Differential Effects of Halothane on Airway Nerves and Muscle

Susumu Korenaga, M.D.,* Kazuo Takeda, M.D.,* Yushi Ito, Ph.D.†

Effects of halothane on the excitation-contraction coupling or neuro-effector transmission in the dog tracheal muscle were observed in vitro in an attempt to clarify the cellular mechanisms involved in anesthetic-induced bronchodilation.

Double sucrose gap, microelectrode, and tension recording methods were used. Application of halothane evoked an initial induction of phasic contraction with no alteration in the electrical membrane properties, and secondarily a reduction in muscle tone with membrane hyperpolarization.

Halothane suppressed the amplitude of the twitch contractions evoked by indirect (nerve mediated) or direct muscle stimulation, the degree of suppression being greater with the former stimulation. The threshold membrane depolarization required for the generation of tension development was increased. In the presence or absence of TES, halothane completely suppressed the generation of an action potential or a local response in the muscle membrane, following stimulation by outward current pulses.

Therefore, halothane has complex actions on Ca++ economy in the tracheal smooth muscle cell, i.e., initial release of Ca++ from the store sites followed by inactivation or a reduction in free calcium ions in the cytoplasm, and/or suppression of the influx of Ca++ across the cell membrane.

Low concentrations of halothane (≤1%) suppressed the amplitude of excitatory junction potential (EJP) without altering the membrane potential, membrane resistance, or muscle sensitivity to acetylcholine. Therefore, this anesthetic probably suppresses the release of transmitter from the nerve terminals. Halothane also suppressed the facilitation phenomena of EJP during repetitive nerve stimulation.

These direct inhibitory effects of halothane on smooth muscle cells and excitatory neuro-effector transmission could account for the potent bronchodilator action of this anesthetic. (Key words: Airway. Anesthetics, volatile: halothane. Muscle: smooth: trachea.)

INHALATION ANESTHETICS such as halothane and di-ethylether prevent and reverse bronchospasm.1 Halothane is effective for treating acute attacks of bronchial asthma severe enough to induce loss of consciousness.2–4 These effects of halothane may be indirect by blocking airway reflexes and/or direct by relaxing the smooth muscle of the airway.2–4 Although halothane is known to block a wide variety of airway reflexes,6–7 relatively little is known of the direct effects of halothane on airway smooth muscle cells.

The electrical membrane properties of dog tracheal smooth muscle cells and neuro-effector transmission have been studied in detail.8–10 To understand the cellular mechanisms involved in anesthetic-induced bronchodilatation, we studied the effects of halothane on excitation-contraction coupling in smooth muscle cells of the dog trachea and, for comparison, we studied the effects of halothane on excitatory neuro-effector transmission.

Materials and Methods

Adult, mongrel dogs of either sex, weighing 13–15 kg, were anesthetized with 30 mg/kg pentobarbital intravenously. Segments of the cervical trachea were excised, and a dorsal strip of transversely running smooth muscle fiber was separated from the cartilage. The mucosa and adventitial areolar tissue were removed carefully. The preparation was then bathed in a modified Krebs' solution of the following ionic concentration (mm): Na+ 137.4, K+ 5.9, Mg++ 1.2, Ca++ 2.5, Cl- 134.0, H₂PO₄⁻ 15.5, and glucose 11.5. The solution was aerated with 97% O₂ and 3% CO₂ to adjust the pH to a range between 7.2–7.3 and to supply the tissue with oxygen.

Intracellular recording of the membrane potential from single cells, thin strips of tissue about 15–20 mm long, 4–5 mm wide, and 0.3–0.4 mm thick were used, and conventional microelectrodes filled with 3 M KCl were inserted from the outer surface of the preparation. To record excitatory junction potentials (EJPs), that is the phasic membrane depolarization of the smooth muscle cells generated by acetylcholine released from the terminals of the vagus nerve, field stimulation of short duration (50 µs) was applied to stimulate the nervous elements (not the smooth muscle cells) in the muscle tissue, by means of a silver needle electrode coated except for the tip (diameter 0.3 mm) with Araldite (CIBA Ltd.), and an indifferent electrode was placed at a distance of 0.5–1.0 cm from the tissue. The chamber for the microelectrode method had a volume of 2 ml, and was perfused at a rate of 3 ml/min at a temperature 35–36°C.

