ORIGINAL ARTICLE

The cellular mechanisms underlying the inhibitory effects of isoflurane and sevoflurane on arginine vasopressin-induced vasoconstriction

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Abstract

Purpose Arginine vasopressin (AVP) is a potent vasoconstrictor that is sometimes used for the treatment of refractory vasodilatory shock. AVP constricts vascular smooth muscle by increasing both intracellular calcium concentration ($[Ca^{2+}]_i$) and myofilament Ca^{2+} sensitivity. However, the modulation of AVP-mediated vasoconstriction by volatile anesthetics remains to be determined. This study investigates the effects of isoflurane and sevoflurane on AVP-induced vasoconstriction and elucidates the underlying mechanisms, with an emphasis on the Ca^{2+}-mediated pathways and Ca^{2+} sensitization pathways of rat aortic smooth muscle.

Methods The effects of isoflurane and sevoflurane on AVP-induced vasoconstriction and on the AVP-induced increase in $[Ca^{2+}]_i$ and Rho activity in rat aorta were investigated by isometric force recording, by measuring $[Ca^{2+}]_i$ using fluorescence dye, and by Western blotting techniques.

Results Arginine vasopressin (10^{-7} M) elicited a transient contractile response that was inhibited by isoflurane and sevoflurane in a concentration-dependent manner. AVP (10^{-7} M) induced a transient increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Isoflurane and sevoflurane also inhibited an AVP-induced increase in $[Ca^{2+}]_i$ in a concentration-dependent manner. AVP (10^{-7} M) increased

the Rho activity that was attenuated by 2 minimum alveolar concentration of sevoflurane ($P < 0.01$), but not by an equipotent concentration of isoflurane.

Conclusion Arginine vasopressin-induced vasoconstriction is mediated by an increase in $[Ca^{2+}]_i$ and by the activation of the Rho-Rho kinase pathway in rat aortic smooth muscle. Although both isoflurane and sevoflurane, at clinically relevant concentrations, attenuate AVP-induced contraction, the cellular mechanisms of their inhibitory effects appear to differ.

Keywords Vasopressin · Isoflurane · Sevoflurane · Artery · Rho kinase

Introduction

Volatile anesthetics reduce systemic blood pressure by the relaxation of the blood vessels and by the inhibition of myocardial contraction. Although these anesthetics are known to attenuate the vasoconstriction induced by many intrinsic neurohumoral factors such as norepinephrine and angiotensin II (Ang II), the underlying mechanisms have been poorly understood. Vascular contraction induced by agonists is primarily regulated by the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). However, the relationship between the muscle tension and the $[Ca^{2+}]_i$ is not linear and depends on the vasoconstrictor. A greater extent of vascular contraction observed for a given change in $[Ca^{2+}]_i$ is defined as an increase in the "Ca^{2+} sensitivity" of the contraction-associated proteins. Protein kinase C (PKC) and Rho kinase are known to promote myofilament Ca^{2+} sensitivity by inhibiting myosin light chain (MLC) phosphatase activity, which leads to increased MLC phosphorylation, resulting a greater contraction for a given

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$[Ca^{2+}]_i$ [1]. We have previously demonstrated the cellular mechanism by which volatile anesthetics attenuate the Ang II-induced contraction of vascular smooth muscle [2–4]. In the series of studies, we showed that sevoflurane inhibited Ang II-induced vasoconstriction mainly by suppressing myofilament Ca^{2+} sensitivity, while not affecting the $[Ca^{2+}]_i$ [2]. By contrast, the inhibitory effect of isoflurane on Ang II-induced vasoconstriction was mainly mediated by the attenuation of $[Ca^{2+}]_i$ [3].

