A Large Blood Pressure–Raising Effect of Nitric Oxide Synthase Inhibition in Humans

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Abstract—In experimental animals, systemic administration of nitric oxide synthase (NOS) inhibitors causes large increases in blood pressure that are in part sympathetically mediated. The aim of this study was to determine the extent to which these conclusions can be extrapolated to humans. In healthy normotensive humans, we measured blood pressure in response to two NOS inhibitors, N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME), the latter of which recently became available for use in humans. The major new findings are 3-fold. First, L-NAME produced robust increases in blood pressure that were more than 2 times larger than those previously reported in humans with L-NMMA and approximated those seen in experimental animals. L-NAME (4 mg/kg) raised mean arterial pressure by 24±2 mm Hg (n=27, $P<0.001$), whereas in subjects who received both inhibitors, a 12-fold higher dose of L-NMMA (50 mg/kg) raised mean arterial pressure by 15±2 mm Hg (n=4, $P<0.05$ vs L-NAME). Second, the L-NAME–induced increases in blood pressure were caused specifically by NOS inhibition because they were reversed by L-arginine (200 mg/kg, n=12) but not D-arginine (200 mg/kg, n=6) and because N^G-nitro-D-arginine methyl ester (4 mg/kg, n=5) had no effect on blood pressure. Third, in humans, there is an important sympathetic component to the blood pressure–raising effect of NOS inhibition. α-Adrenergic blockade with phentolamine (0.2 mg/kg, n=9) attenuated the L-NAME–induced increase in blood pressure by 40% ($P<0.05$). From these data, we conclude that pharmacological inhibition of NOS causes large increases in blood pressure that are in part sympathetically mediated in humans as well as experimental animals. (***Hypertension*. 1999;33:937-942.**)

Key Words: nitric oxide ■ blood pressure ■ L-NMMA ■ L-NAME ■ D-NAME ■ arginine ■ adrenergic receptor blockers

Pharmacological inhibitors of nitric oxide (NO) synthesis, such as N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME), are firmly established to produce both acute and chronic hypertension in many animal species.[1–6] Although this experimental NO-deficient hypertension at first was attributed solely to inhibition of endothelium-dependent vasodilation,[1,7] there is increasing evidence of an important sympathetic neural component.[5,6,8–12] The concept is that neuronally produced NO is part of the signal transduction pathway involved in the restraint of brainstem sympathetic vasomotor outflow and that inhibition of such restraint leads to neurogenic hypertension. The combination of inhibited endothelium-dependent vasodilation plus augmented sympathetic vasoconstriction helps to explain the remarkable severity and chronicity of experimental NO-deficient hypertension.

A major unanswered question is the extent to which these conclusions can be extrapolated from animals to humans. In normotensive humans, unlike many animal species, administration of the specific NO synthase (NOS) inhibitor L-NMMA has produced only small increases in blood pressure of ≈10 mm Hg.[13–16] If this were the maximum elevation in blood pressure that could be achieved by pharmacological NOS inhibition in healthy humans, the NO pathway must be far less important as a regulator of blood pressure in humans than in animals. One possibility is a fundamental species difference. For example, in humans, the NO pathway, although mediating endothelium-dependent vasodilation,[7,17–19] may have little or no effect on sympathetic control of blood pressure.[15,16] Another possibility is that the apparent differences between animal and human studies are not due to species but rather to inadequate NOS inhibition resulting from the low doses of L-NMMA used in humans.

Accordingly, the aim of this study was to address two related questions. First, in normotensive humans, is endogenous NO synthesis such a powerful regulator of blood pressure that transient pharmacological inhibition of this protective mechanism produces a sizable increase in blood pressure? Second, if so, is there an important sympathetic neural component? So as not to underestimate the blood pressure–raising effect of systemic NOS inhibition in the humans, we measured blood pressure during incremental intravenous doses of both L-NMMA and L-NAME, the latter of which is a more potent NOS inhibitor that has only recently

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become available for use in humans. To address the issue of sympathetic mediation of a hypertensive response to NOS inhibition, we tested the degree to which the acute increase in blood pressure is reversed by α-adrenergic blockade.

