CARDIOVASCULAR MEDICINE

Effects of L-arginine on flow mediated dilatation induced by atrial pacing in diseased epicardial coronary arteries

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Accepted 2 October 2002

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Objective: To examine the effects of L-arginine on basal coronary tone and flow mediated dilatation induced by atrial pacing in patients with coronary artery disease and stable angina.
Design: Atrial pacing was performed during intracoronary infusions of normal saline and L-arginine (150 μmol/min) in 8 patients with coronary artery disease and stable angina. The luminal diameter of epicardial coronary arteries was assessed by quantitative angiography.
Results: L-Arginine administration significantly increased the diameter of all the coronary segments and stenoses. During atrial pacing with saline infusion, luminal diameter of the proximal, distal, and stenosis reference segments increased significantly ($p < 0.01$ versus saline) but stenosis diameter did not change. L-Arginine administration did not change the magnitude (NS) of atrial pacing induced dilatation in proximal and distal segments and in coronary stenoses and their reference segments.
Conclusions: Non-stenotic segments of diseased coronary arteries dilate in response to atrial pacing but stenoses do not. L-Arginine dilates coronary segments and stenoses but does not increase the magnitude of the response to atrial pacing in proximal and distal segments and in coronary stenoses and their reference segments. These findings provide evidence that the shear stress responsive mechanism is absent at stenoses but present in non-stenotic segments of diseased coronary arteries. They also indicate a relative deficiency of L-arginine, except in the shear response mechanism.

N itric oxide, a major component of endothelium derived relaxing factors, is synthesised from the amino acid L-arginine by a family of enzymes through the L-arginine-nitric oxide pathway.[1–4] This synthesis of vascular endothelium is responsible for the vasodilator tone that is essential for the regulation of blood flow. L-Arginine is the substrate for nitric oxide synthesis, and previous studies showed that intra-arterial infusion of L-arginine caused forearm vasodilatation, augmented the endothelium dependent forearm vasodilatation, and reversed the defective endothelium dependent vasodilatation caused by low density lipoprotein or hypercholesterolaemia.[5–8]

During exercise and atrial pacing coronary blood flow increases as a result of the microvascular dilatation that is associated with increased myocardial work.[9–12] This microvascular dilatation is probably caused by release of metabolites from the myocardium, and adenosine has been proposed as one possible mediator.[13–15] Whether nitric oxide production is involved is unknown. The L-arginine-nitric oxide pathway is involved in the dilator response of normal epicardial coronary arteries to flow increase consequent upon the microvascular dilatation associated with increased myocardial work. However, there is little information about the effects of coronary disease on L-arginine mediated coronary vasomotor changes in humans.

We have therefore measured epicardial coronary artery diameter during atrial pacing with and without intracoronary L-arginine infusion in patients with chronic stable angina and coronary artery disease.

METHODS

Study population

Eight patients (seven men, one woman, mean (SD) age 57 (9) years) with chronic stable angina, coronary artery disease, and a positive treadmill exercise test result ($\geqslant 0.1$ mV ST segment depression at between 5–7 metabolic equivalents (METS) using the modified Bruce protocol) were studied. Patients were excluded from the study if they had diabetes mellitus, a history of coronary spasm, recent myocardial infarction (< 6 months), left ventricular hypertrophy (on echocardiography), three vessel coronary artery disease, left ventricular dysfunction (left ventricular ejection fraction $< 50\%$), or valvar heart disease. The protocol was approved by the research ethics committee of Hippokration Hospital, Athens University, and each patient gave written and informed consent.

Study protocol

Antianginal medication was stopped 24 hours before the study. The patients were allowed to use sublingual glyceryl trinitrate as necessary but not within three hours of study. Following the diagnostic coronary angiogram, an optimal radiographic projection was selected and kept constant for subsequent angiograms. The artery studied was chosen to comply with the research ethics committee's requirements that coronary stenoses $> 70\%$ be avoided. Eighteen stenoses were examined.

