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Role of Endothelium in the Action of Isoflurane on Vascular Smooth Muscle of Isolated Mesenteric Resistance Arteries

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Background: **It is believed that isoflurane decreases blood pressure predominantly by decreasing systemic vascular resistance with modest myocardial depression. Nevertheless, little information is available regarding the direct action of isoflurane on systemic resistance arteries.**

Methods: **With use of the isometric force recording method, the action of isoflurane on contractile response to norepinephrine, a neurotransmitter that plays a central role in sympathetic maintenance of vascular tone *in vivo*, was investigated in isolated rat small mesenteric arteries.**

Results: **In the endothelium-intact strips, the norepinephrine response was initially enhanced after application of isoflurane (2–5%), but it was subsequently almost normalized to the control level during exposure to isoflurane. However, the norepinephrine response was notably inhibited after washout of isoflurane. In the endothelium-denuded strips, the norepinephrine response was gradually inhibited during exposure to isoflurane (≥ 3%), and the inhibition was prolonged after washout of isoflurane. The isoflurane-induced enhancement of norepinephrine response was still observed after inhibitions of the nitric oxide, endothelium-derived hyperpolarizing factor, cyclooxygenase and lipoxygenase pathways, or after blockade of endothelin-1, angiotensin-II, and serotonin receptors; however, it was prevented by superoxide dismutase.**

Conclusions: **In isolated mesenteric resistance artery, the action of isoflurane on contractile response to norepinephrine consists of two distinct components: an endothelium-dependent enhancing component and an endothelium-independent inhibitory component. During exposure to isoflurane, the former counteracted the latter, preventing the norepinephrine response from being strongly inhibited. However, only the endothelium-independent component persists after washout of isoflurane, causing prolonged inhibition of the norepinephrine response. Superoxide anions may be involved in the enhanced response to norepinephrine.**

ISOFLURANE and sevoflurane are halogenated volatile anesthetics commonly used nowadays in clinical practice. Previous *in vivo* studies have suggested that the overall circulatory effects of these two anesthetics, *i.e.,* systemic hypotension due to both peripheral vasodilation and myocardial depression, are substantially identical.[1–3] Indeed, similarities between these two anesthetics have been observed in some *in vitro* cardiovascular actions.[4,5] However, previous *in vitro* and *in situ* studies have also demonstrated significant differences in their actions on a number of cellular mechanisms controlling cardiovascular function.[6–9] Therefore, the underlying cellular mechanisms of the *in vivo* circulatory effects of these two anesthetics may not necessarily be identical.

We recently reported on the action of sevoflurane on isolated endothelium-intact mesenteric resistance arteries.[10] In that study, neither contractile response to norepinephrine nor that to KCl was inhibited during sevoflurane administration; however, the contractile response to norepinephrine was considerably inhibited for a while (≥ 15 min) after washout of sevoflurane.[10] In addition, the endothelial vasodilator mechanisms mediated by either nitric oxide or endothelium-derived hyperpolarizing factor were inhibited during sevoflurane administration.[10] Thus, we proposed that the direct (*i.e.,* nonneural) vascular action of sevoflurane may not contribute to systemic hypotension during sevoflurane anesthesia but that it may contribute to the prolonged systemic hypotension after sevoflurane anesthesia.[9] In the current study, we determined the action of isoflurane on isolated mesenteric resistance arteries and compared its direct vascular action with that of sevoflurane we previously observed.[10] As in the previous study with sevoflurane,[10] we used rats in the current study because the *in vivo* circulatory effects of these two anesthetics in rats have been well characterized[9,11–13] and appear to be similar to those in humans.[14–16]

Methods and Materials

Tissue Preparation

With approval from the Kyushu University Animal Care and Use Committee (Fukuoka, Japan), as previously detailed,[17,18] either endothelium-intact (+E) or endothelium-denuded (−E) strips (250–400 μm long and 150–200 μm wide) were prepared from the third- or fourth-order branches (approximately 150–200 μm in diameter) of Sprague-Dawley rat (male, 250–320 g) mesenteric arteries, which are known to substantially contribute to systemic vascular resistance.[19,20]

Force Measurement Experiments

Isometric force was measured by attaching the strip to a strain gauge transducer (UL-2 type; Shinko, Tokyo, Japan) as previously detailed.[10,18] In brief, the strip was

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horizontally mounted in a chamber attached to the stage of an inverted microscope, and the resting tension was adjusted to obtain a maximal response to KCl. The solution was changed by infusing it rapidly into one end while aspirating simultaneously from the other end. All experiments were carried out in 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES)-buffered physiologic salt solution containing guanethidine (3 μM) to prevent norepinephrine outflow from the sympathetic nerve terminals. Endothelial intactness was verified by the ability of acetylcholine (ACh, 1 μM) to cause complete (≥ 90%) relaxation during contractions induced by norepinephrine (10 μM). Conversely, functional removal of endothelium was verified by the inability of ACh (10 μM) to cause significant (≥ 10%) relaxation during contractions induced by norepinephrine (10 μM). All experiments were carried out at 35°C to prevent early deterioration of the strips.

Solutions and Drugs

The ionic concentrations of the HEPES-buffered physiologic salt solution were as follows: NaCl, 138 mM; KCl, 5.0 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 1.5 mM; HEPES, 10 mM; and glucose, 10 mM. The pH was adjusted with NaOH to 7.35 at 35°C. The high K^+ (40-mM) solutions were prepared by replacing NaCl with KCl iso-osmotically.

