Halothane decreases calcium sensitivity of rat aortic smooth muscle

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Purpose: To examine the effect of halothane on the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$)-tension relationship of rat aortic smooth muscle.

Methods: Rat aortic rings without endothelia were loaded with the fluorescent Ca^{2+} indicator, Fura PE3-AM, and then mounted in organ baths. The changes in isometric tension and $[\text{Ca}^{2+}]_i$ were measured simultaneously. In one series ionomycin (10 nM - $3 \mu\text{M}$) was added to normal Krebs' solution cumulatively in the absence and presence of halothane (1.5%, 3%). In the other series, CaCl_2 (0.3 - 3 mM) was added to Ca^{2+} -free Krebs' solution including high KCl (50 mM), phenylephrine (100 nM) or prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$, 1 - $3 \mu\text{M}$) in the absence and presence of halothane (1.5%, 3%). The linear part of $[\text{Ca}^{2+}]_i$ -tension relationship was analyzed by a linear regression.

Results: Halothane, 1.5%, had no effect on the normal $[\text{Ca}^{2+}]_i$ -tension relationship obtained with the calcium ionophore, ionomycin (10 nM - $3 \mu\text{M}$), but halothane 3% decreased the slope of the relationship (0.239 ± 0.037 for control and 0.110 ± 0.010 for halothane 3%, $P < 0.05$). Halothane, 1.5% and 3%, did not change the $[\text{Ca}^{2+}]_i$ -tension relationship obtained with CaCl_2 (0.3 - 3 mM) in the presence of high KCl (50 mM) or phenylephrine (100 nM). In contrast, halothane, 3%, inhibited the intercept of $[\text{Ca}^{2+}]_i$ -tension relationship obtained with CaCl_2 (0.3 - 3 mM) in the presence of prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$, 1 - $3 \mu\text{M}$) (45.708 ± 4.233 for control and 26.997 ± 2.522 for halothane 3%, $P < 0.01$).

Conclusion: Halothane decreases the Ca^{2+} sensitivity and that in the presence of PGF_2 .

Objectif : Étudier l'effet de l'halothane sur la relation tension isométrique-concentration calcique cytosolique ($[\text{Ca}^{2+}]_i$) sur le muscle lisse de l'aorte chez le rat.

Méthode : Des anneaux aortiques de rat, sans endothélium, ont été chargés avec un indicateur de fluorescence pour le Ca^{2+} , le Fura PE3-AM, et montés ensuite dans des bains d'organes. Les changements de tension isométrique et de $[\text{Ca}^{2+}]_i$ ont été mesurés simultanément. Dans une série, on a ajouté de l'ionomycine (10 nM - $3 \mu\text{M}$) à la solution Krebs normale, de façon cumulée en l'absence et en présence d'halothane (1,5 %, 3 %). Dans l'autre série, du CaCl_2 ($0,3$ - 3 mM) a été ajouté à la solution Krebs libre de Ca^{2+} incluant une forte concentration de KCl (50 mM), de phényléphrine (100 nM) ou de prostaglandine F_2 (PGF_2 , 1 - $3 \mu\text{M}$) en l'absence et en présence d'halothane (1,5 %, 3 %). La portion linéaire de la relation $[\text{Ca}^{2+}]_i$ -tension a été analysée par une régression linéaire.

Résultats : L'halothane, 1,5 %, n'a pas eu d'effet sur la relation $[\text{Ca}^{2+}]_i$ -tension isométrique normale obtenue avec l'ionophore calcique (ionomycine 10 nM - $3 \mu\text{M}$), mais l'halothane 3 % a fait baisser la pente de la relation ($0,239 \pm 0,037$ pour les témoins et $0,110 \pm 0,010$ pour l'halothane 3 %, $P < 0,05$). L'halothane, 1,5 % et 3 %, n'a pas changé la relation $[\text{Ca}^{2+}]_i$ -tension obtenue avec le CaCl_2 ($0,3$ - 3 mM) en présence d'une forte concentration de KCl (50 mM) ou de phényléphrine (100 nM). Par ailleurs, l'halothane 3 % modifie à la baisse l'ordonnée à l'origine de la relation $[\text{Ca}^{2+}]_i$ -tension obtenue avec le CaCl_2 ($0,3$ - 3 mM) en présence de prostaglandine F_2 (PGF_2 , 1 - $3 \mu\text{M}$) ($45,708 \pm 4,233$ pour les témoins et $26,997 \pm 2,522$ pour l'halothane 3 %, $P < 0,01$).