Generally, in smooth muscle, electrical measurements with intracellular microelectrodes are rather difficult, owing to small size of the muscle fibers, particularly if it is necessary to continuously observe changes produced.
by drugs. Therefore, the sucrose-gap method, which utilizes external electrodes, is widely employed for physiologic and pharmacologic studies, recording changes in the electrical and mechanical properties of smooth muscles simultaneously (see for example Coburn et al. 1975). The development of the double sucrose-gap method made feasible direct stimulation of the smooth muscle cells by passing current through the muscle membrane, or stimulation of the nervous elements in the muscle tissue. Thus, in the present experiments the double sucrose-gap method was used to record the membrane potential and tension development simultaneously in response to the direct (muscle) or indirect (nerve) stimulations. For this purpose, the tracheal smooth muscle tissues were cut at a width of 2.0–2.5 mm and a length of about 20 mm. The chamber in which the preparation was mounted had been described in detail elsewhere. 19 Field stimulation was applied using a pair of electrodes in the center pool of the ap-
paratus, placed in such a manner that a current pulse would pass transversely across the tissue. To produce neurogenic responses, single and repetitive stimulation was applied, using a current pulse of 50–100 μs in duration and about 10–30 V in strength. To observe the depolarization-contraction coupling of the smooth muscle cells, outward current pulses of 3-s duration and 1 to 3 V in strength were applied to the tissue.

Halothane was vaporized into a gas mixture of 97% oxygen and 3% carbon dioxide using Fluotec 3 (Cyprane) to eliminate thymol which is present in the liquid halothane as a stabilizer. The perfusing solution then was bubbled with this gas mixture containing halothane. Throughout the experiments, the concentration of halothane in this gas mixture was monitored by a gas sensor for halothane (Engstrom Emma; Engstrom Medical AB), and the concentration expressed as a volume per cent. The concentrations of halothane in the perfusing solution, when bubbled with 1, 2, 3, and 4% halothane, were 250, 560, 770, and 960 μg/ml, respectively, as measured by gas chromatography using a flame ionization detector. The obtained values (membrane potential, amplitude of contraction, and EJPs) were expressed as means ± SD, and analyzed for significance using Student’s paired t test. N represents the number of trials or samples, prepared from several (four to seven) dogs.

Results

Effects of Halothane on Mechanical Properties of Dog Trachea

Figure 1 shows an example of the effects of halothane (1–3%) on the twitch-type contraction of the dog trachea evoked by nerve stimulation with a short duration (0.5 ms). The amplitude of the twitch contraction increased in proportion to the number of stimuli at a constant stimulus intensity and frequency (20 Hz). These contractions were suppressed completely by pretreatment with either $2 \times 10^{-7}$ M tetrodotoxin (a nerve poison) or $5 \times 10^{-6}$ M atropine, indicating that the twitch responses are caused by stimulation of cholinergic nerves, as reported previously.

Application of halothane evoked a transient increase in the muscle tone and reduced the resting tension and amplitude of twitch contractions in a dose-dependent manner (fig. 1A and B).

The amplitude of the halothane-induced transient contraction was dose-dependent, and the mean values (±SD) were 0.03 ± 0.01 (n = 5) and 0.32 ± 0.05 (n = 4) of the amplitude of the twitch contraction evoked by 25 stimuli at 20 Hz, when 1% and 3% halothane were applied, respectively. After application of atropine ($5 \times 10^{-6}$ M) (fig. 1C) or tetrodotoxin ($2 \times 10^{-7}$ M) (data not shown), the halothane-induced contraction was still apparent, thus indicating that the response resulted from a direct action of halothane on the smooth muscle cells. After pretreatment of the muscle tissue with solution containing 0 mM Ca and 0.5 mM EGTA (calcium chelator; estimated free Ca is less than $10^{-7}$ M), halothane still evoked a phasic contraction, indicating that halothane releases the stored Ca++ in the muscle cells. However, on repetitive application of halothane in the presence of EGTA, the amplitude of the contraction was reduced gradually and the phasic tension disappeared after several applications (fig. 1D). After rinsing the tissue with normal Krebs solution with 2.5 mM Ca, there was a development of phasic tension in response to application of halothane (fig. 1E).

