Anesthesiology
80:1120–1127, 1994

Halothane and Enflurane Constrict Canine Mesenteric Arteries by Releasing Ca^{2+} from Intracellular Ca^{2+} Stores

Masahiro Kakuyama, M.D., Yoshio Hatano, M.D.,† Kumi Nakamura, M.D.,‡ Hiroshi Toda, M.D.,§ Kiyoshi Terasako, M.D.,* Makoto Nishiwada, M.D.,‖ Kenjiro Mori, M.D., F.R.C.A.#*

Background: **Recent studies suggest that volatile anesthetics cause not only vasodilation but also vasoconstriction, depending on the experimental conditions. However, the mechanism of the constrictive effect of volatile anesthetics has not been clarified. The aim of this study was to evaluate the vasoconstrictor effects of halothane, enflurane, and isoflurane and to elucidate the underlying mechanism.**

Methods: **Vascular rings of canine mesenteric arteries were mounted in organ baths, and isometric tension changes were recorded. Changes in intracellular free Ca^{2+} concentration of vascular smooth muscle were examined by using the fluorescent Ca^{2+} indicator fura 2 and a dual-wavelength fluorometer.**

Results: **Halothane (0.75–2.3%) and enflurane (1.7–3.4%), but not isoflurane (1.2–3.5%), induced a concentration-dependent transient contraction, followed by a slight, sustained contraction. Halothane (1.5%)- and enflurane (3.4%)-induced contractions were reduced by endothelial denudation and enhanced by indomethacin (10^{-5} M) treatment but were not affected by L-N^G-nitroarginine (10^{-5} M) or nifedipine (2×10^{-7} M) treatment. Ryanodine (2×10^{-5} M) treatment completely abolished the transient increases in tension and Ca^{2+} concentration. Even in ryanodine-treated arteries, however, both anesthetics induced a slowly developing sustained contraction, and the sustained contraction induced by enflurane (3.4%) was not accompanied by a significant increase in Ca^{2+} concentration.**

Conclusions: **Halothane and enflurane, but not isoflurane, induce vasoconstriction by releasing Ca^{2+} from intracellular stores. Release of a vasodilating prostanoid and endothelium-derived constricting factor may also be involved in the vasoconstrictor effect. Furthermore, increased Ca^{2+} sensitivity of contractile machinery may be involved in the effect of enflurane. (Key words: Anesthetics, volatile: enflurane; halothane; isoflurane. Arteries: mesenteric. Ions: calcium. Ions, calcium channels: ryanodine. Muscle, smooth: sarcoplasmic reticulum.)**

VOLATILE anesthetics, including halothane, enflurane, and isoflurane, are generally considered peripheral vasodilators.[1–4] However, studies *in vitro*[5,6] have suggested that these anesthetics cause vasoconstriction as well, depending on the experimental conditions. Stone and Johns[5] demonstrated that low concentrations of enflurane and isoflurane induced endothelium-dependent vasoconstriction in rat aorta which had been precontracted with phenylephrine. Su and Zhang[6] observed that halothane, independent of endothelium, induced a biphasic change in tension, consisting of an initial slight increase and a subsequent decrease in KCl-precontracted rabbit aorta.

The aim of this study was to evaluate the vasoconstrictive effects of halothane, enflurane, and isoflurane, and to elucidate the responsible mechanism. The concentration of intracellular free Ca^{2+} in vascular smooth muscle was measured with a Ca^{2+}-sensitive fluorescent dye, fura 2, and tension was measured simultaneously. The current study revealed that halothane and enflurane induced two successive phases of contraction and increase in cytosolic Ca^{2+} concentration: a strong, transient phase and a subsequent slight, sustained phase. The mechanism underlying each phase of the contraction was investigated by using inhibitors of NO and prostaglandin synthesis, a Ca^{2+} channel blocker, and a Ca^{2+}-induced Ca^{2+} release (CICR) channel blocker.

* Graduate student in Anesthesia, Kyoto University Faculty of Medicine.

† Professor and Chair of Anesthesiology, Wakayama Medical College.

‡ Associate Professor of Anesthesia, Kyoto University Hospital.

§ Staff Anesthesiologist, Kyoto University Hospital.

‖ Chief Anesthesiologist, Tenri Hospital, Tenri, Nara.

Professor and Chair of Anesthesia, Kyoto University Hospital.

Received from the Department of Anesthesia, Kyoto University Hospital, Kyoto, and the Department of Anesthesiology, Wakayama Medical College, Wakayama, Japan. Accepted for publication January 19, 1994.

Address reprint requests to Dr. Kakuyama: Department of Anesthesia, Kyoto University Hospital, Kyoto 606-01, Japan.

