Nitric Oxide Regulates Basal Systemic and Pulmonary Vascular Resistance in Healthy Humans

Jonathan S. Stamler, MD; Evan Loh, MD; Mary-Anne Roddy, BSN; Kristen E. Currie, BS; Mark A. Creager, MD

Background The endothelium synthesizes and releases a relaxing factor with the physiochemical properties of nitric oxide (NO). However, the role of endothelium-derived NO in the basal regulation of systemic and pulmonary vascular resistance in humans is not known. Our primary objectives were to determine the effects of inhibiting NO synthesis on blood pressure and systemic vascular resistance and to establish the role of endothelium-derived NO in the regulation of normoxic pulmonary vascular tone.

Methods and Results We studied the systemic and pulmonary hemodynamic effects of N\textsuperscript{\textcircled{O}}-monomethyl-L-arginine (L-NMMA, 0.03 to 1.0 mg · kg\textsuperscript{-1} · min\textsuperscript{-1} IV), an NO synthase inhibitor, in 11 healthy volunteers, aged 33 ± 2 years. An arterial cannula and a pulmonary artery catheter were placed in each subject to measure blood pressure, pulmonary artery pressure, and pulmonary capillary wedge pressure. Cardiac output was determined by the Fick technique, and systemic and pulmonary vascular resistances were calculated. Serum NO levels (free and protein bound) were measured by chemiluminescence in 5 subjects. Six of the subjects also received phenylephrine (25 to 100 μg/min IV) to compare the cardiac hemodynamic effects of L-NMMA with those of a direct-acting vasoconstrictor. L-NMMA caused dose-dependent increases in both blood pressure and systemic vascular resistance. At the highest dose of L-NMMA, there was a 15.5 ± 1.3% increase in mean blood pressure and a 63.4 ± 8.2% increase in systemic vascular resistance (each P < .01). Pulmonary vascular resistance increased 39.8 ± 9.4% (P < .01), whereas mean pulmonary artery pressure did not change. Administration of L-NMMA also reduced cardiac output by 27.8 ± 2.9% and stroke volume by 15.4 ± 3.5% (each P < .01). Serum NO levels decreased 65 ± 10% from basal values (P < .05), confirming inhibition of endogenous NO production. Phenylephrine increased blood pressure to a level comparable to that observed with L-NMMA. The decline in stroke volume was greater with L-NMMA than with phenylephrine (P < .01).

Conclusions This study demonstrates that basal release of endothelium-derived NO is directly involved in the determination of systemic vascular resistance and, therefore, blood pressure in healthy humans. In addition, NO regulates basal normoxic pulmonary vascular tone. The complex hemodynamic effects of NO are composite properties of its actions on systemic and pulmonary vascular resistance and cardiac function.

Key Words • endothelium • relaxing factors • blood pressure • N\textsuperscript{\textcircled{O}}-monomethyl-L-arginine

The endothelium plays a central role in the dynamic regulation of vascular tone by synthesizing and releasing a relaxing factor with the physiochemical properties of nitric oxide (NO) or a closely related substance.\textsuperscript{1,2} The biosynthesis of NO in endothelial cells occurs in response to diverse physiological stimuli that perturb intracellular calcium flux.\textsuperscript{3,4} As a result, the amino acid L-arginine is converted enzymatically to NO through oxidation of its guanidine nitrogen.\textsuperscript{3,5} The elucidation of this biochemical mechanism has led to the development of arginine analogues, such as N\textsuperscript{\textcircled{O}}-monomethyl-L-arginine (L-NMMA), which stereospecifically inhibit NO production in vascular tissue.\textsuperscript{3,5} Recent studies have shown that systemic infusion of L-NMMA in laboratory animals increases blood pressure, suggesting that tonic release of NO contributes to vascular resistance in the systemic arterial bed.\textsuperscript{6-10} Although these observations are supported in humans by the finding that limb vascular resistance increases during intrabrachial-arterial infusion of L-NMMA,\textsuperscript{11} inhibitors of NO synthesis have not been administered systemically to healthy subjects. Therefore, the role of endothelium-derived NO in the basal regulation of blood pressure homeostasis is not known.

