

Anesthesiology
 1998; 89:1209-17
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Effects of Halothane and Isoflurane on β -adrenoceptor-mediated Responses in the Vascular Smooth Muscle of Rat Aorta

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Background: Although previous studies have proposed that anesthetics may influence signal transduction systems, their effects on the β -adrenoceptor-mediated system have not been fully characterized in vascular smooth muscle. The aim of this study was to determine how halothane and isoflurane affect β -adrenoceptor-mediated vasodilation in rat aorta and what mechanisms were involved.

Methods: Isometric tension and the intracellular calcium ion concentration ($[Ca^{2+}]_i$) were measured concomitantly in rat aortic strips from which the endothelium was removed. Strips precontracted with norepinephrine were dilated with the β -adrenoceptor agonist, isoproterenol; the adenylyl cyclase activator, forskolin; or the membrane-permeable dibutyryl cyclic adenosine monophosphate (cAMP) with or without halothane or isoflurane. The effects of the anesthetics on each vasodilator were compared with the control responses. β -adrenoceptor binding characteristics and affinity for agonists were determined with [^{125}I]-iodocyanopindolol with and without halothane or isoflurane. Furthermore, concentrations of cAMP induced by either isoproterenol or forskolin were measured with or without the anesthetics using an enzyme immunoassay procedure.

Results: Halothane and isoflurane attenuated vasodilation and $[Ca^{2+}]_i$ decreases induced by isoproterenol, whereas both anesthetics only slightly affected vasodilation and $[Ca^{2+}]_i$ decreases induced by forskolin and dibutyryl cAMP. Halothane and isoflurane had no effect on β -adrenoceptor binding characteristics and affinity for agonists. Three percent halothane or 4% isoflurane significantly reduced cAMP levels induced by isoproterenol but not by forskolin.

Conclusions: Halothane and isoflurane, at clinically relevant concentrations, can interfere with β -adrenoceptor-mediated

responses in the rat aorta at the steps after the agonist-receptor binding but before the adenylyl cyclase activation. (Key words: Catalyst; G protein; ion channel.)

SEVERAL steps in the signal transduction pathway have been reported for relaxation of vascular smooth muscles by β -adrenoceptor agonists. Once the agonist interacts with the β -adrenoceptor, stimulatory G proteins activate adenylyl cyclase, which increases the intracellular concentration of cyclic adenosine monophosphate (cAMP).¹ The increased concentration of cAMP activates a specific protein kinase, protein kinase A, and inhibits calcium ion (Ca^{2+}) influx mainly by acting on inositol 1,4,5-triphosphate or Ca^{2+} -activated potassium channel, resulting in a decrease in cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) and vasorelaxation.² In addition to this mechanism, β agonists induce vasorelaxation through an endothelium-dependent pathway.³

Volatile anesthetics are likely to influence several hydrophobic sites within the cell membrane, particularly membrane-binding proteins such as receptors, G proteins, catalysts, and ion channels.⁴ Recent evidence favors specific sites of action at the level of protein-protein^{5,6} or lipid-protein interactions^{7,8} at clinically relevant concentrations. However, the effects of volatile anesthetics on agonist-receptor bindings, G proteins, catalysts, and ion channels vary. For example, halothane has been shown to inhibit stimulatory G proteins without affecting inhibitory stimulatory G proteins in mice hearts.⁹ Conversely, halothane inhibited inhibitory G proteins without any effect on stimulatory G proteins in rat hearts¹⁰ and failing human hearts.¹¹ Furthermore, halothane antagonized events in the signaling cascade sequence after activation of adenylyl cyclase in rat hearts¹² and adipocytes.¹³

Although volatile anesthetics are suggested to have diverse effects on the signal transduction system of the vascular contraction¹⁴ and relaxation,¹⁵ little information is available about how they affect vasodilation mediated by the β -adrenergic receptor-G protein-effector

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Received from the Department of Anesthesiology, Sapporo Medical University School of Medicine, Sapporo, Japan. Submitted for publication March 26, 1998. Accepted for publication July 7, 1998. Supported by grant-in-aid for scientific research (08671760) from the Ministry of Education, Science and Culture, Japan. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, Atlanta, October 21-25, 1995.

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pathway.¹⁶ In the current study, we investigated the effects of halothane and isoflurane, at clinically relevant concentrations, on the β -adrenoceptor-mediated responses in the vascular smooth muscle of the rat aorta, and we characterized the underlying mechanism of action. We chose rat aorta because it has plenty of α - and β -adrenoceptors and allows us to measure $[Ca^{2+}]_i$ and muscle tension concomitantly.

Materials and Methods

This study was approved by the Committee on Animal Research of the Sapporo Medical University. All experiments were conducted on male Sprague-Dawley rats (average weight, 280 g) that were 7 or 8 weeks old.

