Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity *in vivo*

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Aims To investigate the role of basal nitric oxide (NO) production in regulating large artery stiffness *in vivo*.

Methods Incremental doses of the NO synthase inhibitor L-N^G-monomethyl arginine (LNMMA: 0.1, 0.3, 1.0 and 3.0 mg kg^{-1} min^{-1}) or placebo were infused in eight healthy men. Arterial stiffness was assessed noninvasively by pulse wave analysis.

Results Compared with placebo, infusion of LNMMA led to a dose-dependent increase in mean arterial pressure, peripheral vascular resistance, and aortic and systemic arterial stiffness. There was an accompanying reduction in heart rate and cardiac index. The highest dose of LNMMA resulted in an increase of 25% in AIx (95% confidence limits; 12, 38) and of 16 mmHg in mean arterial pressure (9, 23) compared with infusion of saline.

Conclusions These data indicate functional regulation of large artery stiffness *in vivo* by NO, and may provide new therapeutic strategies for cardiovascular risk reduction.

Keywords: arterial stiffness, LNMMA, nitric oxide, pulse wave analysis

Introduction

Large artery stiffness is an important determinant of cardiovascular risk [1]. Structural components within the arterial wall, mainly collagen and elastin, together with transmural pressure, are key determinants of large vessel stiffness [2, 3]. However, stiffness also depends on smooth muscle tone [4], suggesting a degree of functional regulation of arterial stiffness by local and circulating vasoactive substances. Although endothelium-derived nitric oxide (NO) contributes to resting tone in resistance vessels, whether it also regulates large artery stiffness is unclear. Indeed, data concerning the effect of intra-arterial infusion of the NO synthase inhibitor L-N^G-monomethyl arginine (LNMMA) on local arterial stiffness are conflicting [5, 6].

We hypothesized that inhibition of basal NO synthesis would increase arterial stiffness *in vivo*. Indeed, McVeigh *et al.* [7] have recently reported that systemic infusion of L-N^G nitro-arginine methyl ester (LNAME) reduces small artery compliance in healthy male volunteers. However, they were unable to detect any change in large artery compliance, despite an increase in mean arterial pressure. The aim of the present study was to assess the effect of inhibition of local endogenous NO synthesis on *aortic* and *systemic* arterial stiffness noninvasively using the technique of pulse wave analysis.

Methods

Eight healthy male subjects, mean age 30 years (range 21–42), were studied on two occasions separated by at least 1 week in a double-blind, randomized protocol. The study was approved by the Local Research Ethics Committee, and written informed consent was obtained from each participant. After 30 min supine rest, baseline haemodynamic recordings were made and LNMMA (Clinalfa; Laufelfingen; Switzerland) at 0.1, 0.3, 1.0 and 3.0 mg kg^{-1} min^{-1}, or matching placebo (0.9% saline) were given intravenously. Each dose was infused for 15 min and haemodynamic measurements were made over the last 5 min of this period. Blood pressure was determined with an oscillometric sphygmomanometer (HEM–705CP, Omron Corporation, Japan) and cardiac index by

transthoracic bioimpedance (BoMed; NCCOM3-R7; Irvine, USA). Radial artery waveforms were recorded from the wrist by applanation tonometry and corresponding central artery waveforms were generated and analysed using the technique of pulse wave analysis (SCOR; Atlor Medical, Sydney, Australia), as described in detail previously [8]. In brief, high fidelity peripheral pressure waveforms were recorded by applanation tonometry and a validated transfer function [9–12] was then used to generate corresponding ascending aortic waveforms from which augmentation index (AIx) and the timing of the reflected waveform (T_R) were calculated. Although the transfer function has not been assessed in the context of NO synthesis inhibition, it has been validated for dynamic changes in blood pressure [10–12]. Augmentation index is a measure of the contribution of wave reflection to the aortic waveform and depends on the pulse wave velocity, and magnitude and site of the reflected pressure wave, and thus provides a composite measure of large artery (*systemic*) stiffness [13]. In contrast, the timing of the reflected waveform provides an estimate of aortic pulse wave velocity [14, 15], and thus *aortic* stiffness.

All measurements were made in duplicate, and data were analysed as changes from values at baseline using analysis of variance (ANOVA). Significance was accepted at $P < 0.05$.

Results

Baseline haemodynamics did not differ significantly between the two visits and there was no significant change in any parameter during infusion of placebo. Compared with saline, infusion of LNMMA resulted in a dose-dependent increase in AIx (Figure 1), mean arterial pressure and peripheral vascular resistance, and a corresponding reduction in heart rate, cardiac index and T_R
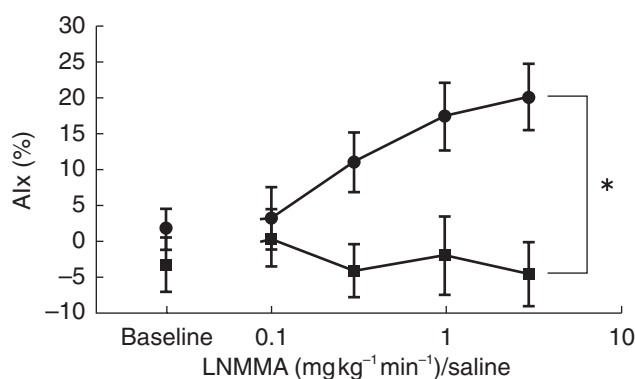

Figure 1 Effect of LNMMA on augmentation index. Effect of incremental infusion of LNMMA (●) and saline (■) on augmentation index (AIx). Values represent means ± s.e. mean; ★$P < 0.001$ (ANOVA); $n = 8$.

