Effect of Inhibition of Nitric Oxide Synthesis on Epicardial Coronary Artery Caliber and Coronary Blood Flow in Humans

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Background. N\textsuperscript{G}-Monomethyl-L-arginine (L-NMMA), a specific inhibitor of nitric oxide synthesis, was used to determine the effects of inhibition of nitric oxide synthesis in the human coronary circulation.

Methods and Results. Twelve patients (mean age, 52±2 years) with normal epicardial coronary arteries were studied. The surface ECG, systemic blood pressure, and coronary venous oxygen saturation (coronary Sv\textsubscript{2}O\textsubscript{2}), an index of coronary blood flow, were monitored continuously. Coronary artery diameter was measured by quantitative arteriography. L-NMMA was given as intracoronary infusions at 2 mL/min via the diagnostic arteriography catheter. In two patients, low doses (0.01 to 5 \textmu mol/min) of L-NMMA were infused into the nondominant right coronary artery. There was no evidence of ischemia in these patients, who were not included in the final analysis. In 10 patients, higher doses of L-NMMA (4, 10, and 25 \textmu mol/min, each for 5 minutes) were infused into the left coronary artery. In six patients, incremental doses of acetylcholine were infused (1, 10, and 100 nMol/min, each for 3 minutes) before and after the L-NMMA infusion. Finally, in all patients, sodium nitroprusside, a nitric oxide donor, was infused. No patient developed myocardial ischemia. The heart rate and systemic blood pressure remained unchanged throughout the infusions. L-NMMA (25 \textmu mol/min), compared with the control saline infusion, caused a significant reduction in distal (-5.9±2.1%, \textit{P}=0.021) but not proximal left anterior descending coronary artery (LAD) diameter and a fall in coronary Sv\textsubscript{2}O\textsubscript{2} from 37.5±2.8% to 34.3±2.8% (\textit{P}=0.019). Sodium nitroprusside dilated the proximal (17.8±6.9%, \textit{P}=0.033) and distal (24.5±6.5%, \textit{P}=0.006) LAD and increased the coronary Sv\textsubscript{2}O\textsubscript{2} to 61.6±5.0% (\textit{P}=0.0002). Acetylcholine caused significant dilatation of the distal (13.8±5.4%, \textit{P}=0.049) but not proximal LAD and a significant increase in coronary Sv\textsubscript{2}O\textsubscript{2} from 36.5±3.5% to 59.2±2.8% (\textit{P}<0.0001). After L-NMMA, acetylcholine-induced dilatation of the distal LAD was abolished, but the rise in coronary Sv\textsubscript{2}O\textsubscript{2} was unchanged.

Conclusions. Inhibition of nitric oxide synthesis in the human coronary circulation caused a decrease in distal basal LAD diameter and basal coronary blood flow assessed by coronary Sv\textsubscript{2}O\textsubscript{2}, indicating that there is a small basal release of nitric oxide in the distal epicardial coronary arteries and resistive vessels. Distal epicardial coronary artery dilation in response to acetylcholine is nitric oxide dependent, but coronary resistive vessel dilatation is not. \textit{(Circulation} 1993;88:43-54)

Key Words: endothelium \textbullet endothelium-derived factors \textbullet acetylcholine

Endothelium-derived relaxing factor (EDRF) was first demonstrated more than a decade ago by Furchgott and Zawadzki\textsuperscript{1} Although the identity of EDRF remains uncertain, it now seems likely that nitric oxide or a closely related compound represents one type of EDRF, because both nitric oxide and EDRF display similar biological and pharmacological properties\textsuperscript{2-4} and nitric oxide is released in sufficient quantities to explain the biological actions of EDRF\textsuperscript{2} Other studies have indicated that nitric oxide cannot account for all the actions of EDRF and that, depending on the species, the vascular bed studied, and the mechanism of activation, there may be more than one type of EDRF\textsuperscript{5-8}

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Nitric oxide is synthesized by endothelial cells from L-arginine\textsuperscript{9} N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) has been shown to inhibit its formation in a concentration-dependent and stereospecific manner.\textsuperscript{10} L-NMMA inhibits endothelium-dependent relaxation,\textsuperscript{11} and it increases the basal tone and attenuates the acetylcholine-induced relaxation in rings of rabbit aorta\textsuperscript{11} and guinea pig pulmonary artery.\textsuperscript{12} It increases the coronary vasculard resistance and attenuates the acetylcholine-induced vasodilatation in the Langendorff-perfused rabbit heart preparation.\textsuperscript{13} In the resting conscious dog, Chu et al\textsuperscript{14} showed that the systemic infusion of L-NMMA, in doses sufficient to significantly increase systemic blood pressure, caused a small decrease in epicardial coronary artery diameter together with a small reduction in coronary blood flow, indicating a rise in coronary vascular resistance resulting from an increase in resistive vessel tone. In contrast, Woodman and Dusting\textsuperscript{15} showed that in the
TABLE 1. Clinical Characteristics and Atherogenic Risk Factors in the 10 Patients of Protocol B

