Diabetes-associated Alterations in Volatile Anesthetic Actions on Contractile Response to Norepinephrine in Isolated Mesenteric Resistance Arteries

Jun Yoshino, M.D.,* Takashi Akata, M.D., Ph.D.,† Kazuhiro Shirozu, M.D.,* Kaoru Izumi, M.D., Ph.D.,‡ Sumio Hoka, M.D., Ph.D.§

ABSTRACT

Background: Clinical concentrations of volatile anesthetics significantly influence contractile response to the sympathetic neurotransmitter norepinephrine although its precise mechanisms remain unclarified. In this study, we investigated its possible alterations in diabetes, as well as its underlying mechanisms.

Methods: Isometric force was recorded in small mesenteric arteries from streptozotocin-induced diabetic and age-matched control rats.

Results: The concentration–response curve for acetylcholine-induced endothelium-dependent relaxation was shifted to the right in diabetic arteries compared with controls. The concentration–response curve for norepinephrine-induced contraction was shifted to the left and upward by both endothelial denudation and diabetic induction. In the presence of endothelium, isoflurane or sevoflurane enhanced norepinephrine-induced contraction in control arteries but not in diabetic arteries; however, in its absence, both anesthetics identically inhibited norepinephrine-induced contraction in both groups. In control arteries, the isoflurane- or sevoflurane-induced enhanced enhancement was not affected by adrenomedullin22–52, calcitonin gene-related peptide8–37, 18β-glycyrrhetinic acid, N²-nitro L-arginine, ouabain, Ba²⁺, indomethacin, losartan, ketanserin, BQ-123, and BQ-788.

Conclusions: In diabetes, vascular responses to acetylcholine, norepinephrine, and volatile anesthetics are altered in mesenteric resistance arteries, presumably reflecting endothelial dysfunction and possibly underlying circulatory instability during administration of either anesthetic. Some endothelial mechanisms that are impaired in diabetes would be involved in the anesthetic-induced enhancement of norepinephrine-induced contraction. However, the vasoregulatory mechanism mediated by adrenomedullin, calcitonin gene-related peptide, angiotensin II, serotonin, or endothelin-1, all of which have been suggested to be impaired in diabetes, would not be involved in the enhancement.

What We Already Know about This Topic

- Hemodynamic instability from volatile anesthetics is more common in patients with endothelial dysfunction, including diabetes, although the causes are not clear

What This Article Tells Us That Is New

- In mesenteric resistance vessels from rats, volatile anesthetics enhanced contractile response to norepinephrine in an endothelium-dependent manner
- This effect was not present in diabetic rats, possibly underlying the hemodynamic instability during the administration of volatile anesthetics

VOLATILE anesthetics affect various aspects of the cardiovascular system and thereby cause, more or less, hemodynamic instability. The most remarkable change is systemic hypotension, which is attributable to attenuated sympathetic nervous system activity, direct (i.e., nonneural) myocardial depression, and direct peripheral vasodilation. However, they rarely cause profound systemic hypotension in healthy younger patients, but often in patients with cardiovascular dysfunction such as patients with old age, heart diseases, hypertension, diabetes mellitus, or sepsis. Many of the cellular and molecular mechanisms regulating vascular tone (e.g., activities of endothelial cells, ion channels, or protein kinases)—that is, potential targets for volatile anesthetics—are altered in those susceptible populations. Thus, vascular response to volatile anesthetics could be altered in those populations, which may account for their enhanced susceptibility to the hypotensive action of volatile anesthetics.

In diabetes mellitus, a number of vasoregulatory mechanisms are altered, including endothelial vasoregulation, β-adrenergic receptor-mediated vasodilation, arachidonic acid-mediated regulation of Ca²⁺-activated K⁺ channels, etc.
ity of various vasoregulatory enzymes (e.g., adenosine 3',5'-cyclic monophosphate-dependent protein kinase, protein kinase C, and phosphodiesterase), and gap junctional permeability. Endothelial dysfunction leads to widespread atherosclerosis, and diabetic patients are at increased risk for heart attacks and strokes. In addition, diabetic patients are associated with higher perioperative morbidity and mortality as well as an increased incidence of hemodynamic instability during general anesthesia. Such hemodynamic instability would be due to diabetes-associated alterations in cardiovascular regulatory mechanisms and the resultant altered cardiovascular response to general anesthetics. However, its precise mechanisms are not fully understood.

In previous studies with isolated vessels, various volatile anesthetics affected vascular reactivity in an endothelium-dependent manner. In isolated mesenteric resistance arteries, isoflurane, sevoflurane, and halothane all enhanced contractile response to the sympathetic neurotransmitter norepinephrine in an endothelium-dependent manner although its precise mechanisms remain to be clarified. The involvement of endothelial vasoactive factors, such as nitric oxide, endothelin-derived hyperpolarizing factor (EDHF), cyclooxygenase products, lipoxygenase products, endothelin-1, angiotensin II, and serotonin, was previously excluded.

