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Bronchodilation by Halothane Is Not Modulated by Airway Epithelium

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Halothane relaxes airway smooth muscles. To test the hypothesis that this relaxation is modulated by airway epithelium, we studied the effects of halothane on isolated second- and third-order canine bronchial rings and second-order canine bronchial segments. Paired rings or segments were examined, with the epithelium removed from one ring or segment of each pair. The bronchial rings were suspended in organ chambers and contracted with 10^{-8}–10^{-3} M acetylcholine (ACh), 10^{-8}–10^{-5} M 5-hydroxytryptamine (5HT), or 0.5–16 Hz electrical field stimulation (EFS, 15 V, 0.5-ms pulse duration). The tissue was contracted in the absence of halothane and during exposure to 1 and 2 MAC halothane. The bronchial segments were perfused intraluminally with physiologic salt solution (PSS) and contracted with 10^{-6} M carbachol added to the tissue-bath PSS. One or 2 MAC halothane was then added to the perfusate. In the absence of halothane, epithelium removal increased the sensitivity of the bronchial rings to ACh and 5HT but not to EFS. Addition of 1 or 2 MAC halothane to the bathing fluid of the rings with or without epithelium decreased the sensitivity of the rings to ACh and 5HT. One MAC halothane decreased the sensitivity of the rings with and without epithelium to EFS. The decrease in sensitivity caused by halothane was not significantly different in rings with or without epithelium for any method of stimulation. In the bronchial segments, relaxations evoked by 1 or 2 MAC were not different in segments with or without epithelium. The time constants of relaxation also were not significantly different for segments with or without epithelium. We conclude that the relaxing effects of halothane on canine bronchial smooth muscle *in vitro* are not modulated by the airway epithelium, and the epithelium does not provide a detectable barrier to the diffusion of halothane from the bronchial lumen to the smooth muscle. (Key words: Acetylcholine. Airways: canine bronchial segments; bronchial rings. Epithelium-derived relaxing factor. Electrical field stimulation. 5-Hydroxytryptamine.)

VOLATILE ANESTHETICS are potent bronchodilators. Halothane, enflurane, and isoflurane relax isolated canine smooth muscle by several mechanisms, including depression of parasympathetic neural pathways innervating airway smooth muscle and a direct effect on the muscle and its receptor systems.[1]

The bronchial epithelium can affect the contraction of underlying airway smooth muscle *in vitro*.[2] When the epithelium is removed, the response to bronchoconstrictor agents is enhanced and the response to bronchodilator agents is reduced,[2] suggesting that the epithelium promotes relaxation of airway smooth muscle. The mechanism of this effect is unclear; possibilities include the production of an epithelium-derived relaxing factor (EpDRF), the identity of which is not known,[3] or the presentation by the epithelium of a barrier to the diffusion of contractile agonists from the lumen to the smooth muscle. Epithelial effects on airway smooth muscle are of possible clinical importance because patients with asthma or viral infections may have epithelial damage or destruction that may contribute to airway hyperreactivity.[4]

Because the response to bronchodilators is modulated by airway epithelium, we hypothesized that the bronchodilating effects of halothane also may be in part modulated by the airway epithelium. If halothane increased the availability of EpDRF or potentiated its action, then the relaxing effect of halothane would be lessened in patients with damaged airway epithelium, *i.e.*, in patients who have the greatest need for bronchodilation. The epithelium also may act as a barrier to the diffusion of halothane, but because of halothane's solubility this possibility does not seem very likely. In this instance, epithelial damage might promote better access of halothane to airway smooth muscle *via* the airway lumen.

To test the above hypotheses, we studied the contractile responses of isolated canine airways to a muscarinic (acetylcholine [ACh]) and to nonmuscarinic stimuli (5-hydroxytryptamine [5HT] and electrical field stimulation [EFS]). These stimuli were chosen because it is known that the concentration-response curves to these stimuli are shifted after the removal of the epithelium.[3] Responses were determined in airways with and without epithelium, in the absence and presence of halothane.

Materials and Methods

The protocol of this study was approved by the Animal Care and Use Committee of the Mayo Clinic. Two sets

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of experiments were conducted using isolated canine bronchi. In the first series bronchial rings were studied to determine the effect of epithelium removal on the ability of halothane to depress bronchial smooth muscle contraction in response to ACh, 5HT, or EFS. In the second series of experiments, bronchial segments perfused with physiologic saline solution (PSS) were studied to determine the effects of epithelium removal on the ability of halothane to relax bronchial smooth muscle contracted with carbachol and to examine the possibility that the epithelium may function as a barrier to the diffusion of halothane.

