Anesthesiology
82:700–712, 1995

Volatile Anesthetic Actions on Contractile Proteins in Membrane-permeabilized Small Mesenteric Arteries

Takashi Akata, M.D., Ph.D., Walter A. Boyle III, M.D.†*

Background: **Volatile anesthetics have been shown to have vasodilating or vasoconstricting actions *in vitro* that may contribute to their cardiovascular effects *in vivo*. However, the precise mechanisms of these actions *in vitro* have not been fully elucidated. Moreover, there are no data regarding the mechanisms of volatile anesthetic action on small resistance arteries, which play a critical role in the regulation of blood pressure and blood flow.**

Methods: **With the use of isometric tension recording methods, volatile anesthetic actions were studied in intact and β-escin–membrane-permeabilized smooth muscle strips from rat small mesenteric arteries. In experiments with intact muscle, the effects of halothane (0.25–5.0%), isoflurane (0.25–5.0%), and enflurane (0.25–5.0%) were investigated on high K^+–induced contractions at 22°C and 35°C. All experiments were performed on endothelium-denuded strips in the presence of 3 μM guanethidine and 0.3 μM tetrodotoxin to minimize the influence of nerve terminal activities. In experiments with membrane-permeabilized muscle, the effects of halothane (0.5–4.0%), isoflurane (0.5–4.0%), and enflurane (0.5–4.0%) on the half-maximal and maximal Ca^{2+}-activated contractions were examined at 22°C in the presence of 0.3 μM ionomycin to eliminate intracellular Ca^{2+} stores.**

Results: **In the high K^+–stimulated intact muscle, all three anesthetics generated transient contractions, which were followed by sustained vasorelaxation. The IC_{50} values for this vasorelaxing action of halothane, isoflurane, and enflurane were 0.47 vol% (0.27 mM), 0.66 vol% (0.32 mM), and 0.53 vol% (0.27 mM), respectively, at 22°C and were 3.36 vol% (0.99 mM), 3.07 vol% (0.69 mM), and 3.19 vol% (0.95 mM), respectively, at 35°C. Ryanodine (10 μM) eliminated the anesthetic-induced contractions but had no significant effect on the anesthetic-induced vasorelaxation in the presence of high K^+. In addition, no significant differences were observed in the dose dependence of the direct vasodilating action among these anesthetics with or without ryanodine at either the low or the high temperature. However, significant differences were observed in the vasoconstricting actions among the anesthetics, and the order of potency was halothane > enflurane > isoflurane. The Ca^{2+}–tension relation in the membrane-permeabilized muscle yielded a half-maximal effective Ca^{2+} concentration (EC_{50}) of 2.02 μM. Halothane modestly but significantly inhibited 3 μM (approximately the EC_{50}) and 30 μM (maximal) Ca^{2+}-induced contractions. Enflurane slightly but significantly inhibited 3 μM but not 30 μM Ca^{2+} contractions. Isoflurane did not significantly inhibit either 3 μM or 30 μM Ca^{2+} contractions.**

Conclusions: **Halothane, isoflurane, and enflurane have both vasoconstricting and vasodilating actions on isolated small splanchnic resistance arteries. The direct vasoconstricting action appears to result from Ca^{2+} release from the ryanodine-sensitive intracellular Ca^{2+} store. The vasodilating action of isoflurane in the presence of high K^+ appears to be attributable mainly to a decrease in intracellular Ca^{2+} concentration, possibly resulting from inhibition of voltage-gated Ca^{2+} channels. In contrast, the vasodilating actions of halothane and enflurane in the presence of high K^+ appears to involve inhibition of Ca^{2+} activation of contractile proteins as well as a decrease in intracellular Ca^{2+} concentration in smooth muscle. (Key words: Anesthetics, volatile: enflurane; halothane; isoflurane. Artery: resistance. Membrane, permeabilization: β-escin. Muscle, smooth: vascular.)**

VOLATILE anesthetics are known to affect cardiovascular stability by causing changes in vascular reactivity, cardiac function, and autonomic reflexes that detect alterations and initiate adjustments in the cardiovascular system.[1–3] Their overall effect is a decrease in mean arterial pressure caused by peripheral vasodilation, myocardial depression, and decreased sympathetic nervous system activity.[1–3] Vasodilating or vasocon-

* Research Associate, Department of Anesthesiology, Washington University School of Medicine; Assistant Professor, Department of Anesthesiology, Kyushu University, Fukuoka, Japan.

† Assistant Professor, Departments of Anesthesiology and of Molecular Biology and Pharmacology, Washington University School of Medicine.

Received from the Department of Anesthesiology Research Unit, Washington University School of Medicine, St. Louis, Missouri. Accepted for publication November 3, 1994. Supported in part by Grant-in-Aid (A) 05771141 from the Ministry of Education, Science, and Culture, Japan (to T.A.). Presented in part at the annual meeting of the American Society of Anesthesiologists, San Francisco, October 15–19, 1994.

Address reprint requests to Dr. Akata: Departments of Anesthesiology and of Molecular Biology and Pharmacology, Washington University School of Medicine, Box 8054, 660 South Euclid Avenue, St. Louis, Missouri 63110-1093 (until November 1, 1995) or Department of Anesthesiology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan (after November 1, 1995).

stricting actions of volatile anesthetics have been demonstrated in isolated aorta and coronary arteries,[4–10] suggesting that direct vascular action may contribute to the cardiovascular effects of volatile anesthetics *in vivo*. In addition, recent studies have demonstrated that volatile anesthetics have vasodilating or vasoconstricting actions on isolated small resistance arteries at clinically relevant concentrations,[11–13] more strongly suggesting that direct actions on vascular tissue do significantly contribute to changes in peripheral vascular resistance *in vivo*.

The precise mechanism(s) of the *in vitro* vasodilating or vasoconstricting actions of volatile anesthetics have not been fully clarified. The involvement of endothelium in the vascular actions of volatile anesthetics have been investigated in several vascular preparations,[4–8,11,12,14–19] and the endothelium dependence of the vasodilating or vasoconstricting actions of halothane, enflurane or isoflurane have been suggested in some preparations including canine coronary and mesenteric arteries and rat aorta.[4,6,14] In addition, several groups of investigators have demonstrated that halothane, isoflurane, enflurane, and sevoflurane can inhibit endothelium-mediated relaxations in a variety of vascular preparations.[12,16–18] However, the lack of effects of endothelial denudation or the endothelium-derived relaxing factor pathway inhibitors on vasodilating actions of isoflurane or halothane has also been documented in some vascular preparations including rat aorta, rabbit basilar and canine cerebral arteries.[7,15,19] Thus, the role of endothelium in the vascular responses to volatile anesthetics appears to be still controversial.

Vasodilating or vasoconstricting actions of volatile anesthetics have also been documented in several endothelium-denuded vascular preparations.[5–8,10,14,15,19,20] Several previous studies that have addressed the mechanisms of the direct vascular smooth muscle actions of volatile anesthetics have suggested that these agents may affect both activation of contractile proteins and intracellular Ca^{2+} mobilization.[5,14,20–24] Su and Zhang demonstrated that halothane slightly depressed maximal Ca^{2+}-activated tension in membrane-permeabilized rabbit aortic tissue,[5] suggesting that inhibition of contractile proteins may contribute to the vasodilating action of halothane. Buljubasic and coworkers, using the patch-clamp method, have demonstrated that halothane (0.75% and 1.5%) and isoflurane (2.6%) inhibit long-lasting Ca^{2+} currents in canine coronary and cerebral arteries, suggesting that volatile anesthetics can decrease intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by inhibiting voltage-gated Ca^{2+} influx.[22,23] More recently, Tsuchida *et al.* have demonstrated that halothane (≥1%) and isoflurane (≥2%) decreased both agonist- and high K^+–induced increases in $[Ca^{2+}]_i$ in rat aortic tissues, and, by using simultaneous measurements of tension and $[Ca^{2+}]_i$, also implied that higher concentrations of the anesthetics may inhibit Ca^{2+}-activation of contractile proteins.[20]

Small splanchnic resistance arteries are known to play a critical role in the regulation of systemic blood pressure and splanchnic blood flow,[25,26] and may be an important site of volatile anesthetic action.[27] In addition, maintenance of splanchnic blood flow during the perioperative period may be important in preventing splanchnic ischemia, which can significantly affect the outcome of critically ill surgical patients by allowing bacterial translocation across the gut wall.[28–30] Although a few previous studies have investigated volatile anesthetic action in isolated large mesenteric arteries,[14,31] little information is available regarding mechanisms of volatile anesthetic action in small splanchnic resistance arteries. Previous studies *in vivo* and *in vitro* have demonstrated significant differences in the vascular actions of volatile anesthetics between large conductance and smaller arteries,[9,27,32–34] indicating the need to further investigate volatile anesthetic action in the small splanchnic resistance arteries.

