Halothane Inhibition of Ion Transport of the Tracheal Epithelium

A Possible Mechanism for Anesthetic-induced Impairment of Mucociliary Clearance

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Significant depression of mucociliary function occurs during general anesthesia. One possible mechanism to account for this effect is a change in ion and water transport across airway epithelium. To determine if anesthetics alter epithelial cell function, we used electrophysiologic techniques to measure the effects of halothane on ion transport of in vitro canine tracheal epithelia. Epithelial tissues were mounted in an Ussing chamber and the short-circuit current (Isc) (a measure of active ion transport) and transepithelial resistance were determined in the absence and presence of halothane. Halothane induced a rapid and reversible decrease in Isc that was dose-dependent. Four percent halothane reversibly decreased Isc from 90 ± 11 to 9 ± 6 μA/cm² (n = 12; P = 0.001) and increased transepithelial resistance. Isoproterenol is a well-known activator of chloride secretion that acts via β-adrenergic receptors and cyclic adenosine monophosphate (cAMP). Pretreatment with isoproterenol or dibutyryl cAMP (a cell permeable analogue of cAMP) increased the percent inhibition of Isc by 4% halothane. These effects are consistent with preferential inhibition of chloride secretion by halothane but rule out a primary action of halothane on the β-adrenergic system. In the presence of indomethacin, which eliminates the contribution of chloride secretion to Isc, 4% halothane induced a much smaller but still significant inhibition. This suggests that sodium absorption is also affected. We conclude that halothane significantly decreases ion and water transport in canine epithelia and that impaired fluid secretion may contribute to decreased mucous clearance in the perioperative period. (Key words: Anesthetics, volatile: halothane. Electrophysiology: chloride secretion; short-circuit current. Lungs: airway; epithelium.)

DURING GENERAL ANESTHESIA the ability to clear airway secretions is impaired.1,2 Inhalation of dry gases may be partly responsible.3,4 However, this impairment occurs even with humidified gases, suggesting that a direct effect of anesthetic agents on the mucociliary system may also be involved.5 A decrease in the ciliary beat frequency with anesthetics has been reported in Tetrahymena5 (a protozoan) and in the canine trachea,7 but the mechanisms by which anesthetics alter ciliary function are not known.

Anesthetics could alter the production of mucus or the secretion or reabsorption of the periciliary fluid layer, which is necessary for normal ciliary function. Because ion channels are of critical importance in fluid secretion by airway epithelia and because anesthetic agents are known to directly modulate channel function in a variety of tissues,6,8 we reasoned that inhibition of mucociliary clearance may be due, at least in part, to inhibition of fluid secretion by airway epithelia.

Electrolyte transport by airway epithelia is believed to control the composition and quantity of periciliary fluid.10,11 This regulation is based on active secretion of chloride into the airway lumen and active reabsorption of sodium. Ion transport produces a small osmotic gradient that drives the secretion or absorption of water.10,11 Transport across epithelial tissues can be readily studied using Ussing chambers. These chambers are designed to allow the two surfaces of the epithelium to be perfused separately, with electrically isolated solutions. Under short-circuit conditions, chloride secretion and sodium reabsorption summate to produce a net current, designated the short-circuit current (Isc), which flows from an apical to a basolateral direction.10,11 Measurement of Isc is a direct means of studying changes in active ion transport by airway epithelia. Ion transport by airway epithelia has been studied extensively in the canine trachea, and we chose this model system to investigate anesthetic related effects.

The β-adrenergic system is a known activator of chloride secretion in canine tracheal epithelium that acts primarily via cyclic adenosine monophosphate (cAMP).12-14 Effects of β-adrenergic stimulation include an increase in the proportion of Isc that is due to chloride secretion and an increased sensitivity to inhibitors of the β-adrenergic pathway. A mechanism by which anesthetics might alter epithelial ion transport is inhibition of the β-adrenergic system, analogous to that described in canine myocardium.15 In this study, therefore, we evaluated the effects of halothane on baseline Isc and on β-adrenergic-stimulated Isc in canine tracheal epithelium. We found that halothane profoundly altered ion transport in this tissue.

