Differential effects of desflurane and halothane on peripheral airway smooth muscle


Summary
Volatile anaesthetics have been shown to have direct relaxant effects on airway smooth muscle. We have examined the effects of 0.9, 1.9, and 2.8 dog MAC of desflurane and halothane on isolated proximal and distal canine airways precontracted with acetylcholine. The proximal and distal airway smooth muscle relaxed with increasing concentration of each anaesthetic in a dose-related manner. Desflurane had a greater relaxant effect than halothane on the proximal airway only at 2.8 MAC. Desflurane relaxed the distal airway to a greater extent than halothane at 1.9 and 2.8 MAC. The distal airway smooth muscle was more sensitive to volatile anaesthetics than the proximal airway smooth muscle with either halothane or desflurane at all concentrations tested. This effect may be a result of differences in cartilage content, myosin content, epithelium-dependent effects, receptor density, myofilament sensitivity to Ca\(^{2+}\), sarcoplasmic reticulum Ca\(^{2+}\) control, or ionic fluxes in the proximal airway compared with the distal airway. The increased sensitivity of airway smooth muscle to desflurane compared with halothane is not known but may be related to possible differences in the effects of Ca\(^{2+}\) homeostasis. (Br. J. Anaesth. 1996; 76: 841–846)

Key words

The bronchodilator effects of desflurane on intraparenchymal airway have not yet been determined. Adverse effects such as coughing and laryngospasm on induction have been associated with desflurane [1]. Although these effects appear to occur in the upper airway, it is not known if they are associated with a detrimental bronchospastic effect distally. In this study we examined the direct effects of desflurane and halothane on the third- and sixth-generation airway. These segments of the peripheral airway are likely to be main sites of airway resistance [2] and airflow regulation [3, 4]. The clinical importance of direct effects of volatile anaesthetics on peripheral airway smooth muscle is not known. However, the direct effects on peripheral airway smooth muscle may be relevant clinically because of the diminishing innervation of airway smooth muscle when proceeding along the bronchial tree distally [5]. In addition, the airway at this level of the bronchial tree is more sensitive and more reactive [3, 6, 7], and therefore the distal airway requires less circulating agonist for a given response than proximally located airway. These factors may allow better control at the peripheral site of airflow regulation through a hormonal component rather than a neural component. This site of airflow regulation may therefore be affected predominately by the direct effects of anesthetics, thereby altering airflow distribution. In this study we have determined the effects of halothane and desflurane on the portion of the airway believed to have a significant role in airflow regulation.

Materials and methods
All experimental procedures were approved by the Animal Care Committee of the Medical College of Wisconsin.

Tissue preparation
The right upper lobe was obtained from 15 mongrel dogs of both sexes (10–15 kg) after halothane anaesthesia and exsanguination. The right upper lobe, chosen to maintain consistency and ease of dissection [8], was placed in ice-cooled Krebs-Ringer bicarbonated solution aerated with 95% oxygen and 5% carbon dioxide. The first intraparenchymal bronchial segment is the third-generation airway and in this study is considered the proximal airway. This airway was dissected carefully and cleaned of lung parenchyma and pulmonary vessels under a binocular microscope. The proximal airway was dissected down to its first bifurcation. The middle third of this proximal airway was cut...
sequentially into 2-mm segments (od 4–6 mm) that excluded side branches. This section of the proximal airway was chosen because it gave the most uniform outer diameter. These segments were then mounted on two tungsten wires (od 0.1 mm), each shaped into an isosceles triangle with the base passing through the lumen.

The distal airway was also selected from the same animal. This airway, obtained after the fifth or sixth bifurcation from the trachea, was dissected carefully and cleaned of lung parenchyma and pulmonary vessels. The airway after the fifth or sixth bifurcation, corresponding to the fifth and sixth airway generation, respectively, was selected based on which airway generation had an outer diameter closest to 1.0 mm. The distal airway was also cut sequentially into 2-mm segments (od 0.7–1.8 mm) that excluded side branches. Based on outer diameter [9] and histology on randomly selected 1-mm segments, these airways were considered bronchioles. These distal segments were then mounted on tungsten wires as described for the proximal airway. During placement of the wires, special care was taken not to damage the luminal surface.

