

## Different Inhibitory Effects of Volatile Anesthetics on T- and L-type Voltage-dependent $Ca^{2+}$ Channels in Porcine Tracheal and Bronchial Smooth Muscles

Michiaki Yamakage, M.D., Ph.D.,\* Xiangdong Chen, M.D.,† Naoki Tsujiguchi, M.D.,‡ Yasuhiro Kamada, M.D.,§ Akiyoshi Namiki, M.D., Ph.D.||

**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  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) and by measuring inward  $Ca^{2+}$  currents ( $I_{Ca}$ ) through voltage-dependent  $Ca^{2+}$  channels (VDCs).

**Methods:** Intracellular concentration of free  $Ca^{2+}$  was monitored by the 500-nm light emission ratio of  $Ca^{2+}$  indicator fura-2. Isometric tension was measured simultaneously. Whole-cell patch clamp recording techniques were used to investigate the effects of volatile anesthetics on  $I_{Ca}$  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  $[Ca^{2+}]_i$ . 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.<sup>1–3</sup> The direct effects of these anesthetics on airway smooth muscle are thought to be ultimately caused by a decrease in intracellular concentration of free  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ),<sup>2,3</sup> a primary regulator of smooth muscle tone.<sup>4</sup> This decrease is, in part, a result of a

blockade of  $Ca^{2+}$  influx through L-type voltage-dependent  $Ca^{2+}$  channels (VDCs).<sup>5</sup> In studies performed *in vivo*,<sup>6,7</sup> variations in airway resistance in the larger, more proximal airway, but not the more distal airway,<sup>8</sup> have been evaluated. In studies performed *in vitro*,<sup>9</sup> 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,<sup>10,11</sup> and a series of studies have shown that there were significant physiological and pharmacologic differences between tracheal and bronchial smooth muscles.<sup>12,13</sup> Mazzeo *et al.*<sup>14,15</sup> 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.*<sup>16</sup> 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  $Ca^{2+}$  entry in tracheal smooth muscle. Recently, Janssen<sup>17</sup> 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 anesthetics<sup>14,15</sup> and to dihydropyridine-sensitive VDC antagonists in proximal and distal airway smooth muscles.<sup>16,18</sup>

This study was conducted to test this hypothesis by simultaneously measuring porcine tracheal or bronchial (third to fifth generation) smooth muscle tension and  $[Ca^{2+}]_i$  using the fluorescence technique<sup>2,4</sup> and by measuring inward  $Ca^{2+}$  currents through VDCs ( $I_{Ca}$ ) using patch clamp techniques.<sup>5,19</sup> We also investigated the inhibitory effects of the volatile anesthetics isoflurane and sevoflurane on these muscle tones with changes in  $[Ca^{2+}]_i$  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-

\* Assistant Professor, † Visiting Research Fellow, ‡ Research Fellow, § Postgraduate Student, || Professor and Chairman.

Received from the Department of Anesthesiology, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan. Submitted for publication August 31, 2000. Accepted for publication December 4, 2000. Supported in part by grant-in-aid No. 12671489 for research from the Ministry of Education, Science, Sports and Culture, Tokyo, Japan, and Incentive grant No. Y-58 for research from Hokksaitac Promotion Association Grant, Sapporo, Japan. Presented in part at the annual meeting of the American Society of Anesthesiologists, San Francisco, California, October 14–18, 2000.

Address reprint requests to Dr. Yamakage: Department of Anesthesiology, Sapporo Medical University School of Medicine, South 1, West 16, Chuo-ku, Sapporo, Hokkaido 060-8543, Japan. Address electronic mail to: yamakage@sapmed.ac.jp. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

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 bicarbonated solution aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. 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.<sup>20</sup>

### *Simultaneous Measurement of Muscle*

#### *Tension and [Ca<sup>2+</sup>]<sub>i</sub>*

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 Ca<sup>2+</sup>, 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 CaCl<sub>2</sub>, 1.0 mM MgCl<sub>2</sub>, 23.9 mM NaHCO<sub>3</sub>, 5.5 mM glucose, and 0.01 mM EDTA. This solution was saturated with a gas mixture of 95% O<sub>2</sub>–5% CO<sub>2</sub> 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 (LVS-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 ( $R_{340/380}$ ) was calculated and used as an indicator of [Ca<sup>2+</sup>]<sub>i</sub>.<sup>2,4</sup>

Physiological salt solution aerated with 95% O<sub>2</sub>–5% CO<sub>2</sub> 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.<sup>2,14–16</sup> Both tissues were contracted with submaximal effect (~ED<sub>95</sub>) 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 pig<sup>21</sup>) or sevoflurane (0.5 [1.4%], 1.0 [2.8%], 1.5 [4.2%]

MAC in the pig<sup>22</sup>). 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 Ca<sup>2+</sup>*

#### *Channel Activity*

We used conventional whole-cell patch clamp techniques<sup>19</sup> to observe inward Ca<sup>2+</sup> currents (I<sub>Ca</sub>) through VDCs. Tracheal and bronchial smooth muscle tissues were minced and digested for 20 min at 37°C in Ca<sup>2+</sup>-free Tyrode solution to which 0.08% (wt/vol) collagenase was added.<sup>5</sup> Cells were dispersed by trituration, filtered through nylon mesh, and centrifuged. The pellet was resuspended in a modified Kraftbrühe solution<sup>23</sup> and stored at 4°C for up to 5 h before use. The modified Kraftbrühe solution contained 85 mM KCl, 30 mM K<sub>2</sub>HPO<sub>4</sub>, 5.0 mM MgSO<sub>4</sub>, 5.0 mM Na<sub>2</sub>ATP, 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<sup>+</sup>–K<sup>+</sup> currents and enhance Ca<sup>2+</sup> currents. The pipette solution contained 130 mM CsCl, 4.0 mM MgCl<sub>2</sub>, 10 mM EGTA, 5.0 mM Na<sub>2</sub>ATP, and 10 mM HEPES (pH adjusted to 7.2 with Tris). The bath solution contained 130 mM tetraethylammonium chloride, 1.0 mM MgCl<sub>2</sub>, 10 mM CaCl<sub>2</sub>, 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 Ca<sup>2+</sup> 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 I<sub>Ca</sub> through L-type VDCs.<sup>17,24</sup> I<sub>Ca</sub> through T-type VDCs was obtained by digital subtraction of I<sub>Ca</sub> obtained at a holding potential of –40 mV from total I<sub>Ca</sub> elicited from a holding potential of –80 mV in the same cell.<sup>24</sup> We also confirmed the presence of two

types of  $I_{\text{CaS}}$  in these cells by pharmacologic identification. The  $\text{Ca}^{2+}$  channel blockers with a dihydropyridine structure in these cells are relatively selective for the  $I_{\text{Ca}}$  through L-type VDCs.<sup>17,18</sup>

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.<sup>25</sup> 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  $\text{Ca}^{2+}$  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.<sup>17</sup> 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 ( $k$ ).<sup>26</sup> Similar to the  $I_{\text{Ca}}$  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.<sup>25</sup> 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  $37^\circ\text{C}$  (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).

