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Comparative Contractile Effects of Halothane and Sevoflurane in Rat Aorta

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Background: **Volatile anesthetic agents have been shown to have contractile effects in vascular tissues during specific conditions. This study compared contractile effects of halothane and sevoflurane in rat aorta treated with verapamil. This study also tried to elucidate the mechanism of the contraction.**

Methods: **Endothelium-denuded rat thoracic aorta was used for recording of isometric tension and measurement of influx of $^{45}Ca^{2+}$. All experiments were performed in the presence of verapamil. In recording of tension, rings were precontracted with a submaximum dose of phenylephrine, followed by exposure to halothane or sevoflurane. For measurement of influx of $^{45}Ca^{2+}$, rat aortic strips were exposed to phenylephrine and then to additional halothane or sevoflurane. Influx of Ca^{2+} was estimated by incubating the strips in $^{45}Ca^{2+}$-labeled solution for 2 min.**

Results: **Halothane (0.5–4.0%) induced contraction in a dose-dependent manner, whereas sevoflurane (1–4%) had no effect on tension. Influx of $^{45}Ca^{2+}$ was strongly enhanced by halothane at 1% and 2%, but only slightly at 4%, and was not affected by 1–4% sevoflurane. SK&F 96365, a blocker of voltage-independent Ca^{2+} channels, abolished contraction and influx of $^{45}Ca^{2+}$ by 1% halothane. Depletion of Ca^{2+} from the sarcoplasmic reticulum with ryanodine or thapsigargin reduced the contraction induced by halothane at 4% but not that at 1% and 2%.**

Conclusion: **Halothane is suggested to cause contraction by enhancing influx of Ca^{2+} *via* voltage-independent Ca^{2+} channels at concentrations up to 2% and by inducing release of Ca^{2+} at 4%. Sevoflurane (1–4%) is devoid of these contractile effects. (Key words: Isoflurane; α-receptor; vascular smooth muscle.)**

THE halogenated volatile anesthetic agents halothane, enflurane, isoflurane, and sevoflurane modify vascular contractility by acting on various mechanisms of contraction. Although they are thought to inhibit vascular contraction, they also can contract vessels depending on the vessel type and on experimental conditions. In resistance arteries, such as mesenteric artery, anesthetic agents cause a transient contraction, which has been clearly explained by release of Ca^{2+} from the sarcoplasmic reticulum (SR).[1–4] In conductance arteries such as aorta, sustained contraction is induced by anesthetic agents when they are precontracted with α-agonists in the presence of blockers of voltage-operated Ca^{2+} channels (VOCCs).[5–7] In these studies in aortae, the preliminary dose of VOCC blockers was considered to mask the inhibitory effect of anesthetic agents on VOCCs,[8,9] revealing a contractile effect of anesthetic agents, which is not fully elucidated however. Namba and Tsuchida[5] suggested a role of the release of Ca^{2+} for the contraction of rat aorta by halothane and isoflurane because of a simultaneous increase in the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). In contrast, by using $^{45}Ca^{2+}$ as a tracer ion, we recently suggested that contraction of rat aorta by isoflurane was attributable to influx of Ca^{2+} through Ca^{2+} channels other than VOCCs and that the role of the release of Ca^{2+} might be negligible.[7]

Vascular smooth muscle cells have heterogenous Ca^{2+} channels, VOCCs, and channels activated by receptor stimulation independent of depolarization. Although the latter channels were first named "receptor-operated Ca^{2+} channels,"[10] we use the term "voltage-independent Ca^{2+} channels" (VICCs) in this article in accordance with recent reviews.[11,12] VICCs can be classified into several groups according to their mode of activation[11,12]: (1) channels tightly coupled to the receptor; (2) depletion-operated Ca^{2+} channels activated when the intracellular Ca^{2+} store is empty; and (3) channels regulated *via*

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soluble messengers. These different types of channels can coexist in the same smooth muscle cell.[13–15] Although VICCs have important roles in contraction,[10] little is known about the effect of anesthetic agents on their activity.

The purpose of this study was to investigate the contractile effect of the other prevailing volatile anesthetic agents halothane and sevoflurane in rat aorta, especially in relation to the activity of VICCs. We conducted tension experiments and measurement of influx of $^{45}Ca^{2+}$ in endothelium-denuded rat aorta in the presence of verapamil.

Methods

Tissue Preparation

The study was approved by the Kyoto University Animal Use Committee. Male Wistar rats (weight, 250–350 g) were anesthetized with pentobarbital, 50 mg/kg given intraperitoneally, and killed by exsanguination. The descending part of the thoracic aorta was isolated and cut into rings 5–6 mm wide. The endothelium was removed by rotating the rings around a rough-surfaced needle. The absence of functional endothelium was confirmed by the inability of 10 μM acetylcholine to inhibit phenylephrine contraction in each ring in tension experiments. In the subsequent experiments throughout, a supramaximal dose (10 μM) of verapamil was included in the bathing solution. This dose of verapamil completely blocked KCl-induced contraction and $[Ca^{2+}]_i$ response in rat aorta,[7,16,17] proving that VOCCs were functionally inhibited. As the effect of the anesthetic agents was observed in the continuous presence of verapamil, it should have been free from the influence of the minor nonspecific effects of verapamil,[17] such as suppression of Na^+ or K^+ channels.

