Direct Vasoconstriction and Endothelium-Dependent Vasodilation

Mechanisms of Acetylcholine Effects on Coronary Flow and Arterial Diameter in Patients With Nonstenotic Coronary Arteries

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With the special technical assistance of Helen Sheehan, RN

An endothelium-dependent vasodilator response to acetylcholine has been described recently in patients with coronary artery disease. Those studies determined responses only of large epicardial arteries. Our study was designed to determine the integrated effects of acetylcholine on epicardial diameter, coronary flow, and vascular resistance. Patients (n=64) with nonstenotic epicardial coronaries underwent coronary angiography with simultaneous recording of coronary flow velocity using a 3F subselective Doppler catheter. Measurements of epicardial arterial cross-sectional area (ECA), velocity, estimated flow (velocity times area), and vascular resistance were made before and after bolus administration of acetylcholine (100 μg i.c.). Similar measurements were made after papaverine (12-15 mg i.c.), a nonendothelium-dependent vasodilator. Acetylcholine resulted in a reduction of ECA of 19±3%, whereas papaverine increased ECA by 9±2%. Estimated flow increased 69±12% after acetylcholine and 147±12% after papaverine. Resistance fell after both agents (acetylcholine, -17±13%; papaverine, -61±2%). Transvascular resistance fell after acetylcholine in all but five patients. These patients had dramatic epicardial artery constriction (40±8% decrease in ECA). The effect of acetylcholine on both ECA and resistance was blocked by atropine (1 mg i.c.). Nitroglycerin (300 μg i.c.) resulted in epicardial dilatation (7.5±2.8%) in the same patients in whom acetylcholine caused constriction (11.2±3.1%). Pretreatment with methylene blue, an inhibitor of endothelium-derived relaxing factor (EDRF), potentiated epicardial artery vasoconstriction with acetylcholine (−25±7% before versus −47±11% after, p<0.01) and resulted in a marked increase in vascular resistance (−24±10 before versus +79±41 after, p<0.05). Methylene blue had no effect on the response to the non-EDRF-dependent vasodilators papaverine and nitroglycerin. These data suggest 1) acetylcholine has differing effects on the epicardial arteries (constriction) and the smaller resistance vessels (dilation), 2) the net response to acetylcholine depends on the interplay between direct vasoconstriction and EDRF-mediated vasodilation, and 3) accurate assessment of the effects of acetylcholine on the coronary circulation requires determination of both coronary flow and epicardial artery diameter. (Circulation 1989;79:1043-1051)

Recent studies strongly suggest an important role for the endothelium in control of vaso-motor tone.1-13 The original observation of Furchgott and Zawadzki14 that acetylcholine and other compounds cause release of factors from the endothelium that induce vasodilation has been corroborated in many species both in vitro and in vivo. More recently, the clinical implications of these observations for patients with coronary artery disease have been investigated.6-12 Normal epicardial arterial segments reportedly dilate and minimally diseased and stenotic segments constrict after acetylcholine infusion. This has been interpreted as evidence for widespread endothelial dysfunction in patients with coronary disease.6-8 To date, only measurements of changes in epicardial coronary arterial diameters have been made in patients during acetylcholine administration."
Our study was undertaken to determine the alterations in coronary blood flow, vascular resistance, and arterial diameters in response to intracoronary acetylcholine. Experiments also were done to determine the mechanism(s) of action for acetylcholine effects on coronary arteries.

**Methods**

**Patient Selection**

Patients selected for this study were undergoing coronary angiography for clinical indications. Patients found to have normal or nearly normal epicardial coronary arteries and who were without valvular heart disease or left ventricular systolic dysfunction were asked to participate. Additional patients undergoing routine evaluation after cardiac transplantation were also asked to participate. All patients gave informed consent. The study protocol was approved by the Human Studies Committee of Virginia Commonwealth University and the research committee of the McGuire Veterans Administration Medical Center.

