

## Direct and Neurally Mediated Effects of Halothane on Pulmonary Resistance In Vivo

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It has been suggested that halothane inhibits contraction of airway smooth muscle *in vivo* mainly by reducing reflex activity in nerves innervating the muscle with only minimal direct effects on the muscle itself. To examine possible mechanisms of action of halothane at clinically relevant concentrations the authors studied the effect of halothane on increases in pulmonary resistance ( $R_L$ ) produced by either vagus nerve stimulation (VNS, which caused neurally mediated constriction) or the inhalation of nebulized acetylcholine (ACh, which directly stimulated the smooth muscle cell) in nine mongrel dogs. The frequency of bilateral VNS and the dose of nebulized ACh were adjusted to produce approximately equal increases in  $R_L$ . Halothane reduced the response to both types of stimulation in a dose-dependent fashion. At halothane concentrations greater than or equal to 0.4 MAC, the VNS response was significantly less than the ACh response. When tetrodotoxin was given to block neural activity, the ACh response was unchanged, confirming that neural activation did not contribute significantly to smooth muscle contraction in response to ACh. The authors conclude that in addition to neurally mediated effects, halothane at clinically used concentrations has significant direct effects on airway smooth muscle stimulated by ACh. The relative importance of each factor *in vivo* should depend on the stimulus that causes contraction of airway smooth muscle. (Key words: Anesthetics, volatile: halothane. Lungs: airway resistance; dynamic compliance; pulmonary resistance. Muscle, smooth: airway. Nerve, vagus: stimulation.)

STUDIES of the effect of halothane on airway smooth muscle tone generally suggest that halothane has actions both on neural reflex pathways and directly on the smooth muscle cell and its receptor systems.<sup>1-5</sup> However, the relative importance of reflex and direct effects is uncertain, as some investigators have found that halothane has only a small direct effect on airway smooth muscle in dogs at clinically relevant halothane concentrations.<sup>4,6</sup>

Halothane and other volatile anesthetics attenuate increases in pulmonary resistance caused by vagus nerve stimulation (VNS) in dogs.<sup>7-9</sup> To separate the neurally mediated from the direct effects of halothane on increases in pulmonary resistance caused by stimulation of the vagal motor pathway, we studied intact mongrel dogs during stimulation of airway smooth muscle in two ways before and during halothane administration. Electrical stimula-

tion of the vagus nerve activated vagal neural efferents,<sup>10</sup> causing acetylcholine (ACh) release from postganglionic parasympathetic nerve endings and smooth muscle contraction. Administration of aerosolized ACh activated the muscle directly at the muscarinic receptor on the muscle membrane, bypassing neural pathways. By comparing the effect of halothane on these two responses, the effect of halothane on each part of the vagal motor pathway *in vivo* could be estimated.

### Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee. Nine mongrel dogs (11-19 kg) were anesthetized with iv chloralose ( $57 \pm 6$  mg/kg [mean  $\pm$  SD]) and urethane ( $566 \pm 61$  mg/kg), anesthetics that have little effect on increases in  $R_L$  produced by vagus nerve stimulation in dogs.<sup>11</sup> This baseline anesthesia was maintained throughout the experiment with repeated doses of chloralose (5 mg/kg) and urethane (50 mg/kg) every 45 min. The dogs were lying supine during the study. A femoral arterial catheter was inserted for measurement of blood pressure and for sampling of arterial blood for blood gas analysis (IL-1302 pH Blood Gas Analyzer, Lexington, MA). After tracheal intubation (9-mm ID) and placement of a pleural balloon as described below, the dogs were paralyzed with vecuronium (0.5 mg/kg iv followed by an infusion of  $0.05 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). The lungs were mechanically ventilated (Harvard 615, Millis, MA) using an inspired oxygen concentration of 21%, a tidal volume of 15 ml/kg, and a breathing frequency adjusted to 12-15 breaths per min to produce normocapnia. These variables were constant for each dog throughout the experiment. Any metabolic acidosis was corrected by sodium bicarbonate (1 mEq/kg) iv to keep the pH above 7.30.

