Effects of Inhibition of Basal Nitric Oxide Synthesis on Carotid-Femoral Pulse Wave Velocity and Augmentation Index in Humans

Andrew D. Stewart, Sandrine C. Millasseau, Mark T. Kearney, James M. Ritter, Philip J. Chowienczyk

Abstract—Aortic stiffness, as measured by carotid-femoral pulse wave velocity (PWV), is a powerful, independent predictor of vascular risk. PWV in muscular arteries is influenced by basal nitric oxide (NO) release. It is not known whether NO also influences carotid-femoral PWV. We examined the effects of an NO synthase inhibitor, N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA), on carotid-femoral PWV and aortic augmentation index (AIx, an indirect measure of arterial stiffness). To control for effects of L-NMMA on distending pressure, we used doses of norepinephrine and dobutamine that caused similar changes in mean arterial blood pressure (MAP). Healthy men (32 to 48 years old, n=8) were studied on 4 occasions and received, in random order, vehicle, L-NMMA (3 mg · kg\textsuperscript{-1} by intravenous bolus followed by 3 mg · kg\textsuperscript{-1} · h\textsuperscript{-1}), norepinephrine (50 ng · kg\textsuperscript{-1} · min\textsuperscript{-1}), and dobutamine (2.5 to 10 µg · kg\textsuperscript{-1} · min\textsuperscript{-1}), each for 30 minutes. PWV and AIx were measured by carotid-femoral PWV and radial tonometry, respectively. L-NMMA and norepinephrine increased MAP by 7.8±1.7 and 9.7±2.1 mm Hg, respectively (each P<0.05 vs vehicle) and increased PWV by 0.7±0.2 and 1.0±0.3 m · s\textsuperscript{-1} (each P<0.01 vs vehicle). Dobutamine, at doses that produced a similar increase in MAP (9.6±2.9 mm Hg), increased PWV by 0.8±0.2 m · s\textsuperscript{-1} (P<0.01 vs vehicle). Changes in PWV caused by the 3 pressor agents were closely correlated with changes in MAP (R>0.99, P<0.0001). L-NMMA and norepinephrine increased AIx, but dobutamine decreased AIx (P<0.01 vs norepinephrine and L-NMMA). Effects of inhibition of basal NO release on carotid-femoral PWV can be explained by the change in MAP that this causes rather than any specific effect of NO inhibition within the aorta. (Hypertension. 2003;42:915-918.)

Key Words: aorta ■ elasticity ■ endothelium ■ nitric oxide ■ nitric oxide synthase

Carotid-femoral pulse wave velocity (PWV), a measure of aortic stiffness,\textsuperscript{1} is a powerful, independent predictor of cardiovascular events and mortality.\textsuperscript{2-4} Aortic stiffening increases peak systolic pressure at the aortic root as a result of diminished systemic compliance and early return of pressure waves reflected from the periphery.\textsuperscript{5,6} This provides a possible mechanism for the increased event rate and mortality associated with aortic stiffening. In addition, carotid-femoral PWV might serve as an integrated measure of the impact of cardiovascular risk factors on age-related changes in aortic structure.\textsuperscript{4,7,8} In muscular arteries, stiffness is influenced by mean arterial blood pressure (MAP, a determinant of transmural distending pressure) and by the tone of arterial smooth muscle (which influences the elastic properties of the arterial wall). In the ovine iliac artery, basal release of nitric oxide (NO) contributes to the functional regulation of stiffness.\textsuperscript{9}

The human aorta is rich in elastin, and with advancing age, increasing relative amounts of collagen, but it also contain vascular smooth muscle, especially in the abdomen.\textsuperscript{10} The extent to which aortic stiffness is influenced by vascular tone and in particular, by basal NO, has not previously been examined in the human aorta. The purpose of the present study was to investigate the functional regulation of central arteries in humans as measured by carotid-femoral PWV. We examined the influence of basal NO synthesis with the NO synthase inhibitor N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA). We used norepinephrine and dobutamine to control for effects of L-NMMA on MAP. In addition to PWV, we also measured aortic augmentation index (AIx), an indirect index of vascular stiffness derived from the contour of the aortic pressure waveform.\textsuperscript{5,11} to determine whether changes in AIx occurred in parallel with those of PWV.

