Anesthesiology
81:641–648, 1994

Interaction between Halothane and the Nonadrenergic, Noncholinergic Inhibitory System in Porcine Trachealis Muscle

Karen S. Lindeman, M.D., Stuart G. Baker, Sc.D.,† Carol A. Hirshman, M.D.‡*

Background: **Volatile anesthetics significantly affect cholinergic neural transmission in the airways and relax airway smooth muscle. Activation of the nonadrenergic, noncholinergic inhibitory neural pathway, which is thought to be mediated by nitric oxide, relaxes human and porcine airways. The purpose of the current study was to determine in the isolated porcine trachealis muscle whether relaxation of airway smooth muscle by halothane is mediated in part by activation of the nonadrenergic, noncholinergic inhibitory system.**

Methods: **Isometric tension was measured in porcine trachealis muscle suspended in tissue baths in the presence of propranalol (10^{-6} M). After stimulation of postsynaptic nicotinic cholinergic receptors with 1,1-dimethyl-4-phenyl-piperazinium iodide (10^{-4} M) to prevent contractile responses to subsequent electrical field stimulation, carbachol (3×10^{-7} M) was added to increase tone. Nonadrenergic, noncholinergic relaxation responses to electrical field stimulation were then measured in the presence of inhibitors of nitric oxide synthase or L-arginine (the substrate for nitric oxide synthase), in the presence and absence halothane.**

Results: **Electrical field stimulation produced frequency-dependent relaxations that were attenuated by inhibitors of nitric oxide synthase (N^G-nitro-L-arginine methyl ester [L-NAME] or N^G-monomethyl-L-arginine, 10^{-4} M). Pretreatment with L-arginine (10^{-4} M) prevented the effect of L-NAME. Halothane (0.5% or 1.0%) neither enhanced nor attenuated nonadrenergic, noncholinergic relaxations in the presence of L-NAME, D-NAME, L-arginine, or D-arginine.**

Conclusions: **Halothane, at concentrations ≤ 1.0%, does not relax porcine airway smooth muscle *in vitro* by activating the nonadrenergic, noncholinergic inhibitory system. (Key words: Anesthetics, volatile: halothane. Lung(s): bronchodilation. Muscle, smooth: airway. Nerve(s): electrical field stimulation; nonadrenergic, noncholinergic inhibitory system. Pharmacology: nitric oxide.)**

VOLATILE anesthetics exert prominent effects in the airways, in large part by inhibiting a wide variety of reflexes.[1–8] Previous studies investigating the effects of volatile anesthetics on neurotransmission have focused on cholinergic pathways, primarily involving the vagus nerve.

Neural stimulation of intrinsic nerves in the airways *in vitro* produces a predominantly cholinergic contractile response. However, in addition to this contractile response, a neural bronchodilator response exists in many species including the human[9–11] and the pig.[12–14] The pathways and neurotransmitters involved in these relaxant responses differ among species. In human and porcine airways, the dominant relaxant innervation is neither adrenergic nor cholinergic and has been called the nonadrenergic, noncholinergic (NANC) inhibitory system.[12,15,16] Several neurotransmitters for the NANC inhibitory system have been identified in a variety of species, but recent studies suggest that nitric oxide (NO) is one important NANC neurotransmitter in human[9–11] and porcine[13] airways.

Both neural and nonneural tissues produce NO from the amino acid L-arginine, but not from its enantiomer D-arginine, *via* NO synthase (NOS). NO directly relaxes airway smooth muscle[17] by activating guanylyl cyclase and increasing intracellular cyclic guanosine 3′5′monophosphate. Several arginine analogs, including N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME), inhibit NOS. The ability of these agents to attenuate NANC relaxations in

* Assistant Professor of Anesthesiology and Critical Care Medicine, The Johns Hopkins University.

† Mathematical Statistician, Biometry Branch, National Cancer Institute.

‡ Professor of Anesthesiology and Critical Care Medicine, Environmental Health Sciences, and Medicine, The Johns Hopkins University.

Received from the Department of Anesthesiology and Critical Care Medicine and Department of Environmental Health Sciences, The Johns Hopkins University, Baltimore, Maryland, and the Division of Cancer Prevention and Control, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. Accepted for publication April 26, 1994. Supported by grant HL02417 from the National Heart, Lung, and Blood Institute.

Address reprint requests to Dr. Lindeman: Department of Environmental Health Sciences, Division of Physiology, The Johns Hopkins Hospital School of Hygiene and Public Health, Room 7006, 600 North Wolfe Street, Baltimore, Maryland 21205.

airway smooth muscle suggests that NO plays a significant role in these responses.[9-11]

The purpose of the current study was to investigate whether halothane-induced relaxation of airway smooth muscle *in vitro* was mediated in part by the NANC inhibitory system or whether halothane inhibited this system. We selected porcine airways because of their known similarities to human airways with respect to the NANC inhibitory system. We first established the role of NO release in NANC responses in porcine trachealis muscle and then investigated whether neural stimulation enhanced or attenuated the effects of NO in the presence of halothane.

