The Hypotensive Effect of L-Arginine Is Associated With Increased Expired Nitric Oxide in Humans*

Sanjay Mehta, MD; Duncan J. Stewart, MD; and Robert D. Levy, MD, FCCP

Endothelial metabolism of L-arginine to L-citrulline and the potent vasodilator, nitric oxide (NO), is important in the regulation of vascular tone and resting BP. L-arginine improves abnormal endothelium-dependent vasodilation in the setting of hypercholesterolemia and has a vasodilatory effect in normal vessels, effects presumed to be mediated through increased endogenous NO production, although this has not been established by direct measurement of NO. In a randomized, placebo-controlled, crossover trial, 10 healthy male subjects received a 30-min infusion of 0.5 g/kg L-arginine hydrochloride. Subjects underwent continuous monitoring of BP and heart rate (HR) as well as intermittent determination of mixed expired NO concentration and plasma L-arginine and L-citrulline levels. Infusion of L-arginine produced a significant fall in mean BP with a peak effect of -9.3±0.9% (p<0.005). The hemodynamic effects of L-arginine were associated with an increase in mixed expired NO concentration (FeNO) of 55±15% (p<0.005) from 15±2 to 21±3 parts per billion (ppb) and an increase in the rate of pulmonary NO excretion of 118±45% (p<0.005), as well as a rise in plasma L-citrulline from 25±4 to 46±5 pmol/L (p<0.005). There was a significant correlation between the hypotensive response to L-arginine and the increase in expired NO (r=-0.68, p<0.05). The hypotensive effect of L-arginine in humans appears to be mediated, at least in part, by NO synthase metabolism of L-arginine and increased endogenous NO production as indicated both by increased plasma L-citrulline and by increased expired NO. (CHEST 1996; 109:1550-55)

Key words: blood pressure; endothelium; endothelium-derived relaxing factor; nitric oxide synthase; vasodilation

Abbreviations: ANOVA=analysis of variance; eNOS=endothelial NOS; FeNO=concentration of NO in expired gas, in parts per billion; L-NMMA=N\(^6\)-monomethyl-L-arginine; NO=nitric oxide; NOS=NO synthase; ppb=parts per billion; VeNO=rate of pulmonary NO excretion in expired gas, in pmol/kg/min

The metabolism of L-arginine to the potent vasodilator, nitric oxide (NO), by NO synthase (NOS) in the vascular endothelium may be essential in the regulation of resting BP.\(^1\) The importance of a basal NO-dependent dilatory influence on vascular tone has been demonstrated through the use of competitive NOS inhibitors such as N\(^6\)-monomethyl-L-arginine (L-NMMA). Parenteral infusion of L-NMMA in animals produces acute hypertension\(^1,2\) and long-term NOS inhibition leads to chronic systemic hypertension.\(^4\) As well, endothelium-dependent vasodilation has been found to be impaired in essential hypertension in humans, suggesting that endothelial dysfunction and inadequate NO production may be important in the pathogenesis of this disorder.\(^5,6\)

The parenteral administration of L-arginine reverses the acute hypotensive effect of NOS inhibition in animals\(^1,7,8\) and has an acute hypotensive effect in humans.\(^8,9\) These hemodynamic effects have been presumed to be the result of increased L-arginine metabolism by endothelial NOS (eNOS) and increased NO production. Recently, several groups have used a very sensitive chemiluminescence technique to measure extremely low, physiologic levels of NO in the parts per billion (ppb) range in the expired gas of animals and humans.\(^10,11\) Thus, we hypothesized that if the hemodynamic effects of L-arginine were mediated by increased endogenous NO production, this should
be reflected by an increase in expired NO. The specific aim of the present study was to determine the effect of L-arginine infusion on expired NO in humans and to relate changes in pulmonary NO excretion to the hemodynamic and metabolic effects of L-arginine.