Methods

Subjects were healthy, male volunteers (n=8; mean age, 40 years; range, 32 to 48 years). All were normotensive and normocholesterolemic (mean±SD total cholesterol, 5.1±0.4 mmol/L) and had normal renal function (serum creatinine, 102±14 µmol/L). No subject had a history of cardiovascular disease, and none was taking prescription or nonprescription drugs or vitamins. Subjects were studied on 4 separate occasions, each separated by at least 6 days. They received L-NMMA, norepinephrine, dobutamine, and saline...
Effect of Saline, L-NMMA, Norepinephrine (NE), and Dobutamine (DOB) on Hemodynamic Measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline (Baseline)</th>
<th>Saline (Baseline)</th>
<th>L-NMMA (Baseline)</th>
<th>L-NMMA (Baseline)</th>
<th>Norepinephrine (Baseline)</th>
<th>Norepinephrine (Baseline)</th>
<th>Dobutamine (Baseline)</th>
<th>Dobutamine (Baseline)</th>
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<td>116±3</td>
<td>116±4</td>
<td>116±4</td>
<td>117±3</td>
<td>123±4†</td>
<td>116±4</td>
<td>136±6†‡</td>
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<tr>
<td>DBP, mm Hg</td>
<td>67±2</td>
<td>66±2</td>
<td>67±3</td>
<td>74±2†§</td>
<td>66±2</td>
<td>73±3†§</td>
<td>71±3</td>
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<tr>
<td>MAP, mm Hg</td>
<td>83±2</td>
<td>81±3</td>
<td>83±3</td>
<td>88±3‡§</td>
<td>83±2</td>
<td>90±3†§</td>
<td>86±3</td>
<td>92±4‡‡</td>
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<tr>
<td>HR, bpm</td>
<td>61±5</td>
<td>60±5</td>
<td>57±3</td>
<td>51±3‡§</td>
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</tr>
<tr>
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<td>7.4±0.3</td>
<td>7.8±0.3‡§</td>
<td>7.7±0.4</td>
<td>8.3±0.4§</td>
<td>7.6±0.2</td>
<td>8.1±0.3§</td>
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<tr>
<td>AIX, %</td>
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<td>5±5*</td>
<td>10±3</td>
<td>15±3‡§</td>
<td>7±3</td>
<td>14±4†§</td>
<td>9±3</td>
<td>2±3†‡</td>
</tr>
</tbody>
</table>

See text for drug dosages and routes of administration. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; and HR, heart rate. All other abbreviations are as defined in text. Values are mean±SE.

*P<0.05, †P<0.01 vs baseline values; ‡P<0.05, §P<0.01 for change from baseline relative to saline vehicle.

Results

Hemodynamic measurements during infusion of saline and vasopressor drugs are summarized in the Table. During infusion of saline, MAP did not change significantly relative to baseline (−2.8±1.9 mm Hg), although there was a tendency for blood pressure to fall during the course of the study. Analysis of responses to drugs was performed relative to saline vehicle. L-NMMA and norepinephrine increased MAP by a similar extent (7.8±1.7 and 9.7±2.1 mm Hg, respectively; each P<0.05; Figure 1). For doses of dobutamine that produced a similar increase in MAP (9.6±2.9 mm Hg), L-NMMA, norepinephrine, and dobutamine all increased PWV (0.7±0.2, 1.0±0.3, and 0.8±0.2 m·s⁻¹ respectively; each P<0.01). There was no significant difference between the effects of L-NMMA, norepinephrine, and dobutamine on PWV. When all doses of dobutamine were included, with the change in MAP incorporated as a covariate, there was no

Analysis

Results were summarized as mean±SE. PWV and AIx responses to the dose of dobutamine that produced an increase in MAP closest to that produced by L-NMMA in that subject were used for the primary analysis. Differences between responses to the vasoactive drugs relative to saline vehicle were sought by repeated-measures ANOVA. In addition, the influence of the drug on PWV, with all doses of dobutamine, with the change in MAP incorporated as a covariate was examined by a general linear model (SPSS version 11.5). P<0.05 was considered significant, and all tests were 2-tailed.