Materials and Methods

Tissue Preparation and Electrophysiology

This study was approved by the Animals Research Committee of The Johns Hopkins University. Tracheas...
were harvested from mongrel dogs after sacrifice by exsanguination. Muscle and connective tissue were dissected from the posterior membranes, and sheets of epithelium from the distal thirds of the tracheas were mounted in an Ussing chamber. The apical and basolateral compartments of the chamber were perfused without recirculation with Hank's balanced salt solutions that contained the following (in millimolar concentrations): NaCl 137, NaHCO₃ 25, KCl 5.4, KH₂PO₄ 0.44, Na₂HPO₄ 0.34, CaCl₂ 1.3, MgSO₄ 0.83, and glucose 5.6. Solutions were equilibrated with 95% O₂/5% CO₂ (pH = 7.4) and were maintained at 37 ± 0.5°C by manually adjusting the voltage applied to electrical heating elements imbedded in the chamber body.

The electrical potential difference across the epithelium was measured using 3 M KCl-agar bridges placed near the two surfaces of the tissue and connected to a custom-made current/voltage clamp instrument via calomel half cells. Current was passed from chlorinated silver wires connected to the chamber halves via 3 M KCl-agar bridges. Iᵥ was measured by automatically passing sufficient current to clamp the electrical potential difference across the epithelium to zero. The transepithelial resistance (Rₑ) was calculated from Ohm's law: \( R = \frac{\Delta V}{\Delta I} \), using the change in current (ΔI) induced by periodic, low-amplitude (5–10 mV; 3-s duration) voltage pulses (ΔV). The solution resistance without tissue was measured and electronically subtracted before each experiment.

**Anesthetic Administration and Analysis**

Halothane was delivered into an O₂/CO₂ mixture with a Fluotec 3 vaporizer (Cyprane Ltd., England). The calibration of this vaporizer was verified by mass spectrometry (Perkin Elmer, Glendale, CA). The gas mixture was bubbled through the Hank's solution that flowed through the chamber. Solution valves machined into the block of the Ussing chamber allowed rapid switching between solution reservoirs that were bubbled with gas mixtures that did or did not contain halothane. In these experiments, changes in the apical and basolateral solutions were made simultaneously in such a way that the halothane concentration was always the same on both sides of the tissue.

During studies with halothane involving dose–response relationships, the concentration of halothane in the gas was increased to 0.5, 1, 2, and 4%. Apical and basolateral sides of the tissue were exposed to these concentrations for 10-min test periods separated by 15-min intervals in halothane-free solutions. During the 15-min control intervals, the halothane-containing solutions were equilibrated by vigorous bubbling with the next halothane concentration. In experiments that involved a single concentration of anesthetic, the perfusion solutions were bubbled for 30 min with 4% halothane before exposure to the tissue. Halothane concentrations in the outflow solutions were measured using gas chromatography (Hewlett-Packard 5880A). The relationship between halothane percentage (vaporizer setting) and concentration measured in the chamber solution is presented in figure 1.

**Protocols**

After 90 min of stabilization under open-circuit conditions, the epithelial tissues were short-circuited and allowed to stabilize for an additional 30 min. Tissues (n = 12) were exposed to 4% halothane for 10 min and were then allowed to recover for 30 min. Following the recovery period the tissues were treated with basolateral isoproterenol (Sigma, St. Louis, MO) (10⁻⁶ M; n = 6) or bilateral dibutyryl cAMP (Sigma) (10⁻⁵ M) (n = 6), and the halothane exposure was repeated. The dose–response to halothane was evaluated as described above in epithelial tissues pretreated with isoproterenol (10⁻⁶ M; n = 6). Responses to 4% halothane in the presence of 10⁻⁵ M indomethacin were measured in tissues from other animals (n = 4) after 1 h of pretreatment with that drug.

The effects of halothane on Iᵥ and Rₑ were recorded and expressed as absolute values and as a percent inhibition. In this report, “control values” refers to measurements of Iᵥ or Rₑ immediately prior to each halothane exposure. Percent inhibition with halothane was calculated as 100 × (1 – halothane/control), where halothane is equal to the experimental value after 10 min of halothane exposure.