Recording of Isometric Tension

The aortic rings were mounted vertically in organ baths filled with 10 ml Krebs–Ringer's solution aerated with a 95% O_2/5% CO_2 gas mixture. Isometric tension was recorded with a force displacement transducer (Sanei, Tokyo, Japan). The rings were placed in the bath with an optimal resting tension of 3 g for 60 min to equilibrate. They were then incubated for 10 min with 10 μM verapamil and contracted with 300 nM phenylephrine. This dose of phenylephrine was shown in our preliminary study to elicit ≈60% of the maximal contraction and was considered to allow enhancement and suppression

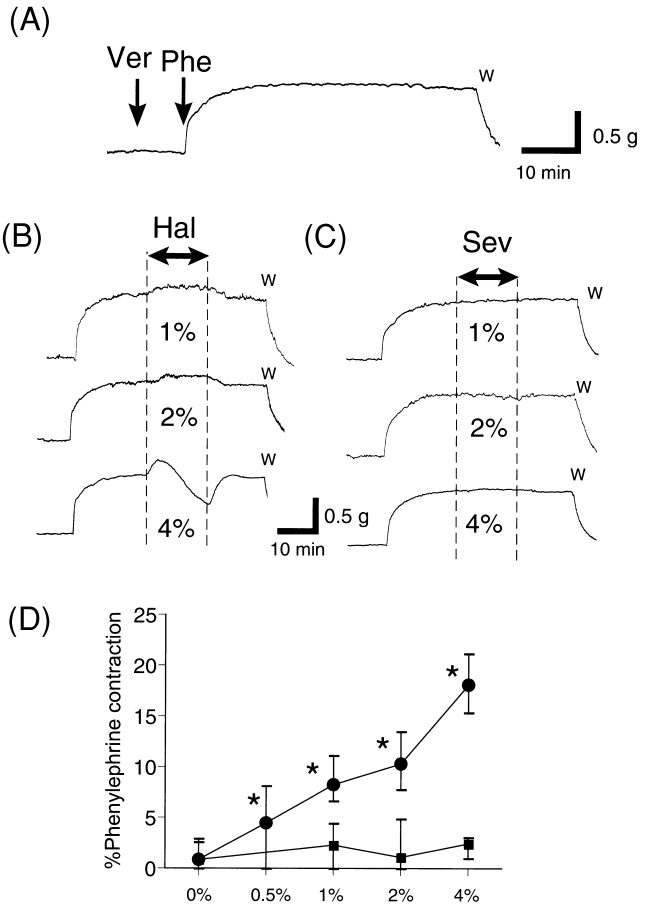

Fig. 1. Effects of halothane and sevoflurane on phenylephrine-induced contraction in rat aorta. (*A*) Rat aortic rings were pretreated with verapamil 10 μM (Ver). Ten minutes later, phenylephrine 300 nM (Phe) was added to evoke sustained contraction. (*B*) Typical responses to subsequent application of 1–4% halothane (Hal). (*C*) Typical responses to 1–4% sevoflurane. (*D*) Maximum tension increment after introduction of halothane (circle) and sevoflurane (square), expressed as percent of contraction by phenylephrine in each ring. Median values with 25th and 75th percentiles were plotted. Each concentration of halothane induced significantly larger tension increment compared with 0% (*$P < 0.05$). n = 16 in each group. W = wash.

of the contraction induced by the anesthetic agents. Phenylephrine induced a sustained contraction, which reached a stable level within 15 min (fig. 1A). Twenty minutes after addition of phenylephrine, halothane or sevoflurane (1–4%) was introduced into the gas aerating the baths. Halothane and sevoflurane were vaporized using Fluotec 3 (Ohmeda, West Yorkshire, United Kingdom) and MK III-ST (Acoma, Tokyo, Japan), respectively, and their concentrations in the gas were adjusted while monitoring with an Atom 303 anesthetic agent monitor (Atom, Tokyo, Japan). In a previous study from our

laboratory in which the concentration of halothane and sevoflurane in the bath solution was assayed by gas chromatography, the anesthetic agents were proven to reach stable levels within 10 min after introduction into the gas.[1,18] Anesthetic agents were maintained for 15 min and then discontinued, after which recovery from their effects were observed for 20 min. The maximum tension increment after introduction of the anesthetic agents was expressed as a percent of the maximum contraction induced by phenylephrine before addition of anesthetic agents in each ring.