In this study, we hypothesized that volatile anesthetics differently influence contractile response to norepinephrine in diabetes. We therefore compared the effects of isoflurane or sevoflurane on contractile response to norepinephrine in small mesenteric arteries between diabetic and age-matched control rats and found that their endothelium-dependent enhancement was inhibited in diabetic rats. This finding suggested that some of the endothelial vasoregulatory mechanisms that are impaired in diabetes are responsible for the enhancement. Thus, we next investigated the possible involvement of adrenomedullin, calcitonin gene-related peptide (CGRP), myoendothelial gap junction, nitric oxide, EDHF, cyclooxygenase products, angiotensin II, serotonin, and endothelin-1 in the enhancement, because the endothelial vasoregulatory mechanism mediated by any of these substances or the intercellular communication via gap junctions has been suggested to be impaired in diabetic subjects.

Materials and Methods

Animal Care and Diabetic Induction

Care of animals conformed to the recommendations of the Helsinki Declaration and procedures described in this study were approved by the Kyushu University Animal Care and Use Committee (Fukuoka, Japan). For induction of diabetes mellitus, 6-week-old male Wistar rats received an intraperitoneal injection of streptozotocin (50 mg/kg) under anesthesia with ether. The rats were then housed in a temperature- and light-controlled environment and had access to food and water ad libitum until the eve of experiments. Control rats were housed under identical conditions. The rats studied were aged 24–27 weeks. In the morning of the experiment day (i.e., after ~12 h of fasting), in addition to body weight, blood glucose was measured colorimetrically using the O-toluidine method (Ascensia Breeze®, Bayer Healthcare AG, Leverkusen, Germany). Streptozotocin-treated rats were considered diabetic and retained for the experiments if their fasting blood glucose concentration was greater than 300 mg/dl (~16.7 mm). In each rat, blood pressure and heart rate were also measured using the tail-cuff method (Muromachi Kikai, Model MK-1030, Tokyo, Japan).

Vessel Preparation and Force Measurements

Under a binocular microscope, an endothelium-intact (+E) or -denuded (−E) strip was prepared from the small mesenteric artery (third-order branch) of the streptozotocin-induced diabetic (24–27 weeks) or age-matched nondiabetic rats, and isometric force was measured by attaching the strip to a strain gauge transducer (UL-2 type, Shinko Co., Tokyo, Japan), as previously detailed. Briefly, the strip was horizontally mounted on a chamber attached to the stage of a microscope, and the resting tension was adjusted to obtain a maximal response to KCl (40 mM). The solution was changed by infusing it into one end while aspirating simultaneously from the other end.

Endothelial removal was verified by the inability of acetylcholine (10 μM) to cause significant (~10%) relaxation during contractions induced by norepinephrine (10 μM).

Solutions and Drugs

The ionic concentrations of HEPES-buffered physiologic salt solutions were as follows: 138 mM NaCl, 5.0 mM KCl, 1.2 mM MgCl₂, 1.5 mM CaCl₂, 10 mM HEPES, and 10 mM (180 mg/dl) glucose. The pH was adjusted to 7.35 with NaOH, at 35°C; all the experiments were performed at 35°C to prevent early deterioration of the thin vascular strips. The high K⁺ (40 mM) solutions were prepared by replacing NaCl with KCl iso-osmotically.

Norepinephrine, acetylcholine, N⁵-nitro L-arginine (LNN), indomethacin, 18β-glycyrrhetinic acid (18β-GA), and streptozotocin were purchased from Sigma Chemical (St. Louis, MO). HEPES and ouabain were purchased from Nacalai Tesque (Kyoto, Japan). Adrenomedullin, and CGRP were purchased from Peptide Institute (Osaka, Japan). Losartan was purchased from Banyu Pharmaceutical (Tokyo, Japan). Ketanserin tartrate was purchased from Research Biochemicals International (Natick, MA). BQ-123 (cyclo[D-a-aspartyl-l-propyl-D-valyl-L-leucyl-D-tryptophyl]) and BQ-788 (N-[N-(2,6-dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl]-1-(methoxycarboxyl)-D-tryptophyl-D-norleucine monosodium) were obtained from Calbiochem (Darmstadt, Germany). Sevoflurane was purchased from Maruishi Pharmaceutical Co. (Osaka, Japan) and isoflurane from Abbott Japan (Tokyo, Japan). All other reagents were of the highest grade of commercially available reagents.
Volatile Anesthetic Delivery and Analysis

Isoflurane and sevoflurane were delivered via calibrated agent-specific vaporizers in line with the air gas aerating the solutions. Each solution was equilibrated with each anesthetic 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 each anesthetic in the physiologic salt solution produced by multiple concentrations of each anesthetic under the same experimental condition; the obtained values were within 90% of theoretical values predicted by the partition coefficient of each anesthetic in Krebs solution or water. Excellent linear relationship was obtained between the aqueous concentrations of each anesthetic (y) and its concentrations (vol%) in the gas mixture (x): that is, isoflurane, \( y = -0.0068 + 0.21x, r = 0.998 \); sevoflurane, \( y = 0.0028 + 0.13x, r = 0.999 \). Therefore, the concentrations produced by 2–5% isoflurane and sevoflurane in the physiologic salt solution can be predicted as 0.42–1.05 and 0.26–0.67 mM, respectively. Previously reported concentrations of isoflurane and sevoflurane in blood sampled from the rat under steady-state anesthesia with 1 minimum alveolar concentration of each anesthetic (1.5 and 2.8% for isoflurane and sevoflurane, respectively) were 0.65 and 0.66 mM, respectively. Therefore, the aqueous concentrations of either anesthetic at 2–5% (which were tested in this study) can be considered as clinical concentrations.

