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Inhibitory Effects of Volatile Anesthetics on K^+ and Cl^- Channel Currents in Porcine Tracheal and Bronchial Smooth Muscle

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Background: **K^+ and Ca^{2+}-activated Cl^- (Cl_{Ca}) channel currents have been shown to contribute to the alteration of membrane electrical activity in airway smooth muscle. This study was conducted to investigate the effects of volatile anesthetics, which are potent bronchodilators, on the activities of these channels in porcine tracheal and bronchial smooth muscles.**

Methods: **Whole-cell patch clamp recording techniques were used to investigate the effects of superfused isoflurane (0–1.5 minimum alveolar concentration) or sevoflurane (0–1.5 minimum alveolar concentration) on K^+ and Cl_{Ca} channel currents in dispersed smooth muscle cells.**

Results: **Isoflurane and sevoflurane inhibited whole-cell K^+ currents to a greater degree in tracheal *versus* bronchial smooth muscle cells. More than 60% of the total K^+ currents in tracheal smooth muscle appeared to be mediated through delayed rectifier K^+ channels compared with less than 40% in bronchial smooth muscle. The inhibitory effects of the anesthetics were greater on the delayed rectifier K^+ channels than on the remaining K^+ channels. Cl^- currents through Cl_{Ca} channels were significantly inhibited by the anesthetics. The inhibitory potencies of the anesthetics on the Cl_{Ca} channels were not different in tracheal and bronchial smooth muscle cells.**

Conclusions: **Volatile anesthetics isoflurane and sevoflurane significantly inhibited Cl^- currents through Cl_{Ca} channels, and the inhibitory effect is consistent with the relaxant effect of volatile anesthetics in airway smooth muscle. Different distributions and different anesthetic sensitivities of K^+ channel subtypes could play a role in the different inhibitory effects of the anesthetics on tracheal and bronchial smooth muscle contractions.**

VOLATILE anesthetics are potent bronchodilators, and the bronchodilation is a result of a direct effect on the airway smooth muscle as well as an indirect effect on the reflex neural pathway.[1–3] One proposed mechanism for the direct relaxant effect of these anesthetics is a decrease in intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$),[4,5] produced mainly by an inhibition of Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs).[6] Most previous studies focused on the direct effects of anesthetics on the larger, more proximal airway; however, the distal airway, especially between the third- and seventh-generation bronchi, is important in the regulation of airflow resistance.[7,8] Brown *et al.*[9] and Mazzeo *et al.*[10,11], using high-resolution computed tomography and by measuring muscle tension, respectively, recently showed that volatile anesthetics have a greater inhibitory effect on distal airway muscle tone than on proximal airway muscle tone. In a more recent study,[12] we demonstrated, by the use of patch clamp techniques, that T-type VDCCs existed in bronchial smooth muscle. The high sensitivity of this channel to volatile anesthetics may be partly responsible for the different reactivities to anesthetics in tracheal and bronchial smooth muscles. However, the mechanisms of the direct inhibitory action of volatile anesthetics on tracheal and bronchial smooth muscle contraction remain to be fully elucidated.

It is well established that membrane depolarization is an important contributor to activation of VDCCs, leading to Ca^{2+} influx into cytosol and subsequent smooth muscle contraction.[13] Contractile agonists such as acetylcholine, histamine, and serotonin depolarize the membranes of airway smooth muscle cells,[14,15] whereas a relaxant agent such as isoproterenol hyperpolarizes the membrane.[16] These observations strongly suggest that alteration in the electrical activity of smooth muscle cell membranes plays an important role in regulation of the contractile and relaxant properties of the cells in various physiologic conditions. The mechanisms of cell membrane depolarization of airway smooth muscle have been demonstrated to be caused by activation of Ca^{2+}-activated Cl^- (Cl_{Ca}) channels and inhibition of K^+ channels.[17–20] Because the Cl^- equilibrium potential in smooth muscle is thought to range from -40 to -20 mV,[21] opening of Cl^- channels can lead to depolarization in resting cells.[17,18] The Cl_{Ca} channel,[18,22] the large-conductance Ca^{2+}-activated K^+ (K_{Ca}) channel,[19,23] and delayed rectifier K^+ (K_{DR}) channel[19,23] have carefully been characterized at the whole-cell or single-channel current levels in airway smooth muscle. Hyperpolarization of cell membranes by volatile anesthetics has been suggested to be one of the mechanisms for the anesthetic action in several cell types.[24–26] However, little is known about the effects of volatile anesthetics on K^+–Cl^- channel activities in airway smooth muscle. Possible different effects of volatile anesthetics on K^+ or Cl^- channel activities in tracheal and bronchial smooth muscles could also explain the regional variations in the inhibitory actions of anesthetics on airway smooth muscle tone.

Therefore, the objective of the current study was to further clarify the effect of volatile anesthetics on K^+ and

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Cl^- channel activities in both proximal and distal airway smooth muscle using whole-cell patch clamp methodology.

Materials and Methods

Isolation of Single Smooth Muscle Cells from Pig Tracheae and Bronchi

The experimental protocol used in this study was approved by the Sapporo Medical University Animal Care and Use Committee (Sapporo, Japan). Using previously described methods,[12] adult pigs of both sexes (*Sus scrofa*, weighing 30–45 kg) were sedated with ketamine (25 mg/kg administered intramuscularly), anesthetized with pentobarbital sodium (7–8 mg/kg administered intravenously), and then killed by exsanguination. The lungs and cervical trachea were removed and placed in ice-cold Krebs-Ringer bicarbonated solution aerated with 95% O_2 and 5% CO_2. The tracheae were excised, and the epithelium, cartilage, and connective tissue were stripped from the smooth muscle. Intrapulmonary bronchi of third to fifth generations were dissected from the surrounding parenchymal tissue, and cartilage and connective tissue were stripped from the smooth muscle. The epithelial layer was removed by gently rolling the tissue across moistened filter paper.