Materials and Methods

Tissue Preparation

Animal protocols were approved by the Animal Care and Use Committee of The Johns Hopkins School of Hygiene and Public Health. Yorkshire pigs (25–35 kg) were sedated with ketamine hydrochloride (700 mg, intramuscular) and anesthetized with pentobarbital sodium (7–8 mg/kg, intravenous). Animals were killed by exsanguination. The cervical trachea was removed and placed in a physiologic salt solution of the following millimolar composition: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.6, KH_2PO_4 1.2, $NaHCO_3$ 25, and D-glucose 11.1. The epithelium was dissected from the trachea, and smooth muscle was cut into rectangular strips (approximately 2 × 8 mm) with small segments of cartilage (2–3 mm) at each end. The strips were suspended in tissue baths with silk threads attached to each segment of cartilage; one end was anchored to the chamber bottom and the other connected to a force transducer (FT03, Grass Medical Instruments, Quincy, MA) to measure isometric force. Trachealis muscle was bathed in the physiologic salt solution at 37°C and bubbled with 95% O_2–5% CO_2 to maintain *pH* at 7.4. Tissues were allowed to equilibrate for 20 min.

Optimum resting length was determined by electrical field stimulation (EFS). An electrical stimulator was connected to two platinum electrodes (20 mm apart) in the tissue bath to produce biphasic, square-wave impulses (30 V, 1 ms, 30 Hz for 10 s). In preliminary studies, these parameters provided maximum responses that were completely abolished by atropine (10^{-6} M) or tetrodotoxin (10^{-6} M), indicating that the contractions were attributable to cholinergic neurotransmission. Initial resting force was set at 1.5 g. Resting force was increased in 0.5 g increments with 15 min rest periods, and the tissues were subjected to EFS. Optimum resting length was defined as the resting length that produced no further increase in active force in response to EFS. The tissues remained at this resting length for the duration of the experiment. Trachealis strips were allowed to equilibrate for 45 min before further manipulation.

Experimental Protocols

Nonadrenergic, Noncholinergic Relaxation. Because we, and others[14] found that porcine airway smooth muscle developed a stable contractile force in response to carbachol or KCl, we selected carbachol as the contractile agonist for all studies involving NANC relaxation. In preliminary experiments, the concentration of carbachol producing half the maximal response was determined by cumulative addition of carbachol to the chambers, in concentrations from 3×10^{-8} to 10^{-4} M. Because the concentration producing half the maximal response varied between 10^{-7} and 10^{-6} M in each of five trachealis strips, 3×10^{-7} M was used in subsequent experiments.

To deplete postganglionic nerves of acetylcholine, postsynaptic nicotinic cholinergic receptors were stimulated with 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP, 10^{-4} M). Propranalol (10^{-6} M) was added to some chambers to prevent adrenergic relaxation. DMPP and propranalol remained in the chamber for the duration of the experiment. Ten minutes later, carbachol (3×10^{-7} M) was added to contract the tissues. When a stable level of tone was established, the tissues were subjected to EFS (30 V, 1 ms, 2–15 Hz, for 10 s every 4 min). At these frequencies contractile responses were blocked with DMPP.[12] In the absence of DMPP, stimulation at these frequencies produced a biphasic response—an immediate contraction followed by a more delayed relaxation.[14]

To demonstrate reproducibility of responses, some tissues were subjected to four consecutive periods of EFS without pharmacologic intervention. Consecutive periods of EFS were separated by a 15-min rest. At the end of each experiment the tissues were fully relaxed with atropine (10^{-6} M).

To demonstrate the neural nature of EFS-induced relaxations, the neurotoxin tetrodotoxin (10^{-6} M) was added to some chambers after the first period of EFS. Fifteen minutes later, EFS was repeated.

Role of Nitric Oxide in Nonadrenergic, Noncholinergic Relaxation. The experimental protocols

were similar to those described above, in that the tissues were subjected to consecutive, identical periods of EFS to produce NANC relaxation.

In three groups of trachealis strips, the effect of inhibition of NOS was investigated by adding either L-NAME (10^{-4} M), its inactive enantiomer D-NAME (10^{-4} M), or L-NMMA (10^{-4} M) to the chambers between the consecutive periods of EFS.

The ability of the NOS substrate to prevent the effects of L-NAME were studied in two sets of trachealis strips. Either L-arginine (10^{-4} M) or its inactive enantiomer D-arginine (10^{-4} M) was added between the first and second consecutive periods of EFS. Between the second and third periods of EFS, L-NAME (10^{-5} M) was added to the chambers.