Experimental Design

After a 60-min equilibration period, each strip was stimulated with 40 mM KCl for 3 min at 7-min intervals so as to obtain reproducible responses. The following experiments were performed after the contractile response to KCl became constant.

In the first series of experiments, to examine the effects of diabetes on endothelium-dependent vasodilator response to acetylcholine, we compared its concentration–response curve in the +E strips between diabetic and control rats. The concentration–response curve was constructed by cumulatively increasing the bath concentration of acetylcholine from 0.1 to 10 \( \mu M \) at 1-min interval—which was sufficient for each concentration of acetylcholine to exert its maximal effect—in the +E strips maximally precontracted with 10 \( \mu M \) norepinephrine.

In the second series of experiments, to examine the effects of diabetes on contractile response to norepinephrine, we compared its concentration–response curve in either +E or −E strips between diabetic and control rats. The concentration–response curve was constructed by cumulatively increasing the bath concentration of norepinephrine from 0.1 to 30 \( \mu M \) at 1-min interval, which was sufficient for each concentration of norepinephrine to exert its maximal (i.e., plateau) effect.

In the third series of experiments, to examine the effects of diabetes on volatile anesthetic actions on contractile response to norepinephrine, we compared the effects of isoflurane (2, 3, and 5%) or sevoflurane (2, 3, and 5%) on contractile response to norepinephrine in either +E or −E strips between diabetic and control rats. The protocols used for this series of experiments were substantially identical to those we previously used in this artery. Briefly, norepinephrine was applied for 3 min at a constant interval (7 min for +E strips; 17 min for −E strips) so as to obtain reproducible responses, and then each concentration of either isoflurane or sevoflurane was applied for 5 min before and during subsequent applications of norepinephrine until steady-state effect was obtained. The concentrations of norepinephrine tested in the +E strips were 2 and 10 \( \mu M \), which almost correspond to a half-maximal effective concentration (EC\(_{50}\)) and a maximal effective concentration (EC\(_{\text{max}}\)), respectively, whereas its concentrations tested in the −E strips were 0.5 and 10 \( \mu M \), which almost correspond to an EC\(_{50}\) and an EC\(_{\text{max}}\), respectively.

In the above experiments with control rats, we confirmed our previous findings that both isoflurane and sevoflurane enhanced contractile response to norepinephrine in an endothelium-dependent manner. In addition, we found that their endothelium-dependent enhancement was attenuated in diabetic rats. These results suggest that some endothelial mechanisms that are impaired in diabetes are involved in the enhancement. Therefore, in the fourth series of experiments with control rats, we investigated the possible involvement of the adrenomedullin, CGRP, and myoendothelial gap junction (i.e., gap junction between vascular smooth muscle and endothelial cells) in the enhancement, because recent evidence has suggested that endothelial vasoregulatory mechanisms mediated by adrenomedullin, CGRP, or intercellular communication through gap junctions are impaired in diabetic subjects. Specifically, we examined the effects of adrenomedullin (22–52) (an adrenomedullin receptor antagonist, 0.3–1 \( \mu M \)), CGRP\(_{3–37}\) (a CGRP receptor antagonist, 0.3–1 \( \mu M \)), and 18\( \beta \)-GA (a gap junction blocker, 3–10 \( \mu M \)) on enhancement in control rats. The concentration of adrenomedullin (22–52) or CGRP\(_{3–37}\), and the preincubation time were decided according to the results of previous experiments in mesenteric vessels. In a previous study with guinea-pig mesenteric arteries, 40 \( \mu M \) of 18\( \beta \)-GA blocked the intercellular electrical coupling between vascular smooth muscle and endothelial cells. However, in our preliminary experiments, 18\( \beta \)-GA at concentrations above 10 \( \mu M \) greatly inhibited contractile response to norepinephrine or KCl (40 mM), consistent with the previous proposal that higher concentrations of 18\( \beta \)-GA may exert nonspecific effects. Thus, the concentrations of 18\( \beta \)-GA tested in this study were 3–10 \( \mu M \).

