N^{ω}-nitro-L-arginine methyl ester selectively inhibits pulmonary vasodilator responses to acetylcholine and bradykinin

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McMAHON, TIMOTHY J., JAMES S. HOOD, JOHN A. BELLAN, AND PHILIP J. KADOWITZ. *N^{ω}-nitro-L-arginine methyl ester selectively inhibits pulmonary vasodilator responses to acetylcholine and bradykinin.* J. Appl. Physiol. 71(5): 2026–2031, 1991.—The effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of endothelium-derived relaxing factor (EDRF) production, on vascular tone and responses were 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 elevated with U-46619, intralobar injections of acetylcholine, bradykinin, sodium nitroprusside, isoproterenol, prostaglandin E_1 (PGE_1), lemakalim, and 8-bromo-guanosine 3',5'-cyclic monophosphate (8-bromo-cGMP) dilated the pulmonary vascular bed. Intravenous administration of L-NAME elevated lobar arterial and systemic arterial pressures without altering left atrial pressure. When U-46619 was infused after L-NAME to raise lobar arterial pressure to levels similar to those attained during the control period, vasodilator responses to acetylcholine and bradykinin were reduced significantly, whereas responses to PGE_1, lemakalim, and 8-bromo-cGMP were not altered, and responses to nitroprusside were increased. There was a small effect on the response to the highest dose of isoproterenol, and pressor responses to BAY K 8644 and angiotensin II were not altered. These results are consistent with the hypothesis that EDRF production may involve the formation of nitric oxide or a nitroso compound from L-arginine and that EDRF production may have a role in the regulation of tone and in the mediation of responses to acetylcholine and bradykinin in the pulmonary vascular bed of the cat.

endothelium-dependent vasodilation; nitric oxide; pulmonary vascular bed; bradykinin

ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF) was first described in 1980 by Furchgott and Zawadski (9). While there is evidence to suggest that more than one factor may cause endothelium-dependent relaxation, it is now widely accepted that EDRF may be NO or a labile nitroso compound (15, 26). NO is synthesized from the amino acid precursor L-arginine in endothelial cells, and L-N^{G}-monomethylarginine (L-NMMA) in a stereospecific manner inhibits NO synthesis from L-arginine (12, 27). L-NMMA increases arterial pressure and regional vascular resistance in a number of laboratory species (2, 5, 26). Pressor responses to L-NMMA are partially inhibited by L-arginine, suggesting that the vasoconstriction is due to a competitive inhibition of NO synthesis from L-arginine (2, 5, 26). In addition to increasing vascular resistance, L-NMMA has been shown to inhibit responses to endothelium-dependent vasodilators but not to nitrovasodilators (6, 24, 30). In recent studies, N^{ω}-nitro-L-arginine (nitroarginine, L-NA) has been reported to be more potent than L-NMMA in inhibiting NO synthesis (18, 24). This arginine analogue has been shown to inhibit EDRF/NO release and decrease guanosine 3',5'-cyclic monophosphate (cGMP) levels in cultured endothelial cells (18). L-NA has been shown to inhibit responses to endothelium-dependent vasodilator agents in the isolated perfused rat mesentery, pulmonary vascular bed of the lamb, and the hindlimb and mesenteric vascular beds of the cat (unpublished observations; 4, 7, 24). However, neither L-NMMA nor L-NA had a significant effect on responses to endothelium-dependent vasodilator agents in the hindlimb vascular bed of the rabbit (25). While L-NA and N^{ω}-nitro-L-arginine methyl ester (L-NAME) have been shown to increase resistance in the pulmonary vascular bed of the rabbit, lamb, and neonatal lamb, L-NMMA and L-canavanine had little or no effect on baseline pulmonary arterial pressures in isolated Krebs albumin- or saline-perfused rat lungs (1, 3, 7, 14, 28). Moreover, L-NA and L-NAME have been shown to reduce vasodilator responses to acetylcholine in the lamb and neonatal lamb while L-NMMA inhibited the pulmonary vasodilator response to bradykinin but not to acetylcholine or histamine in the isolated perfused rat lung (3, 7, 14). However, little if anything is known about the effects of L-NA or L-NAME on the pulmonary vascular bed of the cat. It has been reported that methylene blue, an inhibitor of soluble guanylate cyclase, reduced responses to acetylcholine, bradykinin, and nitrovasodilators in the pulmonary vascular bed of the cat (17). However, the effects of inhibition of NO synthesis on pulmonary vascular responses to endothelium-dependent and endothelium-independent vasodilators have not been determined in the cat. Therefore, the present study was undertaken to investigate the effects of L-NAME on endothelium-dependent and endothelium-independent vasodilator responses, as well as on vasoconstrictor responses, in the pulmonary vascular bed of the cat under conditions of controlled blood flow and constant left atrial pressure.

