Different Inhibitory Effects of Volatile Anesthetics on T- and L-type Voltage-dependent Ca²⁺ Channels in Porcine Tracheal and Bronchial Smooth Muscles

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Background: The distal airway is more important in the regulation of airflow resistance than is the proximal airway, and volatile anesthetics have a greater inhibitory effect on distal airway muscle tone. The authors investigated the different reactivities of airway smooth muscles to volatile anesthetics by measuring porcine tracheal or bronchial (third to fifth generation) smooth muscle tension and intracellular concentration of free Ca²⁺ ([Ca²⁺]i) and by measuring inward Ca²⁺ currents (Ica) through voltage-dependent Ca²⁺ channels (VDCs).

Methods: Intracellular concentration of free Ca²⁺ was monitored by the 500-nm light emission ratio of Ca²⁺ indicator fura-2. Isometric tension was measured simultaneously. Whole-cell patch clamp recording techniques were used to investigate the effects of volatile anesthetics on Ica, in dispersed smooth muscle cells. Isoflurane (0–1.5 minimum alveolar concentration) or sevoflurane (0–1.5 minimum alveolar concentration) was introduced into a bath solution.

Results: The volatile anesthetics tested had greater inhibitory effects on carbachol-induced bronchial smooth muscle contraction than on tracheal smooth muscle contraction. These inhibitory effects by the anesthetics on muscle tension were parallel to the inhibitory effects on [Ca²⁺]i. Although tracheal smooth muscle cells had only L-type VDCs, some bronchial smooth muscle cells (~30%) included T-type VDC. Each of the two anesthetics significantly inhibited the activities of both types of VDCs in a dose-dependent manner; however, the anesthetics had greater inhibitory effects on T-type VDC activity in bronchial smooth muscle.

Conclusions: The existence of the T-type VDC in bronchial smooth muscle and the high sensitivity of this channel to volatile anesthetics seem to be, at least in part, responsible for the different reactivities to the anesthetics in tracheal and bronchial smooth muscles.

VOLATILE anesthetics at clinically relevant concentrations have a potent and direct inhibitory effect on airway smooth muscle.1-5 The direct effects of these anesthetics on airway smooth muscle are thought to be ultimately caused by a decrease in intracellular concentration of free Ca²⁺ ([Ca²⁺]i),2,3 a primary regulator of smooth muscle tone.4 This decrease is, in part, a result of a blockade of Ca²⁺ influx through L-type voltage-dependent Ca²⁺ channels (VDCs).5 In studies performed in vivo,6-7 variations in airway resistance in the larger, more proximal airway, but not the more distal airway,8 have been evaluated. In studies performed in vitro,9,10 the direct effects of anesthetics on the trachea, but only as distal as the segmental bronchus, have been evaluated.

The direct effects of volatile anesthetics on distal airway smooth muscle may be more important clinically. The lung region that is important in regulation of airflow resistance is between the third- and seventh-generation bronchi,10,11 and a series of studies have shown that there were significant physiological and pharmacologic differences between tracheal and bronchial smooth muscles.12,13 Mazzeo et al.14,15 demonstrated, by measuring muscle tension, that volatile anesthetics had a more inhibitory effect on distal airway muscle tone than on proximal airway muscle tone. On the other hand, Croxton et al.16 showed that peripheral airway smooth muscle was more resistant to dihydropyridine-sensitive (L-type) VDC antagonists than was tracheal smooth muscle, indicating that L-type VDCs are the predominant mechanism for Ca²⁺ entry in tracheal smooth muscle. Recently, Janssen17 found T-type VDCs as well as L-type VDCs in canine bronchial smooth muscle cells by using the whole-cell patch clamp technique. We therefore speculated that the difference in distributions of T- and L-type VDCs is related to the difference in reactivities to volatile anesthetics14,15 and to dihydropyridine-sensitive VDC antagonists in proximal and distal airway smooth muscles.16,18

This study was conducted to test this hypothesis by simultaneously measuring porcine tracheal or bronchial (third to fifth generation) smooth muscle tension and [Ca²⁺]i, using the fluorescence technique2,4 and by measuring inward Ca²⁺ currents through VDCs (Ica) using patch clamp techniques.5,19 We also investigated the inhibitory effects of the volatile anesthetics isoflurane and sevoflurane on these muscle tones with changes in [Ca²⁺]i, and on these channels' activities.