Preparation of Rat Aortic Strips

Rats were anesthetized with isoflurane, and the descending thoracic aorta was isolated and placed into a normal physiologic saline solution composed of 136.9 mM NaCl, 5.4 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 23.8 mM $NaHCO_3$, 5.5 mM glucose, and 0.01 mM EDTA and bubbled with 95% oxygen plus 5% carbon dioxide at 37°C, pH 7.4. Fat and connective tissue were removed carefully, and vessels were cut into spiral strips that were about 2 mm wide and 7 mm long. The endothelium was removed by gently rubbing the intimal surface with a moistened cotton swab. The absence of the endothelium was verified by lack of relaxation with acetylcholine (10^{-6} M).

Simultaneous Measurement of Muscle Tension and $[Ca^{2+}]_i$

The $[Ca^{2+}]_i$ was measured as described previously.¹⁷ Briefly, muscle strips were loaded with 10^{-5} M acetoxymethyl ester of fura-2 (fura-2/AM) solution for 3–5 h at room temperature. A noncytotoxic detergent, 0.05% Cremophor EL, was added to solubilize the fura-2/AM in the solution. After the fura-2 loading, muscle strips were washed with physiologic saline solution and bubbled with 95% oxygen plus 5% carbon dioxide at 37°C, pH 7.4. The $[Ca^{2+}]_i$ was measured by a fura-2- Ca^{2+} method using a fluorimeter (CAF-100; Japan Spectroscopic, Tokyo, Japan). The muscle strip was held horizontally in a temperature-controlled organ bath with a 7-ml volume. The solution was aerated with 95% oxygen plus 5% carbon dioxide. One end of the muscle strip was connected to a strain gauge transducer (TB-612T; Nihon Kohden, Tokyo, Japan) to monitor the mechanical activity. The muscle strips were stretched at 5-min intervals in

increments of 0.1 g to achieve optimal resting tension. Optimal resting tension was defined as the minimum amount of stretch required to achieve the largest contractile response to 40 mM KCl and determined in our pilot experiments to be 0.7 g for the muscle strips used in these studies. The optimal resting tension was applied and allowed to equilibrate before the experiments were started. The muscle strip was illuminated alternately at 50 Hz at excitation wavelengths of 340 ± 10 and 380 ± 10 nm, and the amount of 500 ± 20 nm fluorescence induced by 340 nm excitation (F_{340}) and that induced by 380 nm excitation (F_{380}) was measured. The ratio of F_{340} to F_{380} ($R_{340:380}$) was used as an indicator of $[Ca^{2+}]_i$, and the quantitative comparison of $[Ca^{2+}]_i$ was made by taking resting and norepinephrine-stimulated $[Ca^{2+}]_i$ as 0% and 100%, respectively.

After the equilibrating period, 0% (control), 1%, 2%, and 3% halothane or 2% and 4% isoflurane, delivered by a vaporizer, was introduced into the oxygen-carbon dioxide mixture ($n = 6$ each). From our pilot experiments, 10^{-7} M norepinephrine elicited approximately 80% of the maximal norepinephrine-induced contraction both with and without halothane or isoflurane, although the maximum responses were decreased with the anesthetics. Ten minutes after bubbling of one concentration of either anesthetic the muscle strip was contracted with 10^{-7} M norepinephrine, and the changes in muscle tension and $R_{340:380}$ were regarded as the reference point (100%). Five minutes after norepinephrine administration, one of the following drugs was cumulatively administered to the organ bath to induce vasodilation: isoproterenol ($10^{-8.5}$ M to 10^{-7} M), a β -adrenoceptor agonist; forskolin ($10^{-7.5}$ M to 10^{-6} M), an adenylyl cyclase activator; or dibutyryl cAMP (DBcAMP; $10^{-4.5}$ M to 10^{-3} M), a membrane-permeable cAMP.

The concentration of halothane or isoflurane in the gas mixture was monitored continuously by a precalibrated anesthetic agent monitor (model 303; Atom, Tokyo, Japan). The anesthetic concentrations in the bath fluid were measured by gas chromatography with flame ionization detection (GC-17A; Shimadzu, Kyoto, Japan). Gas chromatographic measurements of halothane and isoflurane in the bath fluid were 0.30 ± 0.01 mM, 0.56 ± 0.03 mM, and 0.90 ± 0.03 mM for 1%, 2%, and 3% halothane, and 0.48 ± 0.01 mM and 0.90 ± 0.08 mM for 2% and 4% isoflurane ($n = 6$ each).