In some experiments with halothane, the composition of Krebs–Ringer's solution was modified to control the movement of Ca^{2+}. Reference contraction was obtained in each ring with 300 nM phenylephrine in the presence of 10 μM verapamil. After phenylephrine had been washed out and the tension had returned to its basal level, modified experiments were conducted. Modifications to the Krebs–Ringer's solution were made, by replacing 2 mM Ca^{2+} with 1 mM EGTA or by adding either 30 μM SK&F 96365 (blocker of VICCs), 20 μM ryanodine (blocker of Ca^{2+} release channel of the SR), or 0.1 μM thapsigargin (inhibitor of the SR Ca^{2+}-adenosine triphosphatase). All solutions contained 10 μM verapamil. In either experiment, the increase of maximum tension after addition of halothane was expressed as a percent of the reference contraction induced by phenylephrine.

Experiment on Influx of $^{45}Ca^{2+}$

To estimate the amount of unidirectional influx of Ca^{2+} into the smooth muscle, we used $^{45}Ca^{2+}$ as a tracer ion. Experiments were performed using a method described previously[7,19] with some modifications. Aortic rings were prepared and deprived of endothelium as described earlier. They were then cut open into strips and bathed in HEPES-buffered saline solution (HBSS) containing 10 μM verapamil. Verapamil was included in the solution throughout the experiment. The strips were allowed to equilibrate for 60 min in the solution with continuous aeration with air at 37°C. The strips were then transferred to HBSS containing 300 nM phenylephrine. After 10 min, 0–4% halothane was introduced to the solution through the aeration line. Halothane was vaporized and its concentration monitored as described earlier. The strips were exposed to halothane for up to 20 min. Every 5 min during the course, the rings were transferred to another vial filled with HBSS containing 1 μCi/ml $^{45}Ca^{2+}$ in addition to phenylephrine and halothane. After exposure of the strips to $^{45}Ca^{2+}$ for 2 min, they were bathed in 2 mM EGTA-containing HBSS at 0°C for 50 min to remove extracellular $^{45}Ca^{2+}$ and then blotted and weighed. Intracellular $^{45}Ca^{2+}$ was extracted by incubating the strips in 1.5 ml of 5 mM EDTA at room temperature overnight. $^{45}Ca^{2+}$ in the solution was analyzed with a liquid scintillation counter after adding 3 ml of scintillation cocktail (Scintisol EX-H; Dojindo, Kumamoto, Japan). Data are expressed as the estimated total amount of influx of Ca^{2+} (μmol) per kilogram aorta (wet weight). The number of minutes for each data point represents the time from the beginning of stimulation with phenylephrine until removal from the $^{45}Ca^{2+}$ solution. Therefore, strips were exposed to $^{45}Ca^{2+}$ for the last 2 min of the time indicated. Zero minutes means that strips were exposed to $^{45}Ca^{2+}$ without stimulation by phenylephrine. The same experiment was conducted with sevoflurane (0–4%), but its effect was analyzed only at the time point of 20 min, *i.e.*, after 10 min of exposure to the anesthetic agent.

Solutions and Drugs

The Krebs–Ringer's solution was composed of (in mM) NaCl 120, KCl 5.0, $CaCl_2$ 2.0, $MgCl_2$ 1.0, $NaHCO_3$ 25.0, and dextrose 5.5; the *p*H of the solution was 7.3–7.4 when the solution was aerated with a 95% O_2/5% CO_2 gas mixture. The HEPES-buffered saline solution was composed of (in mM) NaCl 140, KCl 5.0, $CaCl_2$ 2.0, $MgCl_2$ 1.0, dextrose 5.5, HEPES 5.0 and *p*H-adjusted to 7.30–7.35 with NaOH. The drugs used were halothane (Takeda Pharmaceutical Co., Osaka, Japan); sevoflurane (Maruishi Pharmaceutical Co., Osaka, Japan); phenylephrine, ryanodine, thapsigargin, and phentolamine (Sigma Chemical Co., St. Louis, MO); acetylcholine (Daiichi Pharmaceutical Co., Tokyo, Japan); verapamil, EGTA, and EDTA (Nacalai Tesque, Kyoto, Japan), and SK&F 96365 (BIOMOL; Plymouth Meeting, PA).

Statistical Analysis

Data for anesthetic-induced contraction were expressed as median values with 25th and 75th percentiles because the maximum tension increment after anesthetic agents that we measured could not be lower than zero and did not follow a normal distribution. The other data were distributed normally and are expressed as mean ± SD. For comparison between two groups, *t* test was used. For comparison among more than two groups, one-way analysis of variance was used. When data were not distributed normally or when variances were not equal among groups, the Kruskall-Wallis test was used. In either test, if significance ($P < 0.05$) was detected among groups, each group was compared with the con-

trol using Dunnett's test for *post hoc* multiple comparison. Differences at $P < 0.05$ were considered statistically significant.

