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Review article

Cellular mechanisms of airway smooth muscle relaxant effects of anesthetic agents

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Introduction

Studies have been carried out in our department to elucidate the basic mechanisms underlying the actions of anesthetics and the mechanisms of airway smooth muscle contraction and relaxation. We have used various advanced techniques, including electrophysiological, biochemical, and biomolecular techniques [1], to investigate these mechanisms. It is important for anesthesiologists to know how anesthetic agents that are used clinically can affect airway smooth muscle tone, since anesthesia is often performed on patients with airway hyperreactivity, such as patients with asthma or emphysema. Hirshman and Bergman [2] first reviewed the factors influencing intrapulmonary airway caliber during anesthesia. Many studies have since been carried out on direct and indirect effects of anesthetic agents on airway smooth muscle tone using appropriate and sophisticated techniques (Fig. 1). It is essential first to understand the basic physiology of the regulation of airway smooth muscle tone.

Regulation of airway smooth muscle tone

In all types of cells, Ca^{2+} is the most important second messenger for intracellular signal transduction [1]. The intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$) is precisely controlled at a low level, and each cell/tissue/organ can thus perform functions such as secretion and contraction. In a resting condition, $[Ca^{2+}]_i$ is maintained at a level of approximately 100 nM, which is 1/10000 of the extracellular concentration of free Ca^{2+} (approximately 1 mM). As shown in Fig. 2, a large Ca^{2+} concentration gradient is generally maintained by (1) the cell membrane, which consists of a Ca^{2+}-unpermeable lipid bilayer; (2) powerful systems to excrete Ca^{2+} to the extracellular space [Ca^{2+} pump (Ca^{2+}–nH^+ ATPase) and Na^+–Ca^{2+} exchanger]; and (3) intracellular organelles such as the endoplasmic reticulum and mitochondria for Ca^{2+} uptake.

Airway smooth muscle tone is determined by the balance of constrictor and dilator mechanisms, which, in turn, are mediated by receptors and channels on the surfaces of smooth muscle cells [1,2]. Activation of these receptors and channels alters the $[Ca^{2+}]_i$, which itself controls the contractile state of muscle through the Ca^{2+}-dependent stimulation of myosin light chain kinase (MLCK) (Fig. 1, bottom). The active kinase then switches on myosin by phosphorylation of its 20-kDa subunits. However, the sensitivity of Ca^{2+} is not fixed. It is possible under certain circumstances to increase muscle tone without increasing $[Ca^{2+}]_i$ by altering the relationship between $[Ca^{2+}]_i$ and tension [3]. At least two cellular mechanisms contribute to increases in $[Ca^{2+}]_i$: Ca^{2+} release from intracellular stores and Ca^{2+} influx through cell membrane-associated Ca^{2+} channels, especially voltage-dependent Ca^{2+} channels (VDCCs) [1,3]. The mechanisms of Ca^{2+} mobilization depend on the agent inducing the contraction and on the duration of the contractile stimulus. Pharmacological agonists such as acetylcholine initiate contraction by binding to G protein (Gq)-coupled receptors to activate phospholipase C (PLC), which generates inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). The latter activates protein kinase C (PKC), whereas IP_3 mobilizes Ca^{2+} from the sarcoplasmic reticulum. In contrast, agents such as K^+ at high concentrations induce con-