Materials and Methods

Preparation of Vascular Rings

The protocol was approved by the Kyoto University Animal Use Committee. Mongrel dogs (10–15 kg) were anesthetized with intravenous ketamine (10 mg/kg) and killed by exsanguination. The mesenteric arteries of 0.8–1.5-mm outer diameter were isolated and cut into 3-mm–wide rings in a bathing solution. The bathing solution was Krebs' Ringer's solution containing (millimolar): KCl 4.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, NaCl 118.2, $NaHCO_3$ 24.8, and dextrose 10 (*p*H 7.35–7.45). The endothelia of 57 rings were removed carefully by rotating the rings around a needle, and those of the remaining 117 rings were left intact. Endothelial integrity or denudation was functionally confirmed before the experiment by testing acetylcholine (10^{-6} M)-induced relaxation under phenylephrine (10^{-6} M)-precontracted conditions.[7] Vascular rings with more than 60% relaxation or without relaxation were regarded A endothelium-intact or -denuded, respectively, and were used in the following study.

Delivery of Volatile Anesthetics

Halothane, enflurane, and isoflurane were delivered with 95% O_2/5% CO_2 mixed gas through agent-specific vaporizers (Fluotec 3, Enfluratic 3, and Fortec, respectively, Cyprane Keighley, England).[8,9] The concentration of anesthetic in the gas phase were monitored and adjusted with an anesthetic agent monitor (Atom 303, Atom, Tokyo, Japan). The arterial rings were abruptly exposed to a given concentration of an anesthetic by emptying the organ bath and allowing a gentle inward flow of Krebs' solution that had been aerated and equilibrated with the anesthetic for at least 10 min. After the replacement of the solution, anesthetic was supplied through the gas mixture until the tension became stable. In a preliminary study, the concentrations of halothane (1.5%), enflurane (3.4%), and isoflurane (2.3%) in the bath solution were assayed by gas chromatography (Hewlett-Packard 5890A, Palo Alto, CA)[8] and were 0.47 ± 0.02 (n = 3), 1.03 ± 0.01 (n = 6), and 0.56 ± 0.00 (n = 3) mM, respectively, after solution replacement; these concentrations did not change significantly thereafter until cessation of the aeration with anesthetic-containing gas. In contrast, when the bath was aerated with anesthetic-containing gas directly, 3–5 min was required until the concentration in the fluid had reached this level.

Isometric Tension Recordings

For the measurement of isometric tension alone, the arterial rings were mounted in organ baths (37°C) and equilibrated for 60 min in Krebs' Ringer's solution aerated with a mixture of 95% O_2 and 5% CO_2. During this period, the resting tension was adjusted to 2 g. Thereafter, KCl (30 mM)-induced contraction was measured in each arterial ring as a reference value (100%). Each arterial ring was used for one of the following three experiments.

In the first experiment, the concentration–response relationships of halothane (1, 2, and 3 MAC in humans, equivalent to 0.75, 1.5, and 2.3%, respectively), enflurane (1 and 2 MAC in humans, equivalent to 1.7 and 3.4%, respectively), and isoflurane (1, 2, and 3 MAC in humans, equivalent to 1.2, 2.3, and 3.5%, respectively) were determined under resting conditions in endothelium-intact arteries. The arterial rings were exposed to each concentration of halothane, enflurane, or isoflurane in random order, with a recovery period (more than 20 min) between exposures. Other arterial rings were exposed to the aerated solution that did not contain anesthetic.

In the second experiment, halothane (2 MAC)- and enflurane (2 MAC)-induced vasoconstrictions were compared between endothelium-intact and -denuded arterial rings.

In the third experiment, halothane (2 MAC)- and enflurane (2 MAC)-induced tension changes were determined before and after treatment with nifedipine (2×10^{-7} M, a Ca^{2+} channel blocker), L-N^G-nitro-arginine (10^{-5} M, a NO synthesis inhibitor[10]), indomethacin (10^{-5} M, a cyclooxygenase inhibitor), or an equivalent volume of saline (control) for 20 min, or with ryanodine for 30 min. Ryanodine is known to lock the Ca^{2+} channel for CICR of Ca^{2+} stores into the open state.[11] Arterial rings for ryanodine treatment were exposed to ryanodine (2×10^{-5} M) for 30 min, and, 5 min after the beginning of treatment, exposed to caffeine (25 mM) for 5 min to facilitate the effect of ryanodine.[11] Control rings in the ryanodine experiment were not treated with ryanodine but were exposed to caffeine in the same way as were ryanodine-treated arteries.

Measurement of Intracellular Ca^{2+} Concentration

For measurement of the cytosolic Ca^{2+} concentration of vascular smooth muscle, simultaneous with measurement of tension change, arterial rings without endothelium were incubated at 25°C for 3–5 h in Krebs'

solution that contained the fluorescent Ca^{2+} indicator fura 2-AM (2.5×10^{-5} M) and the noncytotoxic detergent Cremophor EL (0.02% vol/vol).[12] After fura 2 loading, the arterial rings were mounted in organ baths as described above and then were rinsed with Krebs' solution for 60 min.

