Nitric oxide: an important role in the maintenance of systemic and pulmonary vascular tone in man

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Aims The aim of this study was to examine whether nitric oxide (NO) has an important role in maintaining basal vascular tone in normal man by examining the effects of nitric oxide inhibition using N^G -monomethyl-L-arginine (L-NMMA) on systemic and pulmonary haemodynamics.

Methods Ten normal male volunteers 26 ± 1.6 years were studied on two separate occasions in a double-blind, placebo controlled crossover study. They were randomised to receive either a continuous infusion of L-NMMA ($4 \text{ mg kg}^{-1} \text{ h}^{-1}$) with a front loaded bolus (4 mg kg^{-1}) or volume matched placebo. Pulsed wave Doppler echocardiography was used to measure cardiac output (CO), mean pulmonary artery pressure (MPAP) and hence systemic vascular resistance (SVR) and total pulmonary vascular resistance (TPR). Measurements were made prior to infusion (t_0) and after 4, 8, and 12 min (t_1 , t_2 and t_3).

Results Infusion of L-NMMA significantly increased mean arterial blood pressure (MAP), SVR and TPR and significantly reduced heart rate (HR), stroke volume (SV) and CO compared to placebo. These effects were observed at t_1 and persisted during the entire infusion period.

Conclusions These results are consistent with a role for basal nitric oxide generation in the maintenance of basal systemic and pulmonary vascular tone in normal man.

Keywords: nitric oxide, pulmonary circulation, systemic circulation

Introduction

Endothelial function is known to be an important determinant of vascular tone although the physiological roles of the endothelium-derived relaxing and constricting factors and their functional antagonism remain the subject of much ongoing research effort. Much interest surrounds a potent short acting vasodilator, nitric oxide, which is generated by the vascular endothelium through the action of nitric oxide synthase [1]. Studies in animals using systemic infusions of nitric oxide synthase inhibitors such as N^G -monomethyl-L-arginine (L-NMMA) have demonstrated an increase in mean arterial pressure and systemic vascular resistance [2, 3]. Evidence in humans suggesting an important role for nitric oxide generation in the maintenance of systemic vascular tone comes from studies demonstrating that infusion of L-NMMA into the brachial artery results in vasoconstriction [4] and systemic infusions of L-NMMA increase systemic vascular resistance [5].

There is however, some debate as to the role of nitric oxide generation in the maintenance of the low pressure pulmonary circulation where conflicting results exist suggesting the possibility of species specificity [6–10]. Interestingly, studies in animals and humans have suggested that impaired endothelial relaxing activity may contribute to the pathogenesis of pulmonary hypertension [11, 12]. To date only Stamler and his colleagues have examined the effects of

nitric oxide inhibition on pulmonary vascular tone in normal man in a dose ranging study [6]. This study examines for the first time in a placebo controlled manner the effects of nitric oxide inhibition on pulmonary vascular tone in normal man using non-invasive pulsed wave Doppler echocardiography.

Methods

Subjects

Ten healthy, non-obese, male volunteers, age (mean $\pm$ s.e.mean) 26 ± 1.6 years were studied. All had normal clinical history and examination, 12-lead electrocardiogram, echocardiogram and haematological and biochemical screen. Subjects refrained from alcohol, tobacco and caffeine for at least 12 h and had taken no medications for at least 1 month before the study. Informed consent was obtained to the study protocol previously approved by the Tayside Committee for Medical Research Ethics and this investigation conforms with the principles outlined in the Declaration of Helsinki.

Protocol

Subjects were studied at the same time of day on two occasions in a randomized, double blind, cross-over design. Subjects then remained supine, turned slightly on the left side throughout the study. After allowing at least 30 min rest to achieve baseline haemodynamic parameters they

received either a continuous infusion of L-NMMA ($4 \text{ mg kg}^{-1} \text{ h}^{-1}$) with a front loaded bolus (4 mg kg^{-1}) given over 2 min or volume matched placebo (0.9% normal saline). Measurements were made prior to infusion (t_0) and after 4, 8, and 12 min (t_1 , t_2 and t_3).

Measurements

For each Doppler derived parameter, the mean of three consistent waveforms recorded at each time point was used for the purpose of analysis. MAP and HR were measured by semi-automatic sphygmomanometer (Vital Signs Monitor; Critikon, Tampa, FL, USA) and averaged over three sequential readings. To measure CO, aortic cross-sectional area (CSA) was first measured by M-mode echocardiography (Vingmed SD50; Vingmed Sound, Horten, Norway) from the left parasternal view at the level of the aortic root. The aortic systolic flow velocity integral (SVI) was then measured by computer analysis of the pulsed-wave Doppler profile of aortic blood flow from the suprasternal notch. Stroke volume ($\text{SV} = \text{SVI} \times \text{CSA}$) and hence CO, as the product of SV and HR, were then calculated on-line. Measurement of pulmonary flow acceleration time (PAT) by pulsed-wave Doppler echocardiography (Vingmed SD50) from the left 3rd/4th intercostal space allowed calculation of mean pulmonary artery pressure (MPAP) using the regression equation $\text{MPAP} = 73 - (0.42 \times \text{PAT})$ which has been shown to have a good correlation with MPAP measured simultaneously during cardiac catheterisation in patients with pulmonary hypertension in both our own hands [13] and those of other workers [14]. The coefficients of variability (CV) for these Doppler measurements in this study were HR: 12.8%; SV: 18.7%; CO: 14.0%; PAT 2.8%; and MAP: 7.1%.

