Anesthesiology
2000; 93:179-88

Low-temperature Modification of the Inhibitory Effects of Volatile Anesthetics on Airway Smooth Muscle Contraction in Dogs

Michiaki Yamakage, M.D., Ph.D., Naoki Tsujiguchi, M.D.,† Jun-ichi Hattori, M.D.,† Yasuhiro Kamada, M.D.,‡ Akiyoshi Namiki, M.D., Ph.D.§*

Background: **Because exposure to low temperature can modify the effect of volatile anesthetics on airway smooth muscle contraction, this study was conducted to investigate low-temperature modifications of the inhibitory effects of isoflurane and sevoflurane on canine tracheal smooth muscle tone by simultaneously measuring the muscle tension and intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$) and by measuring voltage-dependent Ca^{2+} channel activity.**

Methods: **$[Ca^{2+}]_i$ was monitored by the 500-nm light emission ratio of preloaded fura-2, a Ca^{2+} indicator. Isometric tension was measured simultaneously. Whole cell patch clamp recording techniques were used to observe voltage-dependent Ca^{2+} channel activity in dispersed muscle cells. Isoflurane (0–3.0%) or sevoflurane (0–3%) was introduced to a bath solution at various temperatures (37, 34, or 31°C).**

Results: **Low temperature (34 or 31°C) reduced high-K^+–induced (72.7 mM) muscle contraction and increased $[Ca^{2+}]_i$, but it enhanced carbachol-induced (1 μM) muscle contraction with a decrease in $[Ca^{2+}]_i$. The volatile anesthetics tested showed significant inhibition of both high-K^+–induced and carbachol-induced airway smooth muscle contraction, with a concomitant decrease in $[Ca^{2+}]_i$. The inhibition of the carbachol-induced muscle contraction by volatile anesthetics was abolished partially by exposure to low temperature. Volatile anesthetics and low-temperature exposure significantly inhibited voltage-dependent Ca^{2+} channel activity of the smooth muscle.**

Conclusions: **Exposure of airway smooth muscle to low temperature leads to an increase in agonist-induced muscle contractility, with a decrease in $[Ca^{2+}]_i$. The inhibition of voltage-dependent Ca^{2+} channel activity by exposure to low temperature and by volatile anesthetics cam be attributed, at least in part, to the decrease in $[Ca^{2+}]_i$. (Key words: Ca^{2+} Sensitivity; intracellular concentration of free Ca^{2+}; isoflurane; sevoflurane; voltage-dependent Ca^{2+} channel.)**

AN *in vitro* effect of changes in temperature on airway smooth muscle tone has been shown, and it has been found that a decrease in temperature of the muscle enhanced agonist-induced airway smooth muscle contraction.[1-3] Ishii and Shimo[3] speculated, on the basis of muscle tension measurements, that increased responsiveness of airway smooth muscle to acetylcholine with lowered temperature may involve the acceleration of Ca^{2+} release from intracellular storage sites, inhibition of Ca^{2+} extrusion from the cell, or the inhibition of Ca^{2+} reuptake by intracellular storage sites. The exact mechanism of low temperature-induced sensitivity to contractile agonists, however, is unclear. Volatile anesthetics are potent bronchodilators and often are used for severe asthmatic patients.[4] Upper airway smooth muscle in such cases is exposed to cool air during tracheal intubation. The temperature of airway smooth muscle also is decreased during a cardiopulmonary bypass operation and during mild hypothermic therapy,[5,6] and volatile anesthetics sometimes are used for the anesthesia. The decrease in temperature in these situations, therefore, has an effect on the airway smooth muscle tone and might modify the inhibitory effects of volatile anesthetics on the muscle tone.

The aim of the current study was to investigate low-temperature modifications of the inhibitory effects of the volatile anesthetics isoflurane and sevoflurane on canine tracheal smooth muscle tone *in vitro* by simultaneously measuring muscle tension and intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$) and by measuring voltage-

* Instructor.

† Fellow.

‡ Postgraduate Student.

§ Professor and Chairman.

Received from the Department of Anesthesiology, Sapporo Medical University School of Medicine, Sapporo, Japan. Submitted for publication October 11, 1999. Accepted for publication March 3, 2000. Supported in part by a grant-in-aid (No. 10770762) for research from the Ministry of Education, Science and Culture, Tokyo, Japan, and an incentive grant (No. 98-B-02) for research from the Akiyama Research Foundation, Sapporo, Japan. Presented in part at the annual meeting of the American Society of Anesthesiologists, Orlando, Florida, October 17-21, 1998.

Address reprint requests to Dr. Yamakage: Department of Anesthesiology, Sapporo Medical University School of Medicine, South-1, West-16, Chuoku, Sapporo 060-8543, Japan. Address electronic mail to: yamakage@sapmed.ac.jp

dependent Ca^{2+} channel (VDCC) activity. $[Ca^{2+}]_i$ itself primarily controls the contractile state of the muscle,[7] and VDCC is important in regulating the $[Ca^{2+}]_i$.[8,9]

Materials and Methods

The protocol for this study was approved by the Sapporo Medical University Ethical Committee on Animal Research. Adult mongrel dogs weighing 10–15 kg were anesthetized with 10 mg/kg intramuscular ketamine and killed by exsanguination. The tracheas were excised quickly, and the epithelium, cartilage, and connective tissue were stripped from the smooth muscle.

Simultaneous Measurement of Muscle Tension and $[Ca^{2+}]_i$

Tissue preparation was performed according to the previously described method.[8] Briefly, tracheal smooth muscle strips (1 mm wide and 8 mm long) were pretreated with 5 μM acetoxymethyl ester of fura-2, an indicator of Ca^{2+}, in a physiologic salt solution containing 0.02% (vol/vol) cremophor EL for 6 or 7 h at room temperature (22–24°C). The 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. The temperature of the bath solution was monitored continuously using a thermometer (CTM-205; Terumo, 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 $[Ca^{2+}]_i$.[8,9] Contractions were induced by high-K^+ (72.7 mM) and carbachol (1 μM), a potent muscarinic receptor agonist. After the contractions reached a steady state, the temperature of the organ bath was changed from 37°C to 34 or 31°C. In another experiment, the tissues were exposed to a bath solution equilibrated using one of two volatile anesthetics: isoflurane (1.0, 2.0, or 3.0% at the vaporizer) or sevoflurane (1.0, 2.0, or 3.0%), with simultaneous changes in the temperature of the bath solution (34°C or 31°C).