MATERIALS AND METHODS

Study Population

Ten healthy, nonsmoking, white men (34±2 years; mean±SEM) were studied. Three subjects with a smoking history (total exposure, 5 to 10 pack years) had stopped smoking at least 1 year before the study. None of the subjects had a history of hypertension or other cardiovascular disease, nor were they taking any cardiovascular medications. Moreover, none of the subjects had a history suggestive of asthma, and results of baseline spirometry were normal in all subjects (FEV1, 98±1% predicted). The study protocol was approved by an Institutional Clinical Research Ethics Committee and written, informed consent was obtained from all subjects.

Study Protocol

Each subject received sequential 30-min infusions, by peripheral vein, of 0.5 g/kg L-arginine hydrochloride (100 mg/mL; approximately 350 mOsm/L; pH, 5.0 to 6.5) and an equal volume of 1.4% saline solution (approximately 350 mOsm/L; pH, 5.5 to 7.0) in single-blinded, random order. A 90-min recovery period followed the L-arginine infusion. Subjects remained seated during the entire study period.

BP was measured noninvasively via an automated cuff, while heart rate (HR) and arterial oxygen saturation were monitored continuously by digital pulse oximetry. At 15-min intervals during the protocol, mixed expired gas was sampled in triplicate for determination of expired NO while subjects breathed compressed medical air (NO concentration <5 ppb) through a mouthpiece and nonrebreathing two-way valve (model 2700; Hans Rudolph Co; Kansas City, Mo) with noseclips applied. Expiratory flow was measured by pneumotachography and integrated to yield tidal volume and respiratory rate.

NO Determination

Mixed expired NO was determined using a chemiluminescence NO analyzer (NOA 270B; Sievers Medical Instruments Inc; Boulder, Colo), the principle of which has been described in detail elsewhere.12 Briefly, a photomultiplier tube produces an electrical signal proportional to the light generated from a chemical reaction between NO in the sample and ozone in the analyzer. The NO analyzer was calibrated using a reference gas of known NO concentration (Medigs Inc; Montreal, Quebec, Canada). Expired NO is expressed as both the concentration of NO in expired gas (FeNO) in ppb and as the rate of pulmonary NO excretion (VeNO) in pmol/kg/min, calculated as the product of the expired NO concentration and minute ventilation.

Metabolic Studies

Peripheral venous blood was sampled at baseline and every 30 min during hypertonic saline solution infusion, L-arginine infusion, and the recovery period for determination of serum electrolytes, urea, creatinine, and glucose. In addition, plasma concentrations of free L-arginine and L-citrulline were determined by high-pressure liquid chromatography with an amino acid analyzer (119CL; Beckman Instruments Inc; Palo Alto, Calif).

Statistics

Data are expressed as mean values±SEM. Serial hemodynamic and metabolic values with time were analyzed with a repeated measures analysis of variance (ANOVA; SigmaStat; Jandel Scientific; San Rafael, Calif). Significant differences between individual time points were assessed with a Student-Newman-Keuls t test where appropriate. Correlations between hemodynamic and metabolic changes were performed using linear regression analysis by the least-squares method. A two-tailed p value <0.05 was considered statistically significant.

RESULTS

Hemodynamic Effects of L-arginine

Infusion of exogenous L-arginine reduced systolic, mean, and diastolic BP in all subjects with a peak effect on mean BP of -9.3±0.9% (p<0.005) at 32±7 min after start of the L-arginine infusion (Fig 1). Administration of L-arginine was also associated with a slight increase in HR from 69±2 to 73±3 beats/min (p<0.05) coincident with the nadir in mean BP. After termination of L-arginine infusion, BP returned to baseline.

Figure 1. Time course of changes in mean BP, expired NO (VeNO), and plasma L-citrulline during infusion of hypertonic saline solution and L-arginine. Although the hypertonic saline solution control (open circles) had no effect, a 30-min infusion of L-arginine (filled circles) was associated with a reduction in mean BP and increases in expired NO and plasma L-citrulline. L-arginine’s effects were transient, as all parameters returned to baseline during the recovery period. Asterisk=p<0.05 by repeated measures ANOVA and Student’s t test.
levels within 15 min and then remained stable during the recovery period.