Experiments were performed on 24 bronchial rings of second or third order from six mongrel dogs contracted with ACh (6 rings with intact epithelium and exposed to halothane, 6 rings with intact epithelium and in the absence of halothane, 6 rings with epithelium removed and exposed to halothane, and 6 rings with epithelium removed and in the absence of halothane). Forty rings from 10 dogs were stimulated with 5HT and another 24 rings from 6 dogs were stimulated with EFS (table 1). In addition, 12 bronchial segments of second order from another 6 mongrel dogs were studied. The dogs were anesthetized with sodium pentobarbital (30 mg/kg) and killed by exsanguination. The lungs were removed and immersed in PSS of the following composition (millimolar): $MgSO_4$ 0.8, KH_2PO_4 1.2, KCl 3.4, $CaCl_2$ 2.4, NaCl 110.5, $NaHCO_3$ 25.7, and dextrose 5.6. The bronchi were dissected from the lung parenchyma; adherent fat and connective tissue were removed; and paired rings or segments were prepared. The epithelium was removed from one of each pair of rings (4.5–6.5-mm length and 4.0–6.5-mm outer diameter) or segments (23–35-mm length and 4–7-mm outer diameter) by gentle rubbing of the lumen using a cotton swab.[2] The completeness of the epithelium removal was confirmed in all rings and segments by light microscopy (hematoxylin and eosin), which also showed that the submucosa and the smooth muscle had not been damaged by the removal of the epithelium. Rings and segments in which less than 95% of the epithelium had been removed as judged by microscopic examination were not included in the data reported here.

Bronchial Rings

Bronchial rings were placed into 25-ml organ chambers filled with PSS, kept at 37° C and bubbled with a gas mixture consisting of 94% O_2 and 6% CO_2 to maintain a *p*H of 7.40. The rings were suspended horizontally by two stainless steel stirrups between two platinum electrodes. One stirrup was anchored at the bottom of the tissue bath and the other was connected to a force transducer to measure continuously the isometric force (model FT03, Grass Force-Displacement Transducer), which was recorded (model 7418A, Hewlett-Packard). After mounting the rings in the tissue baths, the rings were washed approximately every 5 min with PSS for 1 to 1.5 h. During this time the rings were stimulated for 30 s with EFS (15 V, 25 Hz, 0.5-ms pulse duration); EFS was provided by a direct current amplifier (Mayo Clinic, Section of Engineering) triggered by a stimulator (model 44, Grass). The rings were stretched progressively with the aid of a rack-and-pinion device until the response to EFS reached a stable maximal level (defined as optimal length [L_o]); the length was not changed during the course of the study. All rings were then contracted with 10^{-3} M ACh and the response defined as maximal force. All subsequent force measurements were normalized by expressing them as a percentage of this maximal force.

For tissue from each dog, 1 MAC halothane was added to the gas mixture bubbling through the PSS for 20 min in one pair of rings and 2 MAC halothane was added to a second pair of rings, while a third pair of rings was not exposed to halothane. Concentration-response curves for ACh (10^{-8}–10^{-3} M) or 5HT (10^{-8}–10^{-5} M) or EFS (0.5–16 Hz for 3 min) were obtained simultaneously for all three pairs by cumulatively increasing the drug concentrations in half-log increments or by doubling the frequency of EFS. One method of stimulation was used for each dog studied. Hexamethonium chloride (HXT) (10^{-5}

TABLE 1. Maximal Forces of Contraction, Forces at Optimal Length, and Weights of Bronchial Rings

Stimulus	Force (g)		Force at L_o (g)		Weight (mg)	
	E+	E−	E+	E−	E+	E−
ACh (n = 6)	19.7 ± 4.8*	20.1 ± 3.8*	2.0 ± 0.2	2.0 ± 0.2	90 ± 11	96 ± 20
5HT (n = 10)	13.6 ± 2.5†	12.8 ± 2.0†	2.0 ± 0.2	2.0 ± 0.2	83 ± 37	85 ± 35
EFS (n = 6)¶	9.0 ± 1.7‡§	13.3 ± 3.5‡	2.1 ± 0.1	2.1 ± 0.1	96 ± 22	107 ± 39

Mean ± SD; n = number of dogs.
ACh = acetylcholine; 5HT = 5-hydroxytryptamine; EFS = electrical field stimulation; E+ = epithelium intact; E− = epithelium removed; L_o = optimal length.
* Force of control rings in response to 10^{-3} M ACh.
† Force of control rings in response to 10^{-5} M 5HT.
‡ Force of control rings in response to 16 Hz.
§ Significantly different ($P = 0.04$) from E− (paired *t* test).
¶ Weight not available for one study.