The mounted bronchi and bronchioles were suspended in jacketed baths, temperature controlled at 37 °C. The apex of one triangle was fixed at a point located at the bottom of the bath and the apex of the other triangle was attached to a force transducer (Grass Model FT103, Grass Instruments, Quincy, MA, USA) for measurement of isometric force. All responses were measured by the force transducer and are reported as isometric tension in grams. These rings were then allowed to equilibrate at 37 °C for 1 h in baths containing a 15-ml solution of Krebs–Ringer bicarbonate of the following composition (mmol litre\(^{-1}\)): glucose 11.1; NaCl 118.3; KCl 4.7; CaCl\(_2\) 2.5; MgSO\(_4\) 1.2; KH\(_2\)PO\(_4\) 1.2; NaHCO\(_3\) 28.0; and Ca\(^2+\) disodium edetate 0.026. This solution was aerated with 95 % oxygen and 5 % carbon dioxide to maintain pH 7.40 with a \(p\text{CO}_2\) of 4.7 kPa and \(p\text{O}_2\) greater than 66.7 kPa. These values were assessed by radiometric (ABL 30 Radiometer; Copenhagen, Denmark) methods and verified before electrical field stimulation, before construction of the dose-response curve, during the ED\(_{50}\) response, and during desflurane or halothane administration. In addition, \(p\text{CO}_2\), \(p\text{O}_2\) and anaesthetic concentrations were monitored continuously by an infrared absorption technique (Poet II, Criticare Systems Incorporated, Waukesha, WI, USA, for halothane and Datex, Camonom, Tewksbury, MA, USA, for desflurane). No residual halothane administered from the initial procurement of the lung tissue was detected from the infrared absorption technique at this time. A minimum of 3 h elapsed from the time of tissue procurement to the tissue equilibration, which would be sufficient for any unmeasurable halothane to be dissipated.

**DETERMINATION OF OPTIMAL LENGTH**

Optimal length of the airway was determined by the response to transmural nerve stimulation after incremental changes in airway smooth muscle length. For transmural nerve stimulation, two platinum-plate electrodes were placed parallel to the airway ring (1 cm distance between the electrodes). Electrical impulses were generated by a high current constant voltage stimulator (designed and tested by the Biomedical Engineering Section, Department of Anesthesiology, Medical College of Wisconsin, WI, USA). Based on preliminary experiments, the variables of the voltage stimulator used in this study (15 V, 0.5 ms, 25 Hz) produced maximal indirect airway smooth muscle contraction. This maximal response was abolished by atropine 1 \(\mu\text{mol litre}^{-1}\), indicating that the contraction was not a result of direct electrical stimulation of the muscle [10].

The airway rings were allowed to equilibrate for 1 h after mounting in the jacketed baths. The airway rings were stretched progressively every 10 min, by 0.5-g increments for the proximal airway and 0.25-g increments for the distal airway, until the contractile response to a standard electrical stimulus (15 V, 0.5 ms, 25 Hz) of 30 s duration was maximal. The maximum response was obtained when two consecutive readings were equal. When this maximal response was reached, the airway ring was considered to be at optimal length.

After an additional hour of equilibration, resting tension at optimal length was measured. To block ganglionic nicotinic receptors, hexamethonium chloride 10 \(\mu\text{mol litre}^{-1}\) was then added to each bath and allowed to equilibrate for 30 min before acetylcholine challenge [11].

**ACETYLCHOLINE AND ANAESTHETIC DOSE–RESPONSES**

Acetylcholine cumulative dose–response curves were obtained for both proximal and distal airways by increasing the concentration in log increments every 2 min [12]. The dose range for the proximal airway was 0.01 \(\mu\text{mol litre}^{-1}\) to 10 mmol litre\(^{-1}\), and 0.01 \(\mu\text{mol litre}^{-1}\) to 1 mmol litre\(^{-1}\) for the distal airway. The dose required to reach the maximal plateau response determined the maximum dose (ED\(_{\text{max}}\)) required for each airway group. After obtaining the maximal response in each tissue, a minimum of three bath rinses were performed to wash out the acetylcholine. The tissue rings were allowed to reach baseline resting tension and equilibrate for at least 1 h. The ED\(_{50}\) was calculated from the linear portion (20–80 % of maximum response) of the dose–response curve by linear regression analysis. This dose was then administered to each tissue to obtain a baseline control response before administration of anaesthetic. The control response to the ED\(_{50}\) was allowed to stabilize for 10 min.