Arginine-vasopressin (AVP) is a potent vasoactive peptide hormone that constricts vascular smooth muscle by increasing both $[Ca^{2+}]_i$ [5, 6] and myofilament Ca^{2+} sensitivity [7, 8]. AVP plays an important role in maintaining systemic blood pressure under both physiological and pathophysiological conditions [9]. Moreover, the intravenous infusion of a small dose of AVP has been used to treat refractory vasodilatory shock after cardiopulmonary bypass [10, 11] and to treat anaphylactic shock [12–14] during general anesthesia. However, little information is available regarding the influence of general anesthetics on AVP-induced vasoconstriction. Although Fujihara et al. [15] reported that halothane attenuated an AVP-induced increase in the $[Ca^{2+}]_i$ in vascular smooth muscle cells, the modulatory effects of modern anesthetics on the $[Ca^{2+}]_i$ and Ca^{2+} sensitivity mechanism in response to AVP have never been determined.

We hypothesized that volatile anesthetics might reduce AVP-induced vasoconstriction by affecting the $[Ca^{2+}]_i$ or/ and myofilament Ca^{2+} sensitivity in vascular smooth muscle. To elucidate the cellular mechanism responsible for anesthetic-induced vasodilation, we therefore investigated the anesthetic modulation of the AVP-induced Ca^{2+}-dependent pathway and the Ca^{2+}-independent pathway that regulate Ca^{2+} sensitivity.

Materials and methods

The Wakayama Medical University Animal Care and Use Committee (Wakayama, Japan) approved the experimental protocol.

Isometric force measurement

Male Wistar rats weighing 250–400 g were anesthetized with halothane and exsanguinated by cutting through the common carotid artery. The descending thoracic aorta was dissected, with its surrounding fat and connective tissue carefully removed, and cut into rings 3–4 mm in length. Five to six rings were typically harvested from one rat. The intimal surface was gently rubbed with a stainless steel needle to remove the endothelium. The aortic rings were equilibrated under a resting tension of 3 g in Krebs bicarbonate solution (KBS) with the following composition (in mM) NaCl 118.2, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 24.8, and dextrose 10. The KBS was maintained at a temperature of 37°C and continuously aerated with a mixture of 95% O_2 and 5% CO_2 to keep the pH within the range of 7.35–7.45. Isometric tension was measured as described previously [2, 3]. After 60 min equilibration, the aortic preparations were exposed to KCl (30 mM) to assess their overall contractile responsiveness. Removal of the endothelium was confirmed by the lack of a relaxation response to acetylcholine (10^{-5} M) in rings precontracted with phenylephrine (PE 3×10^{-7} M). Only aortic rings that developed a contractile force of 1.0 g or more in response to KCl (30 mM) and exerted no relaxation response to acetylcholine were used for further experiments.

Arginine vasopressin (10^{-7} M) was used to induce vascular contraction, because this concentration had achieved 80–90% of the maximum contractile response induced by AVP (10^{-5} M) in our preliminary study. To examine the effects of anesthetics on the AVP-induced contraction, five rings from each of six different rats ($n = 6$) were randomly exposed to 0, 1, and 2 minimum alveolar concentration (MAC) of isoflurane or sevoflurane for 15 min before the addition of AVP.

To characterize the contribution of various protein kinases to the AVP-induced contraction, we observed the contractile response to AVP in the absence or presence of the following specific inhibitors: the PKC inhibitor, GF109203X (10^{-6} M); the p44/42 mitogen-activated protein kinase (MAPK) inhibitor, PD98059 (10^{-5} M); and the Rho kinase inhibitor, Y27632 (10^{-6} M). Four rings from each of 8 different rats ($n = 8$) were used for this experiment. Each ring was exposed to only one concentration of an anesthetic or to one inhibitor. The isometric force that developed in response to AVP was expressed as the percentage relative to that induced by KCl (30 mM).

Each anesthetic was delivered to the gas mixture through a calibrated agent-specific vaporizer (Penlon, Abingdon, UK). The concentration of the resulting gas mixture was monitored and adjusted using an Atom 303 anesthetic agent monitor (Atom, Tokyo, Japan). Our previous data using the same experimental system indicated that the concentrations of isoflurane and sevoflurane in KBS, as measured by gas chromatography (Shimadzu, Kyoto, Japan) after 15 min equilibration, were 0.19 ± 0.01 and 0.39 ± 0.01 mM isoflurane at the gas concentrations of 1.2% (1 MAC) and 2.3% (2 MAC), respectively ($n = 8–12$), and 0.17 ± 0.03 and 0.28 ± 0.02 mM sevoflurane at the gas concentrations of 1.7% (1 MAC) and 3.4% (2 MAC), respectively ($n = 8–12$) [3, 16].