**Statistical Analysis**

The effects of 4% halothane before and after treatment were analyzed by paired t tests. The Spearman test of

![Fig. 1. Relationship between volume percent halothane (set on vaporizer) and halothane concentration in the chamber solutions. Symbols denote mean ± SEM (P < 0.001).](image-url)
correlation was used. The data from the dose–response curves were analyzed by analysis of variance. The data are presented as mean ± SEM, with P < 0.05 considered significant.

**Results**

Two hours after mounting, the electrical properties of the epithelium included an $I_{SC}$ of 90 ± 11 μA/cm² and a $R_T$ of 185 ± 20 ohm · cm² (tables 1 and 2). These values are well within the range of previously reported data. Four percent halothane induced a rapid and reversible decrease in $I_{SC}$ to 36 ± 6 μA/cm² ($n = 12$; $P = 0.001$) (fig. 2). This effect was associated with an increase in $R_T$ to 201 ± 21 ohm · cm² ($P = 0.012$). The percent inhibition of $I_{SC}$ by 4% halothane was 56 ± 4% ($n = 12$) and was not correlated ($r = 0.04$) with the control values of $I_{SC}$.

Because of a major role for the β-adrenergic system in regulating $I_{SC}$ in canine tracheal epithelia and because of the suggestion from studies in other tissues that anesthetic agents may inhibit β-adrenergic function, we chose to examine the actions of halothane on epithelia pretreated with a β-adrenergic agonist. Isoproterenol stimulated $I_{SC}$ from 68 ± 12 to 86 ± 11 μA/cm² ($P = 0.019$) with a tendency to decrease $R_T$ that did not reach significance ($P = 0.062$). The magnitude of the change in $I_{SC}$ induced by 4% halothane was significantly different before (40 ± 8 μA/cm²) and after stimulation by isoproterenol (56 ± 10 μA/cm²) (table 1). The percent inhibition of $I_{SC}$ by 4% halothane was significantly enhanced after pretreatment with isoproterenol from 54 ± 6 to 67 ± 4% ($P = 0.01$).

We chose to quantify the dose-dependence of halothane inhibition in tissues pretreated with $10^{-6}$ M basolateral isoproterenol, a condition of greater sensitivity to the anesthetic. The rapid reversibility of halothane effects allowed repeated exposure of each tissue to different concentrations of anesthetic (fig. 3). A clear dose-dependence (fig. 4) was observed in the range of 0.5–4% halothane ($n = 6$; $P = 0.001$). Preliminary experiments have demonstrated qualitatively similar dose–responses in unstimulated epithelia (data not shown).

To evaluate the possibility that inhibition by halothane occurred at a site proximal to adenyl cyclase, we also tested the effects of halothane in the presence of the cell-permeable analogue of cAMP, dibutyryl cAMP. Bilateral perfusion with $10^{-5}$ M dibutyryl cAMP produced increases in $I_{SC}$ similar to those obtained with isoproterenol: $I_{SC}$ increased from 90 ± 13 to 115 ± 11 μA/cm² ($P = 0.014$) (table 2). The percent inhibition of $I_{SC}$ by 4% halothane increased significantly, from 59 ± 4 to 64 ± 3% ($P = 0.01$) after treatment with dibutyryl cAMP.

Preliminary experiments were performed in the presence of bilateral $10^{-6}$ M indomethacin to determine if inhibition of chloride secretion abolished the halothane response. Under these conditions, 4% halothane decreased $I_{SC}$ from 19.8 ± 4.3 to 15.5 ± 3.2 μA/cm² ($P = 0.029$). The mean percent inhibition was 21 ± 1%.

**Discussion**

We have shown that halothane induces a large, rapid, reversible inhibition of $I_{SC}$ in the canine tracheal epithelium. Since $I_{SC}$ is related to ion and water transport, our data suggest that halothane induces profound alterations in fluid secretion or absorption by airway epithelia. This could, in turn, interfere with mucociliary clearance mechanisms in the airway. The $I_{SC}$ represents the sum of

**Table 1. Halothane Effects on Electrophysiologic Properties in the Absence and Presence of Isoproterenol**

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<th>No Isoproterenol</th>
<th>Isoproterenol</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Halothane 4%</td>
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<tr>
<td>$I_{SC}$ (μA/cm²)</td>
<td>76 ± 13</td>
<td>36 ± 10*</td>
</tr>
<tr>
<td>$R_T$ (ohm · cm²)</td>
<td>189 ± 37</td>
<td>198 ± 41*</td>
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</table>

Mean ± SEM, $n = 6$.

