Halothane, Enflurane, and Isoflurane Depress the Peripheral Vagal Motor Pathway in Isolated Canine Tracheal Smooth Muscle

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Volatile anesthetics are potent bronchodilators, but the site of action for the dilation is unclear. To determine the site of action of halothane, enflurane, and isoflurane on the peripheral vagal motor pathway, isolated strips of canine trachealis muscle were stimulated before and during exposure to halothane at 0.3, 1.0, 1.7, or 2.4 MAC, enflurane at 1 MAC, or isoflurane at 1 MAC. The sites and methods of stimulation were: 1) postsynaptic nicotinic cholinergic receptors in the intramural parasympathetic ganglia, with 1,1-dimethyl-4-phenyl-piperazinum iodide (DMPP); 2) preganglionic cholinergic nerve fibers, with electrical field stimulation (EFS); and 3) muscarinic cholinergic receptors of the smooth muscle, with acetylcholine (ACH). The concentration–response curve to DMPP was significantly shifted to the right by 0.3 MAC halothane, whereas 0.3 MAC halothane had no significant effect on the concentration–response curves to ACh and EFS. At concentrations >1 MAC of halothane, enflurane, or isoflurane, concentration–response curves to all three stimuli were shifted significantly to the right; i.e., the contractile responses to ACh, EFS, and DMPP were reduced. At all concentrations of halothane the force of contraction was significantly more reduced during stimulation with DMPP than during stimulation with ACh, and at halothane concentrations ≥1.7 MAC the response to EFS was significantly more reduced than that to ACh. We conclude that halothane, enflurane, and isoflurane attenuated airway constriction by several mechanisms, including 1) reduced excitability of the postsynaptic nicotinic receptors of the intramural parasympathetic ganglia and 2) an effect on the smooth muscle and/or on the muscarinic receptors. At higher concentrations of halothane (≥1.7 MAC), postganglionic nerve function was also affected. (Key words: Anesthetics, volatile: halothane; enflurane; isoflurane. Nerve, vagus: smooth muscle contraction. Parasympathetic nervous system: acetylcholine; intramural parasympathetic ganglia; electrical field stimulation; postganglionic fiber.)

VOLATILE ANESTHETICS and barbiturates attenuate vagally mediated airway constriction in dogs,1–3 but the site of action of the volatile anesthetics on the peripheral vagal motor pathway has not been established. There are several possible sites of action (fig. 1). They may depress neural transmission in the intramural parasympathetic ganglia, as, for instance, halothane depresses synaptic transmission in sympathetic ganglia.4–7 They also may interfere with the conduction or the postsynaptic neurotransmitter release in parasympathetic postganglionic nerve fibers innervating the airway smooth muscle; they may reduce the contractile response of airway smooth muscle8,9 by a direct effect on the smooth muscle; or they may affect the muscle by a combination of these possibilities. Some studies in dogs in vivo suggest that halothane has both neurally mediated and direct relaxing effects on the muscle,10 but other studies have shown little evidence of a direct relaxing effect.11

The current study was designed to determine the sites of action of halothane, enflurane, and isoflurane on the peripheral vagal motor pathway distal to the presynaptic membrane of the parasympathetic ganglia in isolated canine trachealis muscle strips. Three sites of action were studied: 1) the nicotinic cholinergic receptors of the postsynaptic membrane of the parasympathetic intramural ganglion; 2) the postganglionic cholinergic nerve fibers; and 3) the airway smooth muscle and its muscarinic receptors.

Materials and Methods

Tissue Preparation

The protocol for this study was approved by the Animal Care and Use Committee of the Mayo Clinic. Thirty-six mongrel dogs were anesthetized with pentobarbital and killed by exsanguination. A 10-cm segment of trachea was immediately removed and immersed in a preaerated physiologic salt solution (PSS) of the following composition (mM): MgSO₄, 0.8; KH₂PO₄, 1.2; KCl, 3.4; CaCl₂, 2.4; NaCl, 110.5; NaHCO₃, 25.7; and dextrose, 5.6. Six rectangular strips of trachealis muscle (2 mm wide and 10–15 mm long) were dissected from each trachea, and the epithelium was removed mechanically.