M) was added to the PSS 30 min prior to the administration of ACh in order to block stimulation by ACh of the ganglionic cholinergic nicotinic receptors. Atropine (10^{-6} M) was added to the bath 30 min prior to the addition of 5HT, in order to block muscarinic receptors. To inhibit activation of α- and β-adrenergic receptors during EFS, rings were pretreated with phentolamine (10^{-6} M) and propranolol (10^{-6} M).

Bronchial Segments

The segments were mounted horizontally in a 50-ml tissue bath filled with PSS kept at 37° C and bubbled with 94% O_2 and 6% CO_2. Both segments were perfused intraluminally with PSS delivered from a common reservoir by a roller pump (Gilson Minipuls 2) at a rate of 4 ml/min (fig. 1). The contractile force was measured using two stainless steel stirrups passed through the wall of the segments. One stirrup was fixed to a metal rod on the bottom of the chamber, and the other was attached to a force transducer (model FT03, Grass Force-Displacement Transducer). Isometric forces were recorded on a two-channel pen recorder (Kipps and Zonin, World Precision Instruments). The segments were stretched to their L_o at the beginning of the experiment by determining the maximal response to EFS. EFS was supplied by two platinum electrodes mounted parallel on opposite sites of the segments (not shown in fig. 1). The length was not changed during the experiment.

After the determination of L_o, HXT (10^{-5} M) was added to the PSS of the segments 30 min prior to contraction with 10^{-6} M carbachol. A concentration of 10^{-6} M carbachol was chosen because previous studies showed it caused approximately 50% of the maximal contraction evoked by 10^{-3} M carbachol both in segments with and

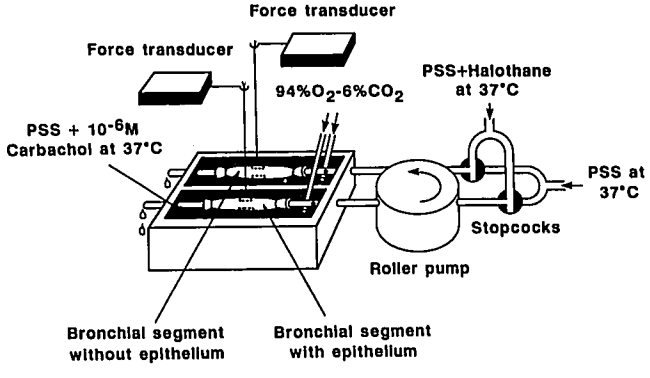

FIG. 1. Two separate organ baths for intraluminal perfusion of bronchial segments. The bronchial segments were perfused with PSS from the same reservoir. Halothane could be added to the PSS perfusing the segments. The segments were bathed in PSS, to which 10^{-6}M carbachol was added to contract the segments to approximately 50% of the maximal contraction. The forces generated by the segments in response to the carbachol were measured by the force transducers.

segments without epithelium. After the contractions to 10^{-6} M carbachol were stable, 1 MAC halothane was added to the PSS perfusing the segments intraluminally. After a stable relaxation in response to halothane (approximately 9 min for segments exposed to 1 MAC halothane and approximately 15 min for 2 MAC), the segments were perfused with PSS containing no halothane, and the contractile force was allowed to recover. The segments were then perfused with PSS containing 2 MAC halothane.

The rate of relaxation of the segments was estimated from the time necessary to reduce the force of contraction after the addition of halothane to the perfusate (time constants of relaxation) to 33% of the response to 10^{-6} M carbachol.

Determination of Halothane Concentrations

The halothane concentration in the gas phase was continuously monitored with a mass spectrometer (model 1100, Perkin Elmer). At the end of the exposure to halothane, the PSS bathing the rings was sampled in 14 studies anaerobically, and the halothane concentration was determined by gas chromatography (model 5880 A, Hewlett-Packard) using an electron capture detector.[5]

Drugs

ACh hydrochloride, HXT chloride, 5HT (creatine sulfate complex), atropine sulfate, DL-propranolol, and carbamyl choline chloride (carbachol) were purchased from Sigma. Halothane was purchased from Ayerst Laboratory, Inc. Phentolamine mesylate was kindly supplied by the Ciba-Geigy Corp.