Materials and Methods

Tissue Preparation

The protocol for this study was approved by the Sapporo Medical University Ethical Committee on Animal Research. Adult pigs of either sex (Sus scrofa, weighing 30–45 kg) were sedated with ketamine (25 mg/kg intra-
muscularly) and anesthetized with pentobarbital sodium (7–8 mg/kg intravenously). The animals were then killed by exsanguination. The lungs and cervical trachea were removed and placed in ice-cold Krebs-Ringer bicarbonate solution aerated with 95% O2 and 5% CO2. 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.

**Simultaneous Measurement of Muscle Tension and ([Ca2+]i)**

Tracheal (1 mm wide and 8 mm long) and bronchial (1 mm wide and 5 mm long) smooth muscle strips were loaded with 5 μM acetoxymethyl ester of fura-2, an indicator of Ca2+, in a physiological salt solution containing 0.02% (vol/vol) cremophor EL for 6 or 7 h at room temperature (22–24°C). The physiological salt solution contained 136.9 mM NaCl, 5.4 mM KCl, 1.5 mM CaCl2, 1.0 mM MgCl2, 23.9 mM NaHCO3, 5.5 mM glucose, and 0.01 mM EDTA. This solution was saturated with a gas mixture of 95% O2-5% CO2 at 37°C (pH ~7.4). Each fura-2-loaded muscle strip was held in a temperature-controlled (37°C) organ bath, and one end of the muscle strip was connected to a strain gauge transducer (IWS-20GA; Kyowa, Tokyo, Japan). Experiments were performed using a fluorescence spectrometer (CAF-100; Japan Spectroscopic, Tokyo, Japan). Excitation light was passed through a rotating filter wheel (48 Hz) that contained 340- and 380-nm filters. The light emitted from the muscle strip at 500 nm was measured using a photomultiplier. The ratio of the fluorescence resulting from excitation at 340 nm to that at 380 nm (R340/380) was calculated and used as an indicator of [Ca2+]i.

Physiological salt solution aerated with 95% O2-5% CO2 was used for the control bath solution, and the airway smooth muscle strips were allowed to equilibrate for 30 min after being mounted in the bath. To establish an optimal length, the resting tension was adjusted to 2 g for tracheal and 1 g for bronchial smooth muscle strips. These values were selected as the optimal values for maximal active force generation determined in preliminary experiments using repeated carbachol contractions and various baseline tensions. Both tissues were contracted with submaximal effect (∼ED50) concentrations of carbachol (1 μM), a stable potent muscarinic receptor agonist. After the contractions had reached a steady state, the tissues were exposed to a bath solution equilibrated with one of two volatile anesthetics: isoflurane (0.5 [0.9% at the vaporizer], 1.0 [1.8%], or 1.5 [2.7%] minimum alveolar concentration [MAC] in the pig21) or sevoflurane (0.5 [1.4%], 1.0 [2.8%], 1.5 [4.2%] MAC in the pig22). Similar to this experiment, the tissue strips were exposed to 1 μM nifedipine, a dihydropyridine-sensitive VDC antagonist, during carbachol-induced contraction.

**Measurement of Voltage-dependent Ca2+ Channel Activity**

We used conventional whole-cell patch clamp techniques19 to observe inward Ca2+ currents (Ica) through VDCs. Tracheal and bronchial smooth muscle tissues were minced and digested for 20 min at 37°C in Ca2+-free Tyrode solution to which 0.08% (wt/vol) collagenase was added. Cells were dispersed by trituration, filtered through nylon mesh, and centrifuged. The pellet was resuspended in a modified Kraftbrühe solution23 and stored at 4°C for up to 5 h before use. The modified Kraftbrühe solution contained 85 mM KCl, 30 mM K2HPO4, 5.0 mM MgSO4, 5.0 mM Na2ATP, 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 adjusted to 7.25 with tris-[hydroxymethyl]-aminomethane [Tris]).

The experiments were performed at 37°C. Micropipettes were pulled from soda lime “hematocrit” tubing (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. Recording solutions were chosen to inhibit Na+–K+ currents and enhance Ca2+ currents. The pipette solution contained 130 mM CsCl, 4.0 mM MgCl2, 10 mM EGTA, 5.0 mM Na2ATP, and 10 mM HEPES (pH adjusted to 7.2 with Tris). The bath solution contained 130 mM tetraethylammonium chloride, 1.0 mM MgCl2, 10 mM CaCl2, 10 mM glucose, and 10 mM HEPES (pH adjusted to 7.4 with Tris). 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 (3–20 MΩ) with slight suction, the patch membrane was disrupted by strong negative pressure. Membrane currents were monitored using a CEZ-2400 patch clamp amplifier (Nihon Kohden, Tokyo, Japan), and the amplifier output 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.

