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Desflurane inhibits endothelium-dependent vasodilation more than sevoflurane with inhibition of endothelial nitric oxide synthase by different mechanisms

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ABSTRACT

The effects of desflurane on endothelium-dependent vasodilation remain uncertain, whereas sevoflurane is known to inhibit it. Endothelium-dependent vasodilation is mainly mediated by endothelial nitric oxide synthase. The effects of desflurane on endothelium-dependent vasodilation were compared with those of sevoflurane, and inhibition mechanisms, including phosphorylation of endothelial nitric oxide synthase and the calcium pathway, were evaluated for the two anesthetics. We hypothesized that desflurane would inhibit endothelium-dependent vasodilation in a concentration-dependent manner more than sevoflurane, with inhibition of a calcium pathway.

Isolated rat aortic rings were randomly assigned to treatment with desflurane or sevoflurane for measurements of the vasodilation ratio. To determine NO production with desflurane and sevoflurane, an in vitro assay was performed with cultured bovine aortic endothelial cells. These cells were also used for measurement of intracellular calcium or Western blotting.

For endothelium-dependent vasodilation, the ratio of vasodilation was more significantly inhibited by 11.4% desflurane than by 4.8% sevoflurane. Inhibition did not between 5.7% desflurane and 2.4% sevoflurane. No inhibitory effect of desflurane or sevoflurane was observed in endothelium-denuded aorta. Desflurane inhibited nitric oxide production caused by stimulation of bradykinin significantly more than sevoflurane. Desflurane had a greater suppressive effect on the bradykinin-induced increase in intracellular calcium concentration than did sevoflurane. Sevoflurane, but not desflurane, inhibited phosphorylation of the serine 1177 residue by bradykinin stimulation.

Desflurane inhibited endothelium-dependent vasodilation more than sevoflurane through inhibition of a calcium pathway. Sevoflurane inhibited endothelium-dependent vasodilation by inhibition of phosphorylation of the serine 1177 residue of endothelial nitric oxide synthase.

1. Introduction

Endothelium-dependent vasodilation is an element forming the basis of the vasodilation response. It has been reported that endothelium-dependent vasodilation is affected in cardiovascular diseases including atherosclerosis, ischemic/reperfusion injury [1,2]. Endothelium-dependent vasodilation is induced by shear stress and humoral factors including acetylcholine, bradykinin. NO serves multiple bioregulatory functions, including endothelium-dependent vasodilation [3]. eNOS is the major source of NO production in the vascular system [4,5], and it is involved in the dysregulation of certain vascular diseases, such as atherosclerosis and hypertension [6,7]. eNOS activity is regulated by several mechanisms, including $[Ca^{2+}]_i$ and serine residue (Ser^{1177}) or threonine residue (Thr^{495}) of eNOS protein [8,9]. Phosphorylation of Ser^{1177} increases calcium sensitivity of the enzyme [10]. Thr^{495} tends to be phosphorylated under non-stimulated conditions.

The effect of volatile anaesthetics on control of vascular tone is

Abbreviations: NO, Nitric oxide; eNOS, Endothelial nitric oxide synthase; $[Ca^{2+}]_i$, Intracellular calcium concentration.

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complex and depends on the agonist and the specific vascular bed [11]. Endothelium-dependent vasodilation is impaired by sevoflurane [12], while the interaction of desflurane with endothelial cells of vessels remains unknown. There are many reports that isoflurane inhibits $[Ca^{2+}]_i$ increase, and isoflurane is structurally analogous to desflurane [13,14]. We hypothesized that desflurane would inhibit endothelium-dependent vasodilation more than sevoflurane by inhibition of a calcium pathway.

2. Methods

2.1. Animals

Approval from the Ethics Committee for Animal Research of Sapporo Medical University was obtained at March 13, 2014 (Permit Number: 13–123). Male Wistar rats weighing 200–300 g were purchased (Japan SLC, Inc. Shizuoka, Japan). All surgical procedures were performed under anaesthesia, and every effort was made to minimize suffering.

