Effects of L- and D-arginine on the basal tone of human diseased coronary arteries and their responses to substance P

D Tousoulis, C Tentolouris, T Crane, G Katsimaglis, C Stefanadis, P Toutouzas, G J Davies

Abstract
Objective—To assess the effects of substance P administration alone and in combination with L- and D-arginine in patients with normal angiograms and in patients with coronary artery disease.

Design—Intracoronary infusions of (a) normal saline, (b) the receptor mediated nitric oxide stimulant substance P (5.6 and 27.8 pmol/min) before and after L- or D-arginine (50 and 150 µmol/min), and (c) glyceryl trinitrate (250 µg bolus) were given to 17 patients with coronary artery disease and stable angina, and to six patients with normal angiograms.

Results—L-arginine administration was associated with significant dilatation of stenoses (p < 0.01) of proximal segments of both “normal” (p < 0.05) and diseased (p < 0.01) arteries, and of distal segments of diseased arteries (p < 0.01). No significant changes were associated with D-arginine administration. Dose dependent dilatation of all segments including stenoses, was observed with substance P both before and after L-arginine infusion (p < 0.01). The magnitude of dilatation of stenoses and all segments of both “normal” and diseased coronaries was greater after L-arginine (p < 0.05) but not D-arginine and substance P infusion, than it was after saline and substance P infusion. Administration of D- or L-arginine did not change the magnitude of substance P induced dilatation.

Conclusions—Diseased and “normal” coronary arteries dilated in response to substance P and L-arginine but were unaffected by D-arginine infusion. The magnitude of the response to substance P was not increased by L-arginine administration, indicating that it is not critically dependent on the availability of substrate for nitric oxide synthase.

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Keywords: endothelium; nitric oxide; coronary artery disease; L-arginine; vasomotor tone

Studies in humans have shown that vascular endothelium plays an important role in the regulation of blood flow by releasing endothelium derived relaxing factors (EDRF). L-arginine, a major component of EDRF, is synthesized from the amino acid L-arginine by a family of enzymes through the L-arginine–nitric oxide pathway. This synthesis of vascular endothelium is responsible for the vasodilator tone that is essential for the regulation of blood flow.

We and other investigators have shown that hypercholesterolaemia and other risk factors for atherosclerosis are associated with impaired endothelium dependent vasodilation in the peripheral or coronary vasculature in animals and humans. It has also been shown that intracoronary infusion of L-arginine causes forearm vasodilation, augments the endothelium dependent forearm vasodilation, and reverses the defective endothelium dependent vasodilatation caused by low density lipoprotein or hypercholesterolaemia. Furthermore, other studies have shown that L-arginine significantly improves the coronary blood flow response to acetylcholine in patients with normal coronary arteries and hypercholesterolaemia. However, it is unknown whether local administration of L-arginine dilates epicardial coronary arteries and improves the endothelium dependent vasodilatation in patients with normal angiograms and in patients with coronary artery disease. We estimated the responses to these substances as a proportion of maximum vasodilator response to the endothelium independent vasodilator glyceryl trinitrate.

Methods

Patients
We studied 17 patients (13 male, four female, mean (SEM) age 59 (8) years) with chronic stable angina, coronary artery disease, and a positive treadmill exercise test result (≥ 0.1 mV ST segment depression) at between 5 and 7 METS using the modified Bruce protocol, and six patients (one male, five female, age 56 (7) years) with atypical chest pain, risk factors for atherosclerosis, and normal coronary angiograms. Coronary arteries were considered normal when they had a smooth angiographic outline in multiple projections, with no irregularity or stenosis. As intravascular ultrasound imaging was not done, it is not possible to exclude the presence of mild atheroma in these angiographically “normal” arteries.
The clinical characteristics of the patients are listed in Table 1. Patients were excluded from the study if they had diabetes mellitus, recent myocardial infarction (<6 months before), left ventricular hypertrophy (on echocardiography), left ventricular dysfunction (left ventricular ejection fraction <50%), or valvar heart disease. Hypercholesterolaemia was defined as a fasting serum total cholesterol of >5.5 mmol/l, or serum triglyceride >1.65 mmol/l. Antianginal drugs were stopped 24 hours before the study. The patients were allowed to use sublingual glyceryl trinitrate as necessary, but no study was performed within three hours of its administration.

The protocol was approved by the research ethics committee and each patient gave written informed consent.

**PROTOCOL**

After the diagnostic coronary angiography, an optimal radiographic projection was selected and kept constant for subsequent angiography. Two ECG leads were monitored continuously throughout the study.

