

Factors Influencing the Direct Actions of Volatile Anesthetics on Airway Smooth Muscle

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Background: The authors hypothesized that the ability of volatile anesthetics to relax airway smooth muscle (AWSM) depends on: 1) the agonist used to produce muscle contraction, 2) the preexisting level of contraction, and 3) the volatile anesthetic used.

Methods: To test the first hypothesis, isolated strips of canine trachealis muscle were precontracted with acetylcholine (ACh), McN-A-343 (McN, another muscarinic agonist), 5-hydroxytryptamine (5HT, a different receptor agonist), or potassium chloride (KCl, which induces contraction by cell depolarization) to 50% of maximal force, then exposed to halothane (0.2–1.6 MAC). To test the second and third hypotheses, other strips were precontracted to 25, 50, 75, and 100% of maximal force with ACh, or to 10, 30, or 50% of maximal force with KCl, and were then exposed to halothane or isoflurane (0.2–1.6 MAC).

Results: For AWSM contracted to 50% of maximal force, the amount of relaxation produced by halothane depended on the agonist used to elicit contraction in the following manner: 5HT = McN > ACh; the muscles contracted with KCl did not relax. For ACh-induced contractions, the absolute amount of relaxation produced by halothane did not depend on the level of precontraction. In muscles contracted with KCl, the volatile anesthetics caused significant relaxation only in muscles contracted to 10% of maximal force. Overall, halothane had a significantly greater relaxing effect as compared with isoflurane during ACh-mediated contractions; the effects of the two agents did not differ significantly during KCl-mediated contractions.

Conclusions: When canine AWSM tone is increased by contractile agonists *in vitro*, the absolute amount of relaxation

produced by halothane depends on the agonist used to elicit contraction, but not the degree of precontraction. In addition, there are small but significant differences between the effects of halothane and isoflurane on ACh-mediated contractions. (Key words: Anesthetics, volatile: halothane; isoflurane. Lung, bronchus: bronchoconstriction. Lung, trachea: canine; smooth muscle. Muscle, smooth: airway; trachea.)

VOLATILE anesthetics relax airway smooth muscle (AWSM) both by depressing reflex neural pathways and by acting directly on the smooth muscle cell.¹⁻⁷ The relative importance of the neural and direct effects of volatile anesthetics in preventing or relieving bronchospasm *in vivo* is unclear, but it appears that these agents have significant direct relaxing effects on AWSM at clinically relevant concentrations.^{1,5,8-10}

Based on current knowledge regarding other bronchodilators, several factors might be expected to modulate the ability of the volatile anesthetics to directly relax AWSM. For example, AWSM tone induced by muscarinic agonists is reduced by β -adrenoreceptor agonists. The degree of this relaxation depends on the initial level of smooth muscle tone produced by the muscarinic agonist; at higher levels of contraction, both the sensitivity and the maximal degree of relaxation to β -adrenoreceptor agonists are reduced as compared with lower levels of contraction.¹¹⁻¹⁴ Thus, the clinical efficacy of these agents may be reduced during severe bronchospasm. In addition, the ability of β -adrenoreceptor agonists to relax AWSM depends markedly on the specific agonist that produces contraction. For example, these drugs have a greater relaxing effect in muscles contracted to a given level of force with 5-hydroxytryptamine as compared with muscles contracted with muscarinic agonists.¹¹ Therefore, the clinical efficacy of β -adrenoreceptor agonists may also depend on the stimulus producing bronchospasm. It is not known if these considerations also apply to relaxation of AWSM produced by volatile anesthetics, the actions of which are not mediated by β -adrenoreceptors.^{3,15} Finally, there is evidence that the specific vol-

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atile anesthetics differ in their ability to relax vascular smooth muscle.¹⁶

The purpose of the current investigation was to evaluate the hypotheses that, when canine tracheal smooth muscle is contracted *in vitro*, the ability of volatile anesthetics to relax the muscle depends on: 1) the agonist used to produce muscle contraction, 2) the preexisting level of contraction, and 3) the volatile anesthetic used.

Materials and Methods

Tissue Preparation

This study was approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. Tracheas were removed from 66 mongrel dogs that were anesthetized with pentobarbital sodium and exsanguinated. The tissue was immediately immersed in a chilled physiologic salt solution (PSS) of the following composition (mM): 0.8 MgSO₄, 1.2 KH₂PO₄, 3.4 KCl, 2.4 CaCl₂, 110 NaCl, 25.7 NaHCO₃, and 5.6 dextrose. After removal of the epithelium and connective tissue, rectangular strips of smooth muscle (10–15 mm long and 2 mm wide) were dissected from the middle of the trachea. Each strip was suspended vertically in a 25-ml water-jacketed tissue bath containing PSS at 37° C and bubbled with 94% O₂/6% CO₂, providing a pH of 7.4, a P_{O₂} of 550 mmHg, and a P_{CO₂} of 36 mmHg (In-

strumentation Laboratories model 1302, Lexington, MA). The strips were connected to strain gauges (Grass FT03, Quincy, MA) for isometric force recording (Hewlett-Packard 7418A, Waltham, MA).

