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Effects of halothane and enflurane on epithelium-dependent contraction and ion transport of canine tracheal epithelium

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Abstract: To gain insight into the cellular mechanisms involved in bronchodilation induced by inhalation anesthetics, we investigated whether halothane and enflurane can modulate functions of airway epithelium, such as epithelium-mediated bronchodilation and transepithelial transport. To measure the isometric tension of the airways, paired rings of canine bronchi (4–6 mm OD), with and without the epithelium were mounted in Krebs-Ringer solution, gassed with 95% O_2 and 5% CO_2, and isometric tension was continuously recorded. To determine transepithelial transport, the posterior membranous portion of the trachea was mounted in Ussing-type chambers and the potential difference (PD), short-circuit current (SCC), and transepithelial resistance (R) were determined.

Halothane and enflurane increased the contractile responses of the trachea to acetylcholine (ACh) in strips either with or without epithelium. However, this enhancement of the contractile responses by volatile agents was much larger with the epithelium than without. Furthermore, halothane tended to gradually increase and then decrease SCC of the trachea, but these changes were not statistically significant. These results indicate that halothane may modulate contractile response of the isolated trachea to ACh, but has no effect on ion transport by airway epithelium. The responsiveness of the trachea may be regulated independently of ion transport by airway epithelium.

Key words: Volatile anesthetics—Epithelium-derived relaxation factor (EpDRF)

Introduction

Bronchospasm, a potentially serious event that occurs in the operating room, is one of the most common and severe complications during anesthesia, especially in patients with bronchial asthma and chronic obstructive pulmonary diseases. It is widely documented that nonspecific airway hyperresponsiveness plays an essential role in bronchial asthma, and airway epithelial damage or a loss of epithelial integrity accounts for airway hyperreactivity [1].

A number of studies in a variety of mammalian species including human indicate that the overlying respiratory epithelium influences the contractile behavior of airway smooth muscle, presumably by releasing factors that inhibit smooth muscle tension. This implies that the epithelium produces an agent which promotes smooth muscle relaxation, and is referred to as an epithelium-derived relaxation factor (EpDRF) [2]. Recently, nitric oxide (NO) has been proven to be an important endothelium-derived relaxing factor (EDRF), producing profound relaxation of vascular smooth muscle [3]. Although it has been speculated that the relaxation factor of the airways is analogous to EDRF generated by vascular endothelium, the nature of EpDRF is not well understood, and it has also been shown that a reduction of EpDRF was associated with bronchial hyperreactivity [1].

Volatile anesthetics, including halothane and enflurane, have been reported to cause bronchodilation, by directly relaxing airway smooth muscles or by inhibiting bronchoconstriction mediated by endogenous neurohumoral substances [4]. However, the possible interactions of halothane or enflurane with the airway epithelium and/or the airway smooth muscle cells have not been investigated in depth because of the difficulty of evaluating these interactions. However, thanks to the development of an in vitro assay system to determine epithelial ion transport [5,6], we have previously demonstrated that various agents such as acetylcholine (ACh) and histamine can modulate ion transport via the airway epithelium [7], which plays a key role in maintaining the effective defense mechanisms in the lungs

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responsible for mucous secretion and mucociliary transport [8]. Although an active electrogenic pump is present in guinea pig and bovine airway smooth muscle [9], there is little information available about the relationship between ion transport and smooth muscle contraction including EpDRF release. Therefore, to establish the cellular mechanisms involved in inhalation anesthetic-induced bronchodilation, we have investigated whether halothane or enflurane can modulate the airway epithelial functions including epithelium-mediated bronchodilation and transepithelial transport to clarify the cellular mechanisms of bronchodilation during inhalation anesthesia.