$I_{SC} =$ short-circuit current; $R_T =$ transepithelial resistance.

* $P < 0.05$ different from control values.
† $P < 0.05$ different from unstimulated values.

**Table 2. Halothane Effects on Electrophysiological Properties in the Absence and Presence of Dibutyryl cAMP**

<table>
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<tr>
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<th>No Dibutyryl cAMP</th>
<th>Dibutyryl cAMP</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Halothane 4%</td>
</tr>
<tr>
<td>$I_{SC}$ (μA/cm²)</td>
<td>104 ± 15</td>
<td>42 ± 7*</td>
</tr>
<tr>
<td>$R_T$ (ohm · cm²)</td>
<td>182 ± 15</td>
<td>204 ± 14*</td>
</tr>
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</table>

Mean ± SEM, $n = 6$.

$I_{SC} =$ short-circuit current; $R_T =$ transepithelial resistance.

* $P < 0.05$ different from control values.
† $P < 0.05$ different from unstimulated values.
active chloride secretion and active sodium reabsorption.\textsuperscript{16} Chloride enters the epithelial cell via a cotransporter in the basolateral membrane and exits the cell through regulated channels in the apical membrane.\textsuperscript{10} Sodium enters through channels in the apical membrane and is extruded on the basolateral side by a pump. The active movement of electrolytes across the epithelium can produce net fluid transport in either direction.\textsuperscript{11} Appropriate regulation of periciliary fluid by alterations in ion transport is believed to be essential for mucociliary clearance.\textsuperscript{10,11}

Our data are most consistent with an inhibition of chloride secretion and concomitant decrease in the volume of airway luminal fluid and increase in fluid viscosity. Two observations support this hypothesis. First, an inhibition of baseline $I_{SC}$ of greater than 50\% by halothane cannot be explained by changes only in sodium transport, since the rate of chloride secretion in this tissue is two to four times that of sodium absorption.\textsuperscript{16,17} Second, isoproterenol is known to stimulate chloride secretion and to increase the ratio of chloride transport to that of sodium.\textsuperscript{17} In the presence of isoproterenol, the percent inhibition of $I_{SC}$ by 4\% halothane was enhanced. Our data suggest that chloride transport is preferentially inhibited. The net effect would be an increase in the viscosity of airway fluid. However, the persistence of an effect in the presence of indomethacin suggests that some inhibition of sodium absorption also occurs, since chloride secretion is negligible under these conditions (table 1 of ref. 18).

The activation of chloride secretion by $\beta$-adrenergic agonists in canine tracheal epithelial tissues has been shown previously.\textsuperscript{10,12} cAMP is an important intracellular second messenger of this system, and the addition of exogenous analogues of cAMP have been shown to mimic the effects of $\beta$-adrenergic agonists on ion transport.\textsuperscript{13,14} In our study, the effect of halothane was not prevented by dibutyryl cAMP, indicating that halothane did not decrease $I_{SC}$ by inhibiting the $\beta$-adrenergic system. A likely alternative is that halothane acts directly on membrane ion channels that are involved in chloride secretion by canine tracheal epithelium.

The pathophysiologic effect of decreased chloride secretion is believed to be inhibition of mucociliary clearance. Such a mechanism has been proposed to explain the pulmonary complications of cystic fibrosis.\textsuperscript{19} The coexistence of our findings with known depression of mu-
ciliary function by inhalation anesthetics suggests that prolonged inhibition of epithelial ion transport may contribute to a higher incidence of early postoperative pulmonary complications in surgical patients. The particular ion transport mechanisms involved and the clinical importance of this effect remain to be established.

The authors thank K. Kubo, Ph.D., Research Associate in The Department of Anesthesiology, for analysis of halothane samples and Laurel Ricucci for editorial assistance.

References