The Action of Sevoflurane on Vascular Smooth Muscle of Isolated Mesenteric Resistance Arteries (Part 1)

Role of Endothelium

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Background: The direct action of sevoflurane on systemic resistance arteries is not fully understood.

Methods: Isometric force was recorded in isolated rat small mesenteric arteries.

Results: Sevoflurane (2–5%) enhanced contractile response to norepinephrine only in the presence of endothelium, but inhibited it in its absence. Sevoflurane still enhanced the norepinephrine response after inhibitions of the nitric oxide, endothelium-derived hyperpolarizing factor, cyclooxygenase and lipoxygenase pathways, or after blockade of either endothelin-1 ET-1), angiotensin-II, or serotonin receptors. Sevoflurane (3–5%) inhibited contractile response to potassium chloride only in the absence of endothelium but did not influence it in its presence. In the endothelium-intact strips, inhibition of the norepinephrine response, which was enhanced during application of sevoflurane, was observed after washout of sevoflurane and persisted for approximately 15 min. In the endothelium-denuded strips, the inhibition of norepinephrine response was similarly prolonged after washout of sevoflurane. However, no significant inhibitions of potassium chloride response were observed after washout of sevoflurane in both the endothelium-intact and the endothelium-denuded strips.

Conclusions: The action of sevoflurane on norepinephrine contractile response consists of endothelium-dependent vasoconstricting and endothelium-independent vasodilating components. In the presence of endothelium, the former predominates over the latter, enhancing the norepinephrine response. The endothelium-independent component persisted after washout of sevoflurane, leading to prolonged inhibition of the norepinephrine response. The mechanisms behind the sevoflurane-induced inhibition of norepinephrine response are at least in part different from those behind its inhibition of potassium chloride response. Nitric oxide, endothelium-derived hyperpolarizing factor, cyclooxygenase products, lipoxygenase products, endothelin-1, angiotensin-II, and serotonin are not involved in the vasoconstricting action. (Key words: Halogenated volatile anesthetics; sympathetic nervous system; systemic hypotension; vascular endothelium.)

THE overall circulatory effects of sevoflurane are similar to those of other halogenated volatile anesthetics, such as halothane, enflurane, or isoflurane; i.e., systemic hypotension caused by systemic vasodilation and myocardial depression.1–5 However, previous in vitro and in situ studies have shown significant differences in the actions on a number of cellular mechanisms that control cardiovascular function among those anesthetics.5–8 The mechanisms behind the in vivo circulatory effects thus seem to be different among those anesthetics.

Vascular endothelium plays an important role in the regulation of peripheral vascular tone in vivo by releasing various vasoactive substances, such as nitric oxide (NO) or endothelium-derived hyperpolarizing factor (EDHF).8–10 In isolated arterial preparations, sevoflurane inhibited both NO-mediated and EDHF-mediated endothelium-dependent vasorelaxations,5,11–15 as was observed with halothane, isoflurane, and enflurane.5,9,12,14 However, clinical significance of such inhibitory action of sevoflurane on ligand-receptor-mediated endothelium-dependent vasorelaxation observed in vitro appears to be unclear.8

The sympathetic nervous system also plays a crucial role in control of vascular tone in vivo.8 However, the direct action of sevoflurane on contractile response to the norepinephrine, sympathetic neurotransmitter, does
not appear to be fully understood. It has been suggested that norepinephrine stimulates the release of NO from vascular endothelium.$^{12,15}$ In addition, norepinephrine was previously shown to hyperpolarize vascular smooth muscle (VSM) cell membrane in the presence of endothelium.$^{16}$ Therefore, NO and EDHF signaling pathways both appear to be involved in the contractile response to norepinephrine in the presence of endothelium. As described previously, sevoflurane inhibits NO and EDHF-mediated vasorelaxations,$^{11,12,17}$ suggesting its ability to inhibit both the NO and the EDHF pathways. Therefore, it may be hypothesized that sevoflurane enhances the contractile response to norepinephrine by inhibiting the NO and/or EDHF pathways.

Voltage-gated Ca$^{2+}$ channel activity also appears to significantly influence vascular tone in vivo.$^{6,18}$ However, less seems to be known about the action of sevoflurane on vascular responses mediated by the voltage-gated Ca$^{2+}$ channels (e.g., potassium chloride [KCl] contraction). Because activation of voltage-gated Ca$^{2+}$ channels is involved in the contractile response to norepinephrine,$^{19,20}$ sevoflurane may influence the contractile response to norepinephrine also through an effect on the voltage-gated Ca$^{2+}$ channels.

The purpose of this study was to address the possibility that mesenteric vasodilation resulting from sevoflurane administration contributes to its hypotensive action. We therefore investigated the action of sevoflurane on contractile response to either norepinephrine or KCl in mesenteric resistance arteries isolated from rats, in which its in vivo circulatory effects have been well-characterized$^{2,5,5}$ and seem to be similar to those in humans.$^{1,21}$ We discuss whether or not the direct and/or endothelium-mediated actions of sevoflurane on VSM observed in this in vitro study contributes to the previously reported hemodynamic changes during sevoflurane anesthesia.$^{2,3,5}$

Materials and Methods

Tissue Preparation

With approval from the Kyushu University Animal Care and Use Committee, male Sprague-Dawley rats (250–320 g) were anesthetized with diethyl ether. The mesenteric tissue was then exposed, rapidly excised, and immediately placed in a disecting chamber filled with oxygenated (2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES)-buffered physiologic salt solution. A transverse strip (250–400 μm in length, 150–200 μm in width) was prepared from the third- or fourth-order small mesenteric artery (≈ 150–200 μm in diameter) with the endothelium intact (+E) or removed (−E). The details of this preparation were reported previously.$^{20,22}$ Briefly, the endothelium was removed by gently rubbing the intimal surface with the round surface of a small pin.$^{20}$ These branches are known to substantially contribute to systemic vascular resistance.$^{8,23}$