Materials and methods

In accordance with institutional Animal Care Committee Standards, 14 mongrel dogs weighing 8–10 kg were killed with sodium pentobarbital (75 mg/kg body weight, i.v.). The trachea were excised and immediately immersed in ice-cold Ringer's solution gassed with 95% O_2-5% CO_2; bronchial segments were dissected free of surrounding tissues, and paired rings (4–5 mm long) were prepared from each segment. In one ring of each pair, care was taken not to touch the epithelial surface, and in the other rings, a cotton swab was inserted into the lumen of the bronchial rings, and the epithelial layer was removed by gently rolling the preparation back and forth. The removal of the epithelium was confirmed histologically. A pair of bronchial rings were suspended in siliconized glass organ chambers, each filled with 25 ml of a modified Krebs-Ringer solution which contained the following ingredients (mmol): NaCl 118.2, KCl4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.1. The solution was gassed with a mixture of 95% O_2 and 5% CO_2 (37°C, pH 7.40–7.49). The preparations were connected to a strain gauge (Gould UTC2), and isometric tension was continuously recorded throughout the experiment.

The rings were progressively stretched to an optimal tension of approximately 1–2 g, which was determined by repeated stimulation with KCl (2×10^{-2}M). Maximal contraction (100%) was measured after stimulation with KCl (5×10^{-2}M), and was used as a reference contraction. After washing with fresh Krebs-Ringer solution, the rings were allowed to equilibrate for 40–60 min before the experiments.

Halothane or enflurane was delivered from a calculated vaporizer to given concentrations of 2% in the 95% O_2-5% CO_2 mixture aerating the Krebs-Ringer solution. To ensure equilibration between the aerating gas carrying 2.0% halothane or enflurane and the bath solution, a high gas flow (at least 300 ml/min) was bubbled into the organ chambers to prevent the aerating gas from immediately escaping into the atmosphere, and the rings were allowed to equilibrate for 40–60 min before the experiments. A high gas flow with equilibration time allowed the concentrations of halothane or enflurane in Krebs-Ringer solution to be maintained, which were confirmed by gas chromatography [10].

Drugs

Acetylcholine chloride (Sigma Chemical, St. Louis, MO, USA), halothane (Takeda Pharma-ceutical Osaka), enflurane (Dainabot, Osaka). The drugs were added to the bath in volumes of 100 µl or less.

Experimental protocols

Two protocols were employed for these experiments. In the first, the effects of exogenous agonists on contraction of the trachea were investigated with and without epithelium. Concentration-effect curves to exogenous agonists were constructed by increasing organ chamber concentrations cumulatively by half-log increments. Concentrations of agonists were not increased until the preceding contraction (or relaxation) was complete. In the second part of the experiment, the effects of halothane or enflurane were evidenced on the ACh-induced concentration of the tracheal rings with and without epithelium. This experiment consisted of two consecutive periods: the first one was a pretreatment period during which tracheal rings with and without epithelium were investigated with increased concentrations of ACh, and the second one was an experimental period in which the effects of halothane or enflurane on this dose-related concentration was examined. Once the concentration reached a plateau, ACh was washed out from the bath and then halothane or enflurane was added to the mixture by gassing the bath solution. The rings were allowed to equilibrate for at least 60 min after induction of halothane or enflurane, and were again exposed to increasing concentrations of Ach.

Ion transport

As previously described [5–7], the posterior membranous portion of the trachea was mounted in Ussing chambers having an exposed surface area of 1.0 cm^2, and PD, SCC, and R were determined. Briefly, PD was measured using 3 M KCl/agar bridges connected via saturated KCl solution to calomel half cell electrodes. Currents were applied using 150 mM NaCl/agar bridges connected via 0.9% NaCl solution to silver-silver chloride wires. SCC was determined by voltage clamping the tissues to 0.0 mV and measured the applied, whereas R was calculated from the change in PD pro-

duced by passing a bipolar current pulse of 10 μA/cm² of 1-s duration. An automatic voltage-current clamp (Bioengineering, University of Iowa, Iowa City, IA, USA) was used for voltage clamping and for current pulse generation. All measurements were corrected for minimal electrode PD offsets and fluid resistance (<10 Ω·cm²), and precautions were taken to minimize edge damage in these preparations.

To confirm that the epithelia used were stable and viable, tissues with the following properties were discarded: resistance less than 100 Ω·cm², or unstable bioelectric parameters during the first 20 min of baseline observation. Halothane or enflurane was added to the gas mixture after PD and R were stabilized for 20–30 min; however, PD was continuously recorded during the desired period except for a brief interruption for measurements of R and SCC.