Because there is a possibility that changes in temperature *per se* can change the affinity of fura-2 for Ca^{2+},[10] we confirmed the effect of temperature on $R_{340/380}$ of the tracheal smooth muscle strips by the use of the skinned fiber technique.[11] The muscle strips (0.1–0.2 mm wide) were superfused for 20 min using a relaxing solution containing 50 μg/ml saponin and 5 μM acetoxymethyl ester of fura-2. The relaxing solution was made with the following composition using the algorithm of Fabiato and Fabiato:[12] Mg-ATP: 7.5 mM; EGTA: 4.0 mM; imidazol: 20 mM; dithiothreitol: 1.0 mM; free Mg^{2+}: 1.0 mM; free Ca^{2+}: 1 nM; creatine phosphate: 10 mM; and creatine phosphokinase: 0.1 mg/ml. After incubation with 0.3 μM free Ca^{2+}, the temperature of the organ bath was changed within the range between 37 and 31°C. $R_{340/380}$, an indicator of $[Ca^{2+}]_i$, did not change within the temperature range tested with or without 1 μM carbachol (n = 4, data not shown).

Measurement of Voltage-dependent Ca^{2+} Channel Activity

We used conventional whole cell patch clamp techniques to observe inward Ca^{2+} currents (I_{Ca}) through VDCCs.[13] Tracheal smooth muscle tissue was minced and digested for 20 min at 37°C in Ca^{2+}-free Tyrode's solution to which 0.08% (wt/vol) collagenase was added.[14] Cells were dispersed by trituration, filtered through nylon mesh, and centrifuged. The pellet was resuspended in a modified Kraftbrühe solution[15] and stored at 4°C for up to 5 h before use. The modified Kraftbrühe solution contained the following: KCl: 85 mM; K_2HPO_4: 30 mM; $MgSO_4$: 5.0 mM; Na_2ATP: 5.0 mM; pyruvic acid: 5.0 mM; creatine: 5.0 mM; taurine: 20 mM; β-hydroxybutyrate: 5.0 mM; and 0.1% (wt/vol) fatty acid–free bovine serum albumin, with pH adjusted to 7.25 with 0.5 M tris[hydroxymethyl]aminomethane.

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). Resistances were 3–5 MΩ if filled with solution. The pipette solution contained the following: CsCl: 130 mM; $MgCl_2$: 4.0 mM; EGTA: 10 mM; Na_2ATP: 5.0 mM; and HEPES: 10 mM, with pH adjusted to 7.2 with tris[hydroxymethyl]aminomethane. The bath solution contained the following: tetraethylammonium chloride: 130 mM; $MgCl_2$: 1.0 mM; $CaCl_2$: 10 mM; glucose: 10 mM; and HEPES: 10 mM, with pH adjusted to 7.4 with tris[hydroxymethyl]aminomethane. An aliquot (approximately 0.5 ml) of the cell suspension was placed in a chamber on the stage of an inverted microscope. A micromanipulator was used to position the patch pipette against the membrane of a tracheal smooth muscle cell. After obtaining a high-resistance seal (> 5 GΩ), 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.

Inward Ca^{2+} currents were elicited by 100-ms depolarizing pulses (−50 to 40 mV) from a holding potential of −80 mV. Leak currents were subtracted, and membrane capacitance and series resistance were compensated. After a stable baseline of peak I_{Ca} was obtained at 37°C, the temperature of the bath solution was changed to 31°C. In another experiment, cells were exposed to a bath solution equilibrated using one of two volatile anesthetics, isoflurane (1.0, 2.0, or 3.0%) or sevoflurane (1.0, 2.0, or 3.0%), with simultaneous change in the temperature of the bath solution from 37 to 31°C. Inactivation curves also were determined, using a double-pulse protocol that consisted of a 3-s prepulse to a potential in the range between −80 and 10 mV, followed by a 100-ms depolarization to 10 mV. The peak change in the current was expressed as a fraction of that obtained using the −80-mV prepulse, and this quantity was least-squares fitted to a Boltzman expression[16] to estimate the potential of half-maximal inactivation and the slope factor.

Bath temperature during the patch clamp recording was controlled using a circular thermofoil heater 115 mm in diameter (MT-1; Narishige), with a 35-mm central opening. Temperature sensing was performed by means of a small insulated thermocouple situated no more than 5 mm from that cell from which recordings were being made. The overall stability of this system was ±0.1°C from the set point.

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

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 (1.0, 2.0, and 3.0% in the gas phase) were 0.26, 0.52, and 0.82 mM, respectively, whereas the mean concentrations of sevoflurane in the solution (1.0, 2.0, and 3.0% in the gas phase) were 0.16, 0.35, and 0.54 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, or between the concentrations of different temperatures of the bath solution in the range between 31 and 37°C (n = 4, data not shown).

Materials

The following drugs and chemicals were used: β-hydroxybutyrate, cremophor EL (Sigma Chemical, St. Louis, MO), acetoxymethyl ester of fura-2 (Dojindo Laboratories, Kumamoto, Japan), type I collagenase (Gibco Laboratories, Grand Island, NY), isoflurane (Ohio Medical, Madison, WI), and sevoflurane (Maruishi Pharmaceutical, Osaka, Japan).