Tension Measurement Experiments

Isometric tension was measured by attaching the strip to a strain gauge transducer (UL-2 type; Shinko Co., Tokyo, Japan), as previously detailed.$^{20,22}$ Briefly, the strip was horizontally mounted in a chamber (0.9 ml) 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 performed in the presence of guanethidine (3 μM)$^{24}$ to prevent norepinephrine outflow from the sympathetic nerve terminals. Endothelial intactness was verified by the ability of acetylcholine (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 acetylcholine (10 μM) to cause significant (≥ 10%) relaxation during contractions induced by norepinephrine (10 μM). Because the thin strips from the small arteries begin to deteriorate a few hours after setup,$^{20}$ all experiments were performed 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; 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 isoosmotically.

Norepinephrine, acetylcholine, N$^{\text{N}}$-nitro-l-arginine (LNN)$\text{A}$, methylene blue, hemoglobin (bovine), indomethacin, nordihydroguaiaretic acid (NDGA), phenidone (1-phenyl-3-pyrazolidinone), superoxide dismutase (SOD), and U46619 (9,11-dideoxy-11α, 9α-epoxymethanoprostagladin F$_{2\alpha}$) were purchased from Sigma Chemical (St. Louis, MO). HEPES, ouabain (g-strophanthin), and tetrodoyllammonium were purchased from Nacalai Tesque (Kyoto, Japan). Sevoflurane was purchased from Kodama Pharmaceutical (Osaka, Japan). BQ-123 (Cyclo(D-$\alpha$-aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl)), BQ-788 (N-$\text{N}$-[N-$\text{N}$-[2,6-
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dimethyl-1-piperidinyl][carbonyl]-4-methyl-L-leucyl]-1-
(methoxy-carbonyl)-D-tryptophyl]-D-norleucine mono-
sodium), and losartan were obtained from Banyu Phar-
aceutical (Tokyo, Japan). Ketanserin tartrate was purchased
from Research Biochemicals International (Natick, MA). All
other reagents were of the highest grade commer-
cially available.

Oxyhemoglobin (HbO₂) was prepared by reducing
commercial bovine hemoglobin containing 75% methem-
oglobin, as previously described. Pure hemoglobin
(oxyhemoglobin) was prepared by adding to a 1-mM
solution of commercial hemoglobin in distilled water, a
10-fold molar excess of the reducing agent, sodium di-
thionite (Na₂S₂O₄). Sodium dithionite was then removed
by dialysis using a cellulose tubing against 200 volumes
of distilled water for 2 h at 4°C. The identification and
final concentrations of oxyhemoglobin were determined
spectrophotometrically.

**Experimental Design**

We first evaluated the effects of sevoflurane (1–5%) on
contractile responses to either norepinephrine (0.5–10
μM) or KCl (40 mM) in both the +E and −E strips. In
most of these experiments, 3 min was enough for the
responses to various concentrations of norepinephrine
or KCl to reach a plateau, and reproducible responses to
them were easily obtained with 7-min intervals. Thus, in
most of these experiments, either norepinephrine or 40
mM K⁺ was applied for 3 min at 7-min intervals. How-
ever, only in experiments with a low concentration (0.5
μM) of norepinephrine in the −E strips, approximately 5
min were necessary for the norepinephrine response to
reach a plateau after exposure to sevoflurane because of
notably inhibited development of force. However, 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. After the response to either norepi-
nephrine or KCl became constant with these protocols,
sevoflurane was applied for 5 or 15 min before and
during subsequent applications of either stimulant until
steady state effect was obtained.

In these experiments, we found that sevoflurane en-
hances the response to norepinephrine in an endothe-
lium-dependent manner. We thus decided to address the
aforementioned possibility that the enhanced norepi-
nephrine response is caused by inhibition of the NO or
EDHF pathways. In this attempt, we first characterized
the endothelium-mediated relaxation caused by either
acetylcholine or histamine, using inhibitors of the NO or
EDHF pathways. Specifically, we evaluated the effects of
LNNA (NO synthesis inhibitor), tetraethylammonium
(K⁺ channel blocker known to inhibit the endothelium-
mediated hyperpolarization²,²⁰), ouabain plus Ba²⁺ (a
combination of a Na⁺–K⁺ adenosine triphosphatase
(ATPase) inhibitor and a K⁺ channel blocker recently
reported to inhibit the EDHF-mediated response in this
artery²⁵), and KCl (40 mM) depolarization (established
intervention to eliminate the influence of EDHF) on
those relaxations. Furthermore, because acetylcholine
has been suggested to stimulate the endothelial produc-
tion of prostacyclin that may cause the release of NO or
hyperpolarize the VSM membrane,²⁶,²⁸ we also evalu-
ated the effects of indomethacin (cyclooxygenase inhib-
itor) on the acetylcholine relaxation. In these experi-
ments, we found that both the acetylcholine-induced and
the histamine-induced vasorelaxations consist of the
NO-mediated and EDHF-mediated components.