#### *Materials*

The following drugs and chemicals were used:  $\beta$ -hydroxybutyrate, cremophor EL, fatty acid-free bovine serum albumin,  $\text{Na}_2\text{ATP}$ , pyruvic acid, creatine, taurine, nifedipine, EGTA (Sigma Chemical, St. Louis, MO), acetoxyethyl ester of fura-2, (Dojindo, Kumamoto, Japan), EDTA (Katayama, Osaka, Japan), 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  $\pm$  SD. For the measurement of  $[\text{Ca}^{2+}]_i$  and muscle tension, carbachol-induced sustained changes in  $[\text{Ca}^{2+}]_i$  (indicated by  $R_{340/380}$ ) and muscle tension were used as references (100%). Changes in measured parameters with exposure to each anesthetic were compared at each point (concentrations or applied potential) using the paired two-tailed  $t$  test. One-way analysis of variance for repeated measurements and the Fisher exact test were used to determine the concentration-dependent effects. In all comparisons,  $P < 0.05$  was considered to be significant.

## Results

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

As has been reported previously,<sup>2,27</sup>  $R_{340/380}$ , an indicator of  $[\text{Ca}^{2+}]_i$ , was rapidly increased by  $1 \mu\text{M}$  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  $[\text{Ca}^{2+}]_i$ . In a bronchial smooth muscle strip (fig. 1B), carbachol similarly increased  $R_{340/380}$  with a concomitant contraction; however, the maximum tension ( $1.1 \pm 0.3$  g) was significantly lower than that ( $4.0 \pm 0.7$  g) of tracheal smooth muscle strips. Isoflurane (1.5 MAC) similarly and significantly decreased both muscle tension and  $[\text{Ca}^{2+}]_i$  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  $[\text{Ca}^{2+}]_i$  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  $[\text{Ca}^{2+}]_i$  are shown in figure 2. In both tracheal and bronchial smooth muscle tissues, the volatile anesthetics tested significantly decreased muscle tension and  $[\text{Ca}^{2+}]_i$  in a dose-dependent manner, and there were no significant differences between these anesthetics in the inhibitory potencies on both muscle tension and  $[\text{Ca}^{2+}]_i$ . The inhibitory effects by these anesthetics were, however,

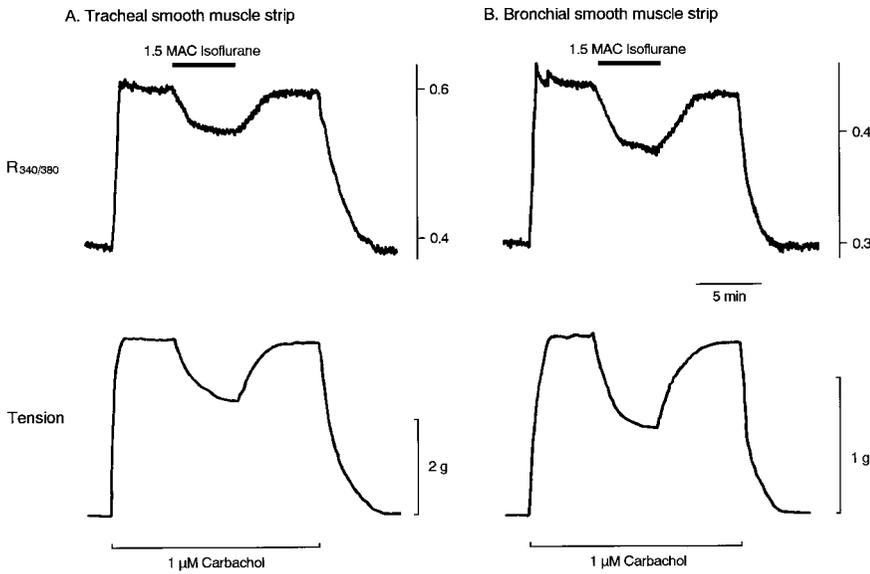


Fig. 1. Representative data of the effects of 1.5 minimum alveolar concentration (MAC) of isoflurane on muscle tension and intracellular concentration of free  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) (indicated by  $R_{340/380}$ ) in  $1 \mu M$  carbachol-stimulated tracheal and bronchial smooth muscles.

significantly greater in bronchial smooth muscles than in tracheal smooth muscles at any MAC tested.

Figure 3 shows the effects of  $1 \mu M$  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  $66 \pm 9\%$  and  $[Ca^{2+}]_i$  by  $97 \pm 4\%$ , respec-

A. Isoflurane

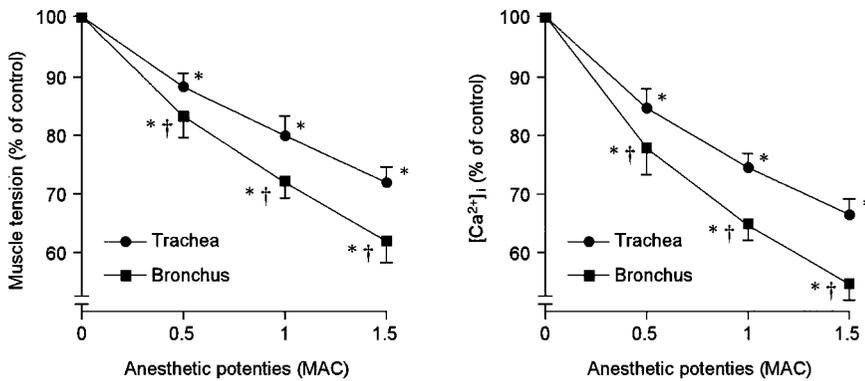
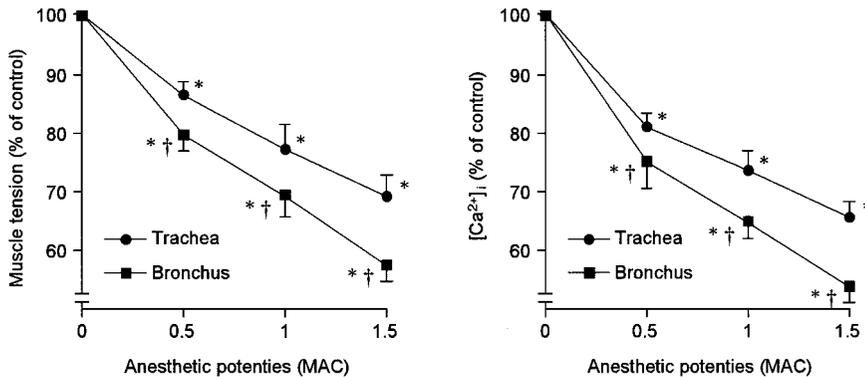
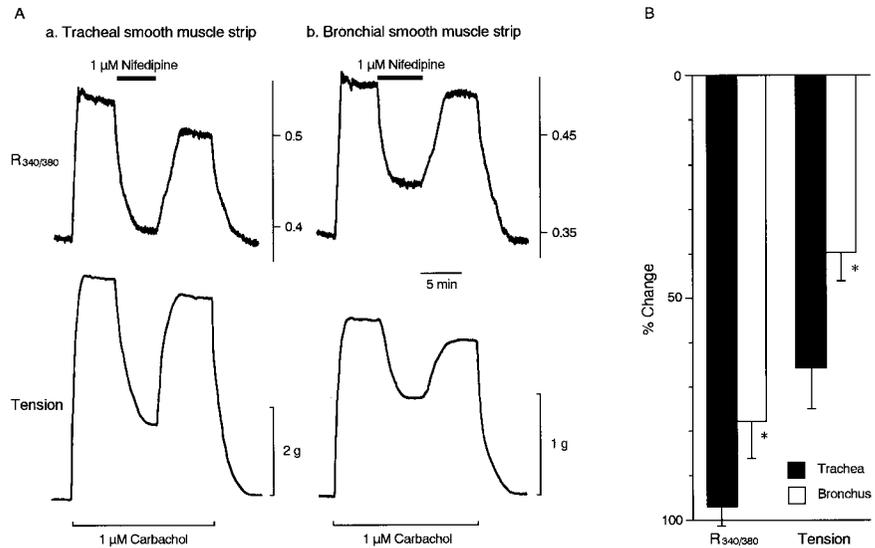