Immediately after dissection, the strips were suspended vertically in 25-ml water-jacketed glass tissue baths containing PSS at 37°C and bubbled with 94–95% O₂ and 5–6% CO₂, providing a pH of 7.40–7.49, a Pco₂ of 32–36 mmHg, and a PaO₂ of 550–560 mmHg (model 1302, Instrumentation Laboratories). One end of each tissue strip was anchored to a hook at the bottom of the bath, and the other end was tied to a force transducer (model FT03D, Grass Medical Instruments). The transducers were mounted on micromanipulators so that the length of the strips could be changed. Isometric force was continuously recorded (model 7418A, Hewlett-Packard).
The strips were washed intermittently with PSS for 2 h, during which time they were stimulated supramaximally (pulse: 0.5-ms duration, 25 Hz, 15 V) by electrical field stimulation (EFS) for 30 s at approximately 5-min intervals. EFS was provided by a direct current amplifier (Section of Engineering, Mayo Clinic) triggered by a stimulator (model 544, Grass Medical Instruments) via two parallel platinum plate electrodes (1 cm × 4 cm). Muscle length was increased progressively after each contraction until the force of contraction reached a maximal response (optimal length); each strip was maintained at this length throughout the experiment. Next, the contractile response of all six strips to $10^{-4}$ M acetylcholine (ACh) was determined. This response was defined as the maximal response, and all subsequent force measurements were expressed as a percentage of this response. ACh then was washed out. At the end of the experiment, the strips were blotted dry on gauze pads and weighed after the ties and excess tissue had been removed.

**Experimental Protocol**

Six strips from each of 36 dogs were studied. In two of the six strips from each dog, concentration–response curves to ACh were obtained in the presence of tetrodotoxin ($10^{-6}$ M), which was added to the bathing solution to prevent coincident stimulation of the intramural parasympathetic ganglia from contributing to the induced contraction. ACh was added cumulatively to the baths ($10^{-9}$–$10^{-5}$ M) in half-log increments, with 5 min allowed for stabilization of the contraction in response to each concentration.

In two other strips in each set of six, frequency–response curves to EFS (pulse: 0.5-ms duration, 15 V, 1-min stimulation period) were obtained. Stimulations at 0.25, 0.5, 1.0, 3.0, 5.0, 10.0, 17.0, and 25 Hz were applied at approximately 3-min intervals. Hexamethonium ($10^{-5}$ M) and propranolol ($10^{-6}$ M) were added to the PSS during EFS to prevent stimulation of the nicotinic cholinergic and $\beta$-adrenoceptors.12

In the remaining two strips, concentration–response curves to 1,1-dimethyl-4-phenyl-piperazinum iodide (DMPP) were obtained by adding DMPP noncumulatively to the bathing solution, in concentrations of $10^{-7}$, $10^{-6}$, $10^{-5.5}$, $10^{-5}$, and $10^{-4}$ M. DMPP stimulates the nicotinic cholinergic receptors of the postsynaptic membranes of the intramural parasympathetic ganglia.13 To avoid desensitization, DMPP was washed out immediately after each contraction had reached a maximum (60 s from the time of administration of DMPP). Thirty minutes were allowed before the addition of the next larger concentration of DMPP. Data from experiments in which the contractile response to $10^{-4}$ M DMPP did not reach at least 20% of the contractile response to $10^{-4}$ M ACh are not reported. In preliminary studies we observed that the response of trachealis muscle strips to DMPP stimulation was consistently greater than that of bronchial smooth muscle, most likely because of higher density of ganglia in larger airways.14 Thus, we chose trachealis and not bronchial muscle strips for this study.