Two ECG leads were monitored continuously throughout the study. All infusions were administered into the coronary artery through an 8 French gauge Judkin angioplasty guiding catheter. All patients received a single five minute infusion of 0.9% saline (2 ml/min by syringe pump), during which atrial pacing at 100 beats/min was initiated after two minutes and increased to 120 beats/minute after three minutes and to 140 beats/min after four minutes. When all haemodynamic parameters had returned to baseline levels (five minutes, on average), a 10 minute infusion of 150 μmol/min L-arginine was administered in saline (2 ml/min by syringe pump), during which pacing at 100 beats/min was initiated after seven minutes increased to 120 beats/min after eight minutes and to 140 beats/min after nine minutes (fig 1). Finally, an intracoronary bolus dose of glyceryl trinitrate (250 μg in 2 ml of saline) was administered. Femoral arterial pressure and heart rate were recorded before pacing and during each pacing increment, during L-arginine infusion alone, and two minutes
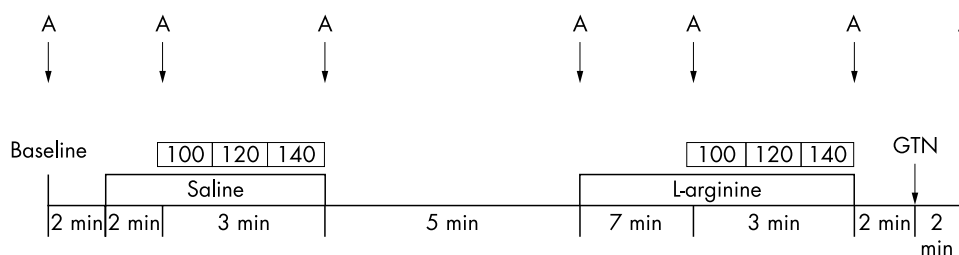
Figure 1 Flow diagram illustrating the study protocol. A, angiogram; GTN, glyceryl trinitrate.

after glyceryl trinitrate administration. Angiography was performed with a hand injection of 6–8 ml non-ionic contrast medium at baseline in different phases of the study, as indicated in fig 1. A maximum pacing rate of 140 beats/min was achieved in each patient without the development of second degree atrioventricular block. Before each angiogram, the catheter was emptied to avoid bolus administration of the infusate.

Quantitative coronary angiography

The arterial segments in each frame were analysed blindly in random order using quantitative computerised analysis with an automated edge contour detection analysis system (CAAS, version 2V2, Pie Data Medical, Maastricht, The Netherlands).[16][17] End diastolic frames from each arteriogram were selected for analysis. The angiographic catheter was used as a scaling device and this, together with pincushion distortion correction, allowed the diameters to be recorded as absolute values (expressed in millimetres). Recorded variables at baseline, during saline infusion, during saline infusion with pacing, during L-arginine infusion, during L-arginine infusion with pacing, and after nitrate administration were as follows:

- the diameter of angiographically normal proximal and distal segments: 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 origin of the second obtuse marginal branch; the proximal right coronary artery diameter was measured just beyond the origin of the artery and the distal diameter just beyond the origin of the posterior descending branch

- the minimum luminal diameter at the site of coronary stenosis

- the diameter of the angiographically normal adjacent segment.

Two proximal segments (one in the left circumflex coronary artery, another in the left anterior descending coronary artery) and at least two distal segments were selected for analysis from each left coronary arteriogram.

Coronary arteriograms were quantitatively analysed 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 $F = 0.3$, p = 0.75).

Statistical analysis

Data are expressed as mean (SEM). Analysis of variance and the Scheffé F test for repeated measures were used to compare serial changes in heart rate and blood pressure and in the diameter of coronary stenoses. To test for differences in the response of proximal and distal segments to atrial pacing, with and without L-arginine, a two way analysis of variance for repeated measures was applied. Associations between responses to atrial pacing and to L-arginine were assessed by performing linear regression analysis and calculating a correlation coefficient. Student's t test was used to compare paired and unpaired data between groups, and the responses to glyceryl trinitrate and to L-arginine. A probability value of p < 0.05 (two tailed) was considered to indicate significance.

RESULTS
Haemodynamic measurements

Systolic blood pressure was 156 (12), 155 (12), 158 (13), 152 (12), and 157 (11) mm Hg during baseline, saline, atrial pacing with saline, L-arginine, and atrial pacing with L-arginine, respectively (NS). Heart rate was 71.1 (1.2), 71.0 (1.2), and 69.7 (2.1) beats/min during baseline, saline, and L-arginine (NS), and 138.4 (0.8) and 138 (0.6) beats/min during atrial pacing with saline and L-arginine, respectively (NS).