A syringe pump was used to give the following sequence of intracoronary infusions in 11 patients with coronary artery disease (nine male, two female) and in six patients with normal angiograms: 0.9% saline (2 ml/min) for two minutes, 5.6 and 27.8 pmol/min of substance P in saline for five minutes each, 50 and 150 µmol/min of L-arginine for eight minutes each, a repeat five minute infusion of 5.6 and 27.8 pmol/min substance P in saline, and finally an intracoronary bolus dose of glyceryl trinitrate (250 µg in 2 ml of saline). In six patients with coronary artery disease (four male, two female) the same protocol was performed, substituting 50 and 150 µmol/min of D-arginine for l-arginine. The lower dose of substance P was selected with reference to the dose–response curve reported by Crossman et al,18 in which maximum segment dilatation occurred at 5.6 pmol/min. The higher dose of 27.8 pmol/min was similar to that used in previous studies.

Femoral arterial pressure and heart rate were recorded during the last 30 seconds of each infusion period. Angiography was performed with a hand injection of 6–8 ml of non-ionic contrast medium at baseline, immediately after each infusion, and two to three minutes after glyceryl trinitrate. Before each angiography, the catheter was emptied to avoid bolus administration of the infusate.

**QUANTITATIVE CORONARY ANGIOGRAPHY**

The arterial segments in each frame were analysed in random order using quantitative computerised analysis with an automated edge contour detection analysis system (Computerised Angiographic Analysis System, Version 2V2; Pie Data Medical, Maastricht, the Netherlands).20 21 End diastolic frames from each arteriogram were selected for analysis. The angiographic catheter was used as a scaling device and this, together with the pincushion distortion correction, allowed the...
diameters to be recorded as absolute values (expressed in mm).

Recorded variables at baseline and after saline, substance P, l-arginine, D-arginine, and nitrate administration were the luminal diameter at the site of stenosis and in non-stenotic proximal and distal segments of “normal” and diseased coronary arteries. The proximal left anterior descending coronary artery diameter was measured just beyond the origin of the artery, and the distal diameter was measured just distal to the second diagonal branch; the proximal left circumflex coronary artery diameter was measured just beyond the origin of the artery and the distal diameter just beyond the posterior descending branch.

Quantitative analysis of coronary arteriograms was carried out by two independent observers, who blindly reanalysed the films at a remote time for reproducibility of the method. No significant intraobserver or interobserver variability was found (analysis of variance F = 0.35, p = 0.82).

Twenty three proximal segments (11 in the left anterior descending coronary artery, 12 in the circumflex coronary artery), 26 distal segments (13 in the left anterior descending coronary artery, 13 in the circumflex coronary artery), and 14 stenoses were analysed from patients with coronary artery disease who received l-arginine. Eleven proximal segments (five in the left anterior descending coronary artery, six in the circumflex coronary artery), 14 distal segments (six in the left anterior descending coronary artery, eight in the circumflex coronary artery), and six stenoses were analysed from patients with coronary artery disease who received D-arginine. Nine proximal segments (four in the left anterior descending coronary artery, four in the circumflex coronary artery, one in the right coronary artery) and 14 distal segments (six in the left anterior descending coronary artery, six in the circumflex coronary artery, two in the right coronary artery) were analysed from patients with normal coronary arteriograms who received L-arginine.

STATISTICAL ANALYSIS
Data are expressed as mean (SEM). When serial changes in the heart rate, blood pressure, and arterial diameter were compared within the group and between the groups, analysis of variance (ANOVA) and the Scheffe F test for repeated measures was used. To test the differences in the response to proximal and distal segments and coronary stenoses to L- or D-arginine, two way ANOVA for repeated measures was applied. Student’s t test was used to compare paired or unpaired data. Probability (p) values of < 0.05 (two tailed) were considered to indicate statistical significance.

Results
In patients with coronary disease who received L-arginine the infusion of 27.8 pmol/min, substance P reduced the systolic blood pressure from (mean (SEM)) 154 (7.3) to 147 (7.7) mm Hg (p < 0.01) and increased the heart rate from 71.3 (1.8) to 79.8 (3.1) beats/min (p < 0.01). The infusion of L-arginine did not affect the systolic blood pressure (152 (7.1) v 150 (7.2) mm Hg before and after infusion, respectively, NS) or heart rate (from 71.6 (1.7) to 72.8 (1.6) beats/min, respectively, NS). After l-arginine administration, 27.8 pmol/min substance P infusion reduced systolic blood pressure to 145 (7.7) mm Hg and increased heart rate to 78.5 (2.4) beats/min (NS, v before L-arginine infusion). In the patients who received D-arginine the infusion of 27.8 pmol/min, substance P reduced the systolic blood pressure from 155 (11.6) to 146 (11.7) mm Hg (p < 0.01) and increased the heart rate from 69.7 (3.7) to 75.0 (3.5) beats/
The infusion of D-arginine did not affect the systolic blood pressure (154 (11.2) vs 157 (11.6) mm Hg before and after infusion, respectively, NS) or heart rate (from 72.1 (2.8) to 70.3 (2.5) beats/min, respectively, NS).

