Influence of basal nitric oxide secretion on cardiac function in man

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- 1 Nitric oxide is recognised as an important biological mediator, which is thought to be involved in cardiovascular homeostasis. The purpose of this study was to investigate the effects of basal nitric oxide synthesis on cardiac function in man, by blocking nitric oxide synthesis with N^G -monomethyl-L-arginine (L-NMMA).
- 2 Eight normal volunteers were studied on two separate occasions. Measurements of heart rate, blood pressure and echocardiographic indices of left ventricular systolic and diastolic function were made at baseline on each day and every 20 min during incremental infusion of L-NMMA (0.1, 0.2, 0.5, 1.0 and 2.0 mg kg⁻¹ h⁻¹) or placebo.
- 3 A trend towards reduction in heart rate was observed with L-NMMA infusion although this did not reach statistical significance, whereas significant increases in both systolic blood pressure (at 2.0 mg kg⁻¹ h⁻¹) and systemic vascular resistance index (at 0.5 mg kg⁻¹ h⁻¹) were seen.
- 4 L-NMMA infusion caused significant reductions in stroke distance and cardiac index, although there was no change in the ratio of end systolic wall stress/end systolic volume index (an afterload independent index of left ventricular systolic performance).
- 5 The isovolumic relaxation time significantly increased with L-NMMA infusion, together with a significant reduction in the 'E' wave flow velocity integral. Reductions in both peak E/A ratio and E/A flow velocity integral ratio were also seen, although these failed to reach statistical significance.
- 6 In conclusion, the basal generation of nitric oxide in man appears to maintain a vasodilated state, and modifies left ventricular diastolic filling parameters.

Keywords nitric oxide left ventricle systole diastole

Introduction

Nitric oxide (NO) is a short lived gas which is now recognised as an important biological mediator. It influences a variety of cellular functions, and affects diverse physiological processes such as arterial vasodilatation [1], neurotransmission [1] and platelet aggregation [2, 3]. NO is synthesised from the guanidino nitrogen of the amino acid L-arginine, this reaction being controlled by the enzyme nitric oxide synthase [4]. Naturally occurring analogues of L-arginine such as N^G -monomethyl-L-arginine [5] (L-NMMA) are competitive inhibitors of nitric oxide synthase [6], and have been demonstrated to produce

systemic vasoconstriction in normal man [7]. Recent evidence suggests that the endocardium modulates the performance of the subjacent myocardium through the release of mediator substances [8, 9] which have effects on both myocardial contraction and relaxation [10]. Although many of these substances are, as yet, unidentified [11] nitric oxide has been shown, in animal models, both to be released from the endocardium, and also to modulate the contractile behaviour of the heart [12]. In man, the NO donor substance sodium nitroprusside has been demonstrated to accelerate left ventricular relaxation, and

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also improve left ventricular diastolic distensibility [13]. However, the effects of basal NO synthesis on cardiac function in man are not known. Therefore we investigated the effects of systemic inhibition of nitric oxide synthesis on cardiac performance using intravenous administration of L-NMMA.

Methods

Subjects

Eight normotensive healthy male volunteers, mean age (s.d.) 22(3) years were studied. Each provided written informed consent, and the study was approved by the Tayside Committee on Medical Research Ethics. Normal health was confirmed by clinical history, physical examination, biochemical and haematological screen, 12 lead electrocardiogram, and echocardiography. All subjects had a high-quality echocardiograph with good apical and parasternal views with high-definition transmitral pulsed-wave Doppler flow velocity profiles as entry criteria.

Protocol

Subjects were studied on two separate days, at least 1 week apart, in a placebo-controlled, randomised, double-blind cross-over fashion. Subjects were asked to refrain from alcohol, caffeine and cigarettes for 24 h and to fast for 2 h before each study day. No restriction on salt intake was imposed, although subjects were asked to make no dietary changes during the period of the study. On both study days subjects reported to the research laboratory, and after voiding urine remained supine and inactive for the entire study period. No food or fluids were consumed until the study was completed. One 18G intravenous cannula was inserted into a forearm vein for infusion of either L-NMMA or placebo.

