Pulmonary Vasodilator Response to Vagal Stimulation Is Blocked by N\textsuperscript{\(\omega\)}-Nitro-L-arginine Methyl Ester in the Cat

T.J. McMahon, J.S. Hood, and P.J. Kadowitz

The effect of N\textsuperscript{\(\omega\)}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of endothelium-derived relaxing factor production, on the vasodilator response to efferent vagal stimulation was investigated in the pulmonary vascular bed of the intact-chest cat under conditions of controlled blood flow and constant left atrial pressure. When pulmonary vascular tone was increased with U46619, efferent vagal stimulation decreased lobar arterial pressure in a stimulus-frequency-dependent manner. The decreases in lobar arterial pressure were enhanced by pretreatment with reserpin, were blocked by atropine, and were not altered by propranolol, indicating that the neurogenic vasodilator response was cholinergic in nature. The decreases in lobar arterial pressure in response to vagal stimulation and to exogenously administered acetylcholine were reduced after administration of L-NAME (100 mg/kg i.v.). Although L-NAME decreased pulmonary vasodilator responses to vagal stimulation and to acetylcholine, responses to adenosine, nicorandil, lemakalin, isoproterenol, prostaglandin E\textsubscript{1}, sodium nitroprusside, and 8-bromo-cGMP, agents that act by a variety of mechanisms, were not decreased. These results are consistent with the hypothesis that efferent vagal stimulation releases acetylcholine, which dilates the pulmonary vascular bed by stimulating the production of nitric oxide or a labile nitroso compound from L-arginine. (Circulation Research 1992;70:364–369)

Endothelium-derived relaxing factor (EDRF), first described in 1980 by Furchgott and Zawadski, is now believed to be nitric oxide (NO) or a labile nitroso compound. NO is released from the amino acid precursor L-arginine in cultured endothelial cells, and N\textsuperscript{\(\omega\)}-monomethyl-L-arginine (L-NMMA) in a stereospecific manner inhibits NO synthesis from L-arginine.\textsuperscript{2,3} L-NMMA has been shown to increase systemic vascular resistance and inhibit responses to endothelium-dependent vasodilators.\textsuperscript{4,5} In recent studies, N\textsuperscript{\(\omega\)}-nitro-L-arginine (nitroarginine, L-NA) has been shown to be more potent than L-NMMA in inhibiting NO synthesis.\textsuperscript{6,7} L-NA and nitroarginine methyl ester (L-NAME) have been shown to increase vascular resistance and inhibit vasodilator responses to endothelium-dependent vasodilator agents such as acetylcholine and bradykinin in the pulmonary and systemic vascular beds.\textsuperscript{8,9} The results of studies in the peripheral and pulmonary vascular beds have provided evidence in support of the hypothesis that the vascular bed is maintained in a dilated state by the tonic release of NO and that NO release plays an important role in mediating responses to endothelium-dependent vasodilator agents.\textsuperscript{4-12} Although studies in species including the cat, rabbit, and lamb have shown that inhibitors of NO synthesis increase basal tone in the pulmonary vascular bed, this has not been observed in the isolated perfused rat lung.\textsuperscript{8,10-14} While the role of EDRF/NO release in response to exogenously administered acetylcholine has received a great deal of attention, little if anything is known about the role of the endothelium in mediating the response to neurogenically released acetylcholine. It has been suggested that parasympathetic coronary vasodilation is mediated by EDRF, since L-NAME specifically inhibits coronary vasodilator responses to acetylcholine and vagal stimulation.\textsuperscript{15} Previous studies indicate that in addition to the presence of adrenergic nerves, the pulmonary vascular bed of the cat is functionally innervated by the cholinergic system, and light and electron microscopic studies suggest the presence of cholinergic-like nerve terminals around small- and medium-sized intrapulmonary arteries.\textsuperscript{16-18} In the pulmonary vascular bed of the cat...
under elevated tone conditions when the integrity of adrenergic terminals is destroyed with 6-hydroxydopamine or nerve terminal catecholamine stores are depleted with reserpine, efferent vagal stimulation decreases pulmonary lobar vascular resistance. The neurogenic pulmonary vasodilator response is stimulus dependent and is blocked by atropine, suggesting that it is muscarinic in nature. However, the role of EDRF/NO release in the neurogenic cholinergic vasodilator response to efferent vagal stimulation in the cat is unknown. The present study was, therefore, undertaken to investigate the effects of L-NAME, an inhibitor of NO synthase, on the response to electrical stimulation of the vagus nerve in the pulmonary vascular bed of the intact-chest cat under conditions of controlled blood flow and constant left atrial pressure.