L-arginine administration (figs 1 and 2, table 2), but not D-arginine administration (fig 1, table 3) was associated with significant dilation of stenoses, of proximal segments of both “normal” and diseased arteries and of distal segments of diseased arteries. Dose dependent dilation of all segments including stenoses was observed with substance P before and after L-arginine infusion and after both L-arginine (table 2, figs 1 and 2) and D-arginine infusions (table 3, fig 1). However, neither D- nor L-arginine administration changed the magnitude of substance P induced dilatation (fig 3, table 3). The dilation was less in the proximal than in the distal segments of diseased arteries (table 2). The magnitude of dilatation of stenoses and all segments of both “normal” and diseased coronaries was greater after L-arginine and substance P infusion than after saline and substance P infusion (table 2).

### Table 2 Reactivity of proximal and distal segments and coronary stenoses to substance (sub) P before and after L-arginine infusion

<table>
<thead>
<tr>
<th>Coronary artery disease</th>
<th>Saline</th>
<th>Sub P1 (5.6 pmol)</th>
<th>Sub P1 (27.8 pmol)</th>
<th>L-arginine (150 µmol)</th>
<th>Sub P2 (5.6 pmol)</th>
<th>Sub P2 (27.8 pmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal (n = 23)</td>
<td>3.08 (0.13)</td>
<td>3.28 (0.14)</td>
<td>3.29 (0.12)</td>
<td>3.27 (0.13)</td>
<td>3.34 (0.15)</td>
<td>3.39 (0.13)†</td>
</tr>
<tr>
<td></td>
<td>(0.6 (0.3)% )</td>
<td>(7.1 (1.3)% )</td>
<td>(8.6 (1.7)% )</td>
<td>(8.5 (1.1)% )</td>
<td>(8.4 (1.5)% )</td>
<td>(12.7 (1.8)% )</td>
</tr>
<tr>
<td>Distal (n = 26)</td>
<td>1.43 (0.05)</td>
<td>1.60 (0.06)*</td>
<td>1.61 (0.07)*</td>
<td>1.50 (0.07)</td>
<td>1.65 (0.07)**</td>
<td>1.70 (0.07)†</td>
</tr>
<tr>
<td></td>
<td>(0.7 (0.5)% )</td>
<td>(14.2 (2.5)% )</td>
<td>(15.3 (2.3)% )</td>
<td>(6.7 (1.3)% )</td>
<td>(15.7 (2.1)% )</td>
<td>(21.8 (2.8)% )</td>
</tr>
<tr>
<td>Stenoses (n = 14)</td>
<td>1.48 (0.14)</td>
<td>1.64 (0.16)</td>
<td>1.72 (0.19)</td>
<td>1.70 (0.18)</td>
<td>1.82 (0.16)†</td>
<td>1.84 (0.19)†</td>
</tr>
<tr>
<td></td>
<td>(0.8 (0.6)% )</td>
<td>(10.9 (2.5)% )</td>
<td>(11.8 (3.3)% )</td>
<td>(18.3 (2.8)% )</td>
<td>(20.3 (2.7)% )</td>
<td></td>
</tr>
</tbody>
</table>

**Normal coronary arteries**

| Proximal (n = 9)        | 3.05 (0.27) | 3.25 (0.25) | 3.40 (0.23) | 3.20 (0.28) | 3.35 (0.24)†† | 3.49 (0.24) |
|                         | (0.9 (0.5)% ) | (8.6 (3.6)% ) | (14.4 (3.5)% ) | (10.4 (3.4)% ) | (12.4 (3.7)% ) | (17.4 (4.1)% ) |
| Distal (n = 14)         | 1.50 (0.04) | 1.68 (0.07)* | 1.71 (0.05) | 1.55 (0.06) | 1.69 (0.01) | 1.79 (0.06)†† |
|                         | (0.6 (0.7)% ) | (13.4 (4.0)% ) | (15.5 (2.3)% ) | (14.6 (3.8)% ) | (20.6 (2.9)% ) | |

Values are mean (SEM) luminal diameter in mm (mean (SEM) percentage change from baseline).