$[Ca^{2+}]_i$ measurement

Endothelium-denuded strips, which were approximately 5 mm long and 3.5 mm wide, were prepared from the isolated rat thoracic aorta. Two or three strips were typically harvested from one rat. The aortic strips were incubated for 6 h at room temperature (20–22°C) in KBS containing acetoxymethyl ester of fura-2 (10^{-5} M). The noncytotoxic detergent, cremophor (0.1%), was added to the solution to increase the solubility of the acetoxymethyl ester. After the loading period, each strip was washed with KBS three times and fixed horizontally in a temperature-controlled (37°C) organ bath that was continuously perfused with KBS bubbled with 95% O_2 and 5% CO_2. A dual-wavelength spectrofluorometer (CAF-110; Japan Spectroscopic, Tokyo, Japan) was used to determine the $[Ca^{2+}]_i$, at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. The 340/380 fluorescence ratio was used as an indicator of $[Ca^{2+}]_i$. Changes in $[Ca^{2+}]_i$ induced by KCl (30 mM) were measured first, and the value was taken as a standard reference (100%). Isoflurane or sevoflurane, at a concentration of 0, 1, or 2 MAC, was introduced into the aerating gas mixture 15 min prior to the addition of AVP (10^{-7} M). Changes in $[Ca^{2+}]_i$ in response to AVP (10^{-7} M) were observed and expressed as a percentage of the reference value.

Measurement of Rho activity

Isometric force measurement study revealed that the AVP-induced vascular contraction was significantly reduced by the Rho kinase inhibitor Y27632, but not by the PKC inhibitor or the MAPK inhibitor. This indicated the possible contribution of the Rho-Rho kinase pathway in the AVP-induced contraction in rat aorta. Therefore, we used Western blotting to measure Rho activity. Strips 3.5 cm in length were excised from the rat descending thoracic aorta and the endothelium was mechanically removed. One strip was obtained from each animal. The strips were incubated in KBS aerated with a mixture of 95% O_2 and 5% CO_2 and were equilibrated for 60 min before the start of the experiment.

Thirty-six strips from 36 different animals were randomly assigned to six groups and incubated for an additional 15 min in one of the following drugs or anesthetics: (1) saline as a basal control; (2) guanosine diphosphate (GDP, 10^{-4} M) as a negative control; (3) guanosine 5′-[γ-thio] triphosphate (GTPγS, 10^{-4} M), a potent stimulator of the Rho-Rho kinase pathway; (4) isoflurane (2 MAC); (5) sevoflurane (2 MAC); (6) no anesthetic or drug. The strips in groups 4, 5, and 6 were then exposed to AVP (10^{-7} M) for 5 min, while those in groups 1, 2, and 3 were not exposed to AVP. Afterwards, all strips were frozen with dry ice. This exposure time was based on the findings from the isometric tension experiment, in which the AVP-induced force development reached its peak level at 5 min after the application of AVP. The frozen aortas were cut into pieces and homogenized in an ice-cold lysis buffer and centrifuged at 16,000g for 15 min at 4°C. The supernatant was collected, and the protein concentration was determined using the bicinchoninic acid method [17]. The samples were used at equal concentrations of total protein in the following experiment.