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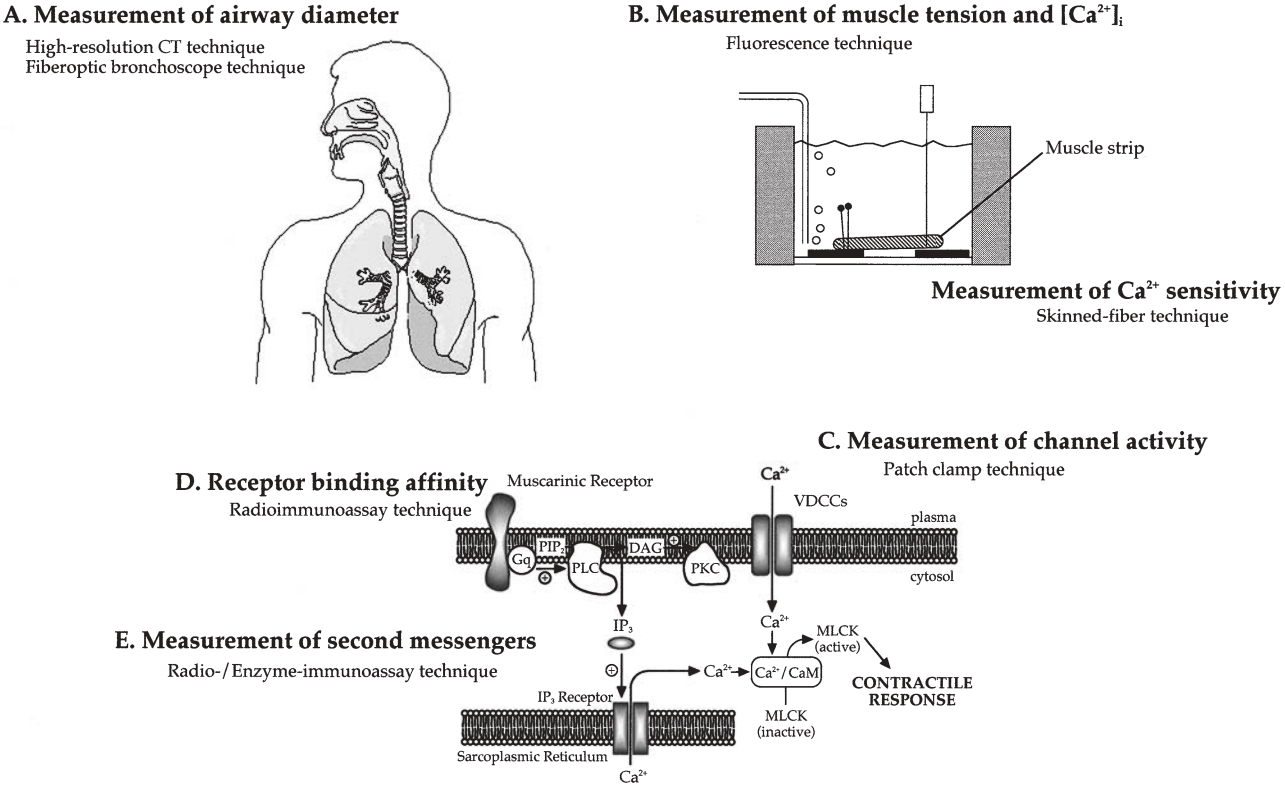

Fig. 1A–E. Techniques used for the study of airway smooth muscle. **A** Using high-resolution computed tomography (*CT*) or a superfine fiberoptic bronchoscope technique, the internal diameter of a small airway can be measured. **B** Using a fluorescence technique, the intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$), a primary second messenger for smooth muscle contraction, and tension of tracheal/bronchial smooth muscles can be measured simultaneously. **C** As an electrophysiological approach, the patch clamp technique enables direct measurement of activities of membrane-associated channels, particularly those of voltage-dependent Ca^{2+} channels (*VDCCs*). **D/E** The effects of anesthetic agents on the other second messengers, cyclic adenosine 3′,5′-monophosphate (AMP) and inositol 1,4,5-triphosphate (IP_3), as well as receptor-binding affinity, can be assessed using immunoassay techniques. *Gq*, G protein; *PIP_2*, phosphatidylinositol 4,5-bisphosphate; *PLC*, phospholipase C; *DAG*, diacylglycerol; *PKC*, protein kinase C; *CaM*, calmodulin; *MLCK*, myosin light chain kinase

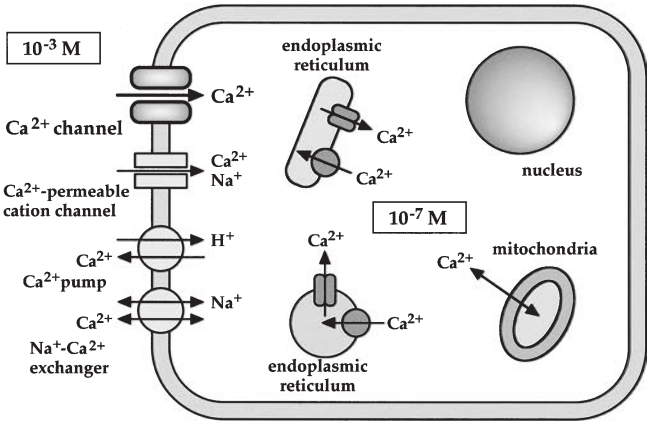

Fig. 2. Regulation and distribution of the intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$). The increase in $[Ca^{2+}]_i$ can be triggered by the release of Ca^{2+} from intracellular stores, especially the *endoplasmic reticulum*, and by influx from the extracellular space through voltage-dependent Ca^{2+} channels (VDCCs) and *Ca^{2+}-permeable cation channels*

traction by depolarizing the cell membrane in a graded manner with resultant activation of VDCCs [4]. However, entry of extracellular Ca^{2+} is necessary for maintenance of contraction under both sets of conditions [5].