(Table 1). Whereas central pulse pressure increased following LNMMA, there was no change in peripheral pulse pressure.

Discussion

The shape of the aortic pressure waveform depends on the pattern of left ventricular ejection, aortic stiffness and also on wave reflection within the arterial tree. AIx is a quantitative measure of the contribution of the reflected pressure wave to the central pressure waveform and depends on the amplitude and velocity of the reflected wave, both of which are influenced by arterial stiffness. Therefore, AIx provides a noninvasive measure of *systemic* arterial stiffness. Similarly, T_R provides an estimate of the aortic pulse wave velocity and thus *aortic* stiffness.

As hypothesized, intravenous infusion of LNMMA significantly increased AIx and T_R, indicating aortic and systemic arterial stiffening. LNMMA is a specific substrate-analogue inhibitor of NO synthase and intra-arterial infusion into the forearm vascular bed reduces forearm blood flow by ∼40%, indicating that there is basal NO production in resistance vessels [16]. Moreover, systemic administration of LNMMA increases mean arterial pressure and reduces heart rate [17]. However, previous data concerning the effect of inhibition of NO synthesis *in vivo* on local arterial stiffness are conflicting. Indeed, both unchanged [6] and reduced [5] radial artery stiffness has been described following intrabrachial administration of LNMMA. Moreover, although McVeigh *et al.* [7] demonstrated increased small artery stiffness following systemic infusion of LNAME, they were unable to detect any alteration in large artery stiffness, which is perhaps surprising since there was an accompanying increase in mean arterial pressure, a key determinant of large artery stiffness.

In the present study, the increase in AIx was accompanied by a fall in heart rate and rise in mean arterial pressure. Although changes in heart rate *per se* do not influence arterial stiffness we have previously shown that AIx, but not T_R, is inversely related to heart rate, due to an alteration in the relative arrival-time of the reflected waveform in the ascending aorta [8]. However, our previous data [8] indicate that the observed change in heart rate only accounts for < 25% of the increase in AIx in the present study. In contrast, distending pressure is a key determinant of arterial stiffness and therefore the accompanying increase in mean arterial pressure may, in part, account for the rise in systemic and aortic stiffness. Indeed, we have previously shown that systemic infusion of angiotensin II or noradrenaline results in an increase in mean arterial pressure, AIx and T_R [18]. Moreover, for a given increase in mean pressure, LNMMA produced a similar increment in AIx and T_R to that observed with angiotensin II and

Table 1 Haemodynamic response to infusion of saline and incremental doses of LNMMA.

| | Saline | | | | | LNMMA (mg kg^{-1} min^{-1}) | | | | | |
	Baseline	*–*	*–*	*–*	*–*	*Baseline*	*0.1*	*0.3*	*1.0*	*3.0*	*Significance*
Mean arterial pressure (mmHg)	86±2	86±2	87±1	86±2	88±2	86±2	91±2	93±2	97±2	105±3	<0.001
Peripheral pulse pressure (mmHg)	47±2	45±2	46±2	47±1	47±2	48±2	48±2	44±2	45±2	45±2	0.3
Central pulse pressure (mmHg)	29±2	28±1	28±1	29±1	29±1	30±1	30±2	31±1	34±2	36±3	0.04
Heart rate (beats min^{-1})	58±2	60±2	59±2	59±2	62±3	60±2	55±1	52±2	49±2	45±2	<0.001
Peripheral vascular resistance (AU)	31±2	30±2	31±2	30±2	32±2	29±2	35±2	39±2	46±3	57±4	<0.001
Cardiac index (l min^{-1} m^{-2})	2.9±0.2	3.0±0.2	2.8±0.2	3.0±0.2	2.8±0.2	3.0±0.1	2.7±0.1	2.4±0.1	2.2±0.1	1.9±0.1	<0.001
T_R (ms)	160±5	160±5	160±5	162±8	160±5	168±6	159±5	162±3	157±5	154±5	<0.001

Values represent means ± s.e. mean; AU = arbitrary units, and significance was determined using ANOVA compared with the placebo phase, $n = 8$. There were no significant differences in baseline values or changes during infusion of saline.

noradrenaline. However, this does not necessarily mean that all the effect of LNMMA is indirect, i.e. passive stretching of vessels in response to a rise in distending pressure, since angiotensin II and noradrenaline may also alter wave reflection and large artery stiffness independently of mean arterial pressure through direct smooth muscle constriction.

Pulse pressure is often regarded as a surrogate measure of large artery stiffness. However, we have previously shown that changes in peripheral pulse pressure do not always predict accompanying changes in central pulse pressure or arterial stiffness [18]. Indeed, in the present study, despite an increase in central pulse pressure and stiffness in response to LNMMA, there was no change in peripheral pulse pressure, which is likely to be due to the observed decline in cardiac index. Therefore, peripheral pulse pressure should not always be considered a reliable indicator of systemic arterial stiffness, especially when investigating the effects of vasoactive drugs.

In conclusion, the present results demonstrate that basal NO synthesis modulates large artery stiffness *in vivo* Therefore, it may be possible to reduce the arterial stiffening associated with ageing and other cardiovascular risk factors, such as diabetes mellitus, either by improving endothelial function or by developing drugs that act selectively on the smooth muscle of large arteries.

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