Fig. 2. Relations between anesthetic potencies (minimum alveolar concentration [MAC]) and percentage of response of muscle tension or intracellular concentration of free  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) (indicated by  $R_{340/380}$ ) in tracheal and bronchial smooth muscles. The volatile anesthetics tested had significantly greater inhibitory effects on bronchial smooth muscles than on tracheal smooth muscles. Symbols represent the mean  $\pm$  SD;  $n = 7$ ; \* $P < 0.05$  versus 0 MAC anesthetic; † $P < 0.05$  versus tracheal smooth muscle.

B. Sevoflurane



**Fig. 3.** Effects of 1  $\mu\text{M}$  nifedipine on muscle tension and intracellular concentration of free  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) (indicated by  $R_{340/380}$ ) in carbachol-stimulated tracheal and bronchial smooth muscles. The inhibitory effects on the muscle tension and  $[\text{Ca}^{2+}]_i$  were significantly greater in tracheal smooth muscles than in bronchial smooth muscles. Bars represent the mean  $\pm$  SD;  $n = 7$ ; \* $P < 0.05$  versus tracheal smooth muscle.



tively) than in bronchial smooth muscle tissue (muscle tension by  $40 \pm 6\%$  and  $[\text{Ca}^{2+}]_i$  by  $78 \pm 8\%$ , respectively) ( $P < 0.01$ ;  $n = 7$ ; fig. 3B).

#### Characteristics of Voltage-dependent $\text{Ca}^{2+}$ 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  $-439 \pm 97$  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  $\mu\text{M}$  nifedipine virtually eliminated the  $I_{\text{Ca}}$  of tracheal smooth muscle cells by 96% ( $n = 7$  each).

The commonly encountered  $I_{\text{Ca}}$ 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  $-372 \pm 63$  pA (range,  $-196$  to  $-679$  pA). In 29% of bronchial smooth muscle cells (24 of 82), however,  $I_{\text{Ca}}$ 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  $\text{Ca}^{2+}$  channel types in some of the porcine bronchial smooth muscle cells. The current-voltage relations of the  $I_{\text{Ca}}$ s shown in figures 5A and 5B are illustrated in figure 5C.  $I_{\text{Ca}}$ 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  $\text{Ca}^{2+}$  current, corresponding to the  $I_{\text{Ca}}$  through transient (T-type) VDCs.<sup>17,24</sup>

We confirmed the presence of two types of  $\text{Ca}^{2+}$  channel currents in bronchial smooth muscle cells by pharmacologic identification. Figure 6 illustrates the effect of 1  $\mu\text{M}$  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  $92 \pm 7\%$ , whereas in the same cells it suppressed the T-type  $I_{\text{Ca}}$  by only  $14 \pm 6\%$  ( $n = 4$ ).

#### Effects of Volatile Anesthetics on T- and L-type $\text{Ca}^{2+}$ 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.<sup>17,24</sup> Sevoflurane significantly and similarly inhibited both  $I_{\text{Ca}}$ 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

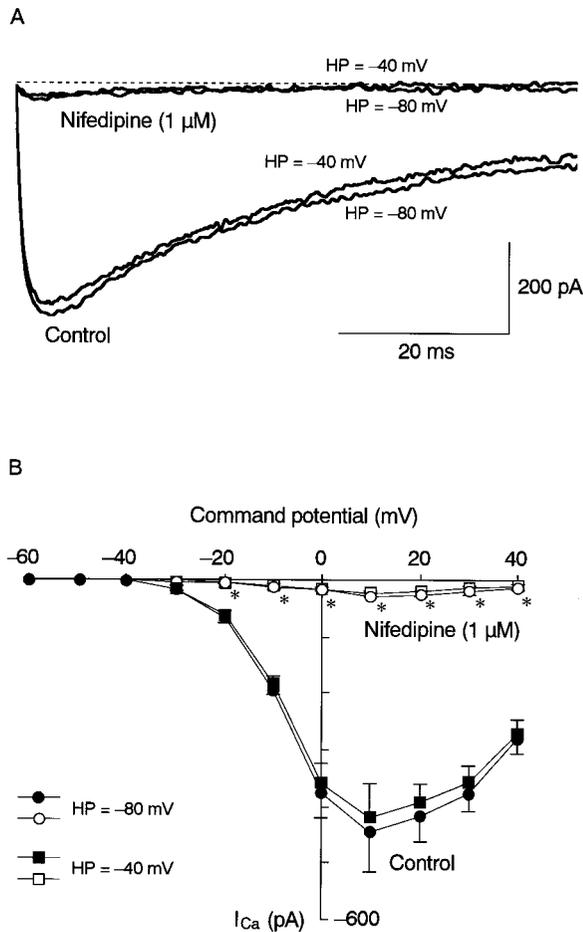


Fig. 4. Effects of 1  $\mu$ M nifedipine on whole-cell inward  $Ca^{2+}$  currents ( $I_{Ca}$ s) in tracheal smooth muscle cells.  $I_{Ca}$ s were elicited from a holding potential (HP) of -80 and -40 mV. The dashed line denotes zero current. Symbols represent the mean  $\pm$  SD;  $n = 7$  each; \*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

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  $I_{Ca}$ s 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 dig-