After these initial measurements, the six strips were washed thoroughly with PSS and allowed to return to resting tensions. In three of the six strips, enfurane (1 MAC), isoflurane (1 MAC), or halothane (0.3, 1.0, 1.7, and 2.4 MAC) was added to the gas that aerated the baths. One MAC for halothane was taken to be 0.87%, for enfurane 2.2%, and for isoflurane to be 1.5%.15 Only one concentration of volatile anesthetic was used for each experiment; the other three strips not exposed to a volatile anesthetic served as controls for the effects of time. After a minimum of 30 min of equilibration with volatile anesthetics, a second complete set of measurements in response to ACh, EFS, and DMPP was obtained.

Approximately 1 h elapsed between the killing of the dog and the completion of the mounting of the muscles in the tissue baths. An average of $70 \pm 14$ min were used to establish the optimal length; $17 \pm 6$ min was necessary to determine the maximal response to $10^{-4}$ M ACh; and
155 ± 21 min was necessary to complete the concentration–response curves for ACh, EFS, and DMPP in the absence of volatile anesthetics. An additional 134 ± 28 min was used to obtain the concentration–response curves in the presence of the volatile anesthetics.

**DATA ANALYSIS**

We compared the contractile response of tissues not exposed to a volatile anesthetic with the response of tissues exposed to a volatile anesthetic. In the muscles exposed to anesthetic, the contractile responses were adjusted for the effect of time according to the formula

\[ C_t = \left( \frac{C_2}{C_1} \right) \cdot C_{VA} \]

in which \( C_t \) = time-adjusted control response from volatile anesthetic muscle; \( C_1 \) = response of control muscle (initial measurement); \( C_2 \) = response of control muscle (measurement in parallel with volatile anesthetic muscle); \( C_{VA} \) = response of volatile anesthetic muscle (initial measurement, *i.e.*, before exposure to the anesthetic).

The effects of the volatile anesthetics on the concentration–response curves to ACh, EFS, and DMPP were tested by repeated-measures of analysis of variance. The effect of increasing the concentration of halothane on the concentration–response curves to ACh, EFS, and DMPP also were examined by repeated-measures of analysis of variance.

To localize the effect of halothane, we determined first the maximal contraction to DMPP in the absence of halothane (fig. 2 (1)). We next determined the contractile response to DMPP in the presence of halothane at the concentration producing the maximal contraction in the absence of halothane (fig. 2 (2)). We then calculated the reduction in the contractile response due to halothane (ΔDMPP). Similar measurements and calculations were performed on the concentration–response curve to ACh and the frequency–response curve to EFS.

Changes in the contractile responses due to halothane, isoflurane, or enfurane were subjected to repeated-measures analysis of variance. Significant differences between the mean reductions in contractile response to DMPP, ACh, and EFS were determined by paired *t* test (two-tailed) with Bonferroni’s correction for multiple comparisons.

Data are expressed as means ± SD. A *P* < 0.05 was considered significant. In all cases, *n* represents the number of dogs.

**DRUGS**

Halothane, enfurane, or isoflurane were added to the aerating gas mixture via an on-line vaporizer (Ohmeda or Cyprane). The concentration of the volatile anesthetic

**TABLE 1. Target and Measured Concentrations of Halothane, Enfurane, and Isoflurane in Gas and Liquid Phase**

<table>
<thead>
<tr>
<th>Volatile Anesthetic</th>
<th>Target Concentration: Gas Phase (MAC)</th>
<th>Measured Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gas Phase (MAC)</td>
<td>Gas Phase (MAC)</td>
</tr>
<tr>
<td>Halothane</td>
<td>0.3</td>
<td>0.35 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.36 ± 0.12 (n = 16)</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>1.00 ± 0.21 (n = 10)</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>1.69 ± 0.11 (n = 18)</td>
</tr>
<tr>
<td></td>
<td>2.59 ± 0.24 (n = 18)</td>
<td></td>
</tr>
<tr>
<td>Enfurane</td>
<td>1.0</td>
<td>2.40 ± 0.32 (n = 18)</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1.0</td>
<td>0.97 ± 0.08 (n = 12)</td>
</tr>
<tr>
<td></td>
<td>1.07 ± 0.06 (n = 11)</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD.
Dash indicates that data were not recorded.
1 MAC = 0.87% for halothane, 2.20% for enfurane, and 1.50% for isoflurane.16

