Nitric Oxide Regulates Local Arterial Distensibility In Vivo

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Background—Arterial stiffness is an important determinant of cardiovascular risk. Several lines of evidence support a role for the endothelium in regulating arterial stiffness by release of vasoactive mediators. We hypothesized that nitric oxide (NO) acting locally regulates arterial stiffness in vivo, and the aim of this experiment was to test this hypothesis in an ovine hind-limb preparation.

Methods and Results—All studies were conducted in anesthetized sheep. Pulse wave velocity (PWV) was calculated by the foot-to-foot methodology from 2 pressure waveforms recorded simultaneously with a high-fidelity dual pressure-sensing catheter placed in the common iliac artery. Intra-arterial infusion of L-arginine (L-NMMA) increased iliac PWV significantly, by 3±2% (P<0.01). Infusion of acetylcholine and glyceryl trinitrate reduced PWV significantly, by 6±4% (P=0.03) and 5±2% (P<0.01), respectively. Only the effect of acetylcholine, however, was significantly inhibited during coinfusion of L-NMMA (P=0.03). There was no change in systemic arterial pressure throughout the studies. Importantly, infusion of L-NMMA or acetylcholine distal to the common iliac artery (via the sheath) did not affect PWV.

Conclusions—These results demonstrate, for the first time, that basal NO production influences large-artery distensibility. In addition, exogenous acetylcholine and glyceryl trinitrate both increase arterial distensibility, the former mainly through NO production. This may help explain why conditions that exhibit endothelial dysfunction are also associated with increased arterial stiffness. Therefore, reversal of endothelial dysfunction or drugs that are large-artery vasorelaxants may be effective in reducing large-artery stiffness in humans, and thus cardiovascular risk. (Circulation. 2002;105:213-217.)

Key Words: blood pressure □ nitric oxide □ arteries □ elasticity

Arterial stiffness is an important, independent determinant of cardiovascular risk.1,2 Structural components within the arterial wall, mainly collagen and elastin, together with transmural pressure, are important determinants of large-vessel stiffness.3,4 Indeed, disruption and fatigue fracture of the elastic load-bearing elements of the arterial wall, resulting in a transfer of stress to the more collagenous fibers, is likely to be responsible for the age-related arterial stiffening observed in most populations.4,5 Smooth muscle tone, however, can also influence the stiffness of the elastic and muscular arteries.6,7 Therefore, a degree of functional regulation of arterial stiffness by circulating and locally generated vasoactive substances seems likely.

The vascular endothelium releases a number of vasoactive substances. One of these, nitric oxide (NO), has a major influence on basal arteriolar tone and blood pressure.8,9 Interestingly, the contribution of NO to resting tone is greater in larger (>200 μm) than in smaller (resistance; <200 μm) vessels,10-12 but whether endogenous NO also regulates large-artery stiffness is, at present, unclear. In dogs, glyceryl trinitrate (nitroglycerin; GTN), which is metabolized to NO within the vascular wall, reduces aortic and large-artery stiffness.13 Similarly, in humans, GTN reduces brachial artery stiffness14 and decreases wave reflection.15 Moreover, drugs that stimulate endothelial NO release, such as acetylcholine (ACh), also reduce muscular artery stiffness in vivo.16,17 Data concerning basal NO and arterial stiffness, however, are unclear. Joannides et al18 reported that intra-arterial infusion of the NO synthase inhibitor Nω-monomethyl-L-arginine (L-NMMA) paradoxically increases brachial artery compliance, but they did not control for changes in limb blood flow directly. This is important because basal NO production is thought to be regulated to a large extent by shear stress, which is itself dependent on blood flow. In contrast, Leeson et al18 found no alteration in radial artery distensibility after intra-arterial infusion of L-NMMA. In contrast to earlier studies,4 however, they also reported that arterial distensibility was unchanged after infusion of a control constrictor, norepinephrine.

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We hypothesized that local endogenous NO generation contributes to the regulation of large-artery stiffness under basal conditions. The aim of this study was to test this hypothesis in vivo in an anesthetized ovine hind-limb preparation by use of intravascular measurement of pulse-wave velocity (PWV) as a well-validated index of arterial distensibility. In addition, we wanted to extend previous work and investigate whether the effect of exogenous ACh on large-artery mechanics was due to NO release.

Methods
All experiments were conducted in adult, crossbred wether sheep between 12 and 18 months old at the University of New South Wales, Sydney, Australia. The study was approved by the University’s Animal Care and Ethics Committee. Anesthesia was induced by intravenous injection of 600 to 900 mg sodium phenobarbitone (Rhone Merieux) and maintained by inhalation of 2% to 3% halothane, administered through a Boyle rebreathing apparatus with an oxygen flow rate of 2 L/min. Animals were breathing spontaneously and were studied in the supine position.