We next characterized the contractile response to nor-
epinephrine, using the aforementioned inhibitors of the
NO or EDHF pathways. Specifically, we evaluated the
effects of endothelial denudation, LNNA, tetraethylam-
monium, and ouabain plus Ba²⁺ on the norepinephrine
response. The concentrations of these inhibitors and the
preincubation time necessary for them to exert their
maximal effects were determined in the aforementioned
experiments. Concentration–response curves for norepi-
nephrine contraction in the presence and absence of
either LNNA or LNNA plus tetraethylammonium were
constructed for the strips with and without endothe-
lium. After the response to 10 μM norepinephrine be-
came constant, lower concentrations of norepinephrine
were applied for a period ranging from 3 to 5 min (until
such response reached a plateau) at 7-min intervals. The
effects of ouabain plus Ba²⁺ were also investigated on
the norepinephrine (10 μM) response in the LNNA-
treated, unrubbed strips. We additionally evaluated the
effects of indomethacin (3–10 μM),²⁸,²⁸ NDGA (0.3–3 μM,²⁸
lipoxygenase inhibitor), and phenidone (10–30 μM)²⁸
lipoxygenase/cyclooxygenase inhibitor) on the norepi-
nephrine response. In these experiments, we found that
both the NO and the EDHF signaling pathways are in-
volved in the contractile response to norepinephrine.

To evaluate the ability of sevoflurane to inhibit the NO
or EDHF pathways, we tested its effects on both the
NO-mediated and the endothelium-mediated vasorela-
xations caused by either acetylcholine or histamine and
found that sevoflurane inhibits both. Several attempts
were made to gain access to the mechanisms causing its
inhibitory action on the endothelium-mediated relaxation. First, we attempted to evaluate the effects of sevoflurane on endothelium-independent relaxation caused by low concentrations of K+; this has recently been proposed as the EDHF.27 We next attempted to evaluate the effects of sevoflurane on the endothelium-dependent relaxation caused by either A23187 or ionomycin, both of which were previously reported to cause the endothelium-dependent hyperpolarization without acting on the endothelial receptors.26 We finally attempted to confirm the previous observation11,13 that SOD (100–300 U/ml) attenuates the inhibitory action of sevoflurane on the NO-mediated relaxation.

These results indeed suggested the possibility that sevoflurane enhances the norepinephrine contractile response by inhibiting the NO or EDHF pathways in this artery. Therefore, we then evaluated the effects of various inhibitors of these pathways, including LNNA, methylene blue, oxyhemoglobin, and tetraethylammonium, on the enhanced response to norepinephrine by sevoflurane. However, sevoflurane still enhanced the norepinephrine response after treatment with these inhibitors. Therefore, we next evaluated a possibility that sevoflurane stimulates the release of an endothelium-derived vasoconstricting factor (EDCF) and thereby enhances the norepinephrine response. Cyclooxygenase products (e.g., thromboxane A2, prostaglandin F2), lipoxygenase products, endothelin-1 (ET-1), angiotensin-II (AT-II), serotonin (5-HT), histamine, adenosine triphosphate (ATP), adenosine diphosphate, and superoxide anions all have been suggested to act as EDCFs.26 In our preliminary experiments, in the –E strips, U46619 (thromboxane–prostaglandin endoperoxide analogue), ET-1, AT-II, and 5-HT all caused significant contractions, both in the presence and the absence of norepinephrine (0.5 μM ≈ 45% effective concentration [EC45]); however, histamine (≤ 300 μM; n = 4), ATP (≤ 10 μM; n = 4) or adenosine diphosphate (≤10 μM, n = 4) did not cause any significant contraction in either presence or absence of the submaximal concentration (0.5 μM) of norepinephrine. We therefore did not evaluate the involvement of histamine, ATP, or adenosine diphosphate in the enhanced norepinephrine response by sevoflurane.

Although sevoflurane did not significantly increase the basal force level in these experiments, there still exists the possibility that sevoflurane enhances the norepinephrine response by stimulating the EDCF release. It should be theoretically possible that the agent enhances the force level only in the presence of agonist stimulation (e.g., in the increased Ca2+ level). In support of this
Sevoflurane from the experimental chamber (i.e., after removal of sevoflurane from the extracellular environment).

**Sevoflurane Delivery and Analysis**

Sevoflurane was delivered via a calibrated sevoflurane vaporizer (Sevotec 3; Ohmeda, Steeton, West Yorkshire, UK) in line with the air gas aerating the HEPES-buffered solutions. Each solution was equilibrated with sevoflurane 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 reported concentrations of sevoflurane in the physiologic salt solution produced by 0.9, 1.9, 2.8 (1 minimum alveolar concentration [MAC] in the Sprague-Dawley rat31) and 3.7% sevoflurane during exactly the same experimental condition;7 and the obtained values were within 95% (95.4–98.5%) of theoretical values predicted by the partition coefficient of sevoflurane in water (0.36 at 37°C). Excellent linear relation was obtained between the aqueous concentrations of sevoflurane (y) and its concentrations (vol%) in the gas mixture (x) (y = 0.0028 + 0.13 x; r = 0.9993).7 Therefore, the concentrations produced by 3 and 5% sevoflurane in the physiologic salt solution in our experiments can be predicted as 0.40 and 0.67 mM, respectively. The latter concentration is almost equal to a recently reported concentration of sevoflurane in blood sampled from this rat during steady state anesthesia with 2.8% (1 MAC in this rat31) sevoflurane; i.e., 0.66 mM.5

**Calculation and Data Analysis**

The acetylcholine relaxation, histamine relaxation, and norepinephrine contraction were assessed at the points at which relaxing or constricting effects reached a maximum. Similarly, the effects of sevoflurane on acetylcholine relaxation, histamine relaxation, norepinephrine contraction, or KCl contraction were assessed at the points at which effects reached a maximum. Changes in force were expressed as the percent value of the reference. The concentration–response data for acetylcholine relaxation, histamine relaxation, or norepinephrine contraction, and some effects of sevoflurane, were fitted according to a logistic model described by De Lean et al.32 The 50% effective concentration (EC50) or the 50% inhibitory concentration (IC50) values were derived from the least-squares fit using the aforementioned model. Because the relation between actual concentrations of sevoflurane 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 percent for the sevoflurane concentration–response relations.