Data analysis

Comparisons were made between active and placebo treatments by repeated measures analysis of variance (ANOVA). Where the overall ANOVA was significant, Duncan's multiple range testing was used to determine differences at individual time points. A P value of less than 0.05 was considered significant and results are expressed as means $\pm$ s.e.mean and where a difference between means is quoted, the 95% confidence (CI) for this difference is given.

Results

Acute administration of L-NMMA was not associated with any adverse effects during the study. Baseline conditions on each study day were similar for all haemodynamic parameters measured. L-NMMA had significant pressor effects compared with placebo, where both SVR ($P=0.02$); mean difference $479 \text{ dyn s cm}^{-5}$ (95% CI 87–872) and TPR ($P=0.02$); 63 dyn s cm^{-5} (95% CI 15–110) were elevated at t_1 and these effects persisted throughout the entire infusion period (Figure 1). Although MAP was significantly increased during infusion of L-NMMA compared with placebo at t_1 ($P=0.03$); mean difference 5 mmHg (95% CI 1–10) an effect that persisted throughout the entire infusion period, MPAP was unchanged. In addition HR ($P=0.003$); mean difference

Figure 1 Systemic and pulmonary pressor effects of L-NMMA and placebo infusions in normal man. Asterisk (*) indicates significant difference between infusion with L-NMMA and placebo at that time point. Cross (+) indicates significant difference from baseline during L-NMMA infusion.

9 beats min^{-1} (95% CI 5–13) and CO ($P=0.02$); mean difference 1.74 l min^{-1} (95% CI 0.33–3.15) were significantly lower during infusion with L-NMMA compared with placebo at t_1 an effect that persisted throughout the infusion period (Table 1).

Discussion

Our results suggest that basal generation of NO is important in the regulation of both systemic and normoxic pulmonary vascular tone in normal man reflected by an increase in both SVR and TPR during inhibition of nitric oxide synthesis.

The systemic effects of NO inhibition in man are well documented. Our results however suggest that in addition that resting normoxic pulmonary vascular tone is dependent on the continuous generation of nitric oxide. In this respect infusion of L-NMMA resulted in a significant increase in TPR which was sustained throughout the entire infusion period. The lack of effect on MPAP is likely to reflect the coexistent reduction in cardiac output. Although this phenomenon has been shown in some lower mammals [7, 8] it has not been demonstrated in either rat [9] or dog models [10]. The results of this study support the findings of Stamler *et al.* [6] who demonstrated a similar effect during a dose ranging study in normal man. In addition, our results

Table 1 Haemodynamic effects of L-NMMA and placebo infusions in normal man.

		t0	t1	t2	t3
HR	P	71 ± 2	69 ± 3	70 ± 4	67 ± 3
(beats min ⁻¹)	L	68 ± 4	60 ± 4* ⁺	59 ± 3* ⁺	57 ± 3* ⁺
SV	P	102 ± 5	104 ± 6	101 ± 4	111 ± 6
(ml)	L	100 ± 6	95 ± 6 ⁺	91 ± 5 ⁺	90 ± 7* ⁺
CO	P	6.92 ± 0.47	6.91 ± 0.48	6.66 ± 0.44	7.21 ± 0.56
(l min ⁻¹)	L	6.34 ± 0.52	5.17 ± 0.5* ⁺	5.10 ± 0.41* ⁺	5.27 ± 0.52* ⁺
MAP	P	87 ± 3	89 ± 3	89 ± 3	87 ± 3
(mmHg)	L	90 ± 3	94 ± 3* ⁺	95 ± 3 ⁺	94 ± 2* ⁺
MPAP	P	11 ± 1	11 ± 1	12 ± 1	11 ± 1
(mmHg)	L	10 ± 1	12 ± 1	12 ± 2	12 ± 1

Absolute values (mean ± s.e.mean). Asterisk (*) indicates significant difference between infusion with L-NMMA (L) and placebo (P) at that time point. Cross (+) indicates significant difference from baseline during L-NMMA infusion.

suggest that these changes persist throughout a more prolonged infusion period.