2.2. Preparation of rat aorta

Wistar rats were anesthetized with isoflurane and killed by decapitation and the descending thoracic aorta was isolated. Special care was taken not to damage the endothelium to have endothelium-intact rings. For the examination that excluded endothelial influence, endothelium was denuded by gently rubbing the luminal surface with a cotton swab. The prepared aorta was cut into rings approximately 5.0 mm in length and 3.5 mm in width. Two to four rings were harvested from each rat. Each aortic ring was held in a 37 °C temperature-controlled organ bath that was continuously perfused with a Krebs bicarbonate solution (KBS) aerated with a mixture of 95% O_2 and 5% CO_2. The constituents of KBS included 118.2 mM NaCl, 4.6 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.8 mM $NaHCO_3$, and 10 mM dextrose. To measure muscle tension, one end of a ring was connected to an isometric force transducer (NEC San-ei Instruments, Tokyo, Japan). The resting tone was set at 3.0 g, which was optimal for inducing maximal constriction in our preliminary experiments. Before the start of the experiments, the aortic rings were allowed to equilibrate for 60 min, during which time the bathing fluid was replaced every 20 min.

2.3. Measurement of isometric tension

Isometric tension was measured as described previously [15,16]. After equilibration, the rings were incubated with 3×10^{-2} M KCl to determine the integrity of vascular smooth muscle cells. Rings that did not develop at least 1.0 g contractile active force were discarded. The presence of an intact endothelium was also confirmed by 10^{-6} M acetylcholine-induced vasodilation reaching more than 60% of 3×10^{-7} M phenylephrine-produced constriction. Rings were incubated with desflurane [5.7%, 11.4%; 1, 2 minimum alveolar concentration (MAC) for normal rats]/sevoflurane (2.4%, 4.8%; 1, 2 MAC for normal rats) for 15 min prior to application of 10^{-6} M acetylcholine following 3×10^{-7} M phenylephrine application (Fig. 1).

To confirm the effects of the anaesthetics on endothelium-independent vasodilation, endothelium-denuded aortic smooth muscle was suspended in the organ bath, and changes in tension in response to 3×10^{-7} M phenylephrine, followed by 10^{-5} M sodium nitroprusside, were recorded. Desflurane and sevoflurane were introduced into the gas mixture using agent-specific vaporizers (for sevoflurane: Vapor 19.3, Drägerwerk, Lübeck, Germany and for desflurane: D-vapor, Drägerwerk). Actual values to volatilize from

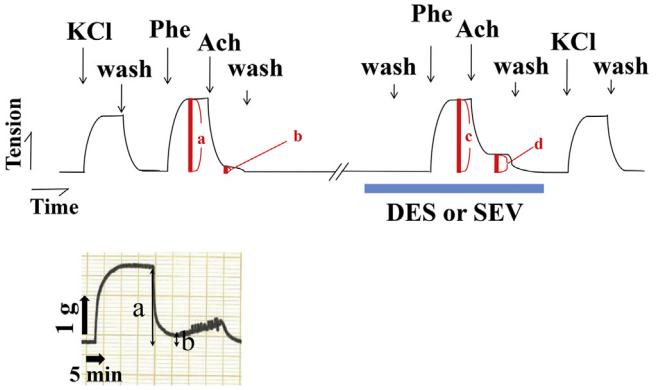

Fig. 1. Typical trace of isometric tension measurement. The aortic rings were incubated with 3×10^{-2} M KCl to determine the integrity of vascular smooth muscle cells. The presence of an intact endothelium was also confirmed by 10^{-6} M acetylcholine (Ach)-induced vasodilation reaching more than 60% of 3×10^{-7} M phenylephrine (Phe)-produced constriction. The aortic rings were incubated with sevoflurane (SEV) or desflurane (DES) for 15 min prior to application of 10^{-6} M acetylcholine following 3×10^{-7} M phenylephrine. The relaxation response prior to application of desflurane or sevoflurane was calculated using the following formula (pre) = (a–b)/a. The relaxation response after application of desflurane or sevoflurane was calculated using the following formula (post)=(c–d)/c. Finally, the ratio of the vasodilation response was calculated as 100 × (pre)/(post) (%).