Materials and Methods

Thirty-eight adult cats, unselected as to sex, weighing 2.2–4.3 kg (mean±SEM, 3.5±0.1 kg) were sedated with ketamine hydrochloride (10–15 mg/kg i.m.) and anesthetized with sodium pentobarbital (30 mg/kg i.v.). The animals were strapped in the supine position to a fluoroscopic table, and supplemental doses of anesthetic were administered as needed to maintain a uniform level of anesthesia. The trachea was intubated with a cuffed pediatric endotracheal tube, and the animals spontaneously breathed room air enriched with 100% O₂. Systemic arterial (aortic) pressure was measured with a catheter inserted into the aorta from a femoral artery, and intravenous injections were made from a catheter positioned in the inferior vena cava from a femoral vein.

For perfusion of the left lower lung lobe a specially designed 28-cm 6F triple-lumen balloon perfusion catheter (Arrow International, Inc., Reading, Pa.) was passed under fluoroscopic guidance from the left external jugular vein into the artery to the left lower lobe. After the cats had been heparinized (1,000 units/kg i.v.), the lobar artery was isolated by distension of the balloon cuff on the perfusion catheter. The lobe was then perfused by way of the catheter lumen beyond the cuff with blood withdrawn from a femoral artery with a perfusion pump (model 1210, Harvard Apparatus, South Natick, Mass.). The perfusion rate was adjusted so lobar arterial perfusion pressure approximated mean pressure in the main pulmonary artery and thereafter was not changed during an experiment. The perfusion rate ranged from 32 to 48 ml/min. Left atrial pressure was measured with a 6F double-lumen catheter (Arrow) or a 5F polyethylene catheter (Cook, Inc., Bloomington, Ind.) passed transeptally into the vein draining the left lower lobe. The catheter tip was positioned so the left atrial pressure port on the distal lumen was 1–2 cm into the lobar vein and the second catheter port was near the venoatrial junction. When necessary, blood could be withdrawn or infused through this second catheter lumen to maintain left atrial pressure constant. All vascular pressures were measured with Spectromed DTX Plus transducers (Spectromed Inc., Oxnard, Calif.) zeroed at right atrial level. Mean vascular pressures obtained by electronic averaging were recorded (model 7 recorder, Grass Instrument Co., Quincy, Mass.). In some experiments a bipolar pacing catheter was placed in the right ventricle under fluoroscopic guidance, and the bradycardia that occurred during vagal stimulation was prevented by means of a digital pulse generator pacemaker (model 4001, Global Specialties Corp., New Haven, Conn.).

In experiments in which adrenergic neuronal blockade was used, the animals were treated with reserpine (Serpasil, CIBA-GEIGY, Summit, N.J.; 1–1.5 mg/kg i.m.) 24 hours before the experiment. The extent of adrenergic neuronal blockade was assessed by comparing responses to the indirect acting agent, tyramine, in control and reserpine-pretreated animals. Pretreatment with reserpine (1–1.5 mg/kg i.m.) 24 hours before the experiment reduced the increase in systemic arterial pressure in response to tyramine (100 µg/kg i.v.) by more than 80%. For efferent vagal stimulation the left cervical vagosympathetic nerve was approached through a midline incision on the ventral side in the midcervical portion of the neck. The nerve was ligated, and a shielded electrode was placed around the distal portion of the ligated nerve. The nerve was stimulated with square-wave 5-msec duration pulses at supramaximal voltages (8–30 V) for 60–90 seconds with a Grass Model SM6 stimulator.

L-NAME hydrochloride (Sigma Chemical Co., St. Louis, Mo.) was dissolved in normal saline immediately before injection. Acetylcholine chloride, sodium nitroprusside, isoproterenol, tyramine, propranolol (all as hydrochloride salts), atropine sulfate, 8-bromo-cGMP (8-Br-cGMP), and adenosine (Sigma) were dissolved in normal saline. The thromboxane receptor agonist U46619 (11α,9α-epoxymethano-9α,11β-dideoxyprostaglandin F2α), prostaglandin E1 (PGE1, Upjohn Co., Kalamazoo, Mich.), and nicorandil (Bekloff and Associates) were dissolved in 100% ethanol at a concentration of 10 mg/ml, and further dilutions were made in normal saline. L-makalim (Smith Kline Beecham, England) was dissolved in 20% ethanol in normal saline at a concentration of 1 mg/ml and diluted in normal saline. All drugs were kept frozen or on ice in brown stoppered glass bottles, and working solutions were prepared on a frequent basis.