Radioligand-binding Receptor Studies

The rats were anesthetized with isoflurane, and the descending thoracic aorta was removed and cleaned of

fat and connective tissue. The plasma membrane preparation, a modification of the method of Shaul *et al.*,¹⁸ was performed at 4°C. Tissue was pooled from three rats for each assay. After harvesting, the aortas were placed in ice-cold 0.25 M sucrose buffer (10 times volume) with 10 mM Tris-HCl (pH 7.4), and the adventitia was dissected off. The sucrose buffer was changed, and the tissue was minced with scissors and homogenized twice with a Polytron homogenizer (Kinematica, Lucerne, Switzerland). The homogenate was centrifuged at 4°C at 1,500g for 10 min, and the resulting supernatant was filtered through two layers of gauze and then centrifuged at 4°C at 100,000g for 30 min. The pellet formed was resuspended in 3 ml of 5 mM HEPES buffer (sodium salt) with 1.0 mM MgSO₄, pH 7.4, yielding a protein concentration of 50–100 μ g/ml measured by the method described by Lowry *et al.*¹⁹

Binding assays were done in 5-ml glass vials that were tightly sealed with a cap to maintain the constant anesthetic concentration. Anesthetic was added to the solution in a liquid form, with a Hamilton microliter syringe. Our pilot experiments showed that the addition of 0.5 μ l halothane or 0.8 μ l isoflurane to 0.5 ml of HEPES buffer at 37°C produced approximately 3% halothane or 4% isoflurane and was maintained for at least 120 min. Actual anesthetic concentrations measured by gas chromatography (model GC-17A, Shimadzu) were 0.87 ± 0.09 mM for halothane and 0.89 ± 0.07 mM for isoflurane ($n = 6$ each). These are equivalent to $2.94 \pm 0.31\%$ halothane and $3.96 \pm 0.32\%$ isoflurane.

β -adrenoceptor binding assays were performed in duplicate using [¹²⁵I]-iodocyanopindolol (2,000 Ci/mmol). The total binding incubation volume was 500 μ l HEPES buffer with 50 μ l [¹²⁵I]-iodocyanopindolol dilution at concentrations ranging from 10 to 100 pM and 100 μ l membrane preparation, in the presence or absence of 10^{-5} M *l*-alprenolol to distinguish nonspecific from specific binding. Incubations were maintained at 37°C with constant shaking for 90 min. The reaction was terminated by rapid dilution with 4 ml of 20 mM KH₂PO₄ buffer, pH 7.4, with 1 mM MgSO₄ at 37°C, and separation of bound and free ligand by filtration through Whatman GF/C filters (Whatman International Ltd., Maidstone, UK) under vacuum, followed by three 4-ml rinses of the filter with the same buffer. Filters were counted for 1 min in a gamma counter (CompuGamma; Wallac, Turku, Finland) with a 70% counting efficiency. The effects of halothane and isoflurane were also studied under identical conditions. Each of the [¹²⁵I]-iodocyanopindolol dilutions as standards was counted in each run under

similar conditions. Specific binding was defined as total binding minus nonspecific binding. The density of receptors (B_{\max}) and the dissociation constant for the ligand (K_D) were determined using linear regression and the Scatchard transformation ($n = 6$ each).

Measurement of Cyclic Adenosine Monophosphate Concentration

Aortic strips were incubated with physiologic saline solution containing 10^{-7} M norepinephrine for 5 min, followed by the administration of 10^{-7} M isoproterenol or $10^{-6.5}$ M forskolin, and no addition for control, with and without the exposure of 3% halothane or 4% isoflurane ($n = 6$ each). The preparations were frozen quickly in liquid nitrogen and stored at -80°C until use. Aortic strips were homogenized in 10 volumes of Hank's balanced salt solution (without calcium and magnesium) containing 5 mM EDTA with a homogenizer (PowerHomogenizer S-203; Ikeda Scientific, Tokyo, Japan). The homogenates were centrifuged at 100g for 10 min at 4°C. Cyclic AMP was extracted from the supernatants by the solid-phase extraction method using Amprep SAX columns (Amersham, Buckinghamshire, UK). The concentrations of cAMP were measured by the Biotrak cAMP enzyme immunoassay system (Amersham). The resulting pellets were solubilized and used to measure protein concentrations by the method described by Lowry *et al.*¹⁹

Statistical Analysis

Data were expressed as mean \pm SD. In smooth muscle tissue, the ratio of fluorescence to autofluorescence is much smaller than that in a single isolated cell. Because of the large amount of background fluorescence, the absolute value of $[\text{Ca}^{2+}]_i$ may not be calculated accurately from the $F_{340}:F_{380}$ ratio.²⁰ To measure $[\text{Ca}^{2+}]_i$ and muscle tension, norepinephrine-induced changes in $[\text{Ca}^{2+}]_i$ and muscle tension 5 min after the start of contraction were used as references (100%). Changes in $[\text{Ca}^{2+}]_i$ and muscle tension produced by isoproterenol, forskolin, or DBcAMP were expressed as a percentage value of those caused by norepinephrine-induced contraction. The effects of halothane and isoflurane on the concentration-response curves to isoproterenol, forskolin, or DBcAMP were assessed by calculating the inhibitory concentration causing 50% relaxation of the contraction to norepinephrine (IC_{50}). The IC_{50} value for each muscle strip was obtained from nonlinear regression according to the Hill equation using Prism software (Graphpad, San Diego, CA) and was presented as log