The pulmonary vasculature is a unique low-pressure system with a special capacity to adapt to local changes in blood flow. Both pulmonary arterial and venous endothelia secrete NO constitutively and in response to a variety of physiological and chemical stimuli.\textsuperscript{12-14} It is well established that impairment of endogenous NO release, whether due to structural endothelial disease or induced pharmacologically, is associated with enhanced responsiveness to vasoconstrictor stimuli in pulmonary vessels.\textsuperscript{14-17} There also is a growing consensus that endothelial dysfunction may contribute to the pathogenesis of pulmonary hypertension.\textsuperscript{13,14,16} Notwithstanding the potential importance of endogenous NO in modulation of pulmonary vascular reactivity, its effects on normoxic basal tone have yielded conflicting results, and species specificity exists.\textsuperscript{10,18-31} Thus, the fundamental question as to whether endothelium-derived NO contributes to the maintenance of normal pulmonary tone in humans remains unanswered.
In this study we administered L-NMMA, in graded fashion, to healthy volunteers. Our primary objectives were to determine the effects of inhibiting NO synthesis on blood pressure and systemic vascular resistance (SVR) and to establish the role of endothelium-derived NO in the regulation of basal normoxic pulmonary vascular tone.

Methods

Subjects
Eleven healthy volunteers participated in this study, including 7 men and 4 women. Their ages ranged from 25 to 41 years and averaged 33±2 years. Health status was determined by history, physical examination, and laboratory analysis to exclude individuals with cardiac or pulmonary disease, hypertension, dyslipidemia, diabetes mellitus, or hematological, renal, or hepatic dysfunction. Only 1 subject smoked cigarettes. None of the subjects were taking any medications. This study was approved by the Human Research Committee of Brigham and Women's Hospital, and each subject gave written informed consent.

Hemodynamic Measurements
Each subject was studied in a 23°C temperature-controlled room in the postabsorptive state. Alcohol, caffeine, and cigarettes were all prohibited within 12 hours of the study. Under local anesthesia and sterile conditions, a 9F Cordis sheath with a side arm was placed percutaneously into an internal jugular vein. A single-lumen, balloon-tipped pulmonary artery catheter was then advanced under pressure guidance to the pulmonary artery to measure mean right atrial (RAP), pulmonary artery (PAP), and pulmonary capillary wedge (PCWP) pressures. In addition, a 20-gauge polyethylene catheter was inserted into a brachial or radial artery of each subject for determination of systolic (SBP), diastolic (DBP), and mean (MBP) blood pressures. Each catheter was attached to a Gould P23 transducer. Zero reference was estimated to be 5 cm vertically beneath the sternal angle of Lewis. Heart rate (HR) was determined from an ECG signal and calculated from the RR interval. All hemodynamic measurements were recorded on a Gould 4600 Physiologic Recorder (Gould, Inc.). Oxygen consumption (\(\dot{VO}_2\)) was measured directly by having the subject breathe into an MRM-2 oxygen consumption monitor (Waters Instruments, Inc.). Arterial and mixed venous oxygen saturations were determined in duplicate from blood withdrawn from the pulmonary artery and brachial or radial artery catheters and assayed with a co-oximeter (CIBA-Corning Diagnostics Corp.). Oxygen content was calculated as oxygen saturation×hemoglobin (g/dL)×1.36 (milliliter of O\(_2\) per gram of hemoglobin)×10. Cardiac output (CO) was determined by the Fick method and calculated as the quotient of \(\dot{VO}_2\) to arterial oxygen content minus mixed venous oxygen content. Stroke volume (SV) was calculated as CO/HR. Cardiac index (CI) and stroke volume index (SVI) were determined by adjusting for surface area (BSA) such that CI=CO/BSA and SVI=SV/BSA. Systemic vascular resistance (SVR) was calculated as [(MBP−RAP)/CO]×80, and pulmonary vascular resistance (PVR) was calculated as [(PAP−PCWP)/CO]×80; each is expressed as dynes per second per cm\(^2\).