The cervical vagus nerves were exposed bilaterally, divided, and stimulating electrodes were applied to the distal nerves.<sup>10</sup> The nerves and electrodes were covered with mineral oil to prevent desiccation. The dog was then placed in an air-conditioned volume-displacement body plethysmograph designed for dogs that measured changes in lung volume using an attached, appropriately damped Krogh spirometer (Emerson, Cambridge, MA) with adequate frequency response to 12 cycles per s.<sup>12</sup> Rectal temperature as measured by a thermistor was maintained between 35-37°C by using a warming mattress. Gas flow at the airway opening was measured by a heated pneumotachograph (Fleisch 1, Instrumentation Associates,

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New York, NY) coupled to a differential pressure transducer (Validyne MP 45, Northridge, CA). Tracheal pressure ( $P_{tr}$ ) was sensed (Statham PM131, Hato Rey, Puerto Rico) through a catheter (PE-200) with its tip positioned 3-cm distal to the tracheal end of the endotracheal tube to avoid distortion of pressure measurements by turbulence at the end of the endotracheal tube. Pleural pressure ( $P_{pl}$ ) was sensed by a flat silastic balloon inserted into the thorax through a small incision in the right sixth intercostal space as previously described.<sup>8</sup> Balloon volume was checked frequently throughout the experiment. Transpulmonary pressure was taken as the difference between  $P_{pl}$  and  $P_{tr}$ . The validity of changes in  $P_{pl}$  as an estimate of change in pleural pressure was tested by obstructing the endotracheal tube during spontaneous breathing and comparing changes in  $P_{pl}$  and  $P_{tr}$ . The maximum difference accepted between the two pressures was  $\pm 10\%$  of the minimum negative tracheal pressure.

A nebulizer (DeVilbiss 645, Somerset, PA) driven by a solenoid-actuated pressure source was used to deliver either normal saline or acetylcholine (Sigma, St. Louis, MO) dissolved in normal saline into the airways. Source pressure and timing were adjusted in each dog to give five breaths of 30 ml/kg tidal volume at 0.2 Hz. The mass mean aerodynamic diameter of the aerosol was  $1.8 \pm 2 \mu\text{m}$ .

Before measurements, each dog was given 2 mg/kg iv propranolol followed by an infusion of  $10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  to prevent  $\beta$ -adrenergic activation caused by stimulation of sympathetic fibers in the vagus nerve during VNS. The adequacy of  $\beta$  blockade was confirmed in each dog by an unchanged heart rate after 100  $\mu\text{g}$  isoproterenol iv.

#### PROCEDURE

The lungs were inflated twice to a  $P_{tr}$  of 25 cm  $\text{H}_2\text{O}$  approximately 10 min before each set of measurements described below. Measurements during VNS were obtained in the following manner. First, five breaths containing nebulized saline were given to provide a standard lung volume history and allow comparison of measurements during VNS with measurements during later administration of ACh. Thirty seconds later, transpulmonary pressure, lung volume, and gas flow were recorded before and during VNS (25-V, 3-ms bipolar pulses; Square Pulse Stimulator, Grass model S44, Quincy, MA) at a stimulation frequency of 15 Hz. VNS was applied for six breaths. Stimulation caused severe bradycardia and hypotension that quickly resolved after stimulation ceased. The dose of nebulized ACh producing an approximately equal increase in pulmonary resistance to that caused by VNS was then determined.<sup>13</sup> Baseline measurements for each dose of ACh were obtained immediately before drug nebulization. Measurements of the response to ACh were obtained 30 s after drug nebulization, a time of maximal response. In each instance, measured values returned to

predrug values over the 10-min period between runs. An initial concentration of 3 mg/ml was given, followed by increases in concentration in increments of 3 mg/ml until the increase in pulmonary resistance caused by ACh approximated the increase provided by vagus nerve stimulation at 15 Hz. The frequency of VNS was then slightly adjusted if necessary to match the magnitude of the ACh response. Both ACh and VNS responses were then obtained in duplicate to confirm that they were approximately equal. The mean concentration of ACh used was  $7.3 \pm 5 \text{ mg/ml}$ , and the mean frequency of VNS was  $12 \pm 4 \text{ Hz}$ .