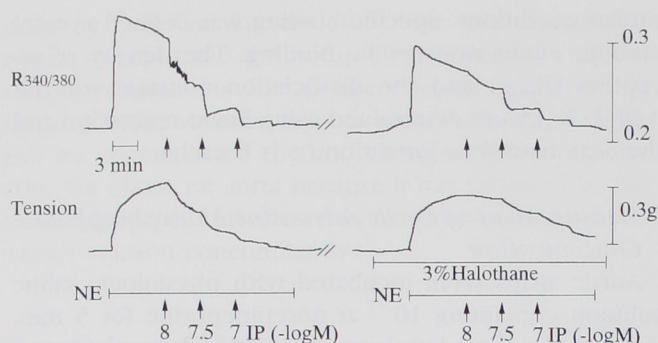


Fig. 1. Original recordings of the $R_{340:380}$ ratio (an indicator of $[Ca^{2+}]_i$) and muscle tension. Norepinephrine evoked sustained increases in muscle tension and $[Ca^{2+}]_i$. Isoproterenol suppressed the increases in muscle tension and $[Ca^{2+}]_i$ in a concentration-dependent manner. Isoproterenol-induced decreases in muscle tension and $[Ca^{2+}]_i$ were blunted in the presence of 3% halothane.

IC_{50} . The mean and SD were calculated from those values. Data were compared using one-way analysis of variance and *post hoc* analysis with Scheffé's F test. The significant value was considered $< 5\%$.

Drugs

The following drugs and chemicals were used: fura-2/AM (Dojindo Laboratories, Kumamoto, Japan), Cremophor EL, norepinephrine, isoproterenol, forskolin, *l*-alprenolol, acetylcholine chloride (Sigma Chemical Co., St. Louis, MO), and DBcAMP (Daiichi, Tokyo, Japan). Forskolin was dissolved in absolute ethanol. The final concentration of ethanol in the bath fluid was $< 0.1\%$, which did not affect either muscle contraction or fura-2- Ca^{2+} signals.

Results

Effects of Halothane and Isoflurane on Isoproterenol-, Forskolin-, and DBcAMP-induced Relaxation

Norepinephrine elicited muscle contraction and the increment of $[Ca^{2+}]_i$. Halothane and isoflurane caused a 10–20% reduction of the contractile response to 10^{-7} M norepinephrine (control, 1%, 2%, 3% halothane; and 2% and 4% isoflurane; 0.29 ± 0.05 g, 0.26 ± 0.04 g, 0.24 ± 0.06 g, 0.24 ± 0.07 g, 0.24 ± 0.06 g, 0.23 ± 0.06 g, respectively). Isoproterenol dose dependently reduced muscle tension and $[Ca^{2+}]_i$ in the muscle strip precontracted with norepinephrine. Figure 1 shows original recordings of fluorescence and force development induced by norepinephrine, followed by dose-dependent

reduction with isoproterenol. These responses were blunted in the presence of 3% halothane.

Figures 2 and 3 show concentration-response curves of halothane and isoflurane against norepinephrine-induced muscle contraction and $[Ca^{2+}]_i$. The effects of halothane and isoflurane on IC_{50} for isoproterenol, forskolin, or DBcAMP are summarized in table 1. Halothane significantly attenuated both muscle relaxation and decreases of $[Ca^{2+}]_i$ induced by isoproterenol in a concentration-dependent manner. Isoflurane also attenuated responses, although the degrees of attenuation were similar between 2% and 4% isoflurane. From the IC_{50} values, 3% halothane was more potent than 4% isoflurane in antagonizing the isoproterenol-induced relaxation, although 3% halothane and 4% isoflurane are approximately equianesthetic concentrations.²¹ In contrast, halothane and isoflurane significantly but slightly augmented the decreases in muscle tension and the changes of $[Ca^{2+}]_i$ induced by forskolin and DBcAMP. These results indicate that halothane and isoflurane can predominantly interfere with both muscle relaxation and decreases of $[Ca^{2+}]_i$ at a step before adenylyl cyclase activation.

Radioligand Binding Studies

β -adrenoceptor binding experiments using [125 I]-iodocyanopindolol in the presence and absence of *l*-alprenolol revealed saturable and high-affinity binding. Scatchard transformation of the data yielded linear plots from which B_{max} and K_d could be determined. Treatment of aortic membranes with 3% halothane or 4% isoflurane had no effect on β -adrenoceptor density and antagonist affinity for iodocyanopindolol (table 2). These results indicate that halothane and isoflurane do not interfere with binding characteristics of the β -adrenoceptors.