\*p < 0.05, **p < 0.01 vs normal saline in both groups.

†p < 0.01, ††p < 0.05 vs the pre-L-arginine responses.

1 = before L-arginine administration; 2 = after L-arginine administration; CA, coronary arteries.

### Table 3 Reactivity of proximal and distal segments and coronary stenoses to substance (sub) P before and after D-arginine infusion

<table>
<thead>
<tr>
<th>Stable angina</th>
<th>Saline</th>
<th>Sub P1 (5.6 pmol)</th>
<th>Sub P1 (27.8 pmol)</th>
<th>D-arginine (150 µmol)</th>
<th>Sub P2 (5.6 pmol)</th>
<th>Sub P2 (27.8 pmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal (n = 11)</td>
<td>2.91 (0.18)</td>
<td>3.07 (0.17)</td>
<td>3.10 (0.17)</td>
<td>2.90 (0.16)**</td>
<td>3.08 (0.17)</td>
<td>3.10 (0.18)*</td>
</tr>
<tr>
<td></td>
<td>(0.9 (0.5)% )</td>
<td>(6.5 (1.1)% )</td>
<td>(8.0 (2.3)% )</td>
<td>(0.8 (1.1)% )</td>
<td>(7.4 (1.7)% )</td>
<td>(7.4 (1.3)% )</td>
</tr>
<tr>
<td>Distal (n = 14)</td>
<td>1.51 (0.05)</td>
<td>1.65 (0.08)</td>
<td>1.70 (0.08)</td>
<td>1.51 (0.05)**</td>
<td>1.66 (0.07)</td>
<td>1.73 (0.08)</td>
</tr>
<tr>
<td></td>
<td>(0.8 (0.6)% )</td>
<td>(10.0 (2.2)% )</td>
<td>(13.6 (2.5)% )</td>
<td>(0.8 (0.7)% )</td>
<td>(10.5 (2.1)% )</td>
<td>(14.7 (2.7)% )</td>
</tr>
<tr>
<td>Stenoses (n = 6)</td>
<td>1.72 (0.10)</td>
<td>1.82 (0.12)</td>
<td>1.86 (0.09)</td>
<td>1.72 (0.10)**</td>
<td>1.84 (0.13)†</td>
<td>1.86 (0.10)*</td>
</tr>
<tr>
<td></td>
<td>(1.6 (1.1)% )</td>
<td>(7.4 (1.6)% )</td>
<td>(10.0 (1.8)% )</td>
<td>(1.7 (0.6)% )</td>
<td>(8.5 (1.2)% )</td>
<td>(10.1 (1.1)% )</td>
</tr>
</tbody>
</table>

Values given are mean (SEM) luminal diameter in mm (mean (SEM) percentage change from baseline).

*\*p < 0.05, **p < 0.01 vs D-arginine infusion; †p = NS in substance P responses before and after D-arginine.

1 = before D-arginine administration; 2 = after D-arginine administration; CA, coronary arteries.
Discussion
In this study we investigated the effects of L- and D-arginine administration on the response of epicardial coronary segments to substance P in patients with normal coronary angiograms and in patients with coronary artery disease. We also compared these responses to those obtained by the administration of endothelium independent vasodilator glyceryl trinitrate. The main findings of the study were first, that all “normal” and diseased coronary segments, including stenoses, dilated in response to substance P and L-arginine but were unaffected by D-arginine infusion; second, that the magnitude of the response to substance P was not increased by L-arginine administration; and third, that a significant proportion of the response to glyceryl trinitrate could be obtained by L-arginine administration.

EFFECTS OF CORONARY DISEASE ON VASODILATOR MECHANISMS
In contrast to concepts derived from pathologi- cal studies suggesting that coronary stenoses are fixed, it is now accepted that they may alter their calibre in response to vasoactive agents. Our observation that glyceryl trinitrate dilates coronary artery stenoses is consistent with previous results, including those of Raffenbeul et al, who reported that stenoses of any degree and site can dilate in response to nitrates. Other investigators have observed that stenoses with the smallest luminal diameters often do not dilate with nitrates. Dilatation in response to nitrates indicates that smooth muscle cells with a preserved contractile mechanism are present within the stenosed segment.