**Instrumentation**

Patients were premedicated with diphenhydramine 50 mg and diazepam 10 mg orally. All other medications were withheld for a minimum of 12 hours before study. Routine coronary angiography was performed before the study protocol.

A 7F bipolar pacing catheter was placed in the right ventricular apex for temporary pacing if needed. A 9F sheath was placed percutaneously in the right femoral artery for continuous arterial pressure monitoring. An 8F angioplasty guiding catheter (Interventional Medical, Danvers, Massachusetts) was advanced to the left coronary ostium. A 3F Doppler velocity catheter (DC-101, Millar Instruments, Houston, Texas) then was passed over a 0.012-in. guide wire (ACS, Temecula, California) into the proximal left anterior descending coronary artery. The Doppler signal then was maximized by carefully positioning the catheter and using an electronic range gate. The signal was considered stable when exaggerated breathing, power injection of contrast through the guide catheter, and coughing did not permanently alter the basal signal. Phasic and mean velocity, aortic pressure, and electrocardiography were recorded continuously on a strip-chart recorder.

Digital angiography was used to assess epicardial artery diameter as described previously. Images were obtained during power injection of 10 ml nonionic contrast medium (Omnipaque 350, Winthrop-Breon) at 10 ml/sec through the guiding catheter. Images were acquired directly to disk at 15 frames/sec in a 512x512x8 bit matrix (DPS 4100-C, ADAC, Milpitas, California).

**Study Protocol**

**Acetylcholine group.** After completion of instrumentation and establishment of the steady state, 45 patients had angiography performed under basal conditions. Velocity again was allowed to return to baseline and acetylcholine (100 μg) was injected through the guide catheter over 5 seconds (assumed concentration at left main flow of 150 ml/min, 4x10^{-5} M). Velocity was monitored continuously and angiography repeated at peak velocity change (30-60 seconds). In addition, all patients had velocity determined before and after a dose of papaverine (12-15 mg i.c.), which causes maximal vasodilation. In three fourths of these patients, epicardial diameters were assessed simultaneously with papaverine. These and seven additional patients then were subjected to one of five different protocols to investigate the mechanism of action of acetylcholine.

**Atropine group.** Five patients received 1 mg i.c. atropine and had repeat basal and acetylcholine determinations.

**Nitroglycerin group.** Thirteen patients had repeat angiography 30-60 seconds after nitroglycerin administration (300 μg i.c.). This was performed after all acetylcholine determinations when velocity had returned to baseline.

**Methylene blue–acetylcholine group.** Five patients had repeat acetylcholine injections after methylene blue infusions. Methylene blue was infused via the guide catheter at 5 ml/min for 10 minutes followed immediately by acetylcholine (100 μg). Estimating left main flow at 150 ml/min, two concentrations of methylene blue were used: 3.4x10^{-6} and 3.4x10^{-5} M. Two patients had determinations after both doses, and three patients received only the lower dose.

**Methylene blue–papaverine group.** Four patients had repeat papaverine injections after methylene blue. Changes in mean pressure and flow velocity were determined for a maximal dilating dose of papaverine before and immediately after infusion of methylene blue at an estimated concentration of 3.4x10^{-6} M.

**Methylene blue–nitroglycerin group.** Five patients had nitroglycerin injections (100 μg i.c.) and angiography after a 10-minute methylene blue infusion at an estimated concentration of 3.4x10^{-6} M.

**Saline control group.** Thirteen patients had continuous recording of coronary velocity during bolus injection of 5 ml 0.9% NaCl before acetylcholine. Angiography was performed to determine epicardial artery diameter before and 2 minutes after saline injection. These patients served as controls.

To further assess the effects of acetylcholine, 20 patients underwent the following subprotocol.

**Acetylcholine–dose response group.** Eight patients had intracoronary infusions of acetylcholine to achieve estimated concentrations of 2x10^{-6} to 3.6x10^{-5} M, and 12 patients had bolus injection of acetylcholine to achieve estimated concentrations of 4x10^{-6} to 4x10^{-5} M. Maximal changes in coronary velocity were recorded for all patients. Patients receiving infusions also had quantitative angiography before and after acetylcholine infusion.