The Rho activity was assayed with an EZ-Detect Rho activation Kit® (Thermo Fisher Scientific, Rockford, IL, USA), used in accordance with the manufacturer's instructions. Rho is active when it binds with GTP and is inactive when it binds with GDP. Activated Rho, but not inactive Rho, specifically binds to rhotekin. The samples were incubated with rhotekin-coated beads for 60 min at 4°C. They were then centrifuged at 7,200g for 30 s to pull down the beads. The supernatant (i.e., unbound material) was discarded. After that, Rho was dissociated from the beads by adding sodium dodecyl sulfate (SDS) sample buffer (125 mM Tris–HCl, pH 6.8; 2% glycerol, 4% SDS; 0.05% bromophenol blue; 0.1% b-mercaptoethanol). The samples were boiled for 5 min, and the protein at equivalent total content (25 mg) was separated by 12% SDS-polyacrylamide gel electrophoresis and electroblotted onto a nitrocellulose membrane. The membranes were incubated with blocking buffer (25 mM Tris–HCl; 3% bovine serum albumin; 150 mM NaCl, pH 7.5) for 2 h at room temperature. They were then incubated with anti-Rho antibody (1:500) overnight at 4°C, followed by incubation with horseradish peroxidase-conjugated antibody (1:2,000) for 1.5 h. The densities of the immunoreactive bands were detected using an enhanced chemiluminescence system (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and were assessed with image analysis software (NIH Image 1.62; Bethesda, MD, USA). Rho activity was expressed as a percentage relative to the level obtained from the basal control group (i.e., exposed to saline).

Materials

All drugs were of the highest purity commercially available. Fura-2 was obtained from DOJINDO Laboratories (Kumamoto, Japan); the secondary antibody, labeled with horseradish peroxidase, was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA); and isoflurane and sevoflurane were obtained from Dinabot (Osaka, Japan). All other drugs were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA).

Data analysis

Statistical analyses were performed using the StatMate software program (Atoms, Tokyo, Japan). The sample size (the n value) represented the number of rats from which aortic rings (for tension measurement), aortic strips (for $[Ca^{2+}]_i$ measurement), or aortas (for Western blotting) had been obtained. Data are presented as medians with the 25th and 75th percentiles. The data were evaluated using the Mann–Whitney's U-test for comparisons between groups and the Kruskal–Wallis test followed by the Newman–Keuls test for multiple comparisons. P values less than 0.05 were considered statistically significant.

Results

Isometric force measurement

Arginine vasopressin (10^{-7} M) induced a rapid and transient contraction of rat aortic smooth muscle, followed by a gradual decline to the resting level (Fig. 1a). The maximum contractile response to AVP was 96% (88–101%) of that induced by KCl (30 mM). It occurred approximately 5 min after the application of AVP. Both isoflurane and sevoflurane inhibited the AVP-induced contraction in a concentration-dependent manner ($n = 6$, each) (Fig. 2). There was no statistically significant difference between the inhibitory effect of isoflurane on the AVP-induced contraction and that of sevoflurane when compared at equipotent concentrations. The AVP-induced contraction

was reduced by the Rho kinase inhibitor Y27632 (10^{-6} M) ($P < 0.01$), but not by the PKC inhibitor GF109230X (10^{-6} M), or the p44/42 MAPK inhibitor PD98059 (10^{-5} M) ($n = 8$, each) (Fig. 3).

$[Ca^{2+}]_i$ measurement

Arginine vasopressin (10^{-7} M) also induced a transient increase in $[Ca^{2+}]_i$, reaching a peak level of 120% (116–127%) of that induced by KCl (30 mM) (Fig. 1b). Isoflurane and sevoflurane attenuated the AVP-induced increase in $[Ca^{2+}]_i$ to similar extents ($n = 6$, each) (Fig. 4).

Measurement of Rho activity

GTPγS (10^{-4} M), a potent Rho-Rho kinase pathway activator, caused an increase in Rho activity. AVP (10^{-7} M) also increased Rho activity, to the same extent as GTPγS. Sevoflurane at 2 MAC significantly suppressed the Rho activity elicited by AVP ($P < 0.01$), while an equipotent concentration of isoflurane failed to influence Rho activity ($n = 6$, each) (Fig. 5).