Methods

General Procedures

Investigational New Drug numbers were obtained from the US Food and Drug Administration for the administration of L-NMMA, L-NAME, N^G-nitro-D-arginine methyl ester (D-NAME), L-arginine, and D-arginine to human subjects. All protocols were approved by the Institutional Review Board of the University of Texas Southwestern Medical Center, and all subjects (28 male and 8 female volunteers, age 21 to 45 years) gave informed written consent to participate. All procedures were in accordance with institutional guidelines.

With the subject supine, blood pressure was measured with an automated sphygmomanometer (Welch Allyn), and the values were averaged over 5-minute periods. Mean arterial pressure (MAP) was calculated as diastolic pressure plus one-third of the pulse pressure. Electrocardiographic recordings were obtained for continuous measurement of heart rate and ST segments.

Drugs

L-NMMA acetate, L-NAME HCl, D-NAME HCl, and D-arginine HCl were purchased from Clinalfa; L-arginine HCl, from Pharmacia; and phentolamine, from CIBA-GEIGY.

Specific Protocols

Protocol 1: Dose-Response Relation Between L-NMMA and Blood Pressure (25 experiments, 5 subjects)

To begin to explore the dose-response relationship between L-NMMA and blood pressure, in our initial series of experiments we measured blood pressure before, during, and after 4 doses of L-NMMA (3, 6, 9, and 12 mg/kg) or vehicle (30 mL of saline), with each dose being infused intravenously over 15 minutes. Each dose of L-NMMA (or vehicle) was administered on separate days, with the order random and the subjects blinded.

Protocol 2: Head-to-Head Comparison of Blood Pressure–Raising Effects of L-NMMA and L-NAME (24 experiments, 12 subjects)

The aim of this protocol was to compare directly the effects of increasing doses of these 2 NOS inhibitors in the same subjects. L-NMMA and L-NAME were administered in random order to the same subjects. First, in 8 subjects on 2 separate days at least 4 days apart, blood pressure responses to intravenous L-NMMA (12 mg/kg over 30 minutes) and L-NAME (2 mg/kg over 30 minutes) were determined. Second, in 4 subjects on 2 separate days at least 4 days apart, blood pressure responses to intravenous L-NMMA (50 mg/kg over 120 minutes) or L-NAME (4 mg/kg over 60 minutes plus 60 minutes of recovery) were determined.

Protocol 3: Time Course and Specificity of Blood Pressure–Raising Effect of L-NAME (42 experiments, 27 subjects)

We measured blood pressure and heart rate before, during, and up to 120 minutes after L-NAME (4 mg/kg, n=27), D-NAME (4 mg/kg, n=5), or vehicle (30 mL of saline, n=6) was infused intravenously over 60 minutes.

To document that NOS inhibition is the specific mechanism underlying an L-NAME–induced increase in blood pressure, at 120 minutes after infusion of L-NAME, we administered either L-arginine (n=12), the natural substrate of NOS, or D-arginine (n=6), the inactive stereoisomer. To control for nonspecific effects, L-arginine (n=6) or D-arginine (n=4) was also infused without prior L-NAME administration (L-arginine given 120 minutes after saline, the vehicle for L-NAME). Both L-arginine and D-arginine were infused intravenously as a 10% solution over 15 minutes to a total dose of 200 mg/kg.

Protocol 4: Effects of α-Adrenergic Receptor Blockade on L-NAME–Induced Increase in Blood Pressure (27 experiments, 9 subjects)

The results of protocols 2 and 3 indicated that blood pressure continues to rise after completion of L-NAME infusion, and this led us to hypothesize that α-adrenergic vasoconstriction contributes to this late elevation in blood pressure, which would be analogous to our findings in rats.[6] To test this, we determined the extent to which the L-NAME–induced increase in blood pressure was sensitive to reversal by α-adrenergic blockade with intravenous phentolamine (0.1 mg/kg infused over 2 minutes, followed by 0.1 mg/kg infused over 10 minutes). In 9 subjects, each studied on 3 separate days, phentolamine was infused either immediately after completion of L-NAME (4 mg/kg) infusion, 90 minutes after L-NAME (4 mg/kg) infusion, or under basal conditions (ie, without L-NAME).

