Isonflurane- and Halothane-mediated Dilation of Distal Bronchi in the Rat Depends on the Epithelium

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Background: Respiratory epithelium releases substance(s) that can modulate bronchoconstriction in response to constrictive agonists and enhance bronchodilation in response to certain bronchodilators. The hypothesis that the bronchodilatory effect of isoflurane and halothane depends on the epithelium was tested in rat distal bronchial segments.

Methods: Wistar rat bronchial segments of the fourth order (diameter approximately 100 μm) were dissected. After preconditioning with 5-hydroxytryptamine, each bronchial segment was exposed to increasing concentrations of 0% to 3% isoflurane or 0% to 3% halothane under four conditions: after epithelial rubbing, after pretreatment with the nitric oxide synthase inhibitor N’-nitro-L-arginine, after pretreatment with the cyclooxygenase inhibitor indomethacin, or with no pretreatment (control). Changes in bronchial diameter were monitored using an in vitro video detection system.

Results: Both isoflurane and halothane produced concentration-dependent bronchodilation (P < 0.001 for either anesthetic; 40% ± 11% [mean ± SD] dilation for 3% isoflurane and 57% ± 10% dilation for 3% halothane). For both anesthetics, bronchodilation was significantly but incompletely attenuated by epithelial rubbing (12% ± 7% dilation for 3% isoflurane [P < 0.01] and 31% ± 10% dilation for 3% halothane [P < 0.01]), by pretreatment with indomethacin (20% ± 8% dilation for 3% isoflurane [P < 0.02] and 21% ± 9% dilation for 3% halothane [P < 0.001]), or by L-NA (9% ± 7% dilation for 3% isoflurane [P < 0.005] and 39% ± 12% dilation for 3% halothane [P < 0.05]). Epithelial rubbing did not impair nitroprusside-associated bronchodilation.

Conclusions: Isonflurane- and halothane-mediated bronchodilation depends at least partially on the epithelium and may involve both a prostanoid and nitric oxide in distal rat bronchi. (Key words: Anesthetics, volatile; halothane; isoflurane. Airways: epithelium; epithelium-derived relaxing factor [EpDRF].)

ANALOGOUS to the endothelial modulation of vascular smooth muscle tone,1 evidence has accumulated that respiratory epithelium may release substances that can modulate bronchial smooth muscle tone. Flavahan et al.2 demonstrated in canine bronchi that removal of epithelium enhanced contractile responses to acetylcholine, histamine, or 5-hydroxytryptamine (5-HT) but reduced the relaxation response to isoproterenol. Similar findings have been reported in other species, including pigs,3 guinea pigs,4 and humans.5

The nature of the epithelium-derived relaxing factors (EpDRFs) has not been determined. It has been suggested that EpDRF may be a prostanoid,6 specifically prostaglandin E2,7,8 Whether EpDRF may be a guanylate cyclase activator such as nitric oxide or carbon monoxide has not been examined systematically.

Volatile anesthetics such as halothane and isoflurane traditionally have been considered potent bronchodilators9 and have even been used to treat status asthmaticus.10,11 In the only previous study that tested whether the bronchodilatory action of a volatile anesthetic depends on the release of EpDRF, Sayiner et al.12 showed, in canine second-order and third-order bronchi, that the bronchodilatory effect of halothane was not epithelium...
dependent. However, as Stuart-Smith and Vanhoutte\textsuperscript{13} have shown, epithelial dependence of the bronchomotor effect varies topographically in canine bronchi: Epithelial dependence of the bronchomotor response to contractile agents such as 5-HT and acetylcholine is seen in the second-order (lobar) and third-order (segmental) bronchi, but not in the fourth-order (subsegmental) bronchi, whereas epithelial dependence of the bronchomotor response to a dilatory agent such as isoproterenol is most prominent in the distal airways. In this investigation, we studied the epithelial dependence of bronchodilatory effects of isoflurane and halothane \textit{in vitro} on isolated rat bronchial segments of the fourth order, in which we hypothesized that the epithelial dependence of the bronchodilatory effect would more likely be present than in more proximal airways. We also tried to determine whether the observed bronchodilatory effects were attenuated by inhibition of cyclooxygenase or NO synthase.