Halothane was then added to the inspired gas in concentrations producing stable end-tidal concentrations corresponding to  $0.12 \pm 0.02$ ,  $0.40 \pm 0.03$ ,  $0.82 \pm 0.03$ , and  $1.24 \pm 0.13 \text{ MAC}$  as measured by a calibrated infrared analyzer (Beckman LB-2, Sensormedics, Anaheim, CA). The concentrations were studied in random order. Twenty minutes were allowed for equilibration at each concentration before measurements were made, and the responses to ACh and VNS were obtained at each concentration.

To determine whether the response to ACh was partially mediated by neural structures such as the parasympathetic ganglia, tetrodotoxin (TTX, Sigma) was used at the end of each experiment to eliminate neural function. TTX 10  $\mu\text{g/kg}$  iv was given and VNS was repeated. In each dog, VNS neither caused bradycardia nor changed pulmonary resistance, confirming the presence of neural blockade. ACh responses were then obtained at the 3 highest halothane concentrations studied in random order. Because the hemodynamic effects of TTX and halothane were eventually fatal in 3 dogs, measurements of ACh response could not be obtained at all halothane concentrations in all dogs.

Following the experiments, the dogs were killed with pentobarbital sodium and the lungs were examined macroscopically. No atelectasis or pneumonia was found in any dog.

#### DATA ANALYSIS

All data were digitally sampled (PDP 11/73, DEC, Maynard, MA) at 100 Hz.  $R_L$  and dynamic lung compliance ( $C_{L,dyn}$ ) were then calculated by a multiple linear regression technique.<sup>8-10</sup> For VNS, prestimulation values of  $R_L$  and  $C_{L,dyn}$  were taken as the mean of the three breaths preceding stimulation.  $R_L$  and  $C_{L,dyn}$  values during VNS were taken as the mean of the last three of six breaths during VNS. For ACh administration, baseline measurements were taken as the mean of three breaths immediately before drug nebulization. Values after ACh were taken as the mean of  $R_L$  and  $C_{L,dyn}$  values over three breaths 30 s after drug nebulization.

Paired comparisons of values for each dog were made with paired student's *t* tests. Multiple paired comparisons

TABLE 1. Pulmonary Resistance,  $\text{cm H}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}^{-1}$

End-Tidal Halothane, MAC	VNS		ACh		ACh after TTX	
	Prestim	Stim	Before	After	Before	After
0	2.54 ± 0.76	6.22 ± 1.38	2.44 ± 0.67	6.32 ± 1.24	—	—
0.1	2.42 ± 0.71	5.97 ± 1.91	2.39 ± 0.74	5.76 ± 1.22	—	—
0.4	2.50 ± 0.86	4.64 ± 1.63	2.37 ± 0.77	5.53 ± 1.69*	2.94 ± 0.38	6.29 ± 1.54* (n = 6)
0.8	2.52 ± 0.75	4.18 ± 1.13	2.40 ± 0.74	5.01 ± 1.17*	2.50 ± 0.45	4.98 ± 0.83* (n = 6)
1.2	2.54 ± 0.72	3.83 ± 0.92	2.49 ± 0.73	4.85 ± 0.94*	2.96 ± 0.57	5.28 ± 1.79* (n = 7)

All values mean ± SD.

VNS = vagus nerve stimulation. ACh = acetylcholine administration. TTX = tetrodotoxin. Prestim = before vagus nerve stimulation. Stim = during vagus nerve stimulation.

\* Significant difference as compared with the response to VNS,  $P < 0.05$ .

were performed with repeated measures analysis of variance (ANOVA). A  $P$  value of less than 0.05 was considered to be significant.