organ baths and glass slides for volatile anaesthetics may differ from theoretical values. Therefore, the concentrations of the anaesthetic agents in KBS were measured by gas chromatography (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) and were determined to be 0.3 (0.1) mM and 0.6 (0.1) mM at sevoflurane concentrations of 2.4% and 4.8%, respectively ($n = 8$), and 0.7 (0.1) mM and 1.4 (0.1) mM at desflurane concentrations of 5.7% and 11.4%, respectively ($n = 8$). It is assumed that the blood concentration of desflurane is equal to the KBS dissolved-desflurane concentration, and the end-expiratory concentration of desflurane is equal to the actual dose of desflurane (%), with dialled 2.4% and 4.8% of sevoflurane equivalent to 1.0% and 2.1%, respectively. Desflurane is easy to volatilize, with dialled 5.7% and 11.4% of desflurane reduced to 3.3% and 6.7%, respectively (sevoflurane up to 360 μmol/L cited in a document of Maruishi Pharmaceutical Co., Ltd.; desflurane 150–200 μg/ml cited in a document of Baxter International Inc.). Since desflurane and sevoflurane volatilize in the organ bath or culture dish, the concentration of these anaesthetics decreased by about half of the original MAC. Thus, these are the clinically relevant concentrations, and the findings are regarded as meaningful.

2.4. Cell culture

Bovine aortic endothelial cells (NM-1 cells, JCRB0155) were purchased from the National Institute of Biomedical Innovation JCRB Cell Bank (Ibaraki, Osaka, Japan). The cells were cultured in a humidified atmosphere of 5% CO_2 at 37 °C in Dulbecco's modified Eagle's medium (D-MEM; American Type Culture Collection, Manassas, VA, USA), which was supplemented with 10% fatal calf serum, penicillin (100 units/ml), and streptomycin (100 μm/ml). The culture medium was replaced with a serum-free medium 24 h before the experiment. The cells were then used for fluorescence microscopy examination including measurement of NO, live cell calcium imaging, and Western blotting.

2.5. Measurement of NO from endothelial cells

The endothelial cells were subcultured to glass slides designed for fluorescence microscopy. The cells were washed with

phosphate-buffered saline (PBS; Gibco, Carlsbad, CA, USA), which contained 154 mM NaCl, 3 mM Na_2HPO_4, and 1 mM KH_2PO_4, adjusted to a pH of 7.4. The cells were incubated 10^{-2} M diaminofluorescein-2 diacetate (DAF-2 DA), 10^{-1} mM L-arginine and then washed twice with PBS. After being washed, DAF-2 DA-loaded cells were replaced with KBS saturated with 11.4% desflurane or 4.8% sevoflurane and incubated for 15 min at 37 °C. Culture slides were placed in a 37 °C temperature-controlled chamber mounted on the stage of a Leica DM IL LED inverted microscope (Leica Camera AG, Wetzlar, Germany) for measurement of the real-time NO production of individual cells. Dynamic changes in the fluorescence intensity of NO production in response to 10^{-6} M bradykinin in the presence or absence of 11.4% desflurane or 4.8% sevoflurane were measured at an excitation wavelength of 515 nm fluorescence and an emission wavelength of 495 nm. After the application of bradykinin, DAF-2 DA fluorescence signals from some definite cells were scanned for 3 min. The ratios of the fluorescence intensity in response to bradykinin (F) and the fluorescence intensity just before the addition of bradykinin (F_0) were compared in the presence and absence of desflurane or sevoflurane.

2.6. Live cell calcium imaging

Similar to determination of NO production, the subcultured endothelial cells were washed with PBS. The cells were incubated 10^{-5} M Fluo-3/AM and then washed twice with PBS. After being washed, Fluo-3/AM-loaded cells were replaced with KBS saturated with 11.4% desflurane or 4.8% sevoflurane and incubated for 15 min at 37 °C. Culture dishes were placed in a 37 °C temperature-controlled chamber mounted on the stage of a Leica DM IL LED inverted microscope. Dynamic changes in the fluorescence intensity of the $[Ca^{2+}]_i$ in response to 10^{-6} M bradykinin in the presence or absence of 11.4% desflurane or 4.8% sevoflurane were measured at an excitation wavelength of 508 nm fluorescence and an emission wavelength of 527 nm. After the application of bradykinin, Fluo-3 fluorescence signals from some definite cells were scanned for 3 min. To eliminate error due to the unevenness of cell size, fluorescence signals were measured at 135 × 135 pixels in each picture. The ratio of the fluorescence intensity was used for comparison, as in the NO production assay.