Changes in epicardial coronary arteries and stenoses

There was no significant change in the luminal diameter of the proximal and distal segments, or in coronary stenoses and their reference segment with saline infusion (table 1). The luminal diameter of the proximal (p < 0.01) and distal (p < 0.01) segments and of the stenosis reference segment (p < 0.01) increased significantly during atrial pacing and saline infusion but did not change at the site of stenosis (table 1). There was a significant (p < 0.01) increase in the luminal diameter of proximal and distal segments and of stenoses and their reference segments with L-arginine infusion before pacing (table 1). All segments and stenoses dilated (p < 0.01) after nitrates (table 1). Distal segments dilated by a greater percentage than proximal segments (p < 0.01) in response to atrial pacing with normal saline or L-arginine and in response

Table 1 Reactivity of proximal and distal segments, and coronary stenoses and their reference segments to intracoronary administration of saline and L-arginine before and during atrial pacing and after nitrates

Segment		Saline	Saline + pacing	Baseline (2)	L-Arginine	L-Arginine + pacing	Nitrates
Proximal	Minimum luminal diameter (mm)	3.37 (0.16)	3.46 (0.16)	3.36 (0.16)	3.53 (0.18)	3.59 (0.2)	3.7 (0.2)
	Change from baseline (%)	0.6 (0.3)	3.4 (0.8)*		4.7 (1.1)*	6.9 (1.5)	10.2 (1.6)*
Distal	Minimum luminal diameter (mm)	1.56 (0.08)	1.67 (0.08)	1.55 (0.08)	1.64 (0.07)	1.73 (0.09)	1.82 (0.08)
	Change from baseline (%)	1.8 (0.08)	8.5 (1.6)*†		6.4 (1.4)*	13.1 (2.3)	17.9 (2.0)*†
Reference segment	Minimum luminal diameter (mm)	3.26 (0.15)	3.42 (0.17)	3.29 (0.16)	3.49 (0.17)	3.62 (0.20)	3.63 (0.20)
	Change from baseline (%)	0.5 (0.5)	5.0 (1.2)*		6.3 (1.6)*	11.2 (2.3)	11.2 (2.5)*
Stenoses	Minimum luminal diameter (mm)	1.50 (0.16)	1.51 (0.18)	1.49 (0.16)	1.61 (0.17)	1.63 (0.17)	1.69 (0.18)
	Change from baseline (%)	1.5 (0.3)	0.5 (1.5)‡		8.7 (2.0)*	10.6 (2.2)	15.3 (3.8)*

Data are mean (SEM). *p<0.01 v saline; †p<0.01 v proximal, ‡p=NS.
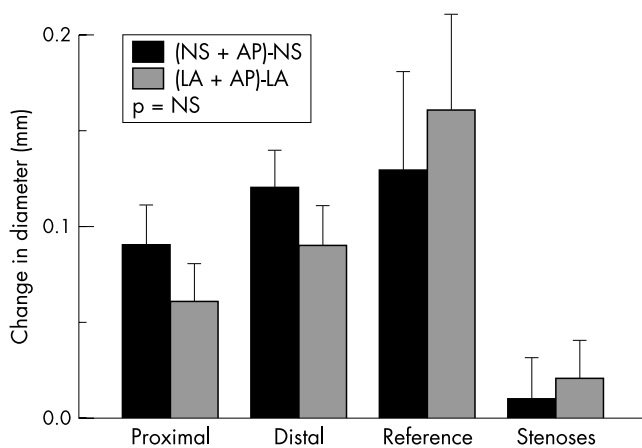
Figure 2 Bar graph showing mean (SEM) change in millimetres of atrial pacing (AP) during saline infusion from normal saline (NS) ((NS+AP)–NS) or atrial pacing during L-arginine (LA) infusion from LA ((LA+AP)–LA) in proximal and distal segments and coronary stenoses and their reference segment. LA administration did not change (not significant) the magnitude of AP induced dilatation in epicardial segments and stenoses.
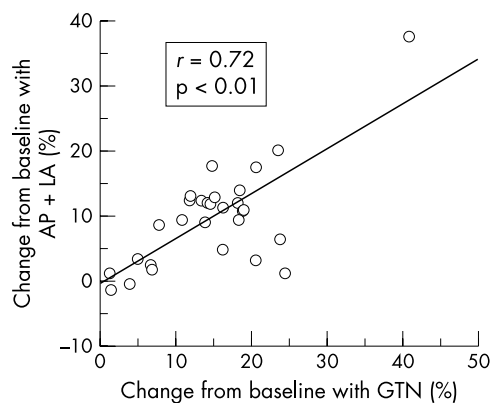
Figure 3 Correlation between glyceryl trinitrate (GTN) and percentage diameter change to L-arginine (LA) after atrial pacing (AP) in epicardial coronary segments. Linear regression analysis shows a significant correlation between these two variables ($r = 0.72$, $p < 0.001$).

to nitrates (table 1). L-Arginine administration did not change the average magnitude of atrial pacing induced dilatation in proximal and distal segments and in coronary stenoses and their reference segments (fig 2). A significant correlation was found between the responses to glyceryl trinitrate and atrial pacing during L-arginine administration (fig 3).