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**Fig. 2.** (A) Method of comparing halothane effects on DMPP- and ACh-induced contractions. The maximal contraction to DMPP (DMPPmax) in the absence of halothane first was determined (1). The reduction in the contractile response due to halothane (ΔDMPP) at the concentration that caused DMPPmax (2) next was determined (ΔDMPP). (B) The reduction in contractile response to ACh due to halothane (ΔACh) at DMPPmax was determined similarly. The reduction in contractile response to EFS due to halothane (ΔEFS) at DMPPmax also was determined (not shown). The Δ values for DMPP, ACh, and EFS were analyzed by repeated-measures analysis of variance for statistical significance.
in the gas mixture was monitored continuously by a mass spectrometer (model MGA-1100, Perkin-Elmer) in all studies except at 1.0 MAC halothane, for which it was monitored intermittently with an acoustic analyzer (Section of Engineering, Mayo Clinic). The concentrations of the volatile anesthetics in the PSS were determined by gas chromatography with an electron-capture detector (model 5880A, Hewlett-Packard) at the end of the experiment. The other drugs or chemicals were ACh chloride, DMPP, hexamethonium chloride, DL-propranolol hydrochloride, and tetrodotoxin (all from Sigma, St. Louis, MO). These drugs or chemicals were added to the PSS in 100-μl aliquots. Concentrations are expressed as molar concentrations in the tissue bath.

**Results**

Mean weight of the muscle strips was 20.9 ± 5.1 mg, and the maximal contractile response to 10⁻⁴ M ACh was 23.3 ± 6.1 g. The mean control maximal contractile response to DMPP was 7.2 ± 2.3 g, and to EFS was 21.7 ± 6.1 g. The concentrations of the volatile anesthetics in the gas phase were close to the target concentrations (table 1), but the concentrations of the volatile anesthetics in the liquid phase were less than expected from their concentrations in the gas phase, suggesting that full equilibration had not occurred.

Halothane (0.3, 1.0, 1.7, or 2.4 MAC), enflurane (1 MAC) and isoflurane (1 MAC) shifted the concentration-response curves to ACh, EFS, and DMPP to the right (figs. 3 and 4), indicating a decreased sensitivity to stimulation. The right shifts were significant at 0.3 MAC halothane only for DMPP (table 2). At halothane concentrations ≥1 MAC, the right shifts were significant for ACh, EFS, and DMPP. 1 MAC enflurane and 1 MAC isoflurane also resulted in significant right shifts of the concentration-response curves for ACh, EFS, and DMPP (table 3).

![Concentration-response curves](image.png)

**FIG. 3.** Concentration-response curves to acetylcholine (ACh, top), electric field stimulation (EFS, middle), and dimethylphenylpiperazinium (DMPP, bottom) in isolated canine trachealis muscle strips in the absence (control [circles]) or presence (squares) of halothane. Data are expressed as percent of maximal response to 10⁻⁴ M ACh. Each point represents mean ± SD; n = 6.
Increasing the concentration of halothane increased significantly the right shift of the concentration–response curves when all halothane concentrations were analyzed (table 4). However, a significant concentration–response relationship for halothane above 1 MAC was observed only for DMPP and EFS, but not for ACh-induced contractions.

The reductions in contractile responses due to halothane, enflurane, and isoflurane were significantly different between DMPP, EFS, and ACh (table 5) at all four concentrations of halothane and at 1 MAC of enflurane and isoflurane. The reductions of the contractile responses to ACh and EFS were not statistically different at 0.3 and 1.0 MAC halothane and at 1.0 MAC enflurane and isoflurane. The contractile responses to DMPP were significantly more depressed than those to EFS and ACh at 0.3 and 1.0 MAC halothane and 1.0 MAC enflurane. At 1.0 MAC isoflurane, only the contractile response to ACh was statistically significant less than to DMPP; the difference between EFS and DMPP was slightly less than significant. In contrast, at 1.7 and 2.4 MAC halothane, the contractile responses to both DMPP and EFS were significantly more depressed than were the responses to ACh. At 1.7 and 2.4 MAC halothane, the reductions in contractile responses to DMPP and EFS were not significantly different.