Experimental Protocol
All subjects rested at least 30 minutes after catheter placement to establish a stable baseline before data collection. During the control period, hemodynamic measurements were repeated every 10 minutes until stable. During this period, vehicle (dextrose 5%) was infused via the side arm of the internal jugular vein sheath. To assess the effect of endothelium-derived NO on basal SVR and PVR, we administered L-NMMA (Calbiochem, Inc) in dosages of 0.01, 0.03, 0.1, 0.3, and 1.0 mg·kg\(^{-1}\)·min\(^{-1}\), each for 3 minutes (by which time the maximal response was achieved), delivered at a rate of 3.9 mL/min, via the side arm of the central venous catheter sheath. The dose range of L-NMMA used systemically in this study was based on knowledge derived from experiments in which L-NMMA was infused intra-arterially at dosages of approximately 1 mg/min for 5 to 20 minutes into the human forearm in our laboratory32 and by others31 and is comparable to that studied in patients with septic shock.32 Hemodynamic measurements were made and blood was collected for arterial and mixed venous oxygen saturation at the end of each infusion period. Steady state was verified by repeating hemodynamic measurements 10 minutes after the last dose of L-NMMA. To compare the hemodynamic effects of L-NMMA with those of a direct-acting vasoconstrictor, 6 of the subjects also received phenylephrine via the central venous catheter at dosages of 25, 50, 75, and 100 μg/min each for 3 minutes at a rate of 0.3 to 1.3 mL/min. Hemodynamic measurements were made and blood was collected for measurements of oxygen saturation after each dose was administered. The phenylephrine infusion was terminated if MBP increased by 20%. Two of the subjects received L-NMMA and phenylephrine on separate days, whereas in 4 subjects, both phenylephrine and L-NMMA were administered in sequence on the same day. In this latter group of subjects, baseline conditions were reestablished before L-NMMA was administered. No adverse effects were observed in any patient during infusion of either L-NMMA or phenylephrine.

Plasma NO Determination
The predominant bioactive reservoir of NO in human plasma is bound to protein thiol groups, and these levels correlate well with endogenous NO production.34 Plasma NO levels were measured in 5 subjects at baseline (before L-NMMA) and immediately after administration of the highest dose of L-NMMA. Platelet-poor plasma was first obtained by centrifugation at 800g for 10 minutes, and plasma NO, free in solution and liberated from protein thiol by photolysis, was assayed by photolysis-chemiluminescence methodology as previously described.34

Statistical Analysis
Results are presented as mean±SEM. Single-factor repeated-measures ANOVA followed by a Scheffé's post-hoc test was used to evaluate the effect of each drug. A repeated-measures ANCOVA was used to compare the hemodynamic responses to L-NMMA and phenylephrine infusions while allowing for correlation due to repeated measures. A paired Student's t test was used to compare plasma NO levels before and after administration of L-NMMA. Statistical significance was accepted at the 95% confidence interval (P<.05).

Results
Effect of L-NMMA on Hemodynamic Measurements
L-NMMA caused dose-dependent increases in both blood pressure and SVR (each P<.01 by ANOVA) (Table). At the highest dose of L-NMMA, there was a 15.5±1.3% increase in MBP and a 63.4±8.2% increase in SVR (Fig 1). The effects on SVR and blood pressure were sustained throughout a 10-minute period of equilibration after termination of the L-NMMA infusion. A dose-dependent increase in PVR also occurred (P<.01 by ANOVA), whereas mean PAP remained essentially unchanged. A plateau (or maximal) effect in the pulmonary dose-response to L-NMMA was not achieved because we chose not to give higher doses in light of limiting systemic hypertension. At the highest dose of L-NMMA, there was a 39.8±9.4% increase in PVR.
Hemodynamic Effects of L-NMMA in Healthy Subjects

<table>
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<tr>
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<th>L-NMMA Dose, mg · kg⁻¹ · min⁻¹</th>
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<tr>
<td></td>
<td>Baseline</td>
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<tr>
<td>SBP</td>
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<tr>
<td>DBP</td>
<td>67±3</td>
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<tr>
<td>MBP</td>
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<td>MPAP</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>SVI</td>
<td>58±4</td>
</tr>
<tr>
<td>SVR</td>
<td>1057±69</td>
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<tr>
<td>PVR</td>
<td>50±9</td>
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L-NMMA indicates N⁵-monomethyl-L-arginine; SBP, systolic blood pressure (mm Hg); DBP, diastolic blood pressure (mm Hg); MBP, mean blood pressure (mm Hg); MPAP, mean pulmonary artery pressure (mm Hg); PCWP, pulmonary capillary wedge pressure (mm Hg); HR, heart rate (beats per minute); CI, cardiac index (L · min⁻¹ · m⁻²); SVI, stroke volume index (mL · min⁻¹ · m⁻²); SVR, systemic vascular resistance (dyne · s⁻¹ · cm⁻⁵); and PVR, pulmonary vascular resistance (dyne · s⁻¹ · cm⁻⁵).