Effects of Halothane and Isoflurane on Isoproterenol or Forskolin-induced Cyclic Adenosine Monophosphate Production

The contents of cAMP in the vascular smooth muscle were directly measured to delineate whether the reduced β -adrenoceptor-mediated responses by halothane and isoflurane could be related to reduced cAMP production. Figure 4 shows the effects of 3% halothane and 4% isoflurane on isoproterenol or forskolin-induced cAMP contents after isoproterenol or forskolin treatment. Isoproterenol increased cAMP contents in the smooth muscle, whereas preincubation of the aortic strips with halothane or isoflurane significantly inhibited these increments. In contrast, both halothane and isoflu-

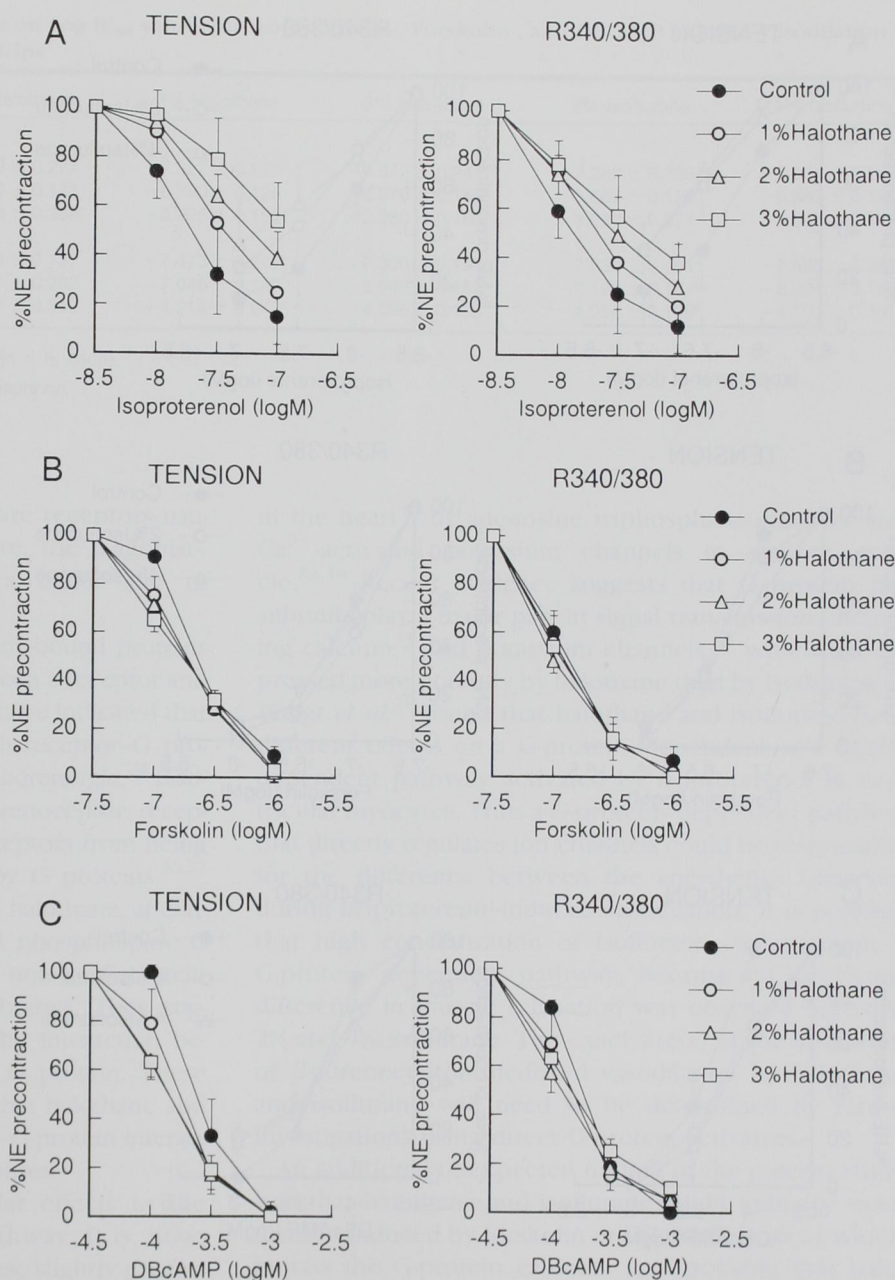


Fig. 2. Effects of halothane (1%, 2%, and 3%) on (A) isoproterenol-, (B) forskolin-, and (C) dibutyryl cyclic adenosine monophosphate (DBcAMP)-induced vasodilation and a decrease of $[Ca^{2+}]_i$ in endothelium-removed strips ($n = 6$ each). Halothane attenuated isoproterenol-induced vasodilation and a decrease of $[Ca^{2+}]_i$ in a dose-dependent manner. In contrast, halothane only slightly enhanced forskolin- and DBcAMP-induced vasodilation and a decrease of $[Ca^{2+}]_i$.

rane did not influence forskolin-induced increases in cAMP contents.

Discussion

This study was designed to determine whether the volatile anesthetics halothane and isoflurane inhibit β -adrenoceptor-mediated responses, and which signal transduction steps are important for the inhibition if they do.