**Discussion**

The major finding of this study is that halothane, enflurane, and isoflurane attenuated vagally induced contractions of isolated canine trachealis muscle strips at several sites along the peripheral vagal motor pathway. Halothane, enflurane, and isoflurane depressed 1) the contractile response of the smooth muscle to ACh and 2) the excitability of the postsynaptic nicotinic cholinergic receptors of the intramural parasympathetic ganglia. At higher concentrations of halothane (≥1.7 MAC), post-ganglionic cholinergic nerve function also was affected.

**Methodology**

Our conclusions about the action of the volatile anesthetics on the peripheral vagal motor pathway are based on the assumption that each of the three stimuli used

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**Table 3. Effect of Enflurane or Isoflurane on the Concentration–Response Curves: P Values**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Enflurane, 1 MAC</th>
<th>Isoflurane, 1 MAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>0.0252</td>
<td>0.0071</td>
</tr>
<tr>
<td>EFS</td>
<td>0.0001</td>
<td>0.0238</td>
</tr>
<tr>
<td>DMPP</td>
<td>0.0004</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

Repeated-measures analysis of variance.

**Table 2. Effect of Halothane on the Concentration–Response Curves: P Values**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>0.3</th>
<th>1.0</th>
<th>1.7</th>
<th>2.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>0.0538</td>
<td>0.0156</td>
<td>0.0001</td>
<td>0.0018</td>
</tr>
<tr>
<td>EFS</td>
<td>0.0675</td>
<td>0.0079</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>DMPP</td>
<td>0.0019</td>
<td>0.0167</td>
<td>0.0008</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Repeated-measures analysis of variance.

**Table 4. Dose–Response to Halothane: P Values**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>0.0201</td>
</tr>
<tr>
<td>EFS</td>
<td>0.0001</td>
</tr>
<tr>
<td>DMPP</td>
<td>0.0262</td>
</tr>
</tbody>
</table>

Repeated-measures analysis of variance.
TABLE 5. Reduction in Contractile Response due to a Volatile Anesthetic for DMPP, ACh, and EFS

<table>
<thead>
<tr>
<th>Volatile Anesthetic</th>
<th>Stimulus</th>
<th>0.3</th>
<th>1.0</th>
<th>1.7</th>
<th>2.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halothane</td>
<td>DMPP</td>
<td>21.7 ± 4.0</td>
<td>34.4 ± 9.0</td>
<td>28.0 ± 6.4</td>
<td>29.1 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>EFS</td>
<td>5.2 ± 9.5*</td>
<td>14.7 ± 11.7*</td>
<td>25.6 ± 6.5</td>
<td>25.3 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>ACh</td>
<td>5.5 ± 6.1*</td>
<td>13.6 ± 8.0*</td>
<td>15.7 ± 3.6‡</td>
<td>13.0 ± 7.0†</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.0008</td>
<td>0.0001</td>
<td>0.0003</td>
<td>0.0003</td>
</tr>
<tr>
<td>Enflurane</td>
<td>DMPP</td>
<td>—</td>
<td>35.0 ± 6.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>EFS</td>
<td>—</td>
<td>17.8 ± 5.9*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>ACh</td>
<td>—</td>
<td>12.9 ± 10.5*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>—</td>
<td>0.0007</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>DMPP</td>
<td>—</td>
<td>25.8 ± 2.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>EFS</td>
<td>—</td>
<td>14.3 ± 10.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>ACh</td>
<td>—</td>
<td>5.3 ± 3.4*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>—</td>
<td>0.0017</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Reductions in contractile responses are expressed in percent of maximal contraction to $10^{-4} M$ ACh. P values for repeated-measures analysis of variance.