Because the pulmonary vascular bed of the intact-chest cat has little if any vasoconstrictor tone under resting conditions when the FIO2 is greater than 0.21, tone in the bed must be actively increased so vasodilator responses can be expressed. In all experiments tone was raised in the control period to an average value of 35±1 mm Hg with an intralobar infusion of U46619. The infusion rate ranged from 40 to 200 ng/min in the control period and 16 to 120 ng/min after the animals were treated with L-NAME. Under conditions of elevated tone in the control period,
responses to efferent vagal stimulation or to intralobar injections of acetylcholine, nicorandil, adenosine, PGE1, 8-Br-cGMP, lemakalim, isoproterenol, and nitroprusside were obtained. The agonists were injected in small volumes directly into the perfusion circuit distal to the pump in a random sequence during the control period. The U46619 infusion was terminated, and lobar arterial pressure was permitted to return to near control value. L-NAME was administered in a dose of 100 mg/kg i.v., which was shown to reduce pulmonary vasodilator responses to acetylcholine in pilot experiments. After the peak increase in lobar arterial pressure in response to L-NAME was attained in 13 ± 1 minutes, the U46619 infusion was resumed if necessary to raise pulmonary vascular tone to a level similar to that attained during the control period. In some experiments, however, L-NAME administration alone was sufficient to raise lobar vascular tone to a level equal to the control level, and in these experiments, U46619 infusion was resumed only later, when lobar arterial pressure had fallen below 30 mm Hg. Responses to vagal stimulation or to intralobar injection of agonists were obtained beginning 20–30 minutes after the administration of L-NAME.

In experiments in which the effects of propranolol or atropine on pulmonary vascular responses to vagal stimulation, acetylcholine, and isoproterenol were investigated, U46619 was used to increase lobar arterial perfusion pressure during both the control and treatment periods, since neither blocking agent changed pulmonary lobar arterial pressure significantly.

Blood gases and pH were measured with an Instrumentation Laboratory Model Micro 13 analyzer. All hemodynamic data are expressed in absolute units and presented as mean ± SEM. Responses represent peak changes, unless otherwise noted. The data were analyzed using a one-way analysis of variance and Scheffe's F test or a paired t test. A value of p < 0.05 was used as the criterion for statistical significance.

**Results**

The effects of L-NAME on pulmonary vasodilator responses to efferent vagal stimulation and acetylcholine are summarized in Figure 1. When tone in the pulmonary vascular bed was raised to an average value of 35 ± 1 mm Hg with an infusion of U46619, vagal stimulation at frequencies of 2–16 Hz decreased lobar arterial pressure in a stimulus-frequency–dependent manner. After administration of L-NAME (100 mg/kg i.v.) when tone in the pulmonary vascular bed had been increased to a level similar to that attained in the control period (36 ± 1 mm Hg), decreases in lobar arterial pressure in response to vagal stimulation at frequencies of 2–16 Hz were reduced significantly (Figure 1). Under elevated tone conditions, decreases in lobar arterial pressure in response to intralobar injections of acetylcholine in doses of 0.03–1 μg were reduced significantly after administration of L-NAME (100 mg/kg i.v.) (Figure 1). The effects of the passage of time and of the saline vehicle for L-NAME on responses to efferent vagal stimulation at frequencies of 4–16 Hz were investigated in three animals, and decreases in lobar arterial pressure in reserpine-pretreated animals averaged 2.7 ± 0.3, 5.1 ± 0.7, and 6.5 ± 1.2 mm Hg during the control period and 3.0 ± 0.6, 4.8 ± 0.4, and 6.5 ± 0.9 mm Hg 30 minutes after administration of the saline vehicle for L-NAME.

Because the vagus is reported to be a mixed nerve containing both sympathetic and parasympathetic fibers, the effects of L-NAME were investigated in animals pretreated with reserpine to impair norepinephrine storage in adrenergic terminals. In animals pretreated with reserpine, decreases in lobar arterial pressure in response to vagal stimulation were enhanced under elevated tone conditions, and responses to vagal stimulation were decreased significantly at stimulus frequencies of 2–16 Hz after treatment with L-NAME (100 mg/kg i.v.) (Figure 1). In these same experiments under elevated tone conditions, vasodilator responses to injections of acetyl-
choline in doses of 0.03-1 µg were reduced significantly after treatment with L-NAME (100 mg/kg i.v.) (Figure 1).

The decreases in lobar arterial pressure in response to vagal stimulation at stimulus frequencies of 2-16 Hz were reduced significantly after administration of atropine (1 mg/kg i.v.) (Figure 2). The decreases in lobar arterial pressure in response to acetylcholine were also decreased significantly after injection of atropine in a dose of 1 mg/kg i.v. (Figure 2).

