Basal NO Locally Modulates Human Iliac Artery Function In Vivo

Matthias Schmitt, Albert Avolio, Ahmad Qasem, Carmel M. McEniery, Mark Butlin, Ian B. Wilkinson, John R. Cockcroft

Abstract—We demonstrated previously that endogenous NO influences large-artery distensibility in the ovine hindlimb. However, the role of basal NO in larger human conduit arteries is controversial. The aim of this study was to investigate whether basal production of NO, acting locally, influences iliac artery distensibility in humans. Distensibility was assessed by intra-arterial measurement of the pulse wave velocity. Eighteen subjects, free of significant coronary or iliac artery disease, were studied after diagnostic cardiac catheterization. Simultaneous pressure waveforms were recorded with a high-fidelity dual-pressure sensing catheter, placed in the common iliac artery during intra-arterial infusion of saline (baseline), glyceryl trinitrate (4 nmol/min), or N\textsubscript{G}-monomethyl-L-arginine (8 and 16 μmol/min). Drugs were infused proximally, via the catheter to perfuse the segment of artery under study, or distally, via the sheath, to control for any reflex changes in flow or sympathetic activation. Velocity was calculated using the foot-to-foot methodology. Six subjects received glyceryl trinitrate and 12 N\textsubscript{G}-monomethyl-L-arginine. There was no change in velocity after infusion of glyceryl trinitrate or N\textsubscript{G}-monomethyl-L-arginine via the sheath. However, infusion of glyceryl trinitrate via the catheter significantly reduced velocity by 31.43±5.80% (mean±SEM; \( P < 0.01; P = 0.02 \) for comparison). Likewise, infusion of the highest dose of N\textsubscript{G}-monomethyl-L-arginine via the catheter significantly increased velocity by 27.25±8.20% (\( P = 0.001; P = 0.02 \) for comparison). Importantly, there was no change in mean arterial blood pressure throughout the studies. These data indicate that under resting conditions, local NO production modulates human iliac artery distensibility and that exogenous NO increases arterial distensibility. (Hypertension. 2005;46:227-231.)

Key Words: arteries ■ nitric oxide ■ arterial stiffness ■ hemodynamics

Aortic pulse wave velocity (PWV), a measure of arterial distensibility, is an important, independent determinant of cardiovascular risk.\(^1\)\(^-\)\(^4\) In addition to the effects of structural components within the arterial wall (mainly collagen and elastin), and mean arterial pressure (MAP), the PWV is also determined by smooth muscle tone.\(^5\)\(^-\)\(^6\) NO, endothelin-1, and natriuretic peptides contribute to resting arteriolar tone in man, and we have shown previously that these endothelium-derived mediators also modulate iliac artery distensibility in the ovine iliac artery.\(^7\)\(^-\)\(^9\) However, the role of endogenous NO in human large arteries is controversial. Although Joannides et al\(^10\) reported that inhibition of basal NO increased brachial artery distensibility, Kinlay et al found the opposite.\(^11\) More recently, Stewart et al\(^12\) investigated the role of NO in regulating carotid-femoral PWV, an indirect measure of aortic distensibility. Although systemic infusion of the NO synthase inhibitor N\textsubscript{G}-monomethyl-L-arginine (l-NMMA) increased PWV, the effect was no greater than that observed by 2 control constrictors, suggesting that there was no pressure-independent effect of l-NMMA, and, therefore, that NO does not influence carotid-femoral PWV in man.

The aim of the present study was to test the hypothesis that NO is important in regulating large artery stiffness in man using local infusions of drugs to modulate the l-arginine NO pathway, thus avoiding the confounding effects of changes in MAP. The methodology was adapted from that used previously in the ovine iliac artery.\(^7\)

Methods

Subjects

Eighteen subjects were studied after diagnostic coronary angiography for evaluation of atypical chest pain. All were free from peripheral vascular disease. Fifteen subjects had no evidence of myocardial ischemia on stress testing, 2 subjects had equivocal stress tests, and 1 had no previous testing. All had normal left ventricular function as assessed by ventriculography or echocardiography. Sixteen subjects had angiographically normal epicardial coronary arteries, including the 2 patients with equivocal stress tests. Two subjects had minor single-vessel coronary artery disease of ≤30% lumen diameter. The demographic data, cardiovascular risk profile, and medication before angiography are outlined in Table 1. All subjects gave written informed consent. The protocol was approved by the Local Research Ethics Committee and conducted in accordance with local regulations.