Discussion

Arginine vasopressin (AVP) is an oligopeptide hormone that is synthesized in the hypothalamus and is secreted from the posterior pituitary grand into the systemic circulation. In addition to its antidiuretic effect, AVP exerts a potent vasoconstrictive effect through the V_1 receptor and

Fig. 1 Typical tracings showing the changes in the isometric tension (a) and in the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ (b) of rat aortic smooth muscle in response to KCl (30 mM) and arginine vasopressin (AVP 10^{-7} M) in the absence (left) and presence (right) of anesthetic. Changes in $[Ca^{2+}]_i$ are presented as ratios of fluorescence measurements at the excitation wavelengths of 340 and 380 nm
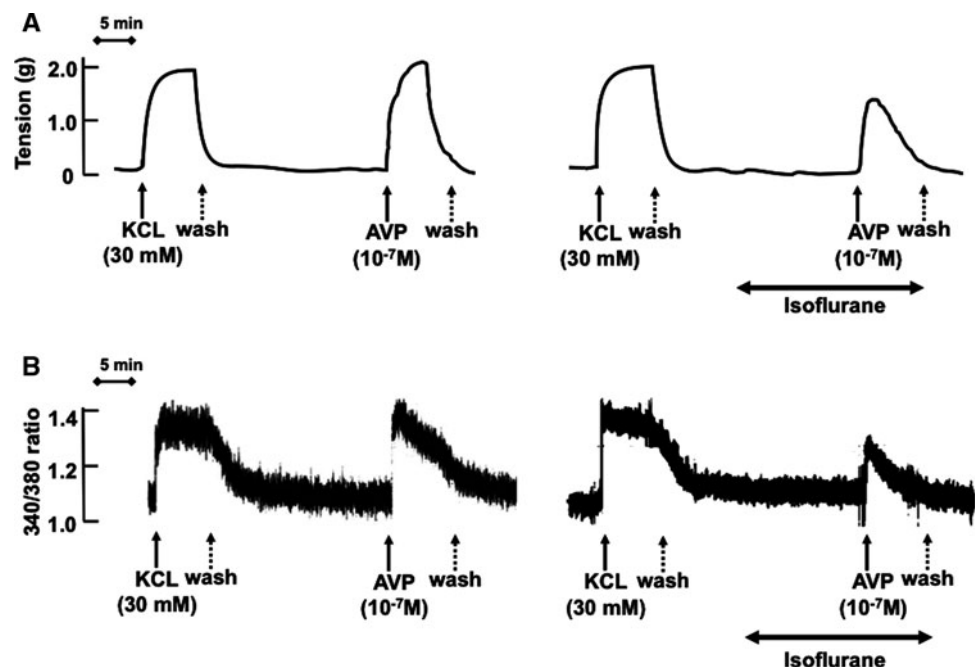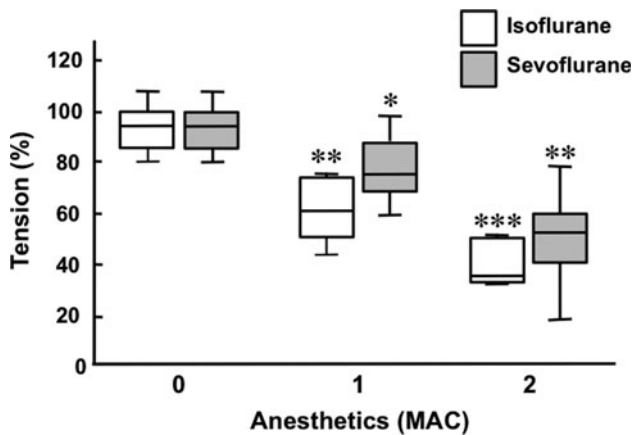
Fig. 2 The effects of isoflurane and sevoflurane on the contractile response to arginine vasopressin (AVP 10^{-7} M). AVP-induced tension changes are expressed as percentages relative to that induced by KCl (30 mM). *Horizontal bars* represent the medians, *boxes* represent the 25th and 75th percentile ranges, and *T-bars* represent the 5th and 95th percentile ranges. *MAC*, Minimum alveolar concentration. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus control ($n = 6$, each)
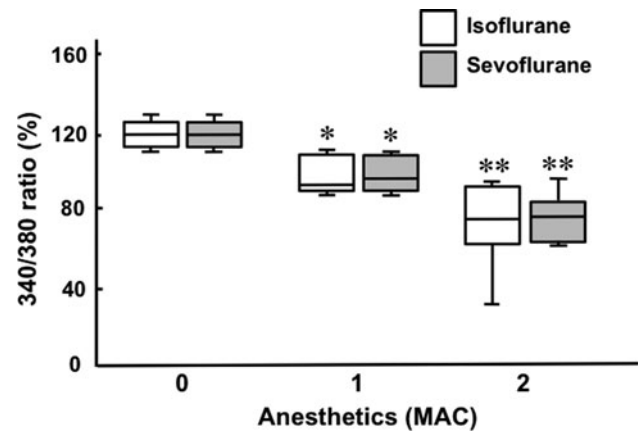