Figure 1. Changes from baseline (relative to saline vehicle) in MAP (ΔMAP), aortic PWV (ΔPWV), and aortic AIx (ΔAIx) during intravenous infusion of L-NMMA (3 mg·kg⁻¹ bolus; 3 mg·kg⁻¹·h⁻¹), norepinephrine (NE, 50 ng·kg⁻¹·min⁻¹), and equipressor doses of dobutamine (DOB, 2.5 to 5 μg·g⁻¹·min⁻¹). *P<0.05, **P<0.01.
significant effect of drug class on PWV. By contrast, there was a close association between change in PWV and change in MAP (R > 0.99, P < 0.0001), for mean change in PWV vs mean change in MAP in each class of drug; Figure 2). L-NMMA reduced heart rate (−6.1 ± 1.1 beats per minute [bpm], P < 0.01), whereas norepinephrine and dobutamine (2.5 to 5 μg · kg⁻¹ · min⁻¹) did not. L-NMMA and norepinephrine increased AIx relative to saline (10.2 ± 1.9% and 13.2 ± 4.4%, respectively, relative to saline; each P < 0.05), whereas dobutamine did not (−4.4 ± 3.2% for equipressor doses; P = NS). The effects of L-NMMA and norepinephrine on AIx differed from that of dobutamine (each P < 0.01).

**Discussion**

Stiffness of muscular arteries is strongly influenced by transmural pressure and hence by MAP (because pressure on the adventitial surface of the artery is usually negligible). In the brachial artery, transmural pressure can be modulated by the application of an external blood pressure cuff, thus allowing effects of vasoactive drugs to be determined independently of the effects on MAP. Studies with this technique show that, independent of transmural pressure, brachial artery elasticity is sensitive to changes in smooth muscle tone induced by NO: the NO donor nitroglycerine decreases stiffness, and inhibition of basal NO synthesis by L-NMMA increases stiffness. Direct infusion of L-NMMA into the sheep iliac artery increased the stiffness of this musculoelastic artery without a significant effect on MAP, suggesting that this artery is also influenced by basal NO release.

Less is known regarding the influence of vascular tone on the functional regulation of large arteries such as the thoracic aorta. This is important, because there are marked differences between muscular and elastic arteries with respect to aging and the association between stiffness and cardiovascular risk. Muscle arteries show little increase in stiffness with age in contrast to elastic arteries. Age-related stiffening of elastic arteries, particularly the aorta, is accelerated in the presence of risk factors for cardiovascular disease. Aortic stiffness, estimated from carotid-femoral PWV, by contrast with the stiffness of muscular arteries, is highly predictive of cardiovascular events and mortality. In the present study, we examined the effects of inhibition of basal NO synthesis on carotid-femoral PWV in healthy men with the use of norepinephrine and dobutamine to control for the effects on MAP.

L-NMMA increased blood pressure, as expected. L-NMMA increased carotid-femoral PWV equivalent to ~8 years of aging, as estimated from the relation found by Avolio et al. However, the increase in carotid-femoral PWV was similar to that produced by equipressor (MAP) doses of norepinephrine and dobutamine. Norepinephrine increases blood pressure mainly through an increase in peripheral resistance, whereas dobutamine, a β₁-selective agonist, acts mainly as a positive inotrope and dilates rather than constricts arterial smooth muscle. An increase in PWV in response to norepinephrine could be caused by an increase in smooth muscle tone in the aorta. However, the increase in PWV in response to norepinephrine was similar to that obtained with dobutamine, which does not constrict vascular smooth muscle. The similarity of the PWV response to the vasopressor agents with diverse mechanisms on the heart and vasculature and the close correlation between change in PWV and change in MAP, irrespective of the drug used, strongly suggest that MAP is the most important determinant of short-term changes in carotid-femoral PWV.

