

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

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**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  $\text{Ca}^{2+}$  concentration of vascular smooth muscle were examined by using the fluorescent  $\text{Ca}^{2+}$  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- $\text{N}^G$ -nitroarginine ( $10^{-5}$  M) or nifedipine ( $2 \times 10^{-7}$  M) treatment. Ryanodine ( $2 \times 10^{-5}$  M) treatment completely abolished the transient increases in tension and  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  concentration.

**Conclusions:** Halothane and enflurane, but not isoflurane, induce vasoconstriction by releasing  $\text{Ca}^{2+}$  from intracellular stores. Release of a vasodilating prostanoid and endothelium-derived constricting factor may also be involved in the vasoconstrictor effect. Furthermore, increased  $\text{Ca}^{2+}$  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.<sup>1–4</sup> However, studies *in vitro*<sup>5,6</sup> have suggested that these anesthetics cause vasoconstriction as well, depending on the experimental conditions. Stone and Johns<sup>5</sup> demonstrated that low concentrations of enflurane and isoflurane induced endothelium-dependent vasoconstriction in rat aorta which had been precontracted with phenylephrine. Su and Zhang<sup>6</sup> 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  $\text{Ca}^{2+}$  in vascular smooth muscle was measured with a  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  channel blocker, and a  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) channel blocker.

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## 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<sub>2</sub> 2.5, KH<sub>2</sub>PO<sub>4</sub> 1.2, MgSO<sub>4</sub> 1.2, NaCl 118.2, NaHCO<sub>3</sub> 24.8, and dextrose 10 (pH 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<sup>-6</sup> M)-induced relaxation under phenylephrine (10<sup>-6</sup> M)-precontracted conditions.<sup>7</sup> Vascular rings with more than 60% relaxation or without relaxation were regarded as 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<sub>2</sub>/5% CO<sub>2</sub> mixed gas through agent-specific vaporizers (Fluotec 3, Enfluratic 3, and Fortec, respectively, Cyprane Keighley, England).<sup>8,9</sup> The concentration of anesthetic in the gas phase was 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)<sup>8</sup> 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<sub>2</sub> and 5% CO<sub>2</sub>. 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<sup>-7</sup> M, a Ca<sup>2+</sup> channel blocker), L-N<sup>G</sup>-nitro-arginine (10<sup>-5</sup> M, a NO synthesis inhibitor<sup>10</sup>), indomethacin (10<sup>-5</sup> 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<sup>2+</sup> channel for CICR of Ca<sup>2+</sup> stores into the open state.<sup>11</sup> Arterial rings for ryanodine treatment were exposed to ryanodine (2 × 10<sup>-5</sup> 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.<sup>11</sup> 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<sup>2+</sup> Concentration*

For measurement of the cytosolic Ca<sup>2+</sup> 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  $\text{Ca}^{2+}$  indicator fura 2-AM ( $2.5 \times 10^{-5}$  M) and the noncytotoxic detergent Cremophor EL (0.02% vol/vol).<sup>12</sup> 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 ( $\pm 5$ ) nm at 128 Hz with a chopping mirror and two monochromators. The fluorescence emitted through a 500 ( $\pm 6$ )-nm bandpass filter was detected with a photomultiplier. Because the fluorescence intensity at 340-nm excitation ( $F_{340}$ ) corresponds to the amount of  $\text{Ca}^{2+}$ -bound fura 2, and that at 380-nm excitation ( $F_{380}$ ) to the amount of  $\text{Ca}^{2+}$ -free fura 2, the  $F_{340}/F_{380}$  ratio correlates well with the cytosolic  $\text{Ca}^{2+}$  concentration.<sup>12,13</sup> In this study, therefore, this ratio was used as a measurement of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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 ( $2 \times 10^{-5}$  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<sup>G</sup>-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, Ku-

mamoto, 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  $\pm$  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-