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Smoking history</th>
<th>BP (mmHg)</th>
<th>Total serum cholesterol (mmol/L)</th>
<th>Serum TG (mmol/L)</th>
<th>Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51/M</td>
<td>Never</td>
<td>125/75</td>
<td>4.9</td>
<td>1.19</td>
<td>4.6</td>
</tr>
<tr>
<td>2</td>
<td>58/F</td>
<td>Never</td>
<td>140/90</td>
<td>4.6</td>
<td>1.30</td>
<td>5.0</td>
</tr>
<tr>
<td>3</td>
<td>55/F</td>
<td>Never</td>
<td>150/85</td>
<td>5.2</td>
<td>1.04</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>52/M</td>
<td>Never</td>
<td>120/70</td>
<td>5.2</td>
<td>0.94</td>
<td>5.0</td>
</tr>
<tr>
<td>5</td>
<td>48/M</td>
<td>Never</td>
<td>125/65</td>
<td>4.3</td>
<td>1.45</td>
<td>4.8</td>
</tr>
<tr>
<td>6</td>
<td>51/F</td>
<td>Never</td>
<td>150/70</td>
<td>4.5</td>
<td>1.46</td>
<td>4.9</td>
</tr>
<tr>
<td>7</td>
<td>47/F</td>
<td>Current, 20 py</td>
<td>110/70</td>
<td>5.1</td>
<td>1.24</td>
<td>4.9</td>
</tr>
<tr>
<td>8</td>
<td>47/F</td>
<td>Never</td>
<td>110/80</td>
<td>4.3</td>
<td>0.90</td>
<td>5.0</td>
</tr>
<tr>
<td>9</td>
<td>44/F</td>
<td>Never</td>
<td>145/80</td>
<td>5.1</td>
<td>0.69</td>
<td>5.2</td>
</tr>
<tr>
<td>10</td>
<td>59/F</td>
<td>Never</td>
<td>140/85</td>
<td>4.8</td>
<td>1.05</td>
<td>5.2</td>
</tr>
</tbody>
</table>

BP, cuff blood pressure; py, pack-years; TG, triglycerides.

anesthetized dog, inhibition of nitric oxide synthesis with the inhibitor N-nitro-L-arginine caused a decrease in epicardial coronary artery diameter without affecting coronary blood flow or coronary vascular resistance despite a significant rise in arterial blood pressure. Vallance et al. showed that in the human forearm, infusion of L-NMMA into the brachial artery substantially reduced basal blood flow and markedly inhibited the acetylcholine-induced increase in blood flow. The effects of L-NMMA were reversed by the infusion of L-arginine but not D-arginine, indicating stereospecific inhibition of nitric oxide synthesis, and also by nitroglycerin. The purpose of this study was to determine the effects of inhibition of nitric oxide production by L-NMMA in the human coronary circulation. The effects of an intracoronary infusion of L-NMMA on epicardial coronary artery basal vasomotor tone and on coronary venous oxygen saturation, an index of coronary blood flow, were investigated. In addition, the effects of L-NMMA on the coronary vascular responses to acetylcholine (an endothelium-dependent vasodilator) and sodium nitroprusside (an endothelium-independent vasodilator) were examined.

Methods

The study was approved by the Research Ethics Committee of Hammersmith Hospital. All patients gave informed and written consent.

Patients

Twelve patients (four men and eight women; mean age, 52±2 years; range, 44 to 61 years) referred for cardiac catheterization were studied. All were undergoing investigation for assessment of atypical chest pain and had exercise tests that were negative at high work loads. All were in sinus rhythm and had normal ECGs. Patients with hyperlipidemia, hypertension, and diabetes mellitus were excluded. One patient smoked but refrained from doing so for 48 hours before the study. No patient with other significant risk factors was studied, so as to minimize the possibility that the patients had early coronary atherosclerosis. All patients had entirely smooth coronary arteries, and the left ventricular angiograms were normal. In seven patients, a noncardiovascular diagnosis was subsequently established; three had musculoskeletal chest pain, one gastritis, one gastric ulcer, one esophageal reflux, and one hyperventilation syndrome; in five patients, symptoms resolved spontaneously. All drug therapy was discontinued at least 48 hours before the studies.

Since L-NMMA (Novabiochem, Nottingham, UK) had not previously been infused into the human coronary circulation and animal studies had shown a variable response of the coronary vascular resistance and coronary blood flow to L-NMMA, we performed an initial dose-ranging study to confirm that L-NMMA would not induce ischemia (protocol A). Vallance et al. had shown that in the human forearm, the intra-arterial infusion of L-NMMA caused a dose-dependent reduction in forearm blood flow of up to 50% with doses of L-NMMA of up to 4 μmol/min. The effects of L-NMMA were reversible with nitroglycerin.

FIG 1. Diagram showing sequence of infusions and timing of angiograms in the 10 patients in protocol B. For the four patients who did not receive acetylcholine, the N\textsuperscript{\textcircled{O}}-monomethyl-L-arginine (LNMA) infusion immediately followed 0.9% saline (N saline) infusion, and the sodium nitroprusside (SNP) infusion immediately followed the LNMMA infusion.
A reduction of blood flow of this magnitude in the human coronary circulation may cause myocardial ischemia. We therefore studied the effects of L-NMMA in two patients, beginning with a very low infusion rate of 0.01 μmol/min into a nondominant right coronary artery (protocol A). After establishing that there was no evidence of myocardial ischemia, we used higher doses of L-NMMA: 4, 10, and 25 μmol/min (protocol B). If resting left coronary artery blood flow is estimated at 125 to 175 mL/min, the estimated maximum coronary arterial concentration of L-NMMA at an infusion rate of 25 μmol/min would be 143 to 200 μmol/L; this is similar to the estimated brachial arterial concentrations (25 to 330 μmol/L) that have been found to reduce human forearm blood flow significantly, by up to 50%.16

Protocol A

Two patients (one man, aged 61 years; one woman, aged 49 years) were studied. Diagnostic coronary arteriography was performed with Omnipaque 350 contrast medium (Nycomed) by the Judkins technique via the right femoral artery. On completion of the diagnostic procedure, the right coronary artery was reintubated with a 7F Judkins catheter (Cordis Corp), through which all infusions were given. A suitable radiographic projection was selected to obtain clearly defined arteriograms of the right coronary artery. The high-resolution 12.7-cm image intensifier (Optimus M200, Phillips) was not moved from this time on. Systemic blood pressure (right iliac artery), heart rate, and ECG were recorded continuously throughout the study.