**Epicardial Artery Diameter and Area**

Arterial diameters were determined by postprocessing of operator selected end diastolic frames using a
totally automated edge detection program (ARTREK, ADAC Laboratories). Mean diameters were taken for a 1–2 cm nonbranching segment. Because all coronary studies were without significant angiographic disease, vessels were assumed to have a circular cross section, and area was calculated as \( \pi r^2 \).

**Velocity Area Index**

To provide an estimate of volumetric coronary blood flow, velocity determinations were combined with the angiographically determined arterial cross-sectional areas. Arteries were without stenoses and were assumed to have a circular cross section. The velocity-area index (VAI) was calculated as:

\[
\text{VAI} = \text{Doppler velocity} \times \pi (D/2)^2
\]

where D is epicardial diameter.

**Coronary Vascular Resistance**

Coronary vascular resistance was calculated as the quotient of the arterial pressure and the VAI.

**Statistics**

Comparisons were performed using paired and unpaired \( t \) tests as appropriate. Values of \( p<0.05 \) were considered significant. Data are expressed as mean±1 SEM.

**Results**

**Patient Characteristics**

Sixty-four patients were studied. Forty-one patients (64%) had undergone cardiac transplantation an average of 13±2 months before study. The average age was 45±11 years for the transplant and 52±10 years for the nontransplant patients. The average left ventricular ejection fraction was 64±2% for the nontransplant patients (range, 55–79%) and 52±2% for the transplant patients (range, 35–69%). Over half of all patients (nontransplant, 75%; transplant, 54%) had echocardiographic evidence of left ventricular hypertrophy.

**Saline Control Patients**

No significant changes in arterial pressure, coronary velocity, or coronary vascular resistance occurred after the injection of saline in 13 patients (Table 1). There was a very small decrease in epicardial artery diameter (−2.1±0.6%, \( p<0.05 \)).

**Dose Dependence of Responses to Acetylcholine**

Coronary flow velocity increased at all doses of acetylcholine estimated to provide final concentrations above 7×10\(^{-7}\) M (Table 2). There were no significant differences between 7×10\(^{-7}\) and 4×10\(^{-5}\) M doses. At 4×10\(^{-7}\) M, little effect was seen. No patients exhibited a biphasic response with increased flow at lower doses and decreased flow at higher doses. All patients continued to achieve a maximal flow level at the highest doses administered. Epicardial diameter decreased to a similar degree at doses ranging from 7×10\(^{-7}\) to 4×10\(^{-5}\) M. In no patient was dilation seen at one dose and constriction seen at another dose.

**Effects of Acetylcholine on Arterial Cross-sectional Area and Vascular Resistance**

Injection of acetylcholine (100 \( \mu \)g, 4×10\(^{-5}\) M) resulted in a significant rise in coronary velocity (105±13%) without a change in arterial pressure (Table 3). Epicardial artery cross-sectional area decreased (−19±3%) (Figure 1). Therefore, the VAI reflected only a 69±12% increase in volume flow (Figure 2). Coronary vascular resistance did not change significantly for the group as a whole (−17±13.4%). The main reason for this was that five patients had striking increases in resistance (average, 164±91%) (Figure 3). These five patients had marked epicardial artery constriction (average, −40±8% area reduction) as shown in Figure 4. If these five patients are excluded, the mean vascular resistance after acetylcholine fell significantly for the remaining 40 patients (−40±3%, \( p<0.0001 \)).