The tracheal and bronchial smooth muscle tissues were minced and digested for 20 min at 37°C in Ca^{2+}-free Tyrode solution, to which 0.08% (wt/vol) collagenase had been added.[12] Cells were then dispersed by trituration, filtered through nylon mesh, and centrifuged. The pellet was resuspended in a modified Kraftbrühe solution[27] and stored at 4°C for up to 5 h before use. The modified Kraftbrühe solution contained 85 mM KCl, 30 mM K_2HPO_4, 5.0 mM $MgSO_4$, 5.0 mM Na_2ATP, 5.0 mM pyruvic acid, 5.0 mM creatine, 20 mM taurine, 5.0 mM β-hydroxybutyrate, and 0.1% (wt/vol) fatty acid-free bovine serum albumin (pH 7.25 with Tris).

Measurements of K^+ and Cl^- Channel Activities

All experiments were performed at 37°C. Micropipettes were pulled from soda lime tubings (GC-1.5; Narishige, Tokyo, Japan) using a brown-flaming horizontal puller (model P-97; Sutter Instrument, Novato, CA). These had resistances of 3–5 MΩ when filled with solution. An aliquot (approximately 0.5 ml) of the cell suspension was placed in a perfusion chamber on the stage of an inverted microscope (IX-70; Olympus, Tokyo, Japan). A micromanipulator was used to position the patch pipette against the membrane of a tracheal or bronchial smooth muscle cell. After obtaining a high-resistance seal (5–20 GΩ) with slight suction, the patch membrane was disrupted by strong negative pressure.

Membrane currents were recorded in the tight-seal whole-cell configuration[28] using a CEZ-2400 patch clamp amplifier (Nihon Kohden, Tokyo, Japan). To measure outward whole-cell K^+ currents (I_K), recording solutions to enhance I_K were used. The bath solution contained 135 mM NaCl, 5.4 mM KCl, 1.0 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES (pH 7.4 with Tris). Niflumic acid (10 μM) was also added to minimize the Cl^- currents (I_{Cl}) through Cl_{Ca} channels ($I_{Cl(Ca)}$).[29] The pipette solution contained 70 mM KCl, 60 mM K^+-glutamate, 1.0 mM $MgCl_2$, 2.5 mM EGTA, 5.0 mM K_2ATP. 1.0 mM $CaCl_2$, and 10 mM HEPES (pH 7.2 with Tris). To measure the whole-cell $I_{Cl(Ca)}$, recording solutions to inhibit I_K were used. The bath solution contained 130 mM tetraethylammonium chloride, 1.0 mM $MgCl_2$, 5.0 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES (pH 7.4 with Tris). The pipette solution contained 130 mM CsCl, 4.0 mM $MgCl_2$, 2.5 mM EGTA, 5.0 mM Na_2ATP, 1.8 mM $CaCl_2$, and 10 mM HEPES (pH 7.2 with Tris); the computer-calculated $[Ca^{2+}]_i$ was approximately 10^{-6} M. The calculated reversal potential for Cl^- (E_{Cl}) was approximately 0 mV. In another experiment, both EGTA and $CaCl_2$ were removed from the pipette solution to show the characteristics of $I_{Cl(Ca)}$. The amplifier output of the membrane currents was low-pass filtered at 2,000 Hz. Leak currents, estimated by appropriate scaling of currents during 20-mV hyperpolarizing pulses, were subtracted from each of these records. Whole-cell I_K was elicited at 5-s intervals by 250-ms depolarizing pulses (−40 to +60 mV) from a holding potential of −70 mV. Whole-cell I_{Cl} was activated by 500-ms depolarizations in 10-mV steps to a series of potentials (−40 to +40 mV) from a holding potential of −70 mV and measured as the slow deactivating tail currents on 500-ms repolarization to the holding potential.

Experimental protocols were performed in control solutions for more than 5 min to obtain a stable baseline. Cells were exposed to a bath solution equilibrated with one of the two volatile anesthetics: isoflurane (0.5 [0.9% at the vaporizer], 1.0 [1.8%], or 1.5 [2.7%] minimum alveolar concentration [MAC][30]) or sevoflurane [0.5 (1.4%), 1.0 (2.8%), or 1.5 (4.2%) MAC[31]]. Charybdotoxin (100 nM), a specific K_{Ca} channel blocker,[32] 4-aminopyridine (5 mM), a K_{DR} channel blocker,[32] and niflumic acid (10 μM), a specific Cl_{Ca} channel blocker,[29] were used to identify the characteristics of membrane currents measured and to investigate the roles of these channels in the effects of the volatile anesthetics on the membrane currents. The temperature-controlled perfusion chamber (MT-1; Narishige) consisted of a glass coverslip bottom, with needles placed for rapid solution changes.[33] After 5-min exposure, the perfusate was switched again to the control solution.

Measurement of Anesthetic Concentrations in the Gas Phase and in the Bath Solution

Anesthetic concentrations were measured according to the previously described method.[33] Briefly, the vapor-

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izers for isoflurane and sevoflurane were calibrated with an infrared anesthetic gas monitor (5250 RGM; Datex-Ohmeda, Madison, WI). Concentrations of the anesthetic agents in bath solution samples were analyzed with a gas chromatograph (GC-17A; Shimadzu, Kyoto, Japan). The mean concentrations of isoflurane and sevoflurane in the solutions were similar to those that had been reported.[33] The mean concentrations of isoflurane in the solution at 37°C (0.9, 1.8, and 2.7% in the gas phase) were 0.25, 0.55, and 0.78 mM, respectively, whereas the mean concentration of sevoflurane in the solution (1.4, 2.8, and 4.2% in the gas phase) were 0.24, 0.56 and 0.82 mM, respectively. Each concentration of the anesthetic had a close linear correlation with each concentration of the agent in the gas phase.

Materials

The following drugs and chemicals were used: fatty acid-free bovine serum albumin, Na_2ATP, pyruvic acid, creatine, taurine, β-hydroxybutyrate, EGTA, nifedipine, charybdotoxin, 4-amonopyridine, niflumic acid (Sigma Chemical, St. Louis, MO), type-I collagenase (Gibco Laboratories, Grand Island, NY), protease (Calbiochem, La Jolla, CA), sevoflurane (Maruishi, Osaka, Japan), and isoflurane (Ohio Medical, Madison, WI). Nifedipine was dissolved in ethanol (0.01% final concentration).