MATERIALS AND METHODS

Twenty-four adult cats unselected as to sex and weighing 3.2–4.4 kg (mean 3.6 ± 0.1 kg) were sedated with ketamine hydrochloride (10–15 mg/kg im) and were anes-

thetized with pentobarbital sodium (30–40 mg/kg iv). 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% O_2. Systemic arterial (aortic) pressure was measured from 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, 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 U/kg iv), 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 Instrument, Millis, MA). The perfusion rate was adjusted so that 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 28 to 45 ml/min. Left atrial pressure was measured with a 6F double-lumen catheter (Arrow International) passed transseptally into the vein draining the left lower lobe. The catheter tip was positioned so that 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 transducers (Spectromed DTX Plus, Oxnard, CA) zeroed at right atrial level. Mean vascular pressures obtained by electronic averaging were recorded on a recorder (model 7, Grass Instrument, Quincy, MA).

In five animals, sodium meclofenamate (2.5 mg/kg iv) was administered before the control injections of bradykinin to inhibit prostaglandin synthesis. The responses to bradykinin in these animals were not significantly different from those recorded in untreated animals, and the data were pooled. In five animals, responses were obtained before and after injection of the vehicle for L-NAME, 0.185 N HCl in normal saline under conditions of elevated pulmonary vascular tone.

L-NAME hydrochloride (Sigma Chemical, St. Louis, MO) was dissolved in normal saline immediately before injection. Acetylcholine chloride, sodium nitroprusside, bradykinin, 8-bromo-cGMP, isoproterenol HCl (Sigma Chemical), angiotensin amide (Ciba-Geigy, Summit, NJ), sodium bicarbonate (Mallinckrodt, Paris, KY), and sodium meclofenamate (Warner Lambert-Parke, Davis, Ann Arbor, MI) were dissolved in normal saline. Prostaglandin E_1 and the thromboxane receptor agonist, U-46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin F_2; Upjohn, Kalamazoo, MI), were dissolved in 100% ethanol at a concentration of 10 mg/ml and diluted in normal saline. Lemakalim (BRL 38227; SmithKline Beecham, Betchworth, Surrey, UK), the pure L-isomeric form of the potassium channel activator cromakalim, was dissolved in 20% ethanol in normal saline at a concentration of 1 mg/ml and diluted in normal saline. BAY K 8644 was dissolved in a 1 ml solution of 1:4 polyoxyethylinated castor oil-50 mM tris (hydroxymethyl)aminomethane hydrochloride (Tris · HCl, pH 7.4). The solution was made up to 8 ml with Tris · HCl (pH 7.4). All drugs were kept frozen or on ice in brown glass bottles, and working solutions were prepared on a frequent basis.

The pulmonary vascular bed of the intact-chest cat has little if any vasoconstrictor tone under resting baseline conditions (17). Therefore, tone must be increased so that vasodilator responses can be expressed. In the present experiments tone was raised in the control period to an average value of 36 ± 1 mmHg with an intralobar infusion of U-46619. Under elevated tone in the control period, responses to intralobar injections of acetylcholine, bradykinin, PGE_1, isoproterenol, sodium nitroprusside, 8-bromo-cGMP, lemakalim, angiotensin II, and BAY K 8644 were obtained. L-NAME was then administered in a dose of 100 mg/kg iv, and in most experiments lobar arterial pressure was raised to similar values by infusions of U-46619 (10–200 ng/min) before and after administration of L-NAME. In seven experiments L-NAME administration alone was sufficient to raise lobar vascular tone to a level equal to the control level, and in these experiments, U-46619 infusion was resumed only later, when lobar arterial pressure had fallen below 30 mmHg. 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 U-46619 infusion was terminated, and lobar arterial pressure was permitted to return to near control value. L-NAME (100 mg/kg iv) was then administered, followed by 0.37 meq NaHCO3/kg iv. After the peak increase in lobar arterial pressure in response to L-NAME was attained, the U-46619 infusion was resumed to raise pulmonary vascular tone to a level similar to that attained during the control period. In the majority of experiments, responses were obtained beginning 15–30 min after the administration of L-NAME.