Figure 2 shows the relationship between the amplitude of twitch tension and the number of stimuli used to evoke twitch contractions in the presence or absence of halothane, where the amplitude of twitch tension evoked by 90 stimuli at 20 Hz in normal Krebs solution was registered as a relative contraction of 1.0.

Halothane suppressed the twitch tension dose-dependently, at any given stimulus condition, and at 4%, halothane completely suppressed the twitch contraction when fewer than 20 repetitive stimuli were given. The amplitude of the twitch contraction evoked by 90 stimuli at 20 Hz was reduced to 61, 34, 21, and 5% of the control.
value, in the presence of 1, 2, 3, and 4% halothane, respectively.

When the same experiments were repeated in the presence of propranolol (10^{-6} M), halothane reduced the twitch contraction to the same extent seen in the normal Krebs solution.

**Effects of Halothane on Membrane and Mechanical Properties**

Halothane, in a concentration of 1%, had no effect on the resting membrane potential of the smooth muscle cell, i.e., the mean resting membrane potential in Krebs solution was \(-57.7 \pm 1.6\) mV (±SD, n = 40), whereas it was \(-58.8 \pm 2.5\) mV (±SD, n = 20, \(P > 0.01\)) in the presence of 1% halothane. On the other hand, in increased concentrations (72%), halothane significantly hyperpolarized the membrane to \(-60.1 \pm 3.1\) mV (n = 20), \(-62.4 \pm 2.0\) mV (n = 20), and \(-63.8 \pm 2.4\) mV (n = 20) in the presence of 2, 3, and 4% halothane, respectively (\(P < 0.01\)).

To assess the effects of halothane on the membrane and mechanical properties of the smooth muscle cells, we used the double sucrose-gap method. Halothane (1%) evoked phasic contraction without significantly affecting the resting membrane potential or input membrane resistance, as measured from the amplitude of electrotonic potentials induced by inward current pulses (3 s in duration). However, with increased concentrations (≥2%), halothane first evoked the phasic contraction, then hyperpolarized the membrane and reduced the input resistance. Figure 3 shows examples of the effects of halothane (4%) on the electrical and mechanical properties of the smooth muscle. Application of halothane evoked a gradual increase in tension, without changing the membrane potential or input resistance of the membrane. After the tension development had reached a peak level, there was a gradual decrease in the muscle tone to below the base line. There was a concomitant and gradual membrane hyperpolarization followed by a reduction in the input resistance of the membrane. The relative reductions in the input membrane resistance observed by application of 2, 3, and 4% halothane were 7 ± 3\% (n = 5), 14 ± 5\% (n = 5), and 21 ± 6\% (n = 5) of the control value, respectively.

To assess the effects of halothane on the mechanical properties of tracheal smooth muscle cells, we observed the relationship between membrane depolarization evoked by outward current pulses (3 s in duration) and the amplitude of the evoked contraction. As previously reported,14 outward current pulses produced no regenerative action potentials. However, a contraction was evoked when the depolarization exceeded 7 mV. The amplitude of the contraction induced by membrane depolarization evoked by outward current pulses increased in proportion to the applied current intensity (fig. 4A).
Fig. 4. Depolarization-contraction relationship observed in the presence or absence of halothane (4%), using four to six dogs. In A and B, upper trace shows the given outward current, median trace the membrane potential, and the lower trace the tension development. (A): control; (B): in the presence of halothane (4%); and (C): graphical presentation of the depolarization-contraction relationship observed in the presence or absence of halothane, where the relative amplitude of contraction recorded by 30 mV depolarization in Krebs solution was taken as 1.0. Each point is the mean value of several experiments, and the vertical bars indicate 2 X SD. (O): control; (●): 1% halothane; (○): 2% halothane; (X): 3% halothane; and (■): 4% halothane.