\section*{Materials and Methods}

\subsection*{Preparation of Bronchial Segments}

In accordance with the standards and with the approval of the institutional animal care and use committee, Wistar rats of either sex, weighing 100 to 150 g, were anesthetized by injecting 40 mg/kg ketamine and 5 mg/kg xylazine intraperitoneally. The lungs were removed quickly and placed in cold (\textdegree{}C) modified Krebs buffer (120 mM NaCl, 5.9 mM KCl, 11.1 mM dextrose, 25 mM NaHCO\textsubscript{3}, 1.2 mM NaH\textsubscript{2}PO\textsubscript{4}, 1.2 mM MgSO\textsubscript{4}, and 2.5 mM CaCl\textsubscript{2}). Fourth-order bronchi were dissected free of surrounding tissues. Each bronchial segment (length, 0.5 - 1.5 mm; diameter, approximately 100 \textmu{}m) was placed in a tissue chamber, cannulated with dual micropipettes (50 - 75 \textmu{}m diameters), and secured with 10-0 sutures. The bronchial segment was bathed continuously with modified Krebs buffer, exposed to a 95\% oxygen - 5\% carbon dioxide mixture, and maintained at 37\degree{}C with a pH of 7.4. The oxygen pressure in the tissue chamber exceeded 400 mmHg, and the bronchial segment was studied in a no-flow state. The bronchial segment was filled with Krebs buffer solution but was not pressurized above atmospheric pressure. All pharmacologic agents were added extraluminally. The bronchial segment was visualized with an inverted phase-contrast microscope (IMT-2; Olympus, Tokyo, Japan) connected to a video camera. The bronchial image was projected onto a television screen (Panasonic, Osaka, Japan). The bronchial lumen internal diameter was measured using an optical-density video detection system (Living Systems Instrumentation, Burlington, VT). Measurements of the lumen diameter were recorded. This tissue chamber system represents an adaptation of the vessel chamber system used for vasomotor studies,\textsuperscript{14} a schematic of which was published previously.\textsuperscript{14}

\subsection*{Stability of the Preparation}

To test for the stability of the bronchial preparation over time, the internal diameters of bronchial segments were followed for 2.5 h. Each segment reached an equilibrium diameter within the first 5 min, and there was neither a spontaneous bronchodilatory nor a bronchoconstrictive tendency during this period.

To test for the stability of bronchomotor responses over time, six bronchial segments (internal diameter, 135 \textpm{} 28 \textmu{}m) were equilibrated in the tissue chamber for 30 min and then subjected successively to 10 \textmu{}M 5-HT, 1 \textmu{}M of the thromboxane analog U-46619, 1 \textmu{}M histamine, and 100 \textmu{}M KCl, with rinsing and re-equilibration for 5 min between interventions. The bronchial segments were subjected to the same set of interventions after 90 min in the tissue chamber. Magnitudes and direction of bronchomotor responses were compared.

In addition, to test for the stability of bronchomotor responses to volatile anesthetics, additional bronchial segments (n = 6; baseline size, 97 \textpm{} 14 \textmu{}m) were preconstricted with 10 \textmu{}M 5-HT and exposed to 2\% isoflurane for 15 min, after 30 min of equilibration and again after 90 min of equilibration with rinsing and re-equilibration in between. Similar studies were performed for 2\% halothane (n = 6; baseline size, 100 \textpm{} 14 \textmu{}m).

\subsection*{Epithelial Denudation}

To produce epithelial denudation, a fine Hamilton wire (Hamilton Co., Reno, NV) was passed repeatedly into the bronchial lumen. Epithelial denudation was confirmed histologically (fig. 1). To further validate our model of epithelial denudation, we compared the dilatory responses of epithelium-intact and epithelium-rubbed bronchial segments to the epithelium-dependent agent isoproterenol\textsuperscript{23,13} and the epithelium-independent agent nitroprusside\textsuperscript{4} after preconstriction with 10 \textmu{}M 5-HT.