Blood gases and pH were measured with an Instrumentation Laboratory analyzer (model micro 13). All hemodynamic data are expressed in absolute units and presented as means ± SE. Mean systemic arterial pressure was not analyzed in experiments in which blood was withdrawn from the left atrium. Responses represent peak changes, unless otherwise noted. The data were analyzed using a one-way analysis of variance and Scheffé's F test or a paired t test. $P < 0.05$ was used as the criterion for statistical significance.

RESULTS

Vasodilator responses to acetylcholine, bradykinin, PGE_1, isoproterenol, nitroprusside, 8-bromo-cGMP, and the K^+_{ATP} channel opener lemakalim were compared when tone in the feline pulmonary vascular bed was raised to similar values with U-46619 and with U-46619 plus L-NAME. When lobar arterial pressure was raised to an average value of 36 ± 1 mmHg with U-46619, injections of acetylcholine, bradykinin, nitroprusside, isoproterenol, PGE_1, 8-bromo-cGMP, and lemakalim into the

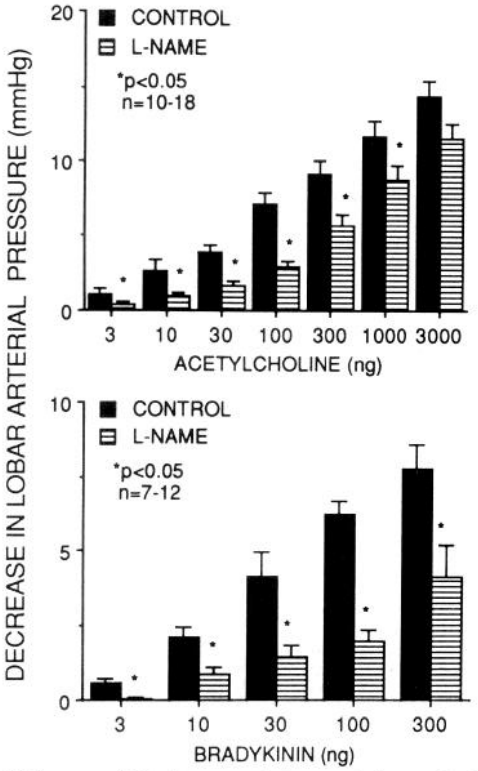

FIG. 1. Influence of N^{ω}-nitro-L-arginine methyl ester (L-NAME) on decreases in lobar arterial pressure in response to acetylcholine and bradykinin. Vasodilator agents were injected directly into perfusion circuit, and responses were determined before and beginning 15–30 min after administration of L-NAME. *n*, No. of experiments. *Response is significantly different from control.

perfused lobar artery caused dose-related decreases in lobar arterial pressure (Figs. 1–3). Injection of L-NAME (100 mg/kg iv) increased lobar arterial pressure from a baseline value of 18 ± 1 mmHg to a peak value of 30 ± 2 mmHg at 12 ± 1 min after injection and systemic arterial pressure from 137 ± 4 mmHg to a peak value of 171 ± 6 mmHg at 21 ± 2 min after injection. Left atrial pressure was unchanged by administration of L-NAME. After lobar arterial pressure had attained a peak value after administration of L-NAME, the U-46619 infusion was again started and the infusion rate adjusted so that an average pressure similar to that obtained during the initial U-46619 infusion was attained. When lobar arterial pressure was increased to an average value of 35 ± 1 mmHg in these experiments, decreases in lobar arterial pressure in response to acetylcholine (3–1,000 ng) and bradykinin (3–300 ng) were reduced significantly (Fig. 1). Although responses to acetylcholine and bradykinin were reduced significantly, decreases in lobar arterial pressure in response to sodium nitroprusside, PGE_1, lemakalim, and 8-bromo-cGMP were not reduced (Figs. 2 and 3). However, there was a small increase in the vasodilator response to nitroprusside that was significantly greater than control at all doses (Fig. 2). Decreases in lobar arterial pressure in response to isoproterenol (3, 10, and 30 ng) were not altered, whereas responses to the β-agonist at the highest dose (100 ng) were reduced significantly (Fig. 2).