Statistical Analysis

Data are expressed as the mean ± SD. For the measurement of $[Ca^{2+}]_i$ and muscle tension, high-K^+-induced sustained changes in $[Ca^{2+}]_i$ (indicated by $R_{340/380}$) and muscle tension at 37°C 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 or Changes in Temperature on Muscle Tension and $[Ca^{2+}]_i$

At 37°C, the ratio $R_{340/380}$, an indicator of $[Ca^{2+}]_i$, was increased rapidly by high K^+ (72.7 mM), with a concomitant contraction (fig. 1). $[Ca^{2+}]_i$ and muscle tension reached their respective peaks within 10–50 s. With carbachol, $[Ca^{2+}]_i$ also increased rapidly to a concentration similar to that induced by high K^+. The amount of muscle contraction with carbachol, however, was more than twice that induced by high K^+, indicating that carbachol induces a greater contraction than does high K^+ at a given $[Ca^{2+}]_i$. Exposure to low temperature (34°C) significantly decreased high-K^+-induced muscle contraction and increase in $[Ca^{2+}]_i$. Low temperature also decreased the carbachol-induced increase in $[Ca^{2+}]_i$; however, it enhanced carbachol-induced muscle contraction. The effects of 3% isoflurane with a decrease in temperature (31°C) are shown in figure 2. Isoflurane significantly and similarly inhibited high-K^+-induced

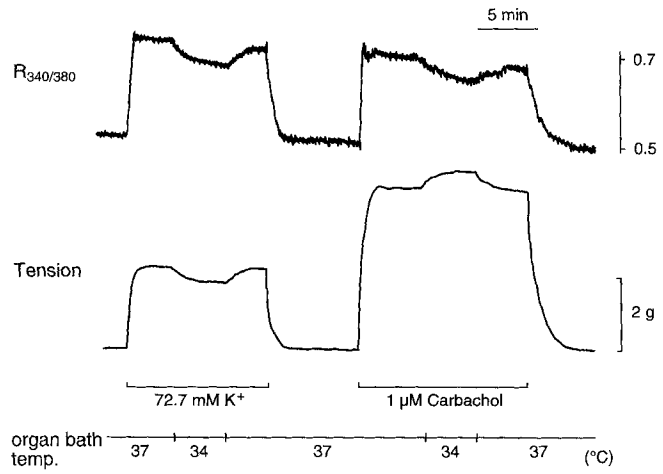

Fig. 1. Changes in intracellular concentration of free Ca^{2+} (indicated by $R_{340/380}$) and muscle tension during contractions induced by high K^+ (72.7 mM) and carbachol (1 μM). Low temperature (34°C) was introduced during the contractions. Exposure to low temperature reduced the carbachol-induced increase in Ca^{2+}, but it enhanced the muscle contraction.

muscle contraction, with an increase in $[Ca^{2+}]_i$, and carbachol-induced muscle contraction, with an increase in $[Ca^{2+}]_i$. The relations between anesthetic concentrations and percentage of response of muscle tension or $[Ca^{2+}]_i$ are shown in figure 3. Linear relations were observed between anesthetic concentrations and percentage of response of muscle tension and between

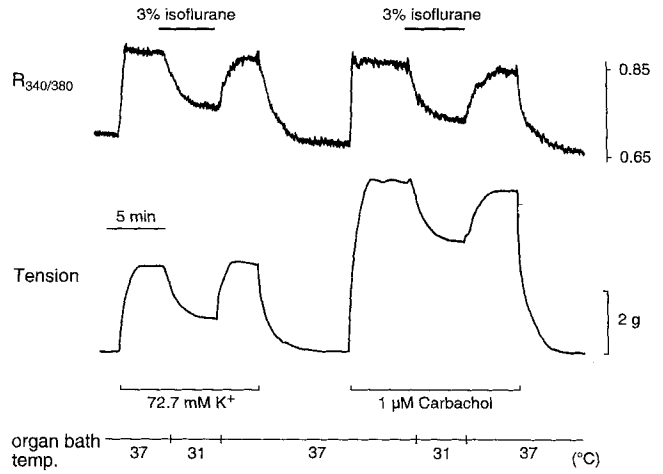

Fig. 2. Changes in intracellular concentration of free Ca^{2+} (indicated by $R_{340/380}$) and muscle tension during contractions induced by high K^+ (72.7 mM) and carbachol (1 μM). Low temperature (31°C) and isoflurane (3%) were introduced simultaneously during the contractions. Isoflurane significantly and similarly inhibited high-K^+– and carbachol-induced muscle contractions, with concomitant inhibition of increases in Ca^{2+}.

anesthetic concentrations and percentage of response of $[Ca^{2+}]_i$. High-K^+-induced muscle contraction and increase in $[Ca^{2+}]_i$ were significantly decreased by low-temperature exposure in a gradient-dependent manner at all concentrations of isoflurane (fig. 3A, n = 7). Similar effects of low-temperature exposure also were seen with sevoflurane (by 10–18% at 31°C, n = 6; data not shown). Exposure to low temperature, however, significantly prevented the inhibitory effect of sevoflurane on carbachol-induced muscle contraction, and the increase in $[Ca^{2+}]_i$ caused by carbachol was inhibited significantly by exposure to low-temperature exposure at all concentrations of sevoflurane (n = 7; fig. 3B). Similar effects of exposure to low temperature were seen with isoflurane (by 11–18% at 31°C, n = 6; data not shown).

Effects of Volatile Anesthetics or Changes in Temperature on I_{Ca} Currents through Voltage-dependent Ca^{2+} Channels

Voltage-pulse protocols were performed in control solutions for more than 5 min to obtain a stable baseline value. Data from cells that showed unstable I_{Ca} amplitudes, less than 100 pA of peak I_{Ca}, or a more than 10% reduction in amplitude during the control recording period were discarded. Technically satisfactory data were obtained from 84 cells studied at the holding potential of −80 mV. In all cases, recordings were performed at two different temperatures (37 and 31°C). At any given temperature, there was sufficient consistency in peak current amplitude and time course to justify the pooling of records according to temperature. At 37°C, I_{Ca} seen in dispersed canine tracheal smooth muscle cells during step depolarization from −80 mV peaked at approximately 10 ms and was inactivated with a time constant in the range between 70 and 100 ms (fig. 4A). During baseline conditions, threshold activation of I_{Ca} occurred at approximately −20 mV, and maximum peak current amplitude was obtained at approximately 10 mV (fig. 4B). The maximum peak I_{Ca} was −439 ± 48 pA (range, −260–−789 pA). The inactivation parameters obtained in 28 cells during control conditions were a potential of half-maximal inactivation of 14.6 ± 3.2 mV and a slope factor of 7.4 ± 1.2 mV. I_{Ca} currents with a similar time course were observed in the inactivation experiments.