Hemodynamic Measurements
Pressure was assessed with a 6F end-hole catheter (Gaeltec) with a 0.46-mm internal lumen and dual high-fidelity pressure sensors located 10 and 60 mm from the distal end. Both sensors were calibrated simultaneously at the start of each experiment with a mercury sphygmomanometer. The analog signal from the pressure control unit was fed directly into a portable microcomputer with a PC Laboratory analog-to-digital converter (AD Instruments) with a sampling rate of 1 kHz. Data were recorded over 20 seconds to allow for variations within the respiratory cycle. Mean arterial pressure (MAP) was calculated from integration of the distal pressure waveform by use of the supplied CHART software (version 4). Data were then exported and resampled at 10 kHz for further analysis with the MATLAB analysis program (Math Works). The transit time was obtained from the foot-to-foot delay between the simultaneously recorded pressure waves (Figure 1) as previously described. The minimum resolution of the system was a difference of 0.1 ms. The iliac PWV was calculated from transit time and the fixed distance between the recording sites (50 mm), which is inversely related to arterial distensibility by the 1922 equation of Bramwell and Hill:

\[
\mathrm{PWV} = \sqrt{\frac{V \cdot \Delta P}{\rho \cdot \Delta V}}
\]

where \(V\) is artery volume, \(\Delta V\) is change in volume, \(\Delta P\) is change in pressure, and \(\rho\) is blood density (assumed to be constant in the present studies). For a distance of 50 mm, the 0.1-ms resolution in transit time provides a PWV resolution of 0.025 m/s (assuming a mean transit time of 14 ms). The repeatability of measurements was high, with a mean±SD difference of 0.006±0.004 m/s between paired samples recorded during the saline infusion period in all 11 sheep. Heart rate was calculated over the measurement period from a simultaneously recorded ECG.

Drugs
All drugs were freshly prepared in an aseptic manner before the start of each experiment, with 0.9% saline used as a diluent. L-NMMA (Clinalfa) was infused at 10 μmol/min in all studies. ACh (Ciba Vision) was infused at 60 and 120 nmol/min and GTN (Schwarz) at 2 and 4 nmol/min.

Protocol
The distal right femoral artery was identified by palpation, and a 20-mm segment of artery was exposed by a limited dissection into which a 7F sheath was inserted. The arterial catheter was then positioned via the sheath in the common iliac artery, with anatomical landmarks used as a guide, and the location was confirmed directly at the end of the experiment. Saline was infused through the sheath and catheter at 1 mL/min for a period of 30 minutes to allow stabilization of the preparation. Baseline measurements of iliac PWV, MAP, and heart rate were then recorded in triplicate or until measurements were stable (within 10% of each other). All drugs were infused for 4 minutes at each dose, at 1 mL/min, and pressure waveforms were recorded over the last 20 seconds of each infusion period. Infusion of drugs through the catheter exposed the arterial segment under study to the drug, whereas infusion through the sheath did not, because this was located distal to the pressure sensors. This methodology, which has been described previously, allows indirect drug effects, such as those produced by changes in flow or reflex activation, to be taken into account.

Pilot Studies
Four sheep were used in the initial dose-ranging studies of ACh (60 to 6000 nmol/min) and GTN (2 and 16 nmol/min). Each sheep received a maximum of 3 different doses of the 2 drugs through the arterial catheter. The initial doses were based on published data, but doses were titrated to produce local and not systemic effects. When hemodynamics returned to baseline, after a minimum period of 30 minutes, L-NMMA was infused through the sheath and then the catheter in turn, at a dose based on previous data.

Main Study
A further 7 sheep were used for the remaining studies. After baseline recordings had been obtained, 2 doses of ACh (60 and 120 nmol/min) were infused through the catheter. After a 15-minute washout period and further baseline recordings, 2 doses of GTN (2 and 4 nmol/min) were infused through the arterial catheter and then, after a further 15-minute washout period, L-NMMA was infused first through the sheath and then the catheter. The highest doses of ACh and GTN were then given in turn through the catheter with concomitant infusion of L-NMMA. To determine whether the response to ACh was due to an effect on the local arterial wall or a distal effect on flow, for example, ACh (120 nmol/min) was infused through the sheath before administration of the initial GTN doses in 4 of the sheep.

Data Analysis
All results are expressed as mean±SD unless otherwise stated. Data were analyzed by paired Student’s t tests and ANOVA, and a value of \(P<0.05\) was considered significant.