Injections of angiotensin II and BAY K 8644 increased lobar arterial pressure, and pressor responses to the peptide and the calcium entry promoting agent BAY K 8644 were not different when tone was increased with U-46619 in the control period or after administration of L-NAME and with the U-46619 infusion (Fig. 4).

Arterial blood gases, measured 5–10 min after administration of L-NAME, were not significantly different from control ($n = 4$), and the effects of the L-NAME vehicle on mean vascular pressures and on vasodilator responses were also investigated in the pulmonary vascular bed of the cat. Injection of the vehicle (0.37 meq/kg hydrochloric acid diluted in 0.9% NaCl solution, 2 ml/kg) at an acid concentration and volume equivalent to those produced by L-NAME (100 mg/kg) in 2 ml/kg 0.9% NaCl produced no significant change in lobar arterial, left atrial, or systemic arterial pressure. Moreover, when lobar arterial pressure was increased to an average value of 35 ± 1 mmHg with U-46619 after administration of the acid vehicle, vasodilator responses to the endothelium-dependent and endothelium-independent vasodilator agents were not significantly different from control (data not shown).

The time course of the inhibitory effects of L-NAME on endothelium-dependent vasodilator responses was also investigated in the feline pulmonary vascular bed. Vasodilator responses to acetylcholine (100 ng) were compared before and at 15-min intervals after the administration of L-NAME (100 mg/kg iv). Vasodilator responses to acetylcholine were significantly different from control 15 min after administration of L-NAME and appeared to decrease to a steady level 45–60 min after administration of L-NAME. Responses did not return to control values in the 120-min period examined (data not shown).

DISCUSSION

Results of the present study show that L-NAME increases systemic and lobar arterial pressures in the intact-chest cat. Inasmuch as blood flow to the lobe was maintained constant and left atrial pressure was unchanged, the increase in lobar arterial pressure reflects an increase in lobar vascular resistance. These data are consistent with results of studies in the pulmonary circulation of the lamb, the fetal lamb, and the rabbit in which L-NAME or L-NA increased pulmonary vascular resistance (1, 7, 28, 31). When taken together, results of studies in the fetal lamb, rabbit, and intact-chest cat are consistent with the hypothesis that tonic release of an EDRF may serve to regulate vascular tone in the pulmonary circulation in the mature animal and in the fetus (1, 7, 28, 31). In addition to increasing lobar vascular resistance in the cat, L-NAME reduced vasodilator responses to acetylcholine and bradykinin. The inhibitory effects of L-NAME were selective, and vasodilator responses to midrange doses of acetylcholine and bradykinin were reduced by >50% at a time when vasodilator responses to sodium nitroprusside, PGE_1, 8-bromo-cGMP, lemakalim, and isoproterenol (3–30 ng) were not reduced.

Although L-NAME did not impair vasodilator responses to agents that act through a variety of mechanisms, responses to the 100-ng dose of isoproterenol were reduced significantly. The mechanism by which L-NAME reduced the response to isoproterenol is uncer-