Inward Ca2+ currents were elicited by 100-ms depolarizing pulses (−60 to +40 mV) from a holding potential of −80 or −40 mV. A holding potential of −40 mV was used to elicit Ica through L-type VDCs. Ica through T-type VDCs was obtained by digital subtraction of Ica obtained at a holding potential of −40 mV from total Ica elicited from a holding potential of −80 mV in the same cell. We also confirmed the presence of two
types of I_{Ca}S in these cells by pharmacologic identification. The Ca^{2+} channel blockers with a dihydropyridine structure in these cells are relatively selective for the I_{Ca} through T-type VDCs.\(^\text{17,18}\)

Voltage-pulse protocols were performed in control solutions for more than 5 min to obtain a stable baseline. Cells were then exposed to bath solution equilibrated with one of two volatile anesthetics: isoflurane (0.5 [0.9% at the vaporizer], 1.0 [1.8%], or 1.5 [2.7%] MAC) or sevoflurane (0.5 [1.4%], 1.0 [2.8%], or 1.5 [4.2%] MAC). The temperature-controlled perfusion chamber (MT-1; Narishige) consisted of a glass coverslip bottom, with needles placed for rapid solution changes.\(^\text{25}\) The chamber volume was approximately 1 ml, and complete solution changes in the chamber could be obtained within 1 min using a peristaltic pump (CTP-3; Iuchi, Tokyo, Japan) attached to the input and output ports. After 6-min exposure, the perfusate was switched again to the control solution.

Inactivation curves were determined, using a double-pulse protocol that consisted of a 3-s prepulse to a potential in the range of −80 to +10 mV, followed by a 100-ms depolarization to +10 mV. To observe the inactivation curve of Ca^{2+} currents through T-type VDCs, we used another double-pulse protocol that consisted of a 3-s prepulse to a potential in the range of −120 to −10 mV, followed by a 100-ms depolarization to −10 mV.\(^\text{17}\) The peak change in the current was expressed as a fraction of that obtained using the −80- or −20-mV prepulse, and this quantity was least-squares fitted to a Boltzman expression to estimate the potential of half-maximal inactivation (V_{1/2}) and the slope factor (k).\(^\text{26}\) Similar to the I_{Ca} experiment, the effects of the volatile anesthetics on the inactivation curves were also shown.

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

Anesthetic concentrations were measured according to the previously described method.\(^\text{25}\) Briefly, the vaporizers for isoflurane and sevoflurane were calibrated using an infrared anesthetic gas monitor (5250 RGM; Datex-Ohmeda, Madison, WI). Concentrations of the anesthetic agents in bath solution samples were analyzed using a gas chromatograph (GC-17A; Shimadzu, Kyoto, Japan). 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 concentrations 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. There were no significant differences between the concentrations of these anesthetics in the perfusion chamber for patch clamp recording and those in the bath solution of a spectrometer (n = 4, data not shown).

**Results**

**Effects of Volatile Anesthetics on Tension and [Ca^{2+}]_i in Tracheal and Bronchial Smooth Muscle Strips**

As has been reported previously,\(^\text{2,27}\) R_{340/380} an indicator of [Ca^{2+}]_j was rapidly increased by 1 μM carbachol with a concomitant contraction in a tracheal smooth muscle strip (fig. 1A). During carbachol-induced contraction, 1.5 MAC isoflurane significantly decreased both the muscle tension and [Ca^{2+}]_j. In a bronchial smooth muscle strip (fig. 1B), carbachol similarly increased R_{340/380} with a concomitant contraction; however, the maximum tension (1.1 ± 0.3 g) was significantly lower than that (4.0 ± 0.7 g) of tracheal smooth muscle strips. Isoflurane (1.5 MAC) similarly and significantly decreased both muscle tension and [Ca^{2+}]_j in a bronchial smooth muscle strip; however, the inhibitory effects of isoflurane on them seemed to be greater than those in a tracheal smooth muscle strip. Sevoflurane had similar inhibitory effects on muscle tension and [Ca^{2+}]_j in both tracheal and bronchial smooth muscle tissues (raw data not shown). The relations between anesthetic potencies (MAC) and percentage of responses of muscle tension and [Ca^{2+}]_j are shown in figure 2. In both tracheal and bronchial smooth muscle tissues, the volatile anesthetics tested significantly decreased muscle tension and [Ca^{2+}]_j in a dose-dependent manner, and there were no significant differences between these anesthetics in the inhibitory potencies on both muscle tension and [Ca^{2+}]_j. The inhibitory effects by these anesthetics were, however,
significantly greater in bronchial smooth muscles than in tracheal smooth muscles at any MAC tested.