\subsection*{Preparation of Sections for Histologic Analysis}

Each bronchial specimen was stained with 2\% toluidine blue and fixed overnight in 2\% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). The next day, the tissue was washed in 0.1 M cacodylate buffer and en-
robed in 2% Agar, which was then re stained in 2% toluidine blue to make the Agar blocks more visible. Blocks were postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h, washed in distilled water, incubated overnight in 2% uranyl acetate in water, washed with distilled water, and then dehydrated through ascending alcohols. Tissue was incubated in propylene oxide (a transitional solvent), infiltrated with several changes of 50% propylene oxide - 50% Epon resin, followed by infiltration and embedding at 60°C in pure Epon. After 48 h of polymerization at 60°C, the blocks were trimmed manually, sectioned at 0.5-µm thickness with an ultramicrotome, and counterstained with 2% toluidine blue.

**Experimental Protocol**

After 30 min of equilibration in the tissue chamber, a baseline measurement of the bronchial lumen diameter was obtained. The bronchus was preconstricted with 10 µM 5-HT, a dose that had been found to produce consistent bronchoconstriction on preliminary studies. Bronchial segments were exposed to increasing concentrations of 0% to 3% isoflurane for 10 min at each concentration or 0% to 3% halothane for 15 min at each concentration by adding the anesthetic to the 95% carbon dioxide - 5% oxygen mixture bubbling the Krebs buffer solution, using an in-line bubble-through vaporizer. Diameter changes at each concentration of the
anesthetic were measured. Previously we showed that in our tissue chamber system, less than 10 min for isoflurane and 15 min for halothane are needed to reach steady-state concentrations after introducing the respective anesthetic in the tissue chamber. We have also shown that the millimolar concentrations and partial pressures of isoflurane and halothane remain consistently proportional to their concentrations in the gas mixture bubbled into the buffer solution. The anesthetic content in the gas mixture was monitored continuously using a Rascal II gas analyzer (Ohmeda, Salt Lake City, UT) that had been calibrated to industrial standards.

At the end of each experiment, the tissue chamber was flushed with fresh Krebs buffer and the bronchus was re-equilibrated at 37°C. Potassium chloride was added to achieve a final concentration of 100 mM, and the bronchial lumen diameter was measured. Only those bronchial segments that constricted by at least 15% in response to potassium chloride at the end of each experiment were considered still viable and included for data analysis. This represented exclusion of any bronchial segment that constricted less than the average amount by more than approximately 1 standard deviation. Potassium chloride has been shown to constrict epithelium-intact and epithelium-denuded bronchial segments equally well.

To determine the epithelial dependence of volatile anesthetic-mediated bronchomotor effect, some bronchial segments were denuded of the epithelium by the technique just described. These segments were equilibrated, preconstricted with 5-HT, and then subjected to a volatile anesthetic, as described previously.

Similarly, other bronchial segments were pretreated with either 10 μM of the cyclooxygenase inhibitor indomethacin or 10 μM of the NO synthase inhibitor Nω-nitro-L-arginine (L-NNA) in the buffer solution, before exposure to a volatile anesthetic, to determine whether a cyclooxygenase product or NO may be implicated as the cause of the bronchomotor effect.

All drugs were dissolved in ultradistilled water, but indomethacin was dissolved in ethanol and water. Ethanol had no significant bronchomotor effect at the concentration used in the study.

Statistical Analysis

No animal contributed more than one bronchial segment to any one experimental group; therefore, the n value for each group represents the number of animals and the number of bronchial segments. Bronchomotor responses to various bronchomotor agents after 30 min of equilibration were compared with responses after 90 min of equilibration using the Student’s t test (two tailed). Dose-response curves of rat bronchial segments to increasing concentrations of either isoflurane or halothane were analyzed by one-way analysis of variance (Scheffé’s linear contrast) to test the null hypothesis that the anesthetic had no concentration-dependent effect on the bronchial lumen diameter. To determine whether isoflurane and halothane had different bronchodilating potencies at equivalent minimum alveolar concentration multiples, a multiple linear regression was performed and a P value was calculated for the identity of the anesthetic as a significant predictor of bronchodilation. The effects of epithelial denudation, pretreatment with indomethacin, or L-NNA on the anesthetic action were analyzed by two-way analysis of variance with a repeated measures factor, with post hoc Newmann-Keuls test for between-groups comparison when the initial analysis of variance yielded a significant P value. In addition, stratified z tests were performed to identify the concentrations at which the between-groups differences were significant. Responses of different experimental groups to 10 μM of the preconstricting agent 5-HT or to viability testing with potassium chloride were compared using the Student’s t test (two tailed). P < 0.05 was considered significant. All statistics were calculated using True Epistat software (Epistat Services, Richardson, TX). All data are presented as means ± standard deviation.