Our group has succeeded in simultaneous measurements of airway smooth muscle tension and $[Ca^{2+}]_i$ during exposure to various anesthetic agents using a fluorescence technique [1,3]. Since 1995, our group has also been using an electrophysiological technique [6] to clarify the effects of anesthetic agents on Ca^{2+} and K^+ channels, which regulate Ca^{2+} influx and membrane potential, respectively. Other intracellular second messengers, cyclic adenosine 3′,5′-monophosphate (cAMP) and IP_3, have been investigated using radioimmuno- and enzyme immunoassay techniques. As in other tissues, the mechanisms of actions of anesthetic agents have been clarified over the past decade using these advanced techniques.

Inhibitory actions of volatile anesthetics on airway smooth muscle

Since volatile anesthetics are potent bronchodilators, many investigators have been trying to clarify the mechanisms underlying their actions [7,8]. Our group, using the Ca^{2+} indicator fura-2, demonstrated that relaxation of contracted tracheal smooth muscle induced by volatile anesthetics at clinically relevant concentrations is associated with a decrease in $[Ca^{2+}]_i$ [3–5]. Although the decrease in $[Ca^{2+}]_i$ caused by volatile anesthetics seems to be the main mechanism underlying the actions of volatile anesthetics, a decrease in Ca^{2+} sensitivity due to inhibition of the activity of PKC has also been reported [3]. Since sustained contraction of airway smooth muscle requires the continuous entry of extracellular Ca^{2+} and since blockade of VDCCs by dihydropyridine-sensitive Ca^{2+} channel blockers suppresses the sustained increase in $[Ca^{2+}]_i$ in agonist-stimulated tracheal smooth muscle [5], it is reasonable to assume that volatile anesthetics can also inhibit VDCC activity. Our group demonstrated that volatile anesthetics have an inhibitory effect on whole-cell inward Ca^{2+} currents through VDCCs of porcine tracheal smooth muscle cells at clinically relevant concentrations [6] and that the potencies of the inhibitory effects of the anesthetics on the currents are closely related to their lipid-phase solubilities (Fig. 3) [9].

Volatile anesthetics also inhibit initial phasic contraction as well as tonic contraction. It has been suggested that transient Ca^{2+} release from the sarcoplasmic reticulum due to an agonist-induced increase in the intracellular amount of IP_3 is important for phasic contraction. Inhibition of IP_3-induced Ca^{2+} release and a decrease in Ca^{2+} content in the sarcoplasmic reticulum caused by volatile anesthetics have been demonstrated [10]. It has also been demonstrated that volatile anesthetics cause a decrease in the amount of agonist-induced IP_3. We also applied these techniques to investigations of vascular smooth muscle [11], platelets [12], neurons [13], and uterine smooth muscle [14,15], and we have clarified the cellular mechanisms of the actions of anesthetics in various kinds of cell types and chronic pain in a neuropathic pain model.

It is known that volatile anesthetics, such as halothane, enflurane, isoflurane, sevoflurane, and desflurane, have strong bronchodilatory effects in vitro on airway smooth muscle [5]. Isoflurane and desflurane, however, are very pungent, and these anesthetics might therefore induce an asthmatic attack by facilitating neurally mediated acetylcholine release during anesthetic induction. Halothane has a proarrhythmogenic property, and care should be taken in the use of this anesthetic when using aminophylline simultaneously. Sevoflurane has a small blood/gas partition coefficient