In this study, we investigated the mechanisms of volatile anesthetic action on small splanchnic resistance arteries. Using isometric-tension recording methods, we first investigated the vasoconstricting and vasodilating actions of halothane, isoflurane, and enflurane in endothelium-denuded tissues precontracted with high K^+. We then investigated the mechanisms of these effects in ryanodine-treated intact smooth muscle and β-escin–permeabilized smooth muscle. This report represents the first description of volatile anesthetic action on Ca^{2+}-activated contractions in membrane-permeabilized resistance arteries.

Materials and Methods

Tissue Preparation for Tension Measurement

After receiving institutional approval for this animal study, Sprague-Dawley rats (200–250 g) were anesthetized with halothane in O_2 after preoxygenation with 100% O_2 for 3–5 min. After obtaining an optimal anesthetic level, the mesenteric tissue was removed and

immediately placed in a dissecting chamber filled with 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES)–buffered physiologic salt solution. The mesenteric artery was then rapidly excised. The distal portion of the third or fourth order branch (0.15–0.20 mm in diameter) of the mesenteric artery was used. Under a binocular microscope, the surrounding connective and fat tissues were removed, the vessel was cut open lengthwise, and the endothelium was removed by gently rubbing the intimal surface with the round surface of a small pin as has previously been described.[35,36] From this "vascular sheet," a transverse strip (150–200 μm in width, 250–400 μm in length) was prepared. Both ends of the strip were then tied with two thin silk threads, and, for the isometric tension measurement, the strip was fixed between one end of a chamber (0.9 ml capacity) and a L-shaped stainless rod connected to the strain-gauge transducer (UL-2, Shinko, Tokyo) as previously reported.[12,35,36] After a 30-min equilibration, the strip was stretched to approximately 1.1 times its resting length (without tension) to obtain a maximal contractile response to high K^+. The functional removal of the endothelium was confirmed by disappearance of endothelium-dependent relaxation by acetylcholine (10 μM). The solution was changed by perfusing it rapidly from one end while aspirating it simultaneously from the other end. Experiments with intact muscle were conducted both at room temperature (22°C) and at 35°C, whereas the experiments with membrane-permeabilized muscle were performed at room temperature (22°C) to prevent deterioration of the permeabilized strips.

Solutions and Drugs

The millimolar ionic concentrations of the HEPES-buffered physiologic salt solution (PSS) for the experiments with intact muscle were as follows: NaCl 138, KCl 5.0, $MgCl_2$ 1.2, $CaCl_2$ 1.5, HEPES 10, glucose 10. The *p*H was adjusted with NaOH to 7.35 at either 22°C or 35°C. The high-K^+ solutions were prepared by replacing NaCl with KCl isoosmotically.

The composition of the relaxing and activating solutions used in the experiments with membrane-permeabilized muscle are listed in table 1, which also shows calculated values of free ion concentrations and total ionic strength in each solution. The *p*H was adjusted with KOH to 7.00 at 22°C. Free ion concentrations and the ionic strengths of the relaxing and activating solutions were calculated by solving multiequilibrium equations with a hydrogen ion activity coefficient 0.75 and association constants between ions (Appendix) as previously used.[37,38] Numerical solution of a set of multiequilibrium equations was achieved by a successive approximation method by computer (Powerbook 170, Apple, Cupertino, CA).

Guanethidine, tetrodotoxin, HEPES, β-escin, and ionomycin were obtained from Sigma Chemical (St. Louis, MO). Ethyleneglycol-bis-(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), piperazine-1,4-bis-(2-ethanesulfonic acid) (PIPES-K_2), and methanesulfonic acid were obtained from Fluka Chemie AG (Buchs, Switzerland). Ryanodine was purchased from Agri Systems International (Wind Gap, PA). Isoflurane and enflurane were obtained from Abbott Laboratories (North Chicago, IL), and halothane was obtained from Ayerst Laboratories (Philadelphia, PA). All other reagents were of the highest grade commercially available.

Experimental Design

Intact Muscle. The effects of halothane, isoflurane, and enflurane on maximum high K^+–induced contractions were studied in endothelium-denuded strips at room temperature (22°C) and at 35°C. To suppress peripheral nerve activities, 3 μM guanethidine and 0.3 μM tetrodotoxin were present in the solutions throughout the experiments. In the experiments at room temperature, the volatile anesthetics were cumulatively (0.25–5.0%) applied to the strip after contractile response to 143 mM K^+ had reached a plateau. Because the 40 mM K^+-induced contractions were often not well maintained for a long period at 35°C, a single concentration (0.125–5.0%) of each anesthetic was applied to the strip after contractile response to 40 mM K^+ reached a plateau. The anesthetics were not cumulatively applied in these experiments (at 35°C).

Because the above experiments revealed that the volatile anesthetics had a vasoconstricting action that resulted from Ca^{2+} release from intracellular Ca^{2+} stores, as previously demonstrated,[11–13] we also examined the effects of volatile anesthetics on ryanodine-treated strips at both temperatures. In these experiments, ryanodine (10 μM) was first applied to the strips with caffeine (20 mM) for approximately 1 min to deplete the Ca^{2+} stores, and the strips were then treated with ryanodine (10 μM)-containing PSS for 25 min. All the experiments with ryanodine were started after 20 mM caffeine–induced contractions were completely abolished.

Membrane-permeabilized Muscle. After measuring steady contractions induced by high K^+, the strips were

Table 1. Composition of Solutions and Calculated Values of Free Ion Concentrations and Total Ionic Strength

Solutions	Total Concentrations (mM)							Calculated Values			
	EGTA	$Ca(Ms)_2$	$Mg(Ms)_2$	KMs	PIPES K_2	ATP	CrP	$[Ca^{2+}]$ (μM)	$[Mg^{2+}]$ (mM)	$[K^+]$ (mM)	Ionic Strength (M)
Relaxing solution	4	0	7	80	20	5	10	0.002	1.680	119.9	0.204
Activating solutions											
pCa = 7.0	4	0.45	7	80	20	5	10	0.101	1.694	119.9	0.204
pCa = 6.5	4	1.09	7	80	20	5	10	0.300	1.716	119.9	0.205
pCa = 6.0	4	2.20	7	80	20	5	10	1.005	1.755	120.0	0.207
pCa = 5.5	4	3.09	7	80	20	5	10	2.992	1.788	120.0	0.208
pCa = 5.0	4	3.62	7	80	20	5	10	10.180	1.810	120.0	0.208
pCa = 4.5	4	3.83	7	80	20	5	10	30.872	1.824	120.0	0.208
pCa = 4.0	4	3.99	7	80	20	5	10	99.282	1.848	120.0	0.209

pCa = −log $[Ca^{2+}]$; EGTA = ethyleneglycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; Ms = methanesulfonate; PIPES = piperazine-*N*-*N'*-bis-(2-ethanesulfonic acid); ATP = adenosine 5'-triphosphate; CrP = creatinine phosphate. The *p*H was adjusted with KOH at 7.0 (22°C).

permeabilized with 50 μM β-escin for 22–24 min at room temperature in relaxing solution.[39–41] Low concentrations of Ca^{2+} ionophores such as ionomycin or A23187 have been used to achieve nonselective and functional depletion of the intracellular Ca^{2+} store.[35,40–43] In fact, in this artery, 0.3 μM ionomycin was effective in abolishing caffeine (20 mM)- and phenylephrine (10 μM)-induced contractions in Ca^{2+}-free (2 mM EGTA) solutions in intact strips, and in abolishing the caffeine contractions in β-escin–permeabilized strips. Therefore, all of the experiments with membrane-permeabilized muscle were performed in the presence of 0.3 μM ionomycin to deplete the intracellular Ca^{2+} stores.

In the first set of experiments with membrane-permeabilized muscle, we determined the *p*Ca–tension relation by applying various concentrations of Ca^{2+} containing activating solutions in a cumulative manner. In another set of the experiments, we examined the effects of halothane, isoflurane, and enflurane at concentrations of 0.5–4.0% on the half-maximal and maximal Ca^{2+}-activated contractions. The volatile anesthetics were applied to the strips in a cumulative manner after the Ca^{2+}-activated contractions reached a plateau (5 min after application of Ca^{2+}); each concentration of each anesthetic was applied for a period of 2 min.

Volatile Anesthetics: Delivery and Analysis

The volatile anesthetics were delivered by agent-specific vaporizers in line with the air gas aerating the solutions in experiments with intact and with membrane-permeabilized muscle. Each solution was equilibrated with the anesthetics for at least 10 min before introduction to the chamber, which was covered with thin glass to prevent the equilibration gas from escaping into the atmosphere. The anesthetic concentrations in the solutions were determined by gas chromatography (fig. 1).