All pharmacologic agents remained in the chambers for the duration of the experiment.

Effect of Halothane on Nonadrenergic, Noncholinergic Relaxation. The experimental protocols were similar to those described above. Between the first and second consecutive periods of EFS, either L-NAME (10^{-5} M), D-NAME (10^{-5} M), L-arginine (10^{-4} M), or D-arginine (10^{-4} M) was added to the chamber. After the second period of EFS, halothane (0.5%) was vaporized with the gas mixture aerating the chamber for 20 min, and the tissues were subjected to a third period of EFS. When EFS was complete, halothane concentration was increased to 1.0%, and the tissues were subjected to a fourth consecutive period of EFS.

Because halothane reduced carbachol-induced tone, in a second series of experiments initial tone was restored. Between the first and second consecutive periods of EFS, either L-arginine (10^{-4} M) or D-arginine (10^{-4} M) was added to the chamber. Halothane (1.0%) was then bubbled through the chamber for 20 min. During this interval, carbachol was incrementally added to restore original carbachol-induced tone. The strips were then subjected to a third period of EFS.

Drugs

A vaporizer (Fluotec 3, Ohio, Madison, WI) was used to vaporize halothane into the gas mixture bubbling through the tissue baths. Concentrations of halothane in physiologic saline solutions were measured by gas chromatography (5880A, Hewlett-Packard, Andover, MA) at the end of the halothane exposure in dose-response experiments. For vaporizer settings of 0.5% and 1.0% these concentrations were 0.24 ± 0.04 and 0.54 ± 0.06 mM, respectively (n = 6).

Carbamylcholine chloride (carbachol), DMPP, propranalol hydrochloride, tetrodotoxin, L-arginine, D-arginine, L-NMMA, L-NAME, and D-NAME (all from Sigma Chemical, St. Louis, MO) were dissolved in the physiologic salt solution. Concentrations described in the text refer to final concentrations in the tissue baths.

Analysis of Results

Reductions in tone in response to EFS were calculated as the percent of maximal relaxation to atropine. Because halothane reduced carbachol-induced tone, percent reduction was calculated based on the level of tone just before EFS in those experiments.

Effects of drugs on NANC relaxations were evaluated as follows. Let y_{ijk} denote the percent reduction of maximal relaxation to atropine, where i, j, and k index tissue, EFS, and drug, respectively. We assume the following model: $y_{ijk} = \alpha_j + \beta_k + \gamma_k + \epsilon_i$, where $\epsilon_{ijk} \sim N(0,\Sigma_i)$. For identifiability we set $\beta_k = \gamma_k = 0$ if k is a control; $\beta_k \neq 0$ and $\gamma_k = 0$ if drug k follows the control (in the experiment); and $\beta_k \neq 0$ and $\gamma_k \neq 0$ if drug k follows another drug. Thus β_k measures the effect relative to control, and γ_k measures the effect relative to the previous drug. To account for the correlation among repeated measures, we make the usual assumption that Σ_i has a compound symmetry structure. Because tissues were exposed to various numbers of drugs, the data were unbalanced. Consequently, we fit the model by using maximum-likelihood methods for unbalanced repeated measures.[18] We report estimates of β_k with 99% confidence intervals (CI). We used 99% rather than 95% CI to lessen the chance of inappropriate inference due to multiple comparisons. If the 99% CI excludes 0, we say that the drug has a significant effect.

We expressed other statistics as mean ± SE.

Results

Nonadrenergic, Noncholinergic Relaxation

Optimum resting force for all tracheal muscle strips was 2.28 ± 0.05 g (n = 79). DMPP (10^{-4} M) produced a transient contraction (0.68 ± 0.08 g, n = 79) that could not be reproduced by an additional dose (10^{-4} M) of DMPP (n = 4). Carbachol (3×10^{-7} M) produced a prompt and stable contraction (2.80 ± 0.20 g, n = 79) of porcine trachealis muscle (fig. 1). In the presence of carbachol, EFS (30 V, 1 ms, 2–15 Hz, for 10 s every 4 min) caused transient, frequency-dependent

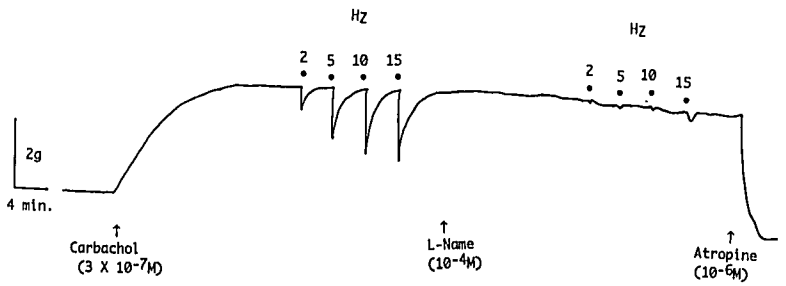

Fig. 1. Relaxation responses to electrical field stimulation (30 V, 1 ms for 10 s at the frequencies shown) in a single porcine tracheal smooth muscle strip after pretreatment with 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP) (10^{-4} M) and propranolol (10^{-6} M), before and after the addition of N^G-nitro-L-arginine methyl ester (L-NAME).

relaxations that did not differ significantly in the presence (41 ± 5% of maximum at 15 Hz, n = 15) or absence (37 ± 5% of maximum at 15 Hz, n = 16) of propranalol (10^{-6} M).