As shown in a representative trace for depolarization from −80 to 10 mV (fig. 4A), the amplitude of I_{Ca} was clearly sensitive to temperature, and exposure to low

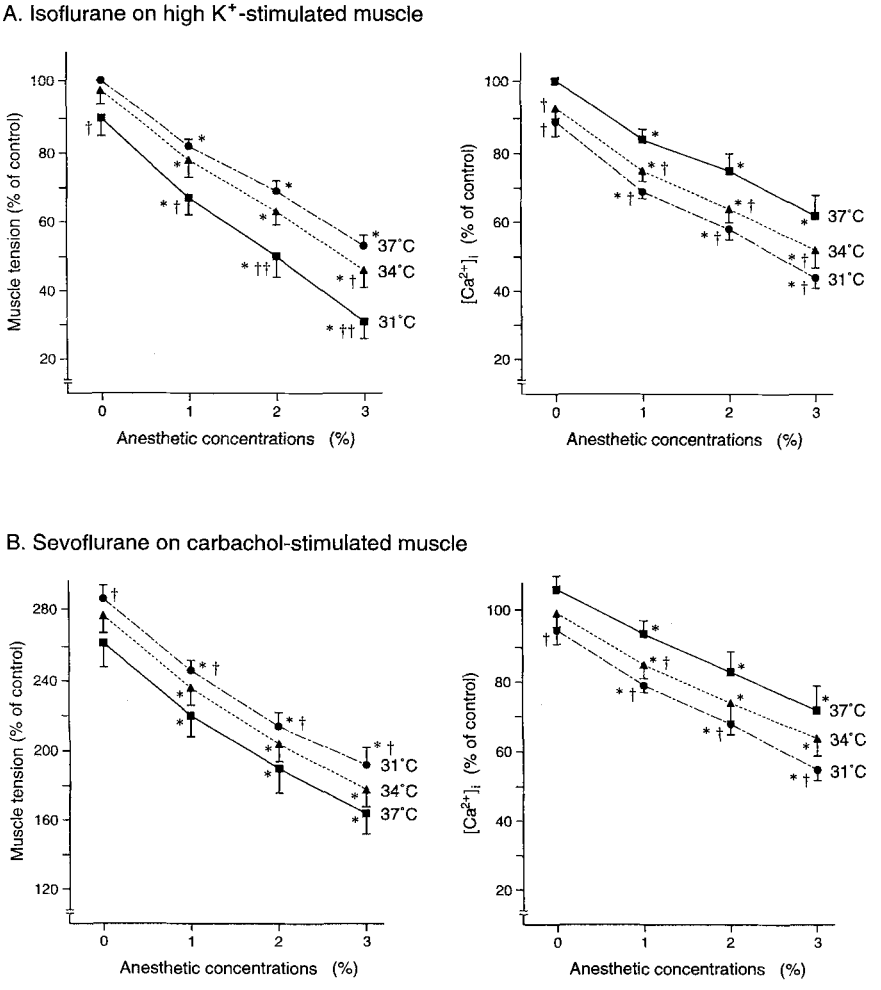

Fig. 3. (*A*) Relations between anesthetic concentrations and percentage of response of muscle tension and between anesthetic concentrations and percentage of response of the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$) in the presence of high K^+ (72.7 mM). Isoflurane significantly decreased muscle contraction and $[Ca^{2+}]_i$ in a dose-dependent manner. High-K^+–induced muscle contraction and increase in $[Ca^{2+}]_i$ were decreased significantly by exposure to low temperature in a gradient-dependent manner at all concentrations of isoflurane. (*B*) Relations between anesthetic concentrations and percentage of response of muscle tension and between anesthetic concentrations and percentage of response of $[Ca^{2+}]_i$ in the presence of carbachol (1 μM). Sevoflurane *per se* significantly decreased muscle contraction and $[Ca^{2+}]_i$ in a dose-dependent manner. Exposure to low temperature significantly prevented the inhibitory effect of sevoflurane on carbachol-induced muscle contraction; the increase in $[Ca^{2+}]_i$ caused by carbachol was inhibited significantly by the low temperature at all concentrations of sevoflurane. Symbols represent the mean ± SD; n = 7; *$P < 0.05$ *vs.* 0% anesthetic, †$P < 0.05$ *vs.* 37°C, ††$P < 0.05$ *vs.* 34°C.

temperature (31°C) inhibited the magnitude of I_{Ca}. Figure 4B shows the relation between peak I_{Ca} and applied potential before and after exposure to low temperature (31°C). Low-temperature exposure significantly inhibited I_{Ca} at step potentials in the range between −10 and 40 mV and decreased peak I_{Ca} at 10 mV by approximately 18% (n = 7). There was no apparent shift in the voltage-dependence of I_{Ca}. We determined the dose-dependence of the inhibition of peak I_{Ca} by each volatile anesthetic tested. Figure 5 shows the relations between the percentage of control peak I_{Ca} at 10 mV and the concentration of each anesthetic, isoflurane (fig. 5A) and sevoflurane (fig. 5B), in the gas phase. Both volatile anesthetics significantly inhibited peak I_{Ca} in a dose-dependent manner (n = 7). Exposure to low temperature significantly inhibited the I_{Ca} at all concentrations of these anesthetics (n = 7).

The effects of the volatile anesthetics isoflurane and sevoflurane at equieffective inhibitory concentrations (2.0 and 3.0%, respectively) on the inactivation curve of I_{Ca} currents are summarized in figure 6 and table 1. Each of these anesthetics shifted the inactivation curve to a more negative potential (n = 7). Exposure to low temperature also significantly shifted the inactivation curve to a more negative potential (n = 7). The induced changes in the potential of half-maximal inactivation brought about both by these anesthetics and by the low temperature were statistically significant in each case. The slope factor was not changed by exposure to either the anesthetics or low temperature.