* Significantly different from DMPP (paired t test).
† Significantly different from DMPP and EFS (paired t test).

(DMPP, EFS, and ACh) was acting on a specific site: nicotinic cholinergic receptors in the postsynaptic membrane of the intramural parasympathetic ganglia; postganglionic cholinergic nerve fibers; and muscarinic cholinergic receptors of the smooth muscle, respectively. The vagal motor pathway to airways can be described as follows (fig. 1). From the central nervous system, preganglionic cholinergic fibers pass down the vagus nerves to the ganglia located in the airway walls, where the fibers form synapses with short postsynaptic fibers that innervate the airway smooth muscle. In the parasympathetic ganglia, preganglionic fibers release ACh, which activates nicotinic cholinergic receptors in the postsynaptic membrane of the ganglia. The latter activation can be modulated by muscarinic receptors (presumably M1 receptors). At the neuromuscular synapse, ACh is released from presynaptic vesicles upon stimulation. This release is attenuated by the activation of M2-muscarinic prejunctional receptors (autoreceptors). The released ACh stimulates the airway smooth muscle, thus inducing contractions presumably via M3 muscarinic receptors.

The validity of the assumption that DMPP specifically stimulates nicotinic cholinergic receptors in the postsynaptic membrane has been discussed in detail by others.

EFS can stimulate the preganglionic and postganglionic parasympathetic nerve fibers, the parasympathetic ganglia, the sympathetic nerve fibers, the nonadrenergic noncholinergic system, and the smooth muscle directly. We have shown previously that smooth muscle contraction does not occur during EFS at the same stimulation variables in the presence of tetrodotoxin; that is, EFS does not stimulate the smooth muscle directly. Stimulation of β-adrenoceptors and nicotinic cholinergic receptors in the parasympathetic ganglia were blocked by propanolol and hexamethonium, respectively. α1- and α2-sympathetic nerve fibers also can be stimulated by EFS; we did not block α1- and α2-adrenoceptors, because EFS does not cause contractions in the presence of propanolol and atropine via these receptors in canine trachealis unless the muscle is precontracted with histamine or serotonin.

The nonadrenergic noncholinergic system is weak or absent in dogs. During stimulation of the muscle with ACh, tetrodotoxin prevented ganglionic stimulation by ACh from contributing to the contractile response. We conclude, therefore, that in this study DMPP stimulated only the postsynaptic membrane of the ganglion; EFS stimulated only the postganglionic cholinergic nerve fibers; and ACh stimulated only the muscarinic receptors in the sacculema.

The average time interval between the killing of the dog and the last measurements was more than 7 h. We decided, therefore, not to repeat control concentration–response curves after the exposure to the volatile anesthetics, since this would have added at least another 2.5 h to the length of the study and thereby increased the chances of tissue deterioration. We chose instead to correct for the effect of time (average correction 2.3 ± 7.3% for ACh, 2.1 ± 1.5% for EFS, and 2.2 ± 1.2% for DMPP). This correction also has the advantage that it avoids the problem of a residual effect of the anesthetic on repeat control concentration–response curves.

**EFFECT OF VOLATILE ANESTHETICS ON PARASYMPATHETIC GANGLIA AND POSTGANGLIONIC CHOLINERGIC FIBERS**

As shown in figure 1, the parasympathetic stimulus travels through the parasympathetic intramural ganglia, the postganglionic fibers to the postjunctional M3 muscarinic cholinergic receptor. If the volatile anesthetics depressed all three sites along the pathway, one would expect the greatest depression of the contraction with DMPP, which stimulated selectively the postsynaptic nicotinic cholinergic receptors. The next largest depression of
VOLATILE ANESTHETICS AND AIRWAY INTERVENTION

