1. Selective ETB-receptor stimulation by S6c at pathophysiological concentrations resulted in only a mild coronary epicardial vasoconstrictor response that was not affected by 10 weeks of high-cholesterol diet, suggesting that the enhanced coronary epicardial vasoconstrictor response to ET-1 in hypercholesterolemia is primarily mediated by non-ETB receptors. Indeed, the short-term intracoronary administration of FR-139317, a selective ETA receptor antagonist, was effective in attenuating the enhanced coronary vasoconstriction elicited by exogenously administered ET-1 at pathophysiological concentrations in this model. However, ET-1 was ineffective in inhibiting the coronary epicardial vasoconstriction associated with acetylcholine infusion. Similarly, short-term intracoronary administration of bosentan, a nonselective ET-receptor antagonist, did not affect the coronary epicardial vasoactive response to acetylcholine, although it did attenuate the severe coronary epicardial vasoconstrictor response to ET-1 after 10 weeks of high-cholesterol diet. These findings suggest that the ET receptor may not mediate the acute epicardial coronary vasoconstrictor response to acetylcholine in experimental hypercholesterolemia.

Previous studies in both experimental hypercholesterolemia in pig\(^6\) and in humans with early atherosclerosis and coronary endothelial dysfunction\(^9\) have demonstrated that circulating ET levels rise during intracoronary infusion of acetylcholine. In light of the potent coronary vasoconstrictor effect of ET,\(^1,5,14\) the vasoconstrictor effect attained with acetylcholine in states of endothelial dysfunction has been presumed to be mediated, at least in part, by ET.\(^17\) Coronary vasoconstriction in the steady state is mediated by both ETA and ETB receptors\(^20–23\) and is significantly attenuated by coinfusion with FR-139317, a selective ETA-receptor antagonist.\(^3\) Thus, we hypothesized that FR-139317 would be effective in attenuating the vasoconstrictor response to acetylcholine in experimental hypercholesterolemia.

Our findings, however, demonstrate that the coronary vasoconstrictor response elicited by acetylcholine cannot be inhibited by short-term administration of a specific ETA antagonist. One could speculate that the selective ETA antagonist is ineffective in blocking the coronary vasoconstrictor

### TABLE 1. Hemodynamic Parameters Measured at Baseline and at 10 Weeks in Phase II

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (n=7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>106±13</td>
<td>102±8</td>
<td>93±7</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>104±9</td>
<td>102±7</td>
<td>99±7</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>3.5±0.4</td>
<td>3.7±0.3</td>
<td>3.7±0.3</td>
</tr>
<tr>
<td>PAP, mm Hg</td>
<td>21±3</td>
<td>20±4</td>
<td>16±2</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>2±1</td>
<td>1±1</td>
<td>1±1</td>
</tr>
<tr>
<td>SVR, mm Hg·min(^{-1}·L^{-1})</td>
<td>22±5</td>
<td>22±2</td>
<td>20±3</td>
</tr>
<tr>
<td>10 weeks (n=7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>80±3</td>
<td>84±3</td>
<td>86±6</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>103±7</td>
<td>101±8</td>
<td>100±7</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>4.9±0.5</td>
<td>5.1±0.5</td>
<td>5.3±0.3</td>
</tr>
<tr>
<td>PAP, mm Hg</td>
<td>19±2</td>
<td>20±4</td>
<td>18±3</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>3±1</td>
<td>4±1</td>
<td>4±1</td>
</tr>
<tr>
<td>SVR, mm Hg·min(^{-1}·L^{-1})</td>
<td>19±2</td>
<td>19±2</td>
<td>18±2</td>
</tr>
</tbody>
</table>

Group IV

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (n=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>122±9</td>
<td>114±10</td>
<td>105±8</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>99±4</td>
<td>91±7</td>
<td>88±5</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>3.9±0.4</td>
<td>3.5±0.2</td>
<td>3.7±0.2</td>
</tr>
<tr>
<td>PAP, mm Hg</td>
<td>22±2</td>
<td>21±3</td>
<td>17±4</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>1±1</td>
<td>1±1</td>
<td>1±1</td>
</tr>
<tr>
<td>SVR, mm Hg·min(^{-1}·L^{-1})</td>
<td>21±3</td>
<td>19±4</td>
<td>18±4</td>
</tr>
<tr>
<td>10 weeks (n=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>84±5</td>
<td>86±7</td>
<td>81±7</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>98±6</td>
<td>95±11</td>
<td>91±6</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>5.4±1</td>
<td>5.5±0.8</td>
<td>5.1±0.2</td>
</tr>
<tr>
<td>PAP, mm Hg</td>
<td>22±2</td>
<td>22±2</td>
<td>19±2</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
<td>5±1</td>
<td>6±1</td>
<td>5±1</td>
</tr>
<tr>
<td>SVR, mm Hg·min(^{-1}·L^{-1})</td>
<td>17±3</td>
<td>18±3</td>
<td>17±2</td>
</tr>
</tbody>
</table>

SVR indicates systemic vascular resistance.