Results

Effect of Anesthetic Agents on Phenylephrine-evoked Contraction and Influx of Ca^{2+}

For a control tension experiment, rat aortic rings pretreated with verapamil were exposed to halothane or sevoflurane (1%, 2%, or 4%; n = 4 each) at their basal tension. No contraction was observed in any of the rings examined (data not shown). We then tried to increase the concentration of anesthetic agent immediately by replacing the bath solution with already saturated solution, a method described by Kakuyama *et al.*[1] to obtain a more definite contraction by halothane. Again, halothane or sevoflurane at 1%, 2%, or 4% failed to evoke any contraction (n = 4 each; data not shown).

Phenylephrine at 300 nM evoked a sustained contraction (fig. 1A), which averaged 0.844 g (SD = 0.209 g, n = 144) and did not differ significantly among groups. In this setting when halothane was introduced into the solution, the precontraction by phenylephrine was enhanced continuously (≤ 2%) or transiently (4%; fig. 1B). Sevoflurane 1–4%, failed to cause any contraction or relaxation (fig. 1C). When the maximum tension increment after use of anesthetic agents was plotted, halothane caused contraction in a linear, dose-dependent manner, whereas sevoflurane had no significant effect (fig. 1D).

The experiment on influx of $^{45}Ca^{2+}$ revealed a stable increase in influx of Ca^{2+} by phenylephrine, which developed within 10 min (fig. 2A). As this influx occurred while VOCCs were blocked, it should be through VICCs activated by phenylephrine. Halothane 1% induced a marked and sustained increase in influx of Ca^{2+}, showing a time course consistent with halothane-induced contraction (fig. 2A). Halothane 4%, however, which induced the largest contraction, caused only a slight and transient increase in influx of Ca^{2+} (fig. 2A). Ten minutes after introduction, the effects of varied concentrations (1–4%) of halothane were compared (fig. 2B). Influx of Ca^{2+} was significantly larger in the 1% and 2% halothane groups than in the control (0%). In contrast, 10 min of exposure to sevoflurane 1–4% did not change influx of Ca^{2+} (fig. 2B). In the subsequent experiments, the mechanism of halothane-induced contraction and influx of Ca^{2+} was investigated.

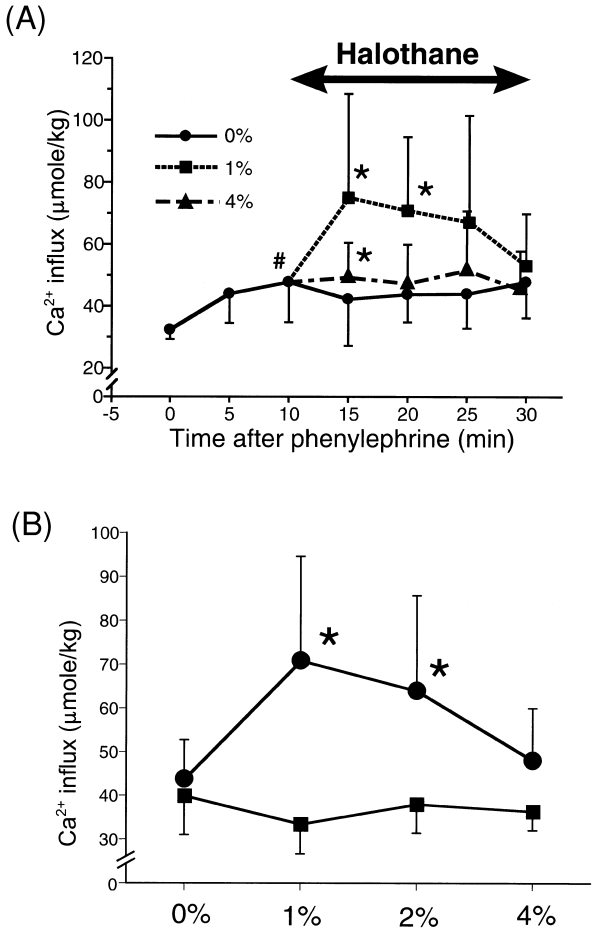

Fig. 2. Effects of halothane on verapamil-resistant influx of Ca^{2+} estimated by measurement of influx of $^{45}Ca^{2+}$. (*A*) Time course of influx of Ca^{2+}. Means ± SDs are shown. Note that strips were exposed to $^{45}Ca^{2+}$ only for the last 2 min before the time indicated in each data point. Stimulation with phenylephrine 300 nM for 10 min induced significantly larger influx of Ca^{2+} than at 0 min (#$P < 0.05$). Halothane 1% and 4%, introduced at this time, further enhanced influx of Ca^{2+} (*$P < 0.05$ compared with 0%). n = 10 in each group. (*B*) Effects of varied concentrations of halothane (circle) and sevoflurane (square) on influx of Ca^{2+} 10 min after introduction (20 min after phenylephrine). Means ± SDs are shown. Halothane 1% and 2% induced significantly larger influx of Ca^{2+} compared with 0% (*$P < 0.05$). n = 10 in each group.