### Results

In the absence of halothane, the increases in  $R_L$  caused by VNS ( $3.68 \pm 0.93 \text{ cm H}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$ ) and ACh ( $3.88 \pm 0.80 \text{ cm H}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s}$ ) were not significantly different ( $P = 0.22$ ; table 1).

Halothane had no significant effect on baseline  $R_L$  values ( $P > 0.05$  by ANOVA before all methods of stimulation; table 1). Halothane caused a dose-dependent decrease in the  $R_L$  response to both VNS and ACh (table 1; fig. 1). At halothane concentrations greater than or equal to 0.4 MAC, the reduction in VNS response was significantly greater than the reduction in ACh response (fig. 1).

The administration of TTX to eliminate neural function did not consistently change the mean response of  $R_L$  to ACh at any halothane concentration ( $P > 0.05$  for each

halothane concentration), although there were small differences in some dogs (fig. 2).

Halothane attenuated the decrease in  $C_{L,dyn}$  caused by both VNS and ACh at concentrations greater than or equal to 0.8 MAC ( $P < 0.05$  as compared with the decrease in the absence of halothane, table 2). ACh always caused a significantly greater decrease in  $C_{L,dyn}$  as compared with VNS ( $P < 0.05$  at all halothane concentrations; table 2). The administration of TTX did not significantly change the  $C_{L,dyn}$  response to ACh at any halothane concentration ( $P > 0.05$  at all concentrations).

There were no significant differences in  $\text{PaO}_2$ ,  $\text{PaCO}_2$ , or  $p\text{H}$  between each condition studied ( $P > 0.10$  for each variable, ANOVA). Both halothane and TTX decreased mean arterial pressure (table 3).

### Discussion

The main finding of this study was that halothane, at clinically relevant concentrations, has significant direct

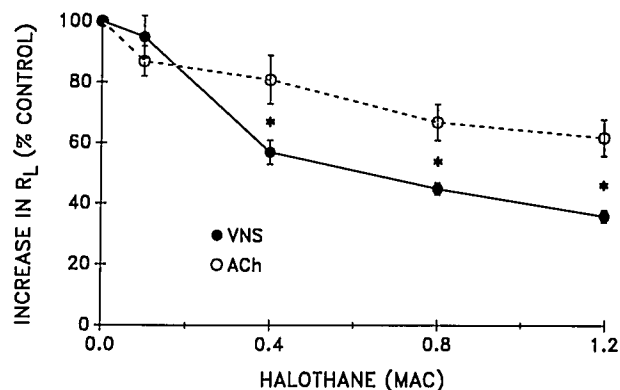


FIG. 1. Increases in pulmonary resistance ( $R_L$ ) during vagus nerve stimulation (VNS) and after acetylcholine (ACh) administration as a function of end-tidal halothane concentration. Values are expressed as a percent of the control response (the response in the absence of halothane), mean ± SE. \*Significant difference between ACh and VNS;  $P < 0.05$ .

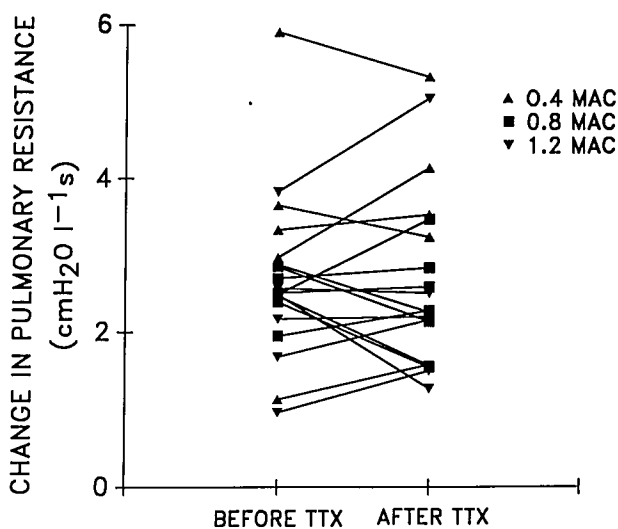


FIG. 2. Change in pulmonary resistance after acetylcholine administration at three end-tidal halothane concentrations before and after the administration of tetrodotoxin (TTX).