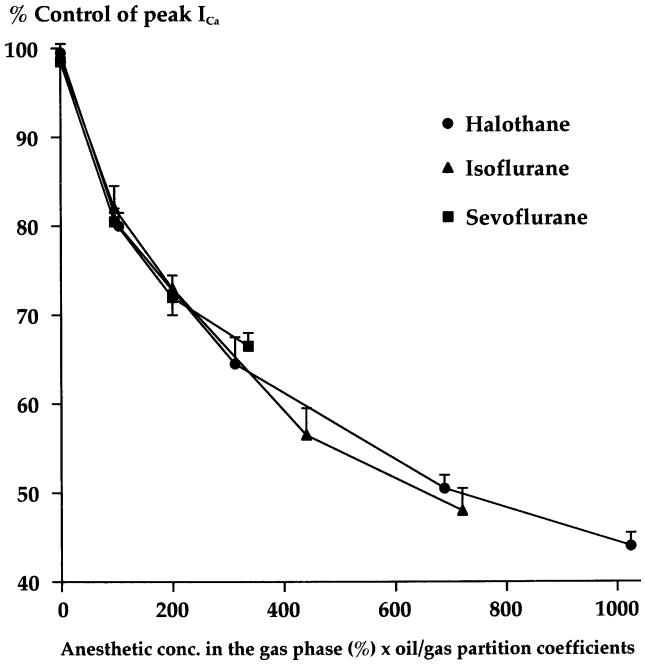

Fig. 3. Relationship between peak inward Ca^{2+} currents (I_{Ca}) relative to the control and concentrations of the volatile anesthetics halothane, isoflurane, and sevoflurane. Symbols represent the means ± SE of data from three to four cells at each concentration. The *x*-axis variable is theoretically proportional to the anesthetic concentration in a lipid phase such as the cell membrane

(0.64), adequate anesthetic potency [1.71% as 1 MAC (maximum alveolar concentration)], and no airway pungency [16]. Therefore, among the currently available volatile anesthetics, sevoflurane is the best anesthetic for low-flow anesthesia (LFA) and for volatile induction and maintenance of anesthesia (VIMA) [17]. An increase in compound A concentration in the anesthetic circuit is one of the main problems during LFA with sevoflurane, but some new soda limes that do not produce compound A are now available [18,19]. Volatile induction of anesthesia using sevoflurane is consequently very smooth and safe [20,21].

Effects of other anesthetic agents and changes in environment on airway smooth muscle

All intravenous anesthetics have to some degree in vitro inhibitory effects on airway smooth muscle tone [22]. As in the case of volatile anesthetics, our group reported that the intravenous anesthetics thiopental, ketamine, and propofol inhibited inward Ca^{2+} currents through VDCCs of porcine tracheal smooth muscle cells in a dose-dependent manner [23]. No intravenous anesthetics showed any apparent shift in the voltage dependence of induced Ca^{2+} currents. However, after

treatment with the Ca^{2+} channel agonist Bay K 8644, thiopental, but not ketamine or propofol, shifted the maximum Ca^{2+} cuurents to more positive potentials. All three anesthetics promoted the inactivated state of the channel at more negative potentials, but propofol was less effective than thiopental or ketamine in this regard.

It has also been reported that the benzodiazepine midazolam inhibited airway smooth muscle contraction by decreasing $[Ca^{2+}]_i$ and that diazepam and midazolam had inhibitory effects on VDCCs [24]. High concentrations of these agents were, however, necessary to inhibit K^+ channels, producing membrane depolarization. It has been reported that the lack of antagonistic effects of their antagonists is related to the non-gamma aminobutyric acid (GABA)-mediated electrophysiological effects of benzodiazepines on airway smooth muscle contractility [24]. Unlike volatile anesthetics, all intravenous anesthetics require rather high concentrations to elicit an inhibitory effect.

Protamine is used to reverse heparin anticoagulation, but it may have a number of adverse effects during this reversal, including bronchoconstriction, which is caused by the generation of anaphylatoxic complements induced by the heparin–protamine complex. In contrast, it has been shown that both protamine and the heparin–protamine complex can inhibit canine tracheal smooth muscle contraction by decreasing $[Ca^{2+}]_i$ in vitro [25]. These agents decrease the agonist-induced increase in $[Ca^{2+}]_i$ by inhibition of VDCCs [25].