**Statistics**

All results are expressed as the mean ± SD, except the results shown in the figures that are expressed as the mean ± SEM for their clarity. The n denotes the number of preparations. The statistical assessment of the data was made by an analysis of variance (one- or two-factor), a Scheffé F test, and a Student t test (paired or unpaired), as appropriate. Comparisons of the effects of acetylcholine, histamine, norepinephrine, and sevoflurane among (or between) treatments were made by two-factor (concentration, treatment) analysis of variance for repeated measures or factorial analysis of variance. When overall differences were detected, individual comparisons among (or between) groups at each concentration were performed by the Scheffé F test. Comparisons of the effects of these agents within each treatment group were made by one-factor (concentration) analysis of variance for repeated measures, and post hoc comparisons were made using the Scheffé F test for multiple comparisons. Similarly, statistical assessment of the data on the time-dependent effects of sevoflurane was made by one- or two-factor analysis of variance, with repeated measures or factorial followed by the Scheffé F test. All other necessary comparisons between two data were made by a two-tailed, paired or unpaired, Student t test after confirming the equal population variances between the groups. A level of P < 0.05 was considered significant.

**Results**

**Effects of Sevoflurane on Contractile Response to Either Norepinephrine or Potassium Chloride**

Sevoflurane (≈ 2%) enhanced submaximal (0.5–1 μM) and maximal (10 μM) contractile responses to norepinephrine in the +E strips; the responses to 0.5, 0.6, and 1 μM norepinephrine were enhanced to 128.4 ± 8.4%, 124.6 ± 15.3%, and 122.9 ± 11.4% of control, respectively, by 3% sevoflurane (P < 0.05; n = 7; data for 10 μM norepinephrine; see fig. 1). However, in the −E strips, sevoflurane (≈ 3%) inhibited submaximal (0.5 μM; ≈ EC50) and maximal (10 μM) contractile responses to norepinephrine (fig. 1). Sevoflurane did not influence contractile response to KCl in the +E strips, whereas it inhibited the response to KCl in the −E strips (fig. 1).
Effects of Sevoflurane on the Endothelium-dependent Relaxation Caused by Acetylcholine or Histamine

Acetylcholine and histamine both produced endothelium-dependent relaxation in the presence of either high K⁺ (40 mM) or norepinephrine (10 μM; fig. 2A). LNNA (100 μM; 60 min) eliminated the acetylcholine relaxation in the presence of KCl (fig. 2A); however, LNNA (100–300 μM; 60 min) only partly inhibited the relaxation caused by either acetylcholine or histamine in the presence of norepinephrine (fig. 2A). Such LNNA-resistant relaxation caused by either acetylcholine or histamine was strongly inhibited or eliminated by tetrachlomethonium (10–30 mM), ouabain (1 mM) plus Ba²⁺ (30 μM), or KCl (40 mM) (fig. 2A). Indomethacin (10 μM; 60 min) did not influence the acetylcholine relaxation (fig. 2A). Sevoflurane inhibited both the LNNA-sensitive and -resistant components of the endothelium-dependent vasorelaxation induced by either acetylcholine or histamine in the presence of norepinephrine (fig. 2B).

The relaxant responses to the low concentrations (10–20 mM) of K⁺ were not constantly observed in our preparations. In addition, both A23187 (≤ 10 μM) and ionomycin (≤ 10 μM) did not cause any significant relaxation in the LNNA (100 μM)-treated, +E strips precontracted with 10 μM norepinephrine (n = 4). We therefore did not further evaluate the effects of sevoflurane on the relaxation induced by either the low concentrations of K⁺ or those Ca²⁺ ionophores.

Superoxide dismutase (100–300 U/ml) did not influence the sevoflurane (3%)-induced inhibition of the acetylcholine (0.03 μM)-induced, LNNA-sensitive relaxations in the +E strips precontracted with 10 μM norepinephrine (P < 0.05; n = 4): control inhibition (64.9 ± 24.5%); inhibition in the presence of 100 U/ml SOD (74.4 ± 17.5%); inhibition in the presence of 300 U/ml (71.9 ± 32.6%).