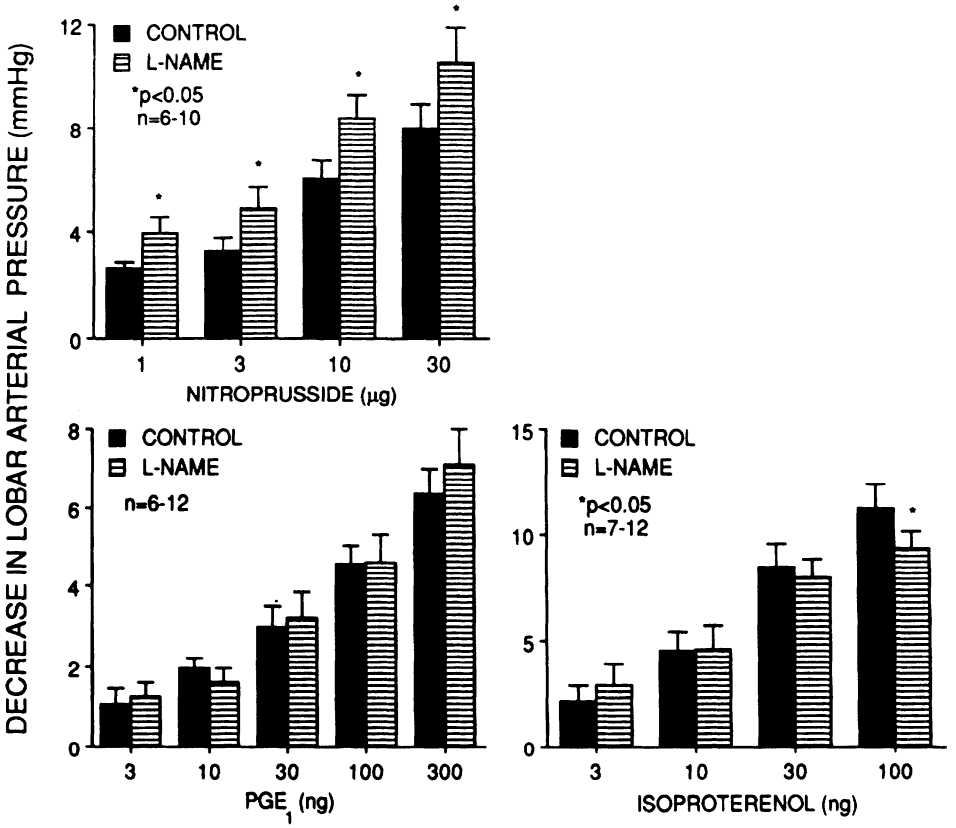

FIG. 2. Influence of L-NAME on decreases in lobar arterial pressure in response to sodium nitroprusside, prostaglandin E_1 (PGE_1), and isoproterenol. Vasodilator agents were injected directly into perfusion circuit, and responses were determined before and beginning 15–30 min after administration of L-NAME. *n*, No. of experiments. *Response is significantly different from control.

tain, and a similar effect has been observed previously with L-NA in the hindlimb vascular bed of the cat (4). Moreover, recent studies indicate that the hindquarters hyperemic vasodilator response to the β-adrenergic agonist, salbutamol, is reduced in the presence of L-NAME, suggesting that vasodilation in response to β-adrenergic agonists may involve the release of endothelium-derived NO, and it has been suggested that under certain conditions in hypertensive animals isoproterenol may release an EDRF (10, 19). Alternatively, a synergistic mechanism has been suggested to exist between cGMP and cAMP in producing vasorelaxation, so that isoproterenol-stimulated vasorelaxation may be diminished when basal release of EDRF is inhibited (13). However, the observation that responses to PGE_1 were not changed suggests that this effect is not seen with all agents that act by increasing adenosine 3′,5′-cyclic monophosphate levels. The significance of the observation that L-NAME had an inhibitory effect on the response to the highest dose of isoproterenol is uncertain.

The observation that vasodilator responses to nitroprusside, 8-bromo-cGMP, and lemakalim are not reduced suggests that L-NAME did not alter endothelium-independent responses mediated by an increase in cGMP levels, activation of cGMP-dependent protein kinase, or the opening of K^+_{ATP} channels (20, 22). These data suggest that the inhibitory actions of L-NAME are selective and provide support for the hypothesis that acetylcholine and bradykinin dilate resistance vessels in the pulmonary vascular bed by an endothelium-dependent mechanism. L-NAME is an arginine analogue that inhibits the release of NO from L-arginine in vitro (29). The selective inhibitory action of L-NAME on pulmonary vasodilator responses to acetylcholine and bradykinin is consistent with studies in isolated vessels and in conscious rats and