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From the Department of Cardiology (M.S., J.R.C.), Wales Heart Research Institute, College of Medicine University Hospital of Wales, Cardiff, UK; Graduate School of Biomedical Engineering (A.A., A.Q., M.B.), University of New South Wales, Sydney, Australia; and Clinical Pharmacology Unit (C.M.M., I.B.W.), University of Cambridge, Addenbrooke’s Hospital, United Kingdom.

Correspondence to Professor John Cockcroft, Department of Cardiology, Wales Heart Research Institute, College of Medicine University Hospital of Wales, Cardiff, UK, E-mail cockcroftjr@cf.ac.uk

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Hemodynamic Measurements

Intravascular pressure measurements were made using custom-made Millar Mikro-Tip 6F end-hole catheters with a 0.46-mm internal lumen, and dual high-fidelity pressure sensors located 10 and 60 mm from the distal end. The analogue signal from the pressure control unit (Millar TC-510) was interfaced with an analogue-to-digital converter (Biopac Systems) and fed into a portable microcomputer, with a sampling rate of 1 kHz. Data were recorded over 20s to allow for variations within the respiratory cycle and then exported and resampled at 10 kHz for further analysis with custom-written MATLAB analysis program (Mathworks). Using this program, systolic diastolic, and pulse pressure and MAP were determined together with the transit time between the 2 pressure waveforms, from the distal sensor, as described previously.7 The minimum resolution of the system was a difference of 0.1 ms. The iliac PWV was calculated from the transit time, as illustrated in Figure 1, and the fixed distance between the recording sites (50 mm). The PWV is inversely related to arterial distensibility by the equation of Bramwell and Hill13; PWV = V/ΔV/ΔP/ρ, where: V=artery volume, ΔV=change in volume, ΔP=change in pressure, and ρ=blood density (assumed to be constant during the study). Heart rate (HR) was recorded simultaneously via a 3-lead ECG. Brachial MAP was assessed at baseline and immediately after each infusion period by oscillometric sphygmonanometer (Dinamap Critikon).

Drugs

All drugs were freshly prepared in an aseptic manner using 0.9% saline as a diluent. Glyceryl trinitrate (GTN; Schwarz) was infused at 4 nmol/min and L-NMMA (Clinalfa) at 8 and 16 mmol/min. All infusions were at 1 mL/min for 5 minutes.

Protocol

All subjects were studied after diagnostic angiography and while resting supine in a temperature-controlled environment. Vasoactive drugs were omitted on the day of the study. The arterial catheter was positioned in the right common iliac artery under radiographic screening, with the tip well below the level of the aortic bifurcation, and most distal sensor at least 5 cm from the sheath (Figure 2). Iliac angiography was performed to rule out presence of iliac artery disease and to select a segment without branching (Figure 3). Saline was then infused for 15 minutes to maintain patency and to allow stabilization of the preparation. Baseline measurements of iliac PWV, iliac blood pressure, and HR were recorded in duplicate. In 6 patients, GTN was infused at 4 nmol/min, first through the sheath and then through the catheter. In another 12 patients, L-NMMA was then infused at 16 mmol/min first through the sheath, and then at 8 mmol/min and 16 mmol/min through the catheter. Infusion of L-NMMA through the catheter exposed the arterial segment under study to the drug, whereas infusion via the sheath did not because the drug is delivered distal to the pressure sensors (Figure 2). Pressure waveforms were recorded during the final 20 s of each infusion.