Characterization of the Contractile Response to Norepinephrine

Norepinephrine produced concentration-dependent (+E, ≥ 1 μM; −E, ≥ 0.3 μM) contraction in both the +E
Fig. 2. (A: a and b) Effects of L-arginine (LNNA), LNNA plus tetraethylammonium (TEA) or LNNA plus ouabain plus Ba2+ on the acetylcholine-induced relaxation in the endothelium-intact (+E) strips precontracted with either potassium chloride [KCl] (40 mM; a) or norepinephrine (NE; 10 μM; b). The IC50 values of the control acetylcholine relaxation in the presence of high K+ and norepinephrine were 0.02 and 0.05 μM, respectively, and those of the acetylcholine relaxation in the presence of norepinephrine after exposure to LNNA (100 μM) and LNNA plus tetraethylammonium (10 μM) were 0.24 and 4.74 μM, respectively. (A: c) Typical examples of the effects of either tetraethylammonium (left) or KCl depolarization (right) on the acetylcholine relaxation in the LNNA-treated +E strips precontracted with norepinephrine. Identical results were obtained in other several strips. (A: d) Effects of LNNA, LNNA plus tetraethylammonium or LNNA plus ouabain plus Ba2+ on the histamine relaxation in the +E strips precontracted with norepinephrine. The IC50 values before and after the LNNA treatment were 3.4 and 12.7 μM, respectively. *P < 0.05 Control groups versus LNNA groups at each concentration. #P < 0.05 Control groups versus groups treated with either LNNA plus tetraethylammonium or LNNA plus ouabain plus Ba2+ at each concentration. (B: a and b) Inhibition by sevoflurane (SEVO) of both the LNNA (100 μM)-sensitive (a) and -resistant (b) relaxations caused acetylcholine in the +E strips precontracted with norepinephrine (10 μM). (B: c) Inhibition by 5% sevoflurane of both the LNNA-sensitive (left; 3 μM, gray; 10 μM, white) and LNNA-resistant (right; 30 μM, black) vasorelaxations caused by histamine in the +E strips precontracted with norepinephrine (10 μM). *P < 0.05 versus control (100%). C = control.
and the \(-E\) strips (figs. 3A and B). The LNNA treatment (100 \(\mu M\); 60 min) enhanced the contractile response to norepinephrine in the \(+E\) strips (figs. 3A and B). The treatment with either tetraethylammonium (10 m\(M\)) or ouabain (1 m\(M\)) plus Ba\(^{2+}\) (30 \(\mu M\)) further enhanced the contractile response to norepinephrine in the LNNA-treated, \(+E\) strips (figs. 3A and B). However, neither LNNA (100 \(\mu M\)) nor LNNA plus tetraethylammonium (10 m\(M\)) influenced the concentration–response curve for norepinephrine contraction in the \(-E\) strips \((P \geq 0.05; \text{fig. 3C})\). The treatment with indomethacin (3–10 m\(M\); 60 min) modestly attenuated the response to norepinephrine in the \(+E\) strips; the norepinephrine (10 \(\mu M\)) contraction was inhibited to 88.9 \(\pm\) 5.8% and 72.0 \(\pm\) 6.6% of control by 3 and 10 \(\mu M\) indomethacin, respectively \((P \leq 0.05; n = 5 \text{ or } 6)\). Both NDGA (0.3–3 \(\mu M\); 15 min) and phenidone (10–30 \(\mu M\); 25 min) also attenuated the contractile response to 10 \(\mu M\) norepinephrine in the \(+E\) strips \((P \leq 0.05; n = 5 \text{–} 7; 0.3 \mu M\) NDGA, 71.8 \(\pm\) 28.6% of control; 1 \(\mu M\) NDGA, 61.6 \(\pm\) 54.9% of control; 10 \(\mu M\) phenidone, 85.0 \(\pm\) 15.2% of control; 30 \(\mu M\) phenidone, 64.9 \(\pm\) 11.5% of control).
Phenidone (100 μM) + TEA (10 mM) 60 NO, EDHF 0.7 ± 0.2 141.7 ± 15.8* 152.2 ± 13.8’ NS 4
MB (3 μM) + HbO₂ (10 μM) + LT 60 NO, EDHF 1.9 ± 1.1 123.6 ± 19.6’ 136.1 ± 28.4’ NS 7
Indomethacin (10 μM) + LT 80 COX, NO, EDHF 1.3 ± 0.3 134.2 ± 30.1’ 141.5 ± 38.8’ NS 6
NDGA (0.3 μM) + LT 80 LOX, NO, EDHF 2.6 ± 4.1 144.4 ± 23.3* 133.1 ± 26.1’ NS 5
NDGA (1 μM) + LT 80 † 8.8 ± 12.0 † 153.0 ± 33.4’ NS 5
Phenidone (30 μM) + LT 60 KCl 10 136.5 ± 34.6’ NS 6
Phenidone (100 μM) + LT 80 † 1.5 ± 1.2 † 136.5 ± 34.6’ NS 6
BQ123 (1 μM) + BQ788 (1 μM) 30 ET receptors 10 128.5 ± 16.9* 136.4 ± 21.1’ NS 7
BQ123 (3 μM) + BQ788 (3 μM) 30 † 10 † 136.5 ± 24.9’ NS 7
Losartan (1 μM) 30 AT-II receptor 10 139.2 ± 46.7* 142.0 ± 31.6’ NS 11
Losartan (3 μM) 30 † 10 † 154.9 ± 54.6’ NS 11
Losartan (10 μM) 30 † 10 † 160.7 ± 44.3’ NS 11
Ketanserin (0.03 μM) 30 5-HT receptor 10 149.5 ± 26.9* 140.8 ± 21.3’ NS 6
Ketanserin (0.1 μM) 30 † 10 † 150.6 ± 34.4’ NS 6
SOD (100 U/ml) 30 Superoxide 10 133.9 ± 15.6* 131.9 ± 19.6’ NS 5
SOD (300 U/ml) 30 † 10 139.6 ± 14.9* 143.8 ± 11.6’ NS 3

Table 1. The Inability of Various Pharmacologic Treatments to Eliminate the Sevoflurane (3%)-induced Endothelium-dependent Enhancement of Contractile Response to Norepinephrine

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Pretreatment Time (min)</th>
<th>Inhibited Pathways/Blocked Receptors</th>
<th>NE Conc. (μM) After Treatment</th>
<th>Enhancement (%) of NE Response</th>
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<td>Control (10 μM NE)</td>
<td>Treatment n</td>
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* P < 0.05 versus control (before sevoflurane; 100%) response to NE in each condition.
† Same as either description or value immediately above the column.

**Effects of Various Pharmacologic Interventions on the Enhanced Contractile Response to Norepinephrine by Sevoflurane in the Presence of Endothelium**

In the LNNA-treated strips, sevoflurane did not influence the maximal response to 10 μM norepinephrine that had notably been enhanced after exposure to LNNA (fig. 4). However, in the LNNA-treated strips, sevoflurane still enhanced the submaximal response to lower concentrations of norepinephrine (0.86 ± 0.53 μM), the amplitude of which was not different from that of the control response to 10 μM norepinephrine (94.9 ± 29.4% of the control; P > 0.05; n = 7; fig. 4). The sevoflurane (3%)-induced enhancement of the response to the low concentrations of norepinephrine after exposure to LNNA was not significantly different from that of the submaximal response to either 0.5, 0.6, or 1 μM norepinephrine in the LNNA-untreated strips (P > 0.05; data shown previously).