The following materials were purchased: norepinephrine, ACh, N^G-nitro L-arginine (LNNA), indomethacin, nordihydroguaiaretic acid (NDGA), superoxide dismutase (SOD), and U46619 (9,11-dideoxy-11α, 9 α-epoxymethanoprostagladin F2α) (Sigma Chemical, St. Louis, MO). HEPES and tetraethylammonium (TEA) (Nacalai Tesque, Kyoto, Japan); ketanserin tartrate (Research Biochemicals International, Natick, MA); and isoflurane (Dainihon Pharmaceutical, Osaka, Japan). Losartan, BQ-123 (Cyclo-[D-α-aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl]), and BQ-788 (N-[N-[N-[2,6-dimethyl-1-piperidinyl]carbonyl]-4-methyl-L-leucyl]-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine monosodium) were obtained from Banyu Pharmaceutical, Tokyo, Japan. All other reagents were of the highest grade commercially available.

Experimental Design

We first examined the effects of isoflurane (1–5%) on contractile responses to either norepinephrine or KCl in both the +E and −E strips. In our preliminary experiments with the +E strips, it was difficult to obtain constant response to lower concentrations (< 1 μM) of norepinephrine for a long period, but it was easy to obtain constant response to 10 μM (maximum) norepinephrine for more than 4 h. Therefore, in the +E strips, we studied the anesthetic effects on the maximal response to 10 μM norepinephrine. In our previous experiments with this artery, both inhibition of the endothelial vasodilator pathways (*i.e.,* nitric oxide [NO] and endothelium-derived hyperpolarizing factor [EDHF] pathways) and endothelial denudation similarly shifted the concentration-response curve for norepinephrine contraction to the left, decreasing the 50% effective concentration value from approximately 1.5 μM to approximately 0.5 μM.[10] The mean amplitude of the 10-μM norepinephrine contraction in the +E strips was almost equal to that of the 0.5-μM norepinephrine contraction in the unrubbed strips treated with inhibitors of the NO and EDHF pathways.[10] Therefore, we examined the anesthetic effects on the submaximal response to 0.5 μM norepinephrine in the −E strips, which was also constant for the long period (greater than 4 h). We used 40 mM KCl to activate voltage-gated Ca^{2+} channels and to eliminate the influence of EDHF. The responses to 40 mM KCl were also constant for the long period (greater than 4 h) in both the +E and −E strips.

Three minutes was sufficient for the responses to either 10 μM norepinephrine or KCl to reach a plateau, and reproducible responses to them were obtained with 7-min intervals. Thus, in experiments with 10 μM norepinephrine or KCl, either norepinephrine or KCl was applied for 3 min at 7-min intervals. However, in experiments with a low concentration (0.5 μM, 45% effective concentration) of norepinephrine in the −E strips, it took approximately 5 min for the norepinephrine response to reach a plateau after exposure to isoflurane, because of strongly inhibited development of force. In addition, with this longer application time, reproducible responses were more easily obtained with a longer interval (17 min) than with the short interval (7 min). Therefore, 0.5 μM norepinephrine was applied for 5 min at 17-min intervals in the −E strips, as done previously.[10] After the response to either norepinephrine or KCl became constant with these protocols, isoflurane was applied for 5 or 15 min before and during subsequent applications of either stimulant, until a steady-state effect was obtained. We also investigated the time course for the recovery of vascular smooth muscle cells from the effects of isoflurane after washout of isoflurane from the chamber.

In the above experiments, we found that isoflurane enhances the norepinephrine response in an endothelium-dependent manner. Since both the NO and EDHF signaling pathways appear to be activated in the contractile response to norepinephrine in this artery,[10] the enhanced contractile response to norepinephrine might be due to inhibition of the NO or EDHF pathway or both. In addition, although isoflurane did not increase the basal force level, it is theoretically possible that isoflurane enhances the contractile response to norepinephrine by stimulating the release of an endothelium-derived vasoconstricting factor.[10] It has been suggested that cyclooxygenase products (*e.g.*, thromboxane A_2, prostaglandin F2α), lipoxygenase products, endothelin-1 (ET), angiotensin-II (AT-II), serotonin (5-HT), histamine, adenosine triphosphate, adenosine diphosphate, and superoxide

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anions all act as endothelium-derived vasoconstricting factors.[21] We thus decided to examine the involvement of these endothelial substances in the vasoconstricting action of isoflurane. In the −E strips, U46619 (thromboxane–prostaglandin endoperoxide analogue), ET-1, AT-II, and 5-HT—but not histamine, adenosine 5′-triphosphate, and adenosine 5′-diphosphate—caused significant contractions in the presence or absence of norepinephrine (0.5 μM, approximately 45% effective concentration).[10] We therefore evaluated the effects of inhibitors of the NO, EDHF, cyclooxygenase, and lipoxygenase pathways; blockers of ET-1, AT-II, and 5-HT receptors; and SOD on the vasoconstricting action, although we did not examine the involvement of histamine, adenosine 5′-triphosphate, or adenosine 5′-diphosphate in the vasoconstricting action.

To examine the involvement of NO, EDHF, cyclooxygenase, and lipoxygenase products in the vasoconstricting action, we evaluated the effects of a cocktail application of 100 μM LNNA (NO synthesis inhibitor), 10 mM TEA (nonselective K^+ channel blocker known to inhibit the endothelium-mediated hyperpolarization[22]), 10 μM indomethacin (cyclooxygenase inhibitor[21]), and 1 μM NDGA (lipoxygenase inhibitor[21]) on the enhanced response to norepinephrine by 3% isoflurane. The concentrations of these inhibitors and the preincubation times were decided on the basis of the results of our recent experiments in this artery[10] and others reported in the literature.[21,23] In other words, the strips were pretreated with these inhibitors for a time considered sufficient for all of them to exert their maximal effects (60 min), and the inhibitors were then applied throughout the remainder of the experiment. Because these inhibitors significantly enhanced the norepinephrine (10 μM) response, the norepinephrine concentration was decreased to produce approximately the same level of tension as the control (10 μM) norepinephrine–induced contraction, as previously done.[10,17] Such low concentrations of norepinephrine were detected in each strip (see Results).