**Comparison With Papaverine**

Epicardial artery area increased (9±2%), arterial pressure fell (−8±1%), velocity and VAI increased (137±12% and 147±12%, respectively), and vascular resistance fell (−62±2%) after papaverine injection (Table 3). All of these changes were significant (\( p<0.0001 \)). Several important differences between the response to acetylcholine and papaverine were

**Table 1. Saline Control Patients**

<table>
<thead>
<tr>
<th></th>
<th>Change from baseline (%)</th>
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<tbody>
<tr>
<td>Epicardial artery diameter</td>
<td>−2.1±0.6*</td>
</tr>
<tr>
<td>Mean aortic pressure</td>
<td>0.9±0.0</td>
</tr>
<tr>
<td>Coronary flow velocity</td>
<td>3.9±1.9</td>
</tr>
<tr>
<td>Velocity-area index</td>
<td>−0.1±2.7</td>
</tr>
<tr>
<td>Coronary vascular resistance</td>
<td>1.8±2.6</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *\( p<0.05 \).

**Table 2. Dose Responses to Intracoronary Acetylcholine**

<table>
<thead>
<tr>
<th>Estimated final acetylcholine concentration (M)</th>
<th>Change coronary flow velocity (%)</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>4×10(^{-7})</td>
<td>2.7±9</td>
<td>3</td>
</tr>
<tr>
<td>7×10(^{-7})</td>
<td>114±39</td>
<td>4</td>
</tr>
<tr>
<td>2–4×10(^{-6})</td>
<td>124±29</td>
<td>6</td>
</tr>
<tr>
<td>1×10(^{-5})</td>
<td>107±15</td>
<td>12</td>
</tr>
<tr>
<td>2×10(^{-5})</td>
<td>85±14</td>
<td>11</td>
</tr>
<tr>
<td>4×10(^{-5})</td>
<td>108±31</td>
<td>10</td>
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<table>
<thead>
<tr>
<th></th>
<th>Change epicardial artery diameter (%)</th>
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<tbody>
<tr>
<td>7×10(^{-7})</td>
<td>−12±7</td>
</tr>
<tr>
<td>2×10(^{-6})</td>
<td>−14±7</td>
</tr>
<tr>
<td>4×10(^{-5})</td>
<td>−14±5</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
observed (Figures 1–3). There was a directionally different effect on epicardial artery area (acetylcholine decreased, papaverine increased; \( p<0.0001 \)). Arterial mean pressure fell only after papaverine. Measured velocity increased after both drugs and was not significantly different (acetylcholine, 105±13; papaverine, 137±12; \( p=NS \)). The VAI (estimate of volumetric flow), however, increased significantly less after acetylcholine (69±12% vs. papaverine, 147±12%; \( p<0.0001 \)). The fall in vascular resistance after acetylcholine (-17±13%) was likewise significantly less than after papaverine (-62±2%; \( p<0.05 \)). If the five outliers with dramatic epicardial constriction after acetylcholine are excluded from these comparisons, the decrease in resistance after acetylcholine (-40±3%) was not different from that after papaverine (-61±2%).

Effect of Atropine

Five patients were challenged with acetylcholine before and after atropine (Table 4). The effects of acetylcholine on epicardial artery diameter and VAI were abolished after atropine. The effect of acetylcholine injection after atropine was not different from the saline control group.

Response to Nitroglycerin

Thirteen patients had an average decrease in epicardial diameter after acetylcholine of 11.2±3.1%. In contrast, nitroglycerin resulted in a 7.5±2.8% increase in diameter (\( p<0.0001 \) versus acetylcholine). A particularly striking example of the degree of vasoreactivity seen in one patient is illustrated in Figure 4.

Effect of Methylene Blue on Acetylcholine Response

Five patients were challenged with acetylcholine before and after methylene blue infusion (Table 5). The vasoconstrictor effects of acetylcholine on epicardial artery diameter were augmented after methylene blue (-25±7% before versus -46±11% after, \( p<0.01 \)). The acetylcholine-induced decrease in vascular resistance observed before methylene blue was replaced by an increase in resistance (-23±10 before versus +79±41 after, \( p<0.05 \)). The marked vasoconstriction seen in one patient is illustrated in Figure 5.
Methylene blue alone had no effect on epicardial artery diameter (-0.8±0.9%, n=4, p=NS).