Figure 3 shows the effects of 1 μM nifedipine on carbachol-induced muscle contraction and increase in \([Ca^{2+}]_i\) in tracheal and bronchial smooth muscle strips. Nifedipine significantly decreased the muscle tension and \([Ca^{2+}]_i\) in both tissues (fig. 3A). However, the inhibitory effects on muscle tension and \([Ca^{2+}]_i\) were significantly greater in tracheal smooth muscle tissue (muscle tension by 66 ± 9% and \([Ca^{2+}]_i\) by 97 ± 4%, respec-
tively) than in bronchial smooth muscle tissue (muscle tension by 40 ± 6% and [Ca\(^{2+}\)]\text{;}\) by 78 ± 8%, respectively) (P < 0.01; n = 7; fig. 3B).

Characteristics of Voltage-dependent Ca\(^{2+}\) Channels in Tracheal and Bronchial Smooth Muscle Cells

The I\(_{\text{Ca}}\) observed in porcine tracheal smooth muscle cells during step depolarizations from −80 mV peaked at approximately 10 ms and was inactivated with a time constant of approximately 50–100 ms (fig. 4A). During baseline conditions, threshold activation of I\(_{\text{Ca}}\) occurred at −20 mV, and maximum peak current amplitude was obtained at +10 mV (fig. 4B). The maximum peak I\(_{\text{Ca}}\) was −439 ± 97 pA (range, −249 to −754 pA). As shown in figure 4A (a superimposed trace), voltage steps from a holding potential of −40 mV to +10 mV elicited similar currents. All tracheal smooth muscle cells tested (n = 56) showed these characteristics. The addition of 1 μM nifedipine virtually eliminated the I\(_{\text{Ca}}\) of tracheal smooth muscle cells by 96% (n = 7 each).

The commonly encountered I\(_{\text{Ca}}\)\(_{\text{s}}\) from bronchial smooth muscle cells were similar to the I\(_{\text{Ca}}\) in tracheal smooth muscle cells as shown in figure 4. In this case, the I\(_{\text{Ca}}\) was maximally activated within 5–10 ms after depolarization and inactivated with a time constant of approximately 50–100 ms. The maximum peak I\(_{\text{Ca}}\) was −372 ± 63 pA (range, −196 to −679 pA). In 29% of bronchial smooth muscle cells (24 of 82), however, I\(_{\text{Ca}}\)\(_{\text{s}}\) were evoked at much more negative potentials and had a more complicated time course as illustrated in figure 5. In this case, membrane depolarizations from a holding potential of −80 mV elicited a rapidly inactivating, low-threshold current at a negative potential (−40 to −20 mV), which was maximally activated at 0 mV (fig. 5A). In contrast, as shown in figure 5B, voltage steps from a holding potential of −40 mV in the same cells elicited only a sustained I\(_{\text{Ca}}\) that resembled the long-lasting current elicited at a holding potential of −80 mV. This sustained I\(_{\text{Ca}}\) was activated at −20 mV with peak activation at +10 mV. The differences between the activation thresholds and the kinetics of inactivation between the two current types suggested the presence of two Ca\(^{2+}\) channel types in some of the porcine bronchial smooth muscle cells. The current–voltage relations of the I\(_{\text{Ca}}\)\(_{\text{s}}\) shown in figures 5A and 5B are illustrated in figure 5C. I\(_{\text{Ca}}\)\(_{\text{s}}\) elicited from the holding potential of −80 mV were larger than those elicited from −40 mV. Subtraction of the latter I\(_{\text{Ca}}\) (holding potential of −40 mV) from the former I\(_{\text{Ca}}\) (holding potential of −80 mV) provided the current–voltage relation of the second Ca\(^{2+}\) current, corresponding to the I\(_{\text{Ca}}\) through transient (T-type) VDCs.