TABLE 2. Dynamic Lung Compliance, ml/cm H<sub>2</sub>O

End-Tidal Halothane, MAC	VNS		ACh		ACh after TTX	
	Prestim	Stim	Before	After	Before	After
0	57 ± 24	48 ± 23	59 ± 25	34 ± 17*	—	—
0.1	56 ± 23	46 ± 23	57 ± 23	35 ± 18*	—	—
0.4	58 ± 23	51 ± 23	60 ± 23	38 ± 17*	52 ± 29	32 ± 18 (n = 6)
0.8	56 ± 23	50 ± 23†	57 ± 23	39 ± 17*†	59 ± 24	36 ± 14 (n = 6)
1.2	58 ± 26	51 ± 23†	58 ± 26	41 ± 22*†	49 ± 25	33 ± 16 (n = 7)

All values mean ± SD.

VNS = vagus nerve stimulation. ACh = acetylcholine administration. TTX = tetrodotoxin. Prestim = before vagus nerve stimulation. Stim = during vagus nerve stimulation.

\* Significant difference as compared with the response to VNS,  $P < 0.05$ .

† Significantly less decrease during stimulation as compared with 0 MAC halothane,  $P < 0.05$ .

relaxing effects on airway smooth muscle constricted by acetylcholine in intact mongrel dogs.

### METHODOLOGY

Before further discussion of these results, the limitations of  $R_L$  measurements as a measure of airway smooth muscle tone should be appreciated. The magnitudes of the two methods of stimulating airway smooth muscle were adjusted to produce equal increases in  $R_L$ . The rationale underlying this protocol was to provide an equal degree of airway muscle contraction by a method which stimulated the muscle directly *via* muscarinic receptors (ACh) and a method that included neural cholinergic pathways (VNS). If the two methods stimulated the same population of airway smooth muscle to the same degree, then the contribution of neurally mediated *versus* direct effect of halothane could be precisely estimated. However,  $R_L$  is a measure of global lung function, and increases in  $R_L$  can be achieved in several ways. For example, measurements of regional airway resistances in dogs have demonstrated that different methods of stimulation (VNS, histamine aerosols, leukotriene aerosols, and others) may produce different responses in the central and peripheral

airways.<sup>14,15</sup> Such regional differences may not be reflected in overall  $R_L$  measurements. These differential responses could be caused by regional differences in receptor density or sensitivity to agonists, or by the difference between the distribution of parasympathetic innervation and the pattern of aerosol deposition. We are unaware of studies of regional airway resistances following acetylcholine administration.

Another factor complicating the interpretation of  $R_L$  measurements is that tissue resistance, not airway resistance, is the principal component of  $R_L$  at this breathing frequency in dogs.<sup>9,15,16</sup> However, we have previously shown that halothane causes similar changes in airway and tissue resistances during VNS in dogs, so that changes in  $R_L$  can be used as an index of changes in airway caliber.<sup>9</sup> Also, changes in tissue resistance during lung stimulation may be caused by airway smooth muscle contraction.<sup>17-19</sup> Finally, it appears that changes in airway and tissue resistances are closely linked so that all methods of lung stimulation studied to date (including VNS and humoral mediators) cause approximately equal increases in airway and tissue resistances.<sup>9,15,17</sup> Thus, it is probable that  $R_L$  is a valid index of airway smooth muscle tone.<sup>9</sup>

The response of  $C_{L,dyn}$  to VNS and the lack of an effect of low concentrations of halothane was comparable to a previous study in closed-chest dogs in our laboratory.<sup>8</sup> The greater decrease in  $C_{L,dyn}$  caused by ACh administration as compared with VNS may be evidence that ACh and VNS produced equal increases in  $R_L$  by different mechanisms. However, we have previously shown in dogs under similar conditions that changes in  $C_{L,dyn}$  caused by VNS are caused by changes in lung elastic properties, not changes in peripheral airway resistance.<sup>9</sup> Therefore, differences in  $C_{L,dyn}$  response *per se* do not imply that ACh and VNS increased  $R_L$  by different mechanisms. Nevertheless, the greater response of  $C_{L,dyn}$  to ACh may imply activation of a different population of airway smooth muscle or parenchymal contractile elements.