The strips were washed in PSS for ~2 h while being supramaximally stimulated (0.5-ms pulse duration, 25 Hz, 15 V) by electrical field stimulation at 5-min intervals. Muscle length was increased after each contraction until the force of contraction reached a maximum. Each strip was maintained at this optimal length for the duration of the experiment. The contractile response to 10⁻⁴ M acetylcholine (ACh), defined as the maximal response, was then determined.

Experimental Protocols

Effects of Contractile Agonist. Four pairs of muscle strips from each of 20 dogs were studied. Pairs of strips from a given dog were contracted with sufficient concentrations of either ACh, McN-A-343 (McN, a muscarinic agonist), 5-hydroxytryptamine (5HT), or potassium chloride (KCl) to produce approximately 50% of the maximal force for that strip. Thus, initial forces were comparable among agonists.^{11,17} Strips that had initial forces not within the range of 40–60% of maximal force were discarded. The mean concentration of each agonist is given in table 1. Muscle strips contracted with ACh and McN were incubated with tetrodotoxin 10⁻⁶ M to prevent any neurally mediated component of contraction.¹ Muscle strips contracted with KCl and

Table 1. Characteristics of Muscle Strips

Agonist	Target Level of Initial Force (% maximal)	Anesthetic Agent	N	Maximal Force (g)	Initial Force (% maximal)	Agonist Concentration
ACh	100	Hal	7	23 ± 7	101 ± 2	100 μM
		Iso	7	25 ± 5	105 ± 4	100 μM
	75	Hal	9	29 ± 4	77 ± 4	3.0 ± 2.4 μM
		Iso	6	28 ± 9	72 ± 3	2.1 ± 1.9 μM
	50	Hal	18	32 ± 9	51 ± 3	0.91 ± 0.51 μM
		Iso	6	24 ± 7	49 ± 4	0.46 ± 0.28 μM
KCl	50	Hal	7	33 ± 4	26 ± 2	0.16 ± 0.10 μM
		Iso	6	26 ± 3	25 ± 4	0.11 ± 0.14 μM
	30	Hal	15	32 ± 8	49 ± 2	23.6 ± 0.7 mM
		Iso	6	25 ± 4	49 ± 6	24.6 ± 1.4 mM
10	Hal	9	32 ± 5	30 ± 2	18.4 ± 1.4 mM	
	Iso	8	25 ± 4	31 ± 2	18.9 ± 0.8 mM	
5-HT	50	Hal	15	32 ± 7	11 ± 2	16.2 ± 1.3 mM
McN	50	Iso	6	24 ± 4	12 ± 4	16.5 ± 0.4 mM
		Hal	8	24 ± 7	49 ± 4	0.65 ± 0.85 μM
		Hal	8	30 ± 12	49 ± 4	0.19 ± 1.0 μM

N = number of pairs of muscle strips analyzed; for a given experimental condition, each pair of strips was obtained from a different dog; ACh = acetylcholine; KCl = potassium chloride; 5-HT = 5-hydroxytryptamine; McN = McN-A-343; Hal = halothane; Iso = Isoflurane.

5HT were incubated with atropine 10^{-6} M to prevent any concurrent muscarinic stimulation. After the force of contraction induced by these agonists had stabilized, halothane in concentrations from 0.2 to 1.6 MAC was added to the gas aerating one tissue bath in each pair. One MAC for halothane was taken as 0.86%.¹⁸ These concentrations were chosen as clinically relevant. The muscle strips were exposed to each concentration of halothane for 5 min, a period sufficient for stable responses. The other muscle strip in each pair did not receive halothane and served as a control for the effect of time.

Acetylcholine and McN activate M_3 subtype muscarinic receptors in canine tracheal smooth muscle;¹⁹ both agonists were studied because they have different receptor occupancy characteristics and different mechanisms of postreceptor signal transduction.^{19,20} 5-Hydroxytryptamine induces smooth muscle contraction by activating another receptor system.^{21,22} Potassium chloride induces contraction by direct depolarization of the smooth muscle cell membrane.²³

Effects of Level of Contraction and Anesthetic Agent. Four pairs of muscle strips from each of another 20 dogs were studied. Pairs of strips from a given dog were contracted with sufficient concentrations of ACh to produce forces equal to approximately 25, 50, 75, or 100% of the maximal contraction to ACh. Another three pairs of muscle strips from each of an additional 26 dogs were contracted with sufficient concentrations of KCl to produce forces equal to approximately 10, 30, or 50% of maximal contraction to ACh. The range of KCl forces was chosen based on the results from the first protocol. Acetylcholine and KCl were chosen as representative agonists operating *via* receptor and nonreceptor mediated pathways, respectively. Strips exposed to ACh were preincubated with tetrodotoxin 10^{-6} M, and strips exposed to KCl were preincubated with atropine 10^{-6} M. Strips that had initial forces not within 10% (expressed as a percentage of maximal force) of the desired initial force were discarded. The mean concentrations used for each agonist to achieve the target levels of force are given in table 1.