Data Analysis

Global differences in the concentration-response curves between rings with or without epithelium and between rings exposed to 1 or 2 MAC halothane were examined by repeated-measures analysis of variance. Differences in the contractile response between segments with or without epithelium and between segments exposed to 1 or 2 MAC halothane were examined by Student's t test for paired observations. P values of <0.05 were considered statistically significant. All data are expressed as means ± standard deviation.

Results

Bronchial Rings

There were no significant differences between the mean maximal forces, forces at L_o, and weights of rings with or without epithelium that were contracted with ACh

or 5HT (table 1). However, the mean maximal force of rings stimulated with EFS was significantly smaller in rings with epithelium than in rings without epithelium ($P = 0.04$). The mean forces at L_o of rings stimulated with EFS and the weights of rings stimulated with EFS were not different in rings with or without epithelium (table 1).

Epithelium removal significantly increased the sensitivity of the bronchial rings to ACh and 5HT both in the presence of 1 or 2 MAC halothane and in the absence of halothane; *i.e.*, the concentration-response curves were significantly shifted to the left (fig. 2). The response to EFS was not significantly affected by the removal of the epithelium (fig. 2). Halothane at 1 or 2 MAC caused significant rightward shifts of the concentration-response curves to ACh (fig. 3A) and 5HT (fig. 3B) and of the frequency-response curve to EFS (fig. 3C). Epithelium removal did not alter the magnitude of the response to halothane. There were no significant differences in the degree of rightward shifts between 1 and 2 MAC halothane for rings contracted with ACh and 5HT. The concentration of halothane in the PSS was 1.1 ± 0.1 MAC (n = 6) for the target concentration of 1 MAC and 2.0 ± 0.2 (n = 8) for the target concentration of 2 MAC.

Bronchial segments

The forces generated in response to 10^{-6} M carbachol were not statistically significantly different for segments with or without epithelium (table 2). One and 2 MAC halothane caused significant reductions in forces of segments contracted with 10^{-6} M carbachol. The relaxation caused by 1 MAC halothane was not significantly different in the segments with or without epithelium (table 2). Relaxation in response to 2 MAC was significantly greater than that caused by 1 MAC both in rings with ($P < 0.02$) and without ($P < 0.025$) epithelium (table 2). However, the relaxation in response to halothane was not significantly different between segments with or without epithelium. The time constants of relaxation in response to exposure to halothane were not significantly different between segments with or without epithelium (table 2, fig. 4).

Discussion

The major finding of this study is that the ability of halothane to attenuate the response of canine bronchial smooth muscle *in vitro* to contractile agonists or to relax contracted muscle is not dependent on the airway epithelium.

This study confirms previous observations that removal of airway epithelium increases the sensitivity of canine bronchial smooth muscle to ACh and 5HT.[2,6–8] Epithelium removal also impairs the relaxation of contracted muscle in response to contractile antagonists under some conditions.[2,7] The mechanisms of these effects are unknown; the major possibilities include: 1) an EpDRF is removed; 2) the epithelium provides a physical barrier to the diffusion of agonists to the smooth muscle[2,6,7,9,10]; and (3) these possibilities, combined, cause the effects.