Changes in extracellular pH have been shown to affect the contractility of airway smooth muscles in vitro, airway smooth muscle tension being increased during alkalosis and decreased during acidosis. Our group demonstrated, using Ca^{2+} and pH fluorescence techniques with fura-2 and 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxy fluorescein (BCECF), that alkalinization significantly increased $[Ca^{2+}]_i$ and enhanced muscle contraction at all concentrations of K^+ but did not alter the relationship between muscle tension and $[Ca^{2+}]_i$ [26]. Acidification significantly decreased $[Ca^{2+}]_i$ without changing muscle tone; hence, the muscle tension—$[Ca^{2+}]_i$ relationship was shifted to the left. These results suggest that changes in extracellular pH can alter airway smooth muscle tone by changing $[Ca^{2+}]_i$ and intracellular pH. Our group has clarified, by using an intracellular perfusion technique, that inward Ca^{2+} currents through VDCCs of airway smooth muscle were decreased by acidosis and increased by alkalosis and that the currents were much more sensitive to changes in intracellular pH than to changes in extracellular pH [27].

Muscarinic agonists such as acetylcholine and carbachol not only activate smooth muscle contraction via M_3 receptor-mediated activation of PLC but also increase airway smooth muscle tone by inhibiting relaxation induced by β-adrenergic agonists. This pathway involves an M_2 muscarinic receptor that is linked to adenylyl cyclase by a pertussis toxin-sensitive G protein (Gi). It is possible that volatile anesthetics relax airway smooth muscle by inhibiting this pathway. Muscarinic stimulation has been shown to have a significant and dose-dependent inhibitory effect on Ca^{2+} currents through VDCCs of dispersed tracheal smooth muscle cells in perforated patch clamp experiments but no effect on the currents in conventional whole-cell experiments [28]. Since an exogenous PKC activator and an activating fragment of PKC both caused inhibition of the currents, as was seen in the case of muscarinic stimulation, we can conclude that the cholinergic effect on VDCCs appears to be mediated by PKC activation.

Using the techniques employed in the studies described above, we also investigated the effects of sodium nitroprusside [29]; a novel Ca^{2+}-channel antagonist, RWJ-22108 [30]; low temperature [31]; and hypoxia [32] on airway smooth muscle, and we believe that the results of these basic experiments have contributed to improvement in the safety of clinical anesthesia.

Different reactivities to volatile anesthetics in proximal and distal airways

Most studies have focused on the direct effects of anesthetics on the larger, more proximal airway because of easier access. However, the distal airway, especially between the third and seventh generation bronchi, is important in the regulation of airflow resistance, and a series of studies have shown that there are significant physiological and pharmacological differences between tracheal and bronchial smooth muscles. Our group demonstrated, using the whole-cell patch clamp technique, that approximately 30% of porcine bronchial smooth muscle cells included T-type VDCCs as well as L-type VDCCs, although tracheal smooth muscle cells had only L-type VDCCs [33]. We also demonstrated that the volatile anesthetics isoflurane and sevoflurane significantly inhibited the activities of both types of VDCCs in a dose-dependent manner; however, the anesthetics had greater inhibitory effects on T-type VDCC activity in bronchial smooth muscle. The existence of T-type VDCCs 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. Isoflurane and sevoflurane inhibited whole-cell K^+ currents to a greater degree in tracheal than in bronchial smooth muscle cells [34]. More than 60% of the K^+ currents in tracheal smooth muscle, but less than 40% in bronchial smooth muscle, appear to be mediated through delayed rectifier K^+ channels. The

inhibitory effects of the anesthetics on the delayed rectifier K^+ channels were greater than those on the remaining K^+ channels. Differences in distributions and anesthetic sensitivities of K^+-channel subtypes might play a role in the difference in inhibitory effects of the anesthetics on tracheal and bronchial smooth muscle contractions. Using the current-clamp technique, the volatile anesthetics isoflurane and sevoflurane induced significant repolarization of depolarized cell membranes in the trachea (from −19.8 to −23.6 mV and −24.8 mV, respectively) and bronchus (from −24.7 to −29.3 mV and −30.4 mV, respectively) without affecting carbachol binding affinity to the muscarinic receptor (Fig. 4) [35]. The repolarizing effect was abolished by a Ca^{2+}-activated Cl^- (Cl_{Ca}) channel blocker, niflumic acid. These results indicate that volatile anesthetic-induced repolarization of airway smooth muscle cell membranes might be due to a change in Cl_{Ca}-channel activity and that the difference in the repolarized effects of the volatile anesthetics could in part contribute to the difference in the effects of volatile anesthetics on tracheal and bronchial smooth muscle contractions.