Fig. 4 The effects of isoflurane and sevoflurane on the increase in intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ induced by arginine vasopressin (AVP, 10^{-7} M). Changes in $[Ca^{2+}]_i$ are presented as ratios of the fluorescence measurements at the excitation wavelengths of 340 and 380 nm and are expressed as percentages relative to that induced by KCl (30 mM). *Horizontal bars* represent the medians, *boxes* represent the 25th and 75th percentile ranges, and *T-bars* represent the 5th and 95th percentile ranges. $*P < 0.05$, $**P < 0.01$ versus control ($n = 6$, each)
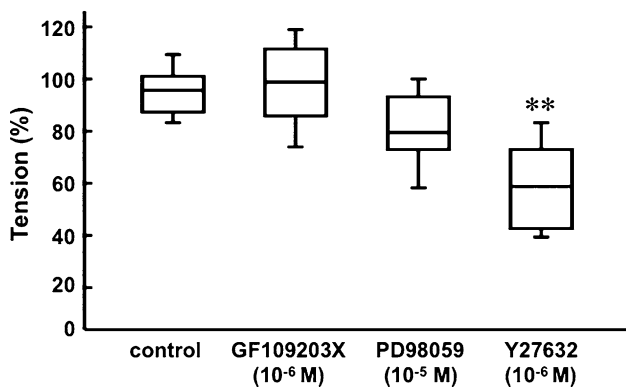

Fig. 3 The effects of protein kinase inhibitors on rat aortic smooth muscle contraction induced by arginine vasopressin (AVP 10^{-7} M). GF109203X (10^{-6} M), PD98059 (10^{-6} M), and Y27632 are selective inhibitors of protein kinase C, p44/42 mitogen-activated protein kinase, and Rho kinase, respectively. The AVP-induced tension changes are expressed as percentages relative to that induced by KCl (30 mM). *Horizontal bars* represent the medians, *boxes* represent the 25th and 75th percentile ranges, and *T-bars* represent the 5th and 95th percentile ranges. $**P < 0.01$ versus control ($n = 8$, each)

plays an important role in regulating the systemic blood pressure under both physiological and pathophysiological conditions [6, 9]. Extrinsic AVP is also used for treating hemodynamic instability in catecholamine-resistant vasodilatory shock, including sepsis [18–20]. AVP is also used to support blood pressure in anaphylactic shock [13–15] and in profound hypotension after cardiopulmonary bypass [11, 12] during general anesthesia. However, little information is available about the modulation of AVP-induced vasoconstriction by anesthetic agents.

As with other receptor-mediated vasoconstrictors, AVP binds to the V_1 receptor on the surface of vascular smooth muscle cells and activates phospholipase C to generate both inositol 1,4,5-triphosphate and diacylglycerol. The former stimulates Ca^{2+} release from the sarcoplasmic reticulum and the latter activates protein kinase C (PKC). AVP also induces an influx of Ca^{2+} from the extracellular space. In turn, PKC increases the Ca^{2+} sensitivity of the contraction-associated protein. Rho kinase and MAPK are also involved in increasing the Ca^{2+} sensitivity through activating a receptor-coupled G-protein [7, 8]. Rho is a small monomeric G-protein and is activated by certain agonists including AVP. Rho kinase is a major downstream effector molecule of Rho and, therefore, Rho activation induces increases in Rho kinase activity that promote myofilament Ca^{2+} sensitivity through MLC phosphatase inhibition [21]. In the present study, the Rho kinase inhibition, by Y27632 (but not by PKC or MAPK inhibitor), of the AVP-induced contraction suggests that the Rho-Rho kinase pathway plays a major role in the regulation of the AVP-induced increase in Ca^{2+} sensitivity, at least in rat aortic smooth muscle.