Conclusion : L'halothane diminue la sensibilité du Ca^{2+} et ce, en présence de la PGF_2 .

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HALOTHANE is a potent direct vasodilator^{1,2} and, combined with a cardiac suppressive effect, influences organ blood flow. In some organs such as the brain, liver and uterus, blood flow is well maintained during halothane anesthesia because decrease in the regional vascular resistance compensates for the reduced cardiac output.^{3,4} Vasodilatation in the brain also causes an increase in intracranial pressure. In the lung, halothane inhibits hypoxic vasoconstriction induced by hypoventilation or atelectasis and may increase intrapulmonary shunt.⁵ Thus, the vasodilating effect of halothane has important clinical implications. However, the mechanisms by which halothane induces direct vasodilatation is not fully understood.

Contraction of vascular smooth muscle is regulated by the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and the sensitivity of contractile elements to $[\text{Ca}^{2+}]_i$ (Ca^{2+} sensitivity). Ca^{2+} sensitivity can be expressed as relationship between $[\text{Ca}^{2+}]_i$ and tension. Recently, a Ca^{2+} -sensitive fluorescent indicator technique has made it possible to observe changes in $[\text{Ca}^{2+}]_i$ and tension of intact vascular smooth muscle simultaneously. This technique has shown that vasoconstrictive receptor agonists such as phenylephrine⁶ and prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$)⁷ increase Ca^{2+} sensitivity. To determine the normal Ca^{2+} sensitivity in the absence of receptor agonists, the Ca^{2+} ionophore, ionomycin or high KCl-induced depolarization has been widely used because they introduce Ca^{2+} into the cytoplasm without acting on receptors. The Ca^{2+} sensitivity obtained with ionomycin is consistent with that obtained with the skinned fibre technique.⁸ However, some studies have demonstrated that high KCl produces stronger contraction than ionomycin in a range of $[\text{Ca}^{2+}]_i$.⁸ This suggests that high KCl-induced depolarization may also increase Ca^{2+} sensitivity. Therefore, ionomycin may be more suitable to determine the normal Ca^{2+} sensitivity than high KCl.

Halothane decreases $[\text{Ca}^{2+}]_i$ by reducing Ca^{2+} influx through Ca^{2+} channels across the sarcolemma.⁹ This suggests that the decrease in $[\text{Ca}^{2+}]_i$ may play a role in halothane-induced vasodilatation. Moreover, some studies using skinned fibre technique have shown that halothane may decrease Ca^{2+} sensitivity. In the rabbit aortic smooth muscle, halothane 3% does not change submaximum Ca^{2+} -induced contraction but slightly decreases maximum Ca^{2+} -induced contraction.¹⁰ In the rat mesenteric artery, halothane, 2% and 4%, decreases submaximum and maximum Ca^{2+} -induced contractions.¹¹ These reports suggest that halothane may induce vasodilatation not only by the decrease in $[\text{Ca}^{2+}]_i$ but also by the decrease in Ca^{2+}

sensitivity. However, the $[\text{Ca}^{2+}]_i$ -tension relationships in the absence and presence of halothane have not been compared directly. The first aim of this study is to determine whether halothane changes the normal $[\text{Ca}^{2+}]_i$ -tension relationship. The second aim is to determine whether halothane changes the Ca^{2+} sensitivity increased by high KCl, phenylephrine or $\text{PGF}_{2\alpha}$.

Methods

Tissue preparation and Fura PE3 loading

The protocol was approved by the Kyoto University Animal Use Committee. Descending thoracic aortas were isolated from Wistar rats (250–300 g) anesthetized with 50 $\text{mg}\cdot\text{kg}^{-1}$ sodium pentobarbital. The aortas were then cut into rings 2.5 mm wide, denuded of endothelia, and incubated with Fura PE3-AM (10 μM , Teflabs, Austin, TX, USA) for 6 h at 25°C. Fura PE3-AM was dissolved in Cremophor EL (0.02%, Sigma Chemical Co., St Louis, MO, USA).