After 1 h of supine bed rest baseline triplicate blood pressure and heart rate measurements were taken using a semiautomatic sphygmomanometer (Dinamap Vital Signs Monitor 1846; Critikon, Tampa, FL, USA) with the cuff being placed around the subjects right arm. Baseline echocardiographic measurements of diastolic filling parameters, systolic function, and M-mode measurements of cardiac dimensions were also taken at this time.

Following baseline measurements, a continuous infusion of either placebo (0.9% (w.v) saline) or L-NMMA (Clinalfa, Laufelfingen, Switzerland) in a similar volume of 0.9% saline was commenced. L-NMMA was given stepwise in increments of 0.1, 0.2, 0.5, 1.0 and 2.0 mg kg⁻¹ h⁻¹ for 20 min at each increment. Repeat measurements of echocardiographic parameters, heart rate and blood pressure were taken during the last 5 min of each dose increment.

Venous blood (10 ml) was withdrawn for plasma endothelin concentrations at baseline, and at maximal infusion rate of L-NMMA or placebo.

Echocardiography

All echocardiographic examinations were performed using a Hewlett-Packard 77020 AC phased array scanner with a 2.5 MHz transducer for the pulsed wave Doppler and the M-mode studies, and a 1.9 MHz non-imaging continuous-wave Doppler probe for measurement of aortic stroke distance. Phonocardiography was performed simultaneously, the position of the phonocardiograph being adjusted to obtain a high quality recording of aortic valve closure. All subjects were studied in the supine position rotated onto the left side and recordings were taken during quiet respiration. All signals were recorded with a simultaneous ECG lead on videotape, and pulsed-wave Doppler recordings were also printed on light-sensitive paper at a speed of 100 mm s⁻¹. For each variable the mean of three beats was analysed.

Using the apical 4 chamber view the Doppler sample volume was placed in the inflow area of the left ventricle between the tips of the mitral valve leaflets and its position adjusted until maximum Doppler flow velocities were recorded. No angle correction was used. From the paper recording the isovolumic relaxation time (IVRT)—the time interval between the first high frequency aortic closure sound and the onset of transmitral flow, the peak early filling velocity ('E' wave) and peak atrial systolic velocity ('A' wave) were measured. Doppler signals were traced using an analysis package built into the echocardiographic unit from which the time velocity integrals of the E and A waves were calculated. The ratios of the peak velocities of the E and A waves (E/A peak), and the ratio of the time velocity integrals of the E and A waves (E/A FVI), were derived from these measurements.

An aortic flow velocity profile was obtained from the suprasternal position and recorded on videotape. Measurement of the time velocity integral (stroke distance) was derived by tracing the Doppler signals in identical fashion to transmitral flow patterns.

To obtain values for stroke volume (SV) we multiplied stroke distance by aortic valve area (determined using M-mode echocardiography at the onset of each study). Cardiac index (CI) was derived by the following equation: $CI = SV \times HR/BSA$ where HR is heart rate and BSA body surface area. To derive information about afterload we calculated systemic vascular resistance index (SVRI) from the following equation: $SVRI = (MAP/CI) \times 80$ where MAP is mean arterial pressure.

To document potential changes in left ventricular size and function posterior wall thickness (PWT) and left ventricular internal diameter (LVID) at end-diastole (d) and end-systole(s) were recorded in the parasternal short axis view at the level of the tips of the mitral valve leaflets. All measurements were taken in accordance with the Penn convention [14]. Left ventricular end systolic volume index was estimated by the cube function formula [15], the accuracy of this method in normal subjects having previously been described [16].

To obtain further information about left ventricular contractile performance (in addition to stroke volume and cardiac index) left ventricular fractional shortening was derived from the formula $(\text{LVIDd}-\text{LVISs})/\text{LVIDd}$.