Electrophysiological characteristics are used to classify Ca^{2+} channels by, for example, the range of membrane potentials over which channels are activated, the kinetics of opening and closing, and the conductance and lifetime of individual channels (Table 1) [36]. Ca^{2+} channels are divided roughly into two classes: high-voltage-activated (HVA) Ca^{2+} channels and low-voltage-activated (LVA) Ca^{2+} channels. HVA Ca^{2+} channels are further divided into L-type, N-type, P/Q-type, and R-type channels, while LVA Ca^{2+} channels consist of only T-type channels. The functions of the L-type Ca^{2+} channel are related to the generation of action potentials and to signal transduction events at the cell membrane. L-type VDCCs are expressed ubiquitously in neuronal, endocrine, cardiac, smooth, and skeletal muscle, as well as in fibroblasts and kidney cells. Because T-type VDCCs are activated at negative membrane potentials close to the resting potential, the T-type channel is thought to be responsible for neuronal oscillatory activity, which has been proposed to be involved in processes such as sleep/wakefulness regulation, motor coordination, and neuronal circuit specification during ontogenesis. In addition, T-type Ca^{2+} channels are involved in pacemaker activity, low-threshold Ca^{2+} spikes, and rebound burst firing. Investigation of the role of T-type channels in other systems (particularly in cardiovascular and endocrine systems) has demonstrated that they are abundant in proliferating cells in both normal and pathological conditions. VDCCs serve as one of the important mechanisms for Ca^{2+} influx into the cells, enabling the regulation of $[Ca^{2+}]_i$. Recent advances both in electrophysiology and in molecular biology have made it possible to observe channel activity directly and to investigate channel functions at molecular levels [37,38]. It has recently become apparent that some hereditary diseases such as hypokalemic periodic paralysis result from calcium channelopathies [39]. The effects of anesthetics on abnormal Ca^{2+} channel activity have not yet been examined.

Technique by the use of a gene expression model

The direct effects of volatile anesthetics on cell membranes involve multiple interactions with the lipid bilayer and proteins, with ion channels being primary targets [40,41]. Numerous K^+-channel currents in the heart have been reported to regulate the duration and repolarization of cardiac action potential. Given the

Table 1. Electrophysiological classification and characteristics of voltage-dependent Ca^{2+} channels.

	T-type	N-type	L-type	P/Q-type	R-type
Voltage dependence	LVA	HVA	HVA	HVA	IVA
Activation range (mV)	−70	−20	−30 to −10	−60	−40
Inactivation range (mV)	−100 to −60	−120 to −30	−60 to −10		
Rate of inactivation[a] (ms)	20–50	50–80	>500		20–40
Single channel conductance (pS)	8	13	25	10–20	14
Ion selectivity	$Ba^{2+} = Ca^{2+}$	$Ba^{2+} > Ca^{2+}$	$Ba^{2+} > Ca^{2+}$	$Ba^{2+} > Ca^{2+}$	$Ba^{2+} > Ca^{2+}$
Blocking by divalent ions	$Ni^{2+} > Cd^{2+}$	$Cd^{2+} > Ni^{2+}$	$Cd^{2+} > Ni^{2+}$	$Ni^{2+} > Cd^{2+}$	
Isoform	α_{1G}, α_{1H}, α_{1I}	α_{1B}	α_{1C}, α_{1D}, α_{1S}	α_{1A}	α_{1B}
Antagonists/blockers	Mibefradil ω-Aga IIIA	ω-CTX GVIA ω-Aga IIIA	DHP PAA BTZ	FTX ω-CTX-MVIIC	ω-Aga IIIA

LVA, low-voltage-activated; HVA, high-voltage-activated; IVA, intermediate-voltage-activated; pS, picosiemens; DHP, dihydropyridine; PAA, phenylalkylamine; BTZ; benzothiazepine; ω-CTX GVIA, ω-conotoxin GVIA from *Conus geographus*, ω-Aga IIIA, ω-agatoxin IIIA; FTX, funnel spider venom toxin; ω-CTX-MVIIC, ω-conotoxin from *Conus magus*