Statistical Analysis

All results are expressed as means±SEM. Data were analyzed by 2-way ANOVA with post hoc comparison to baseline. Pair-wise Spearman rank analysis was performed to assess correlations between iliac MAP and PWV. A P value <0.05 was considered statistically significant.

### TABLE 1. Baseline Characteristics of Subject Group

<table>
<thead>
<tr>
<th>Demographic and Clinical Features</th>
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<tbody>
<tr>
<td>Age, y</td>
<td>59±3</td>
</tr>
<tr>
<td>Sex, male/female, no.</td>
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</tr>
<tr>
<td>Body mass index, kg/m²</td>
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<tr>
<td>Height, m</td>
<td>1.69±0.03</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>85±3</td>
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<tr>
<td>Smoking history, no.</td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>13</td>
</tr>
<tr>
<td>Previous smoker</td>
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</tr>
<tr>
<td>Current smoker</td>
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</tr>
<tr>
<td>Type 2 diabetes, no.</td>
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</tr>
<tr>
<td>History of hypertension, no.</td>
<td>5</td>
</tr>
<tr>
<td>History of hyperlipidemia, no.</td>
<td>4</td>
</tr>
<tr>
<td>Medication, no.</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>9</td>
</tr>
<tr>
<td>ACE inhibitor</td>
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<tr>
<td>Statin</td>
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</tr>
<tr>
<td>β-Blocker</td>
<td>5</td>
</tr>
<tr>
<td>Diuretic</td>
<td>3</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>1</td>
</tr>
<tr>
<td>Nitrate</td>
<td>1</td>
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Continuous data are presented as means±SEM. ACE indicates angiotensin-converting enzyme.

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**Figure 1.** Simultaneous pressure waveforms. The upstroke of the pressure wave in the same patient during saline (A) and during infusion of L-NMMA (16 mmol/min via catheter tip; B). The gap between both waves, assessed on the upstroke at identical pressures, illustrates a decrease in transit time from 10.3 to 5.7 ms.

**Figure 2.** Schema showing infusions. Infusion via the catheter (proximal) and sheath (distal). P1 indicates pressure sensor 1; P2, pressure sensor 2. (This figure was redrawn from McEniery CM, et al8).

MAP was assessed at baseline and immediately after each infusion period by oscillometric sphygmonanometer (Dinamap Critikon).
Results

Infusion of GTN
There was no significant change in MAP or HR during any of the studies. Infusion of GTN via the sheath did not alter PWV, but there was a significant reduction in PWV of 31/11006 6% (from 8.13/11006 0.44 m/s to 5.50/11006 0.47 m/s; \( P<0.01 \); \( P<0.05 \) for comparison with sheath infusion).

Infusion of L-NMMA
Hemodynamic data are presented in Table 2. There was no significant change in brachial or iliac MAP throughout the study. Iliac pulse pressure did not alter after infusion of L-NMMA via the sheath but increased significantly after catheter infusion. However, there was no significant difference between sheath and catheter responses. Compared with the control infusion via the sheath, there was a significant fall of 3 bpm in HR when L-NMMA was infused via the catheter.

There was small, nonsignificant increase in the iliac PWV after infusion of L-NMMA via the sheath (0.88/11006 0.19 m/s; \( P=\text{NS} \)). Infusion of L-NMMA via the catheter led to a significant, dose-dependent increase in PWV (0.94/11006 0.20, and 2.61/11006 0.19 m/s, respectively; \( P=0.001 \)). The increase in PWV remained significant when infusion of equimolar dose through the catheter and sheath were compared (\( P<0.02 \); Figure 4). There was no correlation between the change in iliac PWV and the change in MAP or HR.