Besides the vasodilator response to adrenomedullin or CGRP, vascular responses to nitric oxide, EDHF, cyclooxygenase products, endothelin-1, serotonin, and angiotensin II have been reported to be impaired in diabetes. However, in our previous studies, the enhancement was still evident after inhibitions of the nitric oxide, EDHF, and...
cyclooxygenase pathways, or after blockade of endothelin-1, serotonin, and angiotensin II receptors, suggesting that the enhancement is independent of any of those endothelial vasoactive substances. In the fifth series of experiments with control rats, we attempted to confirm those previous findings, using LNNA (a nitric oxide synthase inhibitor, 100 μM), ouabain plus Ba2+ (a combination of a Na+-K+ adenosine triphosphate inhibitor and a K+-channel blocker previously reported to inhibit the EDHF-mediated response in this artery45; 1 mM and 30 μM for ouabain and Ba2+, respectively), indomethacin (a cyclooxygenase inhibitor,45 10 μM), BQ-123 (a selective ET_A receptor antagonist, 1 μM) plus BQ-788 (a selective ET_B receptor antagonist, 1 μM), losartan (a selective AT-II type 1 receptor antagonist, 1–3 μM), and ketanserin (a 5-HT_2A/5-HT_2C receptor antagonist, 0.03–0.1 μM). Because there exist compensatory crosstalk activities between nitric oxide, EDHF, and cyclooxygenase pathways,44,45 the experiments examining the possible involvement of these three pathways were performed after a combined treatment with LNNA, ouabain, Ba2+, and indomethacin. In addition, experiments examining the possible involvement of endothelin-1, angiotensin II, and serotonin, each of which has been proposed to be an endothelium-derived contracting factor,43 were performed after a combined treatment with BQ-123, BQ-788, losartan, and ketanserin. The rationale for our choice of the concentrations of LNNA, ouabain, Ba2+, indomethacin, BQ-123, BQ-788, losartan, and ketanserin, as well as our choice of the preincubation times for these inhibitors (60 min for LNNA, ouabain, Ba2+, and indomethacin; 25 min for BQ-123, BQ-788, losartan, and ketanserin), were previously detailed.21,22

**Calculation and Data Analysis**

Acetylcholine-induced relaxation and norepinephrine-induced contraction were assessed at the points at which its relaxing or constricting effects reached a maximum. Similarly, the effects of isoflurane or sevoflurane on norepinephrine-induced contraction were assessed at the points at which its effects reached a maximum. Changes in force were expressed as the %value of the reference. The concentration–response data for acetylcholine relaxation or norepinephrine contraction were fitted according to a logistic model described by De Lean et al.46 The EC_{50} or 50% inhibitory concentration values were derived from the least-squares fit using the above model.

The isolated arteries used in this study represented prominent rhythmic oscillations in contractile response to norepinephrine in the +E strips, as reported previously.21,22 We thus evaluated the effects of diabetic induction, volatile anesthetics, or various pharmacological inhibitors on the amplitude and frequency of the oscillations in the +E strips. The amplitude was measured from the top to the bottom of each oscillation, excluding erratic peaks, and averaged for the analyses. For comparison between control and diabetic arteries, the amplitude of the oscillations was evaluated as the %value of the amplitude of norepinephrine-induced contraction, which was defined as the amplitude from the baseline to the middle of the oscillations and evaluated 3 min after the application of norepinephrine (immediately before the washout of norepinephrine). On the other hand, in the analyses of the effects of anesthetics or inhibitors on the amplitude or frequency of the oscillations, changes in the amplitude or frequency were expressed as the %value of the reference (i.e., the control value before application of each inhibitor or each anesthetic).

**Statistical Analysis**

All results are expressed as mean ± SD; n denotes the number of preparations. Data were analyzed using ANOVA, contrast, Scheffe F test, F test, Student t test, and Welch t test. Comparisons within each group were made by one-factor ANOVA for repeated measures, and post hoc comparisons were made using the contrast for multiple comparisons. Comparisons among groups were performed by two-factor ANOVA for repeated measures. When overall differences were detected, individual comparisons among groups at each concentration were performed by the Scheffe F test. All other necessary comparisons between the two groups were made by two-tailed Student t test or Welch t test after the homogeneity of variances was determined by the F test. For all tests, P values of less than 0.05 were considered significant.

All the above analyses were made on a computer using GB-Stat v 6.5.6 PPC® (Dynamic Microsystems, Inc., Silver Spring, MD), PowerStats v 0.9® (Shinko Trading Co Ltd., Tokyo, Japan), or SuperANOVA v 1.1® (Abacus Concepts, Inc., Berkeley, CA).

**Results**

**Characteristics of the Animals**

Fasting blood glucose concentration was higher (P < 0.01) in streptozotocin-treated rats (471 ± 131 mg/dl, 26.2 ± 7.3 mm, n = 31) compared with age-matched control rats (115 ± 15 mg/dl, 6.4 ± 0.8 mm, n = 31). In addition, body weight was lower (P < 0.01) in the streptozotocin-treated rats (316 ± 46 g, n = 31) compared with control rats (550 ± 57 g, n = 31). However, no significant difference (P > 0.05) was found in either mean blood pressure (diabetic, 95.1 ± 5 mmHg, n = 31; control, 97 ± 11 mmHg, n = 31) or heart rate (diabetic, 433 ± 45 bpm, n = 31; control, 416 ± 57 bpm, n = 31) between the two groups.