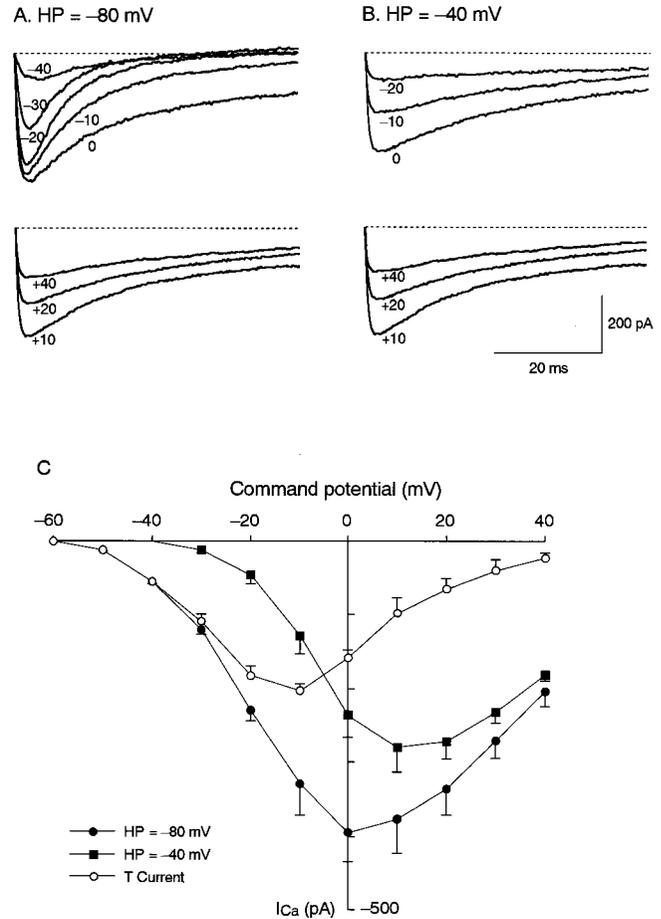


Fig. 5. Two types of inward  $Ca^{2+}$  channel currents ( $I_{Ca}$ s) in single porcine bronchial smooth muscle cells separated by depolarizing pulses from two holding potentials (HPs) (-80 and -40 mV). (A)  $I_{Ca}$ s in response to stepwise depolarizing pulses from an HP of -80 mV. A rapidly decaying  $I_{Ca}$  was elicited at -40 to -20 mV, whereas a long-lasting type of  $I_{Ca}$  was observed at +10 to +30 mV. (B)  $I_{Ca}$ s 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  $\pm$  SD;  $n = 7$ .

ital 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 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 L-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  $I_{Ca}$ s in either tissue at each MAC tested ( $P < 0.05$ ;  $n = 7$ ).

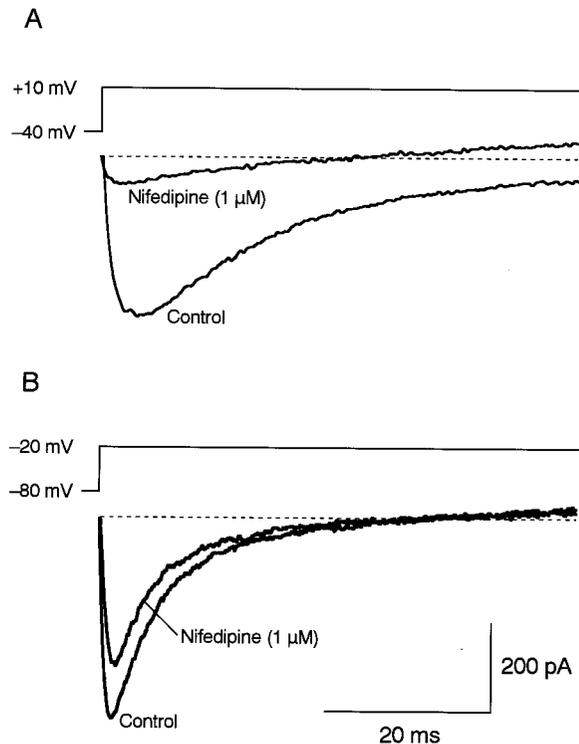


Fig. 6. Superimposed tracings showing the effects of  $1 \mu\text{M}$  nifedipine on the long-lasting (L-type) and transient (T-type)  $\text{I}_{\text{Ca}}$ s in porcine bronchial smooth muscle cells. The L-type  $\text{I}_{\text{Ca}}$  was elicited by depolarizing the cell from a holding potential of  $-40$  to  $+10$  mV (top). T-type  $\text{I}_{\text{Ca}}$  was elicited by depolarizing the cell from  $-80$  to  $-10$  mV (bottom). The dashed line denotes zero current.

The effects of the volatile anesthetics isoflurane and sevoflurane at equi-effective inhibitory potencies (1.0 and 1.5 MAC, respectively) on the inactivation curves of  $\text{I}_{\text{Ca}}$ s are summarized in figure 9 and table 1. The inactivation curve for T-type  $\text{Ca}^{2+}$  current in bronchial smooth muscle cells was obtained by using nifedipine ( $1 \mu\text{M}$ ) to block the  $\text{Ca}^{2+}$  current through L-type VDCs before examining the voltage dependence of inactivation (fig. 9C). Each of these anesthetics shifted the inactivation curve to more negative potentials in either type of airway smooth muscle tissues or in either type of VDCs. The induced changes in  $V_{1/2}$  were indistinguishable between tracheal and bronchial smooth muscle tissues or between T-type and L-type VDCs. The slope factor,  $k$ , was not changed by exposure to each anesthetic.

## Discussion

### *Effects of Volatile Anesthetics and Nifedipine on Muscle Tension and $[\text{Ca}^{2+}]_i$ in Tracheal and Bronchial Smooth Muscles*

One of the major findings of our study is that both isoflurane and sevoflurane had greater inhibitory effects on agonist-induced bronchial smooth muscle contrac-