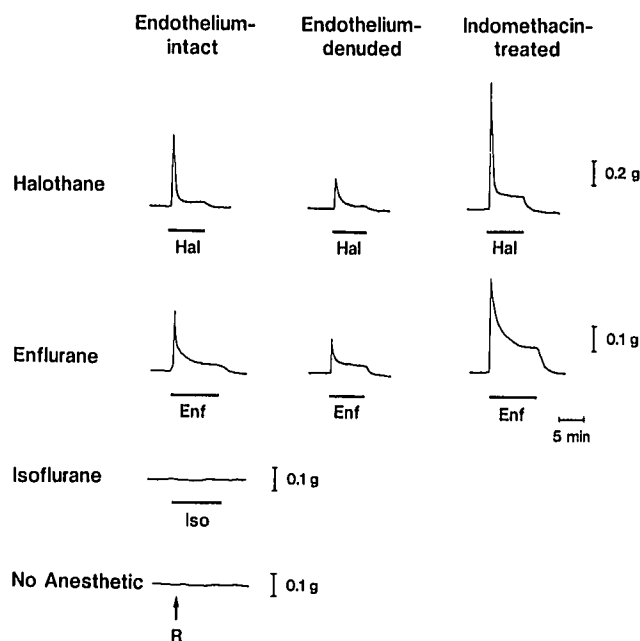


Fig. 1. Typical recordings of halothane (2 MAC)- and enflurane (2 MAC)-induced contractions in endothelium-intact, endothelium-denuded, and indomethacin ( $10^{-5}$  M)-treated arteries. Isoflurane (2 MAC) and replacement of the bathing solution with the aerated solution without anesthetic did not induce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

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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 ( $2 \times 10^{-7}$  M) did not significantly alter either phase (fig. 5).

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

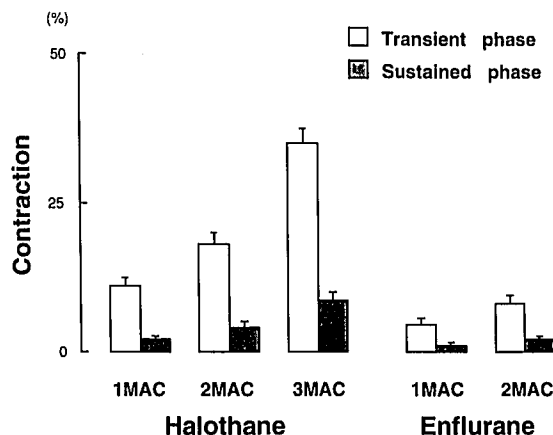


Fig. 2. Tension changes induced by halothane (1–3 MAC) and enflurane (1–2 MAC) ( $n = 6$ , each). KCl (30 mM)-induced contraction was taken as 100%. Isoflurane (1–3 MAC) did not induce contraction.

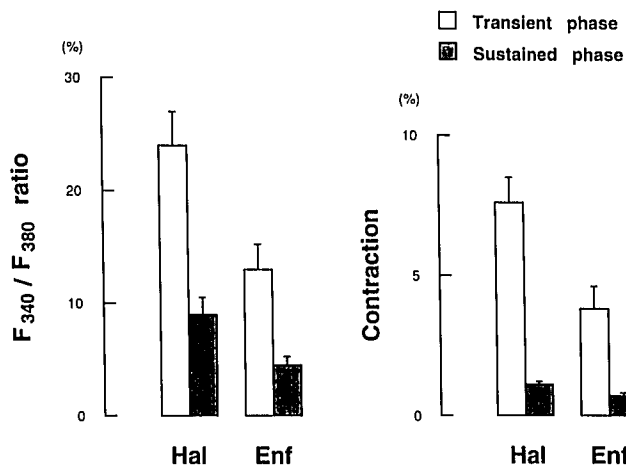


Fig. 3. Changes in the ratio of fluorescence intensity at 340-nm excitation to that at 380-nm excitation ( $F_{340}/F_{380}$ ) and in tension induced by halothane (2 MAC) and enflurane (2 MAC) ( $n = 6$  each). KCl (30 mM)-induced changes were taken as 100%. Hal = halothane (2 MAC); Enf = enflurane (2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