2.7. Western blot analysis

Cultured endothelial cells were treated with 10^{-6} M bradykinin for 1 min in the presence or absence of desflurane or sevoflurane (11.4% and 4.8% for 15 min, respectively). After treatments, cells were homogenized in ice-cold lysis buffer [17], which contained 50 mM HEPES, adjusted to a pH of 7.5, 1% Triton X-100, 50 mM NaCl, 50 mM NaF, 5 mM EDTA, 10 mM sodium pyrophosphate, 1 mM phenylmethanesulfonyl fluoride, 1 mM Na_3VO_4, 100 μg/ml leupeptin, and 10 μg/ml aprotinin. Homogenates were centrifuged at 1.0×10^4 g for 15 min at 4 °C, and the supernatant was collected. The protein concentrations of the supernatant were determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific K. K., Yokohama, Japan) [17]. Equal amounts of total protein were used for every sample in each experiment. Strips were treated with anti-β-actin antibody (1:1000) as a loading control. The proteins were separated by 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto nitrocellulose membranes. The membranes were treated with anti-eNOS antibody (1:1000) and anti-phosphorylated Ser^{1177}-eNOS antibody (1:1000) or anti-phosphorylated Thr^{495}-eNOS antibody (1:1000) and then incubated with horseradish peroxidase-conjugated antibody (1:2000). The densities of the immunoreactive bands were detected using enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and assessed with an image analysis system (ATTO Corporation, Tokyo, Japan). The amount of phosphorylated Ser^{1177}-eNOS or phosphorylated Thr^{495}-eNOS on the membrane fraction is expressed as a percentage of the total fraction.

3. Materials

All drugs were of the highest purity commercially available. Fluo-3/AM and DAF-2 DA were obtained from DOJINDO Laboratories (Kumamoto, Japan) and Carbiochem (Darmstadt, Germany), respectively. Monoclonal antibody against phosphorylated Ser^{1177}-eNOS or polyclonal antibody against phosphorylated Thr^{495}-eNOS antibody and against eNOS antibody, and the secondary antibody labeled with horseradish peroxidase were supplied by Cell Signaling Technology (Danvers, MA, USA). All other drugs were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA).

3.1. Statistical analysis

Statistical analyses were performed with GraphPad Prism (version 7.00., GraphPad Software, San Diego, CA). The D'Agostino and Pearson test was used to test for normality of the ratio of vasodilation for isometric tension. Because other examinations had relatively small numbers, the Shapiro-Wilk test was used to test for normality of the variables. For normally distributed variables, the parameter analyses were based on one-way analysis of variance (ANOVA), followed by multiple comparisons using Tukey's *post hoc* test with adjusted *p* values. Data are presented as mean differences [MD] with 95% confidence interval [CI]. For the NO production experiments with live cell calcium imaging, the sample size was the number of cells observed. For Western blotting, the sample size was the number of times the examination was performed. A value of $P < 0.05$ was considered significant.

From published data obtained in the same experimental model [20], a standard deviation of 12.8% and differences of means between groups of 15% were assumed for detecting the clinically meaningful difference in the inhibition of vasodilation by the two anesthetics. A power analysis demonstrated that a sample size of n = 16 strips of aorta was necessary, assuming a statistical power of 0.9 at a significance level of 0.05.

4. Results

4.1. Inhibitory effects of desflurane and sevoflurane on endothelium-dependent vasodilation of rat aorta

Phenylephrine induced an increase in muscle tension of the rat aorta, and acetylcholine rapidly induced endothelium-dependent vasodilation. Desflurane and sevoflurane inhibited acetylcholine-induced endothelium-dependent vasodilation in a concentration-dependent manner (Fig. 2A). Compared with the control group, this inhibition by desflurane and sevoflurane was significant at concentrations of 11.4% [n = 20 in each group, MD 16.2, CI 12.1 to 20.3, $P < 0.05$] and 4.8% [n = 20 in each group, MD 7.7, 95% CI 3.6 to 11.8, $P < 0.05$], respectively. Inhibition was significant by 5.7% desflurane (n = 20 in each group, MD 6.1, 95% CI 2.0 to 10.2, $P < 0.05$) but not by 2.4% sevoflurane (n = 20 in each group, MD 3.8, 95% CI -0.3 to 7.9, $P > 0.05$) compared with each control group. Compared with the control group, both desflurane and sevoflurane at each of the two doses did not inhibit vasodilation caused by 10^{-5} M sodium nitroprusside in endothelium-denuded aortic smooth muscle (n = 10 in each group) (Fig. 2B).