The identity of the EpDRF and its mechanisms of action

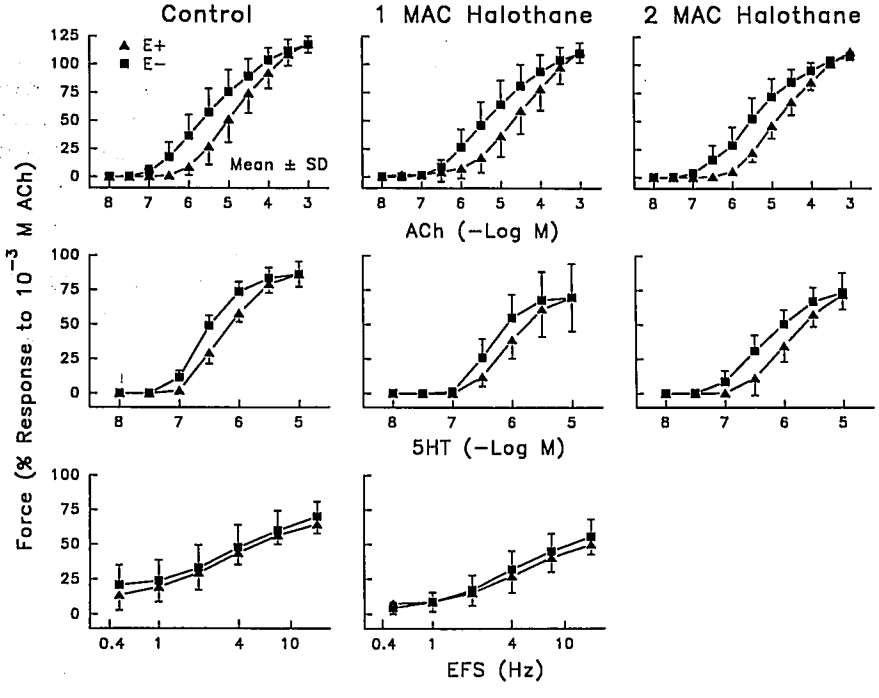

FIG. 2. *Left:* Concentration–response curves for bronchial rings contracted with acetylcholine (ACh), 5-hydroxytryptamine (5HT), or electrical field stimulation (EFS). In the absence of halothane, removal of the epithelium (E−) caused a significant ($P = 0.003$, repeated measures analysis of variance) left shift of the concentration–response curve to ACh; *i.e.*, the contractile force for a given dose of ACh was increased. Removal of the epithelium also caused a significant ($P = 0.002$) leftward shift of the concentration–response curve to 5HT. The frequency–response curve to EFS was not significantly ($P = 0.328$) affected by the removal of the epithelium. *Middle:* 1 MAC halothane did not alter the response to the removal of the epithelium. The concentration–response curves for ACh ($P = 0.021$) and 5HT ($P = 0.004$) were still shifted to the left, whereas the frequency–response curve to EFS was not affected ($P > 0.5$). *Right:* 2 MAC halothane again did not alter the response to the removal of the epithelium. The concentration–response curves for ACh ($P = 0.012$) and 5HT ($P = 0.005$) were still shifted to the left.