contraction would be expected to occur with EFS, which stimulated selectively the postganglionic fibers. The smallest effect would be expected to occur with ACh, which stimulated selectively the M2 receptor. All three volatile anesthetics at ≤1 MAC reduced the contractile response to DMPP more than the response to EFS and ACh; this suggests that the anesthetics at these concentrations depressed the postsynaptic membrane of the intramural parasympathetic ganglia, an effect similar to that of barbiturates. Halothane has a similar effect on the postsynaptic sensitivity of sympathetic ganglia. At concentrations ≥1.7 MAC, halothane reduced the contractile response to EFS more than that to ACh, a result suggesting depression of postganglionic fiber function at these high concentrations. This is consistent with the findings by Korenaga et al. of depression by halothane of excitatory neurotransmitter transmission, as measured by the amplitude of the excitatory junction potential in isolated canine trachealis muscle. The finding that ganglionic transmission was more sensitive than postganglionic function to depression by halothane is consistent with differential sensitivity of the sympathetic system to other anesthetics. The effects of halothane on the parasympathetic ganglia and postganglionic nerve endings are in agreement with the observation in vivo of depression of airway reflexes by halothane.

We did not investigate effects of isoflurane and enflurane at concentrations other than 1 MAC. Because the responses of the canine trachealis muscle at 1 MAC of isoflurane and enflurane were similar to those at 1 MAC of halothane, one may speculate that higher concentrations of these anesthetics would affect the function of postganglionic fibers similarly to higher concentrations of halothane. This assumption remains to be proven.

The underlying mechanisms for the depression of the postsynaptic membrane of the parasympathetic ganglia and postganglionic cholinergic fibers by the volatile anesthetics are unclear. It is possible that in the parasympathetic ganglia, the interaction between nicotinic cholinergic receptors and the guanosine triphosphate (GTP)-binding regulatory proteins (G-proteins) is affected. In both nerves and ganglia, the anesthetics may affect intracellular calcium homeostasis, as halothane does in cardiac myocytes and in vascular smooth muscle cells. It is also possible that volatile anesthetics alter the cell membrane potential, as halothane does in hyperpolarizing canine airway smooth muscle cells and nerves in the CNS of vertebrates.

DIRECT EFFECT OF VOLATILE ANESTHETICS ON SMOOTH MUSCLE

The depression of the contractile response to ACh caused by the volatile anesthetics demonstrates that in addition to the neurally mediated effects, they have direct effects on airway smooth muscle and/or its muscarinic receptors. This conclusion is consistent with the attenuation by halothane of methacholine-induced increases in pulmonary resistance in dogs. It also is consistent with our observation that at clinically used concentrations halothane has a significant direct effect on canine airway smooth muscle in vivo. Again, the underlying mechanisms for the direct effect are unknown. It is possible that the volatile anesthetics interfere with receptor binding, with the intracellular signal transduction, with the contractile proteins, or with a combination of these possibilities.

CLINICAL RELEVANCE

One must be careful in extrapolating the results of this in vitro study of canine trachealis muscle to humans. In vivo, afferent receptors and pathways, the central nervous system, preganglionic cholinergic nerves, and the release of humoral mediators may also be affected. Furthermore, we cannot exclude the possibility that anesthetics affect the smooth muscle of peripheral airways differently than they do the smooth muscle of the trachea. Finally, all of our measurements were done in the absence of airway epithelium; volatile anesthetics may alter epithelial function, which itself can modulate smooth muscle contractility. We believe this to be unlikely, since removal of the epithelium does not affect the response of isolated canine bronchi to halothane.

In isolated canine trachealis muscle, halothane, enflurane, and isoflurane at clinically used concentrations had a direct depressant effect on the smooth muscle or its muscarinic receptors or both. The anesthetics also reduced the sensitivity of the nicotinic cholinergic receptors of the postsynaptic membrane of the intramural parasympathetic ganglia. Halothane, at concentrations ≥1.7 MAC, depressed the function of the postganglionic fibers. At equipotent clinically relevant doses, all three anesthetics appear to have similar effects on peripheral vagal motor pathway and on the contractile responses of isolated canine trachealis muscle.

The authors thank Mrs. Kathleen A. Street and Mr. Gerald Rach for their technical assistance, and Mrs. Janet M. Beckman for secretarial assistance.

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