Desflurane (11.4%) inhibited endothelium-dependent vasodilation significantly more than sevoflurane (4.8%) (n = 20 in each

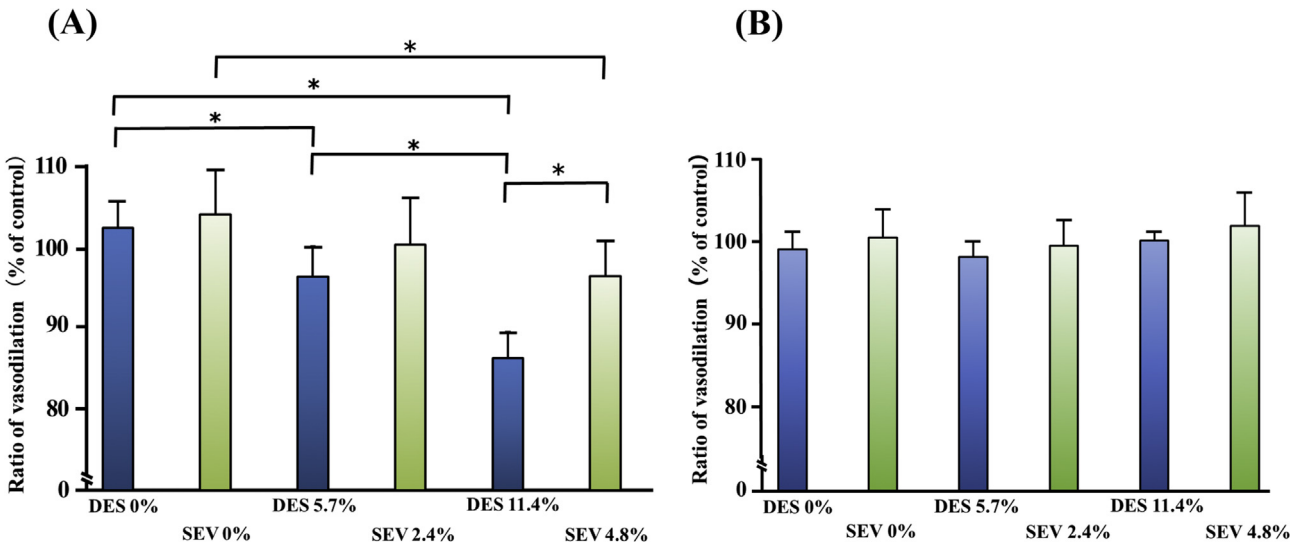

Fig. 2. The effect of desflurane (DES) or sevoflurane (SEV) on endothelium-dependent vasodilation of rat aortic rings (n = 20) **(A)** and on endothelium-denuded aortic rings (n = 10) **(B)**. The aortic rings were incubated with desflurane [5.7%, 11.4%; 1, 2 minimum alveolar concentration (MAC) for normal rats] or sevoflurane (2.4%, 4.8%; 1, 2 MAC for normal rats) for 15 min prior to application of 10^{-6} M acetylcholine following 3×10^{-7} M phenylephrine. To confirm the effects of the anesthetics on endothelium-independent vasodilation, changes in tension in response to 3×10^{-7} M phenylephrine, followed by 10^{-5} M sodium nitroprusside were recorded before and after application of desflurane or sevoflurane. The ratio of vasodilation in the control [no sevoflurane (SEV 0%) or no desflurane (DES 0%)] groups was considered 100%. *$P < 0.05$.

group, MD -10.2, 95% CI -14.2 to −6.1, $P < 0.05$). This inhibition did not differ (n = 20 in each group, $P > 0.05$) between desflurane (5.7%) and sevoflurane (2.4%).

The inhibitory effect of bradykinin-induced NO production by desflurane was greater than that of sevoflurane in bovine aortic endothelial cells.