Responses to EFS were reproducible in three tracheal muscle strips for four consecutive, identical periods of EFS. At 15 Hz, responses achieved 35 ± 4%, 36 ± 4%, and 35 ± 5% of maximum relaxation after the first through fourth consecutive periods of stimulation, respectively.

Tetrodotoxin (10^{-6} M) did not alter carbachol-induced tone but completely abolished EFS-induced relaxations (99% CI 44.68–26.55, n = 5).

Role of Nitric Oxide in Nonadrenergic, Noncholinergic Relaxation

The NOS inhibitor L-NAME (10^{-4} M) did not affect carbachol-induced tone but nearly abolished NANC relaxations (99% CI 25.57–10.91, n = 5) (figs. 1 and 2). D-NAME (10^{-4} M) had no significant effect on responses to EFS (n = 6). L-NMMA (10^{-4} M) also significantly attenuated NANC relaxations (99% CI 14.98–8.49, n = 7) though to a lesser extent than L-NAME (fig. 3).

The substrate for NOS, L-arginine (10^{-4} M), slightly enhanced NANC relaxations (99% CI −2.17 to −8.05, n = 17) compared with control. L-arginine also prevented the inhibitory effect of L-NAME (10^{-5} M) on NANC relaxations (n = 6) (fig. 4B). D-arginine (10^{-4} M) did not significantly affect relaxation responses to EFS (n = 6) (fig. 4A).

Effect of Halothane on Nonadrenergic, Noncholinergic Relaxation

After a carbachol-induced contraction, halothane reduced tone in a concentration-related fashion (20.1 ± 5.3% and 30.3 ± 5.9% reductions with 0.5% and 1.0% halothane, respectively, n = 23) (fig. 5). Halothane did not enhance NANC relaxations. Responses to EFS were not significantly different after halothane (0.5% or 1.0%) in the presence of D-NAME (10^{-5} M) (n = 6) (fig. 6A). Although L-NAME (10^{-5} M) attenuated NANC relaxations (99% CI 21.90–10.57, n = 5), halothane did not reverse this effect (fig. 6B).

Halothane did not inhibit NANC relaxations. Responses to EFS were similar before and after halothane (0.5% or 1.0%) in the presence of D-arginine (10^{-4} M) (n = 6) and L-arginine (n = 6) (fig. 7).

In terms of absolute changes in force, carbachol-induced force before halothane was 2.38 ± 0.28 g, and the maximum EFS-induced relaxation (15 Hz) was 0.68 ± 0.13 g (n = 23). Halothane (1.0%) reduced carbachol-induced force to 1.26 ± 0.20 g, with a maximum EFS-induced relaxation (15 Hz) of 0.40 ± 0.05 g (n = 23).

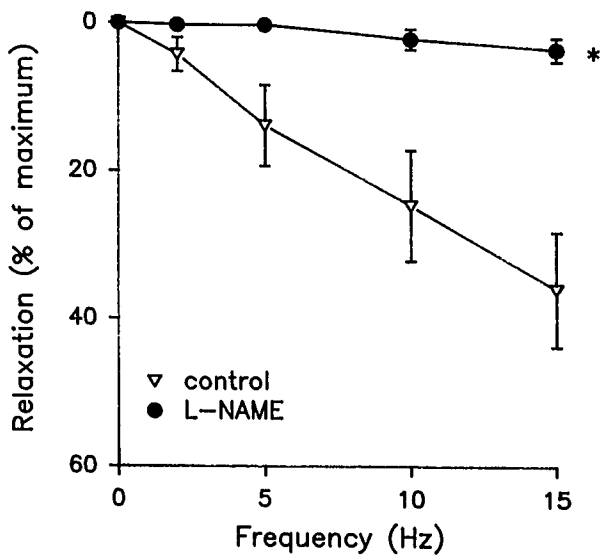

Fig. 2. Mean relaxation responses of porcine trachealis muscle to electrical field stimulation, expressed as a percentage of maximal relaxation to atropine (10^{-6} M), before and after addition of N^G-nitro-L-arginine methyl ester (L-NAME) (10^{-4} M). n = 5. *99% confidence interval excludes zero.