Statistical Analysis

For comparisons of data series containing 1 or 2 measurements, Student's *t* test was used. For comparisons of data series containing >2 measurements, univariate ANOVA for repeated measures, with repeated measures on 1 (time) or 2 factors (time and treatment) was used. Where relevant, multiple comparisons were performed with contrast analysis using the Bonferroni adjustment of the significance level, which was set at $P<0.05$. Results are mean±SE.

Results

L-NAME Causes Larger Increases in Blood Pressure Than L-NMMA

In our initial experiments, the lowest dose of L-NMMA (3 mg/kg) had no effect on MAP (ΔMAP, 3±2 mm Hg; P=NS); 3 higher doses of L-NMMA (6, 9, and 12 mg/kg) all caused significant ($P<0.05$) but modest increases in MAP (8±2, 9±1, and 9±1 mm Hg, respectively). When, in additional subjects, the high dose of L-NMMA (12 mg/kg) was given over 30 minutes, the increase in blood pressure (ΔMAP, 9±1 mm Hg) was similar to the increase observed in the same subjects after a low dose of L-NAME (2 mg/kg) (ΔMAP, 10±1 mm Hg) (Table 1, top). When we increased the total L-NMMA dose to 50 mg/kg and the infusion time to 120 minutes, the pressor response was higher during the second hour of infusion, but when directly compared with the response 120 minutes after the start of L-NAME (4 mg/kg given over the first 60 minutes), the peak increase in MAP was more pronounced with L-NAME than with L-NMMA (ΔMAP, 23±3 vs 15±2 mm Hg ($P<0.05$) (Table 1, bottom).

NOS Inhibition Mediates Large Blood Pressure–Raising Effect of L-NAME

L-NAME (4 mg/kg) produced large and sustained increases in blood pressure that in each subject peaked between 60 and 120 minutes after completion of the infusion (Table 2 and Figure 1). In contrast, neither saline (vehicle) nor D-NAME had any effect on blood pressure (Figure 1). A dose of L-arginine that had no effect on baseline blood pressures largely reversed the L-NAME–induced increase in blood pressure, whereas D-arginine had no effect (Figure 2 and Table 2).

TABLE 1. Summary Data at Baseline and After L-NAME and L-NMMA Infusion

		L-NAME			L-NMMA	
Parameter	Baseline	2 mg/kg 30 min	4 mg/kg 120 min	Baseline	12 mg/kg 30 min	50 mg/kg 120 min
n=8						
Systolic pressure, mm Hg	106±2	113±3*		107±3	113±3*	
Diastolic pressure, mm Hg	64±1	76±3*		60±2	69±2*	
Mean arterial pressure, mm Hg	78±1	88±3*		75±2	84±2*	
Heart rate, bpm	61±1	49±1*		60±2	49±2*	
n=4						
Systolic pressure, mm Hg	105±3		127±6*†	101±2		113±3*
Diastolic pressure, mm Hg	63±2		87±4*†	63±3		79±3*
Mean arterial pressure, mm Hg	77±2		100±5*†	76±2		91±3*
Heart rate, bpm	62±1		46±2*†	62±2		53±1*

*$P<0.001$, baseline vs L-NAME or L-NMMA.
†$P<0.05$, L-NAME vs L-NMMA.

Blood Pressure–Raising Effect of L-NAME Is Partially Reversed by α-Adrenergic Blockade

A dose of phentolamine that had no effect on baseline blood pressures reversed 40% of the peak increase in blood pressure after L-NAME infusion, thereby eliminating the additional late increment in blood pressure occurring after completion of L-NAME infusion (Figure 3). In contrast, when phentolamine was administered immediately after L-NAME (early) rather than 90 minutes after L-NAME (late), a much smaller reduction in MAP was observed (ΔMAP, early vs late, −3±1 vs −8±1 mm Hg; $P=0.02$) (Figure 3).

Symptoms Were Minimal

With L-NMMA, there were no symptoms. With L-NAME, the reported side effects were transient nausea in 5 subjects and fatigue in 8 of the 35 subjects receiving L-NAME on 1 or more occasions. No subject reported headache or chest discomfort, and no ST segment changes were observed. In all subjects, blood pressures returned to baseline within 24 hours of L-NAME administration.