Statistical Analysis.

Data are expressed as mean ± SD. Amplitudes of whole-cell currents (I_K or I_{Cl}) were measured at the end of depolarizations. Changes in the current amplitudes with exposure to each anesthetic and the measured parameters between trachea and bronchus were compared using the paired and unpaired two-tailed *t* test, respectively. One-way analysis of variance for repeated measurements and Fisher exact test were used to determine the concentration-dependent effects. In all comparisons, $P < 0.05$ was considered significant.

Results

Electric Properties of Outward K^+ Currents in Tracheal and Bronchial Smooth Muscle Cells and the Effects of Volatile Anesthetics on These Currents

Figure 1A shows a macroscopic outward I_K obtained from a freshly dispersed porcine tracheal smooth muscle cell dialyzed with a pipette solution containing a $[Ca^{2+}]_i$ of approximately 500 nM, the concentration used to enhance the I_K through Ca^{2+}-activated K^+ channels ($I_{K(Ca)}$). $I_{Cl(Ca)}$ was blocked by 10 μM niflumic acid. The I_K was activated progressively by 250-ms depolarizing pulses from a holding potential of −70 mV to consecutively more-positive membrane potentials. Stepwise depolarization from a holding potential of −70 mV to more than −30 mV elicited an outward I_K with a mean amplitude of 1,760 ± 262 pA at +60 mV (n = 57). The kinetics of the outward K^+ current in this study was similar to that previously reported for I_K currents in airway smooth muscle cells.[19,34] Isoflurane (1.5 MAC) caused an approximately 30% reduction in I_K without an apparent effect on the time course of the current (fig. 1B), an effect that was reversible when the anesthetic was washed out. Sevoflurane showed a similar inhibitory effect (raw data not shown). The same experimental

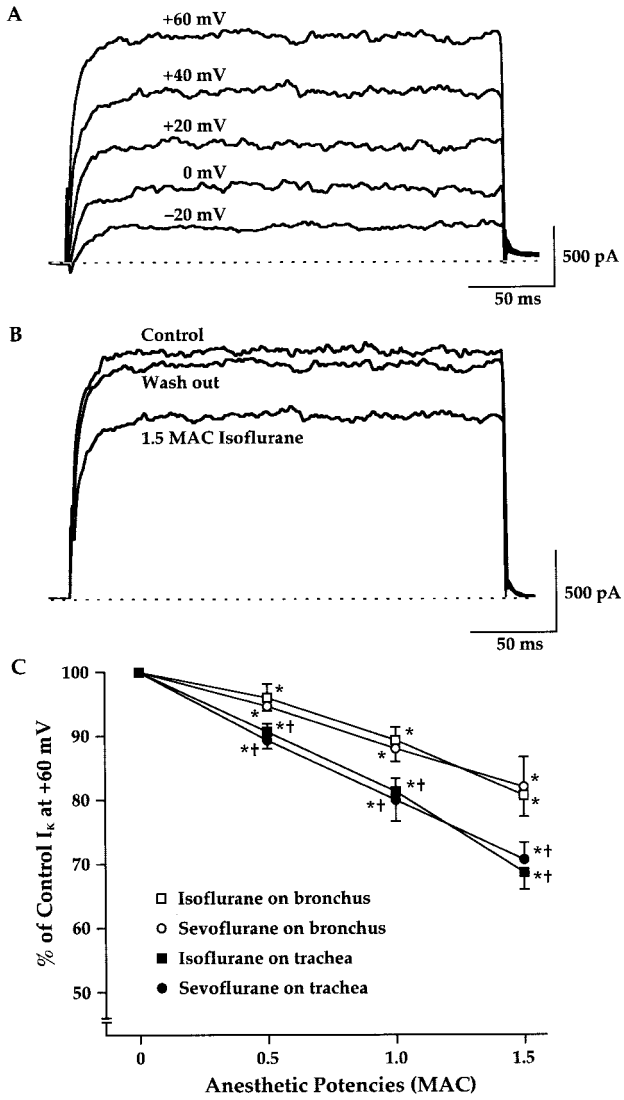

Fig. 1. Effects of volatile anesthetics on whole-cell outward K^+ current (I_K) in tracheal and bronchial smooth muscle cells. (*A*) Representative traces of I_K evoked by depolarizations from a holding potential of −70 to −20, 0, +20, +40, and +60 mV in a tracheal smooth muscle cell. (*B*) Effect of 1.5 minimum alveolar concentration (MAC) isoflurane on the I_K evoked by depolarization from a holding potential of −70 to +60 mV in a tracheal smooth muscle cell. (*C*) Relations between anesthetic potencies (MAC) and I_K amplitude at +60 mV, expressed as a percentage of the control and of tracheal and bronchial smooth muscle cells. Data are expressed as mean ± SD (n = 7 each). *$P < 0.05$ *versus* 0 MAC anesthetic; †$P < 0.05$ *versus* bronchial smooth muscle cells.

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protocol was also performed in bronchial smooth muscle cells. A similar outward I_K with a mean amplitude of 1,376 ± 180 pA at +60 mV (n = 68) was observed, and both volatile anesthetics tested (1.5 MAC) significantly inhibited I_K by approximately 22%. Figure 1C summarizes the relation between amplitude of I_K at +60 mV, expressed as a percentage of the control, and the potencies (minimum alveolar concentration) of the volatile anesthetics tested. Both the volatile anesthetics isoflurane and sevoflurane significantly and dose-dependently inhibited I_K; however, the I_K of tracheal smooth muscle cells was more sensitive to volatile anesthetics than was that of bronchial smooth muscle cells.

