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ORIGINAL ARTICLE

Sevoflurane inhibits angiotensin II-induced Rho kinase-mediated contraction of vascular smooth muscle from spontaneously hypertensive rat

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Abstract
Purpose Angiotensin II (Ang II)-induced vasoconstriction is mediated by changes in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and myofilament Ca^{2+} sensitivity. Protein kinase C- and Rho kinase-mediated signaling pathways are proposed for the regulation of the Ca^{2+} sensitization mechanisms. We have demonstrated that sevoflurane inhibits Rho kinase-mediated contraction of isolated rat aortic smooth muscle. A recent study demonstrated that Rho-kinase mediated Ca^{2+} sensitization was involved in the pathophysiology of hypertension. This study was designed to investigate the effects of sevoflurane on Ang II-induced Rho kinase-mediated vascular contraction in spontaneously hypertensive rats (SHR).
Methods The effects of sevoflurane on vasoconstriction, increase in $[Ca^{2+}]_i$, and membrane translocation of Rho kinase in response to Ang II were investigated in normotensive Wistar–Kyoto rats (WKY) and SHR, using an isometric force transducer, a fluorometer, and Western blotting, respectively.
Results The inhibitory effects of sevoflurane on Ang II (10^{-7} M)-induced contraction were greater ($P < 0.05$) in SHR than in WKY at the highest concentration of sevoflurane (5.1%). Y27632 (3×10^{-7} M), a specific inhibitor of Rho kinase, inhibited the Ang II-induced contraction in SHR, but not in WKY. Sevoflurane did not affect the increases in $[Ca^{2+}]_i$ in response to Ang II in either strain. Ang II stimulated Rho kinase activity in SHR, which was almost abolished by sevoflurane at a concentration of 5.1% ($P < 0.05$).
Conclusions These findings suggest that the inhibition of the Ang II-induced contraction by sevoflurane in SHR may be, at least in part, due to the attenuation of the Rho kinase-mediated signaling pathway.

Keywords Sevoflurane · Hypertension · Artery · Rho kinase

Introduction

Vascular contraction induced by agonists is primarily determined by the level of myosin light chain (MLC) phosphorylation, which is regulated by the balance between Ca^{2+}/calmodulin-dependent myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP). Inhibition of MLCP activity increases MLC phosphorylation for a given intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), and results in the promotion of myofilament Ca^{2+} sensitivity [1]. Rho, a small monomeric G-protein, activates Rho kinase, which inhibits MLCP activity. Thus, the Rho–Rho kinase signaling pathway plays an important role in the Ca^{2+} sensitization mechanism after receptor stimulation [2–4].

Abnormal smooth muscle reactivity and increased vascular resistance contribute to the pathogenesis of hypertension. Alteration of intracellular Ca^{2+} mobilization has been demonstrated in hypertensive models [5–9]. Moreover, increased activity of Rho and Rho kinase expression has been reported in the spontaneously hypertensive rat (SHR) [10, 11]. Inhibition of Rho kinase activity by a specific inhibitor, Y27632, dramatically corrected hypertension in

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rat hypertensive models, but had little effect on the normotensive rat [12]. These findings indicate that the Rho kinase-mediated Ca^{2+} sensitization mechanism is involved in the pathophysiology of hypertension [10–13].

Volatile anesthetics decrease arterial blood pressure—at least in part—by directly dilating the blood vessels [14]. We have demonstrated that sevoflurane inhibits angiotensin II (Ang II)-induced vasoconstriction mainly by suppressing the Ca^{2+} sensitization mechanism [15]. It was also shown that sevoflurane inhibits the vascular contraction induced by GTPγS, a specific activator of the Rho–Rho kinase pathway [16]. These findings suggest that the suppression of the Rho kinase-mediated Ca^{2+} sensitization mechanism may be responsible for the sevoflurane-induced vasodilation and hypotension. Inhibition of the Rho kinase pathway in hypertension might, in part, account for the observation that hypertensive patients are more susceptible to volatile anesthetics than normotensive patients during clinical anesthesia [17]. Indeed, we have previously demonstrated that the inhibitory effects of isoflurane and sevoflurane on Ang II-induced vasoconstriction is enhanced in SHR compared with normotensive control rats [18]. However, a comparison of the inhibitory effects of sevoflurane on Rho kinase activity in SHR and normotensive control rats has never been carried out.

Therefore, the goal of the current study was to elucidate and compare the mechanisms responsible for the inhibitory effect of sevoflurane on the vascular contraction in hypertensive and normotensive rats, with an emphasis on the Rho kinase-mediated Ca^{2+} sensitization mechanism.

Materials and methods

All experimental protocols were approved by the Wakayama Medical University Animal Care and Use Committee.