The effects of propranolol on the response to vagal stimulation were investigated in the pulmonary vascular bed of the cat, and these data are also summarized in Figure 2. After administration of propranolol in a dose of 1 mg/kg i.v. or 0.5 mg/kg directly into the perfused lobar artery, decreases in lobar arterial pressure in response to vagal stimulation and to intralobar injections of acetylcholine were not changed significantly (Figure 2). However, the decreases in lobar arterial pressure in response to intralobar injections of isoproterenol were reduced significantly (Figure 2).

The selectivity of the inhibitory effects of L-NAME on vasodilator responses to vagal stimulation and acetylcholine were assessed by investigating the actions of L-NAME on vasodilator responses to agents that act by a variety of mechanisms, and these results are summarized in Figure 3. Nicorandil and adenosine decreased lobar arterial pressure in a dose-related manner when injected into the perfused lobar artery in a wide range of doses under elevated tone conditions. Midrange doses of isoproterenol, PGE₁, sodium nitroprusside, lemakalim, and the 2 mg dose of 8-Br-cGMP also produced significant decreases in lobar arterial pressure under elevated tone conditions. The decreases in lobar arterial pressure in response to adenosine, lemakalim, isoproterenol, PGE₁, and 8-Br-cGMP were not decreased after administration of L-NAME (100 mg/kg i.v.) (Figure 3). The decreases in lobar arterial pressure in response to nicorandil and sodium nitroprusside were increased significantly after administration of L-NAME (100 mg/kg i.v.) (Figure 3). The effects of L-NAME on lobar arterial, systemic arterial, and left atrial pressures in untreated and in reserpine-pretreated animals are shown in Table 1. In untreated...
and in reserpine-pretreated animals, the administration of L-NAME (100 mg/kg i.v.) caused a significant increase in systemic arterial and lobar arterial pressures without altering left atrial pressure (Table 1). The increases in systemic arterial and lobar arterial pressures in response to L-NAME were not significantly different in untreated and reserpine-pretreated animals (Table 1). Because it is important to compare vasodilator responses at the same or at similar levels of baseline tone in the pulmonary vascular bed, lobar arterial pressure was increased when required and maintained at a value of approximately 35 mm Hg by infusion of U46619 in both untreated and reserpine-pretreated animals.

**Discussion**

Results of the present study demonstrate that efferent vagal stimulation decreases lobar arterial pressure in the intact-chest cat under elevated tone conditions and are consistent with results from previous studies.\(^\text{18}\) Inasmuch as blood flow and left atrial pressure were maintained constant, the decreases in lobar arterial pressure reflect decreases in pulmonary lobar vascular resistance. In the midcervical region the vagus is composed of efferent fibers from both sympathetic and parasympathetic divisions of the autonomic nervous system, and pretreatment with reserpine depletes norepinephrine from adrenergic terminals and enhances the pulmonary vasodilator response to vagal stimulation.\(^\text{18,20}\)

Results of the present investigation extend the findings of previous studies by showing that pulmonary vasodilator responses to efferent vagal stimulation are reduced by L-NAME, an inhibitor of NO synthase.\(^\text{11,18}\) In addition to inhibiting vasodilator responses to efferent vagal stimulation, L-NAME inhibited vasodilator responses to exogenously administered acetylcholine. The inhibitory effects of L-NAME were selective, and responses to vagal stimulation and acetylcholine were decreased at a time vasodilator responses to adenosine, nicorandil, PGE\(_1\), isoproterenol, sodium nitroprusside, lemakalin, and 8-Br-cGMP were not decreased. These data provide support for the hypothesis that vagal stimulation and acetylcholine dilate the pulmonary vascular bed by an endothelium-dependent mechanism. The observation that responses to adenosine, nicorandil, lemakalin, PGE\(_1\), isoproterenol, nitroprusside, and 8-Br-cGMP were not decreased suggests that L-NAME did not impair responses mediated by a variety of mechanisms, including increases in cGMP or cAMP levels, activation of cGMP-dependent protein kinase or adenosine receptors, or opening of ATP-sensitive K\(^+\) channels.\(^\text{9,21-23}\) The enhancement of vasodilator responses to sodium nitroprusside, which acts by stimulating soluble guanylate cyclase, and to nicorandil, which acts by activating soluble guanylate cyclase and by opening ATP-sensitive K\(^+\) channels, in the absence of an effect on the response to 8-Br-cGMP by L-NAME is consistent with results of previous studies in the pulmonary and hind limb vascular beds in the cat and anesthetized rat.\(^\text{9,21-23}\) The mechanism of the enhanced vasodilator response to agents that release NO is uncertain, but it has been hypothesized that the removal of the basal NO-mediated vasodilator tone in the cardiovascular system leads to a "specific supersensitivity" of soluble guanylate cyclase.\(^\text{21}\) The results of the present study with nitroprusside and nicorandil are consistent with the hypothesis that inhibition of NO synthesis with agents such as L-NAME will enhance responses to nitrovasodilators.\(^\text{9,21}\)