Since the above measurements are all dependent on afterload, ventricular performance was estimated at the operating level of systolic wall stress. Meridional end-systolic wall stress was calculated as follows: $(0.334 \times \text{SBP} \times \text{LVIDs})/(\text{PWTs} \times \{1 + \text{PWT}/\text{LVIDs}\})$ [17] where systolic blood pressure (SBP) is considered representative of left ventricular end-systolic pressure. Myocardial contractile performance was then estimated by calculating the ratio of end systolic wall stress to end-systolic left ventricular volume index [18, 19].

Plasma endothelin assay

A 10 ml aliquot of venous blood was collected into a chilled tube for measurement of endothelin. All samples were placed on ice, centrifuged at 4°C and frozen at 20°C until assayed. Samples were measured in a single batch after completion of the study. Assay was performed using a commercially available radioimmunoassay (r.i.a.) kit (Nicholls Institute Diagnostics, San Juan Capistrano, LA, USA) after solid phase extraction on C18 Silica columns. The lower limit of detection was 0.4 pmol l⁻¹ and the intra-assay coefficient of variation was 4.5%. The anti-endothelin antibody was 100% specific for endothelin-1, had 96% cross reactivity with endothelin-3, and 7% cross-reactivity with proendothelin.

Statistical analysis

All data were analysed using the Statgraphics Software package (STSC Software Publishing group, Rockville MD, USA). Analysis of variance repeated at each dose increment, using subject and treatment as within factors, and Bonferroni multiple range tests were performed to determine the significance of L-NMMA-induced changes in haemodynamic and echocardiographic indices, taking significance at the $P < 0.05$ level. Results are described in the tables as mean differences compared with placebo, and the associated 95% confidence limits, and in the figures as mean changes from baseline ($\pm$ s.e. mean).

Results

Baseline parameters (Table 1)

No significant differences in baseline measurements were observed on the L-NMMA study days compared with placebo, except for an increase in the 'E' flow velocity integral of the L-NMMA study day.

Haemodynamic measurements (Table 2, Figure 1)

Infusion of L-NMMA caused a significant increase in systemic vascular resistance at infusion rates above 0.5 mg kg⁻¹ h⁻¹. However, no changes in blood pressure were seen until infusion rates of 2 mg kg⁻¹ h⁻¹ were reached, when there was a significant increase

Table 1 Baseline values

	Placebo	L-NMMA
Heart rate (beats min ⁻¹)	63.5 $\pm$ 3.5	62.1 $\pm$ 2.7
Systolic blood pressure (mm Hg)	113 $\pm$ 3	115 $\pm$ 2
Diastolic blood pressure (mm Hg)	68.4 $\pm$ 3.1	65.9 $\pm$ 2.1
Mean arterial pressure (mm Hg)	85.5 $\pm$ 2.7	86.1 $\pm$ 2.3
Systemic vascular resistance index (dyn s cm ⁻⁵ m ²)	1747 $\pm$ 133	1806 $\pm$ 135
Aortic stroke distance (cm)	19.3 $\pm$ 1.3	19.4 $\pm$ 1.7
Cardiac index (l min ⁻¹ m ²)	4.02 $\pm$ 0.22	3.94 $\pm$ 0.28
IVRT (ms)	64.3 $\pm$ 2.6	66.7 $\pm$ 2.1
Peak E velocity (cm s ⁻¹)	72.6 $\pm$ 4.4	77.2 $\pm$ 3.0
Peak A velocity (cm s ⁻¹)	34.8 $\pm$ 3.5	31.8 $\pm$ 2.1
Peak E/A ratio	2.25 $\pm$ 0.29	2.54 $\pm$ 0.27
E Flow velocity integral (cm)	11.5 $\pm$ 0.4	12.4 $\pm$ 0.4*
A Flow velocity integral (cm)	3.47 $\pm$ 0.38	2.83 $\pm$ 0.16
E/A Flow velocity integral ratio	3.71 $\pm$ 0.54	4.49 $\pm$ 0.29
Left ventricular diastolic internal diameter (cm)	4.90 $\pm$ 0.10	5.05 $\pm$ 0.10
Left ventricular systolic internal diameter (cm)	3.31 $\pm$ 0.10	3.38 $\pm$ 0.09
% Fractional shortening	32.6 $\pm$ 1.3	33.1 $\pm$ 0.9
End-systolic wall stress (10 ³ dyn cm ⁻²)	51.3 $\pm$ 4.7	55.8 $\pm$ 4.9
ESWS/ESVI Index (10 ³ dyn cm ⁻² /(ml m ⁻²))	2.6 $\pm$ 0.2	2.7 $\pm$ 0.1
Plasma endothelin concentration (pmol l ⁻¹)	1.6 $\pm$ 0.2	1.9 $\pm$ 0.2