Volatile anesthetics suppress agonist-induced vasoconstriction by inhibiting the elevation of $[Ca^{2+}]_i$ and by reducing myofilament Ca^{2+} sensitivity [22]. Fujihara and colleagues demonstrated, in cultured rat aortic smooth muscle cells, that halothane attenuated an AVP-induced increase in $[Ca^{2+}]_i$ by inhibiting both Ca^{2+} release from intracellular Ca^{2+} stores and Ca^{2+} influx from the extracellular space [15]. However, the effect of anesthetics on the Ca^{2+} sensitivity mechanism has not yet been
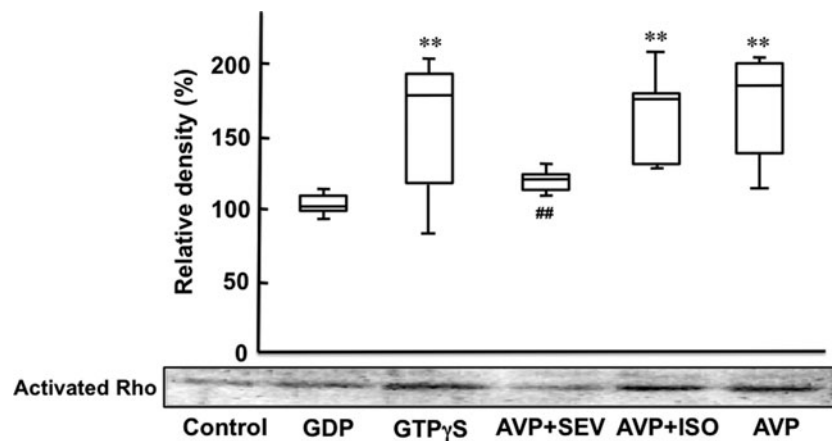
Fig. 5 The effects of isoflurane (*ISO*) and sevoflurane (*SEV*) (at 2 MAC) on the arginine vasopressin (*AVP* 10^{-7} M)-induced increase in Rho activity in rat aortic smooth muscle cells. Rhotekin-bound activated Rho was extracted using the pull down method and measured by Western blotting. Guanosine diphosphate (*GDP* 10^{-4} M) served as a negative control and guanosine 5′-[γ-thio] triphosphate (*GTPγS* 10^{-4} M) served as a positive control. Rho activity was expressed as a percentage relative to the level obtained from the control group (i.e., in the absence of a drug or anesthetic). *Horizontal bars* represent the medians, *boxes* represent the 25th and 75th percentile ranges, and *T-bars* represent the 5th and 95th percentile ranges. **$P < 0.01$ versus control group, ##$P < 0.01$ versus AVP group. ($n = 6$, each)

determined. Moreover, it remains unclear whether the cellular mechanism underlying the effect of isoflurane and sevoflurane is similar to that of halothane. In the present study, both isoflurane and sevoflurane inhibited, as halothane does, an AVP-induced increase in $[Ca^{2+}]_i$. Our previous study demonstrated that isoflurane, but not sevoflurane, suppressed Ang II-induced vasoconstriction by inhibiting $[Ca^{2+}]_i$ in rat aortic smooth muscle [3]. The inhibitory effect of sevoflurane on $[Ca^{2+}]_i$ may depend on the agonist that is being tested.

By contrast, the effect of isoflurane on the Ca^{2+} sensitization mechanism was opposite to that of sevoflurane. Sevoflurane, but not isoflurane, at a clinically relevant concentration, inhibited the AVP-induced increase in Rho activity. This finding is consistent with that from our previous series of studies using Ang II as a vasoconstrictor. Both isoflurane and sevoflurane attenuated the Ang II-induced vascular contraction to the same extent at equipotent concentrations. However, unlike sevoflurane, isoflurane did not affect the Ang II-induced phosphorylation of PKC that mediates the Ca^{2+} sensitization mechanism [2, 3]. We have also demonstrated that sevoflurane, used at the same concentrations as those in the present study, inhibited the GTPγS-induced vascular contraction and activation of the Rho-Rho kinase pathway [16]. Isoflurane appears to lack the ability to affect agonist-induced protein kinase activation. However, isoflurane attenuates tyrosine phosphorylation in response to sodium orthovanadate, a potent protein tyrosine phosphate inhibitor, to a greater extent than does sevoflurane [23]. We cannot exclude the possibility that isoflurane might affect the Ca^{2+} sensitivity mechanism through a pathway other than the