There was no significant difference in the response to 10\(^{-14}\) mol/L acetylcholine among animals receiving normal saline (group III), FR-139317 (group IV), or bosentan (group V) either at baseline (P=.29) or at 10 weeks (P=.92).

### Phase IV

In phase IV, intracoronary infusion of S6c, a selective ETB-receptor agonist, at the same dose of ET-1 infused in phase I, caused only a mild coronary epicardial vasoconstrictor response both at baseline and after 10 weeks of high-cholesterol diet (decrease in CAD of 10±2% and 12±3% at baseline and after 10 weeks, respectively). In contrast to the enhanced response to ET-1 after 10 weeks of high-cholesterol diet observed in phase I, the response to S6c was not enhanced. Both at baseline and at 10 weeks, S6c infusion did not significantly affect systemic hemodynamics (data not shown).

### Discussion

The results of the present studies demonstrate that experimental hypercholesterolemia in the pig resulted in a coronary epicardial

---

### TABLE 2. Coronary Epicardial Dose Response to Cumulative Concentrations of Acetylcholine With and Without ET-Receptor Antagonists at Baseline and at 10 Weeks

<table>
<thead>
<tr>
<th>%ΔCAD*</th>
<th>Acetylcholine Concentration Infused</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10(^{-4}) mol/L</td>
</tr>
<tr>
<td>Baseline: normal saline</td>
<td>3±1</td>
</tr>
<tr>
<td>10 weeks: normal saline</td>
<td>−1±3</td>
</tr>
<tr>
<td>Baseline: FR-139317</td>
<td>5±2</td>
</tr>
<tr>
<td>10 weeks: normal saline</td>
<td>0±1</td>
</tr>
<tr>
<td>Baseline: bosentan</td>
<td>2±3</td>
</tr>
<tr>
<td>10 weeks: bosentan</td>
<td>0±1</td>
</tr>
</tbody>
</table>

*%ΔCAD indicates percentage change in coronary artery diameter in response to drug infusion.
actions of ET-1 in hypercholesterolemia because of the enhanced response to ET observed in hypercholesterolemia and atherosclerosis. Indeed, our preliminary studies (phase I) confirmed that the coronary vasoconstrictor response to ET-1 at pathophysiological concentrations was enhanced in this model of experimental hypercholesterolemia. However, FR-139317 at the dosage used in this study (5 μg · kg⁻¹ · min⁻¹) was successful in significantly attenuating this enhanced vasoconstrictor effect.

Coronary artery ET immunoreactivity is increased in experimental hypercholesterolemia in pigs and in humans with atherosclerosis. It is thus possible that acetylcholine causes a local increase in ET levels, which is of much greater magnitude than the rise in circulating levels. Indeed, circulating concentrations of ET have been shown to correlate poorly with local concentrations because ET is secreted primarily abluminally. Antagonists to ET receptors may be effective in blunting the actions of circulating or exogenously administered ET but may be less effective in inhibiting the local effects of elevated tissue ET levels, as previously reported by Krause and colleagues.