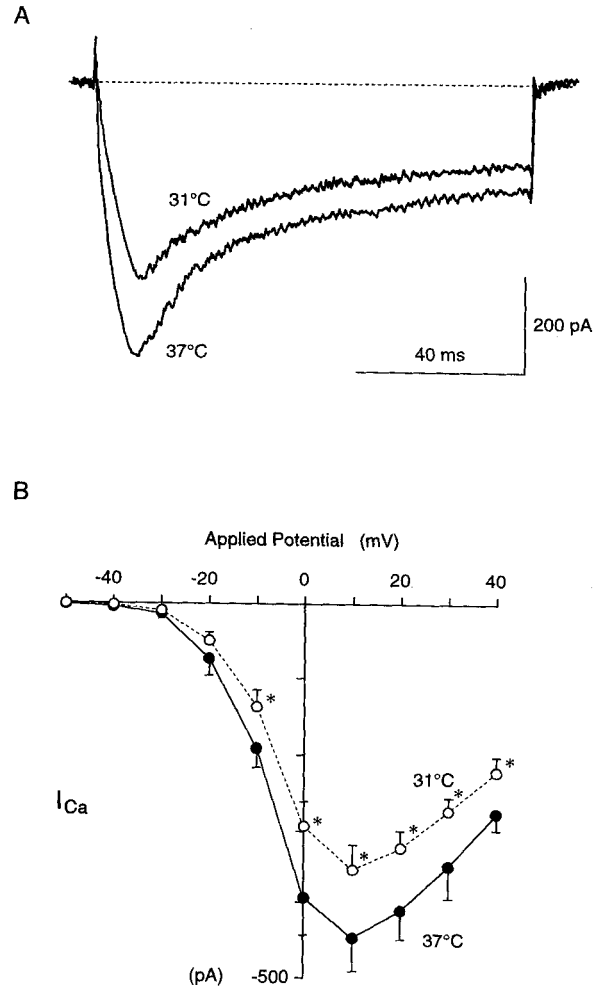

Fig. 4. Effects of exposure to low temperature (31°C) on depolarization-induced inward Ca^{2+} currents (I_{Ca}). (*A*) Typical recordings of I_{Ca} induced by depolarizing pulses to 10 mV at 37 and 31°C. Exposure to low temperature significantly inhibited the magnitude of I_{Ca}. Dashed line = zero current. (*B*) Relation between peak I_{Ca} and applied potential at 37°C (●, solid line) and at 31°C (○, dashed line). Symbols represent mean ± SD; n = 7; *P < 0.05. Exposure to low temperature significantly inhibited I_{Ca} at step potentials in the range of −10 to 40 mV and decreased peak I_{Ca} at 10 mV by approximately 18%.

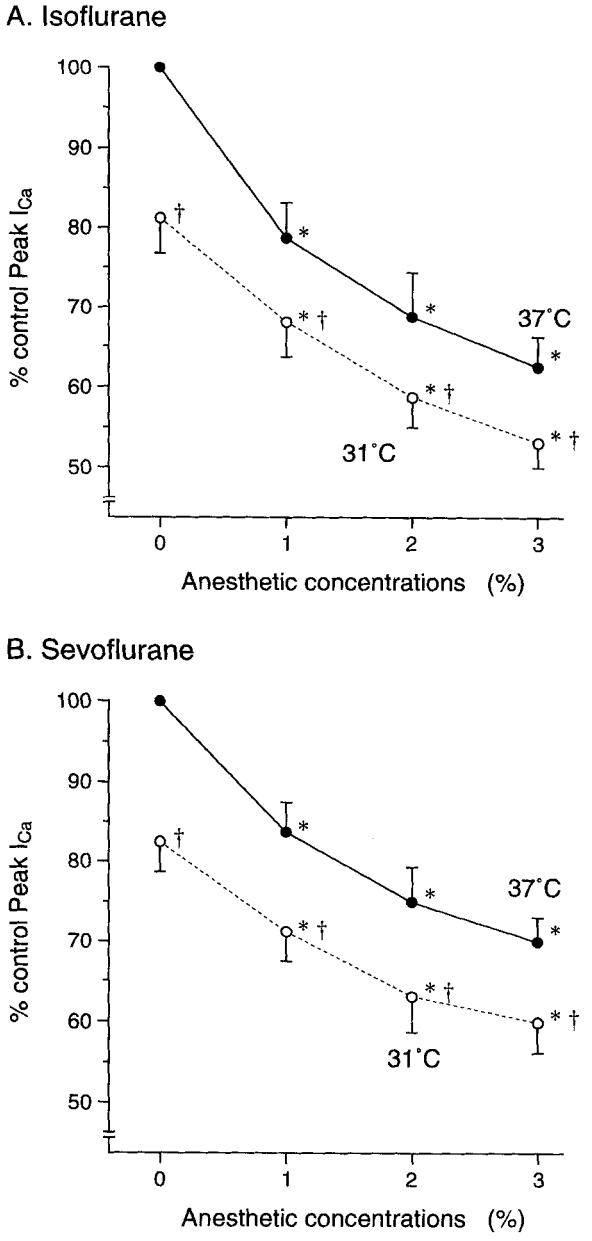

Fig. 5. Relations between peak inward Ca^{2+} currents (I_{Ca}) at 10 mV, expressed as a percentage of control, and the gas-phase concentrations of the anesthetics isoflurane (*A*) and sevoflurane (*B*) at 37°C (●, solid line) and at 31°C (○, dashed line). Symbols represent mean ± SD; n = 7; *P < 0.05 *vs.* 0% anesthetic; †P < 0.05 *vs.* 37°C. Both anesthetics significantly inhibited peak I_{Ca} in a dose-dependent manner. Exposure to low temperature significantly inhibited the I_{Ca} at all concentrations of these anesthetics.