Results

Validation of Epithelial Denudation

Histologic sections of bronchi subjected to repeated intraluminal abrasion showed significant, but incomplete, denudation of the epithelial layer (fig. 1). More vigorous abrasion resulting in complete epithelial denudation produced bronchial smooth muscle injury.

Epithelium-rubbed bronchial segments showed significantly attenuated dilatory response to the epithelium-dependent dilator isoproterenol compared with epithelium-intact segments (fig. 2A) (P < 0.01). But there was no significant difference in the response to the epithelium-intact agent nitroprusside between epithelium-intact and epithelium-rubbed bronchial segments (fig. 2B; P = 0.37). (Isoproterenol — epithelium intact: n = 6; baseline size, 115 ± 21 μm; epithelium-rubbed: n = 6; baseline size, 118 ± 20 μm. Nitropruss-
side—epithelium intact: n = 6; baseline size, 125 ± 13 μm; epithelium rubbed: n = 6; baseline size, 116 ± 16 μm).

Stability of Bronchomotor Responses
We found no significant difference in the respective responses of the bronchial segments to 5-HT, U46619, histamine, or potassium chloride after 30 min of equilibration compared with after 90 min of equilibration (P > 0.10, 0.75, 0.40, 0.59, respectively; fig. 3A). Neither was there a significant difference in the respective responses of the 5-HT–preconstricted bronchial segments to 2% isoflurane or 2% halothane after 30 min of equilibration compared with after 90 min of equilibration (P = 0.32 and 0.85, respectively; fig. 3B).

Bronchomotor Effects of Volatile Anesthetics
We found no significant difference in the magnitude of preconstriction produced by 10 μM 5-HT, among any of the experimental groups (table 1).

Both isoflurane and halothane showed concentration-dependent bronchodilatory effects on 5-HT–preconstricted rat bronchial segments (figs. 4 and 5; P < 0.001 for either anesthetic) (control bronchi—for isoflurane: n = 6; baseline diameter, 129 ± 18 μm; for halothane: n = 7; baseline diameter, 99 ± 24 μm). At equivalent minimum alveolar concentration multiples,17 isoflurane and halothane appeared to be equipotent (P = 0.75; fig. 6).

Isoflurane-mediated bronchodilation was attenuated but not abolished by pretreatment with either indomethacin (n = 7; baseline diameter, 120 ± 21 μm; P < 0.02) or L-NNA (n = 7; baseline diameter, 98 ± 16 μm; P < 0.005) or by epithelial denudation (n = 5; baseline diameter, 108 ± 17 μm; P < 0.01; fig. 4). The attenuating effect of L-NNA pretreatment was significantly greater than that of indomethacin pretreatment (P < 0.05).

Similarly, halothane-mediated bronchodilation was attenuated but not abolished by pretreatment with either indomethacin (n = 7; baseline diameter, 107 ± 24 μm; P < 0.001) or by L-NNA (n = 8; baseline diameter, 94...
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Fig. 3. Comparison of the responses of (A) the bronchial segments to constrictive agents: 10 μM 5-hydroxytryptamine, 1 μM U46619, 1 μM histamine, or 100 mM KCl; and (B) the 5-hydroxytryptamine-preconstricted bronchial segments to 2% isoflurane or 2% halothane after 30 min of equilibration versus after 90 min of equilibration. There was no significant difference between the two time points, demonstrating stability of the bronchomotor response over time.