We further investigated the whole-cell I_K using pharmacologic techniques. Figure 2A shows the result of a typical experiment in a tracheal smooth muscle cell. The addition of 5 mM 4-aminopyridine, a selective K_{DR} channel blocker, significantly decreased I_K, and a supplementary application of 100 nM charybdotoxin, a selective K_{Ca} channel blocker, further decreased the I_K to almost a zero current level. The current–voltage relation for this experiment is shown in figure 2B. Both K^+ channel blockers significantly suppressed the I_K amplitude over the entire voltage range studied without shifting the voltage dependency of the current–voltage relation. The same experimental protocol was performed in bronchial smooth muscle cells, and figure 2C summarizes the inhibitory effects of these K^+ channel blockers on the I_K amplitude at +60 mV in tracheal and bronchial smooth muscle cells. Most of the whole I_K in both tracheal and bronchial smooth muscle cells was occupied by these charybdotoxin- and 4-aminopyridine–sensitive K^+ channels. The 4-aminopyridine–sensitive I_K component was more than 60% in tracheal smooth muscle cells, whereas it was less than 40% in bronchial smooth muscle cells.

The effects of volatile anesthetics tested on each charybdotoxin- and 4-aminopyridine–sensitive current were examined in tracheal and bronchial smooth muscle cells. After application of 5 mM 4-aminopyridine, the remaining current could be considered as the $I_{K(Ca)}$. As shown in figure 3A (top), 1.5 MAC isoflurane significantly decreased the $I_{K(Ca)}$ in a tracheal smooth muscle cell. Similar effects were observed with sevoflurane (raw data not shown), and the same protocol was performed in bronchial smooth muscle cells. Both isoflurane and sevoflurane significantly inhibited $I_{K(Ca)}$, and the inhibitory effects of the volatile anesthetics tested were indistinguishable (fig. 3A, bottom). Although the I_K in the case of application of 100 nM charybdotoxin was considered as the I_K mainly through the K_{DR} channel ($I_{K(DR)}$), it is possible that the $I_{K(Ca)}$ still existed during the condition of high $[Ca^{2+}]_i$ (500 nM). Therefore, in another experiment, we confirmed the characteristics of the I_K using a pipette solution in which $[Ca^{2+}]_i$ was strongly buffered with 10 mM EGTA to minimize the $I_{K(Ca)}$. An application of 100 nM charybdotoxin had no effect on the current (n = 4, data not shown), and 5 mM 4-aminopyridine substantially inhibited the current at +60 mV by more than 90% (n = 4, data not shown). The effects of isoflurane and sevoflurane on the Ca^{2+}-independent $I_{K(DR)}$ were examined in both tracheal and bronchial smooth muscle cells. Both volatile anesthetics significantly inhibited $I_{K(DR)}$, and the inhibitory effects were indistinguishable (fig. 3B). Figure 4 summarizes the rela-

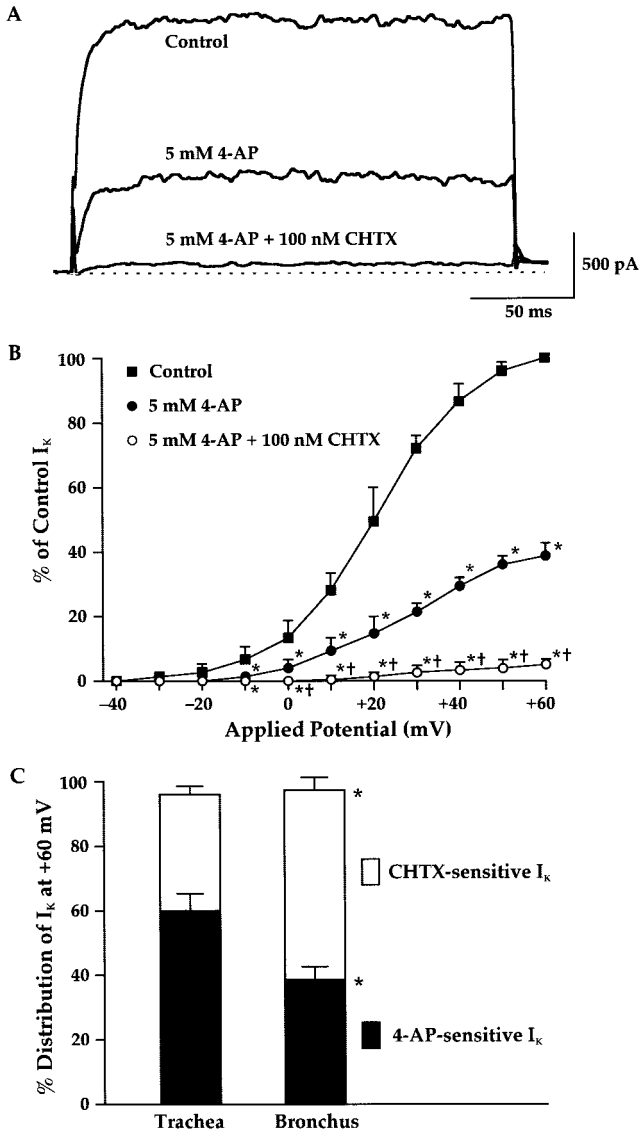

Fig. 2. Effects of 4-aminopyridine (4-AP) and charybdotoxin (CHTX) on the whole-cell outward K^+ current (I_K) in tracheal and bronchial smooth muscle cells. (*A*) Effects of 5 mM 4-AP, a delayed rectifier K^+ channel blocker, and 100 nM CHTX, a Ca^{2+}-activated K^+ channel blocker, on the I_K evoked by depolarization from a holding potential of −70 to +60 mV in a tracheal smooth muscle cell. (*B*) Relative current–voltage relations obtained before and after applications of 4-AP alone and of 4-AP and CHTX in tracheal smooth muscle cells. Symbols represent mean ± SD (n = 7). **P* < 0.05 *versus* control; †*P* < 0.05 *versus* 4-AP. (*C*) Distributions of CHTX- and 4-AP–sensitive I_K in tracheal and bronchial smooth muscle cells. Bars represent mean ± SD (n = 7 each). **P* < 0.05 *versus* trachea.