An additional aim of the present study was to examine the effects of NO synthase inhibition on AIx. AIx has been used as an index of arterial stiffness but is also influenced by ventricular ejection and by the tone of muscular arteries, which influences pressure wave reflection. In the present study, we observed a disassociation between the effects of vasopressor drugs on AIx and carotid-femoral PWV. In agreement with previous studies, L-NMMA and norepinephrine increased AIx, whereas dobutamine, a β₁-selective agonist, acts mainly as a positive inotrope and dilates rather than constricts vascular smooth muscle. By contrast and despite a similar increase in MAP and PWV to that produced by L-NMMA and norepinephrine, dobutamine decreased AIx. This novel finding is not entirely unexpected for an arterial vasodilator in the context of earlier observations that AIx can change independently of aortic PWV, and this reinforces the need for caution in the interpretation of AIx during pharmacologic interventions, as stressed by O’Rourke et al. The different effect of dobutamine on AIx compared with the other 2 pressors could have resulted from a relaxant effect of dobutamine on muscular arteries, thereby reducing pressure wave reflection. An alternative or additional explanation could be increased myocardial contractility, leading to a more rapid rise in aortic pressure in systole. This would increase separation of direct and reflected components of the pressure wave and decrease augmentation of early systolic pressure by pressure wave reflection.

**Limitations of the Study**

There are several limitations to this study. Carotid-femoral PWV reflects vascular stiffness in both the aorta and iliac artery. The iliac artery is more muscular than the aorta, and it is possible that these arteries differ in the extent to which smooth muscle tone influences stiffness. We cannot exclude an influence of endogenous NO release on local stiffness in the iliac artery if this were much less than the passive influence of distending pressure in the aorta. To detect this would require local administration of L-NMMA, as described...
in sheep.9 The importance of carotid-femoral PWV is that it is easily measured and is strongly predictive of coronary artery disease events in humans.2,3

In addition, L-NMMA, norepinephrine, and dobutamine influence systolic blood pressure, diastolic blood pressure, and MAP differently. Systolic blood pressure is determined mainly by stroke volume and aortic stiffness.26 Dobutamine, a positive inotrope, is expected to increase stroke volume and hence, systolic blood pressure more than norepinephrine and L-NMMA (which is a negative inotrope). Given their differing modes of action, it was not possible to cause similar changes in systolic, diastolic, and mean arterial pressure with all 3 agents. Doses of L-NMMA, norepinephrine, and dobutamine were therefore chosen to give similar effects on MAP, a measure of the mean distending pressure throughout the cardiac cycle.

Last, there was a difference in the heart rate response to L-NMMA and the other pressor drugs of ≈6 bpm. PWV exhibits some dependence on heart rate,27 but this occurs only at heart rates >70 bpm, when a change in heart rate of 6 bpm would produce a change in PWV of <0.3 m·s⁻¹. The average heart rate during drug infusions ranged from 51 to 61 bpm. It is unlikely, therefore, that the difference in heart rate for the different drugs had a substantial effect on PWV.

Perspectives

In conclusion, short-term inhibition of basal NO release has an effect on carotid-femoral PWV similar to that of ≈8 years of vascular aging. This is similar to the effects of equipressor (for MAP) doses of norepinephrine and dobutamine, suggesting that the change in MAP accounts for these short-term changes in PWV. There is no evidence of any additional, specific effect of NO inhibition on carotid-femoral PWV. There is a disassociation between the effects of vasoactive drugs on PWV and AIx, consistent with their differing actions on the heart and vascular smooth muscle.

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References


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