Discussion

Compared with the wealth of data from experimental animals, there are large gaps in our understanding of the effects of NOS inhibitors on blood pressure regulation in humans. The recent availability of a new potent NOS inhibitor, L-NAME, provided a new opportunity to probe the blood pressure–raising effect of systemic NOS inhibition in human subjects. The major new findings are 3-fold. First, L-NAME produces robust increases in blood pressure that are more than 2 times larger than those previously reported in humans with L-NMMA and that approximate those seen in experimental animals. Second, the L-NAME–induced increases in blood pressure were caused specifically by NOS inhibition because they were reversed by L-arginine but not D-arginine and because D-NAME had no effect on blood pressure. Third, in humans, there is an important sympathetic component to the blood pressure–raising effect of acute NOS inhibition. α-Adrenergic blockade with phentolamine attenuated the L-NAME–induced increase in blood pressure by 40%. From these data, we conclude that in normotensive humans, pharmacological inhibition of NOS causes large increases in blood pressure that are in part sympathetically mediated.

The blood pressure–raising effect of L-NAME was 2 to 3 times greater than that previously reported in similar studies using L-NMMA.[13–16] When we directly compared these 2 NOS inhibitors, we found L-NMMA to be less potent and to have a much flatter dose-response relation. One possible explanation is that human endothelial cells enzymatically degrade L-NMMA to L-arginine, which would oppose the pressor effect.[20] Regardless of the precise explanation, our data suggest that in humans, the use of relatively low doses of

TABLE 2. Summary Data at Baseline, After L-NAME Infusion, During Recovery, and After Subsequent L-Arginine or D-Arginine Infusion

			Recovery			
Parameter	Baseline	L-NAME 4 mg/kg	60 min	120 min	+ L-Arginine 200 mg/kg	+ D-Arginine 200 mg/kg
Subjects, n	27	27	27	18	12	6
Systolic pressure, mm Hg	108±2	121±2*	127±3*	123±3*	112±2†	130±5*
Diastolic pressure, mm Hg	63±1	83±2*	90±2*	86±2*	68±2*†	90±5*
Mean arterial pressure, mm Hg	78±1	96±2*	102±2*	99±2*	83±2*†	103±5*
Heart rate, bpm	62±2	48±1*	49±1*	50±1*	57±3†	45±2*

*$P<0.01$, baseline vs L-NAME, recovery, L-arginine, and D-arginine.
†$P<0.001$, L-arginine vs D-arginine.

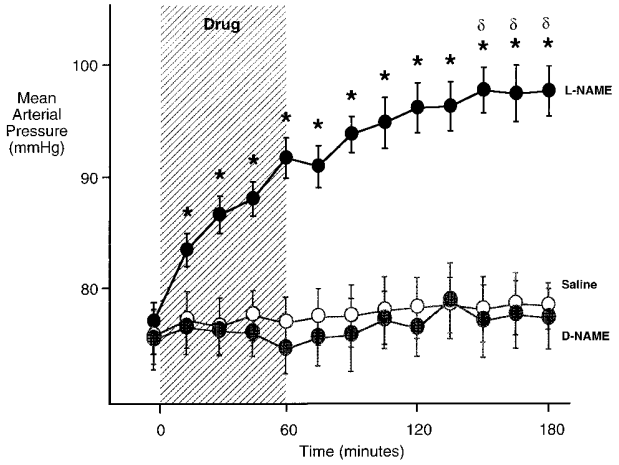

Figure 1. Summary data showing the time course of the blood pressure effects of L-NAME, D-NAME, and saline (vehicle). MAP continued to increase for 2 hours after the end of L-NAME infusion (black symbols, n=14; all subjects in whom blood pressures were obtained at all time points were included). D-NAME (gray symbols, n=5) and vehicle (white symbols, n=6) had no effect on blood pressure. The shaded area denotes the infusion period for all 3 substances. *$P<0.001$ vs time 0 (baseline). δ$P<0.01$ vs time 60 minutes.

L-NMMA has led to an underestimation of the true blood pressure–raising potential of systemic NOS inhibition.