\( P<.01 \) (Fig 2). There was no demonstrable change in PCWP at any dose of L-NMMA.

Administration of L-NMMA resulted in a dose-dependent decline in CI and SVI in all subjects; at the highest dose of L-NMMA, CI decreased by 27.8±2.9% \( (P<.01) \) and SVI decreased by 15.4±3.5% \( (P<.01) \). HR also decreased significantly by 14.2±3.1% \( (P<.01) \).

Plasma NO Levels

As measured by photolysis-chemiluminescence, L-NMMA reduced plasma NO levels by 65±10% \( (P<.05) \), confirming inhibition of endogenous NO production (Fig 3).

Comparative Effects of Phenylephrine and L-NMMA on Cardiac Hemodynamics

The decline in CO and SV in response to L-NMMA may have occurred as a consequence of increased afterload, from withdrawal of sympathetic nervous system activity as a baroreflex response to the increase in systemic blood pressure, or as a direct effect of L-NMMA on cardiac pump function. To distinguish among these possibilities, phenylephrine, an \( \alpha \)-adrenoceptor agonist with systemic and pulmonary vasoconstrictor properties, was infused to achieve a graded increase in systemic blood pressure, resulting in levels similar to those observed with L-NMMA. Both agents reduced CI. However, at comparable levels of blood pressure, the decrease in CI tended to be greater with L-NMMA than with phenylephrine (Fig 4). At the dose of L-NMMA causing a 10 mm Hg increase in SBP, CI decreased 0.75±0.18 L · min⁻¹ · m⁻², whereas a similar increase in blood pressure with phenylephrine reduced CI only 0.56±0.18 L · min⁻¹ · m⁻² \( (P=.08, \) L-NMMA versus phenylephrine). With L-NMMA, the decline in CI occurred because both HR and SVI decreased (Table). Phenylephrine, however, reduced HR at the 50-, 75-, and 100-\( \mu \)g/min dosages (from 63±3 to a nadir of 54±3 beats per minute, \( P<.01 \)) but did not affect SVI at any dose (from a basal value of 52±1 to a nadir of 51±1 mL · min⁻¹ · m⁻², \( P=NS \)). At the doses of L-NMMA and phenylephrine causing a 10 mm Hg increase in blood pressure, SVI decreased 5.5±2.0 and

![Fig 1. Plot of the effect of \( N⁵ \)-monomethyl-L-arginine (L-NMMA) on mean blood pressure (MBP) and systemic vascular resistance (SVR) in 11 healthy subjects. L-NMMA caused a dose-dependent increase in both MBP and SVR. Values are expressed as mean±SEM. *\( P<.05 \), **\( P<.01 \) for each data point compared with baseline value.](image1)

![Fig 2. Plot of the effect of \( N⁵ \)-monomethyl-L-arginine (L-NMMA) on mean pulmonary artery pressure (MPAP) and pulmonary vascular resistance (PVR) in 11 healthy subjects. Values are expressed as mean±SEM. PVR increased significantly only at the L-NMMA dosage of 1.0 mg · kg⁻¹ · min⁻¹. **\( P<.01 \) compared with baseline value. There was no significant change in MPAP.](image2)
The mechanism(s) by which low pressure and vascular resistance are maintained in the pulmonary circulation are poorly understood. The finding that L-arginine analogues increase basal tone in prestimulated pulmonary vascular rings (16, 18) and modulate responsiveness to constrictor stimuli (12, 14) has led to the hypothesis that endothelium-derived NO release is an important etiological factor. However, studies in laboratory animals are divergent with regard to the importance of the effect of endogenous NO on basal pulmonary vascular tone. In the cat, (19, 29) lamb, (15, 27) and ovine fetus, (21) inhibition of the L-arginine--NO pathway is associated with an increase in vascular tone, whereas either variable or no effect has been observed in the rat, (23, 29) rabbit, (24, 28) and dog. (18, 20, 25) In light of species differences, the physiological role of NO in basal regulation of pulmonary tone in humans has remained uncertain. Notably, we observed an increase in PVR in response to systemic infusion of L-NMMA without an effect on cardiac filling pressures, indicating that NO release is an important determinant of resting pulmonary artery tone in humans. The lack of change in PAP may be explained by the concomitant decrease in CO. In addition, most recent data suggest that L-NMMA is a relatively ineffective inhibitor of NO synthase in pulmonary blood vessels, (37) findings compatible with the more pronounced effects seen in the systemic circulation and with the growing evidence for enzyme inhibitor specificity. (3, 5)