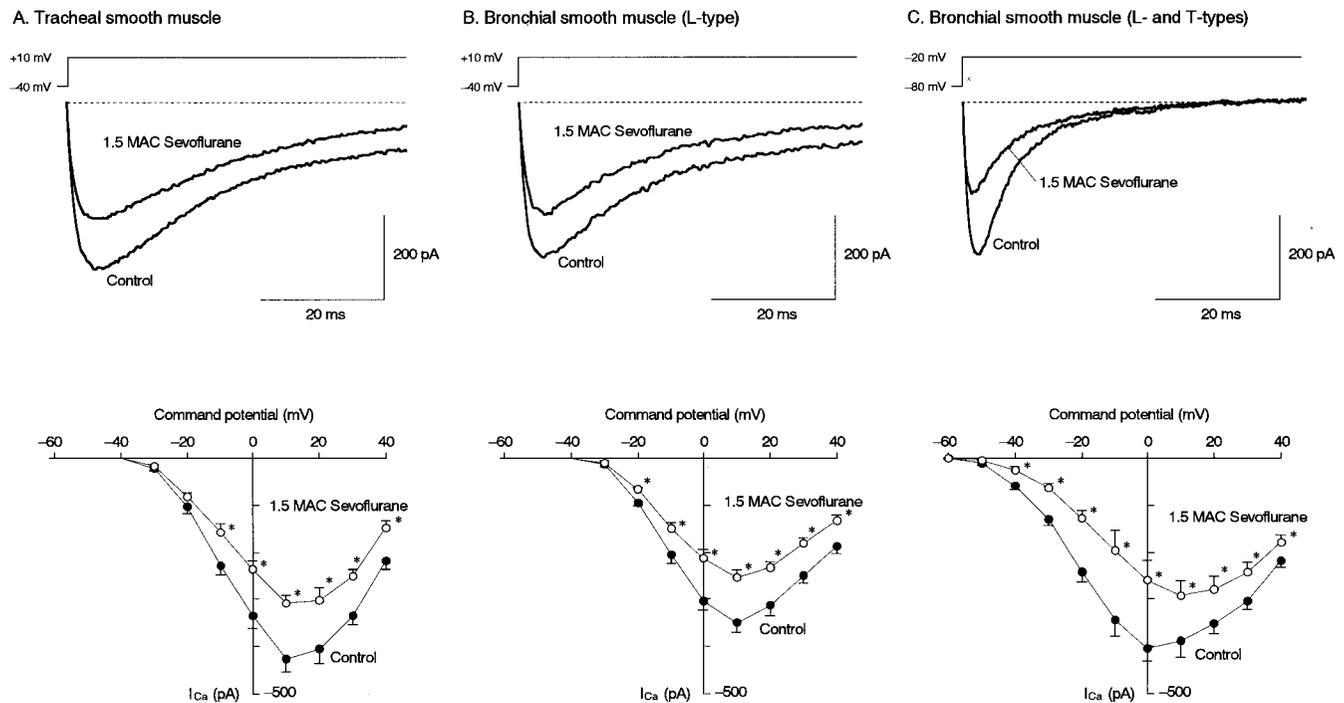
tion than on tracheal smooth muscle contraction (figs. 1 and 2). This result is consistent with that by Mazzeo *et al.*<sup>14,15</sup> These inhibitory effects by the anesthetics on muscle tension were parallel to the inhibitory effects on  $[\text{Ca}^{2+}]_i$ ,  $[\text{Ca}^{2+}]_i$ , which plays a central role in the regulation of airway smooth muscle tone,<sup>4</sup> is regulated by the influx of  $\text{Ca}^{2+}$  through membrane-associated  $\text{Ca}^{2+}$  channels (VDCs and voltage-independent receptor-operated  $\text{Ca}^{2+}$  channels) and by the release of  $\text{Ca}^{2+}$  from intracellular  $\text{Ca}^{2+}$  stores.<sup>28,29</sup> Entry of extracellular  $\text{Ca}^{2+}$  through VDCs is necessary for maintenance of the contraction of airway smooth muscle.<sup>2,4,28-30</sup> Our results therefore indicated that volatile anesthetics had different effects on the  $\text{Ca}^{2+}$  influx through VDCs in tracheal and bronchial smooth muscles.

There are two possible reasons for these different effects on  $\text{Ca}^{2+}$  influx by volatile anesthetics. The anesthetics could have different inhibitory effects on the same type of VDCs, or the anesthetics could have different inhibitory effects on different subtypes of VDCs in tracheal and bronchial smooth muscles. Comparison of nifedipine-induced relaxation responses from tracheal and bronchial smooth muscle preparations contracted by carbachol (fig. 3) suggested that the two tissues responded differently to the dihydropyridine-sensitive (L-type)  $\text{Ca}^{2+}$  channel antagonist. This result is similar to the finding of Croxton *et al.*,<sup>16</sup> and it is consistent with the clinical observations that a dihydropyridine-sensitive  $\text{Ca}^{2+}$  channel antagonist was ineffective in reversing bronchospasm in individuals with asthma.<sup>31,32</sup> We also measured changes in  $[\text{Ca}^{2+}]_i$  with muscle tension and found that the inhibitory effects on muscle tension by nifedipine were parallel to the inhibitory effects on  $[\text{Ca}^{2+}]_i$ . In tracheal smooth muscle,  $1 \mu\text{M}$  nifedipine decreased  $[\text{Ca}^{2+}]_i$  by 97%, whereas the L-type VDC antagonist decreased it by only 78% in bronchial smooth muscle.

The results obtained by using the fluorescence technique in the current study indicate that a dihydropyridine-insensitive and volatile anesthetic-sensitive pathway of  $\text{Ca}^{2+}$  influx may exist in porcine distal airway smooth muscle. To investigate this mechanism, the  $\text{I}_{\text{Ca}}$  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 $\text{Ca}^{2+}$ Currents in Tracheal and Bronchial Smooth Muscle*

As has previously been reported in porcine<sup>5,33</sup> and canine<sup>25,34</sup> tracheal smooth muscle cells, we measured depolarization-induced  $\text{I}_{\text{Ca}}$  in freshly dispersed porcine tracheal smooth muscle cells under ionic conditions designed to inhibit  $\text{K}^+/\text{Na}^+$  currents and to enhance  $\text{Ca}^{2+}$  currents. These  $\text{I}_{\text{Ca}}$ s 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



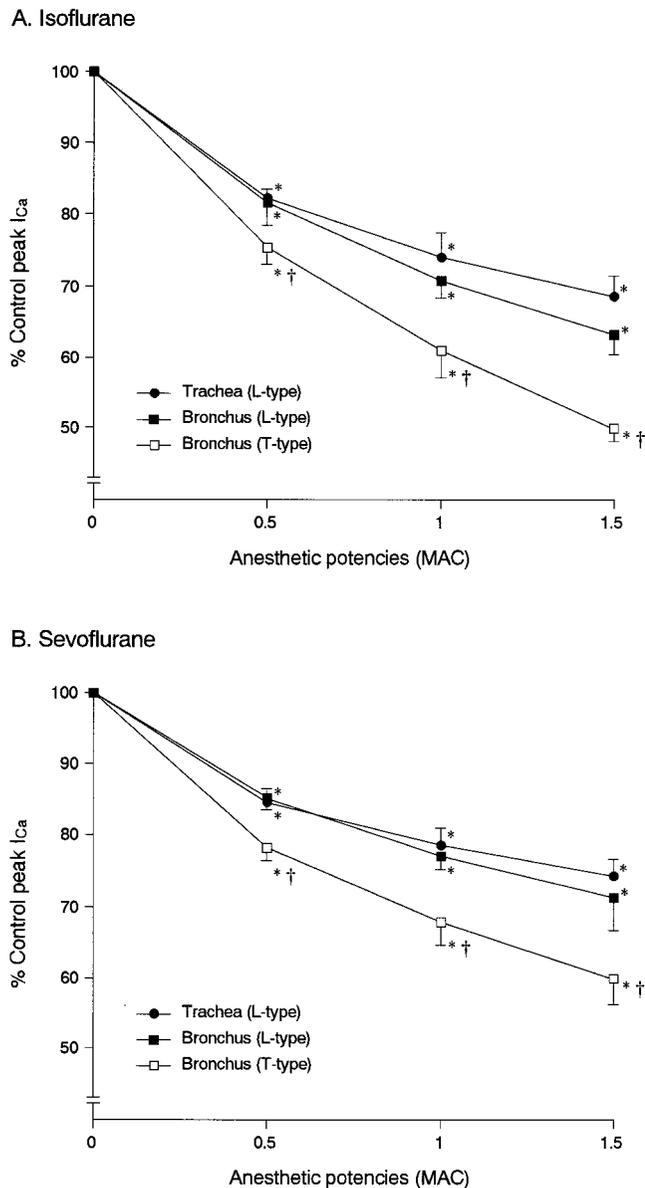
**Fig. 7.** Effect of 1.5 minimum alveolar concentration (MAC) of sevoflurane on whole-cell inward  $\text{Ca}^{2+}$  current ( $I_{\text{Ca}}$ ) in tracheal and bronchial smooth muscle cells. To elicit the  $I_{\text{Ca}}$  through L-type VDCs in tracheal (A) and bronchial (B) smooth muscle cells, depolarizations from a holding potential of  $-40$  mV was used. (C) A stepwise depolarization of bronchial smooth muscle cells from a holding potential of  $-80$  mV was used to activate the mixed  $I_{\text{Ca}}$  carried through both L- and T-type VDCs. Sevoflurane significantly inhibited  $I_{\text{Ca}}$  throughout the voltage range studied in both tracheal and bronchial smooth muscle cells. There was a  $+10$  mV shift of the peak  $I_{\text{Ca}}$  versus command potential curve toward more positive potentials in T- and L-types of bronchial smooth muscle cells. The dashed line denotes zero current (top). Symbols represent the mean  $\pm$  SD (bottom);  $n = 7$  each;  $*P < 0.05$  versus control.