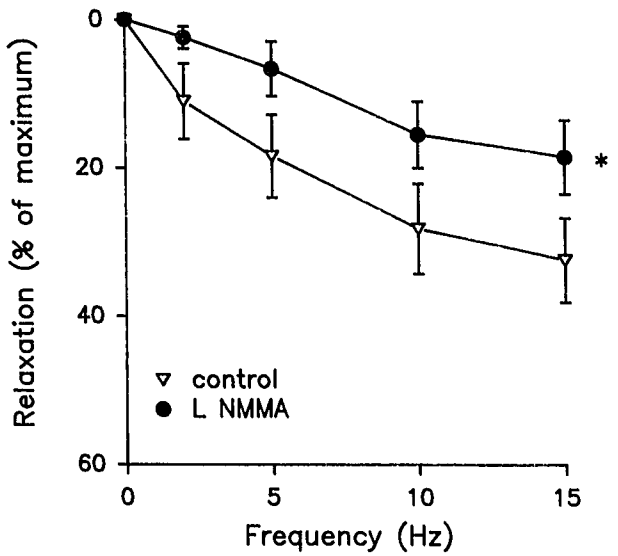

Fig. 3. Mean relaxation responses of porcine trachealis muscle to electrical field stimulation, expressed as a percentage of maximal relaxation to atropine (10^{-6} M), before and after addition of L-NMMA (10^{-4} M). n = 7. *99% confidence interval excludes zero.

In muscle strips in which tone was restored during halothane (1.0%) administration, 3.1 ± 0.53 × 10^{-8} M carbachol was added to the tissue baths to return tension to within 5.1 ± 2.3% (n = 10) of initial carbachol-induced tone. In these strips, halothane (1.0%) did not significantly attenuate NANC relaxations in the presence of either L-arginine (n = 5) or D-arginine (n = 5). In the presence of D-arginine, EFS-induced relaxations (15 Hz) were 36.7 ± 9.6% and 35.9 ± 7.9% of maximum before and after halothane, respectively. In the presence of L-arginine, EFS (15 Hz) relaxed carbachol-induced tone 44.1 ± 9.7% and 45.8 ± 9.0% of maximum before and after halothane, respectively.

Discussion

The results of this study confirm previous evidence of the existence of NANC inhibitory neural pathways in porcine trachealis muscle[12,14] and the importance of NO as a mediator in NANC neurotransmission.[13] We extended these findings to demonstrate that halothane, at the concentrations tested, does not significantly affect formation, transport, or release of NO *via* these nerves in this tissue.

Evidence to support the existence of a NANC inhibitory system in porcine trachealis muscle includes the ability of EFS to induce relaxation of carbachol-induced tone, the ineffectiveness of propranalol in attenuating these relaxations, and the ability of tetrodotoxin to abolish these responses completely. Kannan and Johnson[12] and Mitchell and coworkers[14] obtained similar results by using different stimulus parameters in porcine trachealis muscle with epithelium intact. Because NANC inhibitory responses do not differ significantly in epithelium-intact or epithelium-denuded preparations,[19,20] we chose to remove the epithelium to avoid this potential confounding factor in interpreting the effects of halothane on NANC responses in airway smooth muscle.

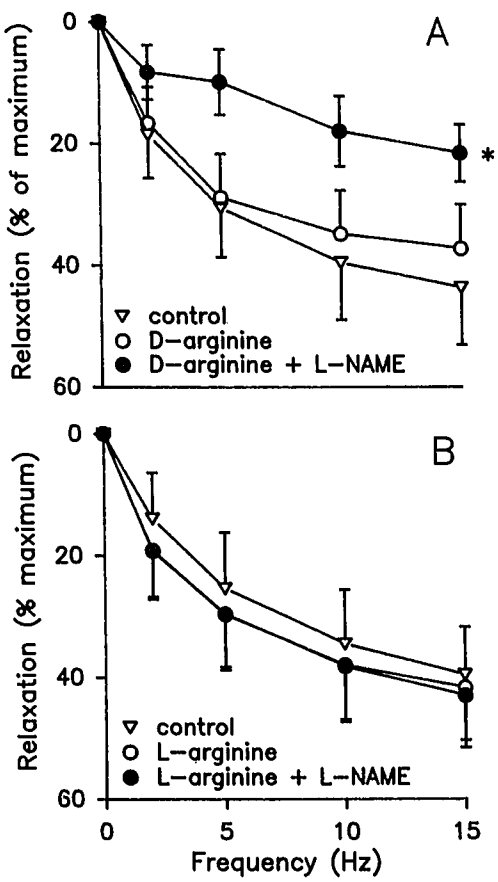

Fig. 4. Mean relaxation responses of porcine trachealis muscle to electrical field stimulation, expressed as a percentage of maximal relaxation to atropine (10^{-6} M). A) Control responses, responses after D-arginine (10^{-4} M), and responses after N^G-nitro-L-arginine methyl ester (L-NAME) (10^{-5} M) + D-arginine. n = 6. B) Control responses, responses after L-arginine (10^{-4} M), and responses after N^G-nitro-L-arginine methyl ester (L-NAME) (10^{-5} M) + L-arginine. n = 6. * 99% confidence interval excludes zero.