We confirmed the presence of two types of Ca\(^{2+}\) channel currents in bronchial smooth muscle cells by pharmacologic identification. Figure 6 illustrates the effect of 1 μM nifedipine, an L-type VDC antagonist, on I\(_{\text{Ca}}\). The L-type I\(_{\text{Ca}}\) was recorded during depolarizing pulses from a holding potential of −40 mV to +10 mV (fig. 6A), whereas the transient I\(_{\text{Ca}}\) was elicited by depolarizing from −80 mV to −20 mV (fig. 6B). Recordings in figures 6A and 6B show that nifedipine decreased the peak amplitude of the L-type I\(_{\text{Ca}}\) by 92 ± 7%, whereas in the same cells it suppressed the T-type I\(_{\text{Ca}}\) by only 14 ± 6% (n = 4).

Effects of Volatile Anesthetics on T- and L-type Ca\(^{2+}\) Channel Currents

Figure 7 shows the effects of 1.5 MAC sevoflurane on whole-cell I\(_{\text{Ca}}\) in tracheal and bronchial smooth muscle cells. To elicit the I\(_{\text{Ca}}\) through L-type VDCs in tracheal and bronchial smooth muscle cells, stepwise depolarizations (−30 to +40 mV) from a holding potential of −40 mV were used. Sevoflurane significantly and similarly inhibited both I\(_{\text{Ca}}\)\(_{\text{s}}\) in tracheal (fig. 7A) and bronchial (fig. 7B) smooth muscle cells without changes in the time course of the currents. The lower figures show the relations...
between peak $I_{Ca}$ and command potential before and after exposure to 1.5 MAC sevoflurane. Sevoflurane significantly inhibited $I_{Ca}$ throughout the voltage range studied. There was no apparent shift in the voltage dependence of induced $I_{Ca}$. As shown in figure 7C, a stepwise depolarization of a bronchial smooth muscle cell from a holding potential of $-80$ mV resulted in the activation of mixed $I_{Ca}$ carried through both L- and T-type VDCs. Sevoflurane decreased $I_{Ca}$ in a bronchial smooth muscle cell without changes in the time course of the currents. Although the anesthetic significantly inhibited $I_{Ca}$ throughout the voltage range studied, there was a +10 mV shift of the peak $I_{Ca}$ versus command potential curve toward more positive potentials (fig. 7C). Isoflurane showed similar inhibitory effects on the $I_{Ca}$ in both tracheal and bronchial smooth muscle cells (data not shown).

We determined the anesthetic potency dependence of the inhibition of peak $I_{Ca}$ by each of these volatile anesthetics. The inhibitions of peak $I_{Ca}$ through T-type VDCs in bronchial smooth muscle cells were obtained by digital subtraction of the currents obtained at a holding potential of $-40$ mV from total currents elicited from an HP of $-80$ mV. A rapidly decaying $I_{Ca}$ was elicited at $-40$ to $-20$ mV, whereas a long-lasting type of $I_{Ca}$ was observed at +10 to +30 mV. (B) $I_{Ca}$ in response to stepwise depolarizing pulses from an HP of $-40$ mV. Only the long-lasting type of $I_{Ca}$ was recorded. The dashed line denotes zero current. (C) Peak current-voltage relations were plotted for total $I_{Ca}$ from an HP of $-80$ mV (closed circles), for L-type $I_{Ca}$ from an HP of $-40$ mV (closed squares), and for T-type $I_{Ca}$ by subtraction of L-type $I_{Ca}$ from total $I_{Ca}$ (open circles). Symbols represent mean ± SD; $n = 7$.

Fig. 4. Effects of 1 μM nifedipine on whole-cell inward Ca$^{2+}$ currents ($I_{Ca}$) in tracheal smooth muscle cells. $I_{Ca}$ were elicited from a holding potential (HP) of $-80$ and $-40$ mV. The dashed line denotes zero current. Symbols represent the mean ± SD; $n = 7$ each; $* P < 0.05$ versus control. These characteristics of $I_{Ca}$ are consistent with L-type voltage-dependent Ca$^{2+}$ channels.

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Fig. 5. Two types of inward Ca$^{2+}$ channel currents ($I_{Ca}$) in single porcine bronchial smooth muscle cells separated by depolarizing pulses from two holding potentials (HPs) ($-80$ and $-40$ mV). (A) $I_{Ca}$ in response to stepwise depolarizing pulses from an HP of $-80$ mV. A rapidly decaying $I_{Ca}$ was elicited at $-40$ to $-20$ mV, whereas a long-lasting type of $I_{Ca}$ was observed at +10 to +30 mV. (B) $I_{Ca}$ in response to stepwise depolarizing pulses from an HP of $-40$ mV. Only the long-lasting type of $I_{Ca}$ was recorded. The dashed line denotes zero current. (C) Peak current-voltage relations were plotted for total $I_{Ca}$ from an HP of $-80$ mV (closed circles), for L-type $I_{Ca}$ from an HP of $-40$ mV (closed squares), and for T-type $I_{Ca}$ by subtraction of L-type $I_{Ca}$ from total $I_{Ca}$ (open circles). Symbols represent mean ± SD; $n = 7$. 