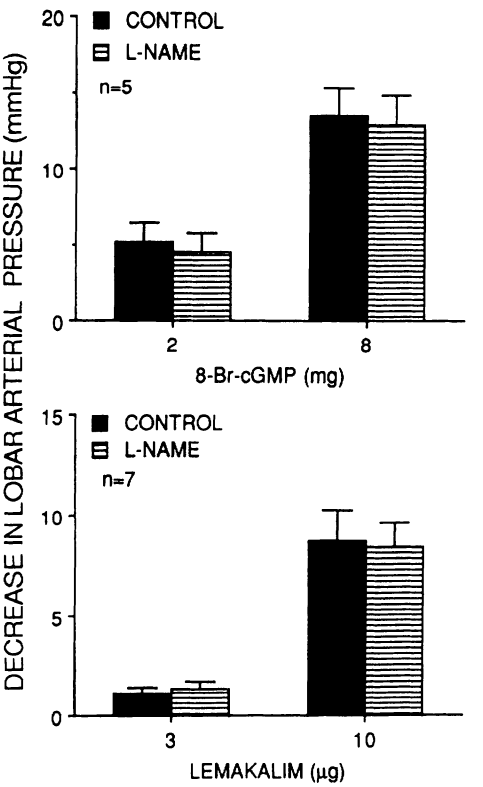

FIG. 3. Influence of L-NAME on decreases in lobar arterial pressure in response to 8-bromo-guanosine 3′,5′-cyclic monophosphate (8-bromo-cGMP) and lemakalim. Vasodilator agents were injected directly into perfusion circuit, and responses were determined before and beginning 15–30 min after administration of L-NAME. *n*, No. of experiments.

provides support for the hypothesis that responses to these agents are dependent at least in part on the release of EDRF/NO from L-arginine (11, 29).

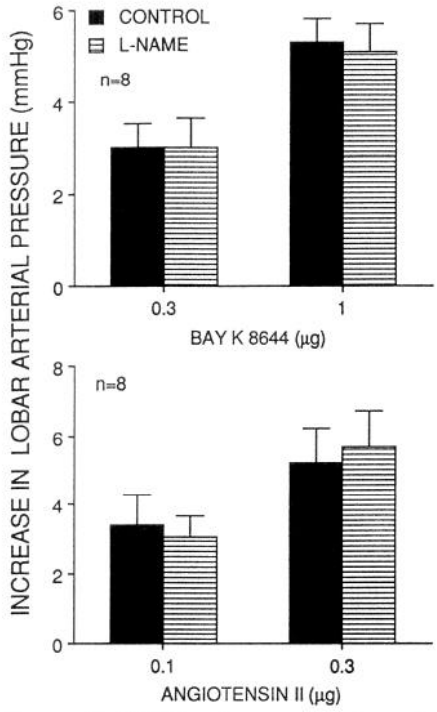

FIG. 4. Influence of L-NAME on increases in lobar arterial pressure in response to BAY K 8644 and angiotensin II. Vasoconstrictor agents were injected directly into perfusion circuit, and responses were determined before and beginning 15–30 min after administration of L-NAME. *n*, No. of experiments.

The enhancement of vasodilator responses to sodium nitroprusside but not to 8-bromo-cGMP in the presence of L-NAME is consistent with previous studies and provides support for the hypothesis that the inhibition of EDRF release induces an enhanced sensitivity of soluble guanylate cyclase to NO (23). Bradykinin and acetylcholine release prostaglandins in some organ systems (8, 21). However, it has been reported that pulmonary vasodilator responses to acetylcholine and bradykinin are not altered by cyclooxygenase inhibitors in the intact-chest cat (16, 21). In the present study, responses to bradykinin and acetylcholine were not inhibited by sodium meclofenamate but were reduced significantly by L-NAME, supporting the hypothesis that EDRF release rather than cyclooxygenase product formation is the major mechanism of action.

Although L-NAME increased lobar vascular resistance and reduced vasodilator responses to acetylcholine and bradykinin, the NO synthesis inhibitor was without significant effect on vasoconstrictor responses to angiotensin II and BAY K 8644. These data may suggest that EDRF release does not limit vasoconstrictor responses in the pulmonary vascular bed of the intact-chest cat. Previous studies in the intact-chest cat have shown that methylene blue, an inhibitor of soluble guanylate cyclase, increased vascular resistance and reduced vasodilator responses to acetylcholine, bradykinin, and the nitrovasodilators (17). Those studies provided support for the concept that cGMP may have a role in the regulation of tone and in the mediation of vasodilator responses to endothelium-dependent agents and to nitrovasodilators (18). The results of the present study extend previous work by showing that EDRF/NO formation may have a role in the regulation of tone and in the mediation of responses to acetylcholine and bradykinin. When taken together, studies with methylene blue and with L-NAME provide support for the concept that cGMP levels may be regulated by the tonic release of EDRF/NO and that acetylcholine and bradykinin may dilate the pulmonary vascular bed at least in part by enhancing the release of EDRF/NO from the endothelium and elevating cGMP levels in resistance vessel elements.