The control infusion was 0.9% saline, and L-NMMA and sodium nitroprusside were dissolved in 0.9% saline. A control infusion of saline was given into the right coronary artery via the Judkins catheter, followed by incremental doses of L-NMMA (0.01, 0.1, 1.0, 2.0, and 5.0 μmol/min, each for 3 minutes), followed by incremental doses of sodium nitroprusside (8, 16, 24, 32, and 40 μg/min, each for 1 minute). An interval of 5 minutes was left between completion of the L-NMMA infusion and the infusion of sodium nitroprusside. All infusions were at the rate of 2

![Cine-arteriogram frames at baseline and during each of the infusions in a representative patient. Panel A: 0.9% saline. Panel B: Acetylcholine 100 nmol/min. Panel C: N⁶-monomethyl-L-arginine 25 μmol/min. Panel D: Sodium nitroprusside.](http://circ.ahajournals.org/)

FIG 2.
Protocol B

Ten pati-"ts (mean age, 51±2 years; range, 44 to 59 years; three men and seven women) were studied. Their risk factor profiles are given in Table 1. Diagnostic coronary arteriography was performed as above. To measure the oxygen saturation of the blood draining from the myocardial territory of the left anterior descending coronary artery, the coronary sinus was intubated with a 4F fiber-optic oximeter catheter (via a left antecubital fossa vein), the tip of which was advanced to the origin of the great cardiac vein. The catheter was connected to an oximeter (IVS 4000, Schwarzer, Picker Instruments) to monitor coronary venous oxygen saturation continuously. With a 7F Judkins catheter engaged in the origin of the left coronary artery, a suitable radiographic projection was selected to obtain clearly defined arteriograms of the left anterior descending coronary artery. The image intensifier was not moved from this time on. The systemic blood pressure (right iliac artery), heart rate, ECG, and coronary venous oxygen saturation were monitored and recorded continuously on analog electromagnetic tape (Store 14DS, Racal Recorders Ltd).

All infusions were given into the left coronary artery via the Judkins catheter, and the infusion rate was 2 mL/min. The sequence of infusions is illustrated in Fig 1. The control infusion was 0.9% saline. L-NMMA, acetylcholine, and sodium nitroprusside were dissolved in 0.9% saline. In 4 of the 10 patients, after the control infusion of saline, incremental doses of L-NMMA (4, 10, and 25 μmol/min, each for 5 minutes) were infused, followed by incremental doses of sodium nitroprusside (8, 16, 24, 32, and 40 μg/min, each for 1 minute). In the remaining six patients, the same protocol was performed with the addition of incremental doses of acetylcholine (1, 10, and 100 nmol/min, each for 3 minutes) infused before and after the L-NMMA infusion. Previous reports have shown that the effects of L-NMMA persist for at least 40 minutes in the human forearm and also for more than 90 minutes in the coronary circulation in the dog.

In this study, therefore, there was a continuing effect of L-NMMA to inhibit nitric oxide synthesis for the 9-minute duration of the acetylcholine infusions after the end of the L-NMMA infusion. In contrast, the effects of intracoronary acetylcholine are very brief, and the interval of 5 minutes between the completion of the second series of acetylcholine infusions and the start of the sodium nitroprusside infusion allowed sufficient time for the effects of acetylcholine to disappear. The infusion of sodium nitroprusside was discontinued immediately when the systemic blood pressure fell. All measurements during the sodium nitroprusside infusion were obtained immediately before the fall in systemic blood pressure. Coronary arteriography was repeated at the end of each infusion period. Representative frames of the coronary arteriograms from one patient are shown in Fig 2.

Data Analysis

Epicardial coronary artery caliber. The epicardial vessel luminal diameter was determined by quantitative analysis of the cine arteriograms using an automated edge detection computer analysis system (Cardiovascular Angiography Analysis System [CAAS], Pie Data Medical). The size of the coronary catheter was used to calibrate the image in millimeters, and correction was made for radiographic pin cushion distortion. Previous studies in this department using this system have shown that reanalysis of the same angiographic frames by blinded observers is highly reproducible and that there is no significant change in measured coronary artery diameter during repeated infusions of 0.9% saline.

Proximal and distal segments of the left anterior descending coronary artery between side branches were analyzed from end-diastolic frames, and the results were expressed in millimeters and as percentage changes in mean vessel diameter from the control arteriogram. Segments analyzed were those that were clearly seen on the coronary arteriogram with no overlapping vessels and with good contrast opacification at end diastole.