After stable contractions were achieved, halothane or isoflurane in increasing concentrations from 0.2 to 1.6 MAC was added to the gas aerating one tissue bath for each pair. One MAC for isoflurane was taken as 1.26%.¹⁸ The muscle strips were exposed to each concentration of halothane or isoflurane for 5 min, a period sufficient for stable responses. The other muscle strip in each pair did not receive an anesthetic and served as a control

for the effects of time. Data for muscles contracted to 50% maximal force and exposed to halothane were used from the first protocol.

Data Analysis

Isometric forces were expressed either as a percent of the maximal force or as a percent of the initial force (before exposure to volatile anesthetic). When used to express data during exposure to volatile anesthetics, the former variable describes absolute reductions in force, whereas the latter variable quantifies the relative degree of relaxation in terms of the initial force. In the muscles exposed to anesthetics, the contractile responses were adjusted for the effect of time according to the formula:

$$C_t = (C_1/C_2)C_{VA}$$

where C_t = time-adjusted response for the volatile anesthetic muscle, C_1 = response of control muscle (initial measurement), C_2 = response of control muscle (measured in parallel with the volatile anesthetic muscle), and C_{VA} = the response of the volatile anesthetic muscle.

Statistical comparisons of initial and maximal forces for each strip were performed using analysis of variance (ANOVA).²⁵ Because the amount of relaxation produced by the volatile anesthetics was often insufficient to calculate conventional indices, such as the 50% effective concentration, the potency of volatile anesthetic in relaxing muscle or the effects of time in control muscle strips was quantified by linear regression of the relationship between force and halothane concentration (or time, for control muscle strips) for individual muscle strips. To determine if the volatile anesthetic (or time, for control muscle strips) had a significant effect on force, the slopes of these individual lines were evaluated using a paired *t* test to test for a significant difference from zero.²⁴ To test for differences in anesthetic effect between agonists or level of contraction, slopes were first compared using ANOVA, then specific differences were evaluated using the Tukey test when appropriate.²⁵ For data concerning the effects of the level of contraction and anesthetic agent, a two-factor ANOVA design was used to test for overall differences in slopes between halothane and isoflurane.

Data are expressed as mean \pm SD. A $P < 0.05$ was considered significant. In all cases, *n* represents the number of muscle strip pairs analyzed for a given experimental condition. For a given condition, each pair of strips was obtained from a different dog. The value

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of *n* is less than the total number of dogs studied in a given protocol because strips that did not achieve initial forces within 10% of the desired initial force were discarded.

Drugs

Halothane (Ayerst Laboratories, New York, NY) or isoflurane (Anaquest, Madison, WI) were added to the aerating gas mixture *via* an on-line vaporizer. The concentration of anesthetic in the gas mixture was monitored continuously by a mass spectrometer (model MGA-1100, Perkin-Elmer, Pomona, CA). The concentration of volatile anesthetics in the PSS was determined by gas chromatography (Model 5880A, Hewlett-Packard) using the method of Van Dyke and Wood.²⁶ The drugs employed were ACh hydrochloride, 5-hydroxytryptamine (creatinine sulfate complex) (both from Sigma, St. Louis, MO), and McN-A-343 (Research Biochemicals, Inc., Natick, MA). Drug concentrations are expressed as the final bath concentrations of the salts. The osmolality of high potassium solutions was maintained by substituting equimolar amounts of KCl for NaCl.

Results

Effects of Contractile Agonist

There were no significant differences in maximal or initial forces among strips contracted with the four agonists to approximately 50% of maximal contraction