In our normotensive human subjects, L-NAME acutely increased blood pressures into the hypertensive range.[21] The largest effect of L-NAME was on diastolic blood pressure, which exceeded 85 mm Hg in 74%, 90 mm Hg in 52%, and 100 mm Hg in 19% of subjects. Diastolic blood pressure did not exceed 109 mm Hg in any subject, and in all subjects, blood pressure returned to baseline values by 24 hours without any deleterious side effects. Given the large hypertensive effect seen with L-NAME at a single dose of 4 mg/kg, it would not have been appropriate to ascertain the maximum increase in blood pressure or the duration of the hypertensive effect that might be achieved with prolonged administration of higher doses. However, the similarity in the data obtained in the present human study with those obtained in several animal studies[2,5,6,22] suggests that in humans, prolonged pharmacological inhibition of NOS would likely produce marked chronic hypertension.

Because L-NAME is a competitive NOS inhibitor, the ease with which its robust hypertensive effect could be reversed by exogenous L-arginine provides the first evidence that the elevated blood pressure was a specific consequence of NOS inhibition. Although L-arginine has been reported to exert nonspecific effects on blood pressure,[23,24] the doses used were more than double those used in our study. We documented the specificity of L-arginine in our experiments by showing that the L-NAME-induced increase in blood pressure was reversed by a low dose of L-arginine that had no effect on baseline blood pressure and unaffected by the same dose of D-arginine. L-NAME also has been reported to exert nonspecific effects on vascular regulation. For example, the alkyl ester moiety has been shown to have affinity for muscarinic receptors in experimental animal preparations,[25] with a resultant weak antimuscarinic effect of L-NAME in vitro[25,26]; however, 2 recent animal studies specifically designed to address this issue could find no evidence of antimuscarinic effects of L-NAME in vivo.[27,28] In our human experiments, we provided further evidence of the specificity of L-NAME by documenting that the same dose of D-NAME had no effect on blood pressure.

An unexpected finding was that in each subject, blood pressure continued to rise for 2 hours after completion of L-NAME infusion. We suspected that this late increase in blood pressure might be sympathetically mediated because our previous rat studies indicated a similar delay in the onset of a sympathetic component to L-NAME-induced increases in blood pressure. Thus, in rats, sympathectomy has no effect on

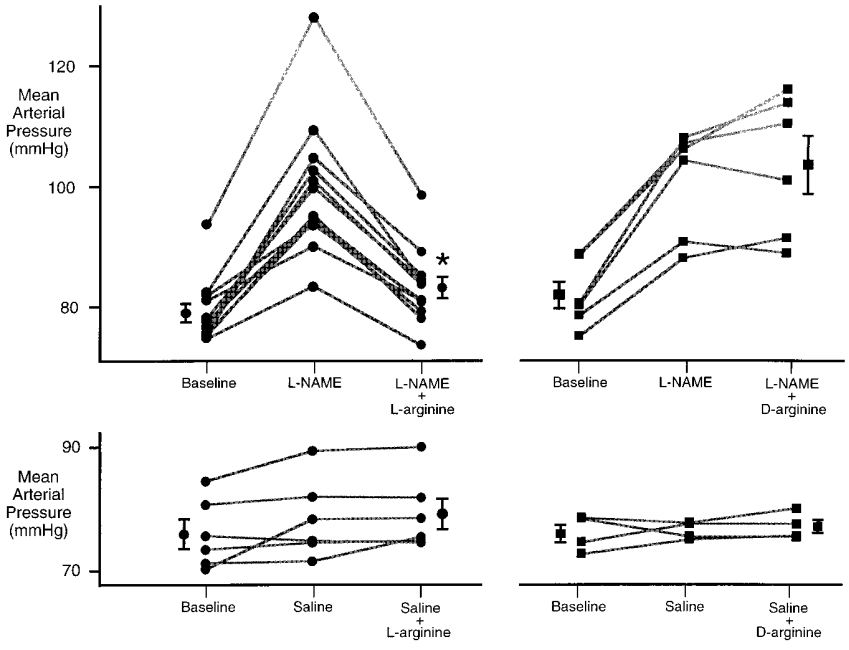

Figure 2. Individual and summary data showing effects of L-NAME and saline (vehicle for L-NAME) plus L-arginine or D-arginine on MAP. The L-NAME–induced increase in blood pressure was reversed by L-arginine (*$P<0.001$) but not by D-arginine.