Results are expressed as means $\pm$ s.e. mean.

Statistical significance * $P < 0.05$ compared with placebo.

Table 2 Changes in haemodynamics with L-NMMA infusion compared with placebo

	Infusion rate (mg kg ⁻¹ h ⁻¹)				
	0.1	0.2	0.5	1.0	2.0
Heart rate (beats min ⁻¹)	0.5 ± 6.5	3.4 ± 5.7	-1.8 ± 3.6	-0.8 ± 6.1	-2.0 ± 5.1
Systolic blood pressure (mm Hg)	-1.4 ± 4.8	-1.9 ± 6.0	-0.3 ± 5.5	-1.1 ± 3.1	2.8 ± 2.8*
Diastolic blood pressure (mm Hg)	-4.2 ± 4.4	-1.8 ± 3.4	-0.1 ± 3.4	0.0 ± 3.7	2.1 ± 3.4
Mean arterial pressure (mm Hg)	-1.8 ± 3.6	-2.2 ± 2.9	0.6 ± 3.9	1.0 ± 3.5	1.8 ± 3.1
Systemic vascular resistance index (dyn s cm ⁻⁵ m ²)	144 ± 299	28 ± 396	319 ± 265*	308 ± 266*	416 ± 211**

Values are means ± 95% confidence intervals for the difference compared with placebo.

Statistical significance **P* < 0.05, ***P* < 0.01.

Figure 1 Changes in systemic vascular resistance index (SVRI) with L-NMMA infusion (●) compared with placebo (○). Infusion rate increasing in increments of 0.1, 0.2, 0.5, 1.0 and 2.0 mg kg⁻¹ h⁻¹. Results are expressed as mean ± s.e. mean. **P* < 0.05, ***P* < 0.01.

in systolic blood pressure. A trend towards reduction in heart rate was observed, although this did not reach statistical significance.

Systolic and diastolic function (Tables 3 and 4, Figures 2, 3 and 4)

A decrease in both stroke distance and cardiac index was observed, at infusion rates of 1.0, and 0.5 mg kg⁻¹ h⁻¹ respectively. However, no significant changes in M-mode cardiac dimensions were seen. Although a trend towards increased end systolic wall stress was seen at L-NMMA infusion rates of 1.0 mg kg⁻¹ h⁻¹ and above the ratio of ESWS/ESVI remained constant throughout.

IVRT significantly increased during L-NMMA infusion at infusion rates of 1.0 mg kg⁻¹ h⁻¹ and above. 'E' flow velocity integral decreased with L-NMMA infusion, accompanied by a similar reduction in peak E velocity although the latter did not reach statistical significance. Both 'A' flow velocity integral and peak 'A' velocity remained constant throughout. As a result strong trends were seen to both decreased E/A flow velocity integral ratio, and peak E/A velocity.

Plasma endothelin

There was no significant difference in plasma endothelin concentrations comparing L-NMMA infusion with placebo (L-NMMA 2.7 ± 0.4 pmol l⁻¹; placebo 2.7 ± 0.4 pmol l⁻¹).