We assumed that VNS produced contraction of airway smooth muscle primarily by acetylcholine release from postganglionic nerves. VNS can also stimulate the release

TABLE 3. Arterial Blood Gases and Blood Pressure

End-Tidal Halothane, MAC	$P_{aO_2}$ , mmHg	$P_{aCO_2}$ , mmHg	pH	MAP, mmHg
Before TTX				
0	113 ± 11	36 ± 2	7.39 ± 0.03	144 ± 13
0.1	114 ± 8	35 ± 3	7.38 ± 0.03	135 ± 14
0.4	114 ± 5	35 ± 4	7.38 ± 0.03	121 ± 16
0.8	113 ± 10	34 ± 2	7.38 ± 0.03	110 ± 26
1.2	114 ± 11	33 ± 3	7.38 ± 0.04	83 ± 31
After TTX				
0.4	109 ± 10	35 ± 3	7.35 ± 0.04	63 ± 26
0.8	112 ± 8	34 ± 3	7.37 ± 0.03	63 ± 11
1.2	112 ± 9	35 ± 4	7.36 ± 0.04	54 ± 19

All values mean ± SD. Values were measured before vagus nerve stimulation and acetylcholine administration.

TTX, tetrodotoxin; MAC, minimal alveolar concentration; MAP, mean arterial pressure.

of other neurotransmitters such as substance P in the airways of guinea pigs.<sup>20</sup> However, the muscarinic antagonists atropine and pirenzepine completely block the response to VNS in dogs,<sup>10</sup> demonstrating that VNS stimulates airway smooth muscle in dogs primarily by a cholinergic mechanism.

TTX was administered to determine whether aerosolized ACh had neurally mediated effects such as ganglionic stimulation in addition to direct effects on muscarinic receptors on airway smooth muscle. No consistent differences in responsiveness between ACh alone and ACh and TTX were found, demonstrating that in the presence of halothane neural activation by ACh did not contribute significantly to airway smooth muscle contraction. This conclusion is valid only if halothane itself does not completely block neural cholinergic transmission. The continued response to VNS in the presence of halothane suggests halothane suppresses but does not eliminate neural transmission.

#### PARTITIONING OF HALOTHANE EFFECTS

These considerations suggest that partitioning of the contribution of neurally mediated and direct effects of halothane on the response to VNS should be done with caution. However, even if ACh and VNS affect different populations of airway smooth muscle, to invalidate such partitioning one must postulate either a different linkage between smooth muscle tone and  $R_L$  or a differential sensitivity to halothane among the two populations. We are unaware of such differences, but the possibility cannot be excluded. With these caveats in mind, our results suggest that during VNS halothane has approximately equal depressant effects on neural transmission and the smooth muscle cell (see Appendix).

These results are consistent with previous investigations both *in vitro* and *in vivo*. The responsiveness of guinea pig and canine airway smooth muscle to histamine and ACh is reduced *in vitro* by halothane.<sup>1-3,5</sup> Although the histamine response is partially dependent on neurally mediated ACh release,<sup>21</sup> reductions in ACh response suggest a direct action on airway smooth muscle. Korenaga *et al.*<sup>3</sup> found that halothane in low concentrations (1-2%) depressed neural transmission and directly suppressed excitation-contraction coupling in canine airway trachealis muscle as measured by responsiveness to membrane depolarization. They also found a small decrease in ACh responsiveness to an unspecified "low concentration" of halothane, although an insufficient number of muscles were studied to draw any statistical conclusions. Recent studies in our laboratory have shown that 1 MAC halothane has both neural and direct effects in canine trachealis muscle *in vitro*, reducing responses to ACh, electrical field stimulation, and stimulation of parasympathetic

ganglia.<sup>22</sup> The barbiturates have similar effects.<sup>23</sup> Halothane also interferes with ganglionic transmission and postganglionic neurotransmitter release in the sympathetic nervous system<sup>24-27</sup> and directly relaxes vascular smooth muscle.<sup>28</sup>