**Effect of Methylene Blue on Papaverine Response**

Methylene blue had no effect on the vasodilator response to a maximal dilating dose of papaverine (Table 6). The responses of coronary flow velocity to papaverine before and after methylene blue were not different (+139±31% before versus +142±34% after). Likewise, the response of coronary vascular resistance was unchanged (-54.7±8.2% before versus -57.0±6.1% after).

**Effect of Methylene Blue on Nitroglycerin Response**

Methylene blue had no effect on the vasodilator response to intracoronary nitroglycerin. Changes in epicardial artery diameter in the seven patients pretreated with methylene blue (7.9±6.6% increase) were no different than those seen without methylene blue pretreatment (7.5±2.8% increase).

**Discussion**

This study describes two important findings. First, acetylcholine has differential effects on large epicardial compared with smaller resistance coronary vessels. Previous investigators have described paradoxical vasoconstriction of epicardial coronaries after acetylcholine injection or infusion.7–9 Our data corroborate these findings but provide new evidence that the effect of acetylcholine may be quite different at the microvascular level. Despite epicardial vasoconstriction after acetylcholine, coronary flow increased markedly in nearly all patients. The only exceptions were patients in whom epicardial artery constriction was extreme. Therefore, resistance vessels dilate in response to acetylcholine. This observation may be explained in two ways. Either the resistance vessels have inherently different responses to acetylcholine or the endothelial dysfunction responsible for epicardial constriction is not found at the resistance vessel level.

There is increasing evidence that coronary vessels of differing sizes react to selected stimuli to different degrees and may even exhibit responses that are directionally opposite.17–19 It is possible that there are also inherent differences in the coronary vascular response to acetylcholine in coronary arteries of different sizes. A more plausible explanation is that endothelial dysfunction exists in the epicardial vessels but is not abnormal in the smaller resistance vessels. Our data show that the epicardial vessels in our patients are capable of vasodilation when stimulated by two non-endothelium-dependent vasodilators (papaverine and nitroglycerin). Thus, we considered it likely that the observed epicardial vasoconstriction after acetylcholine represents a direct effect expressed in the absence of endothelium-derived relaxing factors. In contrast, the observed dilation in the coronary resistance vessels resulted from acetylcholine-induced release of these relaxing factors. Thus, the overall response to acetylcholine is the result of the integration of direct epicardial vasoconstriction plus resistance vessel dilation resulting from release of endothelium-derived relaxing factors.

Although our patients had angiographically normal or minimally diseased coronaries, they were far from truly normal. Many had preexisting hypertension with left ventricular hypertrophy. The heart transplant patients almost certainly had some degree of chronic graft rejection. These underlying abnormalities most likely account for the differences in vasodilator reserve seen in our patients and that previously reported by others.20 The purpose of our study was not to determine the absolute magnitude of normal responses but rather to investigate the integrated effect of acetylcholine on both epicardial and resistance vessels in patients free of obstructing coronary lesions. Our data must not be extrapolated to truly normal patients but rather may serve to
emphasize that early vascular abnormalities are present even in some patients with angiographically normal epicardial coronary arteries.

A second important finding of our study is the demonstration in patients that the action of acetylcholine can be modified by muscarinic cholinergic blockade and by inhibition of endothelium-dependent relaxation. Our data demonstrate in humans the importance of intact muscarinic cholinergic receptors both for the direct vasoconstrictor response and for the release of endothelium-derived relaxing factor. Pretreatment with atropine abolished both epicardial constriction and resistance vessel dilation in our patients. Although well established in vitro, this has not been described previously in humans.