Measurement of isometric tension

Each aortic ring was mounted horizontally in an organ bath containing Krebs' solution (7 ml at 37°C) and connected to a strain gauge transducer (T7-240, Toyo Baldwin, Japan). The solution was gassed with an O_2 95%/CO₂ 5% gas mixture (pH 7.4). The aortic rings were equilibrated at the optimal resting tension of 1.5 g for 30 min, while the fluorescent indicator that had not been taken up into the cells was rinsed out. Following equilibration, each aortic ring was contracted with phenylephrine ($\text{EC}_{\text{max}}=1$ μM , Sigma) or $\text{PGF}_{2\alpha}$ ($\text{EC}_{\text{max}}=10$ μM , Ono Pharmaceutical Co., Tokyo, Japan). The absence of endothelium was confirmed by measuring the response to acetylcholine (10 μM) in the aortic rings submaximally precontracted with phenylephrine ($\text{EC}_{70}=100$ nM) or $\text{PGF}_{2\alpha}$ ($\text{EC}_{70}=1$ –3 μM).

Measurement of $[\text{Ca}^{2+}]_i$

The aortic rings were illuminated with ultraviolet light at 340 and 380 nm and the fluorescence elicited at 500 nm was monitored (as F_{340} and F_{380} , respectively) continuously using a dual-wavelength fluorometer (CAF110, Japan Spectroscopic, Tokyo, Japan).^{12,13} Since the dissociation constant of a fluorescent dye for Ca^{2+} in cytoplasm is controversial, the ratio of F_{340} to F_{380} (F_{340}/F_{380} ratio) was used as an index of the $[\text{Ca}^{2+}]_i$.^{12,14–16} The resting level of tension and F_{340}/F_{380} ratio was regarded as 0% and the increase in tension and F_{340}/F_{380} ratio induced by 30 mM KCl was used as a reference value (100%). Any contraction or increase in F_{340}/F_{380} ratio was expressed as a relative value (%) to the reference value to normalize.^{12,14–16} The F_{340}/F_{380} ratio and tension were measured simultaneously.

Delivery of halothane

Halothane (Takeda Pharmaceutical Co, Osaka, Japan) was delivered with the O₂/CO₂ gas mixture through a vaporizer (Fluotec 3, Cyprane Keighley, England). The concentration of halothane in the gaseous phase was monitored and adjusted with an anesthetic gas monitor (Atom 303, Atom, Tokyo, Japan). The concentration of halothane in Krebs' solution was assayed by gas chromatography.¹²

Experimental protocol

In one series of experiments, ionomycin (10 nM-3 µM, Sigma) was added to normal Krebs' solution cumulatively to obtain the normal [Ca²⁺]_i-tension relationship. Halothane (1.5% or 3%) was added to some aortic rings eight minutes before the cumulative addition of ionomycin.

In the other series of experiments, the aortic rings were treated with Ca²⁺-free Krebs' solution including the Ca²⁺ chelating agent, ethylene glycol bis (b-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA, 2mM, Nacalai Tesque Co., Kyoto, Japan) for five minutes. Subsequently, the bathing solution was replaced by Ca²⁺-free Krebs' solution with EGTA (0.3 mM). Thus, extracellular Ca²⁺ was removed. Two minutes after the replacement, KCl (EC_{max}=50 mM), phenylephrine (EC₇₀=100 nM) or PGF_{2α} (EC₇₀=1-3 µM) was applied. Eight minutes later, CaCl₂ (0.3-3 mM) was given cumulatively to determine the [Ca²⁺]_i-tension relationship in the presence of the vasoconstrictors.⁸ Halothane (1.5% or 3%) was added to some aortic rings eight minutes before the cumulative addition of CaCl₂. At the end of each experiment, the aortic rings were washed out with normal Krebs' solution to con-

firm that the resting level of F₃₄₀/F₃₈₀ ratio and tension was stable. Because more than 3 mM CaCl₂ affected the values of F₃₄₀ and F₃₈₀, up to 3 mM CaCl₂ was used in this study.

Since the Ca²⁺ sensitivity changes time-dependently,¹⁷ F₃₄₀/F₃₈₀ ratio and tension were sampled at five minutes after the cumulative addition of ionomycin or CaCl₂. In our preliminary experiment, the peak level of F₃₄₀/F₃₈₀ ratio had been achieved within five minutes.

Solutions

Krebs' solution comprised (mM) NaCl 127.5, NaHCO₃ 15.5, glucose 10, KCl 4.6, CaCl₂ 2.5, KH₂PO₄ 1.2 and MgSO₄ 1.2. Ca²⁺-free solution was made by exclusion of CaCl₂ from Krebs' solution. High K⁺ solution was made by substituting NaCl with equivalent KCl.

Statistical analysis

The data were expressed as means ± SEM. The F₃₄₀/F₃₈₀ ratio-tension relationships were compared statistically by a linear regression analysis. To fit data to a linear regression, the tail part of data where no contraction was observed was excluded. The slope and intercept values of linear regression were analyzed by one-way analysis of variance and Student's Neuman-Keuls test to evaluate differences between groups. Differences at *P* < 0.05 were considered significant statistically.