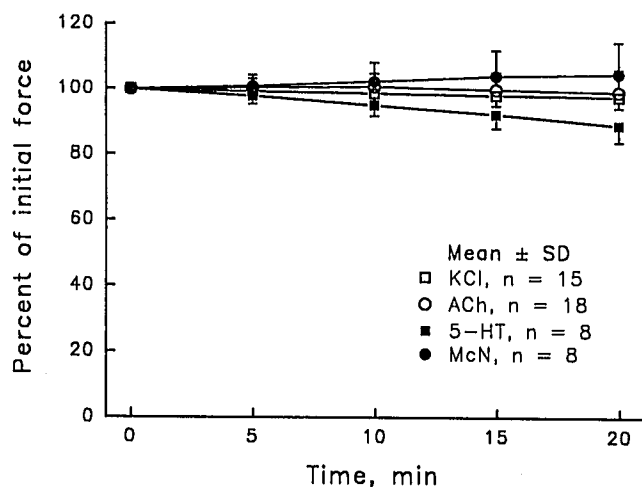


Fig. 1. Isometric force produced over time by control muscle strips precontracted to 50% maximal force over time. ACh = acetylcholine; McN = McN-A-343; 5HT = 5 hydroxytryptamine; KCl = potassium chloride. *n* = number of pairs of muscle strips analyzed; for a given experimental condition; each pair of strips was obtained from a different dog.

Table 2. Relaxation of Muscles Contracted with Different Agonists

Agonist	N	Slope	
		Controls	Halothane
ACh	18	-0.8 ± 4.0	-18.5 ± 9.4*
McN	8	3.0 ± 6.1	-43.1 ± 11.5*†
5-HT	8	-6.6 ± 3.0*†	-46.1 ± 7.5*†
KCl	15	-1.3 ± 2.0	0.7 ± 4.0†

N = number of pairs of muscle strips analyzed; for a given experimental condition, each pair of strips were obtained from a different dog; slope = mean ± SD slopes of relationship between force and halothane concentration in MAC (halothane) or force and time (controls); slopes during halothane are corrected for the effects of time; ACh = acetylcholine; McN = McN-A-343; 5-HT = 5-hydroxytryptamine; KCl = potassium chloride.

* Significant difference from 0, paired *t* test.

† Significant difference from ACh, ANOVA with Tukey's test.

(table 1). In control strips (not exposed to halothane), force did not change over the period of the experiment for strips contracted with KCl, ACh, or McN; strips contracted with 5HT experienced a small but significant spontaneous relaxation over this time (fig. 1; table 2). For muscles exposed to halothane, concentrations of halothane achieved in the PSS were equivalent to 0.2 ± 0.1, 0.4 ± 0.1, 0.8 ± 0.2, and 1.6 ± 0.3 MAC.

The effect of halothane depended significantly on the agonist used to elicit contraction (fig. 2; table 2).

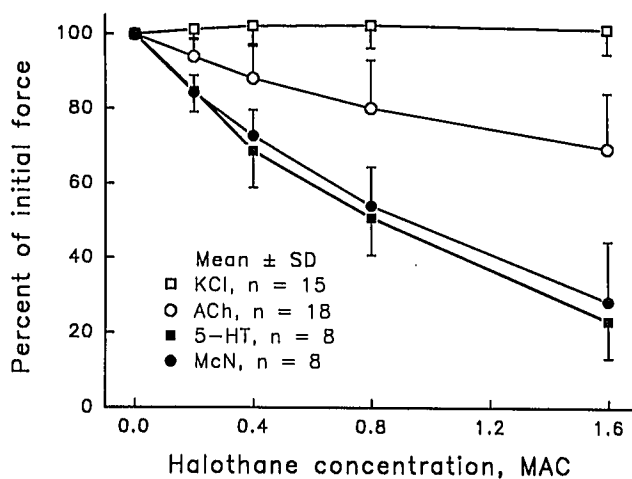


Fig. 2. Isometric force as a percent of initial force during halothane exposure in muscle strips precontracted to 50% of maximal force with four different agonists. ACh = acetylcholine; McN = McN-A-343; 5HT = 5 hydroxytryptamine; KCl = potassium chloride. *n* = number of pairs of muscle strips analyzed; for a given experimental condition, each pair of strips was obtained from a different dog. Values are corrected for the effects of time.

Halothane had no significant effect on strips contracted with KCl. In strips contracted with ACh, McN, and 5HT, halothane produced a significant dose-dependent relaxation. The amount of relaxation produced by halothane, as assessed by the slope of the relationship between force and halothane concentration, depended on the agonist used to elicit contraction in the following manner: 5HT = McN > ACh (table 2).

Effects of Level of Contraction and Anesthetic Agents

There was no significant difference in maximal force among the strips contracted to different levels of initial force (table 1). Actual forces produced by each concentration of agonist corresponded closely to the target levels of 25, 50, 75, and 100% of maximal force for ACh and 10, 30, and 50% of maximal force for KCl (table 1). For muscles exposed to volatile anesthetics, actual concentrations of volatile anesthetic achieved in the PSS were equivalent to 0.2 ± 0.1 , 0.4 ± 0.1 , 0.7 ± 0.2 , and 1.5 ± 0.2 MAC for halothane, and 0.2 ± 0.1 , 0.4 ± 0.1 , 0.8 ± 0.1 , and 1.6 ± 0.2 MAC for isoflurane.