Effect of Removal of Ca^{2+}

To verify the role of influx of Ca^{2+} in halothane-induced contraction, tension experiments were conducted in the absence of extracellular Ca^{2+}. Five minutes after the bath solution was replaced by Ca^{2+}-free Krebs–Ringer's solution, 300 nM phenylephrine was added to the bath, which induced a transient contraction (fig. 3), possibly attributable to release of Ca^{2+} from the SR. As the contraction was not sustained, we had to start

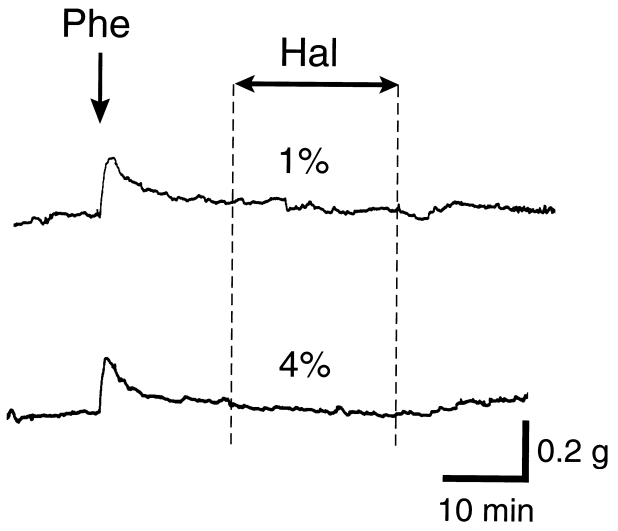

Fig. 3. Typical recordings of tension changes by phenylephrine 300 nM and 1% or 4% halothane in Ca^{2+}-free solution. Phenylephrine (Phe)-induced contraction was only transient, and subsequent application of halothane (Hal), 1% or 4%, revealed no effect.

halothane when the tension was near the basal level. Halothane 1% or 4% induced virtually no change in the tension in any ring (n = 4 each; fig. 3).

Effect of SK&F 96365

To confirm that halothane-induced contraction and influx of Ca^{2+} were mediated by VICCs, the effect of SK&F 96365, a blocker of VICCs,[11] was examined. In vascular smooth muscle, its blocking effect on VICCs has been shown in patch–clamp and contraction studies.[15,20,21] Although it also may have a weak VOCC-blocking action,[21] it should not matter in the presence of the maximal dose of verapamil. Contraction was induced by 300 nM phenylephrine 10 min after adding SK&F 96365 to the bath, which was only transient as in Ca^{2+}-free solution (fig. 4A), suggesting that SK&F 96365 effectively blocked influx of Ca^{2+} *via* VICCs without interrupting release of Ca^{2+}. Again, halothane 1% or 4% induced no change in tension (fig. 4A). The effect of SK&F 96365 was also examined in experiments on the influx of $^{45}Ca^{2+}$. SK&F 96365 30 μM was included in the HBSS from the beginning of the equilibrium period throughout the experiment. Influx of Ca^{2+} was estimated at 0, 10, or 20 min after addition of 300 nM phenylephrine, either with or without exposure to 1% halothane for the last 10 min. Phenylephrine did not significantly increase influx of Ca^{2+} over the time course (fig. 4B). Moreover, halothane did not increase influx of Ca^{2+} at 20 min compared with the control (fig. 4B).

Effect of Ryanodine and Thapsigargin

In another series of tension experiments, we used ryanodine and thapsigargin to block release of Ca^{2+} by halothane. Ryanodine is an open-channel blocker of Ca^{2+}-induced Ca^{2+} release channel, which could abolish halothane-induced release of Ca^{2+} in vascular smooth muscle cells,[1,2,4,22] whereas thapsigargin inhibits Ca^{2+}-adenosine triphosphatase of the SR,[23] both resulting in depletion of stored Ca^{2+}. When ryanodine or thapsigargin was added to the solution, a slowly developing contraction was induced, which has been explained by