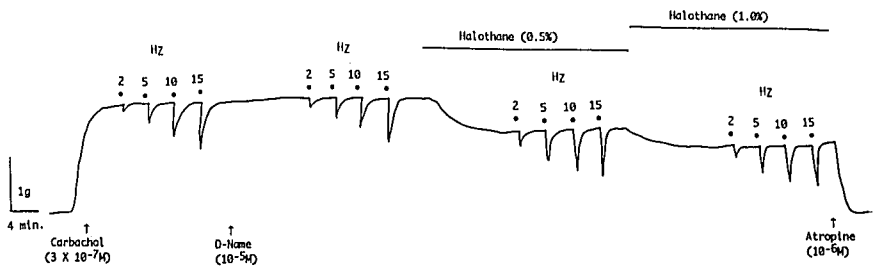

Fig. 5. Relaxation responses to electrical field stimulation (30 V, 1 ms for 10 s at the frequencies shown) in a single porcine tracheal smooth muscle strip after pretreatment with 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP) (10^{-4} M) and propranolol (10^{-6} M), before and after the addition of D-NAME (10^{-5} M). Increasing concentrations of halothane were bubbled into solution as indicated.

Our findings support the central role of NO in NANC inhibitory neurotransmission in porcine trachealis muscle. Two different NOS inhibitors, L-NMMA and L-NAME, but not D-NAME, attenuated NANC relaxations (figs. 1–3). Moreover, the substrate for NOS, L-arginine, but not its inactive enantiomer D-arginine, prevented the inhibitory effect of L-NAME (fig. 4). Interestingly,

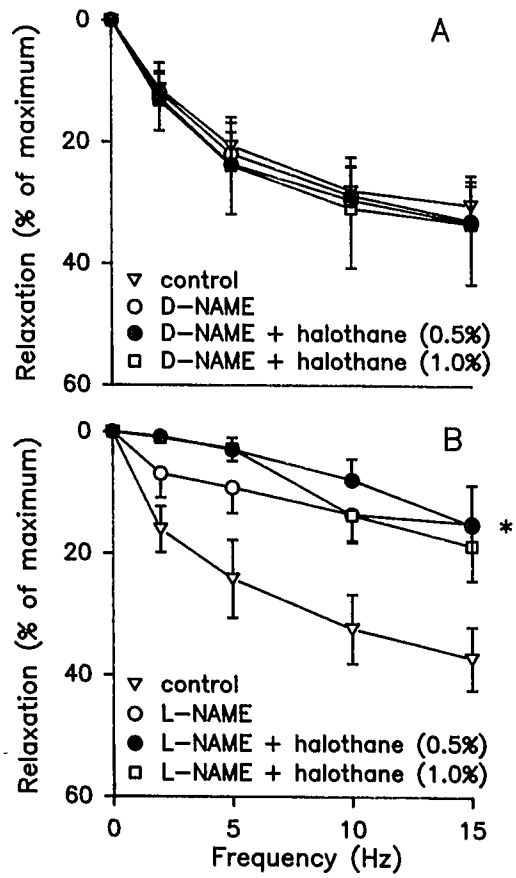

Fig. 6. Mean relaxation responses of porcine trachealis muscle to electrical field stimulation, expressed as a percentage of maximal relaxation to atropine (10^{-6} M). (*A*) Control responses, responses after D-NAME (10^{-5} M), and responses after increasing concentrations of halothane in the presence of D-NAME. n = 6. (*B*) Control responses, responses after N^G-nitro-L-arginine methyl ester (L-NAME) (10^{-5} M), and responses after increasing concentrations of halothane in the presence of L-NAME. n = 5. * 99% confidence interval excludes zero.