Statistical analysis

The results are expressed as means ± SE. Statistical evaluation of the data was by Student's *t*-test for paired or unpaired observations. When $P < 0.05$, means were considered to be significantly different.

Results

Histological examinations

After the experiments were completed, specimens obtained were fixed in 10% formaldehyde and were examined by light microscopy. Figure 1 shows histological findings of bronchial epithelium, including successful removal of the epithelium.

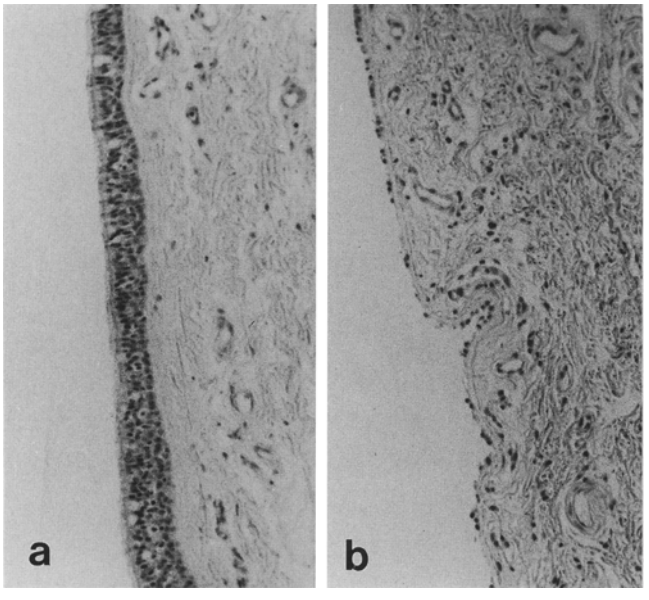

Fig. 1a,b. Histological section demonstrating that gentle mechanical rubbing of luminal surface of canine trachea removes epithelium without damaging the underlying structure. **a** With intact epithelium, **b** without epithelium (H & E, ×200)

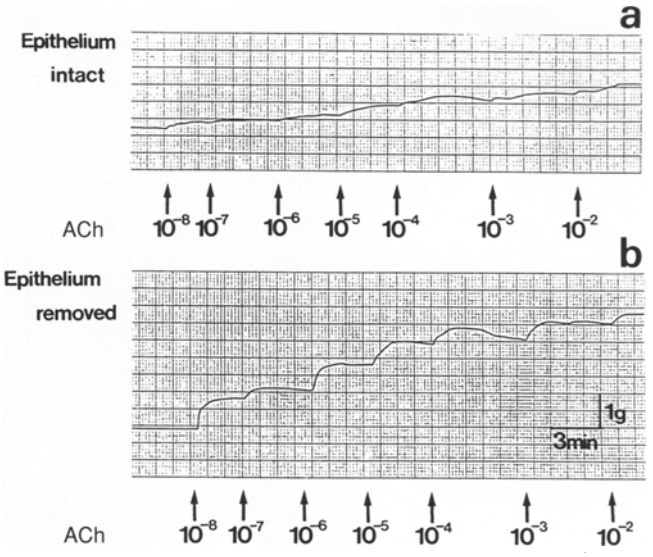

Fig. 2a, b. Cumulative contraction-response relationships to acetylcholine (ACh) in the trachea **a** with epithelium or **b** without epithelium

Effect of epithelium on contractile responses of the trachea to Ach

By using ACh in concentrations ranging from 10^{-8} to 10^{-2} M, cumulative concentration-response relationships in the trachea with or without epithelium were obtained (Fig. 2). In strips without epithelium, the ACh-induced tension was greater than in strips with epithelium intact. By challenging with ACh 10^{-2} M, a concentration which produces maximal contraction, the induced contraction rates in strips with and without epithelium were 74 ± 8% and 140 ± 12%, respectively, compared with those to 50 mM KCl (Fig. 3). Similarly, the reduction of tracheal responses by histamine was also observed when epithelium remained intact (data not shown).