Results

Concentration of halothane in the bathing solution

The concentration of halothane used in this study (1.5% and 3% in the gaseous phase) equated to 0.37 ±

TABLE The effect of halothane on the regression line of F₃₄₀/F₃₈₀-tension relationship in various conditions

Condition			Slope	Intercept
Ionomycin	Control		0.239 ± 0.037	-1.955 ± 8.034
	Halothane	(1.5%)	0.156 ± 0.028	7.391 ± 5.049
	Halothane	(3%)	0.110 ± 0.010*	-3.463 ± 2.343
High KCl	Control		1.142 ± 0.130	10.623 ± 8.234
	Halothane	(1.5%)	0.857 ± 0.161	31.98 ± 7.820
	Halothane	(3%)	0.877 ± 0.110	11.340 ± 5.475
Phenylephrine	Control		0.735 ± 0.125	44.024 ± 4.593
	Halothane	(1.5%)	0.648 ± 0.145	43.363 ± 4.593
	Halothane	(3%)	0.884 ± 0.168	32.572 ± 4.078
PGF _{2α}	Control		0.902 ± 0.140	45.708 ± 4.233
	Halothane	(1.5%)	0.601 ± 0.110	40.715 ± 4.247
	Halothane	(3%)	0.532 ± 0.078	26.997 ± 2.522†

Regression lines were calculated from data on ionomycin (100 nM-3 µM), CaCl₂ (0.3-3 mM) in the presence of high KCl or phenylephrine and CaCl₂ (0.6-3 mM) in the presence of PGF_{2α}.

Values are mean ± SEM. n=20-70.

*P < 0.05 and †*P* < 0.01 for control.

FIGURE 1 Typical recordings of the changes in F_{340}/F_{380} ratio and tension obtained with the cumulative addition of ionomycin (10 nM-3 μ M, every five minutes, $\uparrow$) to normal Krebs' solution (control, A) and the effect of halothane (3%) on the changes in F_{340}/F_{380} ratio and tension (B).

0.01 mM and 0.79 ± 0.01 mM ($n=3$, each), respectively, in Krebs' solution. These concentrations in Krebs' solution are approximately 80% of the theoretical concentrations determined by the Krebs' solution-air partition coefficient at 37°C.¹⁸ Therefore, the concentrations of halothane used in this study were equivalent to 1 and 2 minimum alveolar concentration (MAC) in rats and clinically relevant.¹⁹

Effect of halothane on F_{340}/F_{380} ratio-tension relationship obtained with ionomycin

Figure 1 shows typical recordings of the changes in F_{340}/F_{380} ratio and tension produced by the cumulative addition of ionomycin (10 nM-3 μ M) in the presence and absence of halothane. The F_{340}/F_{380} ratio-tension relationship obtained with ionomycin in the absence and presence of halothane are summarized as Figure 2. Although halothane (1.5%) did not change the F_{340}/F_{380} ratio-tension relationship,

FIGURE 2 The normal F_{340}/F_{380} ratio-tension relationship obtained with the cumulative addition of ionomycin (10 nM-3 μ M) to normal Krebs' solution (control) and the effect of halothane (1.5% and 3%) on the relationship. Values are mean $\pm$ SEM, $n=7-9$.

halothane, 3%, decreased the slope of the relationship ($P < 0.05$, Table).

Effect of halothane on F_{340}/F_{380} ratio-tension relationship in the presence of high KCl, phenylephrine or $\text{PGF}_{2\alpha}$

Figure 3 shows typical recordings of the changes in F_{340}/F_{380} ratio and tension produced by the cumulative addition of CaCl_2 (0.3-3 mM) in the presence of high KCl (50 mM) and the effect of halothane. The F_{340}/F_{380} ratio-tension relationship in the presence of high KCl, phenylephrine or $\text{PGF}_{2\alpha}$ obtained with CaCl_2 and the effect of halothane on the relationship are summarized in Figures 4,6.

The Ca^{2+} sensitivity in the absence of halothane was, in order, phenylephrine = $\text{PGF}_{2\alpha}$ > high KCl > ionomycin ($P < 0.05$, Figures 2,4-6, Table). There was no difference in the maximum values of tension between ionomycin, high KCl, phenylephrine and $\text{PGF}_{2\alpha}$ (Figures 2, 4-6).