**Vasodilator Response to Acetylcholine**

During stimulation with 10 μM (EC_{max}) norepinephrine, the cumulative application of acetylcholine (0.01–10 μM) produced concentration-dependent relaxation in the +E strips from either the control or diabetic rats. However, acetylcholine even at 10 μM did not exert any relaxing action during stimulation with 10 μM (EC_{max}) norepinephrine in the −E strips from either the control or diabetic rats.
In experiments with both control and diabetic rats, the contractile response to norepinephrine was twice as great as that observed in diabetic rats (EC50 12; control, EC50 12; diabetic, EC50 12). In addition, in the E strips, no significant differences (P = 0.31) were observed in the amplitude of norepinephrine-induced oscillations, evaluated as the % value of the amplitude of norepinephrine-induced contraction, between the two groups (diabetic, 30.2 ± 13.8%; control, 34.2 ± 12.5%; n = 12). In addition, in the +E strips, no significant differences (P = 0.89) were observed in the frequency between the two groups (diabetic, 0.082 ± 0.017 Hz, n = 12; control, 0.083 ± 0.018 Hz, n = 12).

**Volatile Anesthetic Actions on Contractile Response to Norepinephrine**

In the +E strips from control rats, the contractile response to norepinephrine (2 and 10 μM) was enhanced (P < 0.05) during exposure to sevoflurane (2–5%) or isoflurane (2–5%; figs. 2 and 3). However, in the +E strips from diabetic rats, it was not enhanced, but little affected during exposure to sevoflurane (2–5%) or isoflurane (2–5%; figs. 2 and 3).

In the +E strips from both the control and diabetic rats, the contractile response to norepinephrine (2–10 μM) was similarly inhibited (P < 0.05) after washout of either anes-
thetic from the chamber; that is, no significant differences were found in the inhibition 5 min after washout of either anesthetic between the control and diabetic rats (figs. 2 and 3). The norepinephrine response was gradually restored to the control level 15–75 min after washout of either anesthetic in the +E strips from both control and diabetic rats (data not shown).

In the −E strips from either control or diabetic rats, the contractile response to norepinephrine (0.5 and 10 μM) was not enhanced but little affected or significantly inhibited in the +E strips from both control and diabetic rats (data not shown).

Fig. 2. Effects of sevoflurane on contractile response to norepinephrine (NE) in endothelium-intact (+E) strips from control (nondiabetic) or diabetic rats. (A, B) Examples in control (A) and diabetic (B) rats. (C, D) Comparisons of maximal (C) and submaximal (D) contractile responses to NE during exposure to sevoflurane (i.e., 5 min after application of sevoflurane; left in each panel) and 5 min after washout of sevoflurane (right in each panel) between control (open columns) and diabetic (closed columns) rats. EC50 = half-maximal effective concentration, n = 12. * P < 0.05 versus control (100%) within each group. # P < 0.05 versus control rats at each concentration.

Fig. 3. Effects of isoflurane on contractile response to norepinephrine (NE) in endothelium-intact (+E) strips from control (nondiabetic) or diabetic rats. (A, B) Examples in control (A) and diabetic (B) rats. (C, D) Comparisons of maximal (C) and submaximal (D) contractile responses to NE during exposure to isoflurane (i.e., 5 min after application of isoflurane; left in each panel) and 5 min after washout of isoflurane (right in each panel) between control (open columns) and diabetic (closed columns) rats. EC50 = half-maximal effective concentration, n = 12. * P < 0.05 versus control (100%) within each group. # P < 0.05 versus control rats at each concentration.
During exposure to sevoflurane (2–5%) or isoflurane (2–5%), and the inhibition was prolonged after washout of either anesthetic from the chamber (figs. 4 and 5). No significant \( P > 0.05 \) differences were found in the inhibition during exposure to either anesthetic, as well as in the inhibition 5 min after washout of either anesthetic between the control and diabetic rats (figs. 4 and 5).

Both sevoflurane and isoflurane inhibited \( P < 0.05 \) both the amplitude and frequency of the norepinephrine (10 \( \mu M \))-induced oscillations in the +E strips from either control and diabetic rats. EC\(_{50} \) = half-maximal effective concentration, \( n = 10. * P < 0.05 \) versus control (100%) within each group.

---

**(Fig. 4)** Effects of sevoflurane on contractile response to norepinephrine (NE) in endothelium-denuded (−E) strips from control (nondiabetic) or diabetic rats. Examples in control (A) and diabetic (B) rats. Comparisons of maximal (C) and submaximal (D) contractile responses to NE during exposure to sevoflurane (i.e., 5 min after application of sevoflurane; left in each panel) and 5 min after washout of sevoflurane (right in each panel) between control (open columns) and diabetic (closed columns) rats.