In summary, the present data demonstrate that the arginine analogue L-NAME, which inhibits NO synthesis in vitro, increases systemic arterial pressure and pulmonary vascular resistance in the intact-chest cat. L-NAME reduced vasodilator responses to acetylcholine and bradykinin but not to endothelium-independent vasodilators that decrease vascular resistance by a variety of mechanisms. These data suggest that L-NAME is a selective inhibitor of EDRF/NO synthesis in the pulmonary vascular bed of the cat and that continuous tonic release of NO serves to maintain the pulmonary vascular bed of the cat in a dilated state.

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REFERENCES

1. ABMAN, S. H., B. A. CHATFIELD, S. L. HALL, AND I. F. MCMURTRY. Role of endothelium-derived relaxing factor during transition of pulmonary circulation at birth. *Am. J. Physiol.* 259 (*Heart Circ. Physiol.* 28): H1921–H1927, 1990.
2. AISAKA, K. S., S. GROSS, O. W. GRIFFITH, AND R. LEVI. N^G-methylarginine, an inhibitor of endothelium-derived nitric oxide synthesis, is a potent pressor agent in the guinea pig: does nitric oxide regulate blood pressure in vivo? *Biochem. Biophys. Res. Commun.* 160: 881–886, 1989.
3. ARCHER, S. L., J. P. TOLINS, L. RAIJ, AND E. K. WEIR. Hypoxic pulmonary vasoconstriction is enhanced by inhibition of the synthesis of an endothelium-derived relaxing factor. *Biochem. Biophys. Res. Commun.* 164: 1198–1205, 1989.
4. BELLAN, J. A., R. K. MINKES, D. B. MCNAMARA, AND P. J. KADOWITZ. N^ωnitro-L-arginine selectively inhibits vasodilator responses to acetylcholine and bradykinin in cats. *Am. J. Physiol.* 260 (*Heart Circ. Physiol.* 29): H1025–H1029, 1991.
5. CHU, A., D. E. CHAMBERS, C.-C. LING, W. D. KUEHL, AND F. R. COBB. Nitric oxide modulates epicardial coronary basal vasomotor tone in awake dogs. *Am. J. Physiol.* 258 (*Heart Circ. Physiol.* 27): H1250–H1254, 1990.
6. CRAWLEY, D. E., S. F. LIU, T. W. EVANS, AND P. J. BARNES. Inhibitory role of endothelium-derived relaxing factor in rat and human pulmonary arteries. *Br. J. Pharmacol.* 101: 166–170, 1990.
7. FINEMAN, J. R., M. A. HEYMANN, AND S. J. SOIFER. N^ω-nitro-L-arginine attenuates endothelium-dependent pulmonary vasodilation in lambs. *Am. J. Physiol.* 260 (*Heart Circ. Physiol.* 29): H1299–H1306, 1991.
8. FURCHGOTT, R. F. Role of endothelium in responses of vascular smooth muscle. *Circ. Res.* 53: 557–573, 1983.
9. FURCHGOTT, R. F., AND J. V. ZAWADZKI. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature Lond.* 288: 373–376, 1980.
10. GARDINER, S. M., A. M. COMPTON, P. A. KEMP, AND T. BENNETT. Regional and cardiac haemodynamic responses to glyceryl trinitrate, acetylcholine, bradykinin, and endothelin-1 in conscious rats: effects of N^G-nitro-L-arginine methyl ester. *Br. J. Pharmacol.* 101: 632–639, 1990.
11. GARDINER, S. M., P. A. KEMP, AND T. BENNETT. Effects of N^G-ni-

tro-L-arginine methyl ester on vasodilator responses to acetylcholine, 5′-*N*-ethylcarboxamodoadenosine or salbutamol in conscious rats. *Br. J. Pharmacol.* 103: 1725–1732, 1991.