DISCUSSION

In this study we examined the effects of saline and L-arginine infusions with and without atrial pacing in patients with coronary artery disease. The results show that coronary stenoses dilated in response to L-arginine but not in response to pacing. The results indicate that angiographically normal proximal and distal segments of diseased epicardial coronary arteries are shear stress responsive, as they dilate in response to atrial pacing, and are not substrate deficient, as the response is not increased by L-arginine administration.

Flow mediated dilatation and endothelial function

Physical exercise induces an increase in both cardiac sympathetic nerve activity and circulating catecholamine concentrations.[18–20] The resulting increase in heart rate and contractility increases myocardial oxygen demand and induces metabolic dilatation of small resistance coronary arteries to augment blood flow and oxygen supply to satisfy this

increased demand.[13] Large epicardial arteries simultaneously dilate, and previous studies[12 21–23] have suggested that this dilatation of the large epicardial coronary arteries is a consequence of the microvascular dilatation. Its mechanism is probably release of endothelium derived relaxing factors caused by increased shear stress related to increased blood flow velocity. Previous studies in vitro and in vivo in animals[11 24–26] have shown that flow mediated dilatation is abolished by removing the endothelium of epicardial arteries. It has been shown that the epicardial coronary artery dilatation in response to the increase in blood flow after temporary coronary artery occlusion is mediated through an endothelium dependent mechanism.[12 27] In a previous study we have shown that epicardial coronary artery dilatation induced by atrial pacing is nitric oxide dependent.[28] In the present study the luminal diameter of the proximal and distal segments and stenosis reference segment increased significantly during atrial pacing and saline infusion but did not change at the site of stenosis.

L-Arginine–nitric oxide pathway in atheromatous coronary arteries

It has been suggested that diseased arteries may be deficient in the substrate L-arginine.[29–32] The substrate deficiency hypothesis is supported by experimental evidence in hypercholesterolaemic rabbits that arginine administration restores cholinergic (nitric oxide dependent) relaxation of thoracic aorta,[33] as well as by clinical studies that show correction of endothelial dysfunction by L-arginine in the coronary microcirculation of hypercholesterolaemic patients and in patients with chest pain and normal coronary arteries.[34 35] Evidence against the hypothesis was obtained by Hirooka and colleagues,[5] 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 L-arginine. By contrast, Drexler and associates[34] found no effects in epicardial coronary arteries but did find a significant increase in blood flow response to acetylcholine in patients with hypercholesterolaemia. In the present study we found that the coronary stenoses dilated in response to L-arginine but did not dilate in response to pacing. Moreover, L-arginine significantly increased the diameter of all the coronary segments. These findings are consistent with a relative deficiency of L-arginine in diseased epicardial coronary arteries. However, the finding that L-arginine did not increase pacing induced dilatation in non-stenotic epicardial coronary artery segments indicates that the substrate concentration is adequate in cells involved in the shear stress response. In response to L-arginine distal segments dilated more that proximal segments, which may due to different local endothelial function.[36]

The possible mechanisms by which L-arginine may cause dilatation in epicardial coronary arteries are as follows: firstly, by reversing inhibitory effects of L-glutamine[37]; secondly, by counteracting the inhibitory effects of asymmetrical dimethyl-L-arginine, a naturally occurring inhibitor of L-arginine[38 39]; thirdly, by its antioxidant actions[40]; fourthly, by stimulating insulin release[41 42]; and lastly, by generating nitric oxide non-enzymatically and non-stereospecifically.[43 44]

A limitation of the present study is that the number of patients is rather small. However, the number of analysed segment is sufficient to draw conclusions. Moreover, the analysed patients were not matched for risk factors, which may have influenced the results.

Conclusions

Non-stenotic segments of diseased coronary arteries dilate in response to atrial pacing but stenoses do not. L-Arginine dilates these segments and stenoses but does not increase the magnitude of the response to atrial pacing. These findings

provide evidence that the shear stress responsive mechanism is absent at stenoses but present in non-stenotic segments of diseased coronary arteries. Furthermore, they provide evidence that the arteries are relatively deficient in L-arginine, except for the shear response mechanism.

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