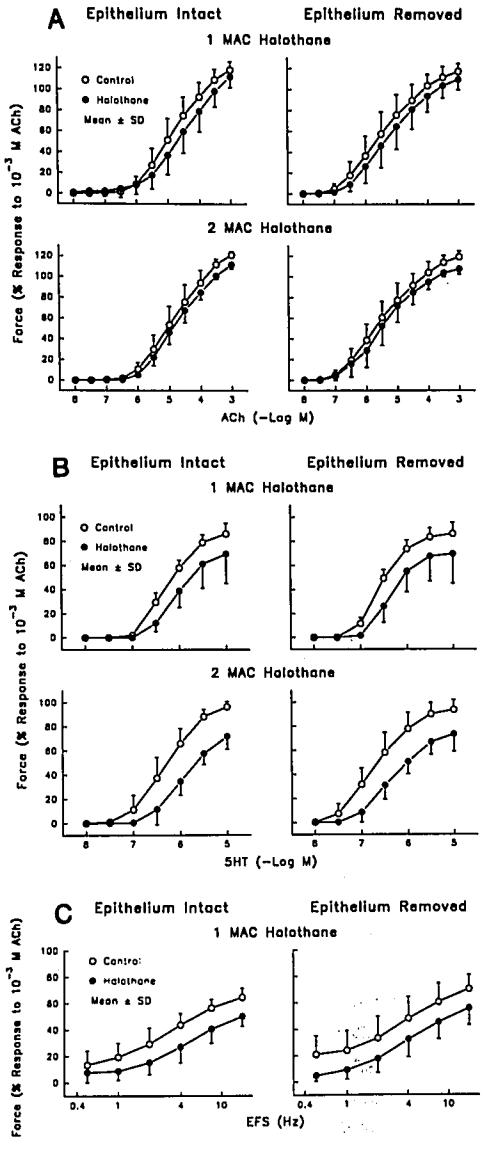

FIG. 3. *A:* Concentration–response curves for bronchial rings contracted with acetylcholine (ACh). 1 MAC (*top*) and 2 MAC (*bottom*) halothane caused significant rightward shifts of the concentration–response curves to ACh ($P = 0.023$ and 0.010, respectively, repeated-measures analysis of variance) in rings with intact epithelium. The control curves for 1 and 2 MAC are slightly different because in two dogs we could examine only 1 or 2 MAC. *Right:* 1 MAC (*top*) and 2 MAC (*bottom*) halothane also caused significant rightward shifts of the concentration–response curves to ACh in bronchial rings with the epithelium removed ($P = 0.004$ and $P = 0.024$, respectively). The data for 1 and 2 MAC were not significantly different. ($P \geq 0.7$ for rings with intact epithelium and rings with the epithelium removed). *B:* Concentration–response curves for bronchial rings contracted with 5-hydroxytryptamine (5HT). 1 MAC (*top*) and 2 MAC (*bottom*) halothane caused significant rightward shifts of the concentration–response curves to 5HT in bronchial rings with intact epithelium ($P = 0.030$ and $P = 0.002$, respectively). *Right:* 1 MAC (*top*) and 2 MAC (*bottom*) halothane caused significant rightward shifts in the concentration–response curves to 5HT in bronchial rings with the epithelium removed ($P = 0.022$ and $P = 0.017$, respectively). The data for 1 and 2 MAC were not significantly different ($P \geq 0.8$ for rings with intact epithelium and rings with the epithelium removed). *C:* Frequency–response curves for bronchial rings stimulated with electrical field stimulation (EFS). 1 MAC halothane caused a significant rightward shift of the frequency–response curve to EFS in bronchial rings with intact epithelium ($P < 0.001$) and in rings with the epithelium removed ($P = 0.011$).

are still unknown.[3] Certain pharmacologic similarities exist between the EpDRF and the endothelium-derived relaxing factor (EDRF). In blood vessels, nitric oxide has been identified as one of the EDRFs, but in airways the EpDRF is not nitric oxide.[11]

Biodegradation of agonists by the epithelium is possible, but it does not appear to be significant, at least for ACh.[2] The effect of epithelium removal is complex, depending on the preexisting tone of the smooth muscle, the size of the airway, and the agonist used to induce contraction.[6,7,12,13] There may also be considerable heterogeneity in responses among species, although all airway tissues (including human) studied to date have demonstrated epithelium-dependent effects.[9,14,15]

Consistent with previous reports,[2] the removal of the epithelium did not affect the initial contractile response to EFS. EFS releases ACh from postganglionic cholinergic nerves within the airway wall; *i.e.*, the ACh released by EFS reaches the smooth muscle without the necessity of penetrating the epithelium. Thus, it appears that the demonstration of epithelial effects requires that the contractile agonist reach the smooth muscle through the ep-

TABLE 2. Forces of Contraction of Bronchial Segments, Relaxation of Force by 1 or 2 MAC Halothane, and Time Constants of Relaxation in Response to Halothane

Halothane (MAC)	Force in Response to 10^{-6} M Carbachol (g)		Relaxation (%)		Time Constant (min)	
	E+	E−	E+	E−	E+	E−
1	5.6 ± 4.1	6.2 ± 5.6	28 ± 17†	26 ± 14‡	3.1 ± 0.5	3.1 ± 1.6
2	5.7 ± 4.3	6.1 ± 5.5	55 ± 27†	57 ± 19‡	4.0 ± 3.5	3.9 ± 1.7

Mean ± SD; n = 6 dogs.
E+ = epithelium intact; E = epithelium removed.
* 1 MAC in dogs was considered 0.87% halothane.[25]

† Significantly different between 1 and 2 MAC ($P < 0.02$).
‡ Significantly different between 1 and 2 MAC ($P < 0.025$).

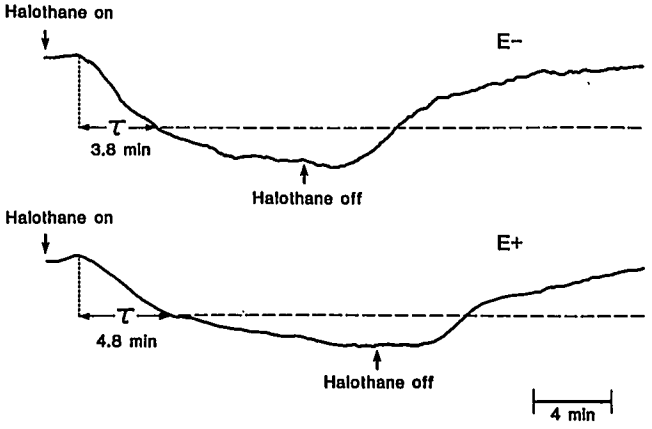

FIG. 4. Tracings of forces produced by bronchial segments without epithelium (E−) or with epithelium (E+). The segments were contracted with 10^{-6} M carbachol to approximately 50% of maximal contraction, and at the points indicated by the first vertical arrows (halothane on) both segments were perfused with PSS equilibrated with 2 MAC halothane. At the points indicated by the second vertical arrows (halothane off), both segments were perfused with PSS. The dashed horizontal line indicates the point in time at which the relaxation of the muscle had reached 66% of its maximum. Note the similar time constants of relaxation of E+ and E− segments.

ithelium *via* the airway lumen. Possible mechanisms include: the agonist stimulates the release of an EpDRF; or the epithelium presents a significant diffusion barrier to the agonist.