The F/F_0 ratio reached its maximum level 60 s after the application of bradykinin. Compared with the control group, bradykinin at 10^{-6} M (n = 7 in each group, MD -0.6, 95% CI -0.9 to −0.4, $P < 0.05$) significantly induced NO production. Desflurane (11.4%) and sevoflurane (4.8%) significantly inhibited bradykinin-induced NO production in bovine aortic cells (n = 7 in each group; desflurane, MD 0.7, 95% CI 0.5 to 0.9, $P < 0.05$; sevoflurane, MD 0.3, 95% CI 0.1 to 0.6, $P < 0.05$). The inhibitory effect of bradykinin-induced NO production was greater for desflurane than for sevoflurane (n = 7 in each group, MD -0.3, 95% CI, −0.6 to −0.1, $P < 0.05$) (Fig. 3A).

Desflurane significantly inhibited the increase in $[Ca^{2+}]_i$ more than sevoflurane in bovine aortic endothelial cells.

The F/F_0 ratio reached its maximum level of 30 s after the application of bradykinin. Bradykinin of 10^{-6} M induced a significant increase in $[Ca^{2+}]_i$ (n = 7 in each group, MD -0.5, 95% CI, −0.8 to −0.2, $P < 0.05$). Desflurane (11.4%) significantly inhibited the bradykinin-induced increase in $[Ca^{2+}]_i$ in bovine endothelial cells (n = 7 in each group, MD 0.6, 95% CI 0.3 to 0.9, $P < 0.05$), whereas sevoflurane (4.8%) did not (n = 7 in each group, MD 0.2, 95% CI -0.1 to 0.5, $P < 0.05$). Desflurane inhibited bradykinin-induced increase in $[Ca^{2+}]_i$ significantly more than sevoflurane (n = 7 in each group, MD -0.3, 95% CI -0.6 to −0.1, $P < 0.05$). (Fig. 3B).

Sevoflurane but not desflurane inhibited bradykinin-induced Ser^{1177}-eNOS phosphorylation in bovine aortic endothelial cells.

Bradykinin stimulated the phosphorylation of Ser^{1177}-eNOS based on the ratio of phosphorylated Ser^{1177}-eNOS to total eNOS (n = 8 in each group, MD -17, 95% CI -21.4 to −12.6, $P < 0.05$). Sevoflurane (4.8%) attenuated bradykinin-induced eNOS phosphorylation (n = 8 in each group, MD 6.9, 95% CI 2.5 to 11.3, $P < 0.05$) (Fig. 4A), whereas desflurane (11.4%) did not (n = 8 in each group, MD -0.5, 95% CI -4.9 to 3.9, $P > 0.05$). Treatment with 10^{-6} M bradykinin for 1 min did not stimulate dephosphorylation of Thr^{495}-eNOS. In addition, both desflurane and sevoflurane did not affect dephosphorylation of Thr^{495}-eNOS (Fig. 4B).

5. Discussion

The key findings of the current study are as follows. Endothelium-dependent vasodilation of the rat aorta was inhibited by desflurane and sevoflurane in a. This inhibitory effect of desflurane (11.4%) was greater than that of sevoflurane (4.8%). Inhibition of the bradykinin-induced $[Ca^{2+}]_i$ increase was greater by desflurane than by sevoflurane in bovine aortic endothelial cells. Western blotting showed that phosphorylation of Ser^{1177}-eNOS was inhibited by sevoflurane, but not by desflurane.

There are few reports showing that desflurane affected endothelium-dependent vasodilation. That sevoflurane inhibited endothelium-dependent vasodilation in the present study does not contradict a past report [18]. There have been some reports that halothane, isoflurane, and enflurane generally suppress endothelium-dependent vasodilation [19]. Because inhibition of vasodilation was not observed in endothelium-denuded rings (Fig. 2B), this suggests that desflurane inhibited endothelial function(s) directly. The eNOS-mediated signal pathway in vascular tissue has been well established [10].

Desflurane causes no inhibition of this phosphorylation (Fig. 4). It has been reported that volatile anesthetics inhibit signal transduction by different mechanisms, similar to the present study. Ishikawa et al. reported that the mechanism behind the inhibitory effect of isoflurane on angiotensin II-induced vascular contraction differs from that of sevoflurane [20]. Since desflurane inhibited endothelium-dependent vasodilation significantly more than sevoflurane (Fig. 2A), this may be one of the reasons why the mechanism varies, in that there was a difference in inhibition between desflurane and sevoflurane.