Changes in the fluorescence intensity were monitored using a dual-wavelength fluorometer specially designed to measure the surface fluorescence of living tissues (CAF-110, Japan Spectroscopic, Tokyo, Japan). The arterial rings were illuminated with excitation light from a xenon lamp (75 W), the wavelength of which was altered between 340 and 380 (± 5) nm at 128 Hz with a chopping mirror and two monochromators. The fluorescence emitted through a 500 (± 6)-nm bandpass filter was detected with a photomultiplier. Because the fluorescence intensity at 340-nm excitation (F_{340}) corresponds to the amount of Ca^{2+}-bound fura 2, and that at 380-nm excitation (F_{380}) to the amount of Ca^{2+}-free fura 2, the F_{340}/F_{380} ratio correlates well with the cytosolic Ca^{2+} concentration.[12,13] In this study, therefore, this ratio was used as a measurement of Ca^{2+} concentration. After determination of KCl (30 mM)-induced changes in the F_{340}/F_{380} ratio and tension, one of the following two experiments was performed on each arterial ring.

In the first experiment, to estimate the effects of halothane (2 MAC) and enflurane (2 MAC), arterial rings were exposed to the anesthetics in random order, with a recovery period (more than 20 min) between exposures. Other arterial rings were exposed to the aerated solution without anesthetics to examine the effects of solution replacement on the F_{340}/F_{380} ratio and tension.

In the second experiment, to test whether anesthetic-induced vasoconstriction is caused by CICR from intracellular Ca^{2+} stores, changes in the F_{340}/F_{380} ratio and tension induced by halothane (2 MAC) and enflurane (2 MAC) before and after treatment with ryanodine (2×10^{-5} M) and caffeine (as described above) were compared.

Drugs and Chemicals

The drugs used were halothane (Takeda Pharmaceutical, Osaka, Japan), enflurane (Dainabot, Tokyo, Japan), isoflurane (Dainabot), L-N^G-nitro-arginine (Sigma Chemical, St. Louis, MO), nifedipine (Bayer, Leverkusen, Germany), ryanodine (Wako Pure Chemical Industries, Osaka, Japan), caffeine (Nacalai Tesque, Kyoto, Japan), fura 2-AM (Dojindo Laboratories, Kumamoto, Japan), and Cremophor EL (Sigma Chemical). Nifedipine, indomethacin, and ryanodine were dissolved in ethanol. The final concentration of ethanol was less than 0.03% (vol/vol) of the bathing solution.

Statistical Analysis

Data are expressed as means ± SE. In each experiment, arterial rings from six or more dogs were used, and those obtained from a single dog were used for corresponding groups (control and treated, or endothelium-intact and -denuded). The data were analyzed statistically by using Student's *t* test for unpaired groups. Differences at $P < 0.05$ were considered significant.

Results

During resting conditions, halothane (1–3 MAC) and enflurane (1–2 MAC) induced a strong, transient contraction that was followed by a slight but sustained contraction (fig. 1). These two phases of contraction were concentration-dependent in the range of concen-

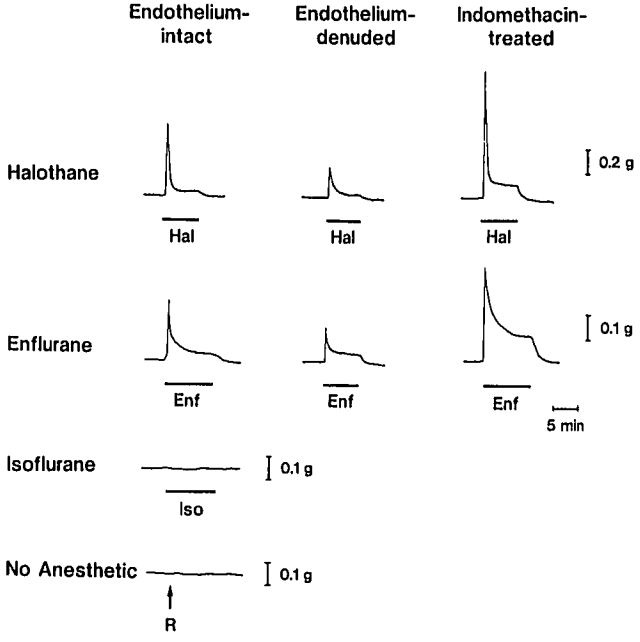

Fig. 1. Typical recordings of halothane (2 MAC)- and enflurane (2 MAC)-induced contractions in endothelium-intact, endothelium-denuded, and indomethacin (10^{-5} M)-treated arteries. Isoflurane (2 MAC) and replacement of the bathing solution with the aerated solution without anesthetic did not induce contraction (n = 6 each). Hal = exposure to halothane; Enf = exposure to enflurane; Iso = exposure to isoflurane; R = replacement of the bathing solution.

trations tested (fig. 2). Isoflurane (1–3 MAC) did not induce any significant change in tension (fig. 1). In fura 2–loaded arteries, halothane (2 MAC) and enflurane (2 MAC) increased the F_{340}/F_{380} ratio, which reflects cytosolic free Ca^{2+} concentration, in a manner parallel with tension (fig. 3). Replacement of the bathing solution with the aerated solution that did not contain anesthetic altered neither tension nor the F_{340}/F_{380} ratio (figs. 1 [bottom trace] and 4C).