Index of left anterior descending coronary artery blood flow. If we make the following assumptions: 1) the great cardiac vein collects the majority of blood draining the left anterior descending coronary artery territory; 2) the predominant determinant of myocardial oxygen consumption is the heart rate–systolic blood pressure product; 3) there was no significant endocardial to epicardial redistribution of blood flow during the study; and 4)

<table>
<thead>
<tr>
<th>TABLE 2. Hemodynamic Measurements During the Saline, L-NMMA, and Sodium Nitroprusside Infusions in 10 Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saline</strong></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Systolic</td>
</tr>
<tr>
<td>Diastolic</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>RPP (bpm · mm Hg)</td>
</tr>
</tbody>
</table>

L-NMMA, N⁵-monomethyl-L-arginine; A, hemodynamic measurements at time of coronary arteriography (dose of sodium nitroprusside 21±2 μg/min); B, peak hemodynamic effects at highest dose of sodium nitroprusside (34±2 μg/min); bpm, beats per minute; RPP, heart rate–systolic blood pressure product. All values are expressed as mean±SEM.

*P<0.01 compared with saline infusion.
L-NMMA and acetylcholine cause no significant change in myocardial contractility in the doses we used, then we can assume that changes in great cardiac vein oxygen saturation reflect changes in myocardial blood flow in the left anterior descending coronary artery territory.

From the indirect Fick principle,

\[ \text{CBF} = \frac{\dot{V}mO_2}{(Cao_2 - Cvo_2)} \]

where CBF is coronary blood flow, \( \dot{V}mO_2 \) is myocardial oxygen consumption, and \( Cao_2 \) and \( Cvo_2 \) are arterial and coronary venous oxygen content, respectively.

**Statistical Analysis**

Statistical analysis was confined to protocol B. All values are expressed as mean±SEM. Changes in left anterior descending coronary artery caliber are expressed as a percentage change from the control saline value. One-way repeated-measures ANOVA was used to compare the effects of the L-NMMA infusions with the control saline infusion on the systemic hemodynamics and the coronary vascular responses. The effects of the highest doses of each agent were compared with the responses during the control saline infusion using paired t tests. Two-way repeated-measures ANOVA was used to compare the dose-response relations to acetylcholine before and after the L-NMMA infusion, followed by paired t tests at each dose with the Bonferroni correction for multiple comparisons. The responses of the proximal and distal coronary artery segments were analyzed separately, because previous studies have shown that there are segmental variations in the responsiveness of the epicardial coronary arteries to vasoactive stimuli. Linear regression analysis was used to correlate the responses of the artery segments to the L-NMMA infusion with the responses to the sodium nitroprusside infusion and to evaluate the relation between the segment diameters and the responses to the various agents. A value of \( P<0.05 \) was considered significant.

**Results**

The procedure was well tolerated by all patients. None of the patients experienced chest pain during the study, and the surface ECG did not change. There were no other side effects.
Protocol A: Effect of L-NMMA on Blood Pressure, Heart Rate, and Right Coronary Artery Diameter

Heart rate and blood pressure remained unchanged during the infusion of 0.9% saline and L-NMMA. At the higher doses of sodium nitroprusside, ≥24 μg/min, there was a fall in systemic blood pressure and a rise in heart rate. There was no change in caliber of either the proximal or distal coronary artery during infusion of L-NMMA 0.01, 0.1, 1.0, and 2.0 μmol/min. In one patient, L-NMMA 5 μmol/min caused a 12% decrease in distal artery caliber (1.78 to 1.57 mm).

Protocol B: Effect of L-NMMA and Sodium Nitroprusside on Left Anterior Descending Coronary Artery Diameter and Coronary Venous Oxygen Saturation

The infusion of 0.9% saline had no effect on the diameter of either the proximal or distal artery segment. The proximal and distal diameters of the left anterior descending coronary artery during the saline infusion were 3.18±0.20 and 1.67±0.08 mm, respectively. The lengths of the proximal and distal segments analyzed were 5.1±0.6 and 9.3±1.5 mm, respectively.

The intracoronary infusion of L-NMMA had no effect on heart rate or arterial blood pressure (Table 2). The changes in left anterior descending coronary artery diameter during the infusions of L-NMMA and sodium nitroprusside compared with saline are shown in Figs 3 and 4. During the infusion of L-NMMA at 25 μmol/min, there was no significant change in proximal left anterior descending coronary artery diameter (−2.0±1.5%, P=0.207), although there was a decrease (−5.9±2.1%, P=0.021) in the distal left anterior de-

TABLE 3. Hemodynamic Measurements During the Saline, Acetylcholine, L-NMMA, and Sodium Nitroprusside Infusions in Six Patients

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Acetylcholine 1</th>
<th>L-NMMA</th>
<th>Acetylcholine 2</th>
<th>Sodium nitroprusside</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>72±4</td>
<td>70±5</td>
<td>69±4</td>
<td>67±5</td>
<td>71±4</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>139±7</td>
<td>142±8</td>
<td>145±9</td>
<td>145±8</td>
<td>136±8</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76±4</td>
<td>76±5</td>
<td>77±4</td>
<td>79±4</td>
<td>74±4</td>
</tr>
<tr>
<td>Mean</td>
<td>99±4</td>
<td>99±6</td>
<td>102±5</td>
<td>104±5</td>
<td>98±6</td>
</tr>
<tr>
<td>RPP (bpm · mm Hg)</td>
<td>9924±1060</td>
<td>9993±985</td>
<td>9846±733</td>
<td>9631±860</td>
<td>9552±743</td>
</tr>
</tbody>
</table>

L-NMMA, N°-monomethyl-L-arginine; Acetylcholine 1, Acetylcholine 2, acetylcholine (100 nmol/min) infusions before and after L-NMMA infusion, respectively; A, hemodynamic measurements at time of coronary arteriography (dose of sodium nitroprusside, 21±2 μg/min); B, peak hemodynamic effects at highest dose of sodium nitroprusside (32±2 μg/min); bpm, beats per minute; RPP, heart rate·systolic blood pressure product. All values are expressed as mean±SEM.