We chose to use a dose of L-NMMA based on findings from previous *in vivo* studies conducted in man to ensure a good profile of nitric oxide inhibition over the whole time course of the study and found comparable systemic haemodynamic effects. Previous work by Haynes *et al.* [5] examined the maximal haemodynamic responses to increments in doses of L-NMMA. He examined on separate days doses of L-NMMA in ascending order (0.03, 0.1, 0.3, 1.0, 3.0 mg kg⁻¹) infused over 60 min and then 3 mg kg⁻¹ L-NMMA infused over 20 min then the same dose infused over 5 min via a peripheral cannula. Although, at doses >1 mg kg⁻¹ there was an apparent decrease in heart rate, cardiac index and an increase in peripheral vascular resistance no effect on blood pressure was observed until a dose of 3 mg kg⁻¹ was given over 20 min when there were isolated increases in both systolic and diastolic blood pressure but these did not occur consistently at similar time points. He and co-workers performed the definitive study using 3 mg kg⁻¹ over 4 min and found maximal increases in mean arterial blood pressure 10 min after the start of the infusion. In a dose ranging study by Stamler *et al.* [6] the haemodynamic responses to dosages of 0.01, 0.03, 0.1, 0.3 and 1.0 mg kg⁻¹ min⁻¹ each for 3 min via a central venous catheter sheath were examined. Although an increase in mean arterial blood pressure was observed at 0.1 mg kg⁻¹ min⁻¹ infused over 3 min no effects were observed on the pulmonary vascular bed until the maximal rate of infusion 1.0 mg kg⁻¹ min⁻¹ given over 3 min. The discrepancy between effects on the systemic and pulmonary vascular beds may be explained by *in vitro* studies suggesting that L-NMMA is a relatively ineffective inhibitor of NO synthase in pulmonary blood vessels [15].

We used a front loaded infusion i.e. a combination of bolus with a continuous infusion to give a good overall profile of NO inhibition over the whole time course of the study. We infused an initial 4 mg kg⁻¹ bolus over 2 min (ie 2 mg kg⁻¹ min⁻¹ for 2 min) with a continuous infusion of 4 mg kg⁻¹ h⁻¹ to ensure that we would achieve maximal NO inhibition during the period under investigation. The maximal haemodynamic changes in this study were noted at 4 min and persisted throughout the entire infusion period. We cannot comment whether haemodynamic changes

occurred earlier than 4 min since the time scale of measurements was influenced partly by the time required for signal acquisition and measurements to be made in triplicate.

In addition for safety considerations we did not feel that a dose escalation above the infusion rate used in this study would be safe because of worries over limiting systemic hypertension and worries over precipitating coronary vascular spasm. The evidence from studies suggests that L-NMMA is a relatively ineffective inhibitor of NO synthase in pulmonary blood vessels [15] and therefore to construct a dose response curve based on the results of previous studies would have meant doubling and quadrupling our dose of L-NMMA and exposing our patients to unwanted risks.

With respect to methodology, we have used non-invasive Doppler-echocardiography to measure haemodynamic changes in the pulmonary circulation. These non-invasive techniques have been shown to be reproducible [16] and the close correlation between Doppler PAT and MPAP as measured by right heart catheter, is well established both in our own hands and those of other workers [13, 14, 17, 18]. Less, however, is known of the correlation between changes in MPAP and PAT. Using regression equations incorporating PAT, Chow *et al.* [19] examined patients before and after pulmonary thromboendarterectomy and although statistically significant the correlation between changes in PAT and catheter measures of MPAP was weak ($r = -0.41$). Beard *et al.* [20], however, showed a good correlation in 11 normal subjects made hypoxic $r = -0.73$. In addition studies performed in normal man using this non-invasive methodology concur with results of invasive studies and we therefore feel that the available evidence supports our view that PAT can be used to assess changes in the pulmonary vascular bed, particularly in normal volunteers where repeated cardiac catheterisation is viewed by some including ourselves as unethical. Doppler derived measures of cardiac output have been shown to be reproducible and correlate well with invasive measures [21]. The major source of error is generally accepted as measurement of the annular diameter and this would be mitigated by the cross-over design of this study.

There is a growing body of evidence suggesting that endothelial dysfunction is important in the development of hypoxic pulmonary hypertension. Endothelium dependent

relaxation is reduced in chronically hypoxic rats [11] and in man in end stage chronic hypoxic lung disease endothelium dependent pulmonary artery relaxation is impaired and in addition the contractile responses to pressor stimuli increased [12]. These patients are known to have elevated levels of pressor substances such as endothelin-1 [22], which has been shown to be potent pressor agent in man *in vivo* [23], and may have significantly greater pressor effects in the context of a dysfunctional endothelium.

In addition to pressor effects we also noted a reduction in HR, CO and SV. This may have been mediated via a baroreceptor reflex and increase in left ventricular afterload although a role for nitric oxide generation in the maintenance of cardiac output remains a possibility.

To conclude, we have shown that basal nitric oxide generation is important in maintaining basal vascular tone. In disease states an imbalance of constricting and relaxing factors may contribute to both systemic and pulmonary vasoconstriction. The development of therapies which are able to deliver relaxing factors such as nitric oxide to the site of endothelial dysfunction holds promise, particularly in chronic hypoxic lung disease where effective therapies are limited.

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