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.<sup>35</sup>

The commonly encountered  $I_{\text{Ca}}$ 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  $I_{\text{Ca}}$ , indicating that these bronchial smooth muscle cells have only L-type VDCs.<sup>17,24</sup> The rest of the bronchial smooth muscle cells (approximately 30%), however, showed different characteristics of  $I_{\text{Ca}}$  during stepwise depolarizations from a holding potential of  $-80$  mV (fig. 5A). As shown in figures 5A and 5C, the  $I_{\text{Ca}}$ s were evoked at much more negative potentials and showed a rapidly inactivating current at a negative potential ( $-40$  to  $-20$  mV). Voltage steps from a holding potential of  $-40$  mV in the same cells elicited only sustained  $I_{\text{Ca}}$ s that resembled the L-type  $I_{\text{Ca}}$  (fig. 5B). 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 I-V relation of the second  $\text{Ca}^{2+}$  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.<sup>17,24</sup>

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  $[\text{Ca}^{2+}]_i$  homeostasis in other tissues because of their transient opening time and their small conductance.<sup>36</sup> 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  $\text{Ca}^{2+}$  influx through these T-type VDCs, which in turn could contribute to excitation-contraction coupling as well as refilling of the internal  $\text{Ca}^{2+}$  stores.<sup>37,38</sup> Furthermore, T-type  $I_{\text{Ca}}$ s are not suppressed during agonist stimulation,<sup>17</sup> as is the case for L-type  $I_{\text{Ca}}$ s.<sup>39,40</sup> The small size of the T-type  $I_{\text{Ca}}$  does not necessarily lessen the possible importance of their contribution to excitation. Accordingly, it is possible that the different responses to nifedipine between



**Fig. 8.** Relations between the percent control peak whole-cell inward  $\text{Ca}^{2+}$  current ( $I_{\text{Ca}}$ ) and the anesthetic potencies (minimum alveolar concentrations [MAC]). The inhibition of peak  $I_{\text{Ca}}$  through T-type voltage-dependent  $\text{Ca}^{2+}$  channels in bronchial smooth muscle cells were obtained by digital subtraction of the currents obtained at a holding potential (HP) of  $-40$  mV from total currents elicited from an HP of  $-80$  mV in the same cells. Symbols represent mean  $\pm$  SD;  $n = 7$ ;  $*P < 0.05$  versus 0 MAC anesthetic;  $\dagger P < 0.05$  versus L-type of either tracheal or bronchial smooth muscle. Each of the two volatile anesthetics significantly inhibited peak  $I_{\text{Ca}}$  in a dose-dependent manner. There was no significant differences between tracheal and bronchial smooth muscles in the inhibitory effects on the L-type  $I_{\text{Ca}}$ ; however, both anesthetics induced greater inhibitory effects on T-type  $I_{\text{Ca}}$ s in bronchial smooth muscles than on the L-type  $I_{\text{Ca}}$ s in either tissue.

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.*,<sup>16</sup> another pathway of dihydropyridine-insensitive receptor-operated  $\text{Ca}^{2+}$  channels<sup>41</sup> may also play a role in the

different responses to nifedipine between tracheal and bronchial smooth muscles, although no evidence suggests that the  $\text{Ca}^{2+}$  influx through receptor-operated  $\text{Ca}^{2+}$  channels are different in these airway smooth muscle tissues.

#### *Effects of Volatile Anesthetics on T- and L-type Voltage-dependent $\text{Ca}^{2+}$ 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  $I_{\text{Ca}}$  in tracheal and bronchial smooth muscle cells and on T-type  $I_{\text{Ca}}$  in bronchial smooth muscle cells separately. Unlike the classical  $\text{Ca}^{2+}$  channel antagonist nifedipine, which has a much greater inhibitory effect on L-type than on T-type  $I_{\text{Ca}}$ s (fig. 6), both isoflurane and sevoflurane had a greater inhibitory effect on T-type  $I_{\text{Ca}}$  than on L-type  $I_{\text{Ca}}$  (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 enflurane.<sup>24</sup> 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.<sup>42</sup> Recent molecular studies have revealed structural heterogeneity between VDCs of different tissues,<sup>43</sup> 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  $\text{Ca}^{2+}$  channels is a direct effect on the channel proteins or whether it is a secondary consequence of, for example, alterations in membrane lipids.<sup>44</sup>

Because of the different properties, such as resting membrane potential, of the distal and proximal airway smooth muscles,<sup>45,46</sup> 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),<sup>46</sup> and this difference is thought to be caused by reduced  $\text{Na}^+$  permeability, not to an increase in  $\text{K}^+$  permeability.<sup>45</sup> However, we have obtained direct evidence that the inhibitory effects of volatile anesthetics on L-type  $I_{\text{Ca}}$ s in bronchial and tracheal smooth muscles are not different (fig. 8). Therefore, the