Results
Effect of L-NMMA
Eleven sheep received intra-arterial L-NMMA, which did not affect MAP (change of 0±1 mm Hg, \(P=0.9\), and 1±1 mm Hg, \(P=0.1\), after infusion through the sheath and catheter, respectively), systolic or diastolic blood pressure, or heart rate (0±4 bpm, \(P=0.7\), and 0±3 bpm, \(P=0.4\), respectively). There was no change in iliac PWV when L-NMMA was infused via the femoral artery sheath (3.71±0.43 versus 3.76±0.41 m/s, \(P=0.1\)). There was a significant increase, however, in the PWV of 4±2% after infusion through the
catheter (sheath versus catheter: 3.76±0.41 and 3.86±0.45 m/s, respectively, \( P<0.01 \)). Doubling the infusion period through the sheath (n=2) or catheter (n=3) did not affect the response to L-NMMA (data not shown).

**Effect of ACh and GTN**

Seven sheep received ACh and GTN through the catheter before and during co-infusion of L-NMMA, and the Table summarizes the effects on iliac PWV, MAP, and heart rate. ACh (60 and 120 nmol/min) reduced PWV by 2±4\% \( (P=0.2) \) and 6±4\% \( (P=0.03) \), respectively. During co-infusion of L-NMMA, however, there was no significant change in PWV in response to the higher dose of ACh \((-1±2\% ; P=0.1) \). Compared with co-infusion of saline, the effect of the highest dose of ACh was significantly reduced by co-infusion of L-NMMA \( (P=0.03, \text{ANOVA}) \) (Figure 2).

Infusion of ACh via the femoral artery sheath (120 nmol/min) in 4 sheep did not result in any significant change in iliac PWV \( (3.72±0.57 \text{ versus } 3.75±0.47 \text{ m/s}, P=0.8) \).

Infusion of GTN (2 and 4 nmol/min) via the catheter significantly reduced iliac PWV \( (2±2\% , P=0.04, \text{ and } 5±2\% , P<0.01, \text{respectively}) \). Co-infusion of L-NMMA with GTN (4 nmol/min) also significantly reduced PWV \( (4±3\% , P=0.03) \), but the response did not differ from that before L-NMMA \( (P=0.6, \text{ANOVA}) \).

**Discussion**

Arterial stiffness is an important determinant of cardiovascular risk.\(^1\)\(^2\) Although structural changes in arteries are thought to be a major factor in the age-related increase in arterial stiffness, several lines of evidence suggest that the endothelium may play an important role in the local functional regulation of stiffness by releasing vasoactive substances, such as NO.\(^3\)\(^4\) Application of potassium cyanide to isolated arterial segments alters vessel stiffness,\(^5\) and arterial stiffness can be modulated by vasoconstrictors and vasodilators, including organic nitrates.\(^6\) Moreover, agonists that stimulate endothelial NO production, such as ACh, reduce arterial stiffness in vivo.\(^7\)\(^8\) Furthermore, endothelial dysfunction, characterized by reduced bioavailability of NO, has been demonstrated in a number of cardiovascular risk factors, including hypercholesterolemia and diabetes mellitus,\(^9\) which are themselves associated with increased arterial stiffness,\(^10\) suggesting that NO may provide a link between such risk factors and increased arterial stiffness.

In the present study, we investigated the importance of basal and stimulated NO production in regulating muscular artery distensibility. We have shown, for the first time, that local arterial distensibility is reduced by blockade of endogenous NO synthesis with the NO synthase inhibitor L-NMMA in the ovine common iliac artery. We have also extended previous observations by demonstrating that the increase in arterial distensibility produced by an endothelium-dependent but not endothelium-independent agonist can be substantially inhibited by L-NMMA.

**Basal NO Synthesis**

L-NMMA was first infused distally (sheath) and then proximally (catheter) to the arterial segment under investigation. This design allowed us to control for the effects of changes in flow and reflex activity within the hind-limb vascular bed and determine whether the response to L-NMMA was a direct or an indirect effect. It also negated the need for separate placebo infusions. Infusion of L-NMMA through the sheath resulted in a small, nonsignificant increase in the iliac PWV, probably due to a reduction in blood flow. There was a significant \( \approx 3\% \) increase in the PWV, however, when L-NMMA was infused through the catheter (compared with infusion via the sheath), indicating arterial stiffening. This was not accompanied by any potentially confounding change in MAP or heart rate, confirming that L-NMMA did not have any systemic hemodynamic action.