**(Fig. 5)** Effects of isoflurane on contractile response to norepinephrine (NE) in endothelium-denuded (−E) strips from control (nondiabetic) or diabetic rats. Examples in control (A) and diabetic (B) rats. Comparisons of maximal (C) and submaximal (D) contractile responses to NE during exposure to isoflurane (i.e., 5 min after application of isoflurane; left in each panel) and 5 min after washout of isoflurane (right in each panel) between control (open columns) and diabetic (closed columns) rats.
**Table 1.** Changes (%) in the Average Amplitude and Frequency of the Norepinephrine-induced Oscillations during Exposure to Sevoflurane or Isoflurane in either Control or Diabetic Arteries

<table>
<thead>
<tr>
<th></th>
<th>Control Arteries</th>
<th>Diabetic Arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amplitude</td>
<td>Frequency</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>67 ± 10†</td>
<td>85 ± 10†</td>
</tr>
<tr>
<td>3%</td>
<td>54 ± 11†</td>
<td>82 ± 11*</td>
</tr>
<tr>
<td>5%</td>
<td>34 ± 16*</td>
<td>79 ± 16†</td>
</tr>
<tr>
<td>Isoflurane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>49 ± 21*</td>
<td>79 ± 13*</td>
</tr>
<tr>
<td>3%</td>
<td>26 ± 14*</td>
<td>70 ± 16*</td>
</tr>
<tr>
<td>5%</td>
<td>14 ± 8</td>
<td>35 ± 21*</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 12).

* P < 0.05 vs. control (100%, before application of either anesthetic).
† P < 0.05 vs. diabetic arteries.

Diabetic or control rats in a concentration-dependent manner (table 1).

**Investigations on the Mechanisms behind the Anesthetic-induced Enhancement of Norepinephrine Response in the Endothelium-intact Strips from Control Rats**

The contractile response to norepinephrine (10 μM) was not affected (P > 0.05) by treatment with adrenomedullin(22–52), CGRP8–37, or 18β-GA (0.3 μM adrenomedullin(22–52), 103 ± 4%, n = 5; 1 μM adrenomedullin(22–52), 105 ± 7%, n = 5; 0.3 μM CGRP8–37, 100 ± 2%, n = 5; 1 μM CGRP8–37, 103 ± 3%, n = 5; 3 μM 18β-GA, 98 ± 6%, n = 8; 10 μM 18β-GA, 112 ± 24%, n = 8). In addition, the enhanced contractile response to norepinephrine by either sevoflurane (5%) or isoflurane (5%) was little affected by treatment with adrenomedullin(22–52) (0.3–1 μM), CGRP8–37 (0.3–1 μM), or 18β-GA (3–10 μM; fig. 6).

The contractile response to norepinephrine (10 μM) was little affected (P > 0.05) by the combined treatment with losartan (1–3 μM), ketanserin (0.03–0.1 μM), BQ-123 (1 μM), and BQ-788 (1 μM); 1 μM losartan + 0.03 μM ketanserin + 1 μM BQ-123 + 1 μM BQ-788, 102.3 ± 13.2%, n = 5; 3 μM losartan + 0.1 μM ketanserin + 1 μM BQ-123 + 1 μM BQ-788, 100.6 ± 11.7%, n = 5. In addition, the enhanced contractile response to norepinephrine by either sevoflurane (5%) or isoflurane (5%) was little affected by the treatment with these receptor blockers (fig. 6).

The contractile response to norepinephrine (10 μM) was greatly enhanced (P < 0.05) by the combined treatment with LNNA (100 μM), ouabain (1 mM), Ba2+ (30 μM), and indomethacin (10 μM; 188.2 ± 38.1% of control, n = 5). Thus, in this condition, the anesthetic effects were examined on contractile response to a lower concentration of norepinephrine (determined in each strip, 1.8 ± 0.5 μM, n = 5), the amplitude of which was identical to that of control contractile response to

---

Fig. 6. Effects of various inhibitors on enhanced contractile response to norepinephrine (NE) by sevoflurane (A) or isoflurane (B) in endothelium-intact strips from control (i.e., nondiabetic) rats. The inhibitors tested in these experiments were adrenomedullin(22–52) (AM22–52, 0.3–1 μM), calcitonin gene-related peptide(8–37) (CGRP8–37, 0.3–1 μM), 18β-glycyrhetinic acid (18β-GA, 3–10 μM), losartan (1–3 μM), ketanserin (0.03–0.1 μM), BQ-123 (1 μM), BQ-788 (1 μM), N5-nitro-L-arginine (LNNA, 100 μM), indomethacin (10 μM), Ba2+ (30 μM), and ouabain (1 mM), n = 5.

* P < 0.05 versus control (100%). NS = not significantly different (P > 0.05).
Table 2. Changes (%) in the Average Amplitude and Frequency of the Norepinephrine-induced Oscillations during Exposure to Various Pharmacological Inhibitors in Control Rats

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Amplitude</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenomedullin</td>
<td>0.3 μM</td>
<td>90 ± 11</td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td>1 μM</td>
<td>111 ± 11</td>
</tr>
<tr>
<td>CGRP B-37</td>
<td>0.3 μM</td>
<td>104 ± 9</td>
</tr>
<tr>
<td>CGRP B-37</td>
<td>1 μM</td>
<td>103 ± 16</td>
</tr>
<tr>
<td>18β-GA</td>
<td>3 μM</td>
<td>82 ± 22*</td>
</tr>
<tr>
<td>18β-GA</td>
<td>10 μM</td>
<td>36 ± 35*</td>
</tr>
<tr>
<td>LKBQ</td>
<td>Low</td>
<td>91 ± 16</td>
</tr>
<tr>
<td>LKBQ</td>
<td>High</td>
<td>95 ± 13</td>
</tr>
<tr>
<td>LOBI</td>
<td>10 μM</td>
<td>41 ± 24*</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 5-8).