[a] Decay time constant

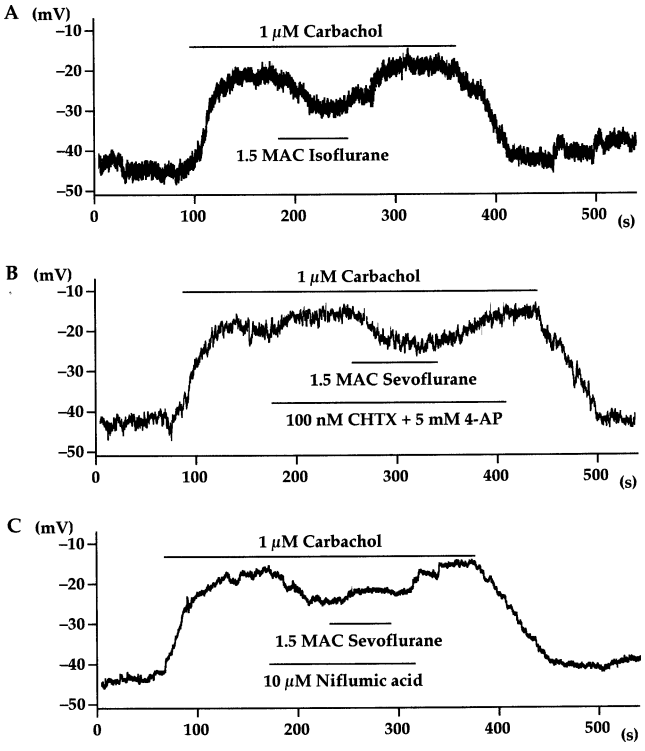

Fig. 4A–C. Representative data of the effects of the volatile anesthetics tested on membrane potentials in tracheal smooth muscle cells. **A** Effect of 1.5 minimum alveolar concentration (*MAC*) isoflurane on carbachol (1 μM)-induced depolarization of the membrane. Isoflurane significantly hyperpolarized the membrane potential. **B** Effect of 1.5 MAC sevoflurane on carbachol (1 μM)-induced depolarization of the membrane pretreated with 100 nM charybdotoxin (*CHTX*), a Ca^{2+}-activated K^+-channel blocker, and 5 mM 4-aminopyridine (*4-AP*), a delayed rectifier K^+-channel blocker. Sevoflurane significantly repolarized the membrane potential, whereas CHTX and 4-AP had little effect on the membrane potential. **C** Effect of 1.5 MAC sevoflurane on carbachol (1 μM)-induced depolarization of the membrane pretreated with 10 μM niflumic acid, a Ca^{2+}-activated Cl^--channel blocker. Sevoflurane had little effect on the membrane potential, whereas niflumic acid significantly hyperpolarized the membrane

important role that K^+ channels have in normal cardiac electrical activity, efforts to determine the effects of anesthetics on the function of these channels have multiplied. Various inhibitory effects of volatile anesthetics on inward rectifier K^+ channels have recently been shown. In contrast, volatile anesthetics appear to have stimulatory effects on cardiac ATP-sensitive K^+ channels. The K^+ current through delayed rectifier K^+ channels (I_K) is important for initiation of repolarization in the heart and therefore plays a major role in control of the duration of the cardiac action potential. There is, however, little information about the interaction between volatile anesthetics and I_K. Two components of I_K have been demonstrated in the human ventricle. One is a very slow activating and deactivating delayed rectifier K^+ current (I_{Ks}). The I_{Ks} represents the predominant repolarizing current during increased heart rate. Recent studies have revealed that the I_{Ks} channel is formed by two transmembrane subunits, coded by KvLQT1 and minK proteins. The KvLQT1 subunit forms the pore of the channel, whereas the minK subunit seems to act as a regulator subunit. Coassembly of these two proteins produces current kinetics resembling that of the native I_K in cardiomyocytes. Mutations of the *KvLQT1* and *minK* genes account for more than 50% of cases of congenital long Q-T syndrome, an inherited cardiac disorder. In most patients with this disorder, a dominant negative effect on the KvLQT1 channel results in a reduction of I_{Ks} amplitude and in prolongation of the Q-T interval in a surface electrocardiogram. Drugs, such as volatile anesthetics, may therefore prolong ventricular repolarization by inhibiting the I_{Ks} and increase the risk of cardiac disorder. We investigated the interaction between the volatile anesthetics isoflurane and sevoflurane and cloned I_{Ks} from normal adult rat hearts expressed in *Xenopus* oocytes [40], and we concluded that the significant inhibitory effect of volatile anesthetics on the cloned I_{Ks} may partly contribute to the clinical observations of prolongation of ventricular repolarization (Q-T interval) caused by the anesthetics (Fig. 5) [41].

In the near future, we plan to use the gene expression technique with the patch clamp technique in studies on airway smooth muscle.