Animals

Wistar–Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were supplied by the Shizuoka Laboratory Animal Center (Shizuoka, Japan). Eleven-week-old male WKY and SHR were used in the current study. Systolic blood pressure (SBP) was recorded in conscious rats by the tail-cuff method, using a model MK-2000 BP monitor for mice and rats (Muromachi Kikai Co., Ltd., Tokyo, Japan), a few days prior to the experiments.

Isometric tension measurement

The rats were anesthetized with halothane and killed by decapitation. The descending thoracic aortas were carefully dissected out, and adherent fat and connective tissues were removed. The prepared aortas were cut into rings 3 mm in length for the tension measurement experiment. The endothelium was denuded by gentle rubbing of the luminal surface with a stainless steel needle. Four to six rings were typically harvested from each rat. The rings were incubated under a resting tension of 3 g in organ chambers containing Krebs bicarbonate solution (KBS) (in mmol/L, NaCl 118.2, KCl 4.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 24.8 and dextrose 10.0). KBS was aerated continuously with a gas mixture of 95% O_2 and 5% CO_2 to keep the pH value within the range 7.35–7.45, and it was maintained at 37°C. Isometric tension was measured as described previously [15, 16, 18].

After a 60-min equilibration period, all the aortic rings were exposed to KBS containing KCl (30 mM) to assess their overall contractile responsiveness, and the values were considered to be the reference (100%). Removal of endothelium was confirmed by the relaxation response to acetylcholine (10^{-6} M) in rings precontracted with phenylephrine (3×10^{-7} M). Only the rings that developed at least 1.0 g of contractile force in response to KCl (30 mM) and exerted no relaxation response to acetylcholine were used for the following experiments.

Initially, the rings of six different animals from each of WKY and SHR ($n = 6$) were exposed to Ang II at concentrations of 10^{-9} to 10^{-6} M to determine the concentration-dependent contractile response. The results showed that Ang II induced almost maximum response at a concentration of 10^{-7} M in rings from WKY and SHR. Thus, the concentration of 10^{-7} M was used for the subsequent experiments. To examine the effect of sevoflurane on Ang II-induced contraction, aortic rings from eight different animals of each strain ($n = 8$) were randomly exposed to 0, 1.7, 3.4 or 5.1% of sevoflurane 15 min prior to the addition of Ang II. To assess the involvement of the Rho–Rho kinase pathway in the Ang II-induced contraction, the rings from each strain ($n = 6$) were incubated in the presence or absence of the specific Rho kinase inhibitor, Y27632 (3×10^{-7} M). Each ring was exposed to only one concentration of Ang II and treated with a single concentration of sevoflurane or Y27632 to avoid the occurrence of tachyphylaxis to Ang II. The change in isometric tension in response to Ang II was expressed as the percentage relative to that induced by KCl (30 mM).

Sevoflurane was introduced into the gas mixture through a vaporizer (Penlon Limited, Abingdon, Oxon, UK). The concentration of the resulting gas mixture was monitored and adjusted using an anesthetic agent monitor (Atom 303, Atom, Tokyo, Japan). Our previous data using the experimental system showed that the concentrations of sevoflurane in the KBS after a 15-min equilibration period, as measured by gas chromatography (Shimadzu Corporation, Kyoto, Japan), were 0.17 ± 0.05, 0.35 ± 0.02 and

0.50 ± 0.03 mM sevoflurane for gas concentrations of 1.7, 3.4, and 5.1%, respectively ($n = 8$–12) [18].

Measurement of intracellular Ca^{2+} concentration

Endothelium-denuded aortic strips, 5 mm long and 3.5 mm wide, were prepared from the isolated rat descending aortas of WKY and SHR. Two or three strips were harvested from each rat. The strips were incubated in KBS containing acetoxymethyl ester of fura-2 (10^{-5} M) (Fura2/AM) for 6 h at room temperature (19–23°C). A noncytotoxic detergent, 0.1% cremophor, was added to the solution to increase the solubility of acetoxymethyl ester. After the loading period, the preparations were repeatedly washed with KBS.

The strip was fixed in a temperature-controlled (37°C) organ bath that was perfused continuously with Krebs bicarbonate solution aerated with a mixture of 95% O_2 and 5% CO_2. Fluorescence measurements were performed using a dual-wavelength spectrofluorometer (CAF-110, Japan Spectroscopic, Tokyo, Japan) at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. The 340–380 nm fluorescence ratio was used as an indicator of $[Ca^{2+}]_i$.

The change in the 340/380 ratio in response to KCl (30 mM) was measured first, and the values were used as a reference standard (100%). Sevoflurane at concentrations of 0, 1.7, 3.4 or 5.1% was introduced into the gas mixture for 15 min, followed by the addition of Ang II (10^{-7} M) into the bathing solution. The change in the 340/380 ratio was expressed as a percentage of the reference value. Aortic strips from eight different animals of each strain ($n = 8$) were used for the $[Ca^{2+}]_i$ measurement experiment, and each strip was exposed to only one concentration of sevoflurane.