Sevoflurane still enhanced the contractile response to norepinephrine after treatment with either LNNA plus tetraethylammonium, LNNA plus tetraethylammonium plus methylene blue plus oxyhemoglobin, indomethacin, NDGA, or phenidone (table 1). Because all of these treatments enhanced (P < 0.05) the response to 10 μM norepinephrine, the effects of these treatments on the vasoconstricting action of sevoflurane were studied for contractions induced by lower concentrations of norepinephrine, the amplitude of which was not significantly different from that of the control 10 μM norepinephrine-induced contraction (P > 0.05; n = 5 or 6; table 1).

Antagonists of either ET-1 (ET_A and ET_B), AT-II type 1, or 5-HT receptors also failed to prevent the enhanced response to norepinephrine by sevoflurane (table 1). SOD (100–300 U/ml) also did not influence the sevoflurane-induced enhancement of norepinephrine response (table 1).

**Persistent Vascular Hyporesponsiveness to Norepinephrine after Washout of Sevoflurane**

Constant responses to either norepinephrine or KCl were observed in either +E or −E strips for more than 4 h in our experiments. As described previously, in the +E strips, the response to norepinephrine was enhanced during application of sevoflurane; however, it was significantly inhibited after washout from the chamber (fig. 5A). This inhibition was prolonged for 15–75 min, depending on the preparations (fig. 5A). Compara-
son of the time-dependent effects of sevoflurane be-
tween two different protocols regarding time for sevoflu-
rane application (i.e., short vs. long application
protocols) indicated that the “postwashout inhibition”
was triggered by the washout of sevoflurane, but not due
to emergence of some “late-onset” inhibition (fig. 5A, d).
Identical postwashout inhibitions were caused by 5%
sevoflurane in the +E strips treated with LNNA, LNNA
plus tetraethylammonium, or LNNA plus tetraethylam-
monium plus indomethacin ($P < 0.05; n = 4–6$).

In the −E strips, sevoflurane, applied after contractile
responses to norepinephrine (0.5 μM) became constant,
inhibited the contractile response to norepinephrine
(fig. 5B). Fifteen minutes was necessary for the inhibi-
tory effect of sevoflurane to reach maximum and steady
state (fig 5B). The inhibition was again prolonged after
washout of sevoflurane from the chamber, and it took
more than 30 min for VSM cells to completely recover
from the inhibition (fig. 5B).

In contrast, the onset of sevoflurane-induced inhibition
of KCl contraction in the −E strips was immediate; five
minutes was enough for its inhibitory effect on contrac-
tile response to KCl to reach maximum and steady state
(fig 5C). No significant inhibition of the KCl contrac-
tion was observed after washout of sevoflurane in either +E
or −E strip (fig. 5C).

Discussion

Relevance to the In Vivo Circulatory Action of
Sevoflurane

The actions of sevoflurane on isolated “endothelium-
intact” resistance arteries observed in this study, as sum-
marized below, do not appear to contribute to systemic
hypotension previously observed during sevoflurane an-
esthesia.$^{1–5}$ First, in the presence of endothelium,
sevoflurane did not inhibit, but enhanced, contractile
response to the sympathetic neurotransmitter norepi-
nephrine. Second, in the presence of endothelium,
sevoflurane did not influence contractile response to
KCl, which is mediated by activation of voltage-gated
$Ca^{2+}$ channels. Finally, sevoflurane inhibited both the
NO-mediated and the EDHF-mediated vasorelaxations.
Rather, our data suggest a possibility that, in the endo-
thelium-intact mesenteric arterial bed, sevoflurane
causes an increase in vascular tone and thereby a de-
crease intestinal blood flow through direct actions on
VSM cells. However, no significant changes in intestinal
blood flow or vascular resistance were observed in this
rat anesthetized with sevoflurane.$^{2,5}$ Therefore, sevoflu-
rane probably has some “indirect” vasodilating actions
on this arterial bed, counteracting the direct vasoco-
stricting action. Centrally and peripherally attenuated
neural excitatory activity may account for such vasodi-

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Fig. 4. Effects of $N^\omega$-nitro-L-arginine (LNNA; 100 μM, 60 min) on the sevoflurane-induced enhancement of contractile response to
norepinephrine (NE). (A) Examples of control enhancement of the response to 10 μM norepinephrine by sevoflurane. Examples of
the effects of sevoflurane on both the maximal (upper, 10 μM) and submaximal (lower, 1.5 μM) responses to norepinephrine in the
LNNA-treated, +E strips. Note notable enhancement of the response to norepinephrine (10 μM) after treatment with LNNA as
indicated by the arrow. The amplitude of contraction induced by 1.5 μM norepinephrine after treatment with LNNA was almost
identical to that of the control (before LNNA) 10-μM norepinephrine contraction. (C) Analyzed data. *$P < 0.05$ versus control within
each group. C = control; +E = endothelium-intact strips; low norepinephrine = low concentrations of norepinephrine (see text).
Fig. 5. (A) Time-dependent effects of sevoflurane on contractile responses to norepinephrine in the endothelium-intact (+E) strips. (a, b) Typical examples. (Upper; a) Shows time control data of the norepinephrine (NE) responses, whereas the lower (b) shows the effects of sevoflurane. Time (min) described (upper, a; lower, b) indicates the time after the first application of norepinephrine and the time after washout of sevoflurane, respectively. Time in parentheses indicates the interval for norepinephrine applications. (c, d) Analyzed data regarding (c) the time-dependent effects of sevoflurane on the norepinephrine response, and (d) the time-dependent effects of 5% sevoflurane on the norepinephrine response, with two different protocols on the application time for sevoflurane (20 vs. 30 min). The data obtained in the experiments in which sevoflurane was applied for 20 min were shown as closed circles (on the x-axis), whereas the data obtained in the experiments in which sevoflurane was applied for 30 min are indicated as open circles (on the x-axis). (B, C) Time-dependent effects of sevoflurane on the response to 0.5 μM norepinephrine in the −E strips (B) and those on the response to potassium chloride (40 mM) in either the +E (open circles) or −E (closed circles) strips (C). *P < 0.05 versus control within each group. #P < 0.05 versus the short-application group 5 min after washout of sevoflurane. Af = time points at which the effect of sevoflurane reached steady state (final application); Ax = x min after application of sevoflurane; C0 = precontrol 1; C1 = precontrol 2; C2 = postcontrol; W = washout of sevoflurane; Wx = x min after washout of sevoflurane.
lating actions. Sevoflurane was recently reported to attenuate this mesenteric arterial tone by inhibiting norepinephrine outflow from the nerve terminals and thereby causing the “in situ hyperpolarization.”5