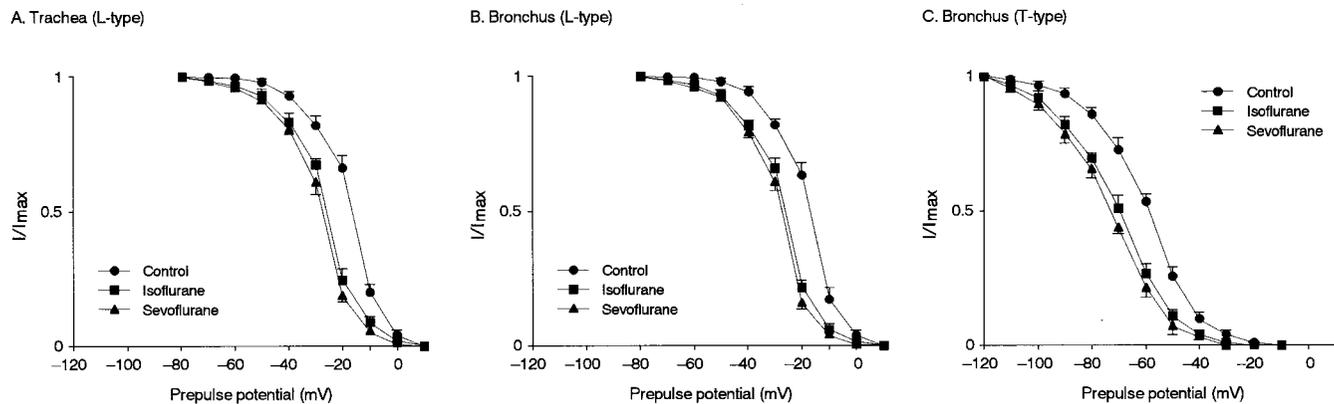


Fig. 9. Effects of the volatile anesthetics isoflurane (1.0 minimum alveolar concentration [MAC]) and sevoflurane (1.5 MAC) at equi-effective inhibitory potencies on the inactivation curves of inward  $\text{Ca}^{2+}$  currents. The inactivation curve for the T-type  $\text{Ca}^{2+}$  current in bronchial smooth muscle cells (C) was obtained by using nifedipine ( $1 \mu\text{M}$ ) to block the L-type  $\text{Ca}^{2+}$  current. Symbols represent mean  $\pm$  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  $\text{Ca}^{2+}$  channels. There was no significant difference in the change of the potential of half-inactivation ( $V_{1/2}$ ) either between the anesthetics or between types of voltage-dependent  $\text{Ca}^{2+}$  channels.

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  $I_{\text{Ca}}$ s. 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  $\text{Ca}^{2+}$  channel antagonists in porcine tracheal smooth muscle cells has been interpreted as evidence of drug-induced stabilization of the inactivated state.<sup>47</sup> 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  $[\text{Ca}^{2+}]_i$ , indicating that the anesthetics have different effects on the  $\text{Ca}^{2+}$  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

Table 1. Effects of the Volatile Anesthetics Isoflurane and Sevoflurane on the Inactivation Parameters of Inward Whole Cell  $\text{Ca}^{2+}$  Currents

	Trachea (L Type)	Bronchus (L Type)	Bronchus (T Type)
Percent inhibition of peak $I_{\text{Ca}}$			
Isoflurane (1.0 MAC)	73.1 $\pm$ 3.3	70.8 $\pm$ 2.5	61.0 $\pm$ 4.5
Sevoflurane (1.5 MAC)	73.5 $\pm$ 3.0	71.2 $\pm$ 6.3	59.5 $\pm$ 3.6
Potential of half inactivation ( $V_{1/2}$ , mV)			
Control	-17.6 $\pm$ 2.9	-16.9 $\pm$ 1.5	-58.7 $\pm$ 4.2†
Isoflurane (1.0 MAC)	-25.2 $\pm$ 2.3*	-25.6 $\pm$ 2.7*	-71.4 $\pm$ 3.1††
Sevoflurane (1.5 MAC)	-26.1 $\pm$ 3.2*	-25.4 $\pm$ 2.2*	-73.4 $\pm$ 4.6††
Slope factor ( $k$ , mV)			
Control	7.6 $\pm$ 0.5	7.4 $\pm$ 0.6	6.1 $\pm$ 0.4†
Isoflurane (1.0 MAC)	7.7 $\pm$ 0.3	7.3 $\pm$ 0.5	6.2 $\pm$ 0.7†
Sevoflurane (1.5 MAC)	7.5 $\pm$ 0.5	7.5 $\pm$ 0.8	6.4 $\pm$ 0.6†