We determined the anesthetic potency dependence of the inhibition of peak $I_{Ca}$ by each of these volatile anesthetics. The inhibitions of peak $I_{Ca}$ through T-type VDCs in bronchial smooth muscle cells were obtained by digital subtraction of the currents obtained at a holding potential of $-40$ mV from total currents elicited from a holding potential of $-80$ mV in the same cells. Figures 8A and 8B show the relations between the percent control peak $I_{Ca}$ and the anesthetic potencies (MAC). Each of the two volatile anesthetics significantly inhibited peak $I_{Ca}$ in a dose-dependent manner. Based on the anesthetic potencies, sevoflurane required somewhat greater concentrations to achieve the same inhibitory effect as that of isoflurane. There was no significant difference between tracheal and bronchial smooth muscles in the inhibitory effects on the $I_{Ca}$ through T-type VDCs; however, both anesthetics induced greater inhibitory effects on $I_{Ca}$ through T-type VDCs in bronchial smooth muscles than they did through L-type $I_{Ca}$ in either tissue at each MAC tested ($P < 0.05$; n = 7).
The results obtained by using the fluorescence technique in the current study indicate that a dihydropyridine-sensitive and volatile anesthetic-sensitive pathway of Ca\(^{2+}\) influx may exist in porcine distal airway smooth muscle. To investigate this mechanism, the I_{Ca} activities through VDCs in smooth muscle cells obtained from porcine tracheae and bronchi were evaluated using the whole-cell patch clamp techniques.

**Electric Properties of Inward Ca\(^{2+}\) Currents in Tracheal and Bronchial Smooth Muscle**

As has previously been reported in porcine and canine tracheal smooth muscle cells, we measured depolarization-induced I_{Ca} in freshly dispersed porcine tracheal smooth muscle cells under ionic conditions designed to inhibit K\(^{+}\)/Na\(^{+}\) currents and to enhance Ca\(^{2+}\) currents. These I_{Ca} showed a threshold and peak activation at −20 mV and +10 mV, respectively (fig. 4). The inactivation parameters V_{1/2} and k were −17.6 and
7.6 mV, respectively (table 1). Based on their time and voltage dependences and their sensitivity to blockade by nifedipine (fig. 4), these currents are presumed to reflect the activity of L-type VDCs.35

The commonly encountered I_{Ca}S from bronchial smooth muscle cells (approximately 70%) were similar to those seen in tracheal smooth muscle cells. Even when the cells were depolarized from a holding potential of either −80 or −40 mV, we observed similar I_{Ca}, indicating that these bronchial smooth muscle cells have only L-type VDCs.17,24 The rest of the bronchial smooth muscle cells (approximately 30%), however, showed different characteristics of I_{Ca} during stepwise depolarizations from a holding potential of −80 mV (fig. 5A). As shown in figures 5A and 5C, the I_{Ca}S were evoked at much more negative potentials and showed a rapidly inactivating current at a negative potential (−250 to −20 mV). Voltage steps from a holding potential of −40 mV in the same cells elicited only sustained I_{Ca}S that resembled the L-type I_{Ca} (fig. 5B). Subtraction of the latter I_{Ca} (holding potential of −40 mV) from the former I_{Ca} (holding potential of −80 mV) provided the I-V relation of the second Ca^{2+} current (fig. 5C). This second current was first noted at −50 or −40 mV with maximum activation at −10 mV. Inactivation parameters V_{1/2} and k were −58.7 and 6.1 mV, respectively (table 1), and these currents were insensitive to nifedipine (fig. 6B). These characteristics are consistent with T-type VDCs.36