*P<0.01 compared with saline infusion.
ascending coronary artery diameter (Figs 3 and 4). The decrease in distal coronary artery diameter was significant compared with the proximal segments \((P=0.035)\). Linear regression analysis of the responses of the 20 segments studied did not reveal a significant correlation between the segment diameter and the response to the L-NMMA infusion. During this period, the coronary venous oxygen saturation showed a small decrease from 37.5±2.8% to 34.3±2.8%, \(P=0.019\) (Figs 3 and 4). Since there was no significant change in the product of heart rate and systolic blood pressure (an index of myocardial work), from 9759±854 to 9881±862 beats per minute · mm Hg (Table 2), these changes in oxygen saturation represent a calculated fall in coronary blood flow of 5.7±1.9%, \(P=0.021\).

Infusion of sodium nitroprusside dilated both the proximal (17.8±6.9%, \(P=0.033\) compared with control) and distal (24.5±6.5%, \(P=0.006\) compared with control) left anterior descending coronary artery (Figs 3 and 4). For each of the distal coronary artery segments, the response to the sodium nitroprusside infusion correlated inversely with the response to the 25-\(\mu\)mol/min L-NMMA infusion \((r=-0.79, P=0.007\), Fig 5). There was an increase in coronary venous oxygen saturation from 37.5±2.8% to 61.6±5.0%, \(P=0.0002\) (Figs 3 and 4), which represents an increase in blood flow of 68±15% \((P=0.0018)\). Measurements were made before a fall in systolic blood pressure occurred (Table 2).

Effect of L-NMMA on the Coronary Vascular Responses to Acetylcholine

There were no significant changes in heart rate, arterial blood pressure, or heart rate–systolic blood pressure product during the various infusions in these six patients (Table 3). During the intracoronary infusion of acetylcholine at 100 nmol/min, dilatation of the distal segments of the left anterior descending coronary artery occurred in all patients (Fig 6); the mean percentage increase in diameter compared with saline control was 13.8±5.4%, \(P=0.049\), and the response to acetylcholine was dose-dependent (Fig 7). The responses of the proximal segments were more variable, with dilatation occurring in four patients and constriction in two. The mean percentage increase in diameter was 7.4±5.9%, \(P=0.27\) (Fig 6), which was significantly less than the response of the distal segments \((P=0.041)\). Linear regression analysis of the responses of the 12 segments studied showed no significant correlation between the segment diameter and the response to acetylcholine either before or after the L-NMMA infusion. The oxygen saturation of the coronary venous blood rose from 36.5±3.5% to 59.2±2.8%, \(P<0.0001\). There was no significant change in the product of heart rate and systolic blood pressure from 9924±1060 to 9993±985 beats per minute · mm Hg (Table 3). The changes in coronary venous oxygen saturation therefore represent a calculated increase in coronary blood flow of 57.0±7.1%, \(P=0.0005\).

During the infusion of acetylcholine at 1, 10, and 100 nmol/min after the L-NMMA infusion, there was no significant change in either proximal \((-3.9±4.0%\) at 100 nmol/min) or distal \((-3.4±5.6%\) at 100 nmol/min) coronary artery diameter compared with the control saline infusion (Fig 7). However, this represented a significant change from the effects of the acetylcholine infusions given before the L-NMMA infusion in both the proximal and distal coronary artery segments \((P=0.023\) and \(P=0.002\), respectively, two-way repeated-measures ANOVA). There was an attenuation of the vasodilator action of acetylcholine and, in some patients, the unmasking of a vasoconstrictor effect (Figs 6 and 7). In three patients, epicardial vessel constriction occurred in both the proximal and distal segments during the infusion of acetylcholine after L-NMMA; this dose had produced proximal segment dilatation in one and distal segment dilatation in all three of them before the L-NMMA infusion (Fig 6). In contrast, the coronary venous oxygen saturation rose significantly, from 36.5±3.5% to 57.0±5.3%, \(P=0.0031\) (Figs 6 and 7) during the acetylcholine infusion after L-NMMA, and the magnitude of this increase was not different from that induced by acetylcholine before L-NMMA. Again, there was no significant change in the heart rate–systolic blood pressure product (Table 3), and the changes in coronary venous oxygen saturation therefore represent a calculated increase in coronary blood flow of 55.4±14.8%, \(P=0.013\).

Discussion

This study demonstrates that, in the human coronary circulation, inhibition of nitric oxide synthesis with L-NMMA causes a small increase in basal tone of the distal segments of the epicardial coronary arteries together with a small fall in basal coronary blood flow. These data indicate a small tonic release of nitric oxide under basal conditions in the distal epicardial coronary arteries and in at least some of the resistive vessels. Sodium nitroprusside, a direct donor of nitric oxide,\(^{29}\) causes dilatation of both the proximal and distal epicardial coronary arterial segments together with an increase in coronary blood flow. Even at high intracoronary doses of sodium nitroprusside, just less than those sufficient to reduce systemic blood pressure, dilatation of the resistive vessels through the nitric oxide–dependent pathway causes only a moderate increase in coronary blood flow (68±15%). L-NMMA inhibits acetylcholine–induced coronary artery dilatation but does not affect acetylcholine–induced increases in coronary blood flow. L-NMMA, in intracoronary doses up to 25 \(\mu\)mol/min, does not change heart rate or arterial blood pressure (Table 2).