Effect of halothane on contractile responses of the trachea to ACh

Paired strips which have similar contractile responses to 50 mM KCl were employed to compare the effects of halothane on ACh-induced contraction of the trachea. After washing, one strip of each pair was exposed to halothane, while the other ring was free from exposure.

Subsequently, the contractile responses of paired strips to ACh were investigated. Figure 4 illustrates representative tracings of effects of halothane on this ACh-induced contraction and findings on intact epithe-

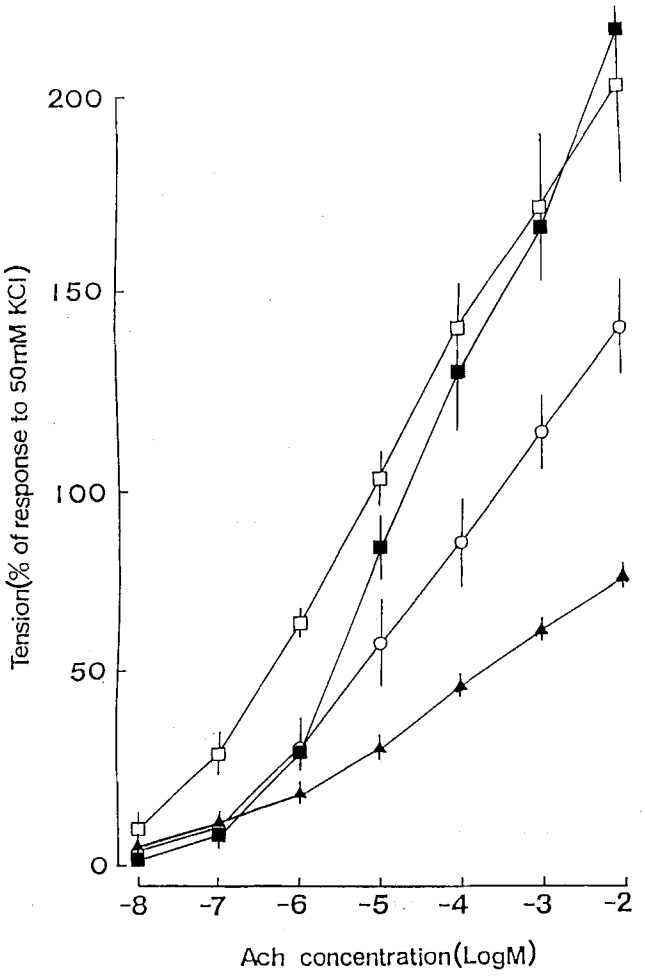

Fig. 3. Effects of epithelium removal and halothane on responsiveness of canine trachea to ACh. *Closed triangles*, rings with epithelium; *open circles*, rings without epithelium; *open squares*, rings with epithelium and halothane; *closed squares*, rings without epithelium and halothane. Values are means ± SE

lium. Halothane increased the contractile responses of the trachea compared with the control (Fig. 3). In the strips with epithelium, maximal tension observed in intact control strips was 74 ± 8% of the potassium-induced referecne contraction while maximal tension in halothane-treated strips was 195 ± 25%. Comparison of integrated areas under dose-response curves demonstrated that halothane treatment significantly enhanced tension generation ($p < 0.05$, Fig. 3). In strips without epithelium, maximal tension in control and halothane-treated strips were 140 ± 12% and 203 ± 24%, respectively (Fig. 3). Comparison of the areas under the curves exhibited that halothane enhanced contraction despite the absence of epithelium (Fig. 3). Similar results were obtained from experiments with enflurane (Fig. 5).