Discussion
Cardiovascular disease is now the leading cause of death worldwide: myocardial infarction and stroke, accounting for >20% of all deaths.14 Atherosclerosis of the blood vessel wall is the final common pathway in the development of these conditions, and a number of predisposing factors for atheroma formation have been identified, including: cigarette smoking, hypercholesterolemia, diabetes mellitus, and hypertension.15 Most traditional cardiovascular risk factors are associated with endothelial dysfunction before the development of manifest atheroma.16 More recently, aortic stiffness, assessed by carotid-femoral PWV, predicts outcome in patients with diabetes,2 and renal disease.1 Moreover, increased PWV has been found in a wide range of cardiovascular risk factors17–19 and even in people with a predominantly sedentary lifestyle, free of any overt cardiovascular disease, in whom PWV was inversely correlated with VO2max.20 Thus, accumulating evidence suggests that large artery stiffness is a key additional and

<table>
<thead>
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<th>TABLE 2. Effect of L-NMMA on Hemodynamics</th>
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<tr>
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<tr>
<td>Iliac PWV, m/s</td>
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<tr>
<td>Iliac SBP, mm Hg</td>
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<tr>
<td>Iliac DBP, mm Hg</td>
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<tr>
<td>Iliac MAP, mm Hg</td>
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<tr>
<td>Iliac PP, mm Hg</td>
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<tr>
<td>Heart rate, bpm</td>
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<tr>
<td>Brachial MAP, mm Hg</td>
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SDB indicates systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure. Values represents means±SEM. Significance values are for the post hoc comparison between L-NMMA infusion via the sheath and 16 nmol/min via the catheter.

\*\( P<0.05 \) for comparison with saline.
Independent predictor of cardiovascular risk. However, the causes of increased arterial stiffness remain unclear. Although degeneration of structural elements and raised MAP may predominate, alterations in smooth muscle tone are also likely to be important. Conditions associated with endothelial dysfunction, such as diabetes and hypercholesterolemia, are also associated with increased arterial stiffness, suggesting that endothelium-derived NO may play a role in the regulation of arterial stiffness. Indeed, recent studies have shown significant positive correlations between endothelial function and arterial stiffness.

Using pulse wave analysis and systemic infusion of L-NMMA, we demonstrated previously that NO plays a role in regulation of large artery distensibility. However, results of this study were difficult to interpret because of changes in systemic blood pressure that would have influenced distensibility. Local infusion of drugs, such as L-NMMA, avoids such confounding factors. Indeed, using such an approach, we demonstrated previously in an ovine hindlimb model that NO, in part, regulates iliac artery distensibility independently of alterations in MAP or HR. We recently extended these studies to demonstrate that nebivolol, a β-blocker that releases NO, also decreases iliac artery distensibility independently of any reduction in blood pressure. The aim of the present study was to use this methodology to test the hypothesis that NO regulates large artery distensibility in man. The main findings were that inhibition of basal NO production increased the PWV, and that infusion of the NO donor GTN decreased PWV. Both these effects were independent of changes in MAP or HR. Interestingly, iliac pulse pressure increased after infusion of L-NMMA, in keeping with an increase in local artery stiffness. Together, these data indicate that NO regulates local iliac artery distensibility in man.

Previous data concerning the role of endogenous NO in modulating large artery distensibility in humans are controversial, not least because of differences in experimental techniques but also the vessels studied. Indeed, the majority of human studies have been performed in the arm. Although the radial and brachial arteries are easily accessible, they may not be the ideal vascular bed in which to study the involvement of basal NO in the physiologic regulation of arterial distensibility. Indeed, unlike the aorta and large arteries of the lower extremities, clinically significant atherosclerosis in the arm vessels is an infrequent process. Intriguingly, brachial artery distensibility also changes little with age, unlike the aorta and femoral arteries, despite reduced brachial endothelial NO production with age. Moreover, the brachiocephalic system contributes little to the overall buffering capacity of the large arteries, unlike the aorto-femoral segment.