To evaluate the involvement of ET-1, AT-II, and 5-HT in the vasoconstricting action, we examined the effects of a cocktail application of 1 μM BQ-123 (selective ET_A receptor antagonist), 1 μM BQ-788 (selective ET_B receptor antagonist), 1 μM losartan (selective AT-II type 1 receptor antagonist), and 0.03 μM ketanserin ($5\text{-}HT_2$/$5\text{-}HT_{1C}$ receptor antagonist) on the enhanced response to norepinephrine by 3% isoflurane. As we recently reported with regard to this artery,[10] the concentrations of BQ-123, BQ-788, losartan, and ketanserin and the preincubation time (25 min) were determined by their ability to eliminate the maximal contractions induced by ET-1 (10 nM), AT-II (3 μM), or 5-HT (3 μM) in the −E strips.

To evaluate the involvement of superoxide anions in the vasoconstricting action, we examined the effects of SOD (15–150 U/ml), a scavenger of superoxide anions, on the enhanced response to norepinephrine by 3% isoflurane. The concentrations of SOD and its preincubation time (25 min) were decided on the basis of findings reported in the literature,[24,25] in which the ability of SOD to inhibit endothelium-dependent vasoconstriction was demonstrated in isolated arteries.

We also evaluated the ability of isoflurane to inhibit the NO and EDHF pathways by testing its effects on both the NO- and EDHF-mediated vasorelaxations caused by ACh. In this artery, the relaxation caused by low concentrations (≤ 0.1 μM) of ACh is exclusively mediated by NO, whereas the relaxation caused by higher concentrations (≥ 0.3 μM) of ACh is mediated by both NO and EDHF.[10] Thus, in this experiment, we evaluated (1) the effects of isoflurane on the NO-mediated (*i.e.*, LNNA-sensitive) relaxation caused by 0.03 μM ACh in the +E strips precontracted with norepinephrine and (2) its effects on the EDHF-mediated (*i.e.*, LNNA-resistant, TEA-sensitive) relaxation caused by 1 μM ACh in the LNNA-treated, unrubbed strips precontracted with norepinephrine.

Isoflurane Delivery and Analysis

Isoflurane was delivered *via* a calibrated isoflurane vaporizer (Forawick; Muraco Medical, Tokyo, Japan) in line with the air gas aerating the HEPES-buffered solutions. Each solution was equilibrated with isoflurane for at least 15 min before introduction to the chamber, which was covered with a thin glass plate to prevent the equilibration gas from escaping into the atmosphere. Using gas chromatography, we previously determined the concentrations of isoflurane in the physiologic salt solution produced by 0.5, 1.0, 1.5, and 3.0% isoflurane under exactly the same experimental conditions,[18,26] and the obtained values were within 92% (91.8–98.5%) of theoretical values predicted by the partition coefficient of isoflurane in Krebs solution (0.55) at 37°C. An excellent linear relation was obtained between the aqueous concentrations of isoflurane (y) and its concentrations (vol%) in the gas mixture (x): $y = -0.0068 + 0.21x$; r [regression coefficient] = 0.998).[26] Therefore, the concentrations produced by 1, 2, 3, and 5% isoflurane in the physiologic salt solution can be predicted as 0.21, 0.42, 0.63, and 1.05 mM, respectively. The aqueous concentration produced by 3% isoflurane (*i.e.*, 0.63 mM) is almost equal to a recently reported concentration of isoflurane (0.65 mM)[9] in blood sampled from this rat under steady-state anesthesia with 1.5% (1 MAC in this rat[27]) isoflurane.

Calculation and Data Analysis

The effects of isoflurane on norepinephrine contraction, KCl contraction, or ACh relaxation were assessed at the points at which its effects reached a maximum. Changes in force were expressed as percentage of the reference. Because the relation between actual concen-

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trations of isoflurane in the solutions and anesthetic concentrations (vol%) in the gas mixture is theoretically linear, the anesthetic concentrations on the x-axis are displayed as vol% for the isoflurane concentration–response relations, as done previously.[10,18]

Statistics

All the results are expressed as mean ± SD. In each series of experiments, the effect of each concentration of isoflurane was examined once in a single strip prepared from one animal. However, it was not always possible to test the effects of all four concentrations of isoflurane in each strip because the high concentrations (≥ 3%) of isoflurane produced prolonged inhibition of vascular reactivity, as shown in Results. Accordingly, although the number of preparations is equal to the number of animals at each data point, the number of total animals required for each series of experiments is not necessarily equal to the number of preparations for each data point. The number of preparations for each data point (n) and that of total animals required for each series of experiments (N) are both described in Results.

The data were statistically assessed by analysis of variance, Scheffé F test, and Student *t* test, as appropriate. Comparisons of the time-dependent effects of isoflurane among concentrations and those of the effects of isoflurane between treatments (short application *vs.* long application) were made by two-factor (time, concentration; concentration, treatment) analysis of variance for repeated measures or factorial analysis. When overall differences were detected, individual comparisons among or between groups at each concentration were performed by means of the Scheffé F test. Comparisons of the effects of isoflurane within each concentration group or each treatment group were made by one-factor (time or concentration) analysis of variance for repeated measures, and post hoc comparisons were made with use of the Scheffé F test for multiple comparisons. Similarly, the effects of SOD on the vasoconstricting action of isoflurane were assessed by one-factor (concentration) analysis of variance for repeated measures, followed by the Scheffé F test. The effects of pharmacologic inhibitors on norepinephrine response and the effects of isoflurane (3%) on norepinephrine response after treatment with the pharmacologic inhibitors were assessed by means of a two-tailed, paired Student *t* test. In the Discussion, comparisons between the current data on isoflurane and our previous data on sevoflurane[10] at each data point were made with a two-tailed, unpaired Student *t* test. A level of $P < 0.05$ was considered significant. All the statistical analyses were performed on a computer with use of Statview J 4.02® software (Abacus Concepts, Berkeley, CA).