In addition, recent studies have indicated that the relative contribution of each ET receptor subtype (ie, ETA and ETB) may be different in pathophysiological states than in the steady state. In diet-induced hypercholesterolemia and atherosclerosis, there is diffuse upregulation of the ETB receptor. Furthermore, we showed that in a canine experimental model of heart failure, which is associated with endothelial dysfunction, there is enhanced coronary vasoconstriction to ETB-receptor activation, albeit primarily at the level of the coronary microcirculation. It is thus possible that the contribution of endogenous ET to acetylcholine-induced coronary epicardial vasoconstriction in hypercholesterolemia is primarily mediated by ETB receptors, rather than by ETA receptors. However, this possibility is unlikely for two reasons: (1) in phase IV of the current study, we demonstrated that selective stimulation of the ETB receptor results in only mild coronary epicardial vasoconstriction, which was not altered after 10 weeks of high-cholesterol diet; and (2) the selective ETB-receptor antagonist FR-139317 significantly attenuated the coronary epicardial vasoconstrictor response to exogenously administered ET-1 in this experimental model of hypercholesterolemia. Accordingly, inhibition of the ETB receptor by bosentan in our study at a dose that attenuated the coronary epicardial vasoconstrictor response to ET-1 failed to affect the coronary epicardial vasoconstrctor response to acetylcholine. This does not negate a role for the ETB receptor in hypercholesterolemia. Indeed, Mathew et al have demonstrated increased vasoconstriction elicited by ETB-receptor stimulation after 10 weeks of high-cholesterol diet, albeit only at the coronary micrcirculation level.

Wang and colleagues recently reported that administration of bosentan, a combined ETA- and ETB-receptor antagonist, preserved acetylcholine-induced endothelium-dependent vasodilation in an isolated rat model of myocardial injury and reperfusion, suggesting that inhibition of both ET receptors, ETA and ETB, may be required to attenuate the coronary vasoconstrictor effects of acetylcholine. In contrast, our studies with bosentan at a dose that induced mild systemic hypotension and blunted the coronary vasoconstrictor response to ET-1 failed to show any effect on the coronary response to acetylcholine. We cannot exclude the possibility that at higher doses of bosentan, an effect might have been attained. However, given the profound effects on systemic hemodynamics with higher doses of bosentan, it would be difficult to evaluate this possibility in vivo. In addition, bosentan may block the endothelial ETB-receptor–mediating vasodilation to a greater extent than the vascular smooth muscle ETB-receptor–mediating vasoconstriction, thus possibly exacerbating the vasoconstrictor response to acetylcholine.

Coronary vasoconstriction in response to acetylcholine is a complex process involving multiple vasoactive agents. Endogenous agents causing vasoconstriction other than ET, such as angiotensin II and prostanoids, are also activated by acetylcholine. Thus, it is possible that the inhibition of only one pathway is not sufficient in blunting acetylcholine-induced coronary vasoconstriction. Recently, ACE inhibitors have been shown to be effective in attenuating coronary endothelial dysfunction both in animal models and in humans. The salutary effects of ACE inhibitors are attributed to their actions on different pathways, including the modulation of endogenous ET concentrations and activity. Drugs were given over a prolonged period in these studies, suggesting an antithrombotic effect. Hence, long-term inhibition of ET, blunting its atherogenic actions, may also be beneficial. Prolonged inhibition of ET may also be necessary to attenuate the time-dependent effects of elevated circulating and local levels of ET on acetylcholine-receptor production. In addition, we recently demonstrated that the coronary artery nitric oxide pathway is attenuated in this porcine experimental model. Thus, attenuating the vasoconstrictor response to acetylcholine may also require an increase in activity of vasodilators such as nitric oxide. Indeed, it is postulated that ACE inhibitors exert their salutary effect in part by increasing nitric oxide activity. Thus, our findings should not be interpreted as negating a role for ET in mediating the vasoconstrictor effect attained with acetylcholine. Rather, they underscore the complexity of this response and hence the need for a therapeutic strategy involving the inhibition of multiple pathways.

In conclusion, the present studies demonstrate that acetylcholine-induced coronary vasoconstriction in a porcine model of hypercholesterolemia is not affected by short-term administration of ET–receptor antagonists. The mechanisms by which ET potentially affects acetylcholine-induced vasoconstriction in hypercholesterolemia remain to be determined.

Acknowledgments

This study was supported by grants from the National Institute of Health (HL–03180–01) and the Mayo Foundation. We thank Dr Terry Op genom (Abbott Laboratories) for the use and supply of FR–139317 and Dr Martine Clozel (Hoffman La Roche) for the use and supply of bosentan (Ro 47–0203).

References


Acute Endothelin-Receptor Inhibition Does Not Attenuate Acetylcholine-Induced Coronary Vasoconstriction in Experimental Hypercholesterolemia
David Hasdai, Patricia J.M. Best, Charles R. Cannan, Verghese Mathew, Robert S. Schwartz, David R. Holmes, Jr and Amir Lerman

*Arterioscler Thromb Vasc Biol.* 1998;18:108-113
doi: 10.1161/01.ATV.18.1.108

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/18/1/108

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
http://atvb.ahajournals.org/subscriptions/