In strips contracted with ACh, both halothane and isoflurane produced significant relaxation at all levels of initial force (fig. 3; table 3). When forces were expressed as a percent of the initial force, the relaxation caused by increasing doses of both volatile anesthetics

was significantly greater at lower levels of initial force (fig. 3). The order of potency of the relaxing effect among the four levels of initial force, as assessed by the slope of the relationship between force and volatile anesthetic concentration, was 25% > 50% = 75% > 100% (fig. 3; table 3) for both volatile anesthetics. However, when forces were expressed as a percent of the maximal force, the absolute change in force caused by increasing doses of volatile anesthetic did not depend on the level of initial force, with the exception of a significantly lesser degree of relaxation for muscles contracted to 100% of maximal force exposed to isoflurane (fig. 3; table 3). When the volatile agents were compared over all levels of initial force, halothane had a significantly greater relaxing effect as compared with isoflurane (table 3).

In strips contracted with KCl, the volatile anesthetics caused significant relaxation only in muscles contracted to 10% of maximal force (fig. 4; table 3). When the volatile agents were compared over all levels of pre-contraction, the effects of halothane and isoflurane did not differ significantly (table 3).

Discussion

The main finding of this study is that, when canine AWSM tone is increased by contractile agonists *in vitro*,

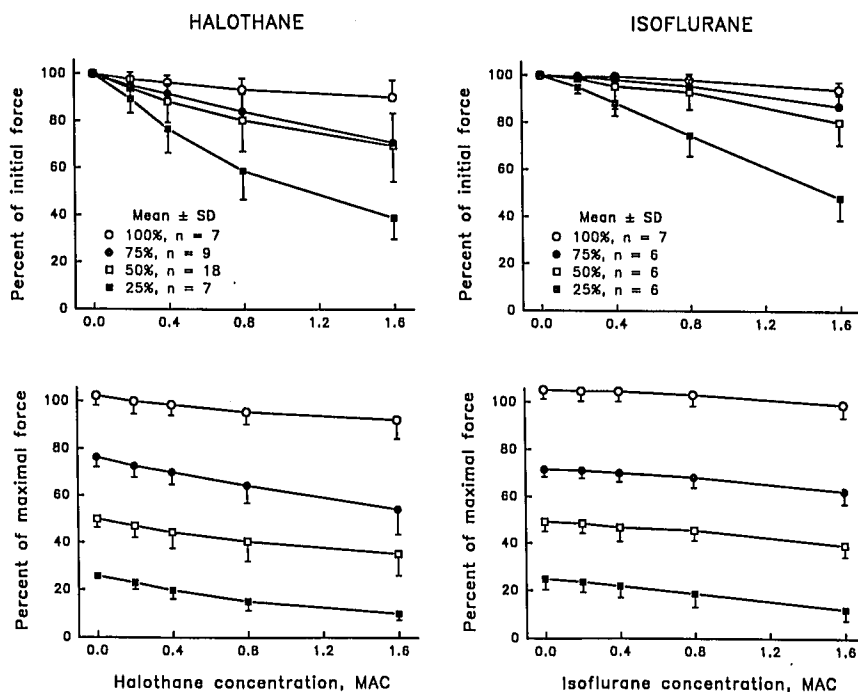


Fig. 3. Isometric force during exposure to volatile anesthetics in muscle strips precontracted to 25, 50, 75, and 100% maximal force with acetylcholine. Left panel: halothane; right panel: isoflurane. In upper panels, force is expressed as a percent of initial force, denoting the relative amount of relaxation for a given level of precontraction; in lower panels, force is expressed as a percent of maximal force, denoting absolute changes in force. Values are corrected for the effects of time. n = number of pairs of muscle strips analyzed; for a given experimental condition, each pair of strips was obtained from a different dog.

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Table 3. Relaxation at Different Levels of Initial Force

Agonist	Level of Initial Force (% maximal)	Halothane			Isoflurane		
		N	Slope		N	Slope	
			% Initial Force	% Max Force		% Initial Force	% Max Force
ACh	100	7	-5.9 ± 4.2*†	-6.9 ± 3.4*	6	-3.7 ± 2.0*†	-3.9 ± 2.0*†
	75	9	-17.9 ± 7.8*	-12.1 ± 4.2*	6	-8.2 ± 2.9*	-5.8 ± 2.0*
	50	18	-18.5 ± 9.4*	-9.0 ± 4.1*	6	-12.3 ± 5.5*	-6.1 ± 2.9*
	25	7	-37.7 ± 4.7*†	-9.7 ± 1.0*	7	-33.0 ± 5.9*†	-8.0 ± 0.8*
KCl	50	15	0.7 ± 4.0	-0.0 ± 1.7	6	2.1 ± 3.4	0.7 ± 1.4
	30	9	-8.6 ± 11.9	-3.1 ± 3.6	8	-1.1 ± 3.7	-0.4 ± 1.2
	10	15	-19.5 ± 18.6*†	-2.1 ± 1.5*	6	-12.6 ± 9.2*†	-1.9 ± 2.0