The transient phases of halothane (2 MAC)- and enflurane (2 MAC)-induced contraction were significantly attenuated by endothelial denudation (fig. 1 and table 1). Treatment with L-N^G-nitro-arginine (10^{-5} M) did not significantly affect either the transient or the sustained phase of halothane (2 MAC)- and enflurane (2 MAC)-induced contraction (table 2). However, indomethacin (10^{-5} M) treatment significantly enhanced both phases of contraction (fig. 1 and table 2). Nifedipine (2×10^{-7} M) did not significantly alter either phase (fig. 5).

Treatment with ryanodine (2×10^{-5} M) and caffeine induced a sustained contraction of 25.1 ± 3.8% (n = 12) of the KCl (30 mM)-induced contraction. In fura 2–loaded arteries, treatment with ryanodine (2×10^{-5} M) and caffeine induced a sustained increase in the F_{340}/F_{380} ratio and in tension (39.0 ± 6.7% and 15.8 ± 2.9%, respectively, of KCl (30 mM)-induced changes; n = 12 each).

After ryanodine treatment, neither halothane (2 MAC) nor enflurane (2 MAC) induced a transient contraction. However, halothane (2 MAC) induced a slow-developing, sustained contraction, smaller than the transient contraction before treatment ($P < 0.01$) but significantly greater than the previous sustained one ($P < 0.01$). The sustained contractions in control and ryanodine-treated groups were 101.7 ± 6.5% and 174.4 ± 19.5% (n = 6 each), respectively, of those before ryanodine treatment. Enflurane (2 MAC) induced a strong, slow-developing, sustained contraction; it was 100.9 ± 5.3% (n = 6) and 948.9 ± 81.4% (n = 6) in control and ryanodine-treated groups ($P < 0.01$), respectively.

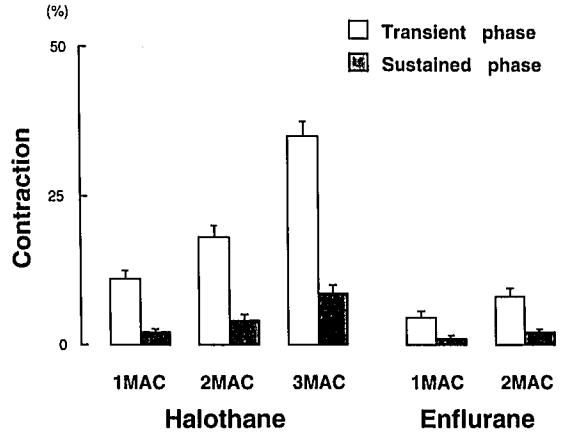

Fig. 2. Tension changes induced by halothane (1–3 MAC) and enflurane (1–2 MAC) (n = 6, each). KCl (30 mM)-induced contraction was taken as 100%. Isoflurane (1–3 MAC) did not induce contraction.

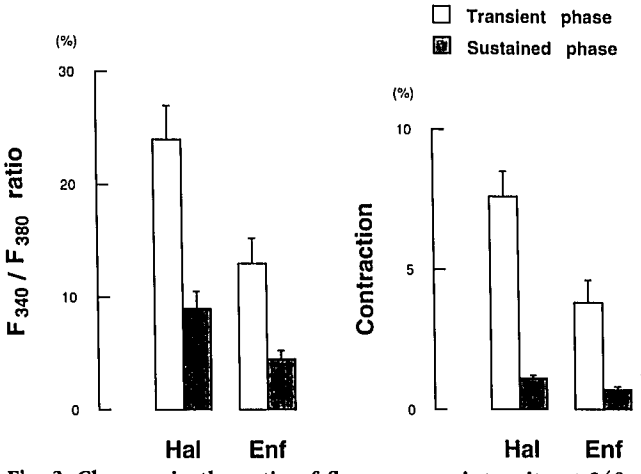

Fig. 3. Changes in the ratio of fluorescence intensity at 340-nm excitation to that at 380-nm excitation (F_{340}/F_{380}) and in tension induced by halothane (2 MAC) and enflurane (2 MAC) (n = 6 each). KCl (30 mM)-induced changes were taken as 100%. Hal = halothane (2 MAC); Enf = enflurane (2 MAC).