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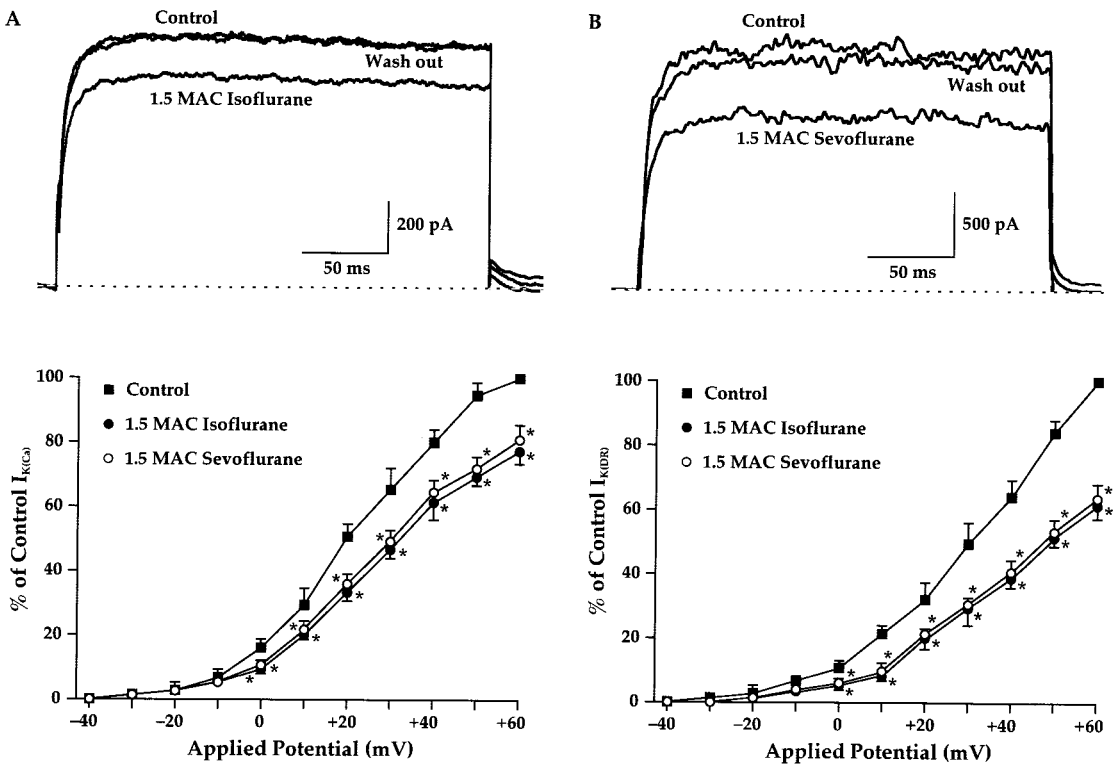

Fig. 3. Effects of volatile anesthetics on K^+ currents through charybdotoxin-sensitive Ca^{2+}-activated K^+ (K_{Ca}) channels ($I_{K(Ca)}$) and 4-aminopyridine–sensitive delayed rectifier K^+ (K_{DR}) channels ($I_{K(DR)}$) in tracheal and bronchial smooth muscle cells. (*A*) Effect of 1.5 minimum alveolar concentration (MAC) isoflurane on the $I_{K(Ca)}$ recorded in a bath solution containing 5 mM 4-aminopyridine by depolarizing pulses from a holding potential of −70 to +60 mV in a tracheal smooth muscle cell (*top*). Relative $I_{K(Ca)}$–voltage relations obtained before and after exposure to volatile anesthetics in tracheal and bronchial smooth muscle cells (*bottom*). (*B*) Effect of 1.5 MAC sevoflurane on the $I_{K(DR)}$ recorded in a bath solution containing 100 nM charybdotoxin by depolarizing pulses from a holding potential of −70 to +60 mV in a bronchial smooth muscle cell (*top*). Relative $I_{K(DR)}$–voltage relations obtained before and after exposure to volatile anesthetics in tracheal and bronchial smooth muscle cells (*bottom*). Data are expressed as mean ± SD (n = 7 each). *$P < 0.05$ *versus* control.

tion between the percentage control of $I_{K(Ca)}$ (fig. 4A) and $I_{K(DR)}$ (fig. 4B) at +60 mV and the anesthetic potencies of the anesthetics tested in tracheal and bronchial smooth muscle cells. Both isoflurane and sevoflurane significantly and dose-dependently inhibited both the $I_{K(Ca)}$ and $I_{K(DR)}$, and the inhibitory effects of the anesthetics tested on each current were indistinguishable. However, the $K_{(DR)}$ channel was more sensitive than was the $K_{(Ca)}$ channel to the volatile anesthetics.

Electric Properties of Whole-cell Ca^{2+}-activated Cl^- Currents in Tracheal and Bronchial Smooth Muscle Cells and the Effects of Volatile Anesthetics on These Currents

As shown in figure 5A, 500-ms depolarizing pulses from a holding potential of −70 mV to a series of potentials elicited an initial inward Ca^{2+} current, which was followed by a sustained current when 130 mM CsCl and no EGTA-Ca^{2+} in the pipette solution and 130 mM tetraethylammonium chloride in the bath solution were used. An inwardly directed tail current was observed after return to the holding potential. The kinetics of this tail current was much slower than those of the inward Ca^{2+} current. A specific Cl_{Ca} channel blocker, niflumic acid (10 μM), substantially blocked the sustained outward current and inward tail current by more than 80% (fig. 5B). Nifedipine (1 μM), a potent L-type VDCC blocker, dramatically inhibited both the Ca^{2+} current and the tail current (fig. 5B). The patterns of the tail current were similar to those reported for Cl_{Ca} in rabbit coronary artery myocytes[35] and rabbit esophageal smooth muscle.[36] Figure 5C shows the current–voltage relation of the I_{Cl} through Cl_{Ca} channels ($I_{Cl(Ca)}$) measured at the end of the clamp steps.