Likewise, pretreatment with an antagonist of endothelium-derived relaxation, methylene blue, greatly potentiated the vasoconstrictor response. This

Table 3. Effects of Acetylcholine and Papaverine

<table>
<thead>
<tr>
<th></th>
<th>Acetylcholine (100 µg i.c.)</th>
<th>Papaverine (12–15 mg i.c.)</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Epicardial artery area (mm²)</td>
<td>8.2±0.5</td>
<td>7.0±0.6</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>110±3</td>
<td>107±3</td>
</tr>
<tr>
<td>Coronary flow velocity</td>
<td>6.8±0.3</td>
<td>13.5±0.8</td>
</tr>
<tr>
<td>Velocity-area index</td>
<td>22.7±2.0</td>
<td>39.1±4.5</td>
</tr>
<tr>
<td>Coronary vascular resistance</td>
<td>7.1±0.8</td>
<td>6.4±1.5</td>
</tr>
<tr>
<td>Coronary vascular resistance (5 outliers excluded)</td>
<td>6.5±0.8</td>
<td>3.8±0.5</td>
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</tbody>
</table>

Values are mean±SEM.

*p<0.0001 before versus after.

†p<0.0001 acetylcholine versus papaverine.

‡p<0.05 acetylcholine versus papaverine.
potentiation was evident both at the epicardial and the resistance vessel levels (Table 4). When acetylcholine was administered after methylene blue, there was often profound vascular constriction and associated ischemic chest pain and electrocardiographic changes (Figure 5). In two cases, atropine was administered to reverse these profound abnormalities.

Methylene blue was first implicated as an inhibitor of vasodilation in 1977 by Katsuki et al. Martin et al.23 demonstrated that 50 μM methylene blue abolished the dilation from acetylcholine but only partially blocked the dilation from nitroglycerin. They concluded that methylene blue was blocking dilation by inhibition of guanylate cyclase, which may not be the case in vascular beds in vivo. In the cerebral microcirculation of the cat, methylene blue specifically blocks endothelium-dependent dilation from acetylcholine without affecting the dilation from nitroglycerin, sodium nitroprusside, or nitric oxide.23 Selective blockade of endothelium-derived relaxing factor-mediated dilation also has been demonstrated in the femoral arteries of dogs in vivo24 and recently in vitro by investigators using cultured bovine aortic endothelial cells.25

Our data are consistent with these recent observations made in animals. Methylene blue resulted in a reversal of the effect of acetylcholine on resistance vessels (from dilation to constriction). The supposition that methylene blue exerts its effect by blocking endothelium-derived relaxing factors is supported by our observation that pretreatment with methylene blue had no effect on the endothelium-independent dilators papaverine26 and nitroglycerin. We interpret our data to indicate that endothelium-derived relaxing factor is released by acetylcholine in patients and has an important vasodilating role even in disease processes that abolish expected epicardial vasodilator responses to acetylcholine. The integration of direct vasoconstrictor and endothelium-dependent vasodilator responses will determine the net response of an intact vascular bed to acetylcholine.

**Potential Limitations**

Our estimation of the local concentration of acetylcholine is important if we are to compare our results with those of other investigators. It is conceivable that the dose of acetylcholine used for this study (estimated blood concentration, \(4 \times 10^{-7}\) M) was so high that direct constriction was inevitable. Similar concentrations have induced vasodilation in other patient populations, and both in vivo and in vitro studies suggest that concentrations greater than \(10^{-4}\) M are required to "override" vasodilation. In our dose-response experiments, we found no evidence that the 100-μg bolus injection (\(4 \times 10^{-5}\) M) caused excessive epicardial artery constriction or limitation of maximal coronary flow velocity. It seems likely that the results obtained in this study represent maximal changes. Qualitatively similar responses also were observed with effective concentrations of acetylcholine nearly two orders of magnitude smaller than the one we used in most of our studies.

A second concern is the time course of the very short-lived effects of acetylcholine and endothelium-derived relaxing factor. Previous animal work has shown disparity between maximal coronary flow changes and epicardial artery diameter changes (the latter being delayed 1–2 minutes).29 To date, human studies have taken diameter measurements at an arbitrary period of time (1–2 minutes) after injection of acetylcholine. We made our determinations at peak velocity change assessed by the on-line Doppler catheter. To ensure that we did not miss potential arterial diameter changes occurring later after the injection, we assessed artery diameter soon after and 2 minutes after injection in eight patients. Epicardial diameter decreased an average of 9.8±4.4% at peak velocity change (20–35 seconds) and was similarly reduced 2 minutes later (12.3±5.4%) when velocity had returned nearly to baseline. Thus, the changes of vascular diameter observed in this study are most likely an accurate estimate of maximal changes. Although flow-dependent vasodilation may have been expected to result in increases in epicardial diameter, there was no evidence that vascular diameter was any different when flow was increased compared with after flow had returned to baseline.