L-NAME is an arginine analogue that inhibits the release of NO from L-arginine.\(^\text{6,7}\) The selective inhibitory effect of L-NAME on pulmonary vasodilator responses to vagal stimulation and to acetylcholine is consistent with the hypothesis that these responses are dependent at least in part on the release of EDRF/NO or a labile nitroso compound from L-arginine. These results are in agreement with previous studies in the coronary circulation of the anesthetized dog, in which L-NAME specifically inhibited coronary vasodilation caused by acetylcholine and vagal stimulation.\(^\text{15}\) L-NAME increased baseline tone, suggesting that basal release of EDRF/NO may serve to maintain the pulmonary vascular bed in a dilated state. These data are consistent with results in the rabbit and lamb but not those in the rat.\(^\text{8,10-14}\)

The reason for the difference in results in the isolated rat lung and in studies in the cat, rabbit, and lamb is uncertain but may suggest that basal release of EDRF is low in the pulmonary vascular bed of the rat.\(^\text{13,14}\) However, the response to hypoxia is enhanced by inhibitors of NO synthase in the isolated perfused rat lung, suggesting that hypoxia releases EDRF/NO and that EDRF/NO may modulate hypoxic pulmonary vasoconstriction in the rat.\(^\text{13,14}\)

### Table 1. Influence of N\textsuperscript{\(\text{\textalpha}\)}-Nitro-L-Arginine Methyl Ester on Mean Vascular Pressures in the Cat

<table>
<thead>
<tr>
<th></th>
<th>Aorta</th>
<th>Lobar artery</th>
<th>Left atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>L-NAME</td>
<td>Control</td>
</tr>
<tr>
<td>All animals (n=38)</td>
<td>119±5</td>
<td>156±6*</td>
<td>19±1</td>
</tr>
<tr>
<td>Untreated animals (n=20)</td>
<td>131±6</td>
<td>166±7*</td>
<td>18±1</td>
</tr>
<tr>
<td>Reserpine-pretreated animals (n=18)</td>
<td>105±8</td>
<td>144±10*</td>
<td>20±2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. L-NAME, N\textsuperscript{\(\text{\textalpha}\)}-nitro-L-arginine methyl ester. *p<0.05 compared with corresponding control.
The source or cell type that releases NO during vagal stimulation is uncertain in the intact pulmonary vascular bed. It is possible that NO may be released from the postganglionic neurons that innervate the pulmonary vascular bed. Stimulation of nonadrenergic, noncholinergic nerves in the canine ileocolonic junction has recently been shown to release a chemically unstable factor that is inactivated by superoxide anion or hemoglobin, inhibited by l-NA, and potentiated by L-arginine, indicating that it may be identical to NO.24 Similarly, hemoglobin and l-NAME inhibited the relaxant response of dog cerebral artery to transmural electrical stimulation, suggesting that NO may act as a primary messenger transmitting information from nerves to cerebroarterial smooth muscle.25 However, nonadrenergic, noncholinergic neurotransmission or the direct release of NO from postganglionic nerves does not appear to play a role in the pulmonary vasodilator response to vagal stimulation in the cat, because the response to vagal stimulation is blocked by atropine. The release of the adrenergic transmitter also does not appear to play an important role in the response to vagal stimulation, since responses are not modified by propranolol in doses that blocked the pulmonary vasodilator response to isoproterenol. Although it is possible that some NO release may come from smooth muscle or other cell types when the vagus is stimulated, it seems likely that the major source of NO is from endothelial cells lining resistance vessel elements in the pulmonary vascular bed. These findings suggest that acetylcholine released from cholinergic terminals in the adventitial-medial zone of intrapulmonary arteries diffuses to the endothelium, where activation of the muscarinic receptor results in release of NO, which activates soluble guanylate cyclase in smooth muscle cells. Although it is difficult if not impossible to prove this hypothesis in the intact lung, the observation that l-NAME blocks the response to vagal stimulation and to exogenous acetylcholine in a selective manner provides support for the concept that neuronally released acetylcholine dilates the pulmonary vascular bed by releasing endothelium-derived NO or labile derivatives of NO from L-arginine.

References

Key Words • endothelium-derived relaxing factor • nitric oxide • neurogenic responses • cholinergic vasodilatation • pulmonary vascular bed
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