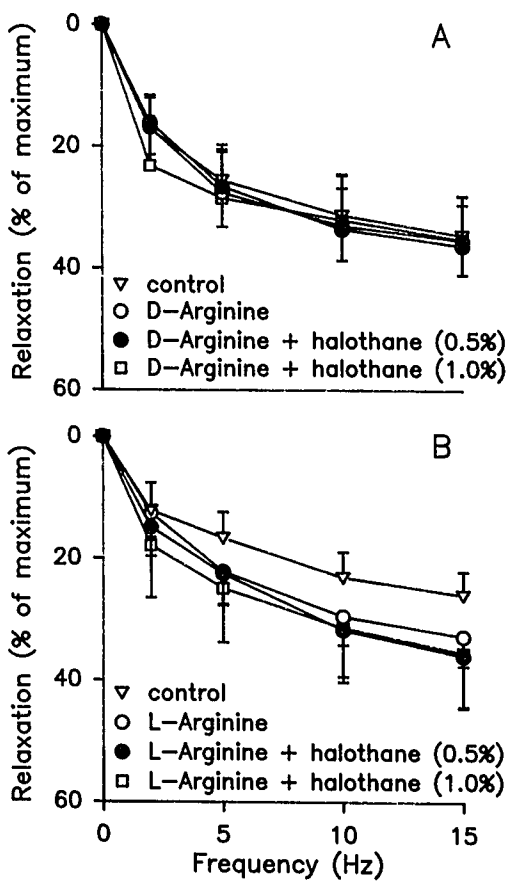

Fig. 7. Mean relaxation responses of porcine trachealis muscle to electrical field stimulation, expressed as a percentage of maximal relaxation to atropine (10^{-6} M). (*A*) Control responses, responses after D-arginine (10^{-4} M), and responses after increasing concentrations of halothane in the presence of D-arginine. n = 6. (*B*) Control responses, responses after L-arginine (10^{-4} M), and responses after increasing concentrations of halothane in the presence of L-arginine. n = 6.

L-arginine, alone, slightly but significantly increased NANC relaxations when compared with controls (figs. 4B and 7B), suggesting that formation of NO is somewhat limited by availability of the substrate. Kannan and Johnson[13] noted a trend toward enhancement of NANC responses in the presence of L-arginine, but this difference was not significant, perhaps because in their study the number of experimental subjects was small. However, these two studies do not support an important role for substrate availability in determining the rate of NO formation.

Volatile anesthetics could affect the NANC inhibitory system in any of several ways. Potentially, part of the bronchodilator action of volatile anesthetics could be mediated by NO release from NANC nerves, although these anesthetics are generally not thought to stimulate neurotransmitter release. If this effect occurred through peripheral, rather than central stimulation of this pathway, then halothane should enhance EFS-induced relaxations alone or in the presence of D-NAME, an inactive enantiomer of L-NAME. Halothane should also reverse the inhibitory effect of L-NAME on NANC relaxations. Our findings do not support this hypothesis. In the presence of D-NAME, inhibitory responses to EFS did not differ before or after halothane (fig. 6A). Furthermore, halothane did not reverse the effect of L-NAME on NANC responses (fig. 6B). Thus, NO release from the NANC system does not play a significant role in bronchodilation by volatile anesthetics *in vitro*.

Alternatively, volatile anesthetics could attenuate NANC relaxations. Potential sites of action of volatile anesthetics in the NANC inhibitory pathway include formation, release, and transport of NO, or antagonism of NO at the target cell. In blood vessels, volatile anesthetics attenuate endothelial-dependent vasodilation,[21-23] which is thought to be mediated by NO. In some vessels this inhibition appears to occur in the synthesis, release, or transport of NO.[21] In other vessels, the mechanism involves an interference with activation of guanylyl cyclase in the smooth muscle cell.[23] For halothane, these effects occur at concentrations $\geq$ 2.0%.[21,23]

Our findings suggest that halothane, at concentrations $\leq$ 1.0%, does not have a similar effect on NO released from the NANC inhibitory system in the airways. In contrast to the marked effects of L-NAME (figs. 2 and 4A), halothane did not attenuate NANC relaxations in the presence of D-arginine (fig. 7). In those experiments, halothane reduced tone by up to 30%. We did not study higher concentrations of halothane because NANC relaxations would be difficult to detect and interpret in the presence of even lower levels of carbachol-induced tone. In addition, because of functional antagonism,[24] this reduction in initial tone might have enhanced EFS-induced relaxations, thus masking an inhibitory effect of halothane. However, in parallel studies in which initial tone was restored by adding more carbachol, halothane did not inhibit NANC relaxations in the presence of either D-arginine or L-arginine. Together, these findings support the idea that halothane, at the concentrations tested, does not interfere with NANC relaxations of airway smooth muscle *in vitro*.

Thus, like human airway smooth muscle, porcine trachealis muscle exhibits an active NANC inhibitory system that is mediated by NO. Halothane, at clinically relevant concentrations, does not affect activity of this system *in vitro*. If these results can be extrapolated to human subjects, the potent neural effects of halothane on airways *in vivo* do not appear to involve the NANC inhibitory system.

The authors thank Kenneth Kubos, Ph.D., Department of Anesthesiology and Critical Care Medicine, The Johns Hopkins University, for analysis of halothane samples.