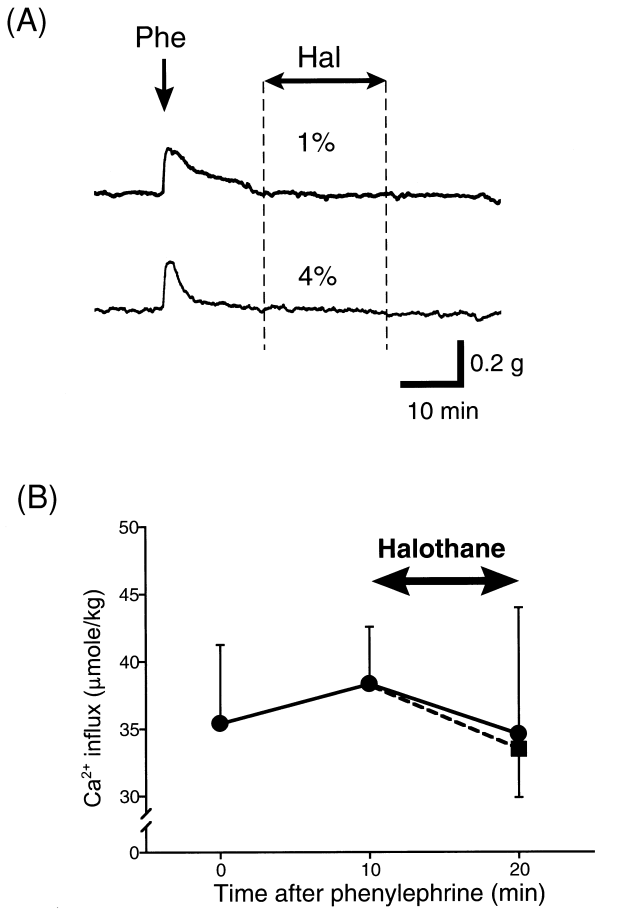

Fig. 4. Effects of SK&F 96365 on halothane-induced contraction and influx of $^{45}Ca^{2+}$. (*A*) Typical recordings of tension changes in the presence of SK&F 96365. As with Ca^{2+}-free solution, phenylephrine (Phe)-induced contraction was transient and halothane (Hal) had no effect on tension. (*B*) Influx of Ca^{2+} at 10 and 20 min after phenylephrine. Means ± SDs are shown. In the control group (circle), strips were exposed to phenylephrine for 20 min. In the halothane group (square), strips were exposed to halothane from 10 min after the addition of phenylephrine. Strips were exposed to $^{45}Ca^{2+}$ for the last 2 min of the time indicated.

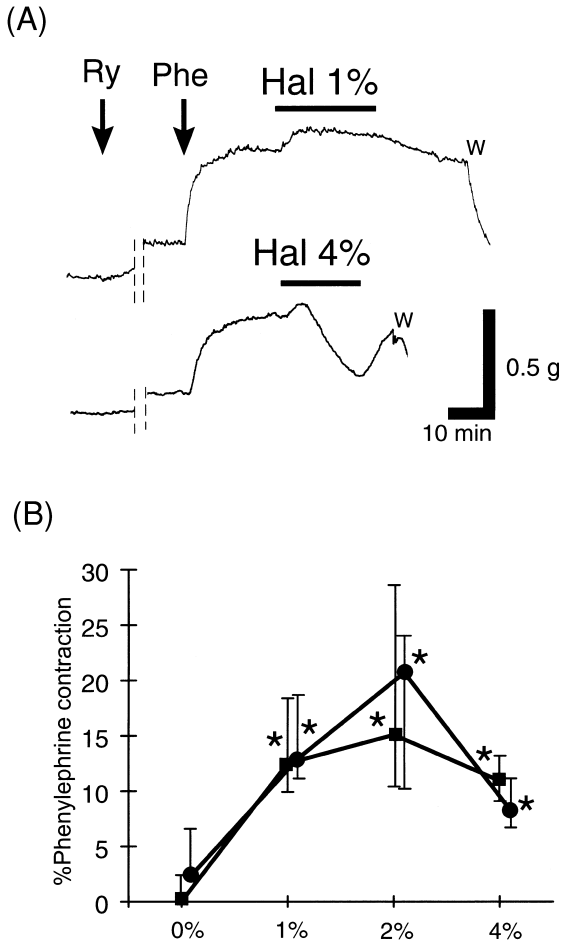

Fig. 5. Effects of ryanodine and thapsigargin on halothane-induced contraction. (*A*) Typical recordings of tension changes. Ryanodine (Ry) 20 μM induced a small sustained contraction. After 40 min, phenylephrine (Phe) 300 nM was added. Halothane (Hal) 1% still induced a sustained contraction, whereas contraction by 4% was smaller than without ryanodine. (*B*) Maximum tension increment after introduction of halothane, expressed as percent of contraction by phenylephrine in each ring. Rings were pretreated with ryanodine (square) or thapsigargin (circle). Median values with 25th and 75th percentiles were plotted. After ryanodine and thapsigargin, 1%, 2%, and 4% halothane induced significantly larger tension increments compared with 0% (*$P < 0.05$). n = 10 in each group. W = wash.

increased leakage of Ca^{2+} from the SR or by influx of Ca^{2+} attributable to SR depletion.[24,25] After 40 min, when the contraction was stable, 300 nM phenylephrine and then halothane were applied to the ring (fig. 5A). Treatment with ryanodine, however, enhanced contraction by halothane up to 2%, but reduced it at 4% (figs. 5A and 5B). Thus, the maximal contraction was obtained at 2% (fig. 5B). Treatment with thapsigargin gave essentially the same result with a similar concentration–response curve (fig. 5B).