The results from the current experiments showed that two types (L- and T-types) of VDCs coexist in some porcine bronchial smooth muscle cells; however, no evidence of the existence of a second VDC in tracheal smooth muscle was obtained. It seems that the presence of T-type VDCs in approximately 30% of bronchial smooth muscle cells cannot completely explain the very different responses to nifedipine in tracheal and bronchial smooth muscles. T-type VDC has been suggested to play a prominent role in the initiation of action potentials rather than in [Ca^{2+}]_{i} homeostasis in other tissues because of their transient opening time and their small conductance.36 However, T-type VDCs induced a window current at potentials ranging from −50 to −10 mV. Depolarization of the cell membrane at potentials in this range would lead to a persistent Ca^{2+} influx through these T-type VDCs, which in turn could contribute to excitation–contraction coupling as well as refilling of the internal Ca^{2+} stores.37,38 Furthermore, T-type I_{Ca}S are not suppressed during agonist stimulation,17 as is the case for L-type I_{Ca}S.39,40 The small size of the T-type I_{Ca} does not necessarily lessen the possible importance of their contribution to excitation. Accordingly, it is possible that the different responses to nifedipine between...
These channels are partly caused by the different electric properties of VDCs in tracheal and bronchial smooth muscles. In addition, as suggested by Croxton et al., another pathway of dihydropyridine-insensitive receptor-operated Ca\(^{2+}\) channels may also play a role in the different responses to nifedipine between tracheal and bronchial smooth muscles, although no evidence suggests that the Ca\(^{2+}\) influx through receptor-operated Ca\(^{2+}\) channels are different in these airway smooth muscle tissues.

Effects of Volatile Anesthetics on T- and L-type Voltage-dependent Ca\(^{2+}\) Channel Activities

Since we confirmed that two types of VDCs coexist in some porcine bronchial smooth muscle cells, we evaluated the inhibitory effects of the volatile anesthetics isoflurane and sevoflurane on L-type \(I_{\text{L}}\) in tracheal and bronchial smooth muscle cells and on T-type \(I_{\text{T}}\) in bronchial smooth muscle cells separately. Unlike the classical Ca\(^{2+}\) channel antagonist nifedipine, which has a much greater inhibitory effect on L-type than on T-type \(I_{\text{T}}\) (fig. 6), both isoflurane and sevoflurane had a greater inhibitory effect on T-type \(I_{\text{T}}\) than on L-type \(I_{\text{L}}\) (figs. 7 and 8). These results are consistent with the results of experiments in which muscle tension and \([\text{Ca}^{2+}]_i\) were measured (figs. 1 and 2). A series of investigations has been conducted to examine the possible actions of volatile anesthetics on different types of VDCs, and it has been shown that the activities of both L- and T-type VDCs in cardiac Purkinje cells appeared to be approximately equally suppressed by halothane, isoflurane, and enfurane. In clonal (GH3) pituitary cells, however, it has been found that there were different sensitivities to the reduction by halothane between T- and L-type VDCs activities. Recent molecular studies have revealed structural heterogeneity between VDCs of different tissues, suggesting that these apparent discrepancies may result from the differences of cell types and species or the experimental conditions used. Taking our current findings into account, there seems to be some information concerning the actions of volatile anesthetics at the level of membrane-associated channels, although it remains to be determined whether the action of the anesthetics on Ca\(^{2+}\) channels is a direct effect on the channel proteins or whether it is a secondary consequence of, for example, alterations in membrane lipids.

Because of the different properties, such as resting membrane potential, of the distal and proximal airway smooth muscles, the different responses of volatile anesthetics on distal and proximal airway smooth muscles is also likely, in part, to be caused by the different effects of the anesthetics on the common L-type VDCs, which are identified extensively in airway smooth muscle. The absolute magnitude of the resting membrane potential is greater in the bronchus (−70 mV) than in the trachea (−60 mV), and this difference is thought to be caused by reduced Na\(^+\) permeability, not to an increase in K\(^+\) permeability. However, we have obtained direct evidence that the inhibitory effects of volatile anesthetics on L-type \(I_{\text{L}}\) in bronchial and tracheal smooth muscles are not different (fig. 8). Therefore, the
substantial inhibitory effects of the anesthetics on T-type VDC activity could, at least in part, be caused by the fact that bronchial smooth muscle is more sensitive to volatile anesthetics than is tracheal smooth muscle.

To further examine the inhibitory actions of these volatile anesthetics on VDCs of tracheal (L-type) and bronchial (T- and L-type) smooth muscle cells, we studied the effects of these anesthetics on steady state, voltage-dependent inactivation of ICas. During prolonged depolarization, a fraction of the VDCs enters an unavailable or “inactivated” state. The degree of steady state inactivation depends on the prepulse potential (fig. 9). Each of the two volatile anesthetics tested significantly shifted the inactivation curves to more negative potentials without changing the sigmoid shapes of the curves. A qualitatively similar shift induced by some dihydropyridine-sensitive Ca2+ channel antagonists in porcine tracheal smooth muscle cells has been interpreted as evidence of drug-induced stabilization of the inactivated state.47 There were no significant differences in the shift of the inactivation curve either between tracheal and bronchial smooth muscles L-type VDCs or between T- and L-type VDCs of bronchial smooth muscle.