Although the actual concentrations of volatile anesthetics in the PSS were not measured in all experiments,

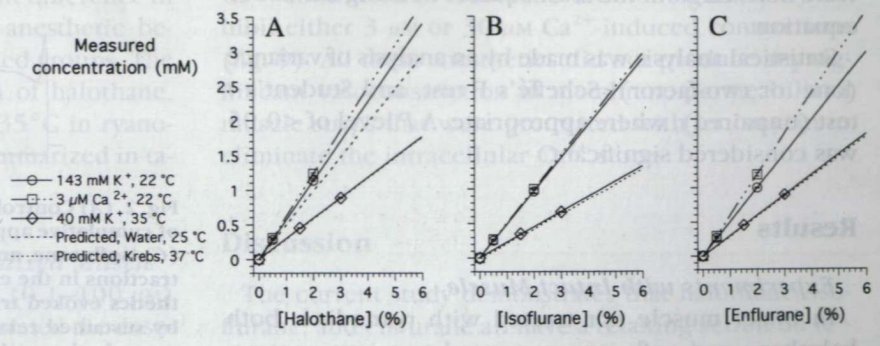

Fig. 1. Measured concentrations of (*A*) halothane, (*B*) isoflurane, and (*C*) enflurane dissolved in various solutions at 22°C or 35°C. The predicted values (dotted lines) were calculated from the water–gas partition coefficient at 25°C (1.20 for halothane) or from Krebs' solution–gas partition coefficients at 37°C (0.75, 0.55 and 0.74 for halothane, isoflurane, and enflurane, respectively).[44] The water–gas or Krebs' solution–gas partition coefficients at low temperature for isoflurane and enflurane were not available. The data were linearly fitted with the least square fit methods. All values are mean ± SEM (n = 4).

the relation between actual concentrations of volatile anesthetics in PSS and anesthetic concentrations (volume percentage) in the gas mixture should be theoretically linear. The anesthetic concentrations on the x-axis are displayed as volume percentage for the volatile anesthetic concentration–response relations.

Calculation and Statistical Analysis

All results were expressed as the means ± standard error of the mean (SEM). The n denotes the number of preparations, and the number of animals is also noted.

The volatile anesthetic–induced relaxation in experiments with intact muscle at both temperatures was expressed as a percent change from the amplitude of high K^+–induced contraction before application of the anesthetics, and the amplitude of volatile anesthetic–induced vasoconstriction at 35°C was expressed relative to the amplitude of the 40 mM K^+-induced phasic contraction (100%). The concentration-dependence of the volatile anesthetic–induced vasoconstriction in experiments with intact muscle at 22°C was not assessed, because the amount of Ca^{2+} in the intracellular Ca^{2+} store was probably not constant during "cumulative" application of volatile anesthetics in these experiments.

In experiments with β-escin–permeabilized muscle, the amplitude of the Ca^{2+}-activated contraction at various time points in the presence and absence of volatile anesthetics was normalized to the amplitude of Ca^{2+} contraction 5 min after application of the activating solution. The effects of volatile anesthetics were then assessed by comparing the data in the presence of volatile anesthetics with the time control data.

The concentration–response data for volatile anesthetic effects or Ca^{2+}-activated contractions were fitted according to a four parameter logistic model described by De Lean *et al.*[45] The EC_{50} (50% effective concentration) or IC_{50} (50% inhibitory concentration) values were derived from the least-squares fit using the above equation.

Statistical analysis was made by an analysis of variance (one- or two-factor), Scheffé's F test, and Student's *t* test (unpaired), where appropriate. A P level of <0.05 was considered significant.

Results

Experiments with Intact Muscle

In intact muscle not treated with ryanodine, both halothane and enflurane generated transient contractions superimposed on the high K^+–induced contractions at 22°C and at 35°C (figs. 2 and 3). Isoflurane generated similar transient contractions at 22°C, but not at 35°C (figs. 2 and 3). The transient contractions caused by volatile anesthetics at both temperatures were followed by significant vasorelaxation (figs. 2 and 3). Ryanodine (10 μM) abolished the volatile anes-

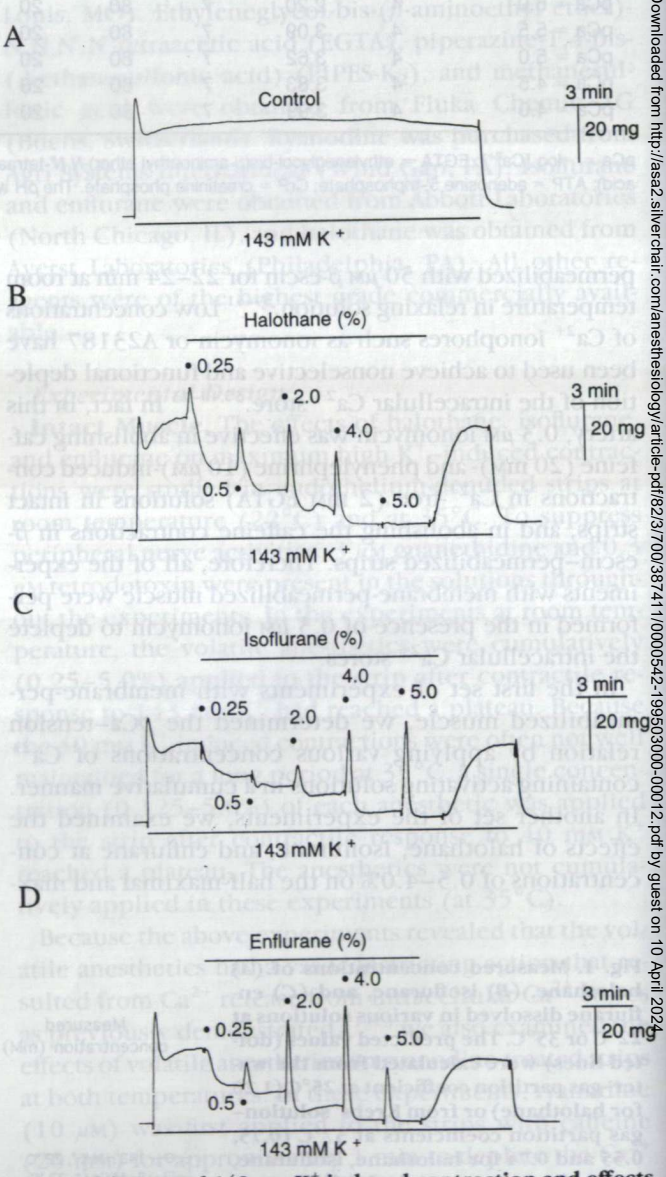

Fig. 2. (*A*) Control 143 mM K^+-induced contraction and effects of cumulative application (0.25–5.0%, 22°C) of (*B*) halothane, (*C*) isoflurane, and (*D*) enflurane on 143 mM K^+-induced contractions in the endothelium-denuded strips. All of the anesthetics evoked transient contractions, which were followed by sustained relaxation. Similar observations were made in several other strips (n = 7–11, six animals).

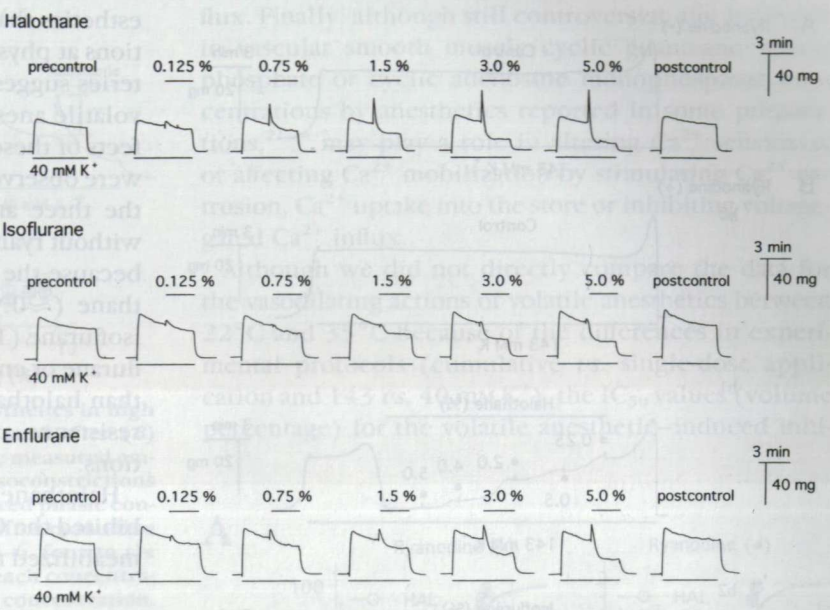

Fig. 3. Effects of various concentrations (0.125–5.0%) of (*A*) halothane, (*B*) isoflurane, and (*C*) enflurane on 40 mM K^+-induced contractions in the endothelium-denuded strips at 35°C. Halothane and enflurane produced vasoconstriction and vasodilation in a concentration-dependent manner, whereas isoflurane produced only vasodilation and not vasoconstriction. Similar observations were made in several other strips (n = 6, six animals).

thetic–induced contractions in the presence of high K^+ at both temperatures (figs. 4–6). Significant differences were observed in the vasoconstricting effects at 35°C among the anesthetics, and the order of potency was halothane > enflurane > isoflurane (fig. 6). Ryanodine treatment did not significantly affect the phasic or tonic components of high K^+–induced contractions at either temperature ($P > 0.05$).