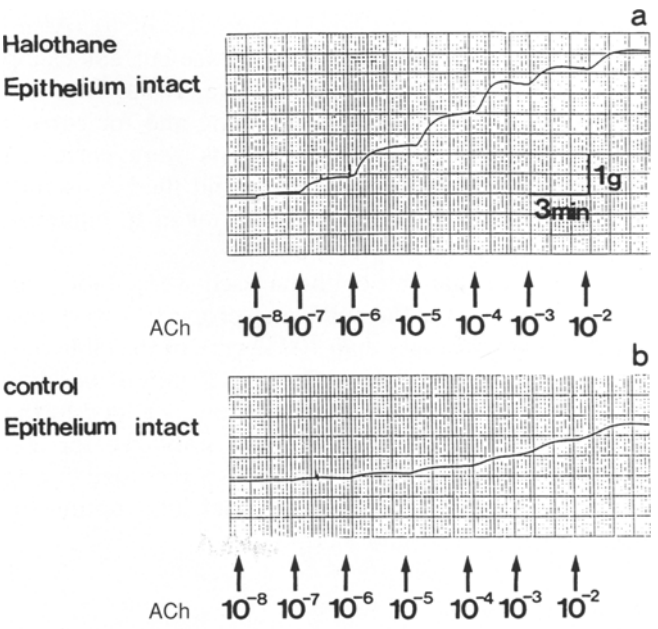

Fig. 4. Representative tracing of effect of halothane on responsiveness of canine trachea with epithelium to ACh. Note the similarity in changes with those in tracheal rings without epithelium (Fig. 2)

Effect of halothane on ion transport by airway epithelium

The bioelectric parameters of airway epithelium were stabilized within 20–30 min after the airway epithelium was mounted. The mean values and standard errors of PD, R and SCC were −6.3 ± 4.2 mV (mucosal surface negative to submucosa), 170 ± 29 Ω·cm² and 41 ± 17 μA/cm², respectively. Figure 6 shows the effects of halothane on SCC, which are thought to be well correlated with the activity of the Na pump in the cell. The addition of halothane gradually increased SCC from an initial value of 41 ± 17 μA/cm² to a peak of 68 ± 18 μA/cm² (means ± SE, $n = 4$) at 60 min, and then progressively decreased the SCC to 28 ± 15 μA/cm² ($P < 0.05$, Fig. 6). This stimulation of SCC was associated with a similar response (an initial increase and then a subsequent decrease) of PD in magnitude and time course (data not shown).

However, these changes of SCC and PD by halothane were not statistically significant. The transepithelial resistance failed to change perceptively during the experiments.

Discussion

The present study demonstrates that gentle mechanical removal of the airway epithelium increases the tension generated by the airway smooth muscle in response to

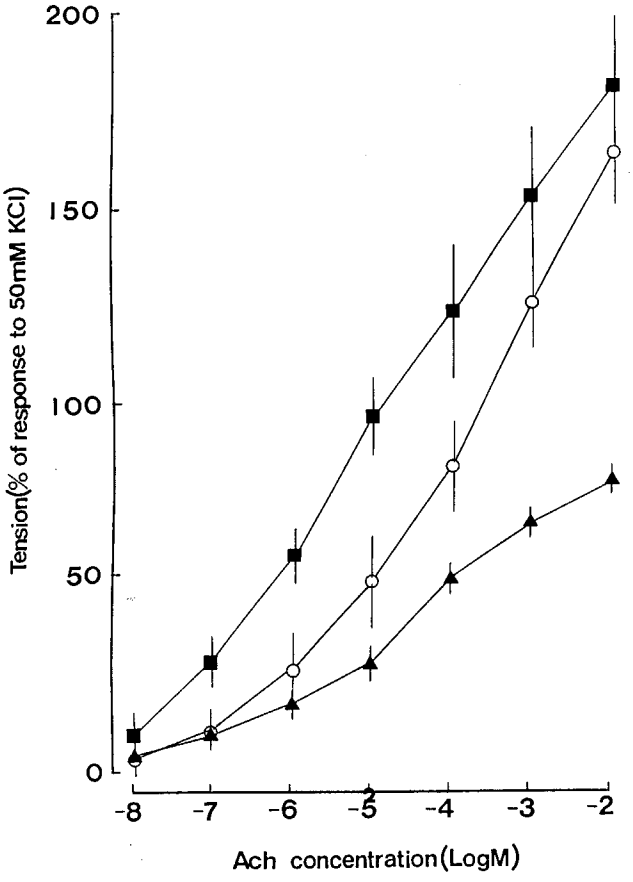

Fig. 5. Effects of enflurane and epithelium removal on responsiveness of canine trachea to ACh. *Closed triangles*, rings with epithelium; *open circles*, rings without epithelium and enflurane; *closed squares*, rings with epithelium and enflurane. Values are means ± SE