± 14 μm; P < 0.05), or by epithelial denudation (n = 6; baseline diameter, 99 ± 20 μm; P < 0.01; fig. 5). Unlike with isoflurane, the attenuating effect of L-NNA pretreatment on halothane-mediated bronchodilation was significantly less than that of indomethacin pretreatment (P < 0.01).

Table 1. Percent Contraction (Percent Decrease in Diameter from Baseline Equilibration Diameter) to KCl (Viability Testing) and to 5-HT 10 μM (Preconstriction) of Various Experimental Groups of Rat Bronchial Segments

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Experimental Condition</th>
<th>N</th>
<th>% Contraction to KCl 100 mM</th>
<th>% Contraction to 5-HT 10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane</td>
<td>Control</td>
<td>6</td>
<td>20.2 ± 5.1</td>
<td>22.8 ± 2.3</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>Indomethacin-pretreated</td>
<td>7</td>
<td>17.9 ± 4.5</td>
<td>22.5 ± 4.3</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>L-NNA-pretreated</td>
<td>7</td>
<td>23.3 ± 4.8</td>
<td>24.5 ± 1.9</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>Epithelium-rubbed</td>
<td>5</td>
<td>19.1 ± 2.5</td>
<td>21.2 ± 1.9</td>
</tr>
<tr>
<td>Halothane</td>
<td>Control</td>
<td>7</td>
<td>22.3 ± 3.0</td>
<td>22.5 ± 2.6</td>
</tr>
<tr>
<td>Halothane</td>
<td>Indomethacin-pretreated</td>
<td>7</td>
<td>21.8 ± 5.6</td>
<td>22.5 ± 3.8</td>
</tr>
<tr>
<td>Halothane</td>
<td>L-NNA-pretreated</td>
<td>8</td>
<td>20.1 ± 3.8</td>
<td>23.1 ± 3.2</td>
</tr>
<tr>
<td>Halothane</td>
<td>Epithelium-rubbed</td>
<td>6</td>
<td>20.1 ± 3.1</td>
<td>21.6 ± 3.0</td>
</tr>
</tbody>
</table>

Data are mean ± SD. There was no significant difference between epithelium-intact and epithelium-rubbed bronchial segments in their responses to KCl. There was no significant difference in the magnitude of 5-HT-mediated precontraction between any of the experimental groups and their respective control group.
We found no significant difference in responses to potassium chloride, used to test bronchial viability, among any of the experimental groups (Table 1). Specifically, there was no significant difference between the epithelium-intact and epithelium-denuded segments.

**Discussion**

The main findings of our study are that bronchodilatory effects of isoflurane and halothane depend at least partially on the epithelium in subsegmental rat bronchi, and that these effects appear to be mediated by both a cyclooxygenase product and NO.

The bronchomotor effects of volatile anesthetics *in vivo* are multiple and complex. All of them increase airway resistance to some extent because of loss of lung volume on induction. However, the overall effect *in vivo* is bronchodilation for isoflurane, halothane, and enflurane in human beings and in dogs. Our *in vitro* finding is consistent with those of earlier *in vitro* studies.

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**Fig. 4.** Relaxation of the 5-hydroxytryptamine–preconstricted bronchial segments to increasing concentrations of isoflurane: control, after epithelial rubbing, after pretreatment with N^6^-nitro-L-arginine (L-NNA), or after pretreatment with indomethacin. Isoflurane-mediated bronchodilation was significantly attenuated after epithelial rubbing (*P* < 0.01) or after pretreatment with L-NNA (*P* < 0.05) or indomethacin (*P* < 0.02). Attenuation after L-NNA was significantly greater than after indomethacin (*P* < 0.05). #P < 0.05 versus control. *P* < 0.05 between L-NNA pretreatment and indomethacin pretreatment. (One minimum alveolar concentration of isoflurane in rats was considered 1.20%.)