Effect of L-Arginine on Expired NO

The baseline mixed expired NO (FeNO) was 14±2 ppb, yielding a calculated pulmonary NO excretion (VeNO) of 88±17 pmol/kg/min. L-arginine infusion was associated with an increase in expired NO in all subjects (Figs 1 and 2), with peak increases in FeNO of 55±15% (p<0.005) and VeNO of 118±45% (p<0.005) between 60 and 75 min after start of the L-arginine infusion. Infusion of hypertonic saline solution had no effect on mean expired NO for the group (Fig 1), although in two individuals, expired NO changed markedly in opposing directions (Fig 2). Minute ventilation also increased by 28±10% from 10.6±1.3 to 13.0±1.5 L/min (p<0.05) following L-arginine infusion but did not change with hypertonic saline solution. The mean arterial oxygen saturation remained stable at 97±1% during both hypertonic saline solution and L-arginine infusions.

Metabolic Effects of L-Arginine

The mean baseline plasma L-arginine concentration was 72±7 μmol/L (normal range for our laboratory, 54 to 134 μmol/L). During L-arginine infusion, the plasma level increased to a peak of 7,640±646 μmol/L (p<0.001) at 30 min and then fell quickly during the recovery period. As well, the plasma L-citrulline level increased from 25±4 to 46±5 μmol/L (p<0.005) at 59±4 min after start of the L-arginine infusion (Fig 1).

Hypertonic saline solution had no metabolic effects but L-arginine infusion produced slight increases in serum potassium level from 4.5±0.1 to 4.9±0.1 mmol/L (p<0.005), serum chloride level from 106±1 to 112±1 mmol/L (p<0.005), and urea nitrogen level from 5.3±0.6 to 7.3±0.5 mmol/L (p<0.005). There was also a mild decrease in the blood glucose level from 6.5±0.4 to 5.0±0.1 mmol/L (p<0.005) and the production of a mild metabolic acidosis with a fall in serum bicarbonate level from 25±1 to 25±1 mmol/L (p<0.005) from baseline to post-L-arginine, respectively.

Metabolic-Hemodynamic Correlations

The hemodynamic response to L-arginine correlated significantly with the increase in expired NO concentration (r=−0.68; p<0.05) but not with the change in VeNO (r=−0.20; p=NS), which reflects changes in both expired NO and minute ventilation. The fall in mean BP with L-arginine did not correlate with either the peak plasma levels or the change in the plasma levels of L-arginine or L-citrulline.

Adverse Effects

The peak hypotensive effect of L-arginine was associated with transient feelings of lightheadedness in one subject and nausea and presyncope in another, all of which resolved spontaneously within 2 min after cessation of L-arginine administration. Moreover, two subjects experienced local irritation and pruritus at the infusion site, one during L-arginine and the other during hypertonic saline solution infusion.

Discussion

This report confirms an important acute systemic hypotensive effect of L-arginine infusion in normal humans. The hemodynamic action of L-arginine was associated with an increase in expired NO and an increase in the plasma level of L-citrulline, suggesting that L-arginine-induced vasodilation is mediated, at least in part, by NOS metabolism and increased NO production.

Exogenous L-arginine, the substrate for NOS, produces vasodilation and improves abnormal endotheli-
um-dependent vasodilation in the setting of hypercholesterolemia both in animals\textsuperscript{13,14} and in humans.\textsuperscript{15} We have reported previously an important pulmonary vasodilatory effect of L-arginine in patients with pulmonary hypertension.\textsuperscript{16} Furthermore, a systemic hypotensive effect of parenteral L-arginine in pharmacologic doses, equal in magnitude to the hemodynamic effect in the present report, has been described previously in humans.\textsuperscript{5}