Discussion

The principal findings in this study were that infusion of L-NMMA caused increases in blood pressure, systemic vascular resistance, a decrease in cardiac output, and a prolongation of isovolumic relaxation.

The increases in blood pressure and systemic vascular resistance are similar to those demonstrated in the study of Haynes and colleagues [7], and suggest that basal generation of nitric oxide in normal man helps maintain a vasodilated state.

The reduction in cardiac output observed probably relates to the increase in afterload, as opposed to a reduction cardiac inotropy. This is supported by two findings (a) the lack of change in the ratio of end-systolic wall stress to end-systolic volume ratio—an index of contractile function which is largely independent of afterload, and (b) that Brady *et al.* demonstrated that, *in vitro*, nitric oxide is actually negatively inotropic [20]. The lack of associated changes in cardiac dimensions is not, altogether surprising since M-mode echocardiography is only a 1-dimensional measurement of a 3-dimensional structure, and therefore any volume changes would have to be large to be detectable with accuracy on the M-mode recordings.

L-NMMA caused a significant prolongation of the isovolumic relaxation time, reduction in the 'E' flow velocity integral and a reduction in peak E/A ratio and E/A flow velocity integral (although these did not reach significance due to the broad confidence intervals associated with these measurements). These changes are similar to those found in instances of impaired myocardial relaxation [21, 22], and suggest that L-NMMA caused alterations in diastolic function—primarily through impairment of myocardial relaxation (a negative lusitropic effect). Doppler echocardiography, although being a well established

Table 3 Changes in systolic function with L-NMMA infusion compared with placebo

	Infusion rate (mg kg ⁻¹ h ⁻¹)				
	0.1	0.2	0.5	1.0	2.0
Stroke distance (cm)	-1.8 ± 2.9	-1.3 ± 2.3	-2.2 ± 2.4	-2.6 ± 1.7**	-2.3 ± 2.0*
Cardiac index (l min ⁻¹ m ²)	-0.44 ± 0.64	-0.10 ± 0.77	-0.62 ± 0.52*	-0.63 ± 0.67	-0.65 ± 0.61*
LVIDd (cm)	-0.1 ± 0.17	-0.1 ± 0.19	0.0 ± 0.35	0.02 ± 0.37	0.06 ± 0.25
LVIDs (cm)	0.0 ± 0.27	-0.01 ± 0.38	-0.16 ± 0.45	0.0 ± 0.30	0.07 ± 0.26
% Fractional shortening	-1.2 ± 5.0	-1.0 ± 7.2	3.7 ± 6.9	0.5 ± 5.9	-0.5 ± 3.3
End systolic wall stress (10 ³ dyn cm ⁻²)	1.5 ± 7.0	1.8 ± 12.2	-5.7 ± 13.7	4.2 ± 9.5	4.6 ± 11.2
ESWS/ESVI (10 ³ dyn cm ⁻² /(ml m ⁻²))	0.04 ± 0.48	0.0 ± 0.55	-0.01 ± 0.45	0.15 ± 0.35	-0.06 ± 0.28