In fura 2–loaded arteries, after ryanodine treatment, halothane (2 MAC) and enflurane (2 MAC) failed to induce a transient change in the F_{340}/F_{380} ratio or tension. However, halothane (2 MAC) induced slow, sustained increases in the F_{340}/F_{380} ratio and tension; the sustained change in the ratio was significantly smaller ($P < 0.01$) but the sustained contraction was greater than that before treatment ($P < 0.05$). Enflurane (2 MAC) induced a slow-developing but prominent contraction, without any change in the ratio (table 3 and fig. 4).

Discussion

In skeletal and cardiac muscle cells, halothane and enflurane are known to cause release of Ca^{2+} from the sarcoplasmic reticulum *via* CICR channels. In skeletal muscle, this phenomenon is believed to be related to

the pathogenesis of malignant hyperthermia.[14,15] In cardiac muscle, the same phenomenon results in a negative inotropic effect, by decreasing the content of Ca^{2+} stores available for contraction.[16–18] Vascular smooth muscle also has intracellular Ca^{2+} stores, which are sensitive to both ryanodine and inositol 1,4,5-triphosphate (IP_3) or only to IP_3. However, it has not been determined whether halogenated anesthetics induce Ca^{2+} release from these vascular smooth muscle stores sufficient to cause vasoconstriction or relaxation.

The current study demonstrated that halothane and enflurane, but not isoflurane, induced a biphasic change in tension, comprising a rapidly developing, transient contraction followed by a slight, sustained contraction. We succeeded in observing the evident transient contraction because we exposed arterial strips to a given concentration of an anesthetic abruptly, by emptying the bath and allowing and inward flow of Krebs' solution that had been aerated with anesthetic-containing gas and equilibrated for more than 10 min. If arteries were exposed to an anesthetic by the direct aeration of the bathing solution with the anesthetic-containing gas, the concentration of the anesthetic dissolved in the bath fluid increased only slowly. In the latter experimental condition, therefore, we could not evaluate the transient phase of increase in Ca^{2+} concentration and tension properly.

Table 1. Contractions Induced by Halothane (2 MAC) and Enflurane (2 MAC) Relative to KCl (30 mM)-induced Contraction in Endothelium-intact and Denuded Arteries

	Endothelium-intact		Endothelium-denuded	
	Transient	Sustained	Transient	Sustained
Halothane (2 MAC)	20.3 ± 2.7 (n = 12)	3.1 ± 0.6	9.5 ± 1.0* (n = 12)	2.3 ± 0.4
Enflurane (2 MAC)	11.1 ± 1.3 (n = 9)	2.3 ± 0.4	5.6 ± 0.7* (n = 9)	1.7 ± 0.1

Values are mean ± SE (%). The absolute values of KCl (30 mM)-induced contractions averaged 2.16 ± 0.14 g and 2.10 ± 0.17 g (n = 21, each), respectively, in endothelium-intact and -denuded arteries.

* $P < 0.01$ *versus* endothelium-intact arterial rings.

In this study, nifedipine (2×10^{-7} M) inhibited neither of the phases of contraction. Ca^{2+} channel blockers such as nifedipine inhibit high K^+-induced contraction strongly by blocking L-type voltage-dependent Ca^{2+} channels. At higher concentrations, they also decrease the contractions induced by α-adrenergic receptor ag-