After stabilization, the halothane or desflurane dose, equivalent to 0.9, 1.9 or 2.8 dog MAC, was selected randomly and allowed to equilibrate in each bath for a minimum of 15 min. After the response stabilized or the minimum equilibration time elapsed, whichever was longer in duration, the response was measured. The anaesthetic vaporizer was then turned off and the tissue was allowed a wash-out period of at least 15 min. A calibration curve with carrier gas flows (oxygen, carbon dioxide) and volatile anaesthetic concentrations used for the
experiments were determined and correlated with gas chromatography samples and infrared absorption analyser (Poet II for halothane or Datex for desflurane) measurements. The infrared absorption measurements correlated with gas chromatography samples after these values (mmol litre\(^{-1}\)) were converted to percentage volume values. The infrared absorption measurements were then used to ensure stable anaesthetic concentrations in the baths throughout the experiments. The infrared absorption analyser intake sampler located in-line with the carrier gas showed that the anaesthetic concentrations in the baths equilibrated in less than 5 min. One dog MAC of halothane is equivalent to 0.88 % [13] and 1 dog MAC of desflurane is equivalent to 7.2 % [14].

### DATA ANALYSIS

The control response for each ring was isometric contraction to the ED\(_{50}\) of acetylcholine before administration of the anaesthetic. Airway smooth muscle relaxation was the change in tension caused by the anaesthetic. This change was expressed as a percentage of the control response. Analysis of variance was performed on the control response to the ED\(_{50}\) of acetylcholine, expressed as a percentage of maximal contraction, between the proximal and distal airway, for each volatile anaesthetic and anaesthetic dose, to confirm that there was no significant difference between the control responses in both tissue groups. The control response to the ED\(_{50}\) of acetylcholine was also compared with the post-control response to the ED\(_{50}\) of acetylcholine using the paired \(t\) test to evaluate potential tissue deterioration or residual anaesthetic effect. To evaluate the differences between proximal and distal airway responses at each anaesthetic group, paired \(t\) tests were used.

Analysis of variance with Scheffé’s test was used to test for significant differences between halothane and desflurane, the three concentrations of each anaesthetic and the tissue groups. \(P < 0.05\) was considered significant. The results are expressed as mean (SD).

### Results

To reach optimal length with the use of micro-manipulators, the proximal airway required 1.0–3.5 g of applied tension and the distal airway 0.50–2.0 g. The average increase in length (3.8 (0.8) mm) needed to reach optimal length in the proximal airway was almost equal to the od (5.0 (0.4) mm). This finding was consistent with that of Stephens, Meyers and Cherniack [15]. The increase in length (1.6 (0.7) mm) needed to reach optimal length in the distal airway was slightly greater than the od (1.0 (0.4) mm). The resting tension of the proximal airway (0.29 (0.12) g) was twice the resting tension of the distal airway (0.13 (0.04) g) (table 1).

### Table 1: Characteristics of proximal and distal airway smooth muscle (mean (SD))

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>Desflurane</th>
<th>Halothane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Distal</td>
</tr>
<tr>
<td>od (mm)</td>
<td>5.1 (0.5)</td>
<td>1.1 (0.3)</td>
</tr>
<tr>
<td>Applied length (mm)</td>
<td>3.6 (0.5)</td>
<td>1.2 (0.3)</td>
</tr>
<tr>
<td>Optimal length (mm)</td>
<td>8.7 (0.8)</td>
<td>2.3 (0.8)</td>
</tr>
<tr>
<td>Resting tension (g)</td>
<td>0.28 (0.32)</td>
<td>0.14 (0.11)</td>
</tr>
<tr>
<td>Response to ED(_{50}) of acetylcholine (g)</td>
<td>5.00 (1.27)</td>
<td>1.91 (0.56)</td>
</tr>
<tr>
<td>Response to ED(_{50}) of acetylcholine ((\mu)mol litre(^{-1}))</td>
<td>2.50 (1.83)</td>
<td>0.85 (0.50)</td>
</tr>
<tr>
<td>ED(_{50}) acetylcholine ((\mu)mol litre(^{-1}))</td>
<td>40.3 (32.5)</td>
<td>5.4 (7.9)</td>
</tr>
</tbody>
</table>

### Table 2: Responses to the ED\(_{50}\) of acetylcholine for proximal and distal airway smooth muscle before and after recovery from halothane and desflurane (mean (SD) percentage of maximal contraction)