Table 4 Changes in diastolic function with L-NMMA infusion compared with placebo

	Infusion rate (mg kg ⁻¹ h ⁻¹)				
	0.1	0.2	0.5	1.0	2.0
Isovolumic relaxation (ms)	-1.7 ± 3.2	-1.2 ± 1.9	-1.7 ± 5.7	7.1 ± 4.7**	6.0 ± 5.7*
Peak E velocity (cm s ⁻¹)	6.5 ± 4.8*	-3.5 ± 6.1	-0.5 ± 6.6	-3.4 ± 5.0	-5.4 ± 9.9
Peak A velocity (cm s ⁻¹)	1.3 ± 8.2	2.4 ± 9.0	2.7 ± 7.9	2.9 ± 9.2	-1.6 ± 6.6
Peak E/A ratio	0.21 ± 0.79	0.20 ± 0.79	-0.08 ± 0.51	-0.08 ± 0.75	-0.25 ± 0.57
'E' flow velocity integral (cm)	0.0 ± 1.7	-0.6 ± 2.1	0.0 ± 1.3	-0.3 ± 1.1	-1.7 ± 1.6*
'A' flow velocity integral (cm)	0.4 ± 0.7	0.4 ± 1.1	0.6 ± 1.1	0.6 ± 1.1	0.5 ± 0.9
E/A flow velocity integral ratio	-0.25 ± 1.07	-0.56 ± 1.52	0.01 ± 1.25	-0.68 ± 1.37	-0.98 ± 1.28

Figure 2 Changes in cardiac index with L-NMMA infusion (●) compared with placebo (○). Means ± s.e. mean. **P* < 0.05. **Figure 3** Changes in the ratio of end systolic wall stress/end systolic volume index (ESWS/ESVI—an afterload independent measure of contractile performance) with L-NMMA infusion (●) compared with placebo (○). Means ± s.e. mean.

and validated technique for the assessment of diastolic function [23–25], is an indirect method which can also be affected by heart rate and loading conditions. Only a small, non-significant reduction in heart rate was observed with L-NMMA compared with placebo. Preload was not directly measured in this study. A reduction in preload can mimic the changes of impaired myocardial relaxation [26, 27]. However, this is unlikely to have occurred since nitrates, and nitroprusside (nitric oxide donors) reduce preload in

normal man [13, 26], and L-NMMA itself has been demonstrated not to affect pulmonary capillary wedge pressure in a group of patients with suspected heart failure [28]. L-NMMA infusion is therefore likely to have either caused no preload changes, or a preload increase, although to our knowledge the effects of L-NMMA on left atrial pressure in normal subjects have not been measured.

Figure 4 Changes in isovolumic relaxation time (IVRT) with L-NMMA (●) compared with placebo (○). Means $\pm$ s.e. mean. * $P < 0.05$, ** $P < 0.01$.

Previous studies assessing the effect of afterload on transmitral Doppler flow profiles have been hampered by methodological difficulties involving reflex neurohormonal and sympathetic activation. The general consensus, however, is that increasing afterload decreases the early filling velocity [29]. The isovolumic relaxation time varies much more predictably, changes being directly related to the natural logarithm of aortic closing pressure [30]. Therefore in the present study the changes in diastolic function seen with L-NMMA infusion could be partially attributable to the changes in afterload. However, the magnitude of changes in the IVRT are much greater

than would be anticipated solely from changes in blood pressure. We did not use a control 'vasoconstrictor' arm, since at present there are no known vasoconstrictors which have been demonstrated not to affect diastolic function [31, 32].

The study did not aim to address the mechanisms of action of L-NMMA, and it is possible that the observed changes were mediated through either neurohormonal or reflex sympathetic activation. In animal models inhibition of nitric oxide synthase increases plasma endothelin concentrations [33], which is a potent vasoconstrictor [34], with positive inotropic and negative lusitropic effects [32]. In the present study no changes in plasma endothelin were observed comparing L-NMMA infusion with placebo. However, since this hormone is thought to have a predominantly local action plasma concentrations are only a crude indicator of endothelin activity at the endothelial level, and thus do not exclude that at least some of the effects of L-NMMA infusion may have been mediated indirectly through endothelin.

It is interesting to speculate on the possible role of nitric oxide in certain disease states. In particular decreased generation of nitric oxide has been postulated to occur in essential hypertension [35–37]. The results of the present study suggest that the elevated blood pressure, may, at least be partially attributable to the decreased generation of nitric oxide.

In summary, the present study demonstrates that inhibition of nitric oxide synthase in normal man increases systemic vascular resistance, decreases cardiac output, and alters diastolic filling patterns.

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