Figures 4A and B shows the effects of halothane (4%) on the depolarization-induced contraction. Halothane suppressed the contraction at any given depolarization of the membrane produced by application of the outward current pulse, and raised the threshold membrane depolarization required to produce the contraction. Figure 4C represents the relationship between the membrane depolarization and contraction in the presence or absence of halothane (1–4%), where the amplitude of the contraction evoked by 30 mV depolarization in Krebs solution
was registered as a relative contraction of 1.0. In the presence of halothane (1%), the resting membrane potential remained unaffected. However, the minimum depolarization required to produce tension development was increased from about 7 mV to about 10 mV. The amplitude of contraction evoked by 30 mV depolarization was reduced to 87, 77, 65, and 56% of the control value in the presence of 1, 2, 3, and 4% halothane, respectively. This means that halothane more effectively suppressed the tension development of the tracheal tissue when indirect (nerve-mediated) muscle stimulation was given (see for example fig. 2).

In the dog trachea, there are no spontaneous action potentials and it is almost impossible, in normal Krebs solution, to elicit action potentials by electrical stimulation. This is largely caused by strong rectifying properties of the cell membrane.\(^8\) The application of tetrathylammonium (TEA) was found to suppress the rectifying properties of the membrane and to evoke action potentials (Ca\(^{++}\) spikes) in response to outward-current pulses.\(^8,14\)

Since as shown in figure 4B, halothane suppressed the local response evoked by strong outward-current pulses, it was of interest to observe the effects of halothane on the action-potentials evoked in the presence of TEA.

In the presence of 5 mM TEA, action potentials with large phasic tension development (about 10 times larger than the evoked contraction by 30 mV depolarization in Krebs solution) were evoked when the membrane depolarization exceeded 10 mV, thus indicating that Ca\(^{++}\) enters the cell during this action potential. As shown in figure 5, halothane (3%) completely suppressed the action potential and reduced the amplitude of the evoked tension development to less than 50% of the value observed in the presence of TEA alone.

These results indicate that halothane suppressed the Ca\(^{++}\) influx across the cell membrane during generation of an action potential in the presence of TEA.

**Effects of Halothane on EJP**

To further assess the mechanisms involved in the inhibitory effect of halothane on the twitch contraction evoked by indirect (nerve-mediated) muscle stimulation, we examined the effects of halothane on excitatory neuro-effector transmission in the dog trachea in the presence of propranolol (10\(^{-6}\) M). Here, we used the double sucrose-gap method to record the excitatory junction potentials (EJPs) followed by development of phasic tension. To obtain EJPs with a constant amplitude for a given stimulus condition, indomethacin (10\(^{-5}\) M) and propranolol (10\(^{-6}\) M) were used throughout. Propranolol blocks adrenergic inhibitory nerves\(^8\) and endogenous and exogenous catecholamines that activate beta-receptors in the nerve terminal of the vagus.\(^14,15\) Indomethacin blocks
production of endogenous prostaglandins E series, which play an important role in feedback inhibitory mechanisms at the cholinergic nerve terminal related to acetylcholine release.

As shown in Figure 6, field stimulation (50 μs in duration) given through electrodes in the center pool of the double sucrose-gap apparatus, produced EJP followed by phasic contraction, and halothane (1–4%) reduced the amplitude of the EJP and phasic contraction. In the presence of 1% halothane, the amplitude of the EJP was reduced to 71% ± 5% (n = 6) of the control value with no significant changes in the membrane potential and input membrane resistance. Figure 7 shows the relationship between the concentration of halothane and changes in amplitude of EJP and in the membrane resistance measured with the double sucrose-gap method.

When the amplitude of the EJP was suppressed by a low concentration of halothane (1%), there was no change in the membrane potential, as measured by microelectrodes and in the membrane resistance of the smooth muscle cells measured with the double sucrose-gap method. Therefore, the effects of halothane (1%) on the sensitivity of the postsynaptic muscle membrane to acetylcholine were also observed. Here, we compared the amplitude of the acetylcholine-induced (10−5 M) contraction before and during application of halothane. The amplitude of the contracture in the presence of halothane was 93 ± 10% (±SD, n = 4) of the control value, albeit not statistically significant (P > 0.05). This would suggest that the chemosensitivity of the postsynaptic smooth muscle cell is not affected by treatment with a low concentration of halothane, and that the reduction in the amplitude of the EJP, as induced by halothane, is caused by the presynaptic action of this drug.