To investigate the effects of volatile anesthetics on the Cl_{Ca} channel activity, a pipette solution containing 2.5 mM EGTA and 1.8 mM $CaCl_2$ was used to fix the $[Ca^{2+}]_i$ to approximately 1 μM because it is possible that the inhibitory action of volatile anesthetics on VDCCs[6,12] have some effect on the Cl_{Ca} channel activity. During this condition, a similar current was evoked by pulses from a holding potential of −70 mV for 500-ms depolarization in both tracheal and bronchial smooth muscle cells. When the current was evoked by a depolarization pulse from a holding potential of −70 to +20 mV, 1 μM nifedipine completely blocked the initial inward Ca^{2+}

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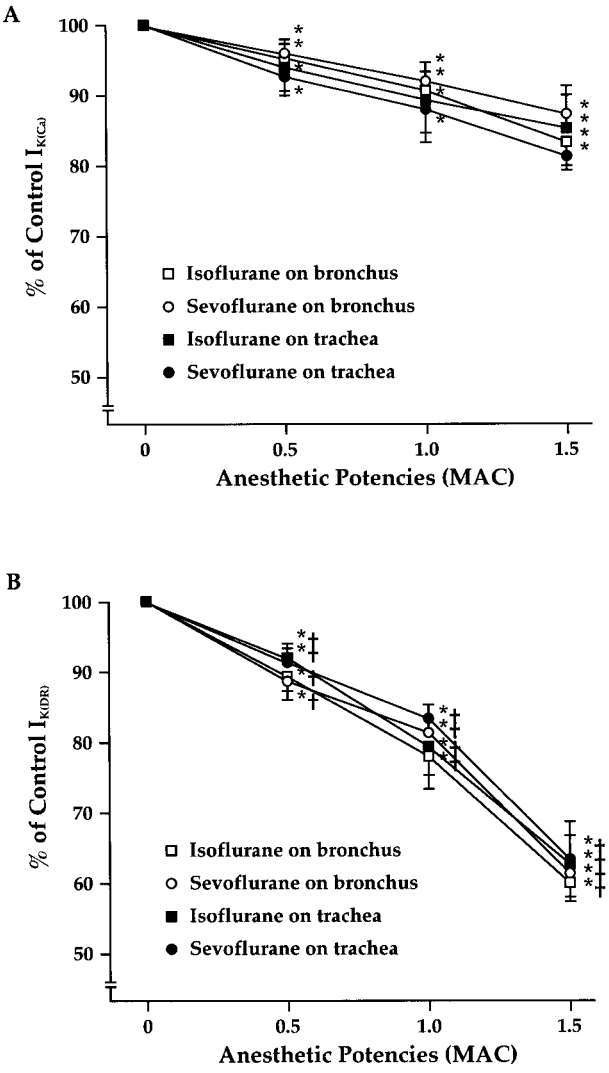

Fig. 4. Relations between anesthetic potencies (minimum alveolar concentration [MAC]) and Ca^{2+}-activated K^+ channel current ($I_{K(Ca)}$) (*A*) and delayed rectifier K^+ channel current ($I_{K(DR)}$) (*B*) at +60 mV, expressed as a percentage of the control, in tracheal and bronchial smooth muscle cells. Data are expressed as mean ± SD (n = 7). *$P < 0.05$ *versus* 0 MAC anesthetic; †$P < 0.05$ *versus* $I_{K(Ca)}$ at the same anesthetic potencies.

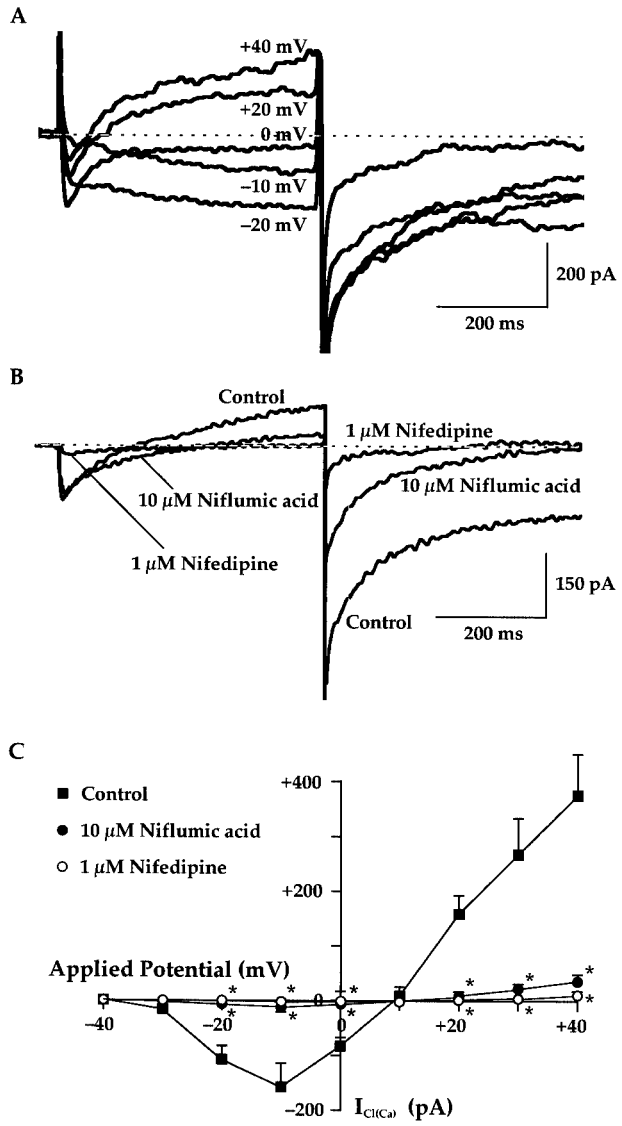

Fig. 5. Whole-cell Ca^{2+}-activated Cl^- channel currents ($I_{Cl(Ca)}$) in bronchial smooth muscle cells recorded in a pipette solution not containing EGTA–Ca^{2+}. (*A*) Depolarization pulses (500 ms) from a holding potential of −70 mV elicited initial inward Ca^{2+} currents (peak at +10 mV), followed by sustained currents (inward at −20, −10, and 0 mV and outward at +20 and +40 mV). (*B*) A specific Ca^{2+}-activated Cl^- channel blocker, niflumic acid (10 μM), substantially blocked the sustained outward and tail currents at +20 mV by more than 80%, and a potent L-type voltage-dependent Ca^{2+} channel blocker, nifedipine (1 μM), dramatically inhibited both the Ca^{2+} current and the tail current. (*C*) Current–voltage relation of the $I_{Cl(Ca)}$ measured at the end of the clamp steps in bronchial smooth muscle cells. Data are expressed as mean ± SD (n = 7 each). *$P < 0.05$ *versus* control.

current but had little effect on the sustained outward $I_{Cl(Ca)}$ (n = 3, data not shown).