Since an inhibitor of nitric oxide synthesis had not previously been infused into the human coronary circulation, we elected initially to infuse very low doses of L-NMMA into the nondominant right coronary artery of the first two patients (protocol A). There were no adverse effects, and in one of these two patients, constriction of the distal right coronary artery was observed at the highest dose (5 \(\mu\)mol/min). We then used higher doses of L-NMMA (4, 10, and 25 \(\mu\)mol/min) into the left coronary artery (protocol B), and if resting left coronary artery blood flow is assumed to be 125 to 175 mL/min, this gives estimated coronary arterial concentrations of L-NMMA of 23 to 200 \(\mu\)mol/L. These are similar to the estimated brachial arterial concentrations of L-NMMA (25 to 330 \(\mu\)mol/L) that were observed by Vallance et al\(^{10}\) to cause a fall in forearm blood flow of up to 50%. Very high doses of L-NMMA were not given; therefore, it is not certain

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caused by direct inhibition of nitric oxide synthesis. In this study, we avoided systemic hemodynamic changes by infusing all the agents directly into the coronary circulation, and the doses of L-NMMA and acetylcholine selected did not significantly change heart rate or systemic blood pressure (Tables 2 and 3).

In previous studies, indirect evidence for the role of nitric oxide production in the human coronary circulation has been obtained with agents that are known to stimulate endothelial nitric oxide production (for example, acetylcholine,23 substance P,24 and serotonin25,30). Intracoronary infusion of each of these agents causes dilatation of normal epicardial coronary arteries in humans, and this effect has been shown to vary along the length of the epicardial vessel23-25,30; the effect at a given dose is more marked in the distal than in the proximal segments.

These reports,23-25,30 together with our data (Figs 3 and 4), suggest a difference in behavior between the proximal and distal epicardial coronary artery segments in response to vasoactive agents. In our study, this difference may either result from a specific difference in basal nitric oxide production and responsiveness between different coronary artery segments or simply reflect a nonspecific reduction of responsiveness of the proximal and larger coronary artery segments to all vasoactive stimuli. In support of this latter view, a large study by Brown et al28 demonstrated an inverse relation between epicardial coronary artery responsiveness and segment diameter for every vasoactive agent, both constrictor and dilator, that they examined. They studied agents acting through both nitric oxide–related mechanisms (eg, sodium nitroprusside) and nitric oxide–independent mechanisms (eg, verapamil). Their findings28 indicate that the differences between proximal and distal vessel responsiveness that we observed may be nonspecific and not confined to the nitric oxide system.

In addition, in the distal epicardial coronary artery segments, we observed a significant inverse correlation between the magnitude of the vasoconstrictor response to the L-NMMA infusion and the vasodilator response to the sodium nitroprusside infusion (Fig 5). The corresponding analysis was not performed for the proximal segments because of the lack of a significant change in proximal segment diameter during the L-NMMA infusion. We speculate that there is variation in basal endogenous nitric oxide activity in the distal coronary artery segments between individual patients. Thus, those patients with a large response to the L-NMMA infusion may have had a high basal nitric oxide activity and only a small incremental response to sodium nitroprusside, a donor of nitric oxide, because the nitric oxide pathway was already substantially activated. In addition, in these patients, the response to sodium nitroprusside may have been attenuated further by the development of tolerance, which recent experimental work has suggested may occur after prolonged activation of the nitric oxide pathway.31 In contrast, the patients with a low basal nitric oxide activity may have had a small response to the L-NMMA infusion but a large response to sodium nitroprusside (Fig 5).

Effect of L-NMMA on Coronary Resistive Vessels

Myocardial work as assessed by the heart rate–systolic blood pressure product remained unchanged through-

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**FIG 7.** Graphs showing effects of the intracoronary infusion of acetylcholine (ACh) on proximal and distal left anterior descending (LAD) coronary artery diameters and coronary venous oxygen saturation before (○) and after (●) the NO2-monomethyl-L-arginine (LNMMA) infusion. Results are mean ± SEM in six patients. *P < 0.05, **P < 0.01 compared with before LNMMA infusion.
out the L-NMMA infusion, and according to the indirect Fick principle, coronary venous oxygen saturation can be used as an index of coronary blood flow. The coronary venous oxygen saturation decreased only slightly during the L-NMMA infusion (Figs 3 and 4). These findings are compatible with a small decrease (5.7±1.9%) in coronary blood flow in response to the L-NMMA infusion, indicating a small increase in coronary resistance caused by a small increase in resistive vessel tone, and are consistent with the measurements reported in the chronically instrumented dog. However, our results contrast with those of Amezgua et al., who demonstrated that in the isolated and buffer-perfused rabbit heart, L-NMMA (with a perfusate concentration of 30 to 100 μmol/L) caused a marked increase in coronary perfusion pressure of up to 120% above baseline, indicating a similar increase in coronary vascular resistance. This finding indicates that, in the buffer-perfused rabbit coronary circulation, there is a high basal effect of nitric oxide. Hemoglobin is an inhibitor of nitric oxide, so when the coronary circulation is perfused with whole blood, the effect of nitric oxide, which is released under basal conditions, would be expected to be substantially attenuated. When the coronary circulation is buffer-perfused, this mechanism of attenuation is removed; therefore, there might be a higher basal effect of nitric oxide under these conditions. Thus, addition of L-NMMA might be expected to cause a much greater relative increase in coronary vascular resistance in the buffer-perfused heart compared with the blood-perfused heart.