* P < 0.05 vs. control (100%).

Discussion

In streptozotocin-treated rats, fasting blood glucose concentration and body weight were significantly higher and lower, respectively, compared with the control rats, indicating successful induction of diabetes by streptozotocin. No significant differences were observed in both blood pressure and heart rate between the control and diabetic rats, implying that the streptozotocin-treated rats had not suffered from hypertension or heart failure, both of which can be associated with diabetes.27,47 However, the endothelium-mediated vasodilator response to acetylcholine was significantly impaired in streptozotocin-treated rats (fig. 1), suggesting that endothelial function had already been impaired 18–21 weeks after the injection of streptozotocin. Because the vasodilator response to acetylcholine in rat small mesenteric artery is mediated by both nitric oxide and EDHF,21 its impairment is likely due to the decreased nitric oxide or EDHF activity associated with diabetes.13 It is believed that the impaired vasoregulatory role of endothelium is an initiating factor in the development of diabetic vascular disease, which is a major cause of morbidity and mortality in the diabetic population.15

In previous experiments with rat small mesenteric arteries,21,48 contractile response to α-adrenergic agonists including norepinephrine was greatly (~1.5–2 fold) enhanced by inhibition of the nitric oxide or EDHF pathway only in the presence of endothelium and not in its absence. Those results suggest that in this artery, in the presence of endothelium, basal, or stimulated release of nitric oxide and EDHF attenuates the contractile response to norepinephrine. In this study, the contractile response to norepinephrine was also enhanced by diabetic induction only in the presence of endothelium and not in its absence, and the concentration–response curve for norepinephrine in the +E strips from diabetic rats was almost overlapped with that in the −E strips from control rats. Thus, the enhancement is also likely due to the diabetes-associated decrease in nitric oxide and EDHF activities. In a previous clinical study,18 increases in blood pressure and systemic vascular resistance after tracheal intubation were much greater in diabetic patients compared with nondiabetic patients. The enhanced contractile response to norepinephrine might underlie such an exaggerated pressor response to noxious stimuli under light anesthesia.

In nondiabetic rats, both sevoflurane and isoflurane enhanced contractile response to norepinephrine in an endothelium-dependent manner, consistent with our previous observation.21,22 In this study, diabetic induction identically affected the sevoflurane- and isoflurane-induced enhancement. Thus, the mechanisms behind sevoflurane-induced enhancement are presumably identical to those behind isoflurane-induced enhancement. As we previously suggested,21,22 the enhancement could be due to inhibition of endothelium-dependent vasodilator mechanisms or stimulation of endothelium-dependent vasoconstrictor mechanisms. Because both the anesthetics did not enhance the norepinephrine response in diabetic rats, the enhancement is likely to be mediated by some endothelial vasoregulatory mechanisms that are impaired in diabetes.

Vascular responses to various endothelium-derived relaxing factors including nitric oxide, EDHF, and prostacyclin, as well as those to various endothelium-derived contracting factors including thromboxane A2, endothelin-1, serotonin, and angiotensin II have been reported to be impaired in diabetes.13,24–26,30 However, in the current experiments with control rats, the enhancement was still evident after inhibition of the nitric oxide, EDHF, and cyclooxygenase pathways, or blockade of endothelin-1, serotonin, and angiotensin II receptors, consistent with our previous findings.21,22 Thus, the enhancement would be, at least in part, independent of nitric oxide, EDHF, cyclooxygenase products, endothelin-1, serotonin, and angiotensin II.

Adrenomedullin, a potent vasodilator peptide widely distributed in various organs, has been shown to be secreted...
from vascular endothelial cells and stimulate adenosine 3',5'-cyclic monophosphate formation in vascular smooth muscle cells, possibly serving as an endothelium-derived relaxing factor and contributing to the regulation of blood pressure in both normal and pathophysiologic states.59 CGRP, the predominant neurotransmitter in the capsaicin-sensitive sensory nerves and the most potent endogenous vasodilator currently known, has also been shown to be synthesized in vascular endothelial cells, possibly acting as an endothelium-derived relaxing factor.50 The myoendothelial gap junctions have been suggested to contribute to endothelial vasoregulation by allowing electrotonical transmission of membrane potential changes from the endothelial cells to the smooth muscle cells.51,52 Recent studies27–30 have suggested that the vasodilator response to adrenomedullin, CGRP, or the intercellular communication via gap junction is impaired in diabetic subjects or in a medium containing high glucose levels. However, our results obtained in control arteries suggest that adrenomedullin, CGRP, or myoendothelial gap junction is not involved in the enhanced contractile response to norepinephrine by sevoflurane or isoflurane.