Halothane reduces the sensitivity of airway smooth muscle to ACh[16] and methacholine[17] *in vivo*. Halothane also reduces the sensitivity of canine trachealis muscle to stimulation by ACh and EFS *in vitro*.[1,18] Halothane had similar effects on bronchial rings in the current study. The effect of halothane was not modulated by the epithelium, since the shifts in stimulus-response curves caused by halothane were not different in rings with or without epithelium. These findings were similar during stimulation with 5HT. Therefore, the results in the bronchial rings indicate that halothane has a significant effect on the sensitivity of the smooth muscle-receptor system to both muscarinic and nonmuscarinic agonists and that this effect is independent of the epithelium.

When a drug is added to tissue baths containing bronchial rings, the drug has access to the airway smooth muscle from the serosal side, from the cut edges of the rings, and through the luminal surface. Thus, the lack of epithelial effects in the bronchial rings does not exclude the possibility that the epithelium may provide a diffusion barrier to halothane. To test for the presence of a diffusion barrier to halothane, bronchial segments perfused with PSS were studied. In this preparation, halothane can reach the smooth muscle in the segments with intact epithelium only by diffusing through the epithelium. Similar preparations have demonstrated that the epithelium can provide a significant barrier to the diffusion of contractile agonists.[10] However, these agonists are relatively large, highly charged molecules, in contrast to halothane, which is a lipid-soluble hydrocarbon. We observed that the steady-state relaxation caused by halothane was not significantly different in the segments with or without epithelium, consistent with the results in the bronchial rings. However, this finding does not exclude a diffusion barrier. Even if a diffusion barrier existed, the halothane concentration in the muscle eventually would equilibrate with the concentration in the bronchial perfusate. To estimate the effects of the epithelium on the diffusion of halothane, the time constants of relaxation in response to a step change in halothane concentration in the perfusate were calculated. The lack of a significant difference in time constants between segments with and without epithelium suggests that the epithelium does not appreciably retard the diffusion of halothane in these segments.

Consistent with previous reports regarding canine trachealis,[1,19] increasing the concentration of halothane above 1 MAC did not further depress the sensitivity of the bronchial rings to ACh. This finding also was true during stimulation with 5HT. It is noteworthy that increasing the halothane concentration from 1 to 2 MAC did produce further relaxation in the bronchial segments precontracted with carbachol. It is possible that the concentration-response relationship of halothane, like that of other antagonists of contraction,[20,21] depends on the agonist used to contract the muscle; *i.e.*, the response to halothane may be different if the muscle is contracted with ACh or with carbachol. It is also possible that the effect of halothane to inhibit the initiation of contractile force (as studied in the bronchial rings) differs from its relaxing effects when added to precontracted muscles (as studied in the bronchial segments), since the biochemical intracellular events causing force initiation and force maintenance are distinct.[22]

These findings have possible clinical significance to the extent that they are applicable to humans. Patients with pulmonary diseases such as asthma or respiratory infections may have damaged or absent airway epithelium.[4] The lack of normal EpDRF function or the removal of a diffusion barrier to contractile agonists has been implicated as a possible mechanism of airway hyperreactivity in these patients.[4] Based on our results, the absence of airway epithelium should not affect the clinically beneficial bronchodilating properties of halothane. In addition, because the epithelium does not present a diffusion barrier to halothane, halothane should diffuse from the airway lumen to the airway smooth muscle in patients with and patients without intact epithelium.

In conclusion, the relaxing effects of halothane on canine bronchial smooth muscle *in vitro* are not modulated by the airway epithelium, and the epithelium does not

provide an appreciable barrier to the diffusion of halothane from the bronchial lumen to the smooth muscle.