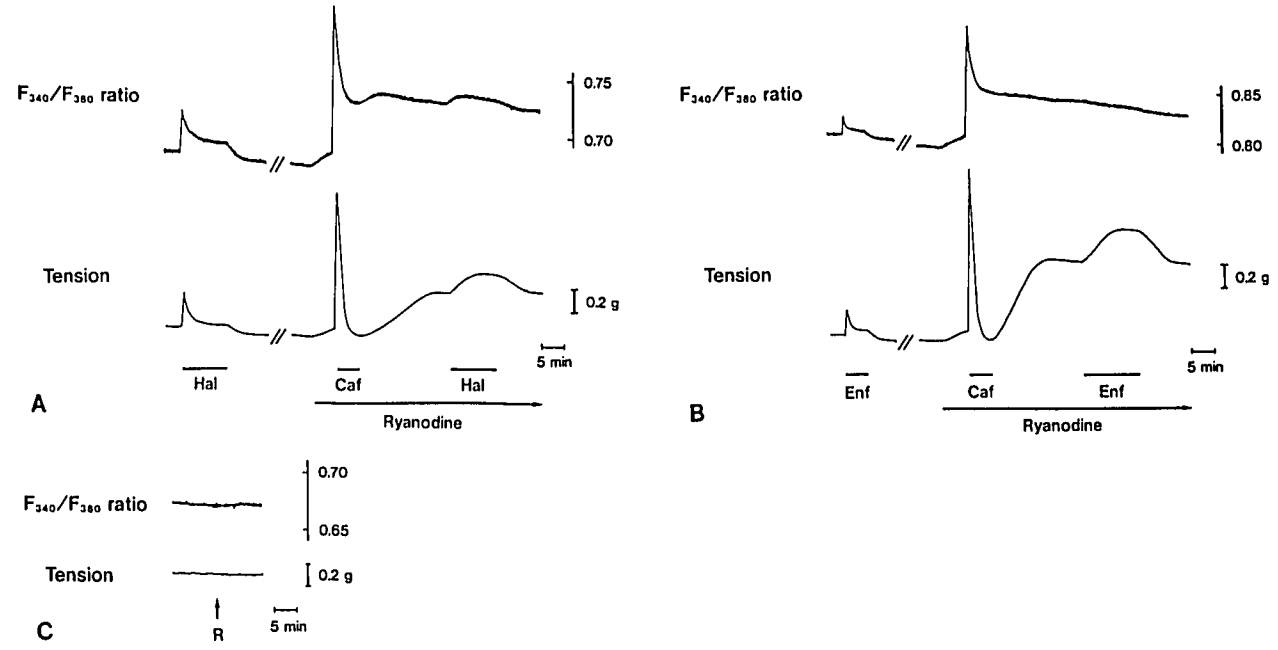

Fig. 4. Typical recordings of changes in the ratio of fluorescence intensity at 340-nm excitation to that at 380-nm excitation (F_{340}/F_{380}) and in tension after exposure to halothane (*A*) and enflurane (*B*) before and after ryanodine treatment in fura 2–loaded arteries. Replacement with the aerated solution without anesthetic did not induce changes in the F_{340}/F_{380} ratio or in tension (*C*) (n = 6 each). Hal = halothane (2 MAC); Enf = enflurane (2 MAC); Caf = caffeine (25 mM); R = replacement of the bathing solution.

onists, serotonin and prostaglandin $F_{2\alpha}$, with strong inhibition of Ca^{2+} influx.[19,20] Therefore, the current finding indicates that these anesthetic-induced contractions are not dependent on Ca^{2+} influx either through L-type voltage-dependent Ca^{2+} channels or through nifedipine-sensitive receptor-operated Ca^{2+} channels.

In skeletal muscle, ryanodine at low concentration locks the CICR channel of the sarcoplasmic reticulum into an open state and at high concentration closes the channel.[21] In the current study, treatment of vascular smooth muscle with ryanodine (2×10^{-5} M) followed by caffeine increased cytosolic Ca^{2+} concentration, indicating that this concentration of ryanodine effectively locked the channel into an open state and thus induced depletion of the ryanodine-sensitive Ca^{2+} stores. The rapidly developing, transient phase of anesthetic-induced increases in tension and cytosolic Ca^{2+} concentration was abolished by ryanodine treatment. This result indicates that the rapid phase of contraction is elicited by Ca^{2+} release from the ryanodine-sensitive Ca^{2+} stores.

The slowly developing, sustained phase of halothane (2 MAC)-induced increase in cytosolic Ca^{2+} concentration also was strongly inhibited, but was not abolished, by ryanodine treatment. This finding suggests that this phase of increase in cytosolic Ca^{2+} concentration is mainly but not totally dependent on Ca^{2+} release from the ryanodine-sensitive Ca^{2+} stores. We speculate that this phase of contraction depends in part on release of Ca^{2+} from ryanodine-insensitive Ca^{2+} stores[22] or on influx of extracellular Ca^{2+} through nifedipine-insensitive Ca^{2+} channels.[23] Despite the strong inhibition of increase in cytosolic Ca^{2+} concentration, halothane (2 MAC) induced a slowly developing, sustained contraction greater than that before ryanodine treatment. Therefore, one can suppose that halothane increased the sensitivity of contractile machinery to Ca^{2+}, or more likely, that ryanodine treatment or the resulting increase in cytosolic Ca^{2+} concentration altered the Ca^{2+} sensitivity.