One other potential confounding factor is the possibility that interference with arachidonic acid metabolism in the endothelial cells or that platelets alters the response to acetylcholine. Miller et al.30 demonstrated that inhibition of prostaglandin synthesis may profoundly alter the response of epicardial arteries with damaged endothelium to acetylcholine (abolished constrictor response). Other investigators, however, failed to see any difference

<table>
<thead>
<tr>
<th>TABLE 4. Effects of Atropine</th>
<th>Change after acetylcholine (%)</th>
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<tr>
<td></td>
<td>Before atropine</td>
</tr>
<tr>
<td>Epicardial artery diameter</td>
<td>-12.8±5.7</td>
</tr>
<tr>
<td>Velocity-area index</td>
<td>70.6±4.3</td>
</tr>
</tbody>
</table>

\(n=5\). Values are mean±SEM. *p<0.01 before versus after. †p<0.0001 before versus after.

<table>
<thead>
<tr>
<th>TABLE 5. Effects of Methylene Blue on Acetylcholine Response</th>
<th>Change after acetylcholine (%)</th>
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<tr>
<td></td>
<td>Before methylene blue</td>
</tr>
<tr>
<td>Epicardial artery area</td>
<td>-25.4±7.2</td>
</tr>
<tr>
<td>Coronary vascular resistance</td>
<td>-23.8±10.4</td>
</tr>
</tbody>
</table>

\(n=7\). Values are mean±SEM. *p<0.01 before versus after. †p<0.05 before versus after.
in flow-induced vasodilation in conscious dogs after prostaglandin synthesis inhibition. Thirty percent of our patients were receiving aspirin (325–975 mg/day orally) before catheterization. We found no significant difference in the response to acetylcholine between patients receiving aspirin (n=11) and those not receiving aspirin (n=29). Epicardial artery area decreased $-14\pm3\%$ in the nonaspirin group and $-22\pm6\%$ in the aspirin group. Coronary vascular resistance decreased to a similar degree in both groups (no aspirin, $-39\pm4\%$; aspirin, $-42\pm5\%$). These data indicate that either prostaglandin synthesis may not have been inhibited by the dose of aspirin being used by our patients or inhibition did not significantly modify the response to acetylcholine in our patients.

In conclusion, our experiments provide clear evidence for differential effects of acetylcholine on epicardial conduit arteries compared with resistance vessels. They provide the first evidence in humans that coronary resistance vessels are under the control of endothelium-derived relaxing factor. Differences in the regulation of large and small coronary arteries and in the impact of various disease processes on this regulation may have important pathophysiologic consequences.

### Acknowledgments
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### References

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**Figure 5.** Representative images of the left coronary system in a patient with normal-appearing coronary images. Upper left: baseline; upper right: 30 seconds after 100 μg i.c. acetylcholine; bottom: 30 seconds after acetylcholine given after pretreatment with methylene blue. Guidewire and Doppler are visible in the left anterior descending coronary.

<table>
<thead>
<tr>
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<th>Change after papaverine (%)</th>
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<tr>
<td></td>
<td>Before methylene blue</td>
</tr>
<tr>
<td>Coronary flow velocity</td>
<td>139±31</td>
</tr>
<tr>
<td>Coronary vascular resistance*</td>
<td>$-54.7\pm8.2$</td>
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</tbody>
</table>

$n=4$.

Values are mean±SEM.

*Calculated as mean pressure/velocity.


KEY WORDS • endothelium-derived relaxing factor • coronary vessels
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