Discussion

Effects of Volatile Anesthetics or Changes in Temperature on Muscle Tension and $[Ca^{2+}]_i$

One of the major findings of our study is that in canine tracheal smooth muscle *in vitro*, exposure of the muscle to low temperature significantly decreased high-K^+-induced muscle contraction and increase in $[Ca^{2+}]_i$, but it enhanced carbachol-induced muscle contraction with a decrease in $[Ca^{2+}]_i$ (fig. 1). Our results are in general agreement with those of studies using various species in which exposure to low temperature has been associated

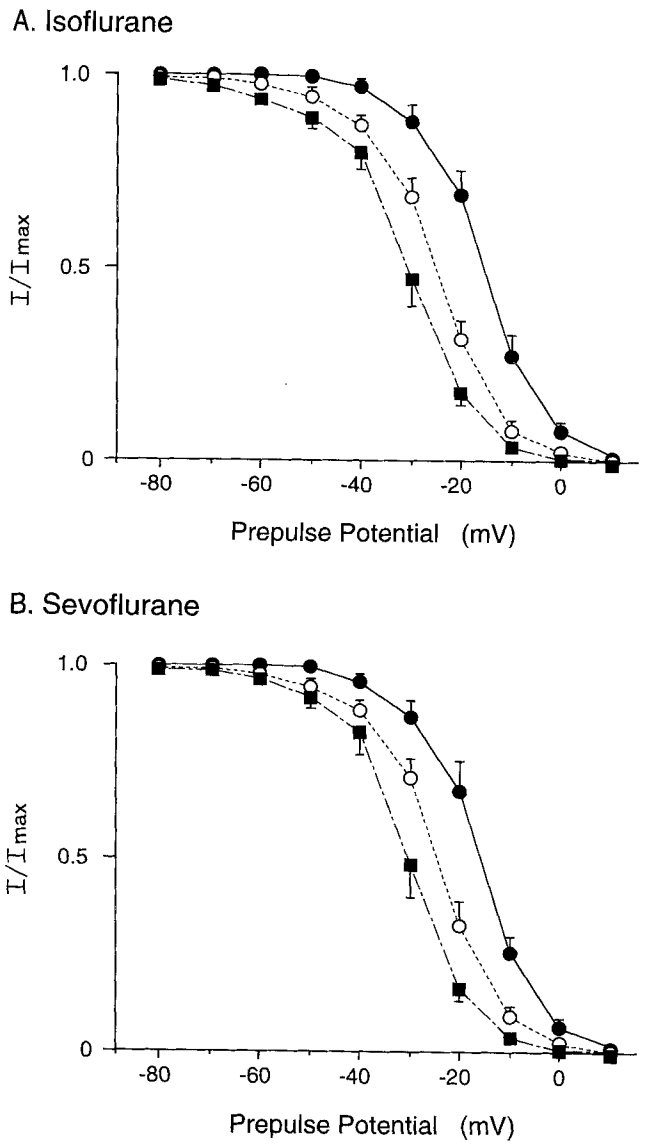

Fig. 6. Effects of the volatile anesthetics isoflurane (*A*) and sevoflurane (*B*) at different temperatures on voltage-dependent steady state inactivation of I_{Ca}. Inactivation curves were generated during control conditions at 37°C (●, solid line) and with one volatile anesthetic at 37°C (○, dashed line) and at 31°C (■, dot-dashed line). Symbols represent the mean ± SD; n = 7.

with an enhancement of the agonist-induced contraction of airway smooth muscle.[1-3,17-19] The results of these studies suggested that the effect of direct exposure to low temperature on airway smooth muscle *per se* has some role in low-temperature-induced airway hyperresponsiveness in a clinical situation. Ishii and Shimo[20] and Freed and Stream,[21] however, showed in *in vivo* studies that exposure of the airway to low temperature decreased the response to contractile agonists in guinea pigs and in dogs, respectively. These apparent discrepancies may result from differences in agonist types, concentrations, *in vivo* or *in vitro* studies, species, or the temperature gradients used in these studies.

Intracellular Ca^{2+} is the primary regulator of smooth muscle contraction.[7,22] $[Ca^{2+}]_i$ is regulated by influx of Ca^{2+} through cell membrane-associated Ca^{2+} channels and by release of Ca^{2+} from intracellular Ca^{2+} stores, especially the sarcoplasmic reticulum.[7] Ishii and Shimo[3] reported that increased responsiveness of the rat airway smooth muscle to acetylcholine with lowered temperature might involve the enhancement of increase in $[Ca^{2+}]_i$, resulting from the acceleration of Ca^{2+} release from the sarcoplasmic reticulum, inhibition of Ca^{2+} extrusion from the cell, or the inhibition of Ca^{2+} reuptake by the sarcoplasmic reticulum. Release of Ca^{2+} from the sarcoplasmic reticulum is, however, important in initiation, not maintenance, of the muscle contraction,[7-9] and we found that the low temperature could enhance the agonist-induced muscle contractility with a decrease in $[Ca^{2+}]_i$ (fig. 1). Therefore, the change in $[Ca^{2+}]_i$ cannot be attributed to the low-temperature-induced contraction of airway smooth muscle shown in this study. Low temperature could increase Ca^{2+} sensitivity in a broad sense. Activation of muscarinic receptors also activates protein kinase C concomitant with an increase in $[Ca^{2+}]_i$. Many investigators,[9,23,24] including us,[8] have shown that protein kinase C activation by muscarinic agonist sensitizes contractile elements to Ca^{2+} or activates a Ca^{2+}-independent mechanism in airway smooth muscles, because carbachol induces a much greater contraction than does high K^+ at a given $[Ca^{2+}]_i$ (fig. 1). Huang *et al.*[25] reported that a potent exogenous protein kinase C activator, phorbol-12,13-diacetate, increased airway smooth muscle tone more at a lower temperature. Therefore, protein kinase C is thought to play a role in regulation of airway smooth muscle contractility at different temperatures. The role of protein kinase C activity in airway smooth muscle tone, however, is controversial.[26,27] Investigations of this possibility, such as measurement of muscle tension in β-escin-permeabilized skinned fiber,[28] must be conducted in the future.