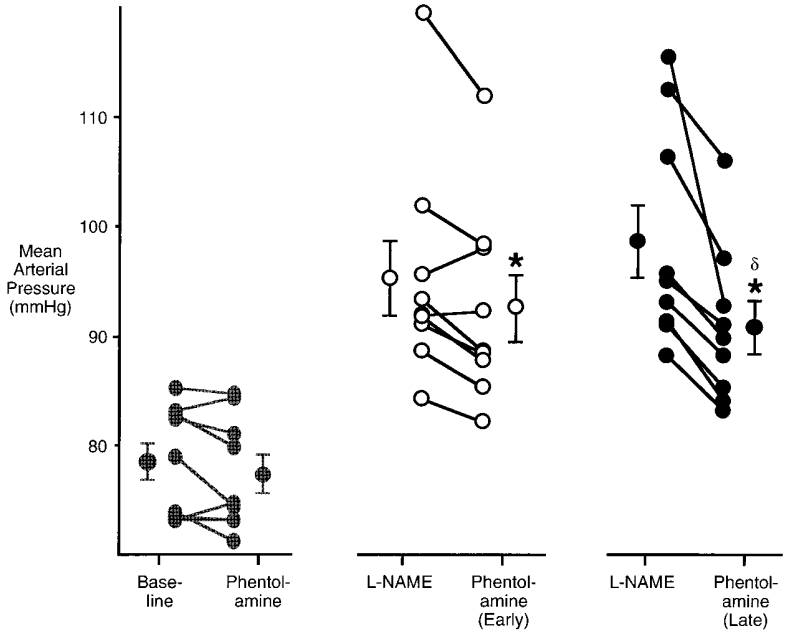

Figure 3. α-Adrenergic component to the blood pressure–raising effect of L-NAME. Individual and summary data showing that phentolamine had no effect on baseline arterial pressure (left, gray symbols) and very little effect immediately after L-NAME infusion (phentolamine early, center, white symbols) but reversed a substantial part of the increase in arterial pressure seen 90 minutes after L-NAME infusion (phentolamine late, right, black symbols). *$P<0.05$ vs before phentolamine. δ$P<0.05$ vs phentolamine response at baseline and vs phentolamine response immediately after L-NAME infusion.

the initial pressor response during the first hour of L-NAME infusion but attenuates the hypertensive response 2 hours later.[6] Similarly, in our human subjects, α-adrenergic blockade had little effect on the initial pressor response to L-NAME even though it eliminated the additional increase in blood pressure over the next 2 hours. The underlying mechanism for a delayed onset of a sympathetic component to L-NAME–induced hypertension is unknown but may be related in part to the time required for systemically administered L-NAME to cross the blood-brain barrier and inhibit NOS in the relevant neuronal pools.[29,30]

The major new concept arising from our studies in conscious rats[5,6] and this study in humans is that, although inhibition of endothelium-dependent vasodilation is the primary mechanism underlying the initiation of the hypertensive response to L-NAME, the sympathetic nervous system plays an important role in the full expression and maintenance of this large blood pressure–raising effect. Because of the delayed onset of the sympathetic component, which contributes to L-NAME–induced hypertension in rats, we have suggested[6] that previous animal studies overlooked a sympathetic component to acute L-NAME–induced hypertension by examining only the first hour of blood pressure response to L-NAME.[6,31] For the same reason, we now suggest that the previous microneurographic studies in humans,[15,16] including our own, underestimated the sympathoexcitatory response to systemic NOS inhibition by examining only the first hour of sympathetic nerve response to a less potent NOS inhibitor, L-NMMA.

In conclusion, the results of the present experiments suggest that in normotensive humans, NO synthesis is such a powerful regulator of blood pressure that transient pharmacological inhibition of this protective mechanism produces large increases in blood pressures. If NO deficiency is shown to be an important cause of human hypertension, as hypothesized,[5,6,11,17–19,32–39] the NO pathway would represent a target for novel pharmacological[37] or gene-based[40] treatment of human hypertension.

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