Only indirect evidence exists in support of NOS metabolism as the presumed mechanism of L-arginine's hemodynamic effects \textit{in vivo}. For example, as in the present report, the production of L-citrulline, a coproduct of NOS metabolism of L-arginine to NO, has been used as a surrogate marker of NOS activity.\textsuperscript{16-18} In addition, both L-arginine's hemodynamic effects and L-citrulline production may be inhibited by substituted L-arginine compounds that are competitive NOS inhibitors.\textsuperscript{3,17-19} The stereospecificity of L-arginine's vascular effects in many studies also suggests a specific, enzyme-dependent mechanism,\textsuperscript{13,14,20} although this stereospecificity may be lost at higher doses.\textsuperscript{21,22} However, the role of NO in mediating L-arginine-induced vasodilation has not yet been confirmed by the direct measurement of NO or its metabolites during L-arginine administration.

The direct measurement of NO in the expired gas of animals and humans using a highly sensitive chemiluminescence technique was first described by Gustafsson et al\textsuperscript{10} in 1991. Despite numerous reports, the site of production of expired NO still remains uncertain. Although some evidence suggests that expired NO derives from either bronchial epithelial\textsuperscript{23,24} or alveolar sources,\textsuperscript{11} very high levels of NO have been observed in the nasopharynx and sinuses, raising the possibility that autoinhalation contributes to expired NO.\textsuperscript{25-27}

The baseline expired NO in our subjects was comparable to published data in normal humans.\textsuperscript{10,11} The observed increase in expired NO with L-arginine infusion is also consistent with the findings of a recent study in which smaller doses of oral L-arginine increased expired NO in humans, although no hemodynamic effect was observed by this group.\textsuperscript{28} The significant and parallel increases in both expired NO and plasma L-citrulline during L-arginine infusion are consistent with the metabolism of exogenous L-arginine by NOS. Furthermore, the increase in expired NO correlated significantly with the magnitude of the systemic hypotensive response to L-arginine. These observations suggest that the increase in expired NO associated with L-arginine infusion may have been derived directly from increased NO production in the systemic vasculature, similar to the increase in expired NO reported with infusion of the NO-releasing nitrovasodilator, nitroglycerin.\textsuperscript{29} It is also possible that the observed changes in expired NO resulting from L-arginine infusion reflect, but do not necessarily derive from, changes in systemic NOS activity.

The increase in expired NO and the hypotensive response to L-arginine infusion demonstrated in the present study indicate that eNOS may be substrate limited \textit{in vivo}. Our findings are consistent with a study on cultured endothelial cells, in which production of NO, reflected by nitrite levels, was submaximal at physiologic L-arginine levels, increased dose dependently with increasing extracellular L-arginine levels, and was not saturated at an L-arginine concentration of 2.5 mmol/L.\textsuperscript{30} Endothelial cell NO production may be enhanced more significantly by supraphysiologic L-arginine levels under certain conditions, for example, in the presence of physiologic levels of L-glutamine.\textsuperscript{31,32} Moreover, L-arginine potentiates endothelium-dependent vascular responses and also directly produces significant vasodilation in the forearm vasculature of healthy human subjects,\textsuperscript{30} indicating the responsiveness of eNOS to L-arginine levels \textit{in vivo}. This physiologic substrate limitation of eNOS may be related to the subcellular compartmentalization of NOS or to complex regulatory mechanisms for L-arginine intracellular transport and metabolism,\textsuperscript{33} for example, circulating endogenous inhibitors of NOS\textsuperscript{34} or inhibitors of endothelial cell resynthesis of L-arginine from L-citrulline.\textsuperscript{31,32}