There are some reports that isoflurane inhibits the $[Ca^{2+}]_i$ increase and affect endothelium-dependent vasodilation [21]. In the present study, desflurane inhibited the $[Ca^{2+}]_i$ increase significantly more than sevoflurane (Fig. 3B). Isoflurane inhibits $[Ca^{2+}]_i$ similar

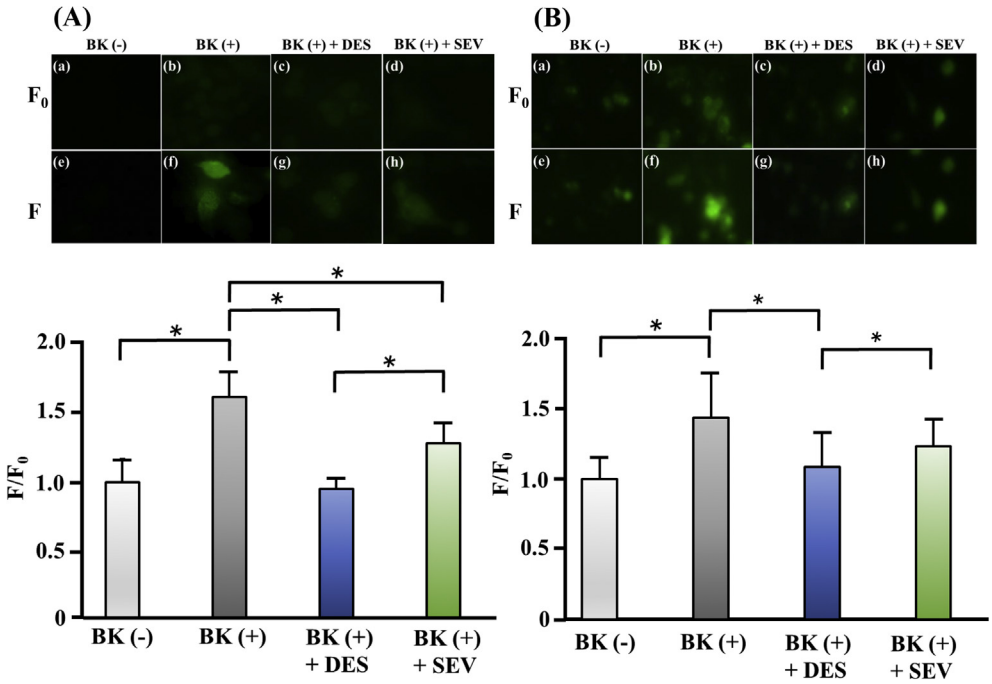

Fig. 3. **(A)** Measurement of NO production (n = 7). The bovine aortic endothelial cells (NM-1 cell) were incubated for 30 min at 37 °C in KBS containing 10^{-2} M diaminofluorescein-2 diacetate (DAF-2 DA) and 10^{-1} mM L-arginine. The cells were replaced with KBS saturated with 11.4% desflurane (DES) or 4.8% sevoflurane (SEV) and incubated for 15 min at 37 °C. Changes in the fluorescence intensity of NO production in response to 10^{-6} M bradykinin were measured. The ratio of the fluorescence intensity in response to bradykinin (F) and the fluorescence intensity just before the addition of bradykinin (F_0) were measured. (a): non-treatment with bradykinin as control; (b)–(d): pretreatment with bradykinin; (e): after 1 min control; (f)–(h): after 1 min treatment with bradykinin. **(B)** Live cell calcium imaging (n = 7). The subcultured bovine aortic endothelial cells were incubated for 30 min at 37 °C in KBS containing 10^{-5} M Fluo-3/AM. The cells were incubated with KBS saturated with 11.4% desflurane (DES) or 4.8% sevoflurane (SEV) for 15 min at 37 °C. Changes in the fluorescence intensity of the intracellular calcium concentration in response to 10^{-6} M bradykinin in the presence or absence of desflurane or sevoflurane were measured. The ratio of the fluorescence intensity was used for comparison, as in the NO production assay. (a): control; (b)–(d): pretreatment with bradykinin; (e): after 1 min control; (f)–(h): after 1 min treatment with bradykinin. *$P < 0.05$.