The concentration–response curves for volatile anesthetic–induced relaxation under various conditions (in the absence or presence of ryanodine; at either low or high temperature) are shown in figure 7. Two factor (concentration [volume percentage], anesthetic) analysis of variance revealed no significant difference in the vasorelaxation among the three volatile anesthetics under each condition. In addition, two-factor (concentration and treatment [with or without ryanodine]) analysis of variance showed no significant difference in the vasorelaxation produced by each anesthetic between the ryanodine-treated and untreated groups. The IC_{50} values for the vasodilating action of halothane, isoflurane, and enflurane at 22°C and 35°C in ryanodine-treated and untreated strips are summarized in table 2.

Experiments with β-Escin–Permeabilized Muscle

Application of various concentrations (0.1–100 μM) of Ca^{2+} to the β-escin–treated strips generated the dose-dependent contractions, and the EC_{50} value for the Ca^{2+}–tension relation derived from the least-squares fit with the logistic equation[45] was 2.02 μM (fig. 8). The maximal Ca^{2+} activated contraction was 1.58 ± 0.25 times that of 143 mM K^+-induced maximal (phasic) contractions before permeabilization (n = 4, four animals), suggesting satisfactory membrane permeabilization.

The effects of volatile anesthetics on 3 μM ($\approx EC_{50}$) and 30 μM (maximal) Ca^{2+}-activated contractions were then examined. The 3 μM Ca^{2+} contraction was well maintained, whereas the 30 μM Ca^{2+} contraction slightly decayed (time control data, fig. 9). Comparison between the volatile anesthetic data and the time control data showed that halothane modestly, but significantly inhibited both 3 μM ($\approx EC_{50}$) and 30 μM (maximum) Ca^{2+}-induced contractions (fig. 9). Enflurane slightly but significantly inhibited 3 μM, but not 30 μM Ca^{2+} contractions. Isoflurane did not significantly inhibit either 3 μM or 30 μM Ca^{2+}-induced contractions (fig. 9). All these anesthetics did not produce any significant vasoconstriction in membrane-permeabilized muscle strips that were pretreated with ionomycin to eliminate the intracellular Ca^{2+} stores.

Discussion

The current study demonstrates that halothane, isoflurane, and enflurane all have a relaxing action on K^+-constricted small splanchnic resistance arteries. This

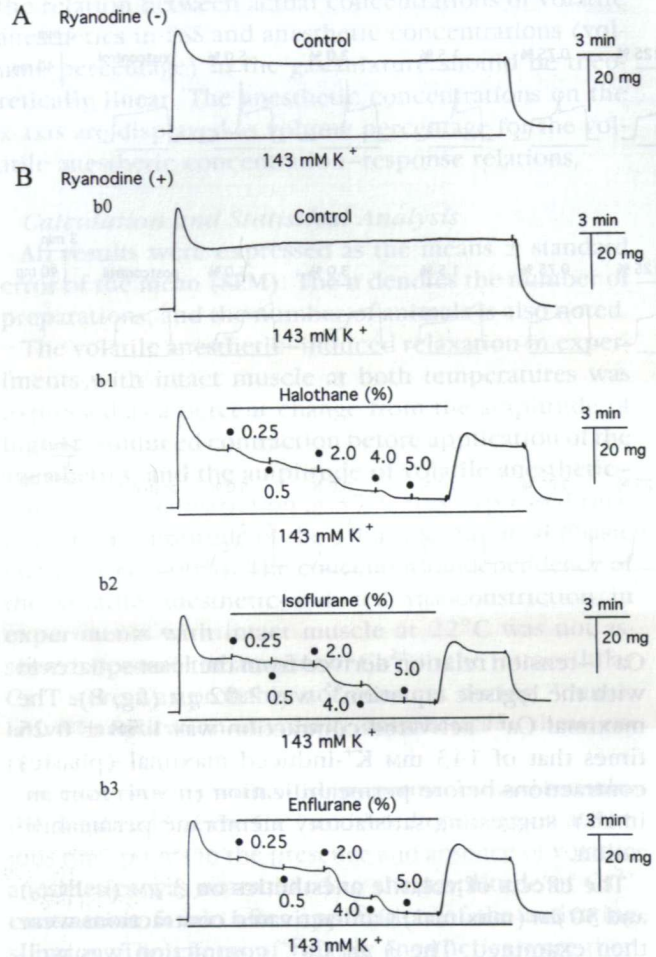

Fig. 4. Effects of ryanodine (10 μM) on volatile anesthetic actions in the 143 mM K^+-treated endothelium-denuded strips at 22°C. (*A* and *B-b0*) The 143 mM K^+ contractions (*A*) before and (*B*) after treatment with ryanodine. (*B-b1, B-b2,* and *B-b3*) Effects of cumulative application (0.25–5.0%) of halothane, isoflurane, and enflurane on 143 mM K^+ contraction in the presence of ryanodine. Ryanodine had little effect on the 143 mM K^+ contraction or the anesthetic-induced vasodilation, but ryanodine eliminated the anesthetic-induced contractions that were observed in the absence of ryanodine (fig. 1). Similar observations were made in several other strips (n = 5, five animals).

action observed at clinically relevant anesthetic concentrations appears to be independent of endothelial or peripheral nerve activities, although this action may be influenced by anesthetic effects on endothelium or peripheral nerves *in vivo*. It is well established that K^+-induced constrictions are caused by activation of voltage-gated Ca^{2+} channels and that these channels play a significant role in maintaining vascular tone *in vivo*. Thus, the effective concentrations of volatile anesthetics that inhibited the high K^+–induced constrictions at physiologic temperature in these resistance arteries suggest that this "direct" vasodilating action of volatile anesthetics contributes to the hypotensive effects of these agents *in vivo*. No significant differences were observed in the direct vasodilating actions among the three anesthetics under any condition (with or without ryanodine; at either 22°C or 35°C). However, because the minimum alveolar concentration of halothane (≈0.90%) is known to be lower than that of isoflurane (1.38%) or enflurane (1.40%) in the rat, isoflurane or enflurane would be a more potent vasodilator than halothane in the high K^+–stimulated splanchnic resistance artery at equivalent anesthetic concentrations.

Halothane and enflurane slightly but significantly inhibited the Ca^{2+}-activated contractions in β-escin–permeabilized muscle, suggesting that the observed relax-

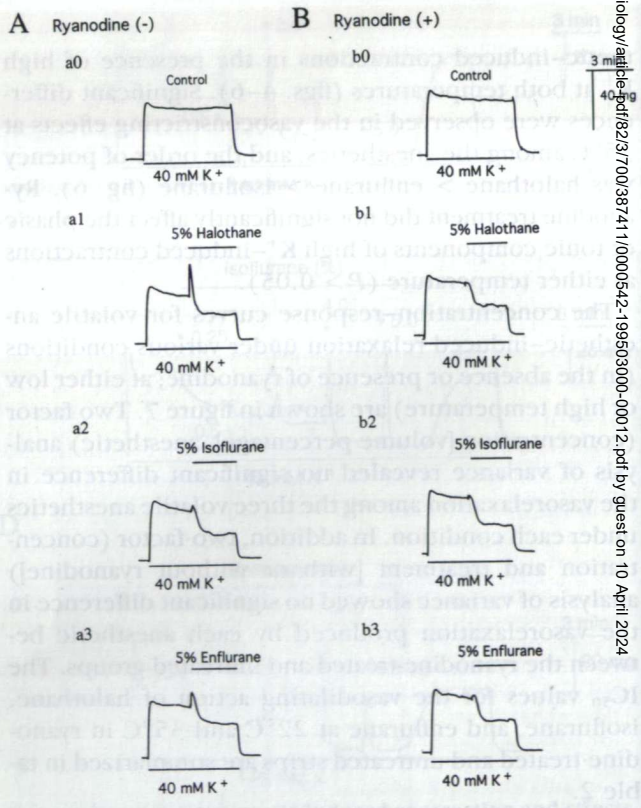

Fig. 5. Effects of 5% of halothane, isoflurane, and enflurane on 40 mM K^+-induced contraction before (*A*) and after (*B*) ryanodine (10 μM) treatment. Ryanodine had little effect on the 40 mM K^+ contraction or the volatile anesthetic–induced vasodilation but eliminated the volatile anesthetic–induced vasoconstrictions. Similar observations were made in other several strips (n = 4, four animals).