12. GOLD, M. E., K. S. WOOD, G. M. BUGA, R. E. BYRNS, AND L. J. IGNARRO. L-Arginine causes whereas L-arginosuccinic acid inhibits endothelium-dependent vascular smooth muscle relaxation. *Biochem. Biophys. Res. Commun.* 161: 536–543, 1989.
13. GRACE, G. C., P. S. MACDONALD, AND G. J. DUSTING. Cyclic nucleotide interactions involved in endothelium-dependent dilation in rat aortic rings. *Eur. J. Pharmacol.* 148: 17–24, 1988.
14. HASUNUMA, K., T. YAMAGUCHI, D. M. RODMAN, R. F. O'BRIEN, AND I. F. MCMURTRY. Effects of inhibitors of EDRF and EDHF on vasoreactivity of perfused rat lungs. *Am. J. Physiol.* 260 (*Lung Cell. Mol. Physiol.* 4): L97–L104, 1991.
15. HOEFFNER, U., M. FELETOU, N. A. FLAVAHAN, AND P. M. VANHOUTTE. Canine arteries release two different endothelium-derived relaxing factors. *Am. J. Physiol.* 257 (*Heart Circ. Physiol.* 26): H330–H333, 1989.
16. HYMAN, A. L., AND P. J. KADOWITZ. Tone-dependent responses to acetylcholine in the feline pulmonary vascular bed. *J. Appl. Physiol.* 64: 2002–2009, 1988.
17. HYMAN, A. L., P. J. KADOWITZ, AND H. L. LIPPTON. Methylene blue selectively inhibits pulmonary vasodilator responses in cats. *J. Appl. Physiol.* 66: 1513–1517, 1989.
18. ISHII, K., B. CHANG, J. F. KERWIN, Z.-J. HUANG, AND F. MURAD. N^{ω}-nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation. *Eur. J. Pharmacol.* 176: 219–223, 1990.
19. LEE, T. F. J., Y. SHIRASAKI, AND G. A. NICHOLS. Altered endothelial modulation of vascular tone in aging and hypertension. *Blood Vessels* 24: 132–136, 1987.
20. LINCOLN, T. M. Cyclic GMP and mechanisms of vasodilation. *J. Pharmacol. Exp. Ther.* 41: 479–502, 1989.
21. LIPPTON, H. L., P. A. NANDIWADA, A. L. HYMAN, AND P. J. KADOWITZ. Influence of cyclooxygenase blockade on responses to isoproterenol, bradykinin and nitroglycerin in the feline pulmonary vascular bed. *Prostaglandins* 28: 253–270, 1984.
22. MINKES, R. K., P. KVAMME, T. R. HIGUERA, B. D. NOSSAMAN, AND P. J. KADOWITZ. Analysis of pulmonary and systemic vascular responses to cromakalim, an activator of K^{+}_{ATP} channels. *Am. J. Physiol.* 260 (*Heart Circ. Physiol.* 29): H957–H966, 1991.
23. MONCADA, S., D. D. REES, R. SCHULZ, AND R. M. J. PALMER. Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. *Proc. Natl. Acad. Sci. USA* 88: 2166–2170, 1991.
24. MOORE, P. K., O. A. AL-SWAYEH, N. W. S. CHONG, R. A. EVANS, AND A. GIBSON. L-N^{G}-nitro-arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilation in vitro. *Br. J. Pharmacol.* 99: 408–412, 1990.
25. MUGGE, A., J. A. G. LOPEZ, D. J. PIEGORS, K. R. BREESE, AND D. D. HEISTAD. Acetylcholine-induced vasodilatation in rabbit hindlimb in vivo is not inhibited by analogues of L-arginine. *Am. J. Physiol.* 260 (*Heart Circ. Physiol.* 29): H242–H247, 1991.
26. PALMER, R. M. J., A. G. FERRIGE, AND S. MONCADA. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature Lond.* 327: 524–526, 1987.
27. PALMER, R. M. J., AND S. MONCADA. A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. *Biochem. Biophys. Res. Commun.* 158: 348–352, 1989.
28. PERSSON, M. G., L. E. GUSTAFSSON, N. P. WIKLUND, S. MONCADA, AND P. HEDQVIST. Endogenous nitric oxide as a probable modulator of pulmonary circulation and hypoxic pressor response in vivo. *Acta Physiol. Scand.* 140: 449–457, 1990.
29. REES, D. D., R. M. J. PALMER, R. SCHULZ, H. F. HODSON, AND S. MONCADA. Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br. J. Pharmacol.* 101: 746–752, 1990.
30. WHITTLE, B. J. R., J. LOPEZ-BELMONTE, AND D. D. REES. Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P, and endothelin in the rat by a specific inhibitor of nitric oxide formation. *Br. J. Pharmacol.* 98: 646–652, 1989.
31. WIKLUND, N. P., M. G. PERSSON, L. E. GUSTAFSSON, S. MONCADA, AND P. HEDQVIST. Modulatory role of endogenous nitric oxide in pulmonary circulation in vivo. *Eur. J. Pharmcol.* 185: 123–124, 1990.