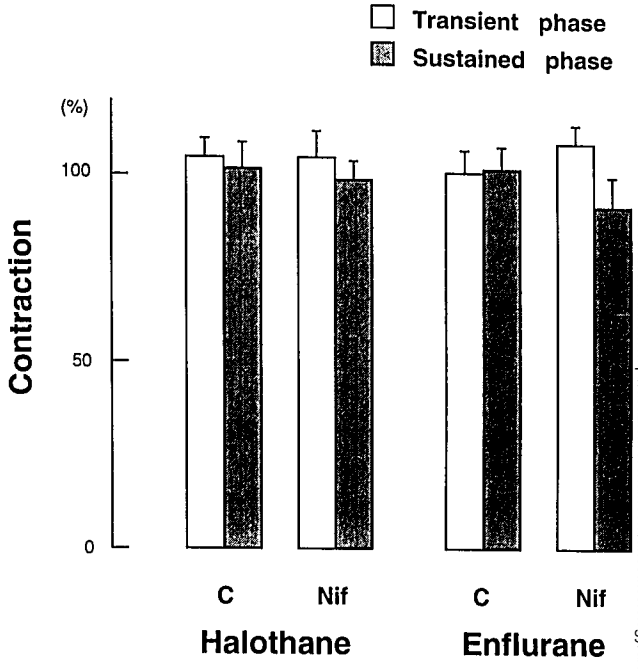

Fig. 5. Modification by nifedipine of halothane (2 MAC)- and enflurane (2 MAC)-induced contractions (n = 6 each). Those before treatment were taken as 100% in each phase. C = control (arterial rings treated with equivalent volume of saline); Nif = rings treated with nifedipine (2×10^{-7} M).

Enflurane (2 MAC), in ryanodine-treated arteries, induced a strong, sustained vasoconstriction without a significant increase in cytosolic Ca^{2+} concentration. This striking finding indicates that increase of cytosolic Ca^{2+} concentration by enflurane in the sustained phase as well as in the transient phase depends on release of Ca^{2+} from the ryanodine-sensitive Ca^{2+} stores and suggests that enflurane or ryanodine treatment, or both,

Table 2. Modification by L-NNA and Indomethacin Treatments of Transient and Sustained Phases of Contractions Induced by Halothane (2 MAC) and Enflurane (2 MAC)

	Halothane (2 MAC)		Enflurane (2 MAC)	
	Transient	Sustained	Transient	Sustained
Control	105.2 ± 5.4	102.2 ± 7.0	100.9 ± 5.7	102.1 ± 5.6
L-NNA (10^{-5} M)-treated	115.7 ± 7.4	107.8 ± 10.0	96.6 ± 4.0	98.8 ± 3.1
Indomethacin (10^{-5} M)-treated	179.5 ± 7.5*	345.9 ± 20.4*	175.2 ± 4.0*	226.9 ± 30.1*

Values are mean ± SE. Halothane (2 MAC)- and enflurane (2 MAC)-induced contractions before treatment were taken as 100% in each phase (n = 6, each).
* $P < 0.01$ *versus* control.

Table 3. Modification by Ryanodine Treatment of Transient and Sustained Phases of Increases in the F_{340}/F_{380} Ratio and Tension Induced by Halothane (2 MAC) and Enflurane (2 MAC) in Fura 2-loaded arteries

	Halothane (2 MAC)		Enflurane (2 MAC)	
	Transient	Sustained	Transient	Sustained
Control				
F_{340}/F_{380} ratio	92.5 ± 3.2	101.3 ± 5.7	98.9 ± 4.3	106.2 ± 4.2
Tension	103.1 ± 3.6	109.0 ± 11.4	108.4 ± 7.2	105.6 ± 5.6
Ryanodine (2×10^{-5} M)-treated				
F_{340}/F_{380} ratio	ND	27.8 ± 8.3*	ND	ND
Tension	ND	236.6 ± 52.2†	ND	729.3 ± 184.5*

Values are mean ± SE. Halothane (2 MAC)- and enflurane (2 MAC)-induced increases in the F_{340}/F_{380} ratio and tension before treatment were taken as 100% in each phase (n = 6, each). ND: not detectable.

* $P < 0.01$ *versus* control.

† $P < 0.05$ *versus* control.

enhanced sensitivity of contractile machinery to Ca^{2+} in the vascular smooth muscle.

The current study revealed that isoflurane lacks the ability to induce vasoconstriction by releasing Ca^{2+} from ryanodine-sensitive stores. Recently, Connelly *et al.*[24] and Frazer and Lynch[25] demonstrated that isoflurane activated skeletal CICR channels but not cardiac CICR channels. Thus, the current study suggests that the sensitivity of the CICR channel in vascular smooth muscle to volatile anesthetics is probably similar to that of the cardiac muscle channel but not to that of the skeletal muscle channel.

It is known that volatile anesthetics inhibit endothelium-dependent relaxation.[8,26,27] In the current study, although removal of the endothelium attenuated anesthetic (2 MAC)-induced contraction, treatment with a NO synthase inhibitor, L-N^G-nitro-arginine, did not affect it. Thus, it appears that inhibition of the basal production of endothelium-derived relaxing factor, or NO, does not significantly contribute to the contraction of arteries at basal tension and that increased production of endothelium-derived constricting factor, such as endothelin, might be involved in the vascular effects of halothane and enflurane. The finding that indomethacin enhanced the contraction implies that these anesthetics stimulate production of a vasodilating prostanoid, such as prostaglandin I_2. This hypothesis is in good agreement with that of Stone and Johns,[5] who demonstrated that halothane, enflurane, and isoflurane enhanced phenylephrine-induced contraction of endothelium-intact rat aorta in the presence of indomethacin.