In our previous study with the rat small mesenteric artery, the isoflurane-induced enhancement of norepinephrine response was eliminated by superoxide dismutase,22 the antioxidant enzyme that catalyzes the dismutation of superoxide anion (O2•−) into hydrogen peroxide (H2O2) and molecular oxygen. Thus, we proposed that isoflurane enhances the norepinephrine response by stimulating the endothelial release of superoxide anion, which was previously reported to act as an endothelium-derived contracting factor.53 However, recent evidence indicates that hydrogen peroxide causes vasorelaxation in various blood vessels including small mesenteric arteries, possibly acting as an EDHF.54–57 Thus, in our previous study,22 superoxide dismutase might nonspecifically eliminate isoflurane-induced enhancement via its hydrogen peroxide-producing action (rather than via a superoxide-scavenging action).

Despite our previous and present struggling investigations,21–23 the mechanisms underlying the endothelium-dependent enhancing action of volatile anesthetics on contractile response to norepinephrine are currently unknown—that is, a mystery in volatile anesthetic vascular pharmacology in mesenteric resistance arteries. There might exist so much redundancy in endothelial vasoregulation that the effects of various inhibitors of endothelial vasoactive substances were masked in our previous and present studies,21–23 or diabetes might activate some vasoregulatory mechanisms that are inactive (or unimportant) in nondiabetic subjects, preventing the enhancement. There also remains a possibility that some as yet unidentified endothelium-derived vasoactive factors are responsible for the enhancement. Further investigations would be necessary to clarify its underlying mechanisms.

Diabetic patients are associated with an increased incidence or degree of hemodynamic instability during general anesthesia.5,18 It was previously reported that autonomic nervous system reflex dysfunction might underlie the increased incidence of hypotension after induction of anesthesia.2 If our results can be extrapolated to humans, the increased incidence or degree of hemodynamic instability during administration of either sevoflurane or isoflurane in diabetic patients might be attributable, in part, to their inability to enhance contractile response to norepinephrine in an endothelium-dependent manner. However, it would not be explained by a difference in their direct action of enhanced contractile response to norepinephrine between nondiabetic and diabetic subjects. As we previously suggested,21,22 the prolonged inhibition of norepinephrine response after washout of sevoflurane or isoflurane (identically observed in the nondiabetic and diabetic rats) may lead to prolonged systemic hypotension after sevoflurane or isoflurane anesthesia.36

The pathogenesis of diabetic angiopathy is complex and multifactorial.58 However, endothelial oxidative stress induced by chronic hyperglycemia, present in both type 1 and 2 diabetes, is believed to play a crucial role in the development of diabetic angiopathy,59,60 and a considerable amount of epidemiologic evidence indicates that there is an association between the level of hyperglycemia and the increased risk of microvascular diseases.58 Indeed, microangiopathy is a common feature of both types of diabetes.60 Thus, vascular reactivity in small vessels could be similarly altered in both types of diabetes, and the current results obtained in small arteries of the streptozotocin-treated rats (i.e., type 1 diabetic model) could be extrapolated to type 2 diabetes. However, further research using type 2 diabetic animal models (e.g., leptin receptor mutant mice) or comparing the vascular response with that of general anesthetics between the two types of diabetes would be necessary to clarify this issue.

The oscillatory contractile activity in resistance vessels would play an important role in fine regulation of tissue blood flow or vascular permeability.1 Previous studies have proposed the involvement of endothelium, nitric oxide, or gap junctions in the generation of the oscillatory contractile activity and reported the ability of volatile anesthetics to inhibit oscillatory contractile activity.1 In support of those previous studies, in this study, norepinephrine-induced oscillations were prominent in the +E strips but almost invisible in the −E strips and inhibited by sevoflurane, isoflurane, 18β-GA, or a cocktail application of LNA, ouabain, Ba2+, and indomethacin. In addition, in this study, diabetic induction did not influence the amplitude or frequency of norepinephrine-induced oscillations, suggesting that they both might not be impaired in diabetic subjects.

In conclusion, in diabetes, vascular responses to acetylcholine, norepinephrine, and volatile anesthetics (i.e., sevoflurane, isoflurane) are altered in mesenteric resistance arteries, presumably reflecting endothelial dysfunction and possibly underlying circulatory instability during administration of either anesthetic. Some endothelial mechanisms that are impaired in diabetes would be involved in the enhanced contractile response to norepinephrine by sevoflu-
rane or isoflurane. However, the endothelial vasoregulatory mechanisms mediated by adrenomedullin, CGRP, myoendothelial gap junction, nitric oxide, EDHF, cyclooxygenase products, endothelin-1, serotonin, or angiotensin II, all of which have been suggested to be impaired in diabetes, would not be involved in the enhancement.

The authors are grateful to Shousoke Takahashi, M.D., Ph.D., Director, Kyushu Medical Center, Fukuoka, Japan, and Masae Yamakawa, B.S., Research Assistant, Kyushu University, Fukuoka, Japan, for their kind encouragement and help regarding this work.

References

28. Tare M, Coleman HA, Parkinson HC: Regulation of vascular smooth muscle relaxation by the endothelium in health and in diabetes (with a focus on endothelium-derived hyperpolarizing factor). Neurophysiology 2003; 35: 256–61