It was recently reported that the systemic hypotension persists after anesthesia with sevoflurane in this rat.5 Recovery of mean arterial pressure was incomplete (≈70–85%) during the postanesthesia period after inhalation of 0.5–1.0 MAC (0.22–0.66 mM in blood) sevoflurane; i.e., after a 15–30 min washout period during which its blood concentration became negligible (0.01–0.02 mM).5 In addition, the in situ hyperpolarization of VSM cell membrane induced by sevoflurane disappeared during the postanesthetic period.5 In our study, the concentrations produced by 3–5% sevoflurane in the physiologic salt solution would be approximately 0.40–0.67 mM. Thus, the persistent hypo responsiveness to norepinephrine after washout of sevoflurane observed in our study may contribute to the prolonged systemic hypotension observed after sevoflurane anesthetic in this rat.5

The direct action of sevoflurane on contractile response to norepinephrine in this artery appears to consist of two distinct components; i.e., an endothelial and a smooth muscle component. The former enhances the norepinephrine response, whereas the latter inhibits it. In the presence of intact endothelium, the endothelial component predominates over the smooth muscle component, enhancing the norepinephrine response. Sevoflurane might inhibit the norepinephrine response in a situation in which the endothelial function is impaired. The endothelial vasoconstricting component immediately emerged after application of sevoflurane and quickly disappeared after its removal, whereas the smooth muscle vasodilating component emerged relatively gradually after application of sevoflurane and persisted after its removal. However, such prolonged inhibition was not observed in the action of sevoflurane on KCl response. This indicates that the mechanisms causing the inhibitory action of sevoflurane on norepinephrine contraction are at least in part different from those on KCl contraction.

Although sevoflurane enhanced the norepinephrine contraction in this resistance artery, previous studies using isolated arteries yielded conflicting results regarding this issue.6,7,11,13,35 In endothelium-intact canine mesenteric arteries, sevoflurane, depending on timing of its application, did not influence or enhance the norepinephrine contraction,11,35 whereas it attenuated the response to norepinephrine or phenylephrine in either the presence or the absence of endothelium in rabbit mesenteric arteries or rat aorta.6,7,13 The observed differences could be caused by either species or regional differences. Unlike in the rat mesenteric arteries, the smooth muscle vasodilating component might predominate over the endothelial vasoconstricting component in those vascular beds.

Effects of Sevoflurane on Endothelium-mediated Vasorelaxation

Sevoflurane inhibited both the LNNA-sensitive and the LNNA-resistant components of either acetylcholine-induced or histamine-induced relaxation. Either tetraethylammonium or ouabain plus Ba2+, both of which have been proposed to inhibit the action of EDHF,27,34 inhibited the latter component. Therefore, the LNNA-resistant component is presumably mediated by EDHF, suggesting the ability of sevoflurane to inhibit the EDHF pathway in addition to the NO pathway. The high concentrations (10–30 mM) of tetraethylammonium, known to act as a nonspecific K+ channel blocker,35,36 probably inhibited K+ channels of both endothelial and VSM cells involved in the EDHF response.27 Conversely, ouabain plus Ba2+ inhibited Na+-K+ ATPase and Ba2+-sensitive K+ channels of VSM cells involved in the EDHF responses.27 In addition, the high concentration (1 mM) of ouabain, known to inhibit K+ channels in VSM,34 might also inhibit those K+ channels.

It was previously proposed that volatile anesthetics, including sevoflurane, interfere with the EDHF-mediated relaxation in addition to the NO-mediated relaxation,12,17 although, to our knowledge, there has been no electrophysiologic evidence showing that the anesthetics inhibit the EDHF-mediated hyperpolarization. In addition, previous studies12,17 evaluated the anesthetic effects only on the acetylcholine relaxation. However, because the volatile anesthetics have been suggested to inhibit acetylcholine signaling by interfering with the muscarinic receptor,37 the anesthetic might globally inhibit the acetylcholine relaxation by acting on the muscarinic receptor in those studies12,17; it was therefore unclear whether the anesthetic inhibited some mechanisms specifically involved in the EDHF response. However, in this study, sevoflurane inhibited both the acetylcholine- and the histamine-induced, LNNA-resistant (i.e., presumed EDHF-mediated) relaxations. This may imply that sevoflurane interferes with the EDHF-mediated response by acting on sites distal to the receptor stimulation. To our knowledge, there is no evidence that volatile anesthetics interfere with the histaminergic re-

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ceptor. To further investigate the site for the inhibitory action of sevoflurane on the EDHF-mediated response, as detailed in Materials and Methods, we attempted to evaluate the effects of sevoflurane on vasorelaxation caused by either \( \text{Ca}^{2+} \) ionophores or the low concentrations of \( \text{K}^+ \). However, we could not perform these experiments because of the reasons described in Results.