To assess the effect of halothane on the Ca^{2+} sensitivity in the presence of high KCl, phenylephrine or $\text{PGF}_{2\alpha}$ statistically, the data obtained with CaCl_2 (0.3-

FIGURE 3 Typical recordings of the changes in F_{340}/F_{380} ratio and tension obtained with the cumulative addition of CaCl_2 (0.3-3 mM, every 5 min, $\uparrow$) to Ca^{2+} -free Krebs' solution in the presence of 50 mM KCl (control, A) and the effect of halothane (1.5%) on the changes in F_{340}/F_{380} ratio and tension (B).

3 mM) in the presence of high KCl or phenylephrine and the data obtained with CaCl_2 (0.6-3 mM) in the presence of $\text{PGF}_{2\alpha}$ were also fitted to linear regression and analyzed (Table). Halothane (1.5% and 3%) did not change the F_{340}/F_{380} ratio-tension relationship in the presence of high KCl (Figure 4, Table). Neither did halothane (1.5% and 3%) change the F_{340}/F_{380} ratio-tension relationship in the presence of phenylephrine (Figure 5, Table). In contrast, although halothane (1.5%) did not affect the F_{340}/F_{380} ratio-tension relationship in the presence of $\text{PGF}_{2\alpha}$, halothane (3%) showed the inhibitory effect on the relationship ($P < 0.01$, Figure 6, Table).

FIGURE 4 The F_{340}/F_{380} ratio-tension relationship obtained with the cumulative addition of CaCl_2 (0.3-3 mM) to Ca^{2+} -free Krebs' solution in the presence of 50 mM KCl (control) and the effect of halothane (1.5% and 3%) on the relationship. Values are mean $\pm$ SEM, $n=6$.

Discussion

The present study has shown that halothane (3%) strongly suppresses the normal $[\text{Ca}^{2+}]_i$ -tension relationship obtained with ionomycin. Our study has also demonstrated the inhibitory effect of halothane (3%) on the $[\text{Ca}^{2+}]_i$ -tension relationship in the presence of $\text{PGF}_{2\alpha}$ in contrast to the results of high KCl and phenylephrine. These results suggest that the inhibitory effect on the Ca^{2+} sensitivity may be an important mechanism of halothane-induced vasodilatation.

The fluorescent Ca^{2+} indicator used in this study, Fura PE3, is a derivative of the well-known fluorescent Ca^{2+} indicator, Fura 2. We used Fura PE3 because Fura PE3 leaks out of cells less than Fura 2 and can therefore be used for prolonged observations of $[\text{Ca}^{2+}]_i$.

The $[\text{Ca}^{2+}]_i$ -tension relationship is usually a nonlinear curve. A conventional approach to analyze the $[\text{Ca}^{2+}]_i$ -tension relationship is the logistic model. To use the logistic regression model, the ceiling values of tension (maximum contraction) is essential. However, we could not confirm whether contraction induced by the last concentration of CaCl_2 used in this study was

FIGURE 5 The F_{340}/F_{380} ratio-tension relationship obtained with the cumulative addition of CaCl_2 (0.3-3 mM, every five minutes) to Ca^{2+} -free Krebs' solution in the presence of phenylephrine (100 nM, control) and the effect of halothane (1.5% and 3%) on the relationship. Values are mean $\pm$ SEM, $n=5-7$.

the maximum contraction because higher concentration of CaCl_2 affected the measurement of F_{340}/F_{380} ratio (see method). Another approach is a linear regression model, because the slope of a logistic curve is almost linear. Therefore, we excluded the tail part of the data where no contraction was developed and analyzed the slope part by using linear regression. The ordinate intercept is a statistical index to compare linear regression, but it must be interpreted with care. If the intercept is a positive value but data are distributed to the right of the axis of ordinate, we cannot say that contraction can be produced when $[\text{Ca}^{2+}]_i$ is lower than the resting level. However, in the case of linear regression in the presence of phenylephrine or $\text{PGF}_{2\alpha}$, the intercept had a positive value and some data were distributed to the left of the ordinate axis. This result suggests that phenylephrine or $\text{PGF}_{2\alpha}$ may produce contraction when $[\text{Ca}^{2+}]_i$ is lower than the resting level. This is consistent with some previous reports^{7,16} and supports the hypothesis that phenylephrine and $\text{PGF}_{2\alpha}$ increase Ca^{2+} sensitivity.