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>Concentration</th>
<th>Airway</th>
<th>Pre-anaesthetic</th>
<th>Post-anaesthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halothane</td>
<td>0.9 MAC</td>
<td>Proximal</td>
<td>40.6 (1.5)</td>
<td>39.4 (2.2)</td>
</tr>
<tr>
<td></td>
<td>1.9 MAC</td>
<td>41.9 (2.2)</td>
<td>38.3 (1.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.8 MAC</td>
<td>45.4 (2.3)</td>
<td>40.3 (2.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9 MAC</td>
<td>Distal</td>
<td>48.9 (1.3)</td>
<td>48.1 (2.2)</td>
</tr>
<tr>
<td></td>
<td>1.9 MAC</td>
<td>47.7 (2.7)</td>
<td>48.9 (3.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.8 MAC</td>
<td>48.6 (1.5)</td>
<td>50.7 (1.8)</td>
<td></td>
</tr>
<tr>
<td>Desflurane</td>
<td>0.9 MAC</td>
<td>Proximal</td>
<td>49.0 (2.3)</td>
<td>49.9 (3.4)</td>
</tr>
<tr>
<td></td>
<td>1.9 MAC</td>
<td>47.1 (2.1)</td>
<td>47.9 (2.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.8 MAC</td>
<td>48.9 (2.4)</td>
<td>47.9 (3.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9 MAC</td>
<td>Distal</td>
<td>45.3 (2.6)</td>
<td>43.6 (2.7)</td>
</tr>
<tr>
<td></td>
<td>1.9 MAC</td>
<td>44.3 (2.7)</td>
<td>43.9 (3.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.8 MAC</td>
<td>44.8 (3.3)</td>
<td>43.5 (3.5)</td>
<td></td>
</tr>
</tbody>
</table>
to the same extent before administration of anaesthetic. The post-experimental responses to the ED50 of acetylcholine were not significantly different from their respective control values, indicating that no tissue deterioration occurred and the anaesthetic effect was reversible.

There was a significant difference between the relaxant effects of 0.9, 1.9 and 2.8 MAC for both desflurane and halothane (figs 1, 2) in the proximal and distal airway tissue groups, indicating a dose–response effect for these anaesthetics. Also, there was a significant difference between the relaxant effects of the proximal and distal airways at each anaesthetic dose within each anaesthetic group (P < 0.003) the average distal airway relaxed 50 % more than the proximal airway). Desflurane relaxed the proximal airway smooth muscle to a greater extent than halothane at 2.8 MAC (P = 0.0001) and the distal airway smooth muscle at 1.9 (P = 0.0007) and 2.8 MAC (P = 0.002). The proximal airway smooth muscle relaxed 13 %, 41 % and 61 % with desflurane and 20 %, 29 % and 41 % with halothane at 0.9, 1.9 and 2.8 MAC, respectively. The distal airway smooth muscle relaxed 34 %, 78 % and 90 % with desflurane and 35 %, 57 % and 67 % with halothane at 0.9, 1.9 and 2.8 MAC, respectively (figs 1, 2).

### Discussion

This study demonstrated that volatile anaesthetics (desflurane and halothane) had a greater bronchodilator effect on distal airway smooth muscle than on the more proximal airway smooth muscle in a dose-dependent manner, similar to the effects of isoflurane [7]. Furthermore, we found that desflurane had a greater direct bronchodilator effect than halothane at the doses tested (greater than 1 MAC for the distal airway smooth muscle and greater than 2 MAC for the proximal airway smooth muscle). Therefore, desflurane had good direct bronchodilator effects on the intraparenchymal airway despite its association with coughing and laryngospasm on induction [1].

To compare the proximal airway, which has a larger diameter and more muscle mass, to the distal airway, isometric tension was measured, thereby allowing the diameter of the airway ring to remain constant. Under these circumstances, changes in tension would be linearly proportional. In addition, the airway smooth muscle rings were evaluated at optimal length. This enables each airway to be compared at the same point on the length–tension curve. Furthermore, the ED50 of acetylcholine for each individual tissue was used as the baseline for comparison. This response maintains each tissue at the same degree of activation and contraction. The ED50 response, when expressed as a percentage of the Emax response, reflects the effects of functional muscle tissue so that it is not necessary to take into account the mass of the tissue.