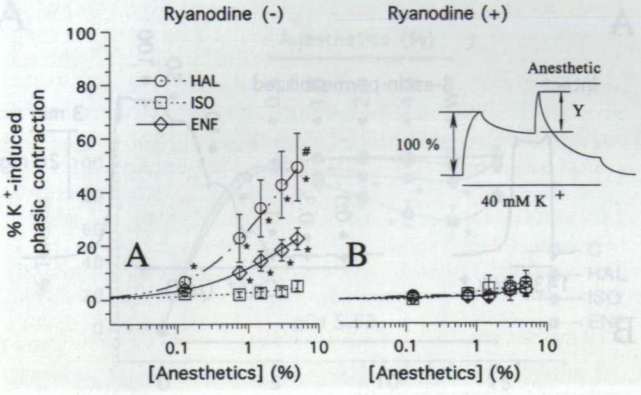

Fig. 6. Vasoconstricting action of volatile anesthetics in high K^+ (40 mM)–preconstricted strips before (*A*) and after (*B*) treatment with ryanodine (10 μM) at 35°C. The measured amplitude (Y) of volatile anesthetic–induced vasoconstrictions (arrow) was normalized to the 40 mM K^+-induced phasic contraction (100%). Vasoconstriction disappears after ryanodine treatment. All values are mean ± SEM (n = 4–6, four to six animals). *$P < 0.05$ *versus* isoflurane group at each concentration. #$P < 0.05$ *versus* enflurane group at each concentration.

ing actions of halothane and enflurane in the presence of high K^+ are attributable in part to inhibition of Ca^{2+} activation of contractile proteins. However, this effect was small relative to the amount of vasodilation observed in the high K^+–constricted intact vessels, suggesting that the vasodilation is caused in large part by depression of increases in $[Ca^{2+}]_i$. At most, only 20% of the relaxing effect of halothane could be attributed to a direct inhibition of Ca^{2+} action on the contractile protein cascade; the rest of the effect is thus presumably caused by decreases in the rise of $[Ca^{2+}]_i$. The complete lack of effect of isoflurane on the Ca^{2+}-activated contractions implies that its relaxing action is attributable almost completely to depression of the rise in $[Ca^{2+}]_i$. The small amount of inhibition of Ca^{2+}-activated contractions by halothane in these small mesenteric arteries is consistent with the slight inhibition of maximal Ca^{2+} activated tension in membrane-permeabilized rabbit aortic tissue.[5] The proposal that volatile anesthetics affect vascular tone by decreasing $[Ca^{2+}]_i$ is consistent with recent investigations using fura-2–loaded vascular tissue in which halothane and isoflurane were shown to decrease both agonist- and high K^+–induced increases in $[Ca^{2+}]_i$.[20] Previous studies have also demonstrated that halothane and isoflurane directly inhibit depolarization-activated long-lasting Ca^{2+} currents in vascular smooth muscle cells,[22,23] suggesting that the $[Ca^{2+}]_i$-reducing effects of volatile anesthetics result at least in part from inhibition of voltage-gated Ca^{2+} influx. Finally, although still controversial, the increases in vascular smooth muscle cyclic guanosine monophosphate or cyclic adenosine monophosphate concentrations by anesthetics reported in some preparations,[21,24] may play a role in altering Ca^{2+} sensitivity or affecting Ca^{2+} mobilization by stimulating Ca^{2+} extrusion, Ca^{2+} uptake into the store or inhibiting voltage-gated Ca^{2+} influx.

Although we did not directly compare the data for the vasodilating actions of volatile anesthetics between 22°C and 35°C because of the differences in experimental protocols (cumulative *vs.* single-dose application and 143 *vs.* 40 mM K^+), the IC_{50} values (volume percentage) for the volatile anesthetic–induced inhi-

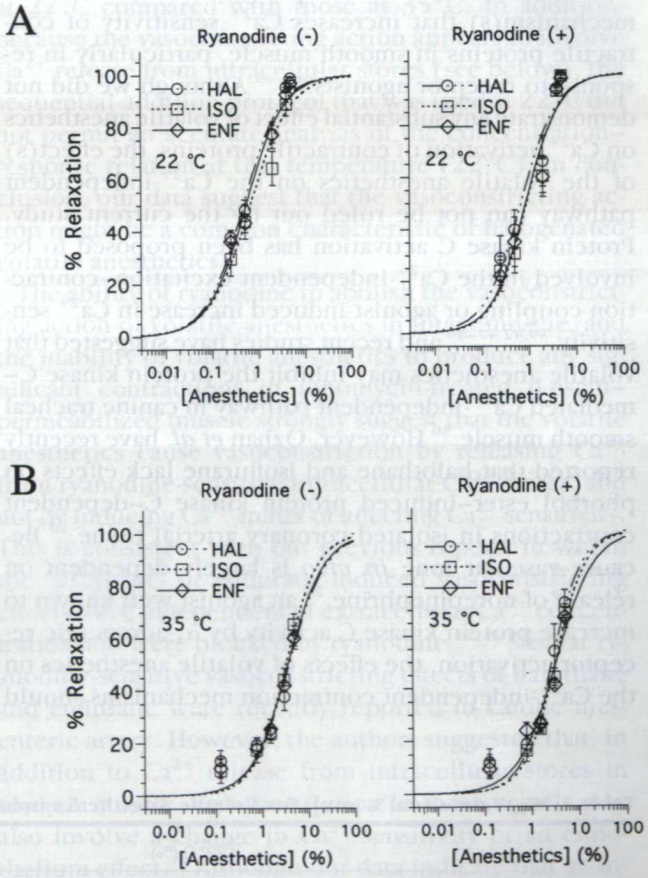

Fig. 7. Vasodilating action of volatile anesthetics in high K^+–treated endothelium-denuded strips in the presence (*right*) or absence (*left*) of ryanodine (10 μM) at (*A*) 22°C and (*B*) 35°C. No significant differences were observed in vasodilating action among three volatile anesthetics under each condition. The IC_{50} values for the vasodilating actions of volatile anesthetics under each condition are shown in table 2. All values are displayed as mean ± SEM (n = 7–11, six animals).

bitions of high K^+–induced contraction were approximately three to six times greater at 35°C than at 22°C. Assuming that the binding of an anesthetic to its active sites is determined by solution concentration (and not partial pressure),[46] then some of this difference can be explained by the approximately 1.5- to 2-fold increase in the solubility of these anesthetics at 22°C compared with 35°C (fig. 1). However, with or without this correction, some of the differences in potency must be related to other temperature-sensitive factors such as the voltage-gated Ca^{2+} channels or enzyme activities involved in smooth muscle contraction.

In addition to the well-known Ca^{2+}-dependent excitation–contraction coupling pathway, recent studies also indicate the existence of either a Ca^{2+}-independent excitation–contraction coupling pathway or some mechanism(s) that increases Ca^{2+} sensitivity of contractile proteins in smooth muscle, particularly in response to receptor agonists.[47–49] Although we did not demonstrate any substantial effect of volatile anesthetics on Ca^{2+} activation of contractile proteins, the effect(s) of the volatile anesthetics on the Ca^{2+}-independent pathway can not be ruled out by the current study. Protein kinase C activation has been proposed to be involved in the Ca^{2+}-independent excitation–contraction coupling or agonist-induced increase in Ca^{2+} sensitivity,[39,42,47–49] and recent studies have suggested that volatile anesthetics may inhibit the protein kinase C–mediated Ca^{2+}-independent pathway in canine tracheal smooth muscle.[50] However, Ozhan *et al.* have recently reported that halothane and isoflurane lack effects on phorbol ester–induced protein kinase C–dependent contractions in isolated coronary arterial tissue.[10] Because vascular tone *in vivo* is largely dependent on release of norepinephrine,[26] an agonist well known to increase protein kinase C activity by α_1-adrenergic receptor activation, the effects of volatile anesthetics on the Ca^{2+}-independent contraction mechanisms should be further investigated to clarify the volatile anesthetic vascular action *in vivo*.