Effects of Volatile Anesthetics or Changes in Temperature on I_{Ca} Currents through Voltage-dependent Ca^{2+} Channels

High-K^+ solution can depolarize the cell membrane of smooth muscle[29] and open VDCCs[30], resulting in a Ca^{2+} influx from the extracellular fluid and muscle contrac-

Table 1. Effects of the Volatile Anesthetics Isoflurane (2.0%) and Sevoflurane (3.0%) at Different Temperatures (37 or 31°C) on the Inactivation Parameters of I_{Ca}

	Isoflurane (2.0%)	Sevoflurane (3.0%)
Percent inhibition of peak I_{Ca} at + 10 mV	69.2 ± 5.2	72.3 ± 3.6
Potential of half inactivation ($V_{1/2}$, mV)		
Control	−15.1 ± 2.6	−15.8 ± 2.4
Anesthetic at 37°C	−25.0 ± 2.2*	−24.3 ± 2.0*
Anesthetic at 31°C	−30.4 ± 2.8*†	−30.0 ± 2.7*†
Slope factor (k, mV)		
Control	7.5 ± 0.8	7.3 ± 0.6
Anesthetic at 37°C	7.2 ± 0.6	7.3 ± 0.5
Anesthetic at 31°C	7.4 ± 0.9	7.4 ± 0.8

Data are expressed as mean ± SD (n = 7). Data were obtained using a 3-s duration prepulse.

* $P < 0.05$, t test comparison with control.

† $P < 0.05$, Fisher test comparison with the other agent.

tion. Sustained contraction of airway smooth muscle requires the continued entry of extracellular Ca^{2+},[31] and blockade of VDCCs suppresses the sustained increase in $[Ca^{2+}]_i$ in carbachol-stimulated tracheal smooth muscle.[8,9] We therefore hypothesized from our results obtained by using the fluorescence technique that the exposure to low temperature could have inhibited the VDCC activity, and we found, by using the whole cell patch clamp technique, that the exposure to low temperature (31°C) inhibited I_{Ca} through VDCCs of freshly dispersed canine tracheal smooth muscle cells without an apparent change in the voltage-dependence of I_{Ca} (fig. 4). These results are consistent with those of previous studies using a variety of species.[32–35] Similar effects on the temperature-dependence of channel conductance and gating kinetics have been reported[34,36,37] and have been attributed to changes in the fluidity of membrane liquids that, in turn, influence the degree and rate of channel opening. The current findings are compatible with the hypothesis that structural changes within the membrane, which occur during thermotropic phase transition, are capable of altering the function of the intramembranous portion of voltage-sensitive calcium channels and leaving unaffected those portions of the channel that do not lie within the membrane *per se*.[34] The volatile anesthetics tested in this study inhibited the activation of I_{Ca} in a dose-dependent manner (figs. 4 and 5). These anesthetics also shifted the inactivation curve of I_{Ca} to a more negative potential (fig. 6). These effects of volatile anesthetics on VDCCs in airway smooth muscle seem to be additive with the effects of low temperature on VDCCs and are consistent with the results of the effects of these anesthetics on $[Ca^{2+}]_i$.

Limitations of This Study

There is a possibility that changes in temperature *per se* could have changed the affinity of fura-2 for Ca^{2+}.[10] We therefore confirmed the effect of temperature on $R_{340/380}$, an indicator of $[Ca^{2+}]_i$, in tracheal smooth muscle strips by the use of the skinned fiber technique,[11] and we found that the $R_{340/380}$ did not change within the temperature range tested (31–37°C). There is another report suggesting that the affinity of a Ca^{2+} chelate compound such as EGTA could be changed by pH.[38] Grynkiewicz *et al.*,[39] however, reported that the intensity of excitation of fura-2 by 500-nm light was not changed by pH excursion. We therefore believe that the measurement of $[Ca^{2+}]_i$ by the use of fura-2 in this study was reliable.

Another uncertainty is the variation in solubilities of volatile anesthetics at different temperatures.[40] We confirmed that the concentrations of the volatile anesthetics in the bath solution were indistinguishable at the different temperatures tested in this study. That is why the replacement of the bath solution in this study was accomplished by changing the inflow perfusate to one that had been bubbled vigorously with each anesthetic at 37°C, and the temperature of the solution was changed during passage through the tube to the bath. We still do not know whether the solubilities of volatile anesthetics in oil or fat (cell membrane) vary at different temperatures. There is a possibility that the change in the solubility of volatile anesthetics in cell membranes at different temperatures plays a role in the low-temperature-induced suppression of VDCC activity.

Exposure to low temperature significantly decreased both high-K^+-induced muscle contraction and increase in $[Ca^{2+}]_i$, but it enhanced carbachol-induced muscle

contraction with a decrease in $[Ca^{2+}]_i$. Volatile anesthetics showed significant inhibition of both high-K^+-induced and carbachol-induced airway smooth muscle contraction, with a concomitant decrease in $[Ca^{2+}]_i$. Exposure to low temperature significantly prevented the inhibitory effect of volatile anesthetics on carbachol-induced muscle contraction; the increase in $[Ca^{2+}]_i$ caused by carbachol was inhibited significantly by the low temperature at all concentrations of the anesthetics. The inhibition of VDCC activity both by exposure to low temperature and by volatile anesthetics can be attributed, at least in part, to the decrease in $[Ca^{2+}]_i$. Low temperature could sensitize contractile elements to Ca^{2+} or activate a Ca^{2+}-independent mechanism if the airway smooth muscle is activated by a muscarinic agonist.[8,9]

References

1. Souhrada M, Souhrada JF: The direct effect of temperature on airway smooth muscle. Respir Physiol 1981; 44:311-23

2. Black JL, Armour CL, Shaw J: The effect of alteration in temperature on contractile responses in human airways in vitro. Respir Physiol 1984; 57:269-77

3. Ishii T, Shimo Y: Cooling-induced supersensitivity to acetylcholine in the isolated airway smooth muscle of the rat. Naunyn Schmiedebergs Arch Pharmacol 1985; 329:167-75

4. Hirshman CA, Bergman NA: Factors influencing intrapulmonary airway calibre during anaesthesia. Br J Anaesth 1990; 65:30-42

5. Milde LN: Clinical use of mild hypothermia for brain protection: a dream revisited. J Neurosurg Anesthesiol 1992; 4:211-5