Removal of the vascular endothelium increases arterial diameter and distensibility,\(^11\) suggesting that the endothelium exerts a “restraining” effect on large-artery stiffness. The vascular endothelium releases a number of vasoactive substances besides NO, however, including endothelin-1 and prostacyclin. Therefore, it is impossible to draw any conclusions regarding the interaction between endothelium-derived NO and large-artery distensibility from such experiments. Nevertheless, previous data concerning the effect of inhibi-
tion of NO synthesis in vivo on arterial stiffness are conflicting but were based on ultrasound-derived indices. We chose to measure PWV because it provides a robust measure of arterial distensibility. Distensibility and PWV are related by the 1922 equation of Bramwell and Hill, which states that PWV is proportional to the square root of distensibility. Our data suggest an ≈6% reduction in distensibility with L-NMMA, other factors being equal, which is a relatively modest change and may have been below the limit of detection by ultrasound. The minimum resolution of our approach was a difference in transit time of 0.1 ms, which equates to a change of ≈0.2% in PWV. The dose of L-NMMA used in the present study (10 μmol/min) was also higher than that used by Joannides et al or Leeson et al (4 μmol/min). Although 4 μmol/min has been widely used in the human forearm, higher doses may well be necessary to inhibit NO production in larger arteries because of higher flow velocities and reduced mixing because of largely laminar blood flow. Indeed, poor mixing of L-NMMA may also explain why conditions that are characterized by reduced NO bioavailability, such as diabetes mellitus, are also associated with increased arterial stiffness at an early stage. Similarly, a 2.7% increase in PWV would be expected to result in an increase of ≈5 mm Hg in pulse pressure. Although this effect is modest, in epidemiological terms, it would be expected to substantially increase cardiovascular risk.

**Stimulated NO Production**

Intra-arterial infusion of ACh reduces large-artery stiffness in humans. The magnitude of this response is reduced in patients with heart failure, who also exhibit reduced resistance-vessel responses to ACh. In neither study was any attempt made to block the effect of ACh with L-NMMA. This is important because ACh stimulates not only endothelial NO production but also the release of a number of other vasodilator substances, including prostacyclin and endothelium-derived hyperpolarizing factor. Therefore, in the present study, we assessed the effect of L-NMMA on the response to ACh as well as GTN, a control endothelium-independent NO donor. As expected, both ACh and GTN produced a dose-dependent reduction in iliac PWV, but only the effect of ACh was inhibited by co-infusion of L-NMMA (Figure 2). The degree of inhibition was ≈80%, which indicates that a substantial proportion of the response to ACh was, indeed, due to NO production. This is in keeping with the relatively greater contribution that NO makes to the vasodilator response to ACh with increasing vessel size. Indeed, the vasodilator response to ACh is almost completely abolished by L-NMMA in coronary and internal mammary arteries but is inhibited by only 40% to 50% in resistance vessels. Importantly, in the present study there was no change in systemic MAP, a major determinant of distensibility, during infusion of ACh or GTN. Moreover, the absence of any change in PWV during infusion of ACh through the femoral sheath excludes the possibility of a change in flow or reflex activity being responsible for the effect of ACh on the iliac artery when infused via the catheter, which is in agreement with previous observations. Although there was a significant reduction in heart rate during co-infusion of ACh and L-NMMA, the change was only 2 bpm, and PWV is not dependent on heart rate.

**Limitations of the Present Study**

The present study was conducted in the ovine iliac artery; therefore, the applicability of the results to human muscular arteries requires confirmation. The ovine and human systemic responses to L-NMMA, however, are similar. The use of general anesthesia may also have influenced our results to some degree, as may the introduction of an arterial catheter. The use of an intravascular catheter to measure PWV, a robust measure of distensibility, however, eliminates inaccuracies in determining the path length and provides a high degree of resolution to detect small but significant differences in transit time. Finally, although distending pressure was constant in the present study (MAP did not change), the observed alterations in distensibility in response to drug infusion have several other potential physiological explanations. These include changes in vessel diameter, wall thickness, or wall stiffness, possibly resulting from an alteration in load distribution to the elastic and collagenous components of the arterial wall accompanying changes in smooth muscle tone. Therefore, we are unable to identify the precise mechanism responsible for changes in distensibility brought about by modulation of the l-arginine–NO pathway.

**Summary**

We have demonstrated, for the first time, that basal NO production influences muscular artery distensibility in vivo and that the effect of ACh on large arteries is mainly NO-dependent. Such findings support the concept of local functional regulation of large-artery stiffness. This may have important implications for the management of patients with increased arterial stiffness, such as those with diabetes mellitus and isolated systolic hypertension, because strategies that improve NO bioavailability or act directly to relax large-artery smooth muscle may prove to be efficient strategies for reducing arterial stiffness and cardiovascular risk.

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