The effects of halothane on the facilitation phenomena of EJP during repetitive nerve stimulation at a high frequency (20 Hz) also were observed. Figure 8 shows the relationship between the number of stimuli at 20 Hz and the amplitude of EJPs in the presence and absence of halothane. Here, the amplitude of the EJP evoked by a single stimulus in the absence of halothane was defined as 1.0. With application of several stimuli at 20 Hz, there was a linear relationship between the number of stimuli and amplitude of EJPs, and the slope of the straight line was 1.8 in normal Krebs solution, thus indicating the presence of facilitation, as already reported. Halothane suppressed the slope of the straight line to 0.6 and 0.3 in concentrations of 3 and 4%. Therefore, the facilitation was suppressed by application of halothane.

**Effects of Halothane on the Contracture Evoked by 5-Hydroxytryptamine**

To observe the effects of halothane on the contracture of tracheal smooth muscle, 5-hydroxytryptamine (5-HT)
was used. In following experiments, muscles were prepared from three to five dogs.

5-HT \((5 \times 10^{-7} \text{ M})\) in the presence of atropine \((10^{-6} \text{ M})\) evoked muscle contraction with a steady increase in tone without fluctuations (fig. 9). Application of halothane \((1-4\%)\) during the tonic contraction produced a relaxation of the contracture, preceded at higher concentrations by an initial phasic contraction.

The amplitude of both the initial contraction or relaxation was dependent on the concentration of halothane. For example, in the presence of 1, 2, and 3\% halothane, the muscle tone was reduced to 80 ± 5\% \((n = 3)\), 27 ± 6\% \((n = 4)\) and 10 ± 4\% \((n = 4)\) of the maximum contraction induced by 5-HT, and at a concentration of 4\%, halothane completely suppressed the 5-HT contracture (fig. 9). This action of halothane was reversible.

To observe changes in the membrane potential and input resistance of the membrane during the relaxation induced by halothane, double sucrose-gap methods were used. As shown in figure 10A, 5-HT \((5 \times 10^{-5} \text{ M})\) depolarized the membrane \((about 20 \text{ mV})\) and evoked a contracture. During the well-maintained membrane depolarization and contracture evoked by 5-HT, application of halothane induced a hyperpolarization of the membrane and a gradual reduction in the muscle tone. A reduction in the membrane resistance, measured from the amplitude of the electrotonic potential, was observed during this membrane hyperpolarization in figure 10B. To confirm the above results, the membrane potential of the smooth muscle cell was measured using microelectrodes. 5-HT depolarized the membrane from \(-60.2 \pm 1.97 \text{ mV} \ (n = 20)\) to \(-44.7 \pm 2.32 \text{ mV} \ (n = 20)\), and application of 4\% halothane hyperpolarized the membrane to \(-57.2 \pm 2.53 \text{ mV} \ (n = 20)\).

**Discussion**

The present results indicate that mechanisms of action of halothane on the airway muscle tissue are complex and involve depression of excitatory neuro-effector transmission as well as direct action on airway smooth muscle cells.

We found that halothane significantly inhibited the development of twitch tension induced by nerve stimulation, to a greater extent than seen with direct muscle stimulation. Halothane \((1\%)\) suppressed the amplitude of EJP, without significantly altering the resting membrane potential or input membrane resistance. Furthermore, the amplitude of the contracture evoked by exogenous acetylcholine was little affected by low concentrations \(<1\%)\) of halothane, thus indicating that the sensitivity of the smooth muscle cells to acetylcholine was little affected, as previously reported.\(^2\) Halothane also suppressed facilitation phenomena during repetitive nerve stimulation, at a high frequency. All these results indicate that halothane reduced the amount of acetylcholine released from the nerve terminals in response to nerve stimulation and there was a reduction in the amplitude of EJPs and twitch contractions. This means that halothane suppresses the final step of the airway reflexes.