N = number of pairs of muscle strips analyzed; for a given experimental condition, each pair of strips was obtained from a different dog; slope = mean ± SD slopes of relationship between force and volatile anesthetic concentration in MAC; all values are corrected for the effects of time; % Initial Force = forces expressed as percent of initial force; % Max Force = forces expressed as percent of maximal force; ACh = acetylcholine; KCl = potassium chloride.

* Significant difference from 0, paired *t* test.

† Significant difference from 50% initial force, ANOVA with Tukey's test.

the *relative* amount of relaxation produced by volatile anesthetics depends on both the agonist used to elicit contraction and the preexisting level of contraction. However, the *absolute* amount of relaxation has little dependence on the preexisting level of contraction. In addition, there are small but significant differences in the effects of halothane and isoflurane on ACh-induced contractions.

Force can be produced in airway smooth muscle by a variety of cellular mechanisms. Agonists such as ACh, McN, and 5HT bind to receptors, activating a cascade of events that releases Ca^{2+} from intracellular stores and promotes the influx of extracellular Ca^{2+} .^{21,22,27-30} The subsequent increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) produces myosin light chain phosphorylation and smooth muscle contraction. Once force is established, other mechanisms apparently operate to maintain force, because muscle tone can be maintained despite falling levels of myosin light chain phosphorylation.³¹ The primary source for increases in $[Ca^{2+}]_i$ may differ, even for agonists that activate the same receptor. Although McN and ACh both activate the same muscarinic receptor subpopulation (M_3) in canine tracheal smooth muscle, the two agonists exhibit different receptor-occupancy characteristics and may increase $[Ca^{2+}]_i$ by different mechanisms; contractions induced by McN appears to depend more on extracellular Ca^{2+} influx as compared with contractions induced by ACh.^{19,20} In contrast to these receptor-mediated mechanisms, KCl directly depolarizes the smooth muscle cell membrane, promoting extracellular Ca^{2+} influx *via* L-type voltage-sensitive Ca^{2+} channels.²³

Given the complexity of these different mechanisms that produce and maintain AWSM tone, it is, perhaps, not surprising that functional effects of bronchodilators on AWSM may not be easily predicted. The effect of volatile anesthetics on AWSM exposed to different contractile agonists is an example of "functional antagonism," defined as the interaction between two agonists that act *via* different mechanisms to produce directly opposing effects on a common effector system.^{27,32} Based on previous studies of other bronchodilators, there are three characteristic features of functional antagonism in AWSM.²⁷ First, the ability of bronchodilators to relax AWSM depends markedly on the agonist eliciting contraction.^{11-13,17,33} Second, the effectiveness of bronchodilators is affected by the initial level of AWSM tone, with less relaxation produced at higher levels of contraction.¹¹⁻¹⁴ Finally, the effect of bronchodilators may depend on whether the agent is given before or after the initiation of contraction.^{34,35}

Although the effects of volatile anesthetics are similar to some features of functional antagonism found in these previous studies, there are also significant differences. In canine tracheal smooth muscle contracted to 50% of maximal force, the degree of relaxation produced by the β -adrenoreceptor agonist isoproterenol is greatest for 5HT and KCl, and least for ACh.¹⁷ In contrast, the volatile anesthetics had no significant effect on KCl-induced contractions at 50% of maximal force. For strips contracted with muscarinic agonists, the absolute amount of relaxation produced by isoproterenol varies considerably with the level of contraction, from complete relaxation at low levels of con-