FIGURE 6 The F_{340}/F_{380} ratio-tension relationship obtained with the cumulative addition of CaCl_2 (0.3-3 mM) to Ca^{2+} -free Krebs' solution in the presence of $\text{PGF}_{2\alpha}$ (1-3 μM , control) and the effect of halothane (1.5% and 3%) on the relationship. Values are mean $\pm$ SEM, $n=6$.

Our study has shown that halothane (3%) reduces the slope of normal $[\text{Ca}^{2+}]_i$ -tension relationship obtained with ionomycin. This suggests that halothane (3%) can decrease the normal Ca^{2+} sensitivity. In contrast to the result of ionomycin, we could not find an inhibitory effect of halothane (3%) on Ca^{2+} sensitivity in the presence of high KCl. Our study suggested that the Ca^{2+} sensitivity in the presence of high KCl may be higher than the normal Ca^{2+} sensitivity obtained with ionomycin. This result supports the previous reports that high KCl-induced depolarization may increase the Ca^{2+} sensitivity.⁸ Therefore, the effect of depolarization may mask that of halothane. However, because our experimental protocols for ionomycin and high KCl were different, further study will be needed to understand the different effects of halothane.

Our results showed that the Ca^{2+} sensitivity in the presence of phenylephrine or $\text{PGF}_{2\alpha}$ is higher than that in the presence of high KCl. This is consistent with the

previous reports that the vasoconstrictive receptor agonists increase the Ca^{2+} sensitivity.^{6,7} Interestingly, statistical analysis of the $[\text{Ca}^{2+}]_i$ -tension relationships demonstrated that halothane (3%) decreases the Ca^{2+} sensitivity in the presence of $\text{PGF}_{2\alpha}$ whilst halothane (3%) does not change Ca^{2+} sensitivity in the presence of phenylephrine. This suggests that the effect of halothane on Ca^{2+} sensitivity may depend on the stimulated receptor type. Phenylephrine and $\text{PGF}_{2\alpha}$ bind to α_1 and FP receptors, respectively. Stimulation of α_1 and FP receptors induces IP_3 generation and increase in $[\text{Ca}^{2+}]_i$. Moreover, the stimulation of α_1 receptor activates protein kinase C and increases the Ca^{2+} sensitivity.^{20,21} However, it is still unclear how stimulation of FP receptor increases Ca^{2+} sensitivity. A recent study suggests that the α_1 and FP receptors may have different signal transduction pathways to increase the Ca^{2+} sensitivity. The study has shown that protein kinase C may not be involved in FP receptor stimulation-induced increase in the Ca^{2+} sensitivity.¹⁶ This may explain the different effects of halothane. Another possible explanation is that halothane may affect the binding affinity of $\text{PGF}_{2\alpha}$ for the FP receptor but not affect the affinity of phenylephrine for the α_1 receptor. We have demonstrated that in human platelets, a high concentration of halothane strongly suppresses the binding affinity of thromboxane A_2 for its receptor.²² However, the effect of halothane on the binding affinity of $\text{PGF}_{2\alpha}$ for the FP receptor in the vascular smooth muscle has not been determined.

It is known that halothane inhibits $\text{PGF}_{2\alpha}$ -induced contraction more than that produced by phenylephrine.²³ Our results may contribute to explaining this and have clinical relevance. Production of $\text{PGF}_{2\alpha}$ increases in intrauterine tissues during parturition and an increase in $\text{PGF}_{2\alpha}$ occurs in maternal plasma.²⁴ $\text{PGF}_{2\alpha}$ induces not only myometrial contraction but also contraction of the uterine artery.²⁵ Therefore, our results may help to explain why obstetric anesthesia with halothane depresses the myometrial contraction and reduces the vascular resistance in the uterus.

To circumvent the problem with time-dependent change in the Ca^{2+} sensitivity, our data were sampled at a constant time (five minutes) after the cumulative addition of ionomycin or CaCl_2 . Therefore, in some experiments, tension was still developing slowly although the peak level of F_{340}/F_{380} ratio had been achieved. This can cause underestimate of the Ca^{2+} sensitivity in the absence of halothane and make it difficult to detect a subtle change in the Ca^{2+} sensitivity in the presence of halothane. This may be one of the reasons why we could not find the significant decrease in the Ca^{2+} sensitivity in the presence of halothane in some series of experiment.

In conclusion, our study has suggested that the inhibitory effect of halothane on the Ca^{2+} sensitivity may contribute to the relaxation of rat aortic smooth muscle contracted with ionomycin or $\text{PGF}_{2\alpha}$.

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