In conclusion, the volatile anesthetics isoflurane and sevoflurane at clinically relevant potencies had greater inhibitory effects on carbachol-induced bronchial smooth muscle contraction than on tracheal smooth muscle contraction. These inhibitory effects on muscle tension induced by the anesthetics were parallel to the inhibitory effects on [Ca2+]i, indicating that the anesthetics have different effects on the Ca2+ influx through VDCs. Although tracheal smooth muscle cells have only L-type VDCs, we have found some bronchial smooth muscle cells (~30%) that have T-type as well as L-type VDCs. Each of the two volatile anesthetics significantly inhibited the activities of both types of VDC in a dose-dependent manner; however, the anesthetics had greater inhibitory effects on T-type VDC activity in bronchial smooth muscle. The existence of the T-type VDC in bronchial smooth muscle and the high sensitivity of this

![Fig. 9. Effects of the volatile anesthetics isoflurane (1.0 minimum alveolar concentration [MAC]) and sevoflurane (1.5 MAC) at equi-effective inhibitory potencies on the inactivation curves of inward Ca2+ currents. The inactivation curve for the T-type Ca2+ current in bronchial smooth muscle cells (C) was obtained by using nifedipine (1 μM) to block the L-type Ca2+ current. Symbols represent mean ± SD; n = 7. Each of the anesthetics significantly shifted the inactivation curve to more negative potentials in either type of airway smooth muscle tissues or in either type of voltage-dependent Ca2+ channels. There was no significant difference in the change of the potential of half inactivation (V1/2) either between the anesthetics or between types of voltage-dependent Ca2+ channels.](image)

Table 1. Effects of the Volatile Anesthetics Isoflurane and Sevoflurane on the Inactivation Parameters of Inward Whole Cell Ca2+ Currents

<table>
<thead>
<tr>
<th></th>
<th>Trachea (L Type)</th>
<th>Bronchus (L Type)</th>
<th>Bronchus (T Type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent inhibition of peak ICa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoflurane (1.0 MAC)</td>
<td>73.1 ± 3.3</td>
<td>70.8 ± 2.5</td>
<td>61.0 ± 4.5</td>
</tr>
<tr>
<td>Sevoflurane (1.5 MAC)</td>
<td>73.5 ± 3.0</td>
<td>71.2 ± 6.3</td>
<td>59.5 ± 3.6</td>
</tr>
<tr>
<td>Potential of half inactivation (V1/2, mV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>−17.6 ± 2.9</td>
<td>−16.9 ± 1.5</td>
<td>−58.7 ± 4.2†</td>
</tr>
<tr>
<td>Isoflurane (1.0 MAC)</td>
<td>−25.2 ± 2.3*</td>
<td>−25.6 ± 2.7*</td>
<td>−71.4 ± 3.1†</td>
</tr>
<tr>
<td>Sevoflurane (1.5 MAC)</td>
<td>−26.1 ± 3.2*</td>
<td>−25.4 ± 2.2†</td>
<td>−73.4 ± 4.6†</td>
</tr>
<tr>
<td>Slope factor (k, mV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.6 ± 0.5</td>
<td>7.4 ± 0.6</td>
<td>6.1 ± 0.4†</td>
</tr>
<tr>
<td>Isoflurane (1.0 MAC)</td>
<td>7.7 ± 0.3</td>
<td>7.3 ± 0.5</td>
<td>6.2 ± 0.7†</td>
</tr>
<tr>
<td>Sevoflurane (1.5 MAC)</td>
<td>7.5 ± 0.5</td>
<td>7.5 ± 0.8</td>
<td>6.4 ± 0.6†</td>
</tr>
</tbody>
</table>

* P < 0.05, t test comparison with control. † P < 0.05, Fisher test comparison with other types of tissues or channels.

MAC = minimum alveolar concentration.
channel to volatile anesthetics seem to be, at least in part, responsible for the different reactivities to the anesthetics in tracheal and bronchial smooth muscles.

References

22. Isenberg G, Klockner U: Calcium tolerant ventricular myocytes prepared by preincubation in a “KB medium.” Pflugers Arch 1982; 395:6–18
27. van Bremmen C: Cellular mechanisms regulating [Ca²⁺], smooth muscle. Annu Rev Physiol 1989; 51:315–29

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