In the current experimental conditions, we did not observe anesthetic-induced vasodilation. However, in arteries *in vitro* in the phenylephrine- or KCl-precontracted condition, which favors observation of the vasodilating effect, and in arteries *in situ*, including most clinical situations, the vasoconstricting effect of the anesthetics is probably overshadowed by their vasodilating effect.

In summary, halothane and enflurane, but not isoflurane, induced a rapidly developing transient contraction in canine mesenteric artery. This was followed by a slight sustained contraction. This biphasic contraction is mainly dependent on Ca^{2+} release from the ryanodine-sensitive Ca^{2+} stores, but contribution of increased sensitivity to Ca^{2+}, increased production of endothelium-derived constricting factor may be also involved in the anesthetic-induced vasoconstriction.

References

1. Price ML, Price HL: Effects of general anesthetics on contractile responses of rabbit aortic strips. ANESTHESIOLOGY 23:16–20, 1962

2. Vatner SF, Smith NT: Effects of halothane on left ventricular function and distribution of regional blood flow in dogs and primates. Circ Res 34:155–167, 1974

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

4. Domenech RJ, Voldes PMJ, Penna M: Coronary vascular resistance during halothane anesthesia. ANESTHESIOLOGY 46:236–240, 1977

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

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

7. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373–376, 1980

8. 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 dogs. ANESTHESIOLOGY 73: 513–517, 1990

9. Toda H, Nakamura K, Hatano Y, Kakuyama M, Nishiwada M, Mori K: Halothane and isoflurane inhibit endothelium-dependent relaxation elicited by acetylcholine. Anesth Analg 75:198–203, 1992

10. Toda N, Minami Y, Okamura T: Inhibitory effect of L-N^G-nitro-arginine on the synthesis of EDRF and the cerebroarterial response to vasodilator nerve stimulation. Life Sci 47:345–351, 1990

11. Iino M, Kobayashi T, Endo M: Use of ryanodine for functional removal of the calcium store in smooth muscle cells of the guinea-pig. Biochem Biophys Res Commun 152:417–422, 1988

12. Sato K, Ozaki H, Karaki H: Changes in cytosolic calcium level in vascular smooth muscle strip measured simultaneously with contraction using fluorescent calcium indicator fura 2. J Pharmacol Exp Ther 246:294–300, 1988

13. Himpens B, Somlyo AP: Free calcium and force transients during depolarization and pharmacomechanical coupling in guinea-pig smooth muscle. J Physiol (Lond) 395:507–530, 1988

14. Nelson TE, Sweo T: Ca^{2+} uptake and Ca^{2+} release by skeletal muscle sarcoplasmic reticulum: Differing sensitivity to inhalation anesthetics. ANESTHESIOLOGY 69:571–577, 1988

15. Ohnishi ST: Effects of halothane, caffeine, dantrolene and tetracaine on the calcium permeability of skeletal sarcoplasmic reticulum of malignant hyperthermic pigs. Biochim Biophys Acta 897: 261–268, 1987

16. Su JY, Kerrick WGL: Effects of halothane on caffeine-induced tension transients in functionally skinned myocardial fibers. Pflügers Arch 380:29–34, 1979

17. Herland JS, Julian FJ, Stephenson DG: Halothane increases Ca^{2+} efflux via Ca^{2+} channels of sarcoplasmic reticulum in chemically skinned rat myocardium. J Physiol (Lond) 426:1–18, 1990

18. Katsuoka M, Ohnishi ST: Inhalation anaesthetics decrease calcium content of cardiac sarcoplasmic reticulum. Br J Anaesth 62: 669–673, 1989

19. Cauvin C, Loutzenhiser R, Breemen CV: Mechanisms of calcium antagonist-induced vasodilation. Ann Rev Pharmacol Toxicol 23:373–396, 1983

20. Ozaki H, Ohyama T, Sato K, Karaki H: Ca^{2+}-dependent and independent mechanisms of sustained contraction in vascular smooth muscle of rat aorta. Jpn J Pharmacol 52:509–512, 1990

21. Meissner G: Ryanodine activation and inhibition of the Ca^{2+} release channel of sarcoplasmic reticulum. J Biol Chem 261:6300–6306, 1986

22. Tsuchida H, Namba H, Yamakage M, Fujita S, Notsuki E, Namiki A: Calcium ion concentration and contraction in the vascular smooth muscle of the rat aorta. ANESTHESIOLOGY 78:531–540, 1993

23. Benham CD, Tsien RW: A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. Nature 328: 275–278, 1987

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

25. Frazer MJ, Lynch C III: Ca^{2+}-release channels of cardiac sarcoplasmic reticulum are activated by halothane but not by isoflurane (abstract). ANESTHESIOLOGY 77:A630, 1992

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

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