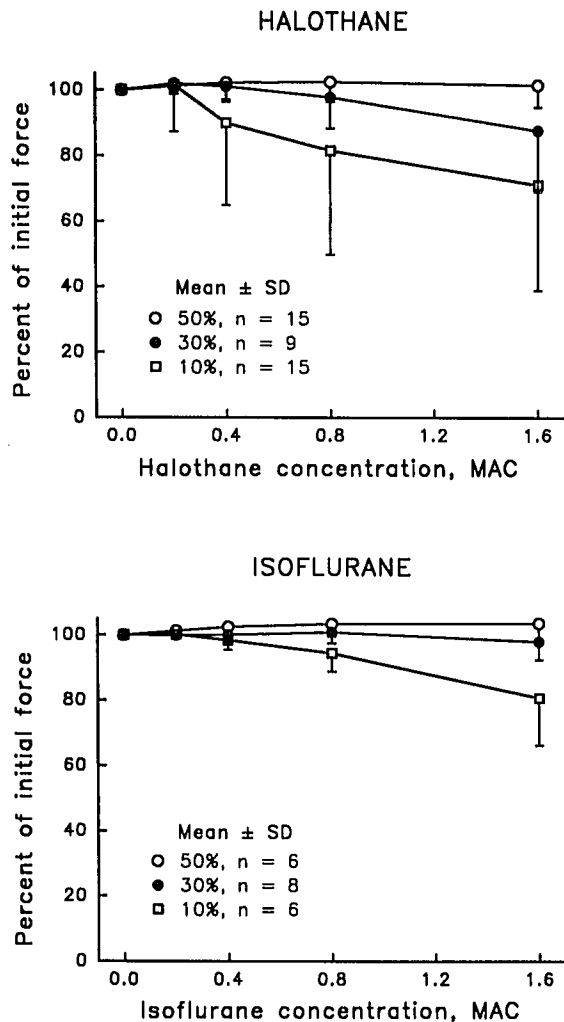


Fig. 4. Isometric force as a percent of initial force during exposure to volatile anesthetics in muscle strips precontracted to 10, 30, and 50% maximal force with potassium chloride. Upper panel: halothane; lower panel: isoflurane. Values are corrected for the effects of time. n = number of pairs of muscle strips analyzed; for a given experimental condition, each pair of strips was obtained from a different dog.

traction to no effect at maximal contraction.^{11,12} In contrast, the absolute amount of relaxation produced by increasing doses of volatile anesthetic did not depend on the level of contraction, with the exception of a significantly lesser amount of relaxation in strips contracted to 100% of maximal force when exposed to isoflurane. These functional differences between the actions of the volatile anesthetics and the β -adrenoreceptor agonists are consistent with previous reports that halothane does not act on smooth muscle by activating β -adrenoreceptors.^{3,15,36}

The present study did not specifically compare the ability of the volatile anesthetics to reverse AWSM contraction with their ability to prevent such contraction. However, such a comparison can be made for ACh-mediated contractions by using data from a previous study in our laboratory.¹ In this study, canine trachealis muscles were prepared in a fashion identical to that in the current study. A concentration-response relationship to cumulative doses of ACh was determined, then was again determined during exposure to halothane or isoflurane (fig. 5). This experiment tested the ability of the volatile anesthetics to prevent the initiation of ACh-mediated contractions. During analysis, the ACh concentration producing approximately 50% maximal contraction was determined. The difference in the force developed in response to this ACh concentration before and during exposure to volatile anesthetics was then calculated (fig. 5). At 50% of maximal contraction, similar concentrations of volatile anesthetics caused similar amounts of relaxation, whether the anesthetic was added before¹ or after (current study) the initiation of contraction (table 4). This finding differs from previous studies of β -adrenoreceptor agonists and calcium channel agonists, which are more effective at reversing agonist-induced contractions than at preventing their development.^{34,35,37,38} It is also of interest that there does not appear to be a dose-response relationship for the prevention of force development above halothane

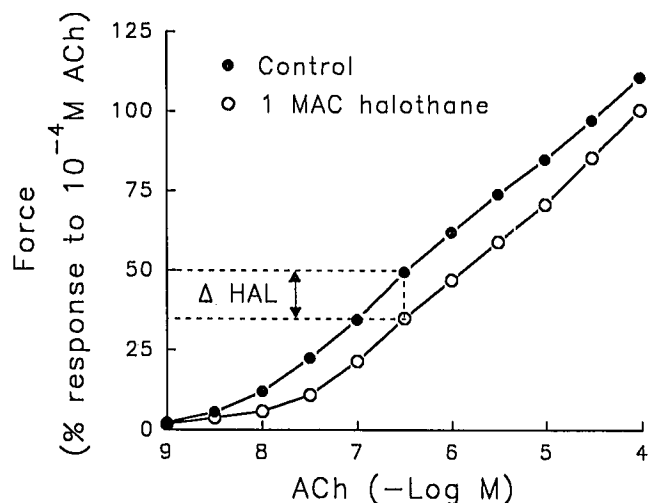


Fig. 5. Example of method used to determine the ability of halothane to prevent contraction in response to acetylcholine (ACh), using data from a representative experiment from Brichant et al.¹ Δ HAL represents reduction caused by halothane in the force developed in response to a given acetylcholine concentration (see text).

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concentrations corresponding to 1 MAC¹ (table 4), whereas such a relationship is present up to concentrations of at least 1.6 MAC when halothane relaxes precontracted muscle (fig. 1, table 4).