In the human forearm, an estimated arterial concentration of L-NMMA similar to that used in our study reduced blood flow by 50% without any change in blood pressure. Thus, in humans, there appears to be considerable variability in response to L-NMMA at similar arterial concentrations between the coronary and forearm circulation. The reasons for this are uncertain but presumably reflect either increased tonic release of nitric oxide in the forearm resistive vessels or a different role for nitric oxide in the peripheral circulation compared with the coronary circulation.

**Effect of L-NMMA on the Coronary Vascular Responses to Acetylcholine**

The intracoronary infusion of acetylcholine has been reported previously to cause dose-dependent dilatation of the epicardial coronary arteries together with an increase in coronary venous oxygen saturation and coronary blood flow; these findings were confirmed in the present study (Figs 6 and 7). L-NMMA inhibited the acetylcholine-induced epicardial coronary artery dilatation of the distal coronary artery segments (Figs 6 and 7); this provides strong evidence that acetylcholine dilates the epicardial coronary arteries by the stimulation of nitric oxide synthesis. L-NMMA did not affect the acetylcholine-induced increase in coronary venous oxygen saturation, however (Figs 6 and 7); this is consistent with the view that the vasodilator effect of acetylcholine on the resistive vessels is independent of nitric oxide synthesis.

Sodium nitroprusside in doses just less than those sufficient to cause a fall in systemic blood pressure induced a calculated increase in coronary blood flow of 68±15%. In contrast, acetylcholine has previously been shown by our group, in similar patients and using the same methods, to cause an increase in coronary blood flow of 167% at a dose of 1000 nmol/min. In the present study, however, the maximal dose of acetylcholine was 1000 nmol/min; we did not use a dose of 1000 nmol/min, because it had been shown previously to induce constriction of normal epicardial arteries that had dilated at the lower dose of 100 nmol/min. Thus, the maximum vasodilator response of the resistive vessels to acetylcholine considerably exceeds that of the resistive vessels to sodium nitroprusside, and this provides further evidence that the vasodilator effect of acetylcholine on the resistive vessels is at least in part independent of nitric oxide. This conclusion is supported by the work of Komar et al., who found that, in the open-chest anesthetized dog, L-NMMA (300 μmol/L) caused complete inhibition of acetylcholine-induced dilatation of arterioles with diameter >120 μm but only partial inhibition of acetylcholine-induced dilatation in arterioles with diameter <120 μm. Woodman and Dusting reported that in the anesthetized dog, N-nitro-L-arginine, another inhibitor of nitric oxide synthesis, completely inhibited the vasodilator response of the epicardial coronary arteries to acetylcholine but only attenuated the acetylcholine-induced increase in coronary blood flow. Similarly, Broten et al. demonstrated in the closed-chest anesthetized dog that the intracoronary infusion of nitro-L-arginine methyl ester only partially inhibited the increase in coronary flow after bolus injection of acetylcholine or vagal stimulation. Although these results differ from our study, since we demonstrated that in the human coronary circulation L-NMMA had no effect on the acetylcholine-induced increase in coronary blood flow (Figs 6 and 7), they indicate that acetylcholine dilates at least some coronary resistive vessels by a mechanism that cannot be completely inhibited by L-NMMA and hence is independent of nitric oxide synthesis.

The failure of L-NMMA to block acetylcholine-induced resistive vessel dilatation (Figs 6 and 7) has several possible explanations. The nitric oxide synthesis pathway in the resistive vessels compared with the epicardial arteries may be less sensitive or even completely insensitive to inhibition by L-NMMA. We consider that it is unlikely to be completely insensitive to inhibition by L-NMMA, because we demonstrated a significant reduction in coronary blood flow during the L-NMMA infusion, indicating at least some inhibition of nitric oxide synthesis in the resistive vessels. In addition, in other studies in which the dose-dependence of inhibition of the nitric oxide pathway has been assessed, concentrations of L-NMMA equivalent to the estimated arterial concentrations in our study (143 to 200 μmol/L) caused near-maximal inhibition of nitric oxide activity. It is possible that acetylcholine dilates the resistive vessels by increasing nitric oxide synthesis as a result of increasing the activity of another nitric oxide synthesis enzyme system that is insensitive to L-NMMA. A more likely explanation, however, is that the vasodilator effect of acetylcholine in the coronary resistive vessels is partly or completely independent of nitric oxide synthesis. It is unlikely to be a direct action on the vascular smooth muscle, because acetylcholine vasoconstricts in the absence of endothelium. Acetylcholine may act upon resistive vessels via the release of
an intermediate vasodilator agent other than nitric oxide from the endothelium. The identity of such an agent remains speculative, but candidates might include adenosine and endothelium-derived hyperpolarizing factor. In our study, it is possible that if higher doses of L-NMMA had been given, we might have observed attenuation of the acetylcholine-induced increase in coronary blood flow. This would have indicated a different sensitivity of the resistive vessels to the effects of L-NMMA compared with the epicardial coronary arteries, because the acetylcholine-induced dilatation of the epicardial arteries was completely inhibited by L-NMMA (Fig 7). We elected not to give higher intracoronary doses of L-NMMA because of a possible increase in systemic blood pressure and vasoconstriction in other vascular beds, in particular in the brain. 