Measurement of Rho kinase translocation

Endothelium-denuded rat aortic strips were bathed in aerated KBS and equilibrated for 60 min before exposure to the agents. One strip was obtained from each animal. Rock-2 (one of the two Rho kinase subtypes) activation was determined by its translocation from the cytosolic to the membrane fraction.

Aortas from 28 different animals of each strain were randomly assigned to four groups ($n = 7$) and incubated with sevoflurane at concentrations of 0, 1.7 or 5.1%, or Y27632 (3×10^{-7} M) for 15 min before the exposure to Ang II. The strips were then exposed to Ang II for 4 min, which was based upon our previous investigations [15, 19, 20], and then rapidly frozen on dry ice. Seven additional aortas of each strain without Ang II and sevoflurane treatment served as a baseline control. The frozen strips were cut into small pieces and homogenized in ice-cold lysis buffer [21]. Homogenates were centrifuged at 13,000*g* for 5 min at 4°C, and the supernatant was collected and centrifuged at 100,000*g* for 60 min at 4°C. The supernatant (cytosolic fraction) was removed, and the pellet (membrane fraction) was resuspended using the same buffer. The protein concentrations of each fraction were determined using the bicinchoninic acid method [22].

Equal amounts of total protein were used for every sample in each experiment. Proteins were separated by 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto nitrocellulose membranes. The membranes were then incubated in blocking buffer overnight at 4°C. The membranes were treated with anti-Rock-2 antibody (1:1000) for 2 h, and then incubated with horseradish peroxidase-conjugated antibody (1:2000) for 1.5 h. Polyclonal antibody against Rock-2 and the secondary antibody labeled with horseradish peroxidase were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA). The densities of the immunoreactive bands were detected using the enhanced chemiluminescence (ECL) system (Amersham Pharmarcia Biotech, Piscataway, NJ, USA). The amount of Rock-2 on the membrane fraction was expressed as a percentage of the total fraction.

Statistical analysis

Statistical analyses were performed using the StatMate software program (Atoms, Tokyo, Japan). Systolic blood pressure measurements of SHR and WKY were expressed as the mean ± SD and compared using the unpaired Student's *t* test. Other data were presented as medians with the 25th and 75th percentiles and evaluated using the Kruskal–Wallis followed by the Newman–Keuls test. The sample size (*n* values) represents the number of rats from which aortic rings (for tension measurements), aortic strips (for $[Ca^{2+}]_i$ measurement), or aortas (for western blotting) were harvested. *P* values of less than 0.05 were considered statistically significant.

Results

Systolic blood pressure was significantly higher ($P < 0.001$) in SHR (187 ± 11 mmHg, $n = 30$) than in WKY (113 ± 8 mmHg, $n = 30$).

Isometric tension measurement

KCl (30 mM) induced a sustained contraction of aortic rings from WKY and SHR, reaching peak values of 1.9 g (1.7–2.3 g) and 1.9 g (1.6–2.3 g), respectively. There was

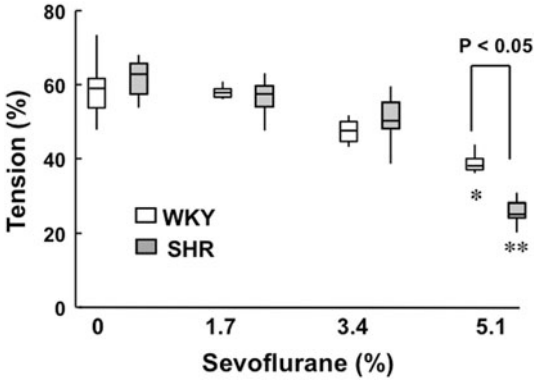

Fig. 1 Inhibitory effect of sevoflurane on Ang II (10^{-7} M)-induced contraction of endothelium-denuded aortic rings from WKY and SHR. Rings were incubated with 1.7, 3.4, or 5.1% sevoflurane for 15 min before treatment with Ang II. Ang II-induced changes in tension were expressed as the percentages relative to those induced by KCl (30 mM). *Horizontal bars* represent medians, *boxes* represent 25th and 75th percentile ranges, and *vertical lines* above and below the *box* represent 5th and 95th percentile ranges. The inhibition of the contraction by sevoflurane at 5.1% was greater ($P < 0.05$) in SHR than in WKY. *$P < 0.05$, **$P < 0.01$ versus sevoflurane 0% ($n = 8$, each)