**Fig. 5.** Relaxation of the 5-hydroxytryptamine–preconstricted bronchial segments to increasing concentrations of halothane: control, after epithelial rubbing, after pretreatment with N^6^-nitro-L-arginine (L-NNA), or after pretreatment with indomethacin. Halothane-mediated bronchodilation was significantly attenuated after epithelial rubbing (*P* < 0.01) or after pretreatment with L-NNA (*P* < 0.05) or indomethacin (*P* < 0.001). Attenuation after L-NNA was significantly less than after indomethacin (*P* < 0.01). #P < 0.05 versus control. *P* < 0.05 between L-NNA pretreatment and indomethacin pretreatment. (One minimum alveolar concentration of halothane in rats was considered 0.88%.)
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Fig. 6. Relaxation of the 5-hydroxytryptamine–preconstricted bronchial segments to increasing concentrations of isoflurane or halothane expressed as minimum alveolar concentration multiples (MAC). The two anesthetics appear to be equipotent bronchodilators at equivalent MAC multiples. (One MAC in rats was considered 1.20% isoflurane or 0.88% halothane.17)

and shows a direct bronchodilatory effect of isoflurane and halothane on rat bronchi.

A role of the respiratory epithelium in modulating bronchoconstriction or enhancing bronchodilation was first suggested by Flavahan et al. They demonstrated in rings of canine bronchi that removal of the epithelium increased contractile responses to acetylcholine, histamine, and 5-HT and decreased relaxation responses to isoproterenol. Subsequent works in the same laboratory and in others produced similar results in several species, including humans.3–5,13,23–25 The structure of the respiratory epithelium changes progressively from pseudostratified, columnar cells of the large airways to thinner, less pseudostratified and simple cuboidal cells of the bronchioles, and finally to squamous cells in the alveoli.25 Because of the varying structure of the respiratory epithelium, the role of epithelium in bronchomotion may vary topographically. In dogs, removing the epithelium enhances the effect of the contractile agonists in the large airways, whereas in small airways the major effect is to decrease relaxation to isoproterenol.13 Such a distinction is not observed in porcine airways. Removal of epithelium has a comparable effect on isoproterenol-mediated relaxation and acetylcholine- or histamine-mediated contraction of porcine airways of different orders.3 Although the role of the epithelium in modulating bronchomotion appears established, its exact nature may vary with the species, airway order, and perhaps experimental conditions.

In the present study, we used isolated rat bronchi of the fourth order, where the pseudostratified epithelium still predominates. One limitation of our experimental preparation is that our denudation technique resulted in incomplete and possibly variable amounts of denudation. Consequently, we cannot state unequivocally that the mild residual bronchodilatory effect of the anesthetics on the rubbed segments was due to incomplete epithelial removal or to an epithelium-independent component of bronchodilation. However, the epithelium rubbed bronchial segments clearly showed significantly attenuated relaxation to isoflurane and halothane and to the known epithelium-dependent agent isoproterenol.2,13 The decrease in bronchodilation with epithelial rubbing was not due to mechanical damage to the muscle layer, because the contractile responses to 10 μM 5-HT and KCl and the dilatory responses to the known epithelium-independent agent nitroprusside of epithelium-rubbed bronchial segments remained intact and comparable to the responses of unrubbed segments. Therefore the bronchodilatory effects of isoflurane and halothane on distal bronchi depend, at least partially, on the epithelium.

In the only previous study examining epithelial dependence of volatile anesthetic-mediated bronchodilation, Sayiner et al.12 examined epithelial dependence of the bronchodilatory effect of halothane in canine bronchi of the second and third orders. They found that the ability of halothane 1 or 2 minimum alveolar concentration to relax carbachol-preconstricted bronchi was not affected by epithelium removal and that halothane caused equivalent rightward shifts of the concentration-response curves to acetylcholine and 5-HT, regardless of whether epithelium was present. Thus they concluded that the bronchodilatory effect of halothane was epithelium independent. In contrast, in our study with fourth-order rat bronchi, the effects of both halothane and isoflurane were strongly epithelium dependent. The apparent disparity between Sayiner et al.’s findings and our own is probably explained by topographic variation in epithelial dependence of bronchomotor effects and by differences in species and experimental methods.