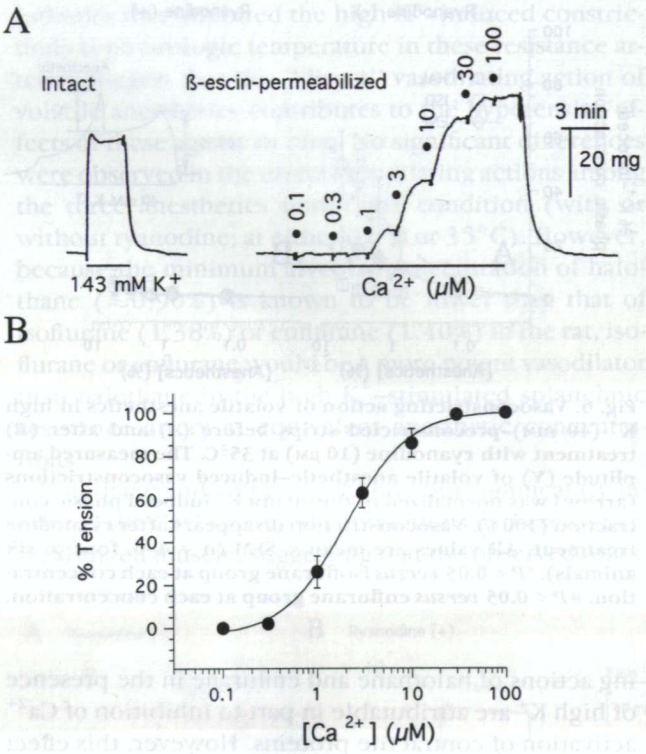

Fig. 8. Ca^{2+}–tension relation in β-escin–permeabilized strips. (*A*) A 143 mM K^+-induced contraction in an intact strip followed by Ca^{2+}-activated contraction in the same strip after β-escin permeabilization. (*B*) The Ca^{2+}–tension relation in β-escin–permeabilized strips. The EC_{50} value derived from the least-squares fit by the logistic equation[45] was 2.02 μM. The magnitude of Ca^{2+}-activated contractions was normalized to maximum (100 μM) Ca^{2+}-activated contraction, and all values represent mean ± SEM (n = 5, five animals).

All three anesthetics also have a significant transient vasoconstricting action that does not require the presence of endothelium or peripheral nerve activity and also occurs at clinically relevant (*i.e.*, anesthetic) con-

Table 2. IC_{50} Values (vol % [mM]) for Volatile Anesthetics-induced Inhibition of High K^+ Contractions

	22°C (%)		35°C (%)	
Anesthetic	Ryanodine (−)	Ryanodine (+)	Ryanodine (−)	Ryanodine (+)
Halothane	0.47 ± 0.05 (0.27 mM)	0.68 ± 0.10 (0.39 mM)	3.36 ± 0.47 (0.99 mM)	2.55 ± 0.23 (0.76 mM)
Isoflurane	0.66 ± 0.09 (0.32 mM)	0.91 ± 0.14 (0.44 mM)	3.07 ± 0.33 (0.69 mM)	2.98 ± 0.28 (0.67 mM)
Enflurane	0.53 ± 0.05 (0.27 mM)	0.87 ± 0.14 (0.44 mM)	3.19 ± 0.41 (0.95 mM)	2.91 ± 0.38 (0.87 mM)

The gas concentrations (%) are expressed as mean ± SEM (n = 4–11, 6 animals). The values in parentheses represent the mean concentrations in high K^+ containing physiologic salt solution expressed in mM. These values were calculated from the data shown in figure 1.

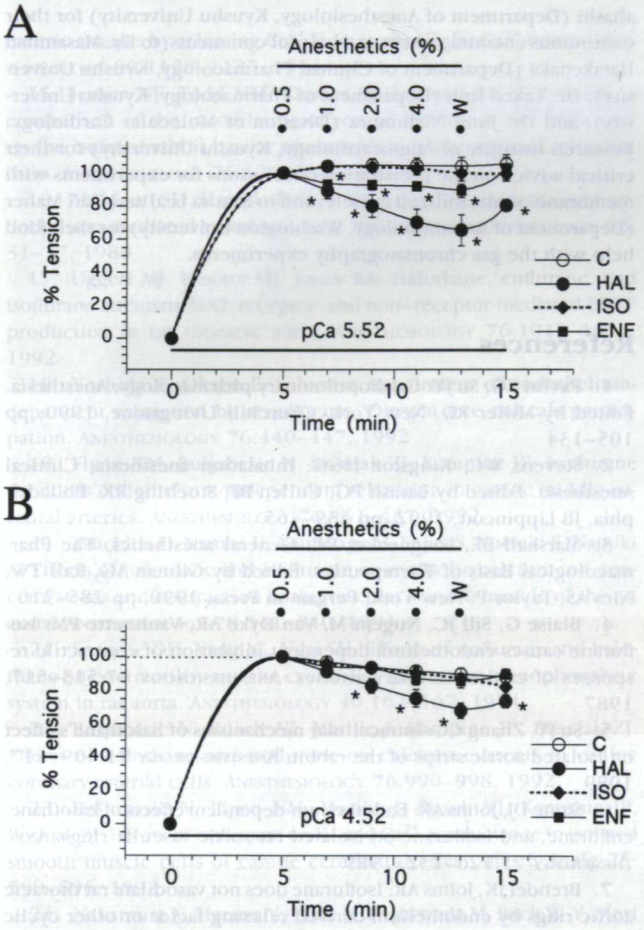

Fig. 9. Effects of cumulative application (0.5–4.0%) of halothane, isoflurane, and enflurane on (*A*) 3 μM (≈EC_{50}) and (*B*) 30 μM (maximal) Ca^{2+}-activated contractions in β-escin–permeabilized strips. Each anesthetic was cumulatively applied to the strips for a period of 2 min at each concentration after the Ca^{2+}-activated contraction reached a plateau (5 min). All values are mean ± SEM (n = 6–10, six to eight animals). *$P <$ 0.05 *versus* time control (open circles) at each time point.

centrations. The vasoconstricting action was independent of the vasodilating action as evidenced by the finding that the relative potency of the anesthetics as vasoconstrictors was unrelated to their vasodilatory potency and the finding that ryanodine abolished the vasoconstricting action with little effect on volatile anesthetic–induced vasodilation. In the high K^+–constricted vessels, the observed vasoconstricting action was only transient, however, and was immediately followed by vasodilation as we have previously reported for halothane in the same vessels[13] and for enflurane in rabbit small mesenteric arteries precontracted with adrenergic agonists.[11] Thus, although vasoconstriction did occur with clinically relevant concentrations of halothane and enflurane, the importance of this effect in altering splanchnic blood flow during anesthesia is uncertain. Of note, the vasoconstricting effect of isoflurane, which has not previously been reported, was observed only at 22°C and not at 35°C, probably because isoflurane is considerably less potent at producing vasoconstriction. Because the solubility of isoflurane (as well as the other anesthetics) was lower at 35°C than at 22°C, the apparent threshold for the vasoconstricting action of isoflurane observed at 22°C (2% ≈ 1 mM) was reached only with the maximum concentration studied at 35°C (*i.e.*, 5% isoflurane; fig. 1). Therefore, the apparent inability of isoflurane to produce vasoconstriction at 35°C may have resulted from the different concentrations achieved in the buffer at 22°C compared with those at 35°C. In addition, because the vasoconstricting action appears to involve Ca^{2+} release from intracellular stores (see below), the sequential-addition protocol that was used at 22°C did not permit an accurate analysis of the concentration–response relation at that temperature (22°C). In conclusion, our data suggest that the vasoconstricting action might be a common characteristic of halogenated volatile anesthetics.

The ability of ryanodine to abolish the vasoconstricting action of volatile anesthetics in intact muscle, and the inability of volatile anesthetics to produce any significant contractions in ionomycin-treated β-escin–permeabilized muscle strongly suggest that the volatile anesthetics cause vasoconstriction by releasing Ca^{2+} from ryanodine-sensitive intracellular Ca^{2+} stores, and not by inducing Ca^{2+} influx or affecting Ca^{2+} sensitivity. This is consistent with our previous reports in which the halothane- or enflurane-induced vasoconstricting effects were independent of extracellular Ca^{2+} concentration and were blocked by ryanodine.[12,13] Similar ryanodine-sensitive vasoconstricting effects of halothane and enflurane were recently reported in canine mesenteric artery. However, the authors suggested that, in addition to Ca^{2+} release from intracellular stores in smooth muscle, vasoconstriction in those vessels may also involve a change in Ca^{2+} sensitivity or an endothelium effect.[14] Although our data indicate that an increase in Ca^{2+} sensitivity is not involved, we did not examine their endothelium dependence. A ryanodine-sensitive intracellular Ca^{2+} store has recently been documented in endothelial cells,[51,52] which may play an important role in the regulation of endothelial function.[53,54] Therefore, it might be possible that volatile

anesthetics affect endothelial function by causing a change in the integrity of the endothelial ryanodine-sensitive Ca^{2+} store, and this endothelial action may modify the volatile anesthetic action on vascular smooth muscle.

The observed order of potency for the Ca^{2+} releasing effects of volatile anesthetics from the ryanodine-sensitive store (halothane > enflurane > isoflurane) is consistent with previous studies in which halothane and enflurane, but not isoflurane, open Ca^{2+} release channels of cardiac sarcoplasmic reticulum[55,56] and stimulate [^{3}H] ryanodine binding to cardiac sarcoplasmic reticulum.[57] However, only isoflurane, and not halothane or enflurane, stimulated [^{3}H] ryanodine binding to skeletal ryanodine receptor.[57] The cardiac ryanodine receptor gene (*RYR2*) or skeletal ryanodine receptor gene (*RYR1*) is known to be different from vascular smooth muscle ryanodine receptor gene (*RYR3*), which is also expressed in brain or lung epithelial cells.[58] Therefore, the *RYR3* presumed present in our tissue appears to resemble *RYR2* more than it does *RYR1*.