Rho-Rho kinase pathway. Although these anesthetics have great structural similarity, their actions on the AVP-induced signaling pathway are not identical. The inhibitory effects of these anesthetics are mediated by some common actions and by agent-specific actions.

The normal plasma level of AVP was reported to be 5-10 pg/ml (approximately 10^{-11} M) in humans [24, 25]. However, AVP therapy in vasodilatory shock, at a dosage of 0.03–0.07 U/min, increases the plasma concentration to a range of 100–290 pg/ml (approximately up to 3×10^{-10} M) [10, 24, 25]. Thus, the AVP concentrations used in the present study are more than 100-fold higher than the clinical concentrations. The primary goal of the present study was to elucidate the cellular mechanism underlying the inhibitory effects of anesthetics on the AVP-induced contraction of vascular smooth muscle. We therefore used a high concentration of AVP to contract isolated arteries. As in the present study, many previous in vitro studies have used a high concentration of AVP to characterize its vasoconstrictive properties [7, 8, 15]. The differences in concentration and the effect of AVP between in vitro studies and the clinical situation may be explained, in part, by the presence of other intrinsic vasoconstrictors such as norepinephrine. It has been demonstrated that AVP potentiates the contractile effect of norepinephrine even at low concentrations at which AVP, per se, would not induce vasoconstriction [26, 27].

The major limitation of the present study is that we used endothelium-denuded aortic preparations. The endothelium plays a significant role in regulating the underlying vascular tone under in vivo conditions. AVP stimulates the release of nitric oxide from the endothelium, and this molecule induces the relaxation of underlying vascular smooth

muscle [28], and volatile anesthetics inhibit this endothelium-dependent vasorelaxation [29]. The presence of the endothelium might have interfered with the interpretation of the findings in the present study. Indeed, a smaller contractile response to a vasoconstrictor and an attenuation of the inhibitory effect of anesthetics on vascular contraction were demonstrated in rat aortas with intact endothelium compared with findings in those without endothelium in our previous study [30] using Ang II as a vasoconstrictive agent. Thus, in the present study, we used endothelium-denuded preparations to focus on vascular smooth muscle. Vascular responsiveness in large conduit arteries including the aorta may be different from that in small resistance arteries that mainly regulate systemic vascular resistance. Several studies have been carried out to examine the contractile response to AVP in small resistance arteries [5, 7, 26]. However, none of these studies has demonstrated direct evidence for an alteration of the protein kinase activity that regulates Ca^{2+} sensitization mechanisms, probably because of insufficient amounts of protein for Western blotting in small arteries. Thus, we used an aortic preparation to attempt to prove the involvement of the Rho pathway using Western blotting. Although the present findings obtained from large conduit arteries may not be directly applied to the clinical situation, the inhibition of AVP-mediated contraction by isoflurane and sevoflurane might be a cause of the hypotension that occurs during general anesthesia in patients in whom AVP is infused to support blood pressure.

In conclusion, AVP elicited a contraction of rat aortic smooth muscle, which was associated with increases in $[Ca^{2+}]_i$ and Rho activity. Isoflurane and sevoflurane, at a clinically relevant concentration, inhibit AVP-induced contraction. However, the cellular mechanism underlying the inhibitory effects of these anesthetics seems to differ. Sevoflurane attenuates both Ca^{2+}-dependent and Rho-Rho kinase-mediated Ca^{2+} sensitization (Ca^{2+}-independent) pathways, while the inhibitory effect of isoflurane is mainly mediated by a Ca^{2+}-dependent pathway.

Conflict of interest None of the authors have any conflicts of interest.

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