Furthermore, halothane may affect tracheobronchial smooth muscle by influencing affenter receptors and the nervous system, central regulatory mechanisms, and autonomic ganglia.\(^5-7\) Thus, the major bronchodilator action of halothane evoked by clinically useful concentrations may be mediated mainly by the depression of airway reflexes.

However, the present results also show that a low concentration of halothane \((1-2\%)\) suppressed the contraction evoked by membrane depolarization produced by application of outward current pulses (direct muscle stimulation), and raised the threshold membrane depolarization required to produce the contraction. These findings indicate that halothane directly suppresses the excitation-contraction coupling in airway smooth muscle cells.

Generally, in smooth muscle cells, the contraction-re-
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Fig. 9. Effects of halothane (1–4%) on the mechanical properties of the dog trachea after pretreatment with 5-hydroxytryptamine (5 × 10⁻⁷ M) and atropine (10⁻⁶ M) applied at the open circles. The filled circles indicate the application and removal of halothane. The triangles indicate the application of 20 repetitive field stimuli at 20 Hz. Lower trace in each shows the resting tension level.

The relaxation cycle is largely dependent on the regulation of ionized free calcium in the cytoplasm, and sources of Ca²⁺ contributing to the activation of contractile proteins are of extra- and intracellular origins. In various smooth muscles, action potentials can be elicited spontaneously or in response to direct or indirect (nerve-mediated) mus-

Fig. 10. Effects of halothane on the electrical and mechanical properties of the dog tracheal muscle during the contracture evoked by 5-HT (5 × 10⁻⁵ M) using the double sucrose-gap method. Open circles indicate application of 5-HT and filled circles the application or removal of halothane. A and B are records from the same preparation.
icle stimulations, and action potentials are thought to be caused by a rapid influx of Ca\(^{++}\) from the extracellular space into the cytoplasm. In the dog trachea, there are no spontaneous action potentials, hence it is impossible to elicit action potentials in physiologic saline by direct or indirect muscle stimulation. However, membrane depolarization of the smooth muscle cells produced by acetylcholine released from the vagus nerve terminal or by outward current pulses increase membrane permeability of Ca\(^{++}\) or induce the mobilization of stored Ca\(^{++}\) in the muscle cells, which in turn results in contractions of the muscle cells. In the presence of TEA, depolarizing current pulses lead to generation of action potentials with characteristics of the Ca spike (Ca-inward current). 14 Thus, TEA greatly enhances the contractile response of the dog tracheal muscle.

Since halothane abolished the local response evoked by strong outward current pulses and completely suppressed the action potential in the presence of TEA, halothane must suppress voltage-dependent inward calcium current. Moreover, halothane (>5%) reduced the resting tension, and increased the threshold membrane depolarization required for the generation of tension development, indicating that halothane either inactivates or reduces free calcium ions in the cytoplasm of smooth muscle cells or suppresses the activity of Ca-binding proteins.

On the other hand, halothane (1%) evoked phasic contractions with no significant changes in the membrane potential or membrane resistance in smooth muscle of the dog trachea. This halothane-induced contraction was observed after pretreatment of the tissue with EGTA (calcium chelator). However, in the presence of EGTA in the bathing solution, repetitive application of halothane induced a gradual decrease in the amplitude of the halothane-induced contractions, and after several applications, halothane-induced contractions were not apparent. After re-loading the tissue with Ca\(^{++}\) by rinsing the preparation with normal physiologic solution containing 2.5 mM Ca\(^{++}\), halothane again evoked a phasic contraction. These results indicate that halothane acts directly on the Ca store sites in tracheal smooth muscle cells, and releases Ca\(^{++}\) from the store sites.

Halothane thus has complex effects on the Ca-economy, and in the initial stage of the drug action, this agent releases Ca\(^{++}\) from the store sites, and in the second stage inactivates or reduces free Ca\(^{++}\) in the cytoplasm of the smooth muscle cells.

In conclusion, the inhibitory effects of halothane on the excitation-neuro-effector transmission in the dog tracheal tissue, and on the excitation-contraction coupling of the smooth muscle cells could account for the potent bronchodilator action of this anesthetics.

References