Results

Effects of Isoflurane on Contractile Response to Norepinephrine

In the +E strips, isoflurane (≥ 2%), applied for 5 min before and during subsequent applications of norepinephrine (10 μM), initially (*i.e.*, 5 min after application of isoflurane) enhanced the contractile response to norepinephrine (fig. 1). However, the enhancement was subsequently attenuated during application of isoflurane, reaching a steady state where the magnitude of norepinephrine response was not significantly different from that before application of isoflurane (fig. 1). However, at this steady state, the rate of rise in force in response to norepinephrine was consistently inhibited by isoflurane (fig. 1Ab). In addition, rhythmic oscillations observed in the contractile response to norepinephrine were consistently inhibited during application of isoflurane (fig. 1Ab). The contractile response to norepinephrine was strongly inhibited after washout of isoflurane (≥ 2%) from the chamber (fig. 1). This inhibition was prolonged for 5–75 min, depending on the preparations (fig. 1); the mean recovery time for the 5% isoflurane-induced postwashout inhibition of norepinephrine response was 40.0 ± 21.4 min (n = 8; N = 13). Comparison of the time-dependent effects of isoflurane between two different protocols regarding time for isoflurane application (*i.e.*, short *vs.* long application protocols) indicated that the postwashout inhibition was indeed triggered by the washout of isoflurane but was not due to emergence of late-onset inhibition (fig. 1Bb).

In the −E strips, isoflurane (≥ 3%), applied for 15 min before and during subsequent applications of 0.5 μM (approximately 45% effective concentration) norepinephrine, inhibited the contractile response to norepinephrine (fig. 2). It took more than 30 min for the inhibitory effect of isoflurane to reach maximum and steady state (fig. 2). The inhibition was prolonged after washout of isoflurane from the chamber, and it took more than 15 min for vascular smooth muscle cells to completely recover from the inhibition (fig. 2). Identical results were obtained in other experiments in which isoflurane (1–5%) was applied for 15 min before and during subsequent applications of 10 μM (maximum) norepinephrine (fig. 2).

Figure 2C shows comparison of the effect of isoflurane on contractile response to norepinephrine in the presence or absence of endothelium in two different protocols with regard to time for pretreatment with isoflurane (*i.e.*, 5 *vs.* 15 min). As shown in figure 1, when isoflurane was applied to the +E strips for 5 min before and during a subsequent application of norepinephrine, the norepinephrine response was significantly enhanced during application of isoflurane (fig. 2C). However, as shown in figure 2C, when isoflurane was applied to the +E strips for the longer time (15 min) before and during a subse-

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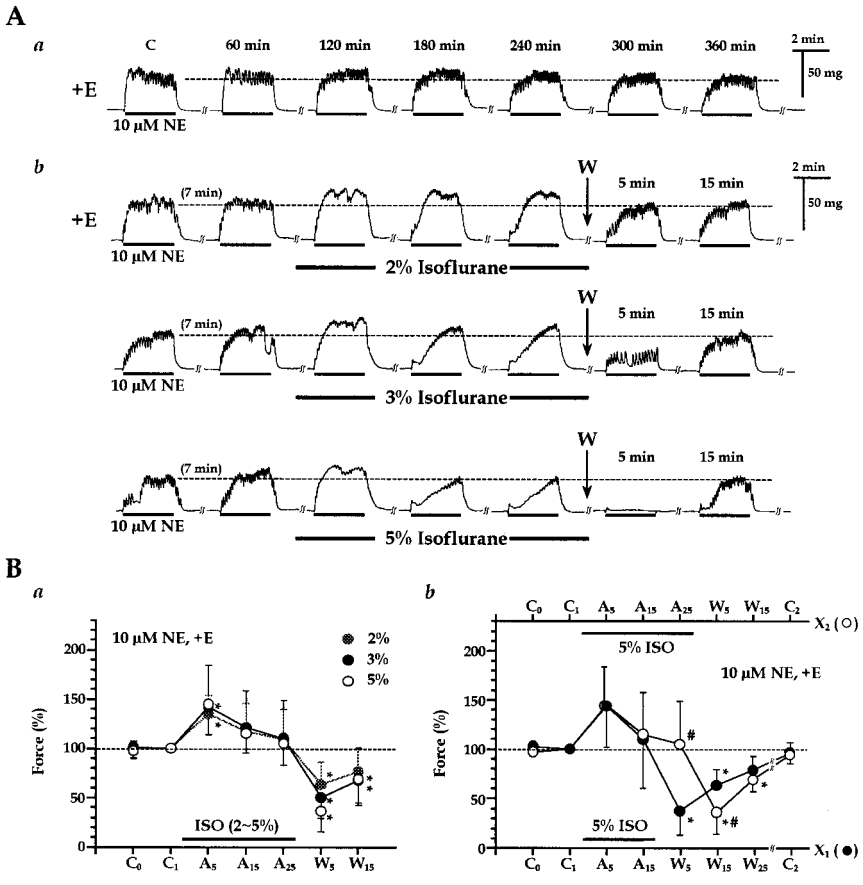