During these conditions, isoflurane (1.5 MAC) significantly reduced the nifedipine-insensitive $I_{Cl(Ca)}$ in a tracheal smooth muscle cell during a depolarization pulse to +40 mV (fig. 6A). Sevoflurane induced a similar inhibitory effect on the current (raw data not shown). Figure 6B shows the current–voltage relation of the $I_{Cl(Ca)}$ with or without each volatile anesthetic tested in tracheal smooth muscle cells. Both volatile anesthetics, isoflurane and sevoflurane, significantly suppressed the $I_{Cl(Ca)}$ amplitude for the entire voltage range studied. Similar experiments were performed in bronchial smooth muscle cells, and figure 6C summarizes the relation between the percentage control of $I_{Cl(Ca)}$ at +40 mV and the anesthetic potencies of isoflurane and sevoflurane in tracheal and bronchial smooth muscle cells. Both the volatile anesthetics tested significantly and dose-dependently inhibited the $I_{Cl(Ca)}$, and the inhibitory effects of the anesthetics tested were indistinguishable both between tracheal and bronchial smooth muscle cells and between isoflurane and sevoflurane.

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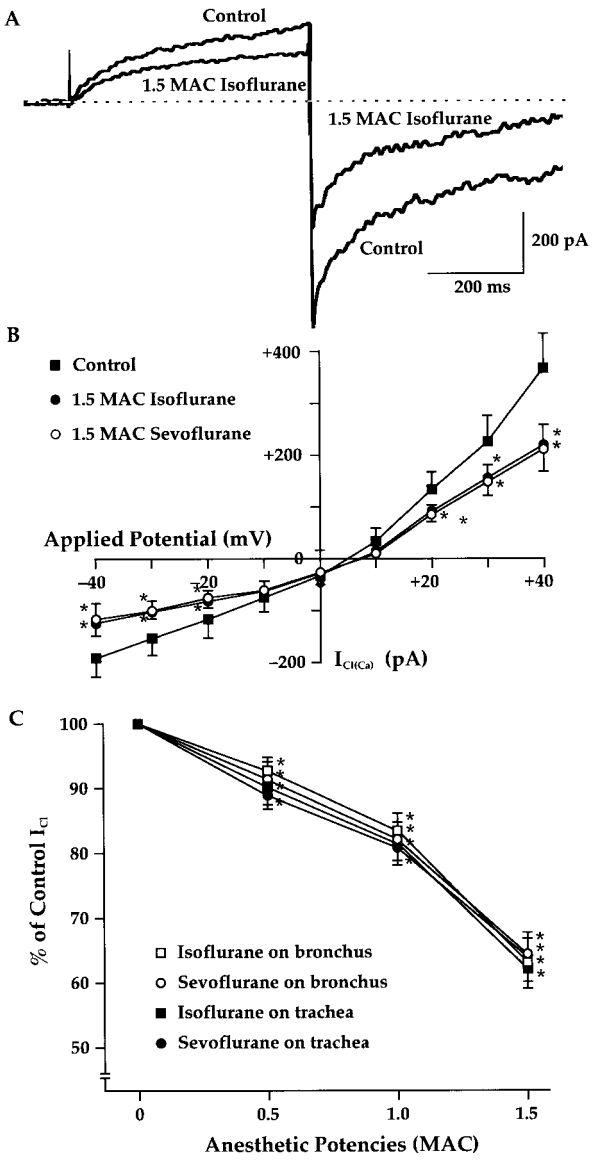

Fig. 6. Effects of volatile anesthetics on Ca^{2+}-activated Cl^- (Cl_{Ca}) channel currents ($I_{Cl(Ca)}$) recorded in a pipette solution containing 1.8 mM $CaCl_2$ and 2.5 mM EGTA. (*A*) Effects of 1.5 minimum alveolar concentration (MAC) isoflurane on $I_{Cl(Ca)}$ evoked by +40-mV depolarizing pulses in a tracheal smooth muscle cell. (*B*) Cl_{Ca} current–voltage relation obtained before and after exposure to 1.5 MAC isoflurane and 1.5 MAC sevoflurane in bronchial smooth muscle cells. (*C*) Relations between anesthetic potencies (MAC) and $I_{Cl(Ca)}$ at +40 mV in both tracheal and bronchial smooth muscle cells. Data are expressed as mean ± SD (n = 7 each). *$P < 0.05$ *versus* 0 MAC anesthetic.

Discussion

Effects of Volatile Anesthetics on Whole-cell K^+ Currents in Tracheal and Bronchial Smooth Muscle Cells

In this experiment, whole-cell I_K was recorded in freshly dispersed porcine tracheal and bronchial smooth muscle cells with a pipette solution containing 2.5 mM EGTA and 1.0 mM $CaCl_2$ (calculated $[Ca^{2+}]_i$ = approximately 500 nM), and the kinetics of the I_K was similar to that previously reported in airway smooth muscles (fig. 1).[19,34] The importance of K^+ conductance for the maintenance of normal electrical behavior in smooth muscle has long been known.[37,38] K^+ channels appear to play important roles in setting resting membrane potential and in limiting electrical responses to excitatory stimuli.[39,40] With the K^+ equilibrium potential at −80 to approximately −90 mV, the opening of K^+ channels causes membrane hyperpolarization, leading to airway smooth muscle relaxation. Volatile anesthetics are potent bronchodilators; therefore, the hyperpolarization of the cell membrane by anesthetic-induced activation of K^+ channels has been suggested to be one of the main mechanisms for the relaxant effects. The current study demonstrated, disappointingly, that the volatile anesthetics tested induced inhibition of K^+ channels in both tracheal and bronchial smooth muscle cells. Although the inhibition of K^+ channels can depolarize the cell membrane, leading to muscle contraction, it is speculated that a muscarinic receptor agonist, carbachol, already and substantially inhibited the whole K^+ channel currents in our experimental conditions.[17–20]