opening, 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  $101.7 \pm 6.5\%$  and  $174.4 \pm 19.5\%$  ( $n = 6$  each), respectively, of those before ryanodine treatment. Enflurane (2 MAC) induced a strong, slow-developing, sustained contraction; it was  $100.9 \pm 5.3\%$  ( $n = 6$ ) and  $948.9 \pm 81.4\%$  ( $n = 6$ ) in control and ryanodine-treated groups ( $P < 0.01$ ), respectively.

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.<sup>14,15</sup> In cardiac muscle, the same phenomenon results in a negative inotropic effect, by decreasing the content of  $\text{Ca}^{2+}$  stores available for contraction.<sup>16-18</sup> Vascular smooth muscle also has intracellular  $\text{Ca}^{2+}$  stores, which are sensitive to both ryanodine and inositol 1,4,5-triphosphate ( $\text{IP}_3$ ) or only to  $\text{IP}_3$ . However, it has not been determined whether halogenated anesthetics induce  $\text{Ca}^{2+}$  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 an 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

**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**

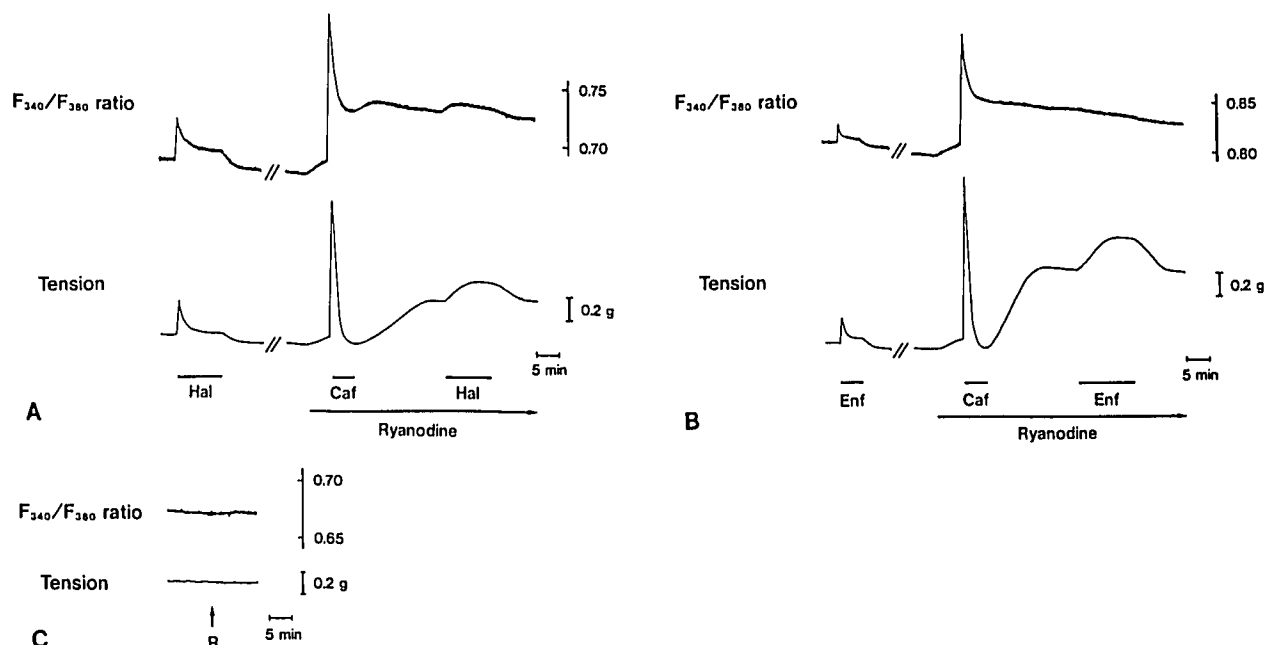
	Endothelium-intact		Endothelium-denuded	
	Transient	Sustained	Transient	Sustained
Halothane (2 MAC)	20.3 ± 2.7 (n = 12)	3.1 ± 0.6	9.5 ± 1.0*	2.3 ± 0.4 (n = 12)
Enflurane (2 MAC)	11.1 ± 1.3 (n = 9)	2.3 ± 0.4	5.6 ± 0.7*	1.7 ± 0.1 (n = 9)