Fig. 1. Time-dependent effects of isoflurane (ISO) on responses to norepinephrine (NE; 10 μM) in the endothelium-intact (+E) strips. (*A*) Examples of (*a*) time control and (*b*) the effects of isoflurane. Time (min) (*upper, a; lower, b*) indicates that after the first application of norepinephrine and that after washout of isoflurane (W), respectively. Time in parentheses indicates the interval for norepinephrine applications (C = control). Note the (1) constant responses to norepinephrine, which was applied for 3 min at 7-min intervals, observed for 6 h; (2) inhibition of norepinephrine-induced rhythmic oscillations during application of isoflurane; (3) concentration-dependent inhibition of rate of rise in force in response to norepinephrine during application of isoflurane; and (4) concentration-dependent inhibition of the norepinephrine response observed after washout of isoflurane. Identical time control data were obtained for the other four strips. (*B*) Analyzed data. (*a*) Time-dependent effects of isoflurane on the norepinephrine response. The data on 1% isoflurane were omitted from this figure for clarity; its effect is shown in figure 2C (mean ± SD; n = 8; N = 13; **P* < 0.05 *vs.* control [C_1]). (*b*) Time-dependent effects of isoflurane on the norepinephrine response with two different protocols for time of application of isoflurane (20 *vs.* 30 min). The data obtained in the experiments in which isoflurane was applied for 20 and 30 min are denoted by the closed circles (on the X_1 axis) and open circles (on the X_2 axis), respectively (mean ± SD; n = 8; N = 8; **P* < 0.05 *vs.* control [C_1] within each group; #*P* < 0.05 *vs.* the short application group at each time point). A_x = x min after application of isoflurane; C_0 = precontrol 1; C_1 = precontrol 2; C_2 = postcontrol; W_x = x min after washout of isoflurane.

quent application of norepinephrine, the norepinephrine response was not significantly enhanced during application of isoflurane. In contrast, when isoflurane was applied to the −E strips for 5 min before and during a subsequent application of norepinephrine (0.5 and 10 μM), the norepinephrine response was not significantly inhibited during application of isoflurane (fig. 2C). However, when isoflurane was applied to the −E strips for the longer time (15 min) before and during a subsequent application of norepinephrine (0.5 and 10 μM), the norepinephrine response was significantly inhibited during application of isoflurane (fig. 2C).

Effects of Isoflurane on Contractile Response to KCl

In the +E strips, lower concentrations (≤ 3%) of isoflurane, applied for 5 min before and during subsequent applications of KCl (40 mM), did not significantly influence the contractile response to KCl (fig. 3). However, as shown in figure 3A, a low concentration (1%) of isoflurane enhanced the contractile response to KCl in some of the +E strips, although its mean effect did not reach statistical significance. Only higher concentrations (≥ 3%) of isoflurane significantly inhibited the KCl response (fig. 3).

In the −E strips, isoflurane (≥ 2%), applied for 5 min before and during subsequent applications of KCl (40 mM), significantly inhibited the contractile response to KCl in a concentration-dependent manner (fig. 3).

In contrast to the difference between the +E and −E strips in time dependence of the effect of isoflurane on norepinephrine response, the time dependence of the inhibitory effect of isoflurane on KCl response observed in the +E strips was similar to that observed in the −E strips (fig. 3). Namely, the onset of isoflurane-induced inhibition of KCl response was immediate in both the +E and −E strips; 5 min was sufficient for its inhibitory effect on KCl response to reach maximum and steady state, regardless of the presence of endothelium (fig. 3). In addition, no significant inhibition of the KCl response was observed after washout of isoflurane in either +E or −E strips (fig. 3).

Effects of Various Pharmacological Interventions on the Enhanced Contractile Response to Norepinephrine by Isoflurane in the Presence of Endothelium

The treatment with LNNA + TEA + NDGA + indomethacin enhanced the response to 10 μM norepinephrine (186 ± 35.2% of the control; $P < 0.05$; n = 5; N =

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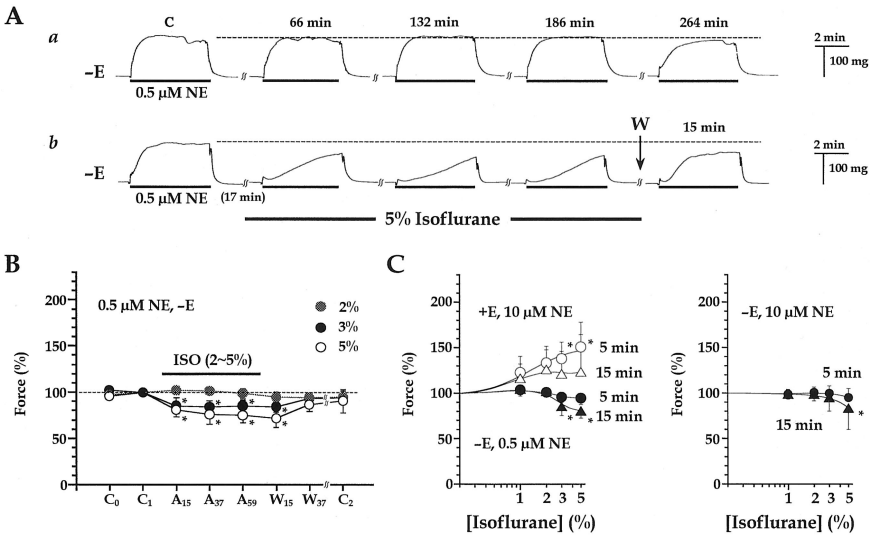