Our measurements do not permit evaluation of the mechanisms responsible for these characteristics of functional antagonism demonstrated by the volatile anesthetics in AWSM. Like many other bronchodilators, volatile anesthetics attenuate increases in $[Ca^{2+}]_i$ during force initiation in both AWSM³⁹ and vascular smooth muscle,^{40,41} and reduce $[Ca^{2+}]_i$ during force maintenance.⁴² Yamakage⁴² provided evidence that, during carbachol-induced contraction, at least a portion of this reduction in $[Ca^{2+}]_i$, and subsequent relaxation, is caused by blockade of L-type voltage-dependent calcium channels. Because KCl directly depolarizes the smooth muscle cell membrane, promoting extracellular Ca^{2+} influx *via* these channels,²³ it might be expected that halothane should relax KCl-induced contractions. This behavior was observed at low levels of active force (10%). However, the volatile anesthetics had no significant effect on KCl-induced contractions at higher levels of active force (30 and 50% of maximal force). Halothane also does not affect force developed in response to maximal KCl stimulation in guinea pig trachealis.⁸ It is possible that halothane changed $[Ca^{2+}]_i$, which we did not measure, during KCl-mediated contractions. However, because force did not change, this would imply that halothane simultaneously changed the sensitivity of the contractile apparatus to $[Ca^{2+}]_i$, which seems unlikely.⁴³ It is more likely that the fundamental differences in the biochemical mechanisms responsible for contraction produced in response to carbachol and KCl²⁷ preclude the application of anesthetic mechanisms from one method of stimulation to another. Furthermore, anesthetic effects may be spe-

cific to the type of smooth muscle examined, as the volatile anesthetics reduce tone induced by KCl in vascular smooth muscle.^{16,43}

Our results concerning the lesser effects of isoflurane at equal MAC levels on ACh-mediated contractions are similar to those in vascular smooth muscle.¹⁶ Consistent with these observations, isoflurane is less effective in attenuating agonist-induced increases in $[Ca^{2+}]_i$,⁴⁰ and has a lesser effect on ion channel currents in vascular smooth muscle cells as compared with halothane.⁴⁴ It should be noted, however, that the differences in the present study were small, and that the two agents do not differ in their ability to attenuate increases in pulmonary resistance caused by vagus nerve stimulation in living dogs.⁶

Clinical Relevance. Information from measurements of isometric force developed by smooth muscle *in vitro* should be applied to intact animals or humans only with caution. For example, AWSM contraction *in vivo* is not isometric, and the relationship between a given change in AWSM tone and subsequent changes in airway diameter is complex.⁴⁵ Nevertheless, such measurements remain important and widely used tools to compare the pharmacologic effects of different drugs.²⁷

As previously discussed,⁵ the relative importance of direct and neurally mediated effects of the volatile anesthetics in relaxing AWSM *in vivo* depends on many factors, including the stimulus producing AWSM contraction. To the extent that direct effects of anesthetics on AWSM are physiologically significant *in vivo*, the present results suggest that these direct actions would be more important when AWSM is stimulated by an endogenous mediator such as 5HT, released in response to an immunologic reaction (*i.e.*, anaphylactic or anaphylactoid reactions), as compared with stimulation by neurally derived ACh (*i.e.*, during reflex broncho-

Table 4. Comparison of Anesthetic Effects of ACh-mediated Contractions, with Anesthetics Added before and after the Initiation of Contraction

Anesthetic Agent	Anesthetic Added before Contraction ¹			Anesthetic Added after Contraction		
	Anesthetic Concentration (MAC)	Control Force (% maximal)	Δ HAL (% maximal)	Anesthetic Concentration (MAC)	Initial Force (% maximal)	Anesthetic-Induced Relaxation (% maximum)
Halothane	0.3	52 ± 5	4 ± 4	0.2	50 ± 4	3 ± 2
	1.0	50 ± 4	15 ± 8	0.8	50 ± 4	10 ± 6
	1.7	51 ± 2	14 ± 4	1.6	50 ± 4	15 ± 7
	2.4	50 ± 3	12 ± 8	—	—	—
Isoflurane	1.0	52 ± 5	6 ± 2	0.8	49 ± 4	4 ± 4

Δ HAL = reduction in force developed in response to a given acetylcholine concentration caused by halothane (see fig. 5 for details).

spasm). It may also be of significance that, although the effect of volatile anesthetics at clinically relevant concentrations on ACh-induced contractions is relatively small, the absolute amount of relaxation either does not depend (halothane) or has little dependence (isoflurane) on the level of contraction. Thus, unlike isoproterenol,¹² the volatile anesthetics should still relax AWSM during severe bronchospasm; even this small action may have significant effects on airway resistance under these conditions.⁴⁵ Finally, the greater effect of halothane on ACh-induced contractions as compared with isoflurane may confer some advantages on this drug in the prevention or treatment of bronchospasm, although it is unclear that the magnitude of this difference is sufficient to be clinically significant.

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