Although it has been reported that there are many kinds of K^+ channel subtype in other tissues, only K_{Ca} and K_{DR} have carefully been identified in airway smooth muscle cells using whole-cell or single-channel patch clamp techniques.[19,23] Adenosine triphosphate–activated K^+ channel has also been shown to exist in airway smooth muscle[41]; however, simultaneous application of K_{Ca} and K_{DR} channel blockers substantially diminished the whole K^+ currents in this study, and adenosine triphosphate–activated K^+ channels can open when the intracellular concentration of adenosine triphosphate is less than 3 mM[41] (5 mM in the pipette solution in this study). Furthermore, it has been suggested that the opening of the adenosine triphosphate–activated K^+ channel is not involved in halothane's relaxant effect on airway smooth muscle.[42]

Because the resting membrane potential is more negative in the bronchus (−70 mV) than in the trachea (−60 mV)[43] and because both isoflurane and sevoflurane had a greater inhibitory effect on whole-cell I_K in tracheal than in bronchial smooth muscle cells (fig. 1), we further examined the effects of the anesthetics on each $I_{K(Ca)}$ or $I_{K(DR)}$ using a pharmacologic technique. The concentrations of K^+ channel blockers used in this study (5 mM for 4-aminopyridine and 100 nM for charybdotoxin) were roughly equivalent to the drug concentrations required to maximally inhibit the I_K through K_{DR} and K_{Ca} channels, respectively.[44,45] In whole-cell studies of dissociated airway smooth muscle cells, a prominent Ca^{2+}-sensitive outward current has been observed by several investigators,[19,40,46] but the magnitude of the current varies depending on the $[Ca^{2+}]_i$ used. The open-state probability of K_{Ca} channels was found to be low in resting conditions ($[Ca^{2+}]_i$ = approximately 100 nM)[19,40]; there-

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fore, rather high $[Ca^{2+}]_i$ (500 nM) was used in the current study, the concentration being similar to the $[Ca^{2+}]_i$ during the condition of agonist-stimulated smooth muscle.[47] During this condition, the $I_{K(Ca)}$ component in bronchial smooth muscle cells was greater than 60%, whereas it was less than 40% in tracheal smooth muscle cells (fig. 2). This result is consistent with the finding reported by Snetkov *et al.*,[46] that the voltage-dependent outward K^+ current was mainly caused by activation of K_{Ca} channels in human bronchial smooth muscles during a physiologic condition. The different distributions of the two components of I_K in tracheal and bronchial smooth muscle are still not clear. However, it could explain the greater inhibitory effects of the volatile anesthetics on whole I_K in tracheal than in bronchial smooth muscle cells, because the K_{DR} channel component was greater in tracheal than bronchial smooth muscle cells and the inhibitory effects of volatile anesthetics tested were greater on the K_{DR} than on K_{Ca} channel currents (figs. 2 and 4).

Effects of Volatile Anesthetics on Whole-cell Cl^- Currents in Tracheal and Bronchial Smooth Muscle Cells

Our previous experiments have extensively characterized the inward Ca^{2+} currents through VDCCs in porcine airway smooth muscle.[6,12,33] All of these studies were performed using a pipette solution containing 10 mM EGTA and 0 mM Ca^{2+}, and no tail currents were seen on the repolarization after application of depolarizing pulses. When EGTA was eliminated from the pipette solution, we observed a more complicated trace, as illustrated in figure 5. When currents were evoked by depolarization pulses from a holding potential of −70 to +20 mV, nifedipine (1 μM), an L-type VDCC blocker, blocked the initial inward, sustained outward, and inwardly directed tail currents. On the other hand, niflumic acid (10 μM), a specific Cl_{Ca} channel blocker, significantly decreased the sustained outward and inwardly directed tail currents but had no effects on the initial inward currents. Because the initial inward currents are thought to be Ca^{2+} currents, the sustained currents are consistent with $I_{Cl(Ca)}$ described at the whole-cell current level in rabbit ventricular myocytes[48] and rabbit smooth muscle cells.[35,36] The $I_{Cl(Ca)}$ observed in this study is thought to contribute to the regulation of membrane potential in airway smooth muscle. It has been shown that changes in Cl_{Ca} channel activity was involved in the acetylcholine-induced depolarization in cells from human[17] and guinea-pig[18] airway smooth muscles. The responses of the Cl_{Ca} channels are considered to be mediated by agonist-induced increase in $[Ca^{2+}]_i$.[17,18] Because the Cl^- equilibrium potential in smooth muscle is thought to range from −40 to −20 mV,[21] opening of Cl^- channels leads to depolarization in resting cells,[17,18] but to hyperpolarization in cells that have already been depolarized to potentials more positive than the equilibrium potential.

With regard to the physiologic role of Cl_{Ca} channels in airway smooth muscle, we examined the effects of the volatile anesthetics isoflurane and sevoflurane on the currents. The volatile anesthetics had similar inhibitory effects on Cl_{Ca} channel activity in tracheal and bronchial smooth muscle cells. The inhibitory effects of the volatile anesthetics on the Cl^- channels would hyperpolarize or repolarize the cell membrane and lead to relaxation of airway smooth muscle. However, the similar inhibitory effects of volatile anesthetics on Cl_{Ca} channel activities in tracheal and bronchial smooth muscle cells indicate that the effects of volatile anesthetics on Cl_{Ca} channels could not contribute to regional differences in the inhibitory effects of volatile anesthetics on tracheal and bronchial smooth muscle contractions.

In conclusion, the volatile anesthetics isoflurane and sevoflurane significantly inhibited Cl^- currents through Cl_{Ca} channels, and the inhibitory effect is consistent with the relaxant effect of volatile anesthetics in airway smooth muscle. Different distributions and different anesthetic sensitivities of K^+ channel subtypes could play a role in the different inhibitory effects of the anesthetics on tracheal and bronchial smooth muscle contractions.

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