Summary and future survey

Direct interactions between perioperative changes in the environment (including anesthetic exposure) and airway smooth muscle tone have been clarified in considerable detail over the past decade. However, the clinical significance of the results of these basic studies is another matter. Hirshman's group developed a Basenji-Greyhound dog model of asthma in 1980; however, the effects of only local anesthetics have been investigated using this model. Further investigations are needed to clarify the interactions between anesthetic agents and airway smooth muscle using easily available and reliable asthmatic/chronic obstructive pulmonary disease (COPD) models, especially in view of the fact that asthma/COPD mortality rates are increasing worldwide. A gene expression technique should also be used in the investigation of airway smooth muscle.

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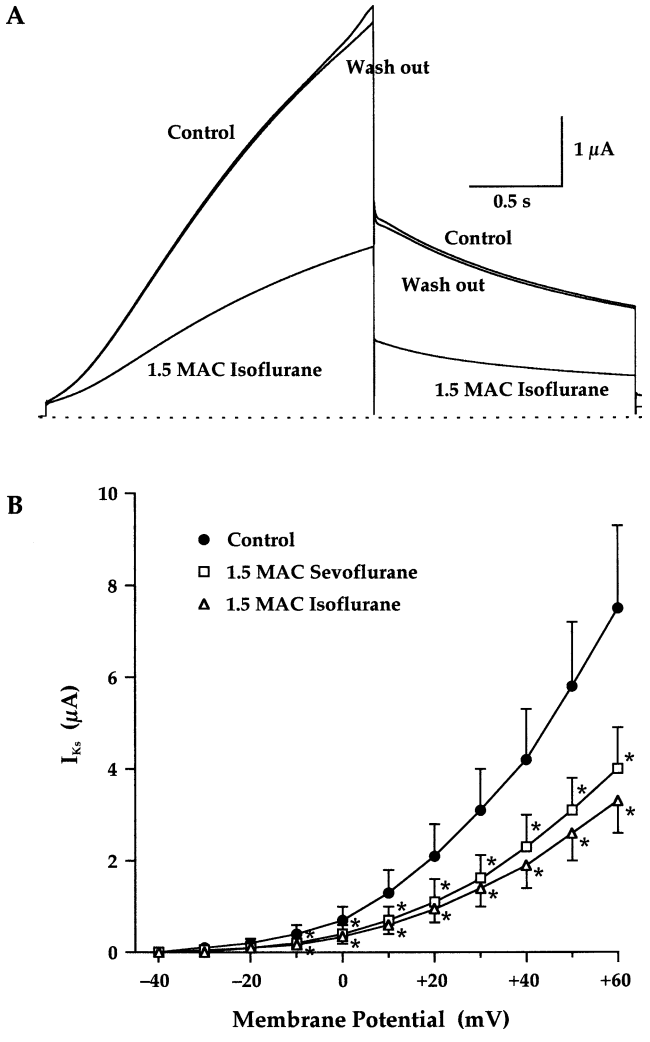

Fig. 5A,B. Effects of the volatile anesthetics isoflurane and sevoflurane on cloned delayed rectifier K^+ currents (I_{Ks}) expressed in *Xenopus* oocytes. **A** Typical recordings of I_{Ks} induced by depolarizing pulses to +40 mV from a holding potential of −80 mV. Exposure to 1.5 minimum alveolar concentration (*MAC*) isoflurane significantly inhibited the magnitude of I_{Ks}. *Dashed line* denotes zero current. **B** Current-voltage relationships obtained before and after exposure to 1.5 *MAC isoflurane* or 1.5 *MAC sevoflurane*. Symbols represent means ± SD (n = 7 each). *Asterisk*, $P < 0.05$ vs control

107, 1994); (2) grants-in-aid (Nos. 10770762, 1998; 12671489, 2000; and 15591648, 2003) for research from the Ministry of Education, Culture, Sports, Science and Technology of Japan; (3) an incentive grant (No. 98-B-02, 1998) for research from the Akiyama Foundation; (4) a grant-in-aid (No. 058, 1999) for research from the Hokkaido Foundation for the Promotion of Scientific and Industrial Technology; (5) an incentive grant (No. III-27, 2000) for research from the Uehara Memorial Foundation; (6) a grant-in-aid (No. A-14, 2001) for research from the Japan Research Foundation for Clinical Pharmacology; and (7) a subsidy as part of the Yamamura Memorial Prize (2002) from the Japanese Society of Anesthesiologists.

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