Influx of Ca^{2+} without Phenylephrine

To examine the possibility that halothane specifically acts on α-receptors, we conducted an experiment on the influx of $^{45}Ca^{2+}$ without phenylephrine. Phentolamine, an α-receptor antagonist, was included at 10 μM in the HBSS from the beginning of the equilibrium period. Influx of Ca^{2+} was estimated either without any treatment or after 10 min of exposure to 1% halothane. In either group, the rings were incubated in solutions containing $^{45}Ca^{2+}$ for the last 2 min. Estimated influx of Ca^{2+} was significantly higher in halothane-exposed strips (44.6 ± 17.5 μmol/kg, n = 16, $P < 0.05$) than in control strips (33.6 ± 7.0 μmol/kg, n = 16). The 1% halothane-evoked fraction of influx of Ca^{2+} was 11.0 μmol/kg, much less than that in the presence of phenylephrine (27.1 μmol/kg). This appeared to be consistent with the result of the tension experiment, in which halothane alone could not induce contraction without precontraction.

Discussion

In rat aortic rings pretreated with verapamil, halothane at 0.5–4.0% enhanced phenylephrine-evoked contraction in a linear, dose-dependent manner, whereas 1–4% sevoflurane had no effect. The result with halothane is compatible with a previous study in which halothane enhanced norepinephrine-induced contraction in verapamil-treated rat aorta.[5] Although the study showed a simultaneous increase in $[Ca^{2+}]_i$ by using a Ca^{2+} indicator dye, this technique does not define the mechanism of increased $[Ca^{2+}]_i$, which might be induced by anesthetic agents either through influx of Ca^{2+},[7] release of Ca^{2+},[4,6,26] inhibited extrusion of cytosolic Ca^{2+},[27,28] or decreased uptake of cytosolic Ca^{2+} into the SR.[4] We performed an experiment on the influx of $^{45}Ca^{2+}$ because it was a useful method for evaluating influx of Ca^{2+} alone, unaffected by movement of Ca^{2+} inside the cell.[19] The results of our experiment on the influx of $^{45}Ca^{2+}$ demonstrated that halothane 1% and 2% increased the influx of Ca^{2+}, which had been submaximally preactivated by phenylephrine. The pathway of influx of Ca^{2+} should be VICCs because VOCCs had been functionally inhibited by verapamil. Halothane 1% induced an even larger influx of Ca^{2+} (27.1 μmol/kg) than phenylephrine (15.5 μmol/kg) despite that it

caused only ≈10% of phenylephrine-induced contraction. This puzzling disproportion may be attributable to their opposite effects on Ca^{2+} sensitivity of the contractile protein, *i.e.*, sensitization with α-receptor stimulation[16,17] and desensitization with halothane.[3,29] In contrast, influx of Ca^{2+} induced by 4% halothane was unexpectedly low, yielding a curious parabolic-shaped dose–response curve different from that of contraction. In addition, 1–4% sevoflurane had no effect on influx of Ca^{2+}, which was consistent with its tension result.

We postulated that the transient contraction induced by 4% halothane was attributable to release of Ca^{2+} and tried to confirm this hypothesis. In previous studies, contraction by halothane has been ascribed to release of Ca^{2+} from the SR, because the contraction was resistant to removal of extracellular Ca^{2+}[4,29] or was abolished by ryanodine.[1–4,22] In this study, 1% or 4% halothane did not evoke contraction in Ca^{2+}-free solution; however, this did not necessarily prove that halothane did not release Ca^{2+} from the SR, because soaking the ring in EGTA-containing solution should have reduced the basal $[Ca^{2+}]_i$ and depleted intracellular Ca^{2+} stores,[30] which is an untoward condition to observe release of Ca^{2+} by halothane. We therefore performed an experiment in normal solution using drugs that deplete the store of Ca^{2+}. Ryanodine and thapsigargin reduced the contraction induced by 4% halothane but not that by 1% and 2% halothane. Although it has been suggested that smooth muscle cells may have a store of Ca^{2+} that is resistant to ryanodine but sensitive to inositol 1,4,5-trisphosphate,[31] thapsigargin is considered to deplete both types of store.[23] Therefore our results suggest that release of Ca^{2+} from the SR plays an important role in contraction by halothane at 4% but not at concentrations of ≤ 2%. After depletion of the store of Ca^{2+}, the dose–response curve of contraction became more similar to that of halothane-induced influx of $^{45}Ca^{2+}$ and could be considered to reflect the activity of influx of Ca^{2+} more directly than that without depletion of the store. A minor discrepancy between the experiments on tension and influx of $^{45}Ca^{2+}$ may be pointed out; 4% halothane still could cause contraction after depletion of the store, whereas it could not cause a large influx of Ca^{2+}. This finding can be explained, however, by the different way of measurement; contraction was measured at its maximal point and influx of Ca^{2+} at the fixed time, 10 min after use of halothane.