Previous clinical studies have shown that angiographically normal coronary arteries dilate in response to the intracoronary infusion of acetylcholine, suggesting that endothelial function is intact. Diseased arteries were found to constrict in response to acetylcholine and this has been interpreted as evidence of endothelial dysfunction. The finding of our study that substance P combined with L-arginine causes dilatation of diseased coronary arteries comparable in magnitude to that induced by nitrates suggests that the nitric oxide pathway remains intact. Furthermore, as substance P acts on nitric oxide synthase through endothelial cell surface receptors our results also indicate the presence of functioning endothelium in these diseased segments.

L-ARGININE AND NITRIC OXIDE
The L-isomer of arginine is a substrate for both the endothelial cells and inducible isoforms (in macrophages, foam cells, and smooth muscle cells) of the enzyme nitric oxide synthase. These enzymes convert L-arginine to citrulline and nitric oxide. Nitric oxide reduces vascular smooth muscle tone by stimulation of soluble granulyl cyclase. Nitric oxide also has antioxidant effect. However, when it combines with equimolar amounts of superoxide, peroxyni- trite is formed, which is a strong oxidant. Inducible nitric oxide synthase can produce large amounts of nitric oxide and is present in human atherosclerotic lesions. It has been suggested that diseased arteries may be deficient in the substrate L-arginine. Apart from reducing nitric oxide production, substrate deficiency could lead to the generation of superoxide by both inducible and endothelial nitric oxide synthase. The substrate deficiency hypothesis is supported by experimental evidence in hypercholesterolaemic rabbits that arginine administration restores cholinergic (nitrergic) dysfunction of both epicardial coronary arteries and coronary microvasculature in cardiac transplant recipients and in patients with atherosclerosis. Evidence against the hypothesis was obtained by Hirooka et al, who found no effect of L-arginine in the vasomotor response of diseased coronary arteries to acetylcholine in patients. However, in their study they infused only 50 µmol/min of L-arginine.

The results of our study are consistent with stimulation of nitric oxide synthase by L-arginine administration. However, this effect was found in proximal segments of “normal arteries” and diseased arteries (including the site of stenosis), suggesting that it is either a physiological response or that it requires only minimal coronary disease which could be present in the proximal segments in the patients with normal coronary angiograms. An alternative explanation to the stimulation of nitric oxide synthase would be a physiological action on vascular smooth muscle which augments its responsiveness to nitric oxide or other endogenous vasodilator mechanisms.

THE MECHANISMS OF ACTION OF SUBSTANCE P
Substance P is a neuropeptide found in sensory neurons of the peripheral nervous system, vagus, some sympathetic ganglia, and the perivascular nerves of small arterioles in the human heart. It dilates both conductive and resistance vessels by an endothelium dependent mechanism which involves the production of nitric oxide and possibly also of an endothelium derived hyperpolarising factor. Crossman et al demonstrated an increase in epicardial coronary artery diameter in patients with normal angiograms and in patients with coronary artery disease, and a significant increase in the coronary sinus blood oxygen saturation in response to intracoronary substance P infusion in patients with angiographically normal coronary arteries. In our study, both normal and diseased arteries dilated in response to substance P, but the response was greater in the distal segments of diseased vessels than in those of “normal” vessels.

Our results also showed that L-arginine (the substrate for nitric oxide synthase) did not alter the magnitude of the response to substance P of epicardial coronary artery segments in patients with normal angiograms or in patients with minimal coronary disease which could be present in the proximal segments in the patients with normal coronary angiograms.
with coronary artery disease. These results are consistent with those of Panza et al., who found that acetylcholine induced forearm vasodilatation was not altered by intra-arterial infusion of L-arginine at 40 μmol/min in hypertensive patients. In contrast, Drexler et al. showed no effects in epicardial coronary arteries, but a significant increase in blood flow response to acetylcholine in patients with hypercholesterolemia. These investigators concluded that L-arginine improved endothelial dysfunction at the level of resistance vessels by increasing the production of endothelium-derived nitric oxide. Furthermore, stimulation of endogenous nitric oxide production might provide further evidence that the nitric oxide pathway is intact in diseased segments and at stenoses, the luminal diameter reached the maximum capacity of the contractile mechanism to relax in response to direct stimulation by glyceryl trinitrate. It provides further evidence that the nitric oxide pathway is intact in those diseased arteries.

**CONCLUSIONS**

Both substance P and nitric oxide synthesize substrate, L-arginine, dilate epicardial coronary arteries in patients with risk factors and normal coronary angiograms and in patients with coronary artery disease. This suggests that the provision of substrate increases the basal activity of nitric oxide synthesize in human coronary arteries. The magnitude of the response to substance P was not increased by L-arginine administration, indicating that it is not critically dependent on the availability of substrate for nitric oxide synthesis.

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