In conclusion, halothane, isoflurane, and enflurane have direct vasoconstricting and vasodilating actions that are independent of endothelial or nerve terminal activities in rat small mesenteric arteries. Although the clinical relevance of the transient vasoconstricting action is uncertain, the vasodilating action at clinically relevant concentrations suggests that it may contribute significantly to the vascular effects of volatile anesthetics *in vivo*. Compared with their vasodilating actions in high K^+–constricted vessels, the relatively small effects of these anesthetics on Ca^{2+}-induced contractions in β-escin–permeabilized muscle suggest that these anesthetics have little or no effect on Ca^{2+} activation of contractile proteins. Thus, the direct vasodilation in high K^+–treated vascular smooth muscle must be largely explained by effects on intracellular Ca^{2+} mobilization. The "direct" vasoconstricting action of volatile anesthetics depends on the Ca^{2+} release from the ryanodine-sensitive Ca^{2+} store and does not appear related to Ca^{2+} influx or any change in the Ca^{2+} sensitivity of contractile proteins. This is the first investigation to demonstrate volatile anesthetic action on Ca^{2+} activation of contractile proteins in the resistance arteries that are important in the regulation of systemic blood pressure and splanchnic blood flow.

The authors are grateful to Professor Alex S. Evers (Department of Anesthesiology, Washington University) and Professor Shosuke Takahashi (Department of Anesthesiology, Kyushu University) for their continuous encouragement and helpful comments; to Dr. Masamitsu Hatakenaka (Department of Clinical Pharmacology, Kyushu University), Dr. Takeo Itoh (Department of Pharmacology, Kyushu University), and Dr. Junji Nishimura (Division of Molecular Cardiology, Research Institute of Angiocardiology, Kyushu University) for their critical advice on the preparation of solutions for experiments with membrane-permeabilized muscle; and to Sandra Leal and Gail Maher (Department of Anesthesiology, Washington University) for their kind help with the gas chromatography experiments.

References

1. Pavlin EG, Su JY: Cardiopulmonary pharmacology, Anesthesia. Edited by Miller RD. New York, Churchill Livingstone, 1990, pp 105–134

2. Stevens WC, Kingston HGG: Inhalation anesthesia, Clinical Anesthesia. Edited by Barash PG, Cullen BF, Stoelting RK. Philadelphia, JB Lippincott, 1992, pp 439–465

3. Marshall BE, Longnecker DE: General anesthetics, The Pharmacological Basis of Therapeutics. Edited by Gilman AG, Rall TW, Nies AS, Taylor P. New York, Pergamon Press, 1990, pp 285–310

4. Blaise G, Sill JC, Nugent M, Van Dyke AR, Vanhoutte PM: Isoflurane causes endothelium-dependent inhibition of contractile responses of canine coronary arteries. ANESTHESIOLOGY 67:513–517, 1987

5. Su JY, Zhang CC: Intracellular mechanisms of halothane's effect on isolated aortic strips of the rabbit. ANESTHESIOLOGY 71:409–417, 1989

6. Stone DJ, Johns AR: Endothelium-dependent effects of halothane, enflurane, and isoflurane on isolated rat aortic vascular rings. ANESTHESIOLOGY 71:126–132, 1989

7. Brendel JK, Johns AR: Isoflurane does not vasodilate rat thoracic aortic rings by endothelium-derived relaxing factor or other cyclic GMP–mediated mechanisms. ANESTHESIOLOGY 77:126–131, 1992

8. Witzeling TM, Sill JC, Hughes JM, Blaise GA, Nugent M, Rolie K: Isoflurane and halothane attenuate coronary artery constriction evoked by serotonin in isolated porcine vessels and in intact pigs. ANESTHESIOLOGY 73:100–108, 1990

9. Hatano Y, Nakamura K, Yakushiji T, Nishiwada M, Mori K: Comparison of the direct effects of halothane and isoflurane on large and small coronary arteries isolated from dog. ANESTHESIOLOGY 73: 513–517, 1990

10. Ozhan M, Sill JC, Atagunduz P, Martin R, Katusic ZS: Volatile anesthetics and agonist-induced contractions in porcine coronary artery smooth muscle and Ca^{2+} mobilization in cultured immortalized vascular smooth muscle cells. ANESTHESIOLOGY 80:1102–1113, 1994

11. Akata T, Takahashi S, Kodama K, Tominaga M, Taniyama T: Effects of volatile anesthetics on rhythmic smooth muscle activity in rabbit mesenteric arteries (abstract). ANESTHESIOLOGY 77:A676, 1992

12. Akata T, Nakashima M, Kenji K, Boyle WA III, Takahashi S: Effects of volatile anesthetics on acetylcholine-induced relaxation in the rabbit mesenteric resistance artery. ANESTHESIOLOGY 82:188–204, 1995

13. Boyle WA III, Maher G: Endothelium-independent vasoconstricting and vasodilating actions of halothane on rat mesenteric resistance blood vessels. ANESTHESIOLOGY 82:221–235, 1995

14. Kakuyama M, Hatano Y, Nakamura K, Toda H, Terasako K, Nishiwada M, Mori K: Halothane and enflurane constrict canine mes-

enteric arteries by releasing Ca^{2+} from intracellular Ca^{2+} stores. ANESTHESIOLOGY 80:1120–1127, 1994

15. Jensen NF, Todd MM, Kramer DJ, Leonard PA, Warner DS: A comparison of the vasodilating effects of halothane and isoflurane on the isolated rabbit basilar artery with or without intact endothelium. ANESTHESIOLOGY 76:624–634, 1992

16. Muldoon SM, Hart JL, Bowen KA, Freas W: Attenuation of endothelium-mediated vasodilation by halothane. ANESTHESIOLOGY 68: 31–37, 1988

17. Uggeri MJ, Proctor GJ, Jones RA: Halothane, enflurane, and isoflurane attenuate both receptor- and non–receptor-mediated EDRF production in rat thoracic aorta. ANESTHESIOLOGY 76:1012–1017, 1992

18. Yoshida K, Okabe E: Selective impairment of endothelium-dependent relaxation by sevoflurane: Oxygen free radicals participation. ANESTHESIOLOGY 76:440–447, 1992

19. Flynn NM, Buljubasic N, Bosnjak ZJ, Kampine JP: Isoflurane produces endothelium-independent relaxation in canine middle cerebral arteries. ANESTHESIOLOGY 76:461–467, 1992

20. Tsuchida H, Namba H, Yamakage M, Fujita S, Notsuki E, Namiki A: Effects of halothane and isoflurane on cytosolic calcium ion concentrations and contraction in the vascular smooth muscle of the rat aorta. ANESTHESIOLOGY 78:531–540, 1993

21. Sprague DH, Yang JC, Ngai SH: Effects of isoflurane and halothane on contractility and the cyclic 3′,5′-adenosine monophosphate system in rat aorta. ANESTHESIOLOGY 40:162–167, 1974

22. Buljubasic N, Rusch NJ, Marijic J, Kampine JP, Bosnjak ZJ: Effects of halothane and isoflurane on calcium currents in canine coronary arterial cells. ANESTHESIOLOGY 76:990–998, 1992

23. Buljubasic N, Flynn NM, Marijic J, Rusch NJ, Kampine JP, Bosnjak ZJ: Effects of isoflurane on K^+ and Ca^{2+} conductance in isolated smooth muscle cells of canine cerebral arteries. Anesth Analg 75: 590–596, 1992

24. Nakamura K, Hatano Y, Toda H, Nishiwada M, Baek W-Y, Mori K: Halothane-induced relaxation of vascular smooth muscle: A possible contribution of increased cyclic GMP formation. Jpn J Pharmacol 55:165–168, 1991

25. Stephenson RB: The splanchnic circulation, Textbook of Physiology. Edited by Patton HD, Fuchs AH, Hille B, Scher AM, Steiner R. Philadelphia, WB Saunders, 1989, pp 911–923

26. Guyton AC: Textbook of Medical Physiology. 7th edition. Philadelphia, WB Saunders, 1986, pp 220–243

27. Longnecker D, Harris P: Microcirculatory actions of general anesthetics. Fed Proc 39:1580–83, 1980

28. Gianotti L, Alexander JW, Fukushima R, Childress CP: Translocation of candida albicans is related to the blood flow of individual intestinal villi. Circ Shock 40:250–257, 1993