Ramsey et al investigated the effects of NO on human iliac artery distensibility in health and chronic heart failure by infusing adenosine or the endothelium-dependent agonist acetylcholine (ACh). Whereas adenosine increased distensibility in controls and heart failure patients, changes in arterial distensibility after intra-arterial ACh were attenuated in chronic heart failure patients. However, they did not assess the contribution of basal NO to resting large artery distensibility. Moreover, ACh causes endothelial cells to release prostacyclin and endothelium-derived hyperpolarizing factor in addition to NO, and therefore, the role of NO, per se, in the observed changes is unclear. Therefore, in the present study, we used GTN as a specific NO donor and L-NMMA as an inhibitor of basal NO production and have provided firm evidence that NO modulates iliac distensibility in man. Interestingly, changes in the iliac artery were larger than those we reported previously in the ovine model, suggesting that inhibition of NO synthesis results in a similar degree of arterial stiffening as aging 20 years.

Our results stand in contrast to those of Stewart et al, who examined the effects of systemic administration of L-NMMA on carotid-femoral PWV. In an attempt to control for the pressor effects of systemic NO synthase blockade, they also infused equipressor doses of noradrenaline and dobutamine and concluded that the increase in PWV produced by L-NMMA was solely explained by the changes in MAP. One obvious explanation for the differences between these 2 studies is the vessel studied. Stewart et al assessed carotid-femoral PWV, which includes the iliac and even more muscular femoral arteries. Differences in the techniques used may also be partly responsible because Stewart et al used surface estimation of PWV rather than intra-arterial determination, which may have limited their accuracy and thus the ability to resolve small differences between agents. Indeed, similar studies in animals using intra-arterial measurement of aortic PWV showed a greater increase in aortic PWV after NO synthase inhibition than after phenylephrine, a control constrictor. An alternative explanation is that norepinephrine and dobutamine had a direct effect on arterial smooth muscle or the endothelium, leading to direct stiffening of the artery as well as an increase in MAP in an analogous fashion to that produced by L-NMMA.

Limitations

The large arteries increase in muscularity moving away from the heart, becoming less "elastic." Therefore, the common iliac artery is more similar to the abdominal aorta than the ascending aorta. Nevertheless, the iliofemoral arterial segment still makes a significant contribution to cyclic pressure buffering and is routinely included in measurement of “aortic” PWV, when carotid and femoral sites are used for recording waveforms, as is the case with the SphygmoCor and Complior systems.

Patients undergoing diagnostic coronary angiography must not be considered “normal,” even in absence of significant epicardial coronary artery disease and in absence of a (potentially false) positive stress test. With this in mind, and given the cardiovascular risk factors outlined in Table 1, it is likely that the findings of this study, if anything, quantitatively underestimate the true effect of basal NO on regulation of resting iliac artery distensibility in individuals without cardiovascular risk factors. Finally, there was a small but statistically significant change in HR after infusion of L-NMMA. It has been suggested that PWV and arterial distensibility may exhibit some dependence on HR. However, the magnitude of the change (a fall of 3 bpm) in the present study was small, and a fall in HR would be expected to reduce stiffness, leading us to underestimate the true NO effect.

Perspectives

Conventional cardiac risk factors, of which the majority not only impair endothelial function but also change the composition and thickness of the arterial wall, do not fully explain the incidence of coronary artery disease and cardiovascular events. Risk
stratification and therapy based solely on these conventional risk factors will exclude a population who may benefit from lifestyle and risk factor modification. Arterial stiffening carries a number of serious adverse hemodynamic consequences, including a rise in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection in pulse pressure, which tends to be more pronounced in the aorta than in the brachial artery because of wave reflection.

Whatever the mechanism, arterial stiffening remains an important and independent predictor of cardiovascular risk, which will assume greater prominence because of the predicted growth in the older population of Western countries in future years. Here we show that endogenous NO locally modulates human iliac artery distensibility.

Assessment of arterial distensibility has the potential to identify patients at risk for later development of overt cardiovascular disease at an earlier stage than current clinically used techniques. Assessment of arterial distensibility, using various techniques, is likely to gain an important role in risk assessment and stratification. It may also be used for monitoring of therapeutic interventions, such as modulation of the NO pathway, especially in conditions associated with increased arterial stiffness.

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References
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