The reduction in VNS response caused by halothane is consistent with previous studies.<sup>7-9</sup> Halothane also reduces airway responsiveness *in vivo* to specific antigen,<sup>4,29</sup> histamine,<sup>6,30</sup> and methacholine.<sup>4</sup> Two of these studies have attempted to separate reflex from direct effects of halothane on airway smooth muscle stimulation *in vivo* in dogs.

Hirshman *et al.*<sup>4</sup> showed that halothane depressed responsiveness of pulmonary resistance to both *Ascaris* antigen and methacholine in the basenji-greyhound dog model of asthma. They assumed that antigen challenge constricted airway smooth muscle by both reflex and direct effects<sup>31</sup> and that methacholine acted directly on the smooth muscle. Halothane, 1.5 MAC, caused approximately equal depression of the  $R_L$  response to aerosol methacholine 0.30 mg/ml and the response to antigen. In previous investigations on the same population of dogs, these investigators administered intravenous atropine<sup>31</sup> or lidocaine<sup>32</sup> to eliminate the reflex effects of antigen stimulation. In these studies, atropine and lidocaine reduced the  $R_L$  response to antigen less than halothane did in the study of Hirshman *et al.* Hirshman *et al.* concluded that halothane antagonized antigen-induced increases in  $R_L$  by depression of airway reflexes and by a small direct action on airway smooth muscle.

In a second study of basenji-greyhound dogs, Shah and Hirshman<sup>6</sup> studied the effects of halothane on the response of pulmonary resistance to aerosol histamine. Histamine, like *Ascaris* antigen, also has direct and reflex effects on the airways.<sup>21</sup> In contrast to the previous study using *Ascaris* antigen, they could find no difference between reductions in response caused by halothane 1.5 MAC and reductions in response caused by atropine given to block reflex effects. Furthermore, they found that the addition of halothane to dogs receiving atropine caused only a small additional decrease in  $R_L$  response, although the number of dogs studied (four) was insufficient to draw statistical inferences. As they could not demonstrate a direct effect of halothane on the response of pulmonary resistance to histamine, they concluded that the major action of halothane on histamine-induced bronchoconstriction was on airway reflexes.

The contrasting results of these two studies may suggest that the action of halothane depends on the applied stimulus. The reduction in methacholine responsiveness caused by halothane found by Hirshman *et al.*<sup>4</sup> is consistent with our ACh results and confirms that halothane in clinically relevant concentrations can have significant direct effects on airway smooth muscle stimulated with musca-

rinic agonists. However, partitioning of the effects of halothane into neural reflex and direct components should be applied to other methods of stimulating airway smooth muscle only with caution. We studied only the motor limb of reflex pathways by using VNS. Reflex airway stimulation also involves afferent receptors, afferent neural pathways, and central synapses. Halothane may also affect these other limbs of airway reflexes in addition to depressing the peripheral vagal motor pathway. In support of this concept, Holtzman *et al.*<sup>11</sup> found that afferent pathways and central synapses were even more sensitive to depression by barbiturates than was the peripheral vagus motor pathway. Thus, to the extent that halothane depresses neural transmission in afferent pathways and central synapses,<sup>33</sup> the effect of halothane on reflex neural pathways would be amplified and would assume a greater importance in antagonizing reflex bronchoconstriction. Direct actions of halothane on airway smooth muscle can only be significant if sufficient neural traffic reaches the smooth muscle cell. Therefore, the relative importance of neurally mediated and direct effects of halothane in antagonizing bronchoconstriction probably depends strongly on the applied stimulus, and this stimulus must be carefully specified when discussing the mechanism of action of halothane. For example, stimuli such as irritation of the tracheal mucosa by an endotracheal tube constrict airway smooth muscle predominantly through reflex pathways. The ability of halothane to depress neural transmission would be of primary importance in antagonizing constriction caused by these stimuli. Stimuli such as humoral mediators released in response to an immunologic reaction or in asthmatics may also have direct effects on the smooth muscle cell. Direct effects of halothane on the smooth muscle cell would then assume greater importance.