We found that the volatile anesthetics used at clinically relevant concentrations can interfere with responses mediated by the β -adrenoceptor agonist, isoproterenol. Halothane and isoflurane impaired isoproterenol-induced vasodilation and a decline of $[Ca^{2+}]_i$, whereas they only slightly affected forskolin- and DBcAMP-induced vasodilation and a decrease of $[Ca^{2+}]_i$. This finding is consistent with the additional result that both anesthetics inhibited isoproterenol-induced, but not for-

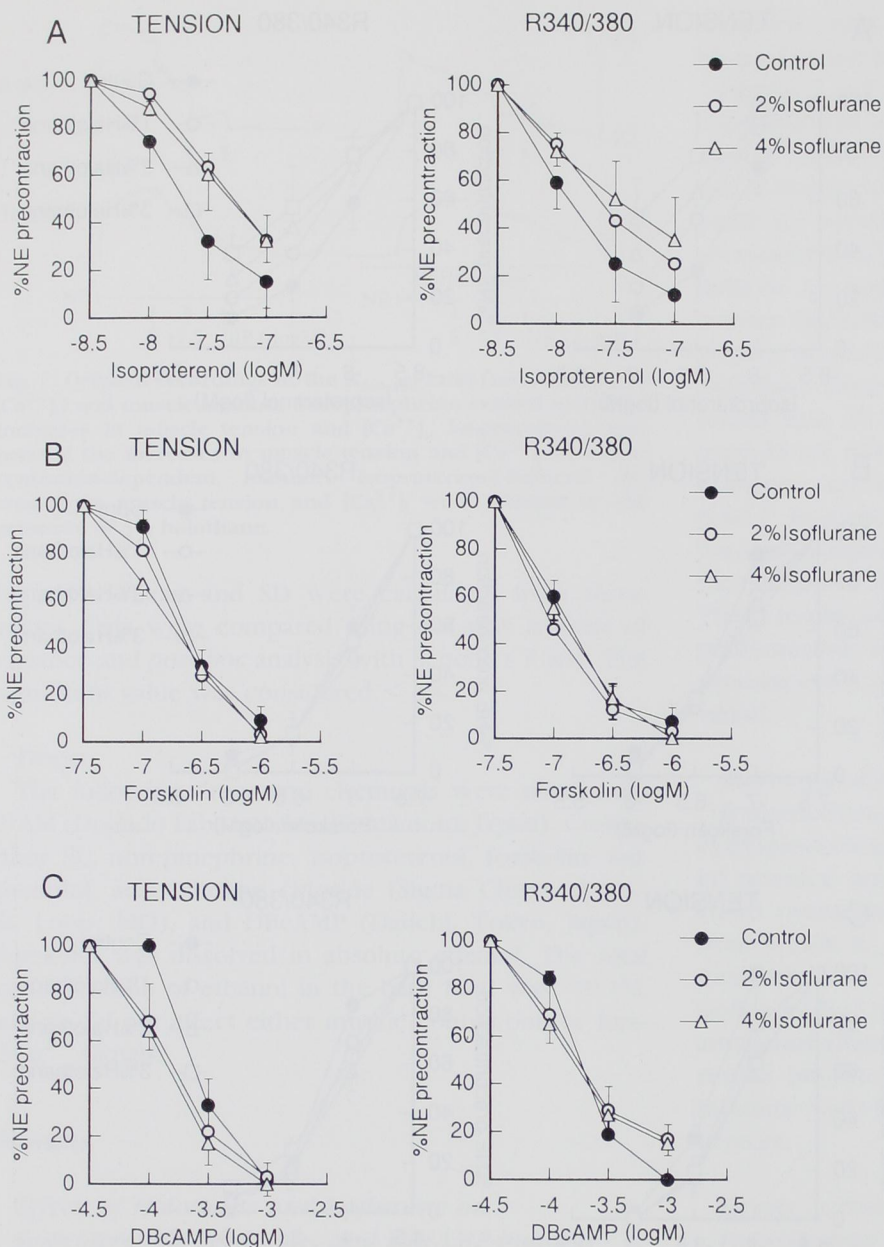


Fig. 3. The effects of isoflurane (2% and 4%) on (A) isoproterenol-, (B) forskolin-, and (C) dibutyryl cyclic adenosine monophosphate (DBcAMP)-induced vasodilation and a decrease of $[Ca^{2+}]_i$ in endothelium-removed strips ($n = 6$, each). Isoflurane attenuated isoproterenol-induced vasodilation and a decrease of $[Ca^{2+}]_i$, although 2% and 4% isoflurane had similar effects on them. In contrast, isoflurane only slightly enhanced forskolin- and DBcAMP-induced vasodilation and a decrease of $[Ca^{2+}]_i$.

skolin-induced, increases in cAMP contents. Furthermore, both agents did not interfere with the ligand-binding property to the β -adrenoceptor. These results indicate that halothane and isoflurane predominantly interfere with β -adrenoceptor-mediated responses at the step between the β -adrenoceptor and adenylyl cyclase; the logical site would therefore include G proteins.