The observed lack of effect of SOD on the inhibitory action of sevoflurane on NO-mediated relaxation is contradictory to previous studies\(^{11,13} \) that propose that sevoflurane impairs the NO-mediated relaxation by generating superoxide free radicals and thereby inactivating NO. Although the previous studies were performed in a hyperoxic environment (95% \( \text{O}_2 \)), our experiments were performed in a nonhyperoxic environment (i.e., air). Therefore, sevoflurane might fail to generate a significant amount of superoxide free radicals in our study. Our results are rather consistent with a previous study\(^{38} \) which showed the inability of sevoflurane to chemically interact with NO and its ability to directly inhibit NO production in endothelial cells.

**The Endothelium-dependent Vasoconstricting Action of Sevoflurane**

The observed effects of LNNA, tetrathylammonium, and ouabain plus \( \text{Ba}^{2+} \) on contractile response to norepinephrine indicate that both the NO–cyclic \( 3',5' \)-guanosine monophosphate and EDHF pathways are involved in the norepinephrine response, consistent with previous proposals\(^{12,15,16} \). In addition, as discussed previously, the observed effects of sevoflurane on the endothelium-mediated relaxation suggest its ability to inhibit both the NO and the EDHF pathways in this artery. It is therefore conceivable that sevoflurane enhances the contractile response to norepinephrine by inhibiting the NO or the EDHF pathway. However, sevoflurane still enhanced the norepinephrine response after inhibition of these pathways. Sevoflurane may inhibit some cellular mechanisms specifically involved in the NO-mediated or EDHF-mediated responses induced by acetylcholine or histamine but not by norepinephrine. Although LNNA eliminated the sevoflurane-induced enhancement of the maximal response to norepinephrine (10 \( \mu \text{m} \)), we do not believe that inhibition of the NO pathway accounts for the enhanced response to norepinephrine by sevoflurane. Because the response to 10 \( \mu \text{m} \) norepinephrine was already notably enhanced, possibly saturated, after exposure to LNNA (at application of sevoflurane), sevoflurane probably failed to further enhance such nearly saturated response to norepinephrine. Our results also indicate that the vasoconstricting action of sevoflurane is not specific to higher (e.g., 10 \( \mu \text{m} \)) concentrations of norepinephrine. Although we do not deny the involvement of inhibition of the NO pathway in the enhanced norepinephrine response by sevoflurane, we believe that such involvement is minimal, if there is any. We rather believe that some mechanism other than inhibition of the NO pathway is mainly attributable to the enhanced response to norepinephrine.

Comparison of the effects of sevoflurane on KCl (40 \( \mu \text{m} \)) response between +E and –E strips indicate the presence of an endothelium-dependent vasoconstricting component in its action on KCl response. Because endothelial function is globally inhibited in the presence of KCl (40 \( \mu \text{m} \)) depolarization because of a lack of hyperpolarization caused by \( \text{K}^+ \) channel opening and a resultant reduced driving force for transmembrane \( \text{Ca}^{2+} \) influx into endothelial cells, the endothelium-mediated action of sevoflurane could be inhibited to some extent in the strips depolarized with KCl. Thus, sevoflurane would fail to enhance the KCl response in the presence of endothelium. However, because sevoflurane still exerts the endothelium-dependent vasoconstricting action in the KCl-depolarized strips, the aforementioned idea is supported: that the enhanced contractile response by sevoflurane is not caused by inhibition of the EDHF pathway.

Volatile anesthetics were previously proposed to stimulate the release of EDCF\S such as cyclooxygenase products or oxygen-derived free radicals and thereby enhance contractile responses.\(^{39,40} \) However, the observed lack of effect of indomethacin, phenindone, NDGA, or SOD on the vasoconstricting action of sevoflurane indicates that cyclooxygenase products, lipoxygenase products, or oxygen-derived free radicals are not involved in the action. In addition, sevoflurane still enhanced the norepinephrine response after blockade of ET-1, AT-II, or 5-HT receptors, suggesting that neither of these receptor agonists is involved in the vasoconstricting action. Sevoflurane may enhance the norepinephrine response by stimulating the release of some other unidentified EDCFs or by inhibiting some unidentified endothelium-mediated vasodilatory mechanisms activated by norepinephrine. Further investigations are necessary to elucidate the mechanisms.

**Summary**

Sevoflurane significantly influences contractile response to norepinephrine in isolated mesenteric resis-
tance arteries, and its action consists of an endothelial and a smooth muscle component. The former enhances the norepinephrine response, whereas the latter inhibits it. In the presence of intact endothelium, the endothelial component predominates over the smooth muscle component, causing enhancement of the norepinephrine response. However, only the smooth muscle component persists after washout of sevoflurane, leading to the prolonged inhibition of the norepinephrine response and possibly contributing to the prolonged systemic hypotension after sevoflurane anesthesia. Such persistent hyporesponsiveness to norepinephrine is caused by an effect on some cellular mechanism specifically involved in the norepinephrine response, but not in the KCI response. Although our results indicate significant involvement of both the NO and the EDHF pathways in the norepinephrine response and the ability of sevoflurane to inhibit both the NO-mediated and the EDHF-mediated responses to either acetylcholine or histamine, sevoflurane still enhanced the norepinephrine response after inhibition of these pathways. This suggests that the enhanced response to norepinephrine by sevoflurane is not caused by inhibition of either the NO or the EDHF pathway, and also that sevoflurane inhibits some cellular mechanisms specifically involved in the NO-mediated or EDHF-mediated responses induced by acetylcholine or histamine (but not by norepinephrine). Finally, cyclooxygenase products, lipoxygenase products, oxygen-derived free radicals, ET-1, AT-II, or 5-HT are not involved in the vasoconstricting action of sevoflurane.

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