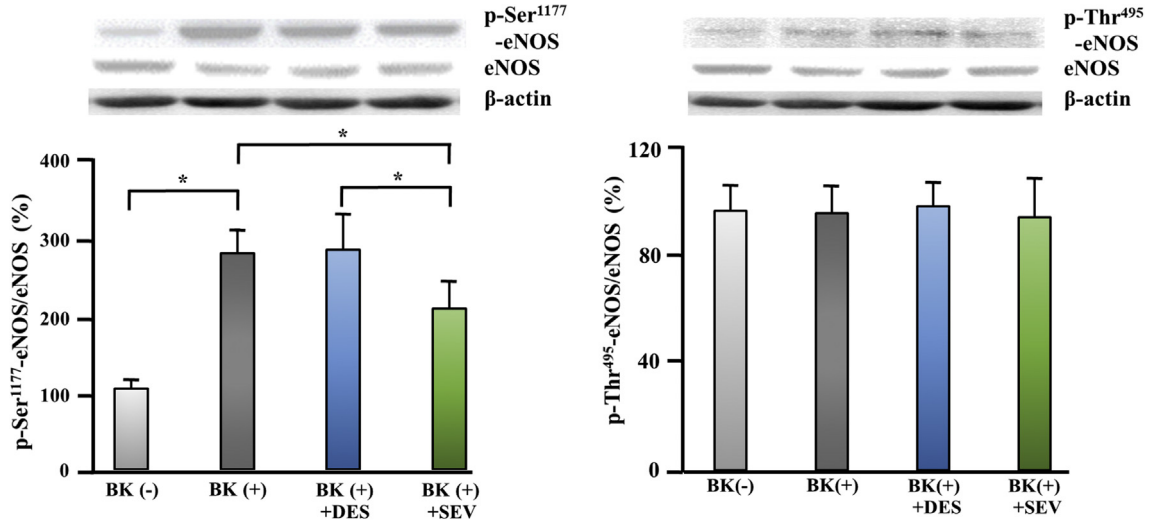

Fig. 4. The effect of desflurane (DES) and sevoflurane (SEV) on bradykinin (BK)-stimulated phosphorylation of serine^{1177}-endothelial nitric oxide synthase (Ser^{1177}-eNOS) (n = 8) **(A)** and threonine^{495}-eNOS (Thr^{495}-eNOS) (n = 8) **(B)** in bovine aortic endothelial cells. The cultured bovine aortic endothelial cells were treated with 10^{-6} M bradykinin for 1 min in the presence or absence of 11.4% desflurane or 4.8% sevoflurane for 15 min at 37 °C. The phosphorylated Ser^{1177}-eNOS or Thr^{495}-eNOS band was detected using a specific antibody with Western blotting. The amount of phosphorylated Ser^{1177}-eNOS or phosphorylated Thr^{495}-eNOS on the membrane fraction is expressed as a percentage of the total fraction. BK (−), no treatment with bradykinin; BK (+), treatment with 1×10^{-6} M bradykinin. *$P < 0.05$.

to desflurane, perhaps because they are structurally analogous.

The major limitation of this study is that the MACs of volatile anesthetics may differ when using samples of two different species. However, Eger reported that MACs are remarkably similar between different species [22]; thus, based on this report, isometric tension measurement and other biochemical examinations in the present study were performed with the same volatile anaesthetic dose. Second, using separate species for isometric tension measurement and other examinations in this study may have caused different results. Not only rat aorta but bovine endothelial cells were used,

because the quantity of the protein necessary for Western blot analysis could be collected, and the cell count necessary for NO or calcium imaging could be obtained. Furthermore, examination of both aortic vessels was considered to have many common points. Third, the vasoconstriction and vasodilation of peripheral vessels including muscular arteries are important functions in the regulation of organ perfusion. In this regard, different results may have been obtained had aortas been used for the present study. However, isometric tension measurement using the rat aorta is an established technique, and it is thought that it is acceptable as a vasodilation model mediated by NO[23].

In summary, the present findings suggest that sevoflurane inhibits endothelium-dependent vasodilation caused by inhibition of phosphorylation of Ser^{1177}-eNOS protein, whereas desflurane inhibits vasodilation by inhibition of calcium signal transduction of eNOS activity. These different mechanisms might reflect the different extents of the inhibitory effects of desflurane and sevoflurane on endothelium-dependent vasodilation.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2017.11.017.

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