Epithelial dependence of isoflurane- and halothane-mediated bronchodilation appeared to be mediated by both NO and a prostanooid. Coupled release of or synergism between NO and a prostanooid has been described in many biological systems. Examples include agonist-induced vasodilation,20 flow- or shear stress–induced vasodilation,27 and modulation of platelet aggregation.28 Modulation of bronchomotion appears to be another
system in which NO and a prostanoid work in conjunction.

The fact that EpDRF or one form of EpDRF may be a prostanoid has been demonstrated in guinea pigs, dogs, and humans. Specifically, prostaglandin E2 appears to be the most important mediator in dogs and humans with prostaglandin I2 possibly providing a synergistic effect. The role of prostaglandin E2 or I2 has been disputed in guinea pigs. Our findings are consistent with the fact that in rats, a prostanoid may be one of the EpDRFs.

Whether NO may be an EpDRF has not been examined specifically before. There is some suggestive evidence supporting this postulate, however. Ilhan and Sahin used a bioassay to show that tracheal epithelium can produce a relaxing factor, which is not a product of cyclooxygenase and which can relax vascular smooth muscle. Furthermore, NO donors such as sodium nitroprusside and 5-nitrosodiethylamine have been shown to relax bronchial smooth muscles of pigs and humans; this is further corroborated in rats in our study. However, whether bronchodilation by NO is by activation of guanylate cyclase and generation of cyclic guanosine monophosphate (as in the case of vascular smooth muscle) or by an unknown, cyclic guanosine monophosphate-independent mechanism has been debated.

Whereas the bronchodilatory effect of isoflurane and halothane may be partially mediated via generation of NO, the effects of the volatile anesthetics on NO-cyclic guanosine monophosphate pathway in other tissue systems may be somewhat different. In the vascular system, the dilatory effect of isoflurane on conductance vessels has been reported to depend on the endothelium in the coronary circulation of rabbits and dogs, but independent in the rat aorta and canine cerebral arteries. Further, volatile anesthetics have been demonstrated to attenuate agonist-induced NO-mediated vasodilation in both conductance arteries and resistance arteries, although the exact site of attenuation has been debated. Finally, volatile anesthetics have been suggested to suppress the formation or action of NO in the neural tissues, thereby producing an anesthetic state. The relation between volatile anesthetics and NO is complex and variable, depending on the tissue type and perhaps on species and experimental conditions.

Although the bronchomotor effects of both isoflurane and halothane are epithelium dependent, the relative contributions of a cyclooxygenase product and NO appear different for the two anesthetics. Namely isoflurane-mediated bronchodilation is more dependent on NO than on a bronchodilatory prostanoid, whereas the opposite is true for halothane. Therefore, even though the two anesthetics appeared to be equipotent bronchodilators at equivalent minimum alveolar concentrations in our model, they may have different potencies in circumstances of selective prostanoid or NO suppression.

In certain patients with bronchial hyperreactivity, such as those with asthma or exposure to an allergen or respiratory virus, focal epithelial damage or inflammation may be apparent. A decrease in epithelial production of EpDRF may contribute to bronchial hyperreactivity, along with other causes such as a decrease in baseline airway caliber, autonomic dysfunction, and alterations in the bronchial smooth muscle itself. We can speculate that to the extent that the bronchodilatory effects of isoflurane and halothane are epithelium dependent, these anesthetics may not be as effective in patients with respiratory epithelial dysfunction in dilating the airways as in healthy persons. If epithelial dependence of volatile anesthetic-mediated bronchodilation is prominent primarily in distal airways even in humans, any beneficial effect of the anesthetics in dilating asthmatic airways may be evident mainly in the proximal airways. Further studies are needed for cross-species validation and using bronchial segments with preexisting airways disease.

In summary, we have shown in rat bronchial segments of the fourth order that isoflurane and halothane have direct, concentration-dependent bronchodilatory effects and that these effects depend at least partially on the epithelium. NO and a prostanoid may mediate the bronchodilatory effects of these anesthetics.

The authors thank Dan Brown for assistance in preparing bronchial sections for histologic analysis, Hang Lee, Ph.D., of Harvard Medical School for help with the statistical analysis, and Leo Hannenberg for assistance with computer software.

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