It is possible that the increased ED50 of acetylcholine in the proximal airway was caused by cartilage. However, the ED50 of acetylcholine between airways with and without papain-softened cartilage were not found to be different [16]. Also, isometric tension measured in bronchial smooth muscle with cartilage compared with bronchial smooth muscle with the cartilage removed was not significantly different [17]. This suggests that the presence of cartilage does not oppose contraction. Furthermore, cartilage does not oppose relaxation but may actually facilitate it. When airway smooth muscle is contracted, cartilage plates and collagen fibres between these plates frequently reverse their orientation by almost 180° [18]. This orientation and the elastic nature of cartilage results in a tendency to spring outward, as described by Olsen and colleagues [18]. Agents that relax airway smooth muscle such as volatile anaesthetics would then allow the cartilage to spring outward, facilitating the tendency for relaxation. Despite this tendency, the distal airway, which lacks cartilage, relaxed to a greater extent than the proximal airway.
To minimize the difference in gas diffusion time, lung parenchyma and vascular tissues were removed carefully and the airway rings were cut into small segments. In addition, airway smooth muscle was allowed to equilibrate with the anaesthetic for a minimum of 15 min, or until the tissue response stabilized, to ensure that each tissue reached the same anaesthetic saturation point. Therefore, the effects on airway smooth muscle contraction could be normalized and compared.

Previous studies have examined the bronchodilator effect of anaesthetics by conventional techniques, such as measuring lung resistance (RL) in vivo [19–21], a value indicative of proximal airway resistance [22], or by in vitro techniques, such as measuring isometric force [11, 23, 24]. The results of these studies have shown that the indirect [21, 23, 24] and direct effects [11, 19, 20, 25] of anaesthetics are responsible for bronchodilatation. Warner and colleagues, using aerosolized acetylcholine, examined the direct effects of halothane on changes in pulmonary resistance and found a decrease in pulmonary resistance at 1.2 MAC from a baseline pulmonary resistance and found a decrease in the direct effects of halothane on changes in muscle tension and intracellular Ca²⁺ in canine trachea, Jones and colleagues [25] found that halothane reduced both of these variables. Halothane was also found to suppress tracheal smooth muscle contractions produced by direct muscle stimulation and increase the threshold for muscle activation [26].

In vivo studies have evaluated variations in global lung resistance (RL), which is indicative of changes in the more proximal airway and may be insensitive to changes in the more distal airway [21]. In addition, RL is a function of airway resistance and tissue resistance, with tissue resistance being the principal component of RL [27]. However, it has been shown that RL can be used as an index of changes in airway calibre [27], although it is not known if this index is homogeneous throughout the lung. Studies performed in vitro examined the direct effects of anaesthetics on the trachea and in some, only as far distally as the segmental bronchus. The results of these in vivo and in vitro studies indicated that volatile anaesthetics have a significant bronchodilator effect on proximal airway smooth muscle [11, 19, 20, 21]. Furthermore, the effects of 1 MAC of halothane may depress neural transmission and smooth muscle to an equal extent [19].

The clinical importance of the direct bronchoconstriction effects of volatile anaesthetics on airway smooth muscle may be more important than previously thought. Earlier studies focused on the direct effects of anaesthetics on the larger, more proximal, airway rather than the distal airway, which is the main contributor to airway resistance. However, in a more recent study, Brown, Zerhouni and Hirshman [28], using high resolution computer tomography, examined the effects of isoflurane and halothane on proximal and distal airways constricted with histamine. Their data suggested that administration of isoflurane and halothane (>1.1 MAC) resulted in greater bronchodilatation in airways less than 3 mm compared with airways greater than 7 mm. Mazzeo and colleagues confirmed that the volatile anaesthetic isoflurane had a differential effect on airway smooth muscle and that the distal airway smooth muscle was significantly more sensitive to the bronchodilating effects of isoflurane [7]. In this study, we also demonstrated that desflurane and halothane in vitro had a similar heterogeneous effect on airway smooth muscle.

The increased bronchodilator effect in the distal airway could be a result of several possibilities. This differential effect could be caused by structural or physiological differences, or both, in the bronchus compared with the bronchiolus. Further investigations are needed to define the factors involved in the differential effect of anaesthetics on airway smooth muscle and the greater relaxant effect of desflurane on airway smooth muscle in general. Discovering the reason for the differential effect of anaesthetics may help to develop a better understanding of anaesthetic actions on airway smooth muscle in the normal patient and in those with bronchospastic disease. This understanding may also lead to improvement and development of more rational drug therapies for the treatment of patients with reactive airway disease.

Acknowledgement

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References