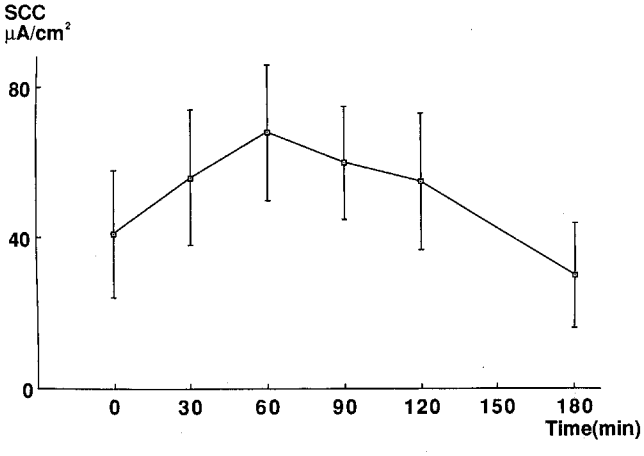

Fig. 6. The time-course changes in short circuit current (*SCC*) of canine tracheal epithelium by halothane. Values are means ± SE These changes were not statistically significant

ACh, and the potent inhalation anesthetics, halothane or enflurane, enhanced contractile responses of the airway smooth muscle to ACh both with and without epithelium. Furthermore, halothane initially tended to increase SCC and then decrease it gradually, but did not affect the SCC of airway epithelium significantly when its bioelectric properties were measured using Ussing-type chambers.

Airway epithelium of several species including the dog has been recently reported to release EpDRF which reduces the contractile responses of airway smooth muscle [2]. According to one plausible hypothesis regarding airway hyperresponsiveness in asthmatic subjects, it is indicated that the loss of EpDRF in the airways of asthmatic patients enhances the responses to inhaled bronchoconstricting agents including ACh and histamine [1, 11].

Potent inhalational anesthetics are knwon to dilate preconstricted areas in the airways. Halothane, enflurane, and isoflurane attenuate antigen-induced bronchoconstriction in the sensitized canine airway [4, 12]. However, little information is available about the direct effect of inhalational anesthetics on the airway epithelium.

In the present study, halothane or enflurane enhanced the contractile responses of the airway smooth muscle to ACh. These responses were consistent with some in vitro findings [13, 14], but not consistent with the concept that halothane clinically exerts a dilating effect on airway smooth muscle. Although the mechanisms involved in halothane-induced bronchodilation are known to include the blockade airway reflexes, directly relaxing airway smooth muscle, inhibiting mediator release, and enhancing β-adrenergic tone [12, 15], this discrepancy may be ascribable to the excited trachea independent of control of central nerve system, giving rise to totally different clinical features.

Another possibility is that halothane might inhibit the airway epithelial function and inhibit the release of EpDRF, eventually increasing the contractile response of the airway smooth muslce in vitro. In this study, halothane and enflurane enhanced the contractile response to ACh both with and without epithelium, but this augmentation was much larger with the epithelium than without (Figs. 3, 4). Therefore, to determine whether halothane would inhibit functions of airway epithelium, we investigated the effects of halothane on other functions of airway epithelium, namely ion transport. Although halothane has a tendency to initially increase SCC, which appears to be correlated well with the activity of Na pump in the cell, and to subsequently decrease SCC, these changes were not statistically significant even though measurement of contractile response of airway smooth muscle was initiated 40–

60 min after exposure to volatile anesthetics to ensure equilibration between the aerating gas and the organ chambers. To our knowledge, this is the first report about effects of halothane on ion transport by airway epithelium. However, contractile response of airway smooth muscle appears to be regulated independently of ion transport by airway epithelium, although there is evidence that a sodium electrogenic pump is present in airway smooth muscle [9]. Very recently, Nijkamp et al. [16] demonstrated that NO synthesis inhibitors induce airway hyperresponsiveness in the guinea pig in vivo and in vitro. Therefore, further study, especially of NO synthesis of airway epithelium by halothane, remains to be conducted to confirm whether this cellular mechanism is responsible for bronchodilation during inhalation anesthesia.

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