\*  $P < 0.05$ ,  $t$  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

- Hirshman CA, Bergman NA: Factors influencing intrapulmonary airway calibre during anaesthesia. *Br J Anaesth* 1990; 65:30-42
- Yamakage M: Direct inhibitory mechanisms of halothane on canine tracheal smooth muscle contraction. *ANESTHESIOLOGY* 1992; 77:546-53
- Jones KA, Wong GY, Lorenz RR, Warner DO, Sieck GC: Effects of halothane on the relationship between cytosolic calcium and force in airway smooth muscle. *Am J Physiol* 1994; 266:L199-204
- Ozaki H, Kwon S-C, Tajimi M, Karaki H: Changes in cytosolic  $\text{Ca}^{2+}$  and contraction induced by various stimulants and relaxants in canine tracheal smooth muscle. *Pflügers Arch* 1990; 416:351-9
- Yamakage M, Hirshman CA, Croxton TL: Volatile anesthetics inhibit voltage-dependent  $\text{Ca}^{2+}$  channels in porcine tracheal smooth muscle cells. *Am J Physiol* 1995; 268:L187-91
- Warner DO, Vettermann J, Brichant JF, Rehder K: Direct and neurally mediated effects of halothane on pulmonary resistance in vivo. *ANESTHESIOLOGY* 1990; 72:105-63
- Hirshman CA, Edelstein G, Peetz S, Wayne R, Downes H: Mechanism of action of inhalational anesthesia on airways. *ANESTHESIOLOGY* 1982; 56:107-11
- Moreno RH, Hogg JC, Paré PD: Mechanics of airway narrowing. *Am Rev Respir Dis* 1986; 133:1171-80
- Korenaga S, Takeda K, Ito Y: Differential effects of halothane on airway nerves and muscle. *ANESTHESIOLOGY* 1984; 60:309-18
- Shioya T, Munoz NM, Leff AR: Effect of resting smooth muscle length on contractile response in resistance airways. *J Appl Physiol* 1987; 62:711-7
- McNamara AE, Müller NL, Okazawa M, Arntorp J, Wiggs BR, Paré PD: Airway narrowing in excised canine lungs measured by high-resolution computed tomography. *J Appl Physiol* 1992; 73:307-16
- Barnes PJ, Basbaum CB, Nadel JA: Autoradiographic localization of autonomic receptors in airway smooth muscle: Marked differences between large and small airways. *Am Rev Respir Dis* 1983; 127:758-62
- Shioya T, Pollack ER, Munoz NM, Leff AR: Distribution of airway contractile responses in major resistance airways of the dog. *Am J Pathol* 1987; 129:102-8
- Mazzeo AJ, Cheng EY, Stadnicka A, Bosnjak ZJ, Coon RL, Kampine JP: Topographical differences in the direct effects of isoflurane on airway smooth muscle. *Anesth Analg* 1994; 78:948-54
- Mazzeo AJ, Cheng EY, Bosnjak ZJ, Coon RL, Kampine JP: Differential effects of desflurane and halothane on peripheral airway smooth muscle. *Br J Anaesth* 1996; 76:841-6
- Croxton TL, Fleming C, Hirshman CA: Expression of dihydropyridine resistance differs in porcine bronchial and tracheal smooth muscle. *Am J Physiol* 1994; 267:L106-12
- Janssen IJ: T-type and L-type  $\text{Ca}^{2+}$  currents in canine bronchial smooth muscle: Characterization and physiological roles. *Am J Physiol* 1997; 272:C1757-65
- Hisada T, Kurachi Y, Sugimoto T: Properties of membrane currents in isolated smooth muscle cells from guinea-pig trachea. *Pflügers Arch* 1990; 416:151-61
- Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ: Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* 1981; 391:85-100
- Stuart-Smith K, Vanhoutte PM: Airway epithelium modulates the responsiveness of porcine bronchial smooth muscle. *J Appl Physiol* 1988; 65:721-7
- Tranquilli WJ, Thurmon JC, Benson GJ: Anesthetic potency of nitrous oxide in young swine (*Sus scrofa*). *Am J Vet Res* 1985; 46:58-60
- Manohar M, Parks CM: Porcine systemic and regional organ blood flow during 1.0 and 1.5 minimum alveolar concentrations of sevoflurane anesthesia without and with 50% nitrous oxide. *J Pharmacol Exp Ther* 1984; 231:640-8
- Isenberg G, Klockner U: Calcium tolerant ventricular myocytes prepared by preincubation in a "KB medium." *Pflügers Arch* 1982; 395:6-18
- Eskinder H, Rusch NJ, Supan FD, Kampine JP, Bosnjak ZJ: The effects of volatile anesthetics on L- and T-type calcium channel currents in canine cardiac purkinje cells. *ANESTHESIOLOGY* 1991; 74:919-26
- Yamakage M, Tsujiguchi N, Hattori J, Kamada Y, Namiki Y: Low-temperature modification of the inhibitory effects of volatile anesthetics on airway smooth muscle contraction in dogs. *ANESTHESIOLOGY* 2000; 93:179-88
- Langton PD, Burke EP, Sanders KM: Participation of Ca currents in colonic electrical activity. *Am J Physiol* 1989; 257:C451-60
- Yamakage M, Kohro S, Kawamata T, Namiki A: Inhibitory effects of four inhaled anesthetics on canine tracheal smooth muscle contraction and intracellular  $\text{Ca}^{2+}$  concentration. *Anesth Analg* 1993; 77:67-72
- Somlyo AP, Himpens B: Cell calcium and its regulation in smooth muscle. *FASEB J* 1989; 3:2266-76
- van Breemen C: Cellular mechanisms regulating  $[\text{Ca}^{2+}]_i$  smooth muscle. *Annu Rev Physiol* 1989; 51:315-29
- Yang CM, Yo Y-L, Wang Y-Y: Intracellular calcium in canine cultured tracheal smooth muscle cells is regulated by  $\text{M}_3$  muscarinic receptors. *Br J Pharmacol* 1993; 110:983-8
- Gonzalez JM, Morice RC, Bloom K, Akers S, Raizner AE, Stevens PM: Inhibition of airway reactivity by nifedipine in patients with coronary artery disease. *Am Rev Respir Dis* 1983; 127:155-7
- Williams DO, Barnes PJ, Vickers HP, Rudolf M: Effect of nifedipine on bronchomotor tone and histamine reactivity in asthma (report). *Br Med J* 1981; 283:348
- Yamakage M, Hirshman CA, Croxton TL: Inhibitory effects of thiopental, ketamine, and propofol on voltage-dependent  $\text{Ca}^{2+}$  channels in porcine tracheal smooth muscle cells. *ANESTHESIOLOGY* 1995; 83:1274-82
- Yamakage M, Matsuzaki T, Tsujiguchi N, Honma Y, Namiki A: Inhibitory effects of diazepam and midazolam on  $\text{Ca}^{2+}$  and  $\text{K}^+$  channels in canine tracheal smooth muscle cells. *ANESTHESIOLOGY* 1999; 90:197-207
- Worley JF III, Kotlikoff MI: Dihydropyridine-sensitive single calcium channels in airway smooth muscle cells. *Am J Physiol* 1990; 259:L468-80
- Meldolesi J, Pozzan T: Pathways of  $\text{Ca}^{2+}$  influx at the plasma membrane: Voltage-, receptor-, and second messenger-operated channels. *Exp Cell Res* 1987; 171:271-83
- Bourreau JP, Kwan CY, Daniel EE: Distinct pathways to refill ACh-sensitive internal  $\text{Ca}^{2+}$  stores in canine airway smooth muscle. *Am J Physiol* 1993; 265:C28-35
- Janssen IJ, Sims SM: Emptying and refilling of  $\text{Ca}^{2+}$  store in tracheal myocytes as indicated by ACh-evoked currents and contraction. *Am J Physiol* 1993; 265:C877-86
- Wade GR, Barbera J, Sims SM: Cholinergic inhibition of  $\text{Ca}^{2+}$  current in guinea-pig gastric and tracheal smooth muscle cells. *J Physiol (Lond)* 1996; 491:307-19
- Yamakage M, Hirshman CA, Croxton TL: Cholinergic regulation of voltage-dependent  $\text{Ca}^{2+}$  channels in porcine tracheal smooth muscle cells. *Am J Physiol* 1995; 269:L776-82
- Murray RK, Kotlikoff MI: Receptor-activated calcium influx in human airway smooth muscle cells. *J Physiol (Lond)* 1991; 435:123-44
- Herrington J, Stern RC, Evers AS, Lingle CJ: Halothane inhibits two components of calcium current in clonal (GH3) pituitary cells. *J Neurosci* 1991; 11:2226-40
- Hofmann F, Biel M, Flockerzi V: Molecular basis for  $\text{Ca}^{2+}$  channel diversity. *Annu Rev Neurosci* 1994; 17:399-418
- Terrar DA: Structure and function of calcium channels and the actions of anesthetics. *Br J Anaesth* 1993; 71:39-46
- Ito Y, Inoue T: Contracture and change in membrane potential produced by sodium removal in the dog trachea and bronchiole. *J Appl Physiol* 1989; 67:2078-86
- Inoue T, Ito Y: Characteristics of neuro-effector transmission in the smooth muscle layer of dog bronchiole and modifications by autacoids. *J Physiol (Lond)* 1986; 370:551-65
- Yamakage M, Hirshman CA, Namiki A, Croxton TL: Inhibition of voltage-dependent  $\text{Ca}^{2+}$  channels of porcine tracheal smooth muscle by the novel  $\text{Ca}^{2+}$  channel antagonist RWJ-22108. *Gen Pharmac* 1997; 28:689-94