Fig. 2. (*A, B*) Time-dependent effects of isoflurane (ISO) on responses to norepinephrine (NE; 0.5 μM) in the endothelium-denuded (−E) strips. (*A*) Examples of (*a*) time control and (*b*) the effects of isoflurane. Time (min) (*upper, a; lower, b*) indicates the time after first application of norepinephrine and the time after washout of isoflurane (W), respectively. Time in parentheses indicates the interval for norepinephrine applications (C = control). Note constant response to norepinephrine, which was applied for 5 min at 17-min intervals, observed for more than 4 h. Identical time control data were obtained for the other four strips. (*B*) Analyzed data. The data on 1% isoflurane were omitted from this figure for clarity; its effect is shown in *C* (mean ± SD; n = 5; N = 11; **P* < 0.05 *vs.* control [C_1] within each group; A_x = x min after application of isoflurane; C_0 = precontrol 1; C_1 = precontrol 2; C_2 = postcontrol; W_x = x min after washout of isoflurane. (*C*) Comparison of the effect of isoflurane on norepinephrine response in either the endothelium-intact (+E; open symbols) or endothelium-denuded (−E; closed symbols) strips between two different protocols for time of pretreatment with isoflurane (*i.e.*, 5 min [circles] *vs.* 15 min [triangles]). (*Left*) Data for 10 μM norepinephrine response in the +E strips (n = 6; N = 6) and 0.5 μM norepinephrine response in the −E strips (n = 5; N = 13). (*Right*) Data for 10 μM norepinephrine response in the −E strips (n = 14; N = 14) (mean ± SD; **P* < 0.05 *vs.* control [100%] within each group).

5). In the unrubbed strips treated with these inhibitors, isoflurane (3%) still enhanced the contractile response to a lower concentration of norepinephrine (3.4 ± 3.0 μM), the amplitude of which was not different from that of the control response to 10 μM norepinephrine ($P > 0.05$; n = 5; N = 5) (fig. 4A).

The treatment with losartan + ketanserin + BQ123 + BQ788 did not influence the response to 10 μM norepinephrine (83.9 ± 15.7% of the control; $P > 0.05$; n = 5; N = 5). Isoflurane (3%) still enhanced the response to 10 μM norepinephrine in the unrubbed strips after treatment with these receptor blockers (fig. 4A).

The treatment with SOD did not influence the response to 10 μM norepinephrine (103.4 ± 5.7, 107.8 ± 13.8, and 100.0 ± 29.8% of the control after treatment with 15, 50, and 150 U/ml SOD, respectively; $P > 0.05$; n = 5; N = 5). Isoflurane (3%) failed to enhance the response to 10 μM norepinephrine in the unrubbed strips after treatment with the higher concentrations of SOD (≥ 50 U/ml) (fig. 4B).

Effects of Isoflurane on the Endothelium-dependent Relaxation Caused by ACh

Isoflurane (≥ 1%) inhibited relaxation caused by 0.03 μM ACh in the +E strips precontracted with 10 μM norepinephrine (fig. 5). Isoflurane (≥ 3%) also inhibited relaxation caused by 1 μM ACh in the unrubbed strips precontracted with 10 μM norepinephrine after exposure to LNNA (100 μM, 60 min) (fig. 5).

Discussion

The effects of isoflurane on vascular response to norepinephrine, KCl, or ACh observed in this study were substantially identical to those of sevoflurane that we previously observed in this resistance artery.[10] Thus, as we previously proposed with regard to sevoflurane,[10] the direct (nonneural) action of isoflurane on vascular smooth muscle tissues (including endothelium) does not appear to contribute to systemic hypotension during isoflurane anesthesia.[9,11,28] However, the direct action of isoflurane on vascular smooth muscle tissues may contribute to the prolonged systemic hypotension after isoflurane anesthesia.[9]

The current results indicate that isoflurane has opposing actions on contractile response to norepinephrine, *i.e.*, an endothelium-dependent enhancing and an endothelium-independent inhibitory action. The former emerges immediately after application of isoflurane, remains during its application, and disappears soon after its removal. By contrast, the latter gradually emerges after application of isoflurane and persists after its removal. The norepinephrine response during application of isoflurane is determined by the net balance between these two components. As a result, in the +E strips, the norepinephrine response is enhanced immediately after application of isoflurane, subsequently normalized to the control level at steady state during its application, and notably inhibited after its washout. This proposal is substantially identical to our previous proposal concerning sevoflurane,[10] although there are some differences in these anesthetics in terms of their action on norepinephrine response.

In our experiments, the aqueous concentrations produced by 3% isoflurane (0.63 mM) and 5% sevoflurane (0.67 mM) are almost equal to the respective anesthetic concentrations in blood sampled from this rat under

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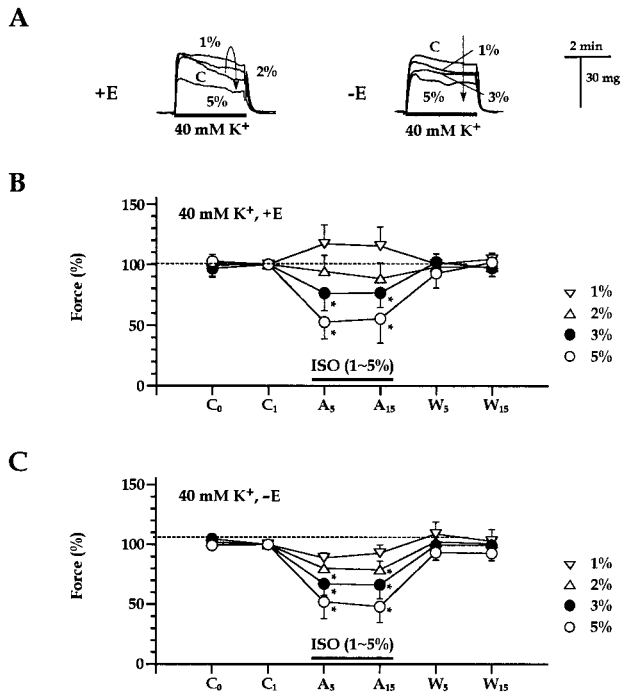