6. Kataoka K, Yanase H: Mild hypothermia: A revived countermeasure against ischemic neuronal damages. Neurosci Res 1998; 32:103-17

7. Somlyo AM, Himpens B: Cell calcium and its regulation in smooth muscle. FASEB J 1989; 3:2266-76

8. Yamakage M: Direct inhibitory mechanisms of halothane on canine tracheal smooth muscle contraction. ANESTHESIOLOGY 1992; 77: 546-53

9. Ozaki H, Kwon S-C, Tajimi M, Karaki H: Changes in cytosolic Ca^{2+} and contraction induced by various stimulants and relaxants in canine tracheal smooth muscle. Pflügers Arch 1990; 416:351-9

10. Lattanzio FA Jr: The effects of pH and temperature on fluorescent calcium indicators as determined with chelex-100 and EDTA buffer systems. Biochem Biophys Res Commun 1990; 171:102-8

11. Ito Y, Itoh T: Effects of isoprenaline on the contraction-relaxation cycle in the cat trachea. Br J Pharmacol 1984; 83:677-86

12. Fabiato A, Fabiato F: Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. J Physiol Paris 1979; 75:463-505

13. 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

14. Yamakage M, Hirshman CA, Croxton TL: Volatile anesthetics inhibit voltage-dependent Ca^{2+} channels in porcine tracheal smooth muscle cells. Am J Physiol 1995; 268:L187-91

15. Isennberg G, Klockner U: Calcium tolerant ventricular myocytes prepared by preincubation in a "KB medium." Pflügers Arch 1982; 395:6-18

16. Langton PD, Burke EP, Sanders KM: Participation of Ca currents in colonic electrical activity. Am J Physiol 1989; 257:C451-60

17. Bratton DL, Tanaka DT, Grunstein MM: Effects of temperature on cholinergic contractility of rabbit airway smooth muscle. J Appl Physiol 1987; 63:1933-41

18. Macquin-Mavier I, Benichou M, Lorino AM, Lorino H, Istin N, Harf A: Influence of body temperature on histamine-induced bronchoconstriction in guinea pigs. J Appl Physiol 1989; 66:1054-8

19. Bethel RA, Tanaka DT, Mitchell LS, Curtis SP, Leff AR, Irvin CG: Effect of cooling on the responsiveness of canine tracheal smooth muscle. Am Rev Respir Dis 1990; 142:1402-6

20. Ishii T, Shimo Y: Cooling-induced subsensitivity to carbachol in the tracheal smooth muscle of the guinea-pig. Naunyn Schmiedebergs Arch Pharmacol 1986; 332:219-23

21. Freed AN, Stream CE: Airway cooling: Stimulus specific modulation of airway responsiveness in the canine lung periphery. Eur Respir J 1991; 4:568-74

22. van Breemen C, Saida K: Cellular mechanisms regulating $[Ca^{2+}]_i$ smooth muscle. Annu Rev Physiol 1989; 51:315-29

23. Park S, Rasmussen H: Activation of tracheal smooth muscle contraction: synergism between Ca^{2+} and activators of protein kinase C. Proc Natl Acad Sci USA 1985; 82:8835-9

24. Menkes H, Baraban JM, Snyder SH: Protein kinase C regulates smooth muscle tension in guinea-pig trachea and ileum. Eur J Pharmacol 1986; 122:19-27

25. Huang C-K, Munakata M, Baraban JM, Menkes H: Protein kinase C and tracheal contraction at low temperature. J Pharmacol Exp Ther 1987; 243:270-80

26. Bremerich DH, Warner DO, Lorenz RR, Shumway R, Jones KA: Role of protein kinase C in calcium sensitization during muscarinic stimulation in airway smooth muscle. Am J Physiol 1997; 273:L775-81

27. Bremerich DH, Kai T, Warner DO, Jones KA: Effect of phorbol esters on Ca^{2+} sensitivity and myosin light-chain phosphorylation in airway smooth muscle. Am J Physiol 1998; 274:C1253-60

28. Kai T, Bremerich DH, Jones KA, Warner DO: Drug-specific effects of volatile anesthetics on Ca^{2+} sensitization in airway smooth muscle. Anesth Analg 1998; 87:425-9

29. Casteels R, Phil D: Electro- and pharmacomechanical coupling in vascular smooth muscle. Chest 1980; 78:150-6

30. Bolton TB: Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev 1979; 3:606-718

31. Bourreau J-P, Abela AP, Kwan CY, Daniel EE: Acetylcholine Ca^{2+} stores refilling directly involves a dihydropyridine-sensitive channel in dog trachea. Am J Physiol 1991; 261:C497-505

32. Nobile M, Carbone E, Lux HD, Zucker H: Temperature sensitivity of Ca currents in chick sensory neurones. Pflügers Arch 1990; 415:658-63

33. Cota G, Siri NL, Stefani E: Calcium-channel gating in frog skeletal muscle membrane: effect of temperature. J Physiol (Lond) 1983; 338: 395-412

34. Rosen AD: Temperature modulation of calcium channel function in GH_3 cells. Am J Physiol 1996; 271:C863-8

35. Van Lunteren E, Elmslie KS, Jones SW: Effects of temperature on calcium current on bullfrog sympathetic neurons. J Physiol (Lond) 1993; 466:81-93

36. Hagiwara S, Yoshii M: Effect of temperature on the anomalous rectification in the membrane of the egg of the starfish, Mediaster aequalis. J Physiol (Lond) 1980; 307:517-27

37. Narahashi T, Tsunoo A, Yoshii M: Characterization of two types of calcium channels in mouse neuroblastoma cells. J Physiol (Lond) 1987; 383:231-49

38. Horrison SM, Bers DM: Correction of proton and Ca association constants of EGTA for temperature and ionic strength. Am J Physiol 1989; 256:C1250-6

39. Grynkiewicz G, Poenie M, Tsien RY: A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 1985; 260:3440-50

40. Allott PR, Steward A, Flook V, Mapleson WW: Variation with temperature of the solubilities of inhaled anaesthetics in water, oil and biological media. Br J Anaesth 1973; 45:294-300