Despite the significant correlation between the hypotensive response to L-arginine and the increase in expired NO, no such relationship was found between changes in expired NO and plasma levels of L-arginine or L-citrulline. The supraphysiologic increase in plasma L-arginine levels by approximately 100-fold following L-arginine infusion was likely greater than that necessary to saturate all L-arginine-binding receptor and transport sites, and thus a lack of correlation of peak L-arginine levels with either hemodynamic or metabolic (eg, expired NO) end points was not surprising. The lack of correlation between these changes in either BP or expired NO with changes in plasma L-citrulline highlights the presence of alternate pathways for metabolism of L-arginine, as well as the possibility of other NO-independent mechanisms for some of L-arginine's effects. As we have shown previously, much of the exogenous L-arginine is likely metabolized through the hepatic urea cycle, which also yields L-citrulline.\textsuperscript{16} Thus, changes in peak levels of L-citrulline may reflect metabolism of L-arginine through both the NOS pathway and the hepatic urea cycle, and therefore may not correlate directly with measures of NO production or with the hemodynamic effects of L-arginine.

Despite an apparent relationship between expired NO and systemic NOS activity, there was a discrepancy
in the timing of the peak hypotensive effect of exogenous L-arginine and the time to peak changes in expired NO and plasma L-citrulline. A similar discrepancy was observed previously between the timing of peak pulmonary vasodilation with L-arginine infusion in patients with pulmonary hypertension and the time to peak plasma L-citrulline levels. This timing discrepancy and the lack of correlation between some of the hemodynamic and metabolic effects of L-arginine suggest that, at higher doses, parts of the hemodynamic actions of L-arginine may not be dependent on substrate loading of eNOS and increased endothelial NO production. L-arginine may have vascular related effects to release of other hormones such as glucagon, prolactin, growth hormone, and insulin. For example, L-arginine stimulates insulin release, as supported by the observed decline in blood glucose level, and increased plasma insulin levels may have a vasodilatory effect, such as during intra-arterial insulin infusion in man. However, release of insulin is unlikely to fully explain L-arginine’s hemodynamic effects, as only L-arginine appears to potentiate endothelium-dependent vasodilatory responses, whereas both L- and D-arginine stimulate insulin release. Similarly, L-arginine may have other direct, NO-independent hemodynamic effects that are mediated through release of histamine, a potent vasodilator in the forearm vascular bed.

Other factors may also have modified expired NO levels during L-arginine infusion. For example, an effect of minute ventilation on pulmonary NO excretion has been described, consisting of a linear increase in NO excretion with increasing ventilation. However, this effect is small over the physiologic range of resting ventilation. As well, although L-arginine infusion was associated with a significant increase in minute ventilation, this change was small relative to the change in the pulmonary NO excretion rate (28% ± 10% vs 118% ± 45%, respectively; p < 0.01) and is therefore unlikely to account for the rise in expired NO associated with L-arginine infusion. It is also unlikely that the increase in expired NO was the result of increased endothelial shear stress due simply to volume loading during L-arginine infusion, as no significant change in expired NO was observed with the saline solution control of equal tonicity and volume.

In contrast to saline solution infusion, L-arginine was associated with minor metabolic changes that may have altered expired NO, although to our knowledge, there are no published data to suggest this. For example, infusion of L-arginine, as the hydrochloride salt, induced a mild metabolic acidosis, perhaps partly responsible for the stimulation of increased minute ventilation. In addition, as previously recognized, L-arginine infusion is associated with an increase in blood urea nitrogen level, a mild elevation in serum potassium level, and both increases and decreases in blood glucose levels.

In summary, parenteral L-arginine has an acute systemic hypotensive effect in normal humans, which appears to be at least partly mediated by increased endogenous NO production, presumably by systemic vascular eNOS. In addition, our findings are consistent with the emerging concept that changes in expired NO, amenable to direct and noninvasive monitoring, may be a useful reflection of changes in systemic NO production. This method might be useful in further investigation of the various pathophysiologic roles postulated for the L-arginine-NO pathway.

ACKNOWLEDGMENTS: The authors gratefully acknowledge the technical expertise of the staff of the Desmond N. Stoker Pulmonary Function Laboratory of the Royal Victoria Hospital.

REFERENCES

CHEST / 109 / 6 / JUNE, 1996  1555