Fig. 3. Effects of isoflurane (ISO) on contractile responses to KCl (40 mM). (*A*) Examples of the effects of isoflurane in either endothelium-intact (+E, *left*) or -denuded (−E, *right*) strips. The arrows indicate the directions of the action of isoflurane (C = control). (*B, C*) Analyzed data on the time-dependent effects of isoflurane on the KCl responses in the +E (*B*) and −E (*C*) strips (mean ± SD; n = 6; N = 6; **P* < 0.05 *vs.* control [C_1] within each group; A_x = x min after application of isoflurane; C_0 = precontrol 1; C_1 = precontrol; W_x = x min after washout of isoflurane).

steady-state anesthesia at 1 MAC (1.5% isoflurane[27] and 2.8% sevoflurane[29]): 0.65 mM and 0.66 mM, respectively.[9] Thus, in the following discussion, we compare the current data on 3% isoflurane with our previous data on 5% sevoflurane.[10] In the +E strips, the norepinephrine response was initially enhanced after application of either isoflurane or sevoflurane; however, this enhancement was subsequently attenuated during application of each anesthetic, presumably because of the emergence of the endothelium-independent (smooth muscle) component. As a result, at steady state (15 min after application of either anesthetic), the magnitude of norepinephrine response returned to the control level during exposure to isoflurane (fig. 1), whereas it was still enhanced ($P < 0.05$; approximately 40%) during exposure to sevoflurane.[10] Namely, during exposure to isoflurane, the smooth muscle component eventually counteracted the endothelial component, preventing the magnitude of norepinephrine response from being enhanced. By contrast, during exposure to sevoflurane, the endothelial component predominated over the smooth muscle component throughout, enhancing the norepinephrine response.[10] However, in the −E strips, the inhibition of the magnitude of the norepinephrine response observed 15 min after application of isoflurane was not significantly different from that observed 15 min after application of sevoflurane[10] ($P > 0.05$). Furthermore, in the +E strips, the rate of rise in force in response to norepinephrine was consistently inhibited during exposure to isoflurane (fig. 1), but it was influenced little during exposure to sevoflurane.[10] However, in the −E strips, the rate of rise in force in response to norepinephrine was similarly inhibited by both anesthetics (fig. 2).[10] Thus, it seems reasonable to postulate that the endothelium-dependent vasoconstricting action of sevoflurane is more potent than that of isoflurane in comparison at the equi-MAC concentration.

Isoflurane inhibited the KCl response at only higher concentrations (≥ 3%) in the +E strips but at lower concentrations (≥ 2%) in the −E strips. In addition, a low concentration (1%) of isoflurane appeared to enhance the KCl response in the +E strips, although its degree did not reach statistical significance. These indicate that the endothelium-dependent vasoconstricting component exists also in its action on KCl response. The endothelial function is considered globally inhibited during 40-mM KCl depolarization because of the lack of endothelial cell hyperpolarization due to K^+ channel opening and the reduced driving force for transmembrane Ca^{2+} influx into endothelial cells. Thus, if the endothelium-dependent action is in part mediated by the Ca^{2+} influx, the action will be attenuated during the depolarization, a circumstance possibly explaining the observed inability of isoflurane to significantly enhance the KCl response in the +E strips. Unlike in the norepinephrine response, neither the postwashout inhibition nor the prolonged inhibition was observed in the action of isoflurane on KCl response, indicating that the mechanisms of inhibition of the norepinephrine response are at least in part different from the mechanisms of inhibition of the KCl response. The observed action of isoflurane on KCl response was also identical to the previously observed action of sevoflurane on KCl response[10]; no significant difference was observed in the effect on KCl response in either +E or −E strips between 3% isoflurane and 5% sevoflurane ($P > 0.05$).

Isoflurane enhanced the norepinephrine response after inhibition of the NO and EDHF pathways, indicating that the enhanced norepinephrine response by isoflurane is at least in part independent of either the NO or EDHF pathway. Since isoflurane inhibited both the NO-mediated and EDHF-mediated relaxation caused by ACh, isoflurane presumably inhibits some cellular mechanisms specifically involved in the NO- or EDHF-mediated responses induced by ACh but not by norepinephrine or β-adrenergic agonists (*e.g.*, endothelial phospholipase C activity). Although it has been suggested that isoflurane stimulates the release of vasoactive prostanoids from endothelium,[30,31] isoflurane still enhanced the norepinephrine response after inhibition of both cyclooxygenase and lipoxygenase in the current study, suggesting that the action is at least in part independent of either

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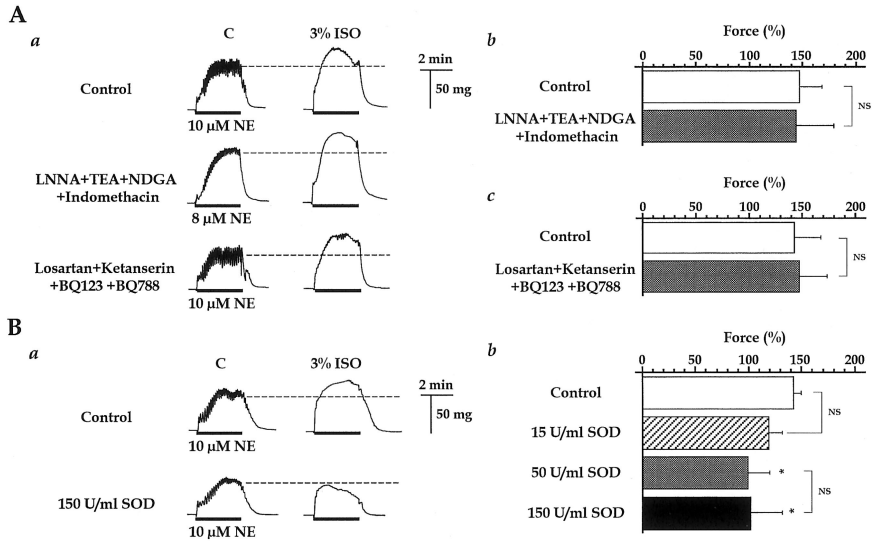