There have been conflicting reports detailing the effects of anesthetics on ligand-receptor binding. Marty *et*

*al.*²² have reported that halothane decreases β -adrenoceptor density by 10% without changing its affinity in lymphocyte membrane. In contrast, halothane had no effect on β -adrenoceptor binding to brain^{23,24} and cardiac tissue.^{25,26} The latter reports are consistent with our result that halothane and isoflurane had no effects on V_{max} and K_d in the ligand-receptor binding study. In addition, even if a 10% reduction in the number of available receptors was elicited by volatile anesthetics, it would result in little attenuation of the β -adrenoceptor-

ANESTHETICS AND β -ADRENOCEPTORS**Table 1. Effect of Halothane and Isoflurane on Log IC₅₀ values for Isoproterenol-, Forskolin-, and DBcAMP-induced Vasodilation and a decline of [Ca²⁺]_i in the Rat Aortic Strips**

	Control	1% Halothane	2% Halothane	3% Halothane	2% Isoflurane	4% Isoflurane
Tension						
Isoproterenol	-7.857 ± 0.306	-7.502 ± 0.274	-7.327 ± 0.225*	-6.872 ± 0.243*†	-7.282 ± 0.239*	-7.331 ± 0.227*
Forskolin	-6.659 ± 0.083	-6.750 ± 0.141	-6.790 ± 0.126	-6.870 ± 0.158*	-6.667 ± 0.129	-6.882 ± 0.190*
DBcAMP	-3.424 ± 0.235	-3.808 ± 0.238*	-3.995 ± 0.193*	-3.990 ± 0.168*	-3.932 ± 0.371	-4.048 ± 0.253
[Ca²⁺]_i						
Isoproterenol	-8.149 ± 0.347	-7.733 ± 0.137	-7.473 ± 0.242*	-7.309 ± 0.198*	-7.630 ± 0.401*	-7.508 ± 0.210*
Forskolin	-6.905 ± 0.102	-7.011 ± 0.269	-7.046 ± 0.132	-6.948 ± 0.182	-7.162 ± 0.161*	-6.924 ± 0.164
DBcAMP	-3.695 ± 0.135	-4.031 ± 0.312	-4.214 ± 0.248*	-4.086 ± 0.133*	-4.054 ± 0.138*	-4.102 ± 0.107*

Values are log IC₅₀ and presented as means ± SD (n = 6, each).

IC₅₀ = 50% relaxation of the contraction to norepinephrine.

* P < 0.05 versus control.

† P < 0.05 versus 4% isoflurane.

mediated responses because many spare receptors usually exist in the membranes. Therefore, the β -agonist-receptor coupling should not be a major site of inhibition by the volatile anesthetics.

The G protein is a family of membrane-bound proteins involved in the signal transduction between a receptor and an intracellular effector. Several studies have indicated that volatile anesthetics could interfere with receptor-G protein interactions in the muscarinic,²⁷ α 2-adrenergic,²⁸ β -adrenergic,²⁶ 5-HT_{1A}, and adenosine adrenoceptor receptors.²⁹ Volatile anesthetics inhibited receptors from being converted into the high-affinity state by G proteins.^{26,27} Furthermore, Rooney *et al.*³⁰ found that halothane, at clinically relevant concentrations, inhibited phospholipase C activity stimulated by β agonists, but not by G-protein activators, in turkey erythrocyte membranes. They suggested that halothane would modify the interaction between the activated receptor and the G protein. These previous reports support our findings that halothane and isoflurane would modulate the receptor-G-protein interactions in β -adrenoceptor-mediated responses.

Halothane and isoflurane had similar effects in the steps of the β -adrenergic signaling pathway. It is, however, possible that halothane could cause slightly greater suppression of isoproterenol-induced vasodilation than isoflurane despite the similar inhibition of cAMP production and [Ca²⁺]_i by both anesthetics. This difference, therefore, might be attributed to the [Ca²⁺]_i-tension relation rather than the cAMP-[Ca²⁺]_i one. The difference could not be explained by the diverse effect of both anesthetics on the intracellular Ca²⁺ storage sites,^{17,31} because decreases in [Ca²⁺]_i were similar between the anesthetics during vasodilation.