To confirm that halothane-induced influx of Ca^{2+} was mediated by VICCs, we examined the effect of SK&F 96365, a blocker of VICCs, in the experiments on tension and influx of $^{45}Ca^{2+}$. SK&F 96365 blocked the sustained phase of contraction induced by phenylephrine to evoke a phasic contraction similar to that in a Ca^{2+}-free solution. Contraction induced by 1% or 4% halothane was also abolished. It was considered, however, that this condition may not be favorable for contraction by halothane, because it should deplete intracellular Ca^{2+} by blocking Ca^{2+} replenishment as in Ca^{2+}-free solution. We then tested SK&F 96365 in experiments on the influx of $^{45}Ca^{2+}$. This completely abolished influx of Ca^{2+} by phenylephrine and 1% halothane. This is an additional finding that supports that halothane activates VICCs. SK&F 96365 is a nonspecific blocker of VICCs.[11] As subtype-specific blocker is not yet established, we could not determine the subtype of VICCs sensitive to halothane.

The mechanism of VICC activation by halothane was not fully examined in this study. Halothane should not act on the α-receptor as an agonist because 1% halothane was able to activate influx of Ca^{2+} even in the presence of phentolamine. In addition, it is unlikely that halothane augments the binding of phenylephrine to the α-receptor, because it has been shown that halothane did not increase, but rather inhibited, the binding of radioligands to α-receptors obtained from rat brain[32] or rabbit myometrium.[33] It seems, however, that activation of VICCs by halothane is not independent of the signal transduction system from the α-receptor to VICCs, because the amount of influx of Ca^{2+} activated by halothane was higher in the presence of phenylephrine. Halothane might enhance activation of the signaling pathway distal to the level of the receptor, *i.e.*, G protein, intracellular second messengers, or the channel protein itself. Another possibility is that halothane evokes release of Ca^{2+}, thus depleting stored Ca^{2+} and stimulating influx of Ca^{2+} *via* a depletion-operated mechanism. This has been suggested to account for the activated pathway of influx of Ca^{2+} induced by halothane in canine airway smooth muscle.[34] This may not be a primary mechanism of VICC activation in rat aorta, however, considering that abolishment of release of Ca^{2+} by ryanodine or thapsigargin did not inhibit contraction by 1–2% halothane. It is necessary to elucidate the signal transduction pathway from the α-receptor to the channel before further conclusions can be made.

In previous studies using mesenteric arteries, low concentrations (< 0.75%) of halothane were shown to induce contraction by stimulating release of Ca^{2+} even in a resting state.[1,2,4] In contrast, in the current study using rat aorta, release of Ca^{2+} was detected only when rings

were precontracted in the presence of verapamil and the concentration of halothane was increased to 4%. The different responses might be ascribable to the different Ca^{2+} regulatory machinery in these two tissues. The vascular smooth muscle from different size of vessels and tissue bed can display different forms of pharmacomechanical coupling mechanisms, such as relative roles of VICCs, VOCCs, and the SR as the source of Ca^{2+}, sensitivity of the SR to halothane, the content of Ca^{2+} in the SR, and the Ca^{2+}-buffering capacity of the cytosol. Although influx of Ca^{2+} has not been examined directly in studies using mesenteric arteries, some of the results have raised the possibility that halothane stimulates influx of Ca^{2+} through the cell membrane.[1,4] Therefore, it is possible that influx of Ca^{2+} induced by halothane is not a specific phenomenon in aortic tissues but a common underlying mechanism in other vascular tissues. In contrast, some studies that have monitored $[Ca^{2+}]_i$ in A7r5, a cultured smooth muscle cell line established from rat aorta, have shown that halothane suppressed rather than stimulated vasopressin-activated influx of Ca^{2+}.[35,36] The discrepancy might be attributable to the absence of VOCC blockers in their studies in addition to the differences in the cell type, vasoconstrictor, or technique used. The current study appears to be the first to demonstrate directly the activation of the VICCs by halothane in vascular smooth muscle.

The effect of halothane on influx of Ca^{2+} was similar to that of isoflurane shown in our previous study.[7] Isoflurane induced influx of Ca^{2+} resistant to nifedipine and verapamil, and its effect was not linearly dose-dependent, showing a maximal effect at 2.3%. Most anesthetic agents may exert their effects on VICCs through common mechanisms. The distinct feature of contraction by halothane is that release of Ca^{2+} also contributed to its effect, unlike with isoflurane. In contrast, sevoflurane seems completely devoid of these effects within a clinically relevant concentration range (1–4%). Although sevoflurane has been shown to suppress KCl-induced contraction in coronary arteries,[18] vasodilating action was abolished by verapamil in this study. Therefore, it can be assumed that the direct vascular effect of sevoflurane is mediated mainly by suppression of VOCCs and that it is inactive with the other contractile mechanisms found for halothane or isoflurane.

Halothane is suggested to have dual contractile effects on verapamil-treated precontracted rat aorta, with sustained contraction at up to 2% attributable mainly to influx of Ca^{2+} *via* VICCs and transient contraction at 4% attributable mainly to release of Ca^{2+} from the SR. In contrast, sevoflurane had no contractile effect. Although these anesthetic agents have VOCC-blocking action in common, they are different in exerting contractile effects. The contractile effect of halothane is weak in rat aorta, hardly visible in normal conditions. The overall consequence of this effect on the other vascular tissues and the systemic circulation may be more profound, however.

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