Fig. 4. Effects of various pharmacologic inhibitors on the isoflurane (ISO; 3%)–induced enhancement of the norepinephrine (NE) response in the endothelium-intact strips. (*A*) Effects of either N^G-nitro L-arginine (LNNA) plus tetraethylammonium (TEA) plus nordihydroguaiaretic acid (NDGA) plus indomethacin or losartan plus ketanserin plus BQ-123 plus BQ-788. (*a*) Examples of control enhancement (*upper*), the effects of LNNA plus TEA plus NDGA plus indomethacin (*middle*), and the effects of losartan plus ketanserin plus BQ-123 plus BQ-788 (*lower*). (*b, c*) Analyzed data on the effect of LNNA plus TEA plus NDGA plus indomethacin (*b*) and on the effect of losartan plus ketanserin plus BQ123 plus BQ788 (*c*) (mean ± SD; n = 5; N = 5; NS = not significantly different [$P > 0.05$]). (*B*) Effects of superoxide dismutase (SOD, 15–150 U/ml). (*a*) Examples of control enhancement (*upper*) and the effects of SOD (150 U/ml) (*lower*). (*b*) Analyzed data [not significantly different [$P > 0.05$]; mean ± SD; n = 5; N = 8; *$P < 0.05$ *vs.* control).

enzyme. In addition, isoflurane still enhanced the norepinephrine response after blockade of ET-1, AT-II, or 5-HT receptors, suggesting that neither receptor agonist is involved in the action.

In this study, isoflurane failed to enhance the norepinephrine response after treatment with SOD. Since it is believed that the exogenously applied SOD (molecular weight, ≥ 33,000) does not enter the endothelial cells,[21,32–34] its mechanism of action is probably the extracellular inactivation of superoxide anions ($\cdot O_2^-$).[21,35] Therefore, the extracellular $\cdot O_2^-$, presumably released from the endothelial cells, appears to be involved in the vasoconstricting action. In aerobic cells, the $\cdot O_2^-$ can be generated from oxygen by action of xanthine oxidase or of various cellular enzymes such as cyclooxygenase, lipoxygenase, cytochrome P_{450}, or flavin enzymes.[33,36] Vascular endothelial cells, which contain these enzymes, also can generate the $\cdot O_2^-$.[37,38] In addition, the $\cdot O_2^-$ was previously shown to have a direct vasoconstrictor action and act as an endothelium-derived vasoconstricting factor.[24,35] In this study, both cyclooxygenase and lipoxygenase inhibitors did not affect the enhanced norepinephrine response by isoflurane. Thus, in this artery, isoflurane may enhance the norepinephrine response by acting on some endothelial enzyme system other than the cyclooxygenase or the lipoxygenase system and thereby stimulating the endothelial release of $\cdot O_2^-$.

Because the $\cdot O_2^-$ can inactivate the NO and activation of the NO pathway is involved in the norepinephrine response in this artery,[10,39] it is also conceivable that isoflurane enhances the norepinephrine response by generating the $\cdot O_2^-$ *via* some endothelial mechanisms and thereby inactivating the NO. However, this seems unlikely because isoflurane still enhanced the norepinephrine response after inhibition of the NO synthesis. The amount of the generated $\cdot O_2^-$ might not be sufficient for the $\cdot O_2^-$ to inactivate the NO but might be sufficient for the $\cdot O_2^-$ to serve as an endothelium-derived vasoconstricting factor.

In conclusion, in isolated rat mesenteric resistance arteries, isoflurane at clinical concentrations significantly influences contractile response to the sympathetic neurotransmitter norepinephrine, and its action consists of an endothelial and a smooth muscle component. The former enhances the norepinephrine response, whereas the latter inhibits it. During application of isoflurane, the endothelial component predominates over or counteracts the smooth muscle component, enhancing the norepinephrine response or preventing the norepinephrine

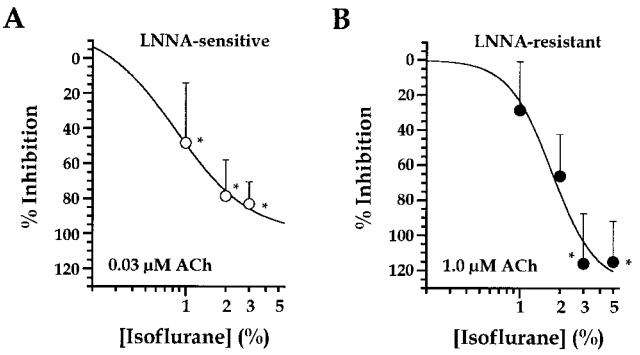

Fig. 5. Effects of isoflurane on endothelium-dependent relaxation caused by acetylcholine (ACh) in the presence of norepinephrine (NE, 10 μM). (*A*) Effects of isoflurane on the N^G-nitro L-arginine (LNNA)–sensitive vasorelaxation caused by 0.03 μM ACh in the endothelium-intact strips. The vasorelaxation caused by 0.03 μM ACh was completely inhibited by 5% isoflurane in two of these strips (not shown) (mean + SD; n = 5; N = 5; *$P < 0.05$ *vs.* control [0%]). (*B*) Effects of isoflurane on the LNNA–resistant vasorelaxation caused by 1 μM ACh in the LNNA-reated, unrubbed strips (mean ± SD; n = 3; N = 3; *$P < 0.05$ *vs.* control [0%]).

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response from being inhibited. Only the smooth muscle component persists after washout of isoflurane, leading to the prolonged inhibition of the norepinephrine response.

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