G proteins coupled with β -adrenoceptors can act on sodium channels, calcium channels, and adenylyl cyclase

in the heart³² or adenosine triphosphate-sensitive and Ca²⁺-activated potassium channels in smooth muscle.^{33,34} Recent evidence suggests that G protein β - γ subunits play a major part in signal transmission, including calcium³⁵ and potassium channels,³⁶ which are depressed more potently by halothane than by isoflurane.³⁷ Weigt *et al.*³⁸ found that halothane and isoflurane have different effects on a G-protein-dependent and cAMP-dependent pathway activated by isoproterenol in ventricular myocytes. Thus a G-protein-dependent pathway that directly regulates ion channels could be responsible for the difference between the anesthetics observed during isoproterenol-induced vasodilation. It is possible that high concentration of isoflurane can augment a G-protein-dependent pathway, because no significant difference in muscle relaxation was observed between 2% and 4% isoflurane. The exact site(s) of the inhibition of β -adrenoceptor-mediated vasodilation by halothane and isoflurane will need to be determined by future investigations using direct G-protein activators.

An additional unexpected finding of the current study was that halothane and isoflurane might enhance vasodilation induced by forskolin or DBcAMP, both of which bypass the G-protein cascade. It is possible that halothane and isoflurane could also influence the sites after

Table 2. Effect of Halothane and Isoflurane on β -Adrenoceptor Binding Characteristics in the Rat Aortic Membranes

	B _{max} (fmol/mg protein)	K _d (pM)
Control	34.6 ± 4.7	22.1 ± 4.9
3% halothane	35.4 ± 2.8	22.4 ± 3.9
4% isoflurane	36.6 ± 5.3	24.8 ± 3.8

Data are mean ± SD from six experiments. Statistical significance was not evident using ANOVA.

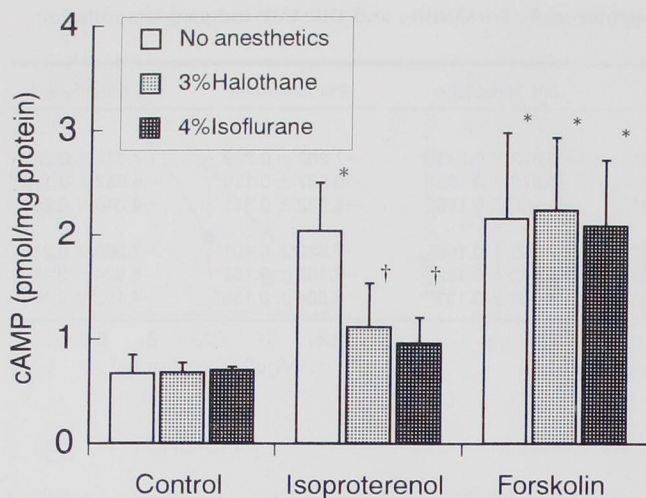


Fig. 4. Effects of 3% halothane (0.9 mM) and 4% isoflurane (0.9 mM) on the smooth muscle content of cyclic adenosine monophosphate (cAMP) induced by 10^{-7} M isoproterenol or $10^{-6.5}$ M forskolin ($n = 6$ each). Isoproterenol and forskolin increased cAMP levels in the smooth muscle. Isoflurane-induced, but not forskolin-induced, increases in cAMP content were attenuated by halothane and isoflurane. *Significant differences from the respective control value. †Significant differences from no anesthetics. $P < 0.05$, and $n = 6$ each.

cAMP elevation. This stimulatory effect may be related to the potential anesthetic dilator properties. Volatile anesthetics are known to depress cardiac function and smooth muscle contractility,^{14,39} mainly because they antagonize the L-type Ca^{2+} channels⁴⁰ and decrease the Ca^{2+} sensitivity in skinned fiber preparations.⁴¹ Therefore, halothane and isoflurane might enhance forskolin- and DBcAMP-mediated vasodilation, probably because of the direct modulation of the Ca^{2+} influx, the sensitivity of the contractile elements by both anesthetics, or both. However, further investigations are needed because this enhancement was small.

Halothane has diverse effects on β -adrenoceptor-G protein-adenylyl cyclase interactions.⁹⁻¹³ These different effects of halothane on the β -receptor-G protein-adenylyl cyclase system may result not only from the differences of the distribution or the amount of G proteins depending on each species or tissues but also from the molecular and functional diversity of G-protein-stimulated adenylyl cyclases.⁴²

In conclusion, we found that halothane and isoflurane, at clinically relevant concentrations, interfere with β -adrenoceptor-mediated responses in rat aortic smooth muscles. Halothane and isoflurane inhibit the β -adrenoceptor-mediated vasodilation but only slightly affect cAMP-mediated vasodilation. Furthermore, both anes-

thetics reduced the isoproterenol-induced, but not forskolin-induced, increases in cAMP. Halothane and isoflurane interfere with β -adrenoceptor-mediated responses at the levels occurring after agonist-receptor binding but before adenylyl cyclase activation.

The authors thank Professor Akiyoshi Namiki, M.D., Ph.D., Chairman, Department of Anesthesiology, Sapporo Medical University School of Medicine, for the direction of this research and review of the manuscript, and Paul A. Murray, Ph.D., Director, Center for Anesthesiology Research, The Cleveland Clinic Foundation, for reviewing the manuscript.

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