Several studies have described the response to intracoronary acetylcholine of angiographically normal coronary arteries in patients selected in a manner similar to those in our study. Newman et al examined the effects of acetylcholine on proximal and distal segments of angiographically normal coronary arteries. They reported that although acetylcholine caused dose-dependent dilatation of distal coronary artery segments at the same doses of acetylcholine as were used in our study, there was no change in proximal coronary artery segments. In addition, they showed that coronary artery segments that dilated in response to acetylcholine in low doses, which we used in our study, were constricted as the infusion concentration increased. Thus, intracoronary acetylcholine may have both vasodilator and vasoconstrictor effects on the same angiographically normal epicardial coronary artery, depending on both the infusion concentration and the vessel segment being studied. Ludmer et al reported dilatation in coronary artery segments that were similar in caliber to the distal artery segments in our study, but these authors did not state the site of the segments or report the effects of acetylcholine on larger coronary artery segments. Werns et al observed that the response to acetylcholine of coronary artery segments slightly smaller in caliber than the proximal segments in our study was variable in that 9 of 14 segments dilated and 5 of 14 constricted, although the overall response was dilatation. Yasue et al reported that in young patients, there was no change in proximal vessel caliber, although dilatation of the distal left anterior descending coronary artery occurred in response to intracoronary acetylcholine in a concentration similar to that used in our study. The findings of these studies are consistent with our data. In our study, acetylcholine caused dilatation of the proximal coronary artery segments in four patients and constriction in two. In contrast, it caused dilatation of the distal coronary artery segments in all six patients (Fig 6). The more consistent vasodilator effect of acetylcholine on the distal coronary artery segments was statistically significant compared with the proximal segments. It is likely that the variability of the overall response of the epicardial coronary artery segments to acetylcholine as a function of the infused dose can be explained by local variations in the balance between its opposing vasomotor effects, namely, vasodilatation caused by stimulating the endothelial release of EDRF and vasoconstriction caused by direct stimula-

tion of smooth muscle. Others have proposed that the epicardial vessel dilatation in response to acetylcholine can be adequately explained as a flow-induced phenomenon; if this were the case, it now seems likely that such flow-induced dilatation would be a result of flow-stimulated increased production of nitric oxide. Our findings are consistent with both of these explanations. Inhibition of nitric oxide synthesis with L-NMMA inhibited the acetylcholine-induced vasodilatation of the epicardial coronary arteries but not the increase in coronary venous oxygen saturation.

It has been suggested that constriction of atherosclerotic vessels in response to acetylcholine is indicative of endothelial dysfunction. Our data are consistent with this view, although at least some EDRF-mediated mechanisms remain intact in atherosclerotic vessels, because dilatation of human epicardial vessels in vivo in response to substance P, an endothelium-dependent dilator without a direct vasoconstrictor effect, is preserved in atherosclerotic vessels.

Acetylcholine has variable effects in different vascular beds, vessel types, and species. In the human forearm, L-NMMA infused into the brachial artery markedly attenuated the increase in forearm blood flow induced by acetylcholine, suggesting that increased nitric oxide synthesis may be the mechanism by which acetylcholine produces resistive vessel dilatation in the forearm. An alternative explanation is that the forearm conduit arteries, ie, the brachial artery and its major branches, may have become so constricted after the L-NMMA infusion that an increase in flow after resistive vessel dilatation with acetylcholine was prevented, but this seems unlikely, because resting flow was reduced by no more than 50%.

Limitations of the Study

It is possible that some of the patients had early coronary atherosclerosis, and this may affect endothelial function. To minimize this likelihood, only those patients who were free of significant cardiovascular risk factors were included, apart from one patient who smoked (Table 1), but this patient had a dilator response to acetylcholine. Early coronary atherosclerosis is associated with loss of the epicardial coronary artery dilator response to acetylcholine. In our study, this response was preserved in the distal epicardial artery segments in all six patients and in the proximal segments in four of the six patients in whom it was assessed (Fig 6). Therefore, it is likely that this group of patients was free from significant coronary atherosclerosis.

Coronary venous oximetry was selected in preference to intracoronary Doppler to measure coronary blood flow in this study because coronary arterial instrumentation may cause substantial endothelial denudation and nitric oxide is derived from the endothelium. The calculated changes in blood flow during the acetylcholine infusions in our study are consistent with those found by others using intracoronary Doppler. Coronary venous oxygen saturation is an indirect method that gives a continuous measurement of coronary blood flow but that may be affected by factors other than coronary blood flow, and we took care to control for these. In particular, possible interference from right atrial reflux into the coronary sinus was avoided by advancing the fiber-optic catheter tip far into the coro-
Conclusions

Inhibition of nitric oxide synthesis in the coronary circulation of humans by the intracoronary infusion of L-NMMA decreases distal epicardial coronary artery basal diameter and basal coronary venous oxygen saturation, an index of coronary blood flow. These findings indicate that basal vasomotor tone in the epicardial coronary arteries and some coronary resistive vessels is at least in part maintained by the synthesis of nitric oxide. Acetylcholine-induced epicardial coronary artery dilatation is nitric oxide dependent, although at least some of its vasodilator effects on the resistive vessels appear to be independent of nitric oxide synthesis.

Acknowledgment

This work was supported by a grant from the British Heart Foundation.

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Circulation. 1993;88:43-54
doi: 10.1161/01.CIR.88.1.43

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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