In conclusion, halothane at clinically relevant concentrations reduces neural transmission in the vagus nerve and directly depresses contraction of the smooth muscle cell caused by ACh in dogs. The relative importance of each mechanism should depend on the stimulus that causes airway smooth muscle constriction.

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*Appendix*

To estimate the relative contribution of neurally mediated and direct effects of halothane on the attenuation of airway smooth muscle contraction, the vagal motor pathway was considered to consist of a neural pathway connected to effector sites in the smooth muscle cell that caused contraction *via* muscarinic receptors (fig. 1 A). We assumed that halothane attenuated neural transmission by a fraction equal to  $f_{nt}$  and attenuated smooth muscle contraction to a given stimulus presented to the muscarinic receptors on the cell by a fraction  $f_m$ . The fraction of any nerve stimulation that reached the receptor on the smooth muscle cell was thus equal to  $1 - f_{nt}$ . The fraction of this neural stimulation that actually activated the muscle at the effector site was equal to the product of  $1 - f_{nt}$  and  $1 - f_m$ . This product  $(1 - f_{nt}) \cdot (1 - f_m)$  could be estimated as the response of  $R_L$  to VNS as a fraction of the control response to VNS in the absence of halothane. For example, at 0.8 MAC, this product was estimated

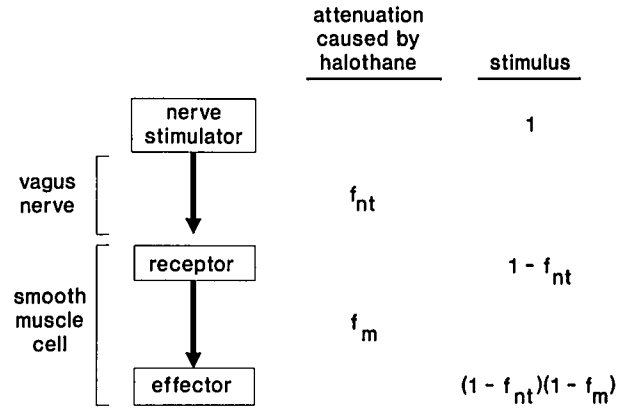


FIG. 1A. Diagram showing the scheme used to estimate neurally mediated and direct effects of halothane on the attenuation of airway smooth muscle contraction. The amount of stimulation applied to the vagus nerve is given an arbitrary value of 1.  $f_{nt}$  = fractional attenuation of neural transmission by halothane;  $f_m$  = fractional attenuation by halothane at or distal to the receptor.

as  $[(4.18-2.52)/(6.22-2.54)]$ , equal to 0.45 (values obtained from table 1). The value of  $1 - f_m$  at any halothane concentration could be estimated as the response of  $R_L$  to ACh as a fraction of the control response to ACh. The values of  $f_{nt}$  and  $f_m$  could then be calculated. Table 1 A shows these quantities at halothane concentrations of 0.4, 0.8, and 1.2 MAC. At each concentration,  $f_m$  and  $f_{nt}$  were similar, suggesting that halothane had approximately equal depressant effects on neural transmission and the smooth muscle cell.

TABLE 1A. Direct and Reflex Effects of Halothane

End-Tidal Halothane, MAC	$(1 - f_{nt}) \cdot (1 - f_m)$ (VNS response)	$1 - f_m$ (ACh response)	$f_m$	$f_{nt}$
0.4	0.57	0.81	0.19	0.30
0.8	0.45	0.67	0.33	0.33
1.2	0.36	0.62	0.38	0.42

$f_{nt}$  = fractional attenuation of neural transmission by halothane.  $f_m$  = fractional attenuation of direct smooth muscle response by halothane.