References

1. Warner DO, Brichant J-F, Rehder K: Direct and neurally mediated effects of halothane on pulmonary resistance *in vivo*. ANESTHESIOLOGY 72:1057–1063, 1990

2. Brichant J-F, Gunst S, Warner DO, Rehder K: Halothane, enflurane, and isoflurane depress the peripheral vagal motor pathway in isolated canine tracheal smooth muscle. ANESTHESIOLOGY 74:325–332, 1991

3. Coon RL, Kampine JP: Hypocapnic bronchoconstriction and inhalation anesthetics. ANESTHESIOLOGY 43:635–644, 1993

4. Hickey RF, Graf PD, Nadel JA, Larson CP: The effects of halothane and cyclopropane on total pulmonary resistance in the dog. ANESTHESIOLOGY 31:334–343, 1969

5. Hirshman CA, Edelstein G, Peetz S, Wayne R, Downes H: Mechanism of action of inhalational anesthesia on airways. ANESTHESIOLOGY 56:107–111, 1982

6. Lloyd TC: Reflex effects of lung inflation and inhalation of halothane, ether, and ammonia. J Appl Physiol 45:212–218, 1978

7. Shah MV, Hirshman CA: Mode of action of halothane on histamine-induced airway constriction in dogs with reactive airways. ANESTHESIOLOGY 65:170–174, 1986

8. Korenaga S, Takeda K, Ito Y: Differential effects of halothane on airway nerves and muscle. ANESTHESIOLOGY 60:309–318, 1984

9. Bai TR, Bramley AM: Effect of an inhibitor of nitric oxide synthase on neural relaxation of human bronchi. Am J Physiol (Lung Cell Mol Physiol) 8:L425–L430, 1993

10. Ellis JL, Undem BJ: Inhibition by L-N-^{G}-nitro-L-arginine of non-adrenergic-noncholinergic-mediated relaxations of human isolated

central and peripheral airways. Am Rev Respir Dis 146:1543–1547, 1992

11. Ward JK, Belvisi MG, Fox AJ, Miura M, Tadjkarimi S, Yacoub MH, Barnes PJ: Modulation of cholinergic neural bronchoconstriction by endogenous nitric oxide and vasoactive intestinal peptide in human airways in vitro. J Clin Invest 92:736–743, 1993

12. Kannan MS, Johnson DE: Functional innervation of pig tracheal smooth muscle: neural and non-neural mechanisms of relaxation. J Pharmacol Exp Ther 260:1180–1184, 1991

13. Kannan MS, Johnson DE: Nitric oxide mediates the neural nonadrenergic, noncholinergic relaxation of pig tracheal smooth muscle. Am J Physiol 262:L511–L514, 1992

14. Mitchell HW, Sparrow MP, Tagliaferri RP: Inhibitory and excitatory responses to field stimulation in fetal and adult pig airway. Pediatr Res 28:69–74, 1990

15. Richardson J, Beland J: Nonadrenergic inhibitory nervous system in human airways. J Appl Physiol 41:764–771, 1976

16. Taylor SM, Pare PD, Schellenberg R: Cholinergic and nonadrenergic mechanisms in human and guinea-pig airways. J Appl Physiol 56:958–965, 1984

17. Gao Y, Vanhoutte PM: Attenuation of contractions to acetylcholine in canine bronchi by an endogenous nitric oxide-like substance. Br J Pharmacol 109:887–891, 1993

18. Jennrich RI, Schluchter MD: Unbalanced repeated-measures models with structured covariance matrices. Biometrics 42:805–820, 1986

19. Rhoden K, Barnes PJ: Epithelial modulation of NANC and VIP-induced responses: Role of neutral endopeptidase. Eur J Pharmacol 171:247–250, 1989

20. Watson N, Maclagan J, Barnes PJ: Vagal control of guinea pig tracheal smooth muscle: Lack of involvement of VIP and nitric oxide. J Appl Physiol 74:1964–1971, 1993

21. Muldoon SM, Hart JL, Bowen KA, Freas W: Attenuation of endothelium-mediated vasodilation by halothane. ANESTHESIOLOGY 68: 31–37, 1988

22. Stone DJ, Johns RA: Endothelium-dependent effects of halothane, enflurane, and isoflurane on isolated rat aortic vascular rings. ANESTHESIOLOGY 71:126–132, 1989

23. Hart JL, Jing M, Bina S, Freas W, Van Dyke RA, Muldoon SM: Effects of halothane on EDRF/cGMP-mediated vascular smooth muscle relaxations. ANESTHESIOLOGY 79:323–331, 1993

24. Torphy TJ, Rinard GA, Reitow MG, Mayer SE: Functional antagonism in canine tracheal smooth muscle: Inhibition by methacholine of the mechanical and biochemical responses to isoproterenol. J Pharmacol Exp Ther 227:694–699, 1983