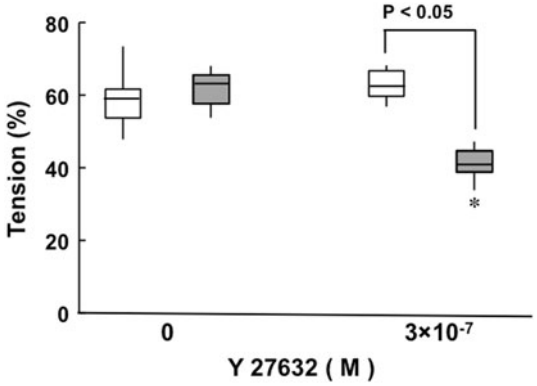

Fig. 2 Effects of Y27632 on the Ang II (10^{-7} M)-induced contraction of endothelium-denuded rat aortic rings from WKY and SHR. The endothelium-denuded aortic rings from WKY and SHR were incubated with Y27632 (3×10^{-7} M) for 15 min before the addition of Ang II. *Horizontal bars* represent medians, *boxes* represent 25th and 75th percentile ranges, and *vertical lines* above and below the *box* represent 5th and 95th percentile ranges. Ang II-induced changes in tension were expressed as the percentage relative to those induced by KCl (30 mM). The inhibitory effect of Y27632 (3×10^{-7} M) was greater ($P < 0.05$) in SHR than in WKY. *$P < 0.05$ versus control ($n = 6$, each)

no statistically significant difference in the KCl-induced contractions of WKY and SHR. Ang II induced a rapid, transient contraction which was followed by gradual decline above the baseline level in aortic rings from both WKY and SHR. The contractile response to Ang II was concentration dependent, and the maximum contraction was obtained at 10^{-6} M in both strains.

Sevoflurane inhibited the Ang II (10^{-7} M)-induced contraction in aortic rings from WKY and SHR. When comparing WKY and SHR, the extent of the inhibitory effects of sevoflurane on the contractile response to Ang II was significantly greater ($P < 0.05$) in SHR than in WKY at the highest concentration of 5.1% (Fig. 1). Y27632 (3×10^{-7} M) inhibited the contractile response to Ang II in SHR, but not in WKY ($P < 0.05$) (Fig. 2).

Ca^{2+} measurement

The KCl (30 mM)-induced increase in $[Ca^{2+}]_i$ was similar in WKY and SHR. Ang II (10^{-7} M) elicited a transient elevation of $[Ca^{2+}]_i$ in aortic smooth muscle from both strains, and there was no significant difference in the Ang II-induced increase in $[Ca^{2+}]_i$ between WKY and SHR. Sevoflurane did not significantly affect the Ang II-induced increases in $[Ca^{2+}]_i$ up to a concentration of 5.1% in either strain (Fig. 3).

Measurement of Rho kinase translocation

Immunoreactive bands with molecular weights of 150 kDa, which corresponded to Rock-2, were detected in the cytosolic and membrane fractions. Ang II (10^{-7} M) did not influence the Rock-2 expression in the membrane fraction in WKY, while it significantly facilitated membrane translocation of Rock-2 in SHR ($P < 0.01$) (Fig. 4).

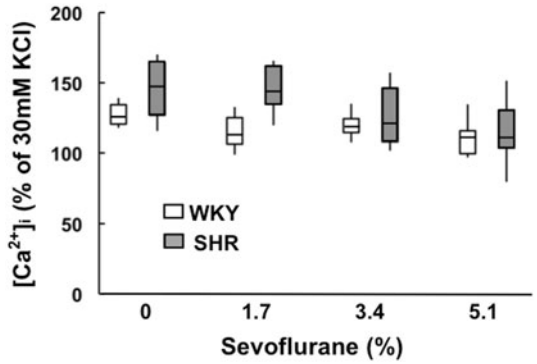

Fig. 3 Effects of sevoflurane on the Ang II (10^{-7} M)-elicited increase in $[Ca^{2+}]_i$ in rat aortic strips from WKY and SHR. The endothelium-denuded rat aortic strips from WKY and SHR were treated with the fura-2 solution and then exposed to 1.7, 3.4, or 5.1% sevoflurane for 15 min. *Horizontal bars* represent medians, *boxes* represent 25th and 75th percentile ranges, and *vertical lines* above and below the *box* represent 5th and 95th percentile ranges. The 340–380 nm fluorescence ratio was used as an indicator of $[Ca^{2+}]_i$ and expressed as the percentage relative to that induced by KCl (30 mM) ($n = 8$, each)

Neither sevoflurane up to 5.1% nor Y27632 (3×10^{-7} M) affected the translocation of Rock-2 in WKY. By contrast, sevoflurane at 5.1% almost reversed ($P < 0.05$) the Ang II-stimulated translocation of Rock-2 to the baseline control level in SHR. Y27632 also abolished