29. Haglund U: Systemic mediators released from the gut in critical illness. Crit Care Med 21(suppl):S15–S18, 1993

30. Landow L: Splanchnic lactate production in cardiac surgery patients. Crit Care Med 21(suppl):S84–S91, 1993

31. Kobayashi Y, Yoshida K, Noguchi M, Wakasugi Y, Ito H, Okabe E: Effect of enflurane on contractile reactivity in isolated canine mesenteric arteries and veins. Anesth Analg 70:530–536, 1990

32. Sill JC, Bove AA, Nugent M, Blaise GA, Dewey JD, Grabau C: Effects of isoflurane on coronary arteries and coronary arterioles in the intact dog. ANESTHESIOLOGY 67:513–517, 1987

33. Bollen BA, McKlveen RE, Stevenson JA: Halothane relaxes preconstricted small and medium isolated porcine coronary artery segments more than isoflurane. Anesth Analg 75:9–17, 1992

34. Conzen PF, Habazetti H, Vollmar B, Christ M, Baier H, Peter K: Coronary microcirculation during halothane, enflurane, isoflurane, and adenosine in dogs. ANESTHESIOLOGY 76:261–270, 1992

35. Akata T, Yoshitake J, Nakashima M, Itoh T. Effects of protamine on vascular smooth muscle of rabbit mesenteric artery. ANESTHESIOLOGY 75:833–846, 1991

36. Akata T, Kodama K, Takahashi S. Effects of heparin on the vasodilator action of protamine in the rabbit mesenteric artery. Br J Pharmacol 109:1247–1253, 1993

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

38. Iino M: Tension responses of chemically skinned fibre bundles of the guinea-pig taenia caeci under varied ionic environments. J Physiol (Lond) 320:449–467, 1981

39. Kobayashi S, Kitazawa T, Somlyo AV, Somlyo AP: Cytosolic heparin inhibits muscarinic and alpha-adrenergic Ca^{2+} release in smooth muscle. J Biol Chem 264:17997–18004, 1989

40. Satoh S, Rensland H, Pfitzer G: Ras proteins increase Ca^{2+}-responsiveness of smooth muscle contraction. FEBS Lett 324:211–215, 1993

41. Itoh T, Suzuki S, Kuriyama H: Effects of pinacidil on contractile proteins in high K^+-treated intact, and in β-escin-treated skinned smooth muscle of the rabbit mesenteric artery. Br J Pharmacol 103: 1697–1702, 1991

42. Kitazawa T, Kobayashi S, Horiuti K, Somlyo AV, Somlyo AP: Receptor-coupled, permeabilized smooth muscle. J Biol Chem 264: 5339–5342, 1989

43. Itoh T, Kanmura Y, Kuriyama H: A23187 increases calcium permeability of store sites more than of surface membranes in the rabbit mesenteric artery. J Physiol (Lond) 359:467–484, 1985

44. F. Renzi BA, Waud BE: Partition coefficients of volatile anesthetics in Krebs solution. ANESTHESIOLOGY 47:62–63, 1977

45. De Lean AP, Munson PJ, Rodbard D: Simultaneous analysis of families of sigmoidal curves: Application to bioassay, radioligand assay, and physiological dose-response curves. Am J Physiol 235: E97–E102, 1978

46. Franks NP, Lieb WR: Molecular and cellular mechanisms of general anaesthesia. Nature 367:607–614, 1994

47. Nishimura J, Moreland S, Ahn HY, Kawase T, Moreland RS, Breemen van C: Endothelin increases myofilament Ca^{2+} sensitivity in alpha-toxin-permeabilized rabbit mesenteric artery. Circ Res 71:951–959, 1992

48. Morgan KG, Khalil RA, Suematsu E, Katsuyama H: Calcium-dependent and calcium-independent pathways of signal transduction in smooth muscle. Jpn J Pharmacol 58(suppl 2):47–53, 1992

49. Nishimura J, Khalil RA, van Breemen C: Agonists-induced vascular tone. Hypertension 13:835–844, 1989

50. Yamakage M, Kohno S, Kawamata T, Namiki A: Inhibitory effects of four inhaled anesthetics on canine tracheal smooth muscle contraction and intracellular Ca^{2+} concentration. Anesth Analg 77: 67–72, 1993

51. Ziegelstein RC, Spurgeon HA, Pili R, Passaniti A, Cheng L, Corda S, Lakatta EG, Capogrossi MC: A functional ryanodine-sensitive intracellular Ca^{2+} store is present in vascular endothelial cells. Circ Res 74:151–156, 1994

52. Kida K, Kondoh W, Horio S, Hisayama T, Moritoki H: Effects of ryanodine and cyclopiazonic acid on ACh-induced, endothelium-

dependent responses in rat thoracic aorta (abstract). Jpn J Pharmacol 61:73P, 1993

53. Nilius B. Ion channels and regulation of transmembrane Ca^{2+} influx in endothelium, Ion Channels of Vascular Smooth Muscle Cells and Endothelial Cells. Edited by Sperelakis N, Kuriyama H. New York, Elsevier Science, 1991, pp 317–325

54. Kukovetz WR, Graier WF, Groschner K: Contribution of agonist-induced hyperpolarization to Ca^{2+} influx and formation of EDRF in vascular endothelial cells. Jpn J Pharmacol 58(suppl 2):213–219, 1992

55. Connelly TJ, Coronado R: Activation of the Ca^{2+} release channel of cardiac sarcoplasmic reticulum by volatile anesthetics. ANESTHESIOLOGY 81:459–469, 1994

56. Frazer MJ, Lynch C III: Ca release channels of cardiac sarcoplasmic reticulum are activated by halothane, but not by isoflurane (abstract). ANESTHESIOLOGY 77:A630, 1992

57. Connelly TJ, Hayek R-E, Rusy BF, Coronado R: Volatile anesthetics selectively alter [^{3}H] ryanodine binding to skeletal and cardiac ryanodine receptors. Biochem Biophys Res Commun 186:595–600, 1992

58. McPherson PS, Campbell KP: The ryanodine receptor/Ca^{2+} release channel. J Biol Chem 268:13765–13768, 1993

Appendix

The binding constants used to calculate the compositions of the solutions for the experiments with membrane-permeabilized muscle are as follows, where ATP = adenosine triphosphate and CrP = creatinine phosphate; other abbreviations are defined in main text. $[HEGTA^{3-}]/[H^+][EGTA^{4-}] = 2.88 \times 10^9$, $[H_2EGTA^{2-}]/[H^+][HEGTA^{3-}] = 7.08 \times 10^8$, $[H_3EGTA^-]/[H^+][H_2EGTA^{2-}] = 4.79 \times 10^2$, $[H_4EGTA]/[H^+][H_3EGTA^-] = 1.00 \times 10^2$; $[HATP^{3-}]/[H^+][ATP^{4-}] = 8.91 \times 10^6$, $[H_2ATP^{2-}]/[H^+][HATP^{3-}] = 1.12 \times 10^4$, $[H_3ATP^-]/[H^+][H_2ATP^{2-}] = 1.00 \times 10^1$, $[H_4ATP]/[H^+][H_3ATP^-] = 1.00 \times 10^1$; $[CaEGTA^{2-}]/[Ca^{2+}][EGTA^{4-}] = 5.01 \times 10^{10}$, $[CaHEGTA^-]/[Ca^{2+}][HEGTA^{3-}] = 2.14 \times 10^5$; $[CaATP^{2-}]/[Ca^{2+}][ATP^{4-}] = 9.55 \times 10^3$, $[CaHATP^-]/[Ca][HATP^{3-}] = 6.31 \times 10^1$; $[CaCrP]/[Ca^{2+}][CrP^{2-}] = 1.41 \times 10^1$, $[MgEGTA^{2-}]/[Mg^{2+}][EGTA^{4-}] = 1.62 \times 10^5$, $[MgHEGTA^-]/[Mg^{2+}][HEGTA^{3-}] = 2.34 \times 10^3$; $[MgATP^{2-}]/[Mg^{2+}][ATP^{4-}] = 2.11 \times 10^4$, $[MgHATP^-]/[Mg^{2+}][HATP^{3-}] = 5.50 \times 10^2$; $[MgCrP]/[Mg^{2+}][CrP^{2-}] = 2.0 \times 10^1$, $[HCrP]/[H^+][CrP^{2-}] = 3.80 \times 10^4$, $[H_2CrP]/[H^+][CrP^{2-}] = 5.01 \times 10^2$; $[KATP^{3-}]/[K^+][ATP^{4-}] = 7.94$, $[K_2ATP^{2-}]/[K^+][KATP^{3-}] = 0.6$, $[KHATP^{2-}]/[K^+][HATP^{3-}] = 0.6$, $[NaATP^{3-}]/[Na^+][ATP^{4-}] = 8.8$, $[Na_2ATP]/[Na^+][NaATP^{3-}] = 8.5$, $[NaHATP^{2-}]/[Na^+][HATP^{3-}] = 5.0$; $[H\ PIPES^-]/[H^+][PIPES^{2-}] = 6.31 \times 10^6$.