**Effect of L-NMMA on CO**

Systemic inhibition of NO production was associated with a decline in CO and SV. Similar results have been reported in dogs, rats, and sheep. (8-10, 20, 38) The most plausible explanations include a baroreceptor reflex response to increases in arterial pressure, depression of SV due to increased afterload (without concomitant change in cardiac filling pressures), a basal myocardial NO requirement for normal contractile function, and myocardial ischemia due to coronary vasoconstriction. We exclude the last possibility with reasonable confidence as continuous ECG monitoring did not reveal ischemic changes and all subjects were free of chest pain; furthermore, intracoronary L-NMMA infusions in dogs (38) and humans (39) have not been associated with ischemia. It seems likely that the increase in blood pressure initiated a baroreceptor reflex, resulting in withdrawal of sympathetic efferent activity and augmentation of vagal activity, since HR decreased. The decrease in CO, however, seemed greater than we might have anticipated simply from withdrawal of sympathetic activity. We queried whether the increased afterload depressed SV. To address these possibilities further, phenylephrine was infused to increase arterial pressure to a level comparable to that resulting from L-NMMA. We observed a modest decline in CO and no significant change in SV in the subjects who received phenylephrine. Indeed, the decrease in SV was substantially less than that which occurred with comparable pressor doses of L-NMMA. Our findings are consistent with a report in intact dogs in which depression of CO by L-NMMA exceeded that observed with phenylephrine. (38) These observations suggest that the effect of L-NMMA on cardiac function is multifactorial and cannot be explained solely by the increase in blood pressure. Recognizing that the comparisons made are limited by the
Effect of L-NMMA on Plasma NO Levels

We have recently shown that NO exists in human plasma predominantly in adduct form with the thiol group of albumin.44 Most recent data derived from plasma and other systems suggest that levels of S-nitrosothioli are in the range of 0.1 to 0.5 μM, approximately two orders of magnitude higher than free NO.45 These S-nitrosoproteins possess vasorelaxant activity at physiologically relevant concentrations and are envisioned to play a bufferlike function, serving as a source and a sink for free NO to modulate rapid changes in vasomotor tone.34,47 In rabbits, infusion of L-NMMA is associated with a decrease in plasma NO content that parallels the increase in blood pressure.34 That we demonstrate the same for humans in this study both confirms the inhibition of endogenous NO synthesis and supports the notion that S-nitrosoprotein levels may be used to monitor changes in NO production in other diverse pathophysiological states.

Conclusions

Our findings have several fundamentally important implications. The conclusion that NO is continuously released to maintain normal pulmonary artery tone, systemic arterial pressure, and CO suggests that compromise of the normal constitutive mechanisms of NO synthesis may have direct pathophysiological consequences. As proposed by Moncada and colleagues,48 certain classes of (essential) hypertension may be better classified as states of “hypovasodilatation”; a defect in NO production in certain patients with hypertension is consistent with this proposal. By analogy, alterations in NO synthesis transport or signaling may be important etiological factors in the development of pulmonary “hypertension,” consequent cor pulmonale, and even compromise in left ventricular systolic function. Importantly, the endothelium and endocardium are not the sole sources of constitutive NO production.3-5 Thus, in addition to dysfunction of the endothelium, primary abnormalities of the “nitrinergic” (nonadrenergic, noncholinergic) nervous system47 and perhaps of the platelet or neutrophil48 deserve consideration in the pathogenesis of abnormal vascular reactivity and cardiac pump function.

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