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References

1. Brichant J-F, Gunst SJ, Warner DO, Rehder K: Halothane, enflurane and isoflurane depress the peripheral vagal motor pathway in isolated canine tracheal smooth muscle. ANESTHESIOLOGY 74:325–332, 1991
2. Flavahan NA, Aarhus LL, Rimele TJ, Vanhoutte PM: Respiratory epithelium inhibits bronchial smooth muscle tone. J Appl Physiol 58:834–838, 1985
3. Stuart-Smith K, Vanhoutte PM: Epithelium-derived relaxing factor, Airway Smooth Muscle: Modulation of Receptors and Response. Edited by Agrawal DK, Townley RG. Boca Raton, CRC Press, 1990, pp 129–145
4. Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T: Damage of the airway epithelium and bronchial reactivity in patients with asthma. Am Rev Respir Dis 131:599–606, 1985
5. Van Dyke RA, Wood CL: Binding of radioactivity from ^{14}C-labeled halothane in isolated perfused rat livers. ANESTHESIOLOGY 38: 328–332, 1973
6. Stuart-Smith K, Vanhoutte PM: Heterogeneity in the effects of epithelium removal in the canine bronchial tree. J Appl Physiol 63:2510–2515, 1987
7. Stuart-Smith K, Vanhoutte PM: Epithelium, contractile tone, and responses to relaxing agonists in canine bronchi. J Appl Physiol 69:678–685, 1990
8. Gao Y, Vanhoutte PM: Removal of the epithelium potentiates acetylcholine in depolarizing canine bronchial smooth muscle. J Appl Physiol 65:2400–2405, 1988
9. Pavlovic D, Fournier M, Aubier M, Pariente R: Epithelial vs. serosal stimulation of tracheal muscle: Role of epithelium. J Appl Physiol 67:2522–2526, 1989
10. Munakata M, Huang I, Mitzner W, Menkes H: Protective role of epithelium in the guinea pig airway. J Appl Physiol 66:1547–1552, 1989
11. Munakata M, Masaki Y, Sakuma I, Ukita H, Otsuka Y, Homma Y, Kawakami Y: Pharmacological differentiation of epithelium-derived relaxing factor from nitric oxide. J Appl Physiol 69: 665–670, 1990
12. Stuart-Smith K, Vanhoutte PM: Airway epithelium modulates the responsiveness of porcine bronchial smooth muscle. J Appl Physiol 65:721–727, 1988
13. Lev A, Christensen GC, Ryan JP, Wang M, Kelsen SG: Epithelial modulation of trachealis muscle tension is calcium and temperature dependent. J Appl Physiol 67:713–719, 1989
14. Fernandes LB, Paterson JW, Goldie RG: Co-axial bioassay of a smooth muscle relaxant factor released from guinea-pig tracheal epithelium. Br J Pharmacol 96:117–124, 1989
15. Raeburn D, Hay DWP, Farmer SG, Fedan JS: Epithelium removal increases the reactivity of human isolated tracheal muscle to methacholine and reduces the effect of verapamil. Eur J Pharmacol 123:451–453, 1986
16. Warner DO, Vettermann J, Brichant J-F, Rehder K: Direct and neurally mediated effects of halothane on pulmonary resistance in vivo. ANESTHESIOLOGY 72:1057–1063, 1990
17. Hirshman CA, Edelstein G, Peetz S, Wayne R, Downes H: Mechanism of action of inhalational anesthesia on airways. ANESTHESIOLOGY 56:107–111, 1982
18. Korenaga S, Takeda K, Ito Y: Differential effects of halothane on airway nerves and muscle. ANESTHESIOLOGY 60:309–318, 1984
19. Shah MV, Hirshman CA: Mode of action of halothane on histamine-induced airway constriction in dogs with reactive airways. ANESTHESIOLOGY 65:170–174, 1986
20. Koenig SM, Mitchell RW, Kelly E, White SR, Leff AR, Popovich KJ: β-adrenergic relaxation of dog trachealis: Contractile agonist-specific interaction. J Appl Physiol 67:181–185, 1989
21. Russell JA: Differential inhibitory effect of isoproterenol on contractions of canine airways. J Appl Physiol 57:801–807, 1984
22. Dillon PF, Aksoy MO, Driska SP, Murphy RA: Myosin phosphorylation and the cross-bridge cycle in arterial smooth muscle. Science 211:495–497, 1981
23. Eger EI II, Brandstater B, Saidman LJ, Regan MJ, Severinghaus JW, Munson ES: Equipotent alveolar concentrations of methoxyflurane, halothane, diethyl ether, fluroxene, cyclopropane, xenon and nitrous oxide in the dog. ANESTHESIOLOGY 26:771–777, 1965