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

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

the transient phase of increase in  $\text{Ca}^{2+}$  concentration and tension properly.

In this study, nifedipine ( $2 \times 10^{-7}$  M) inhibited neither of the phases of contraction.  $\text{Ca}^{2+}$  channel blockers such as nifedipine inhibit high  $\text{K}^{+}$ -induced contraction strongly by blocking L-type voltage-dependent  $\text{Ca}^{2+}$  channels. At higher concentrations, they also decrease the contractions induced by  $\alpha$ -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.<sup>19,20</sup> 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.<sup>21</sup> In the current study, treatment of vascular smooth muscle with ryanodine ( $2 \times 10^{-5}$  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<sup>22</sup> or on influx of extracellular  $Ca^{2+}$  through nifedipine-insensitive  $Ca^{2+}$  channels.<sup>23</sup> 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 in-

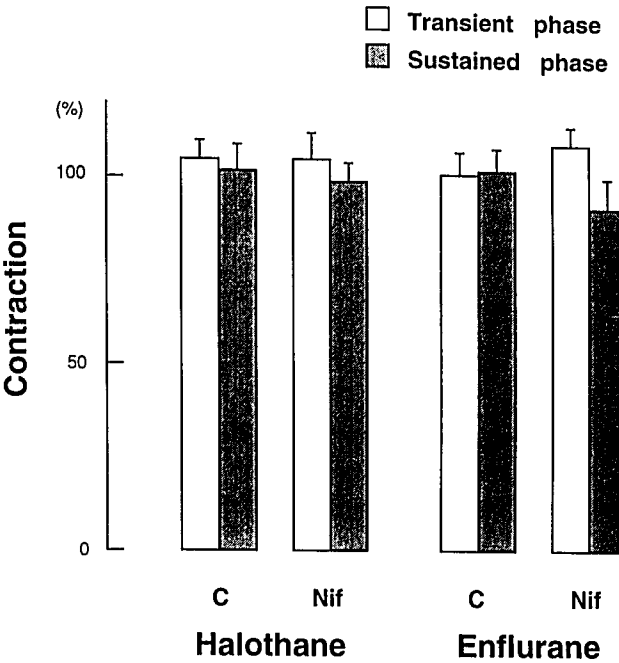


Fig. 5. Modification by nifedipine of halothane (2 MAC)- and enflurane (2 MAC)-induced contractions ( $n = 6$  each). Those before treatment were taken as 100% in each phase. C = control (arterial rings treated with equivalent volume of saline); Nif = rings treated with nifedipine ( $2 \times 10^{-7}$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

crease in cytosolic  $Ca^{2+}$  concentration altered the  $Ca^{2+}$  sensitivity.

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 ( $2 \times 10^{-5}$ 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.*<sup>24</sup> and Frazer and Lynch<sup>25</sup> 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.<sup>8,26,27</sup> In the current study, although removal of the endothelium attenuated anesthetic (2 MAC)-induced contraction, treatment with a NO synthase inhibitor, L-N<sup>G</sup>-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<sub>2</sub>. This hypothesis is in good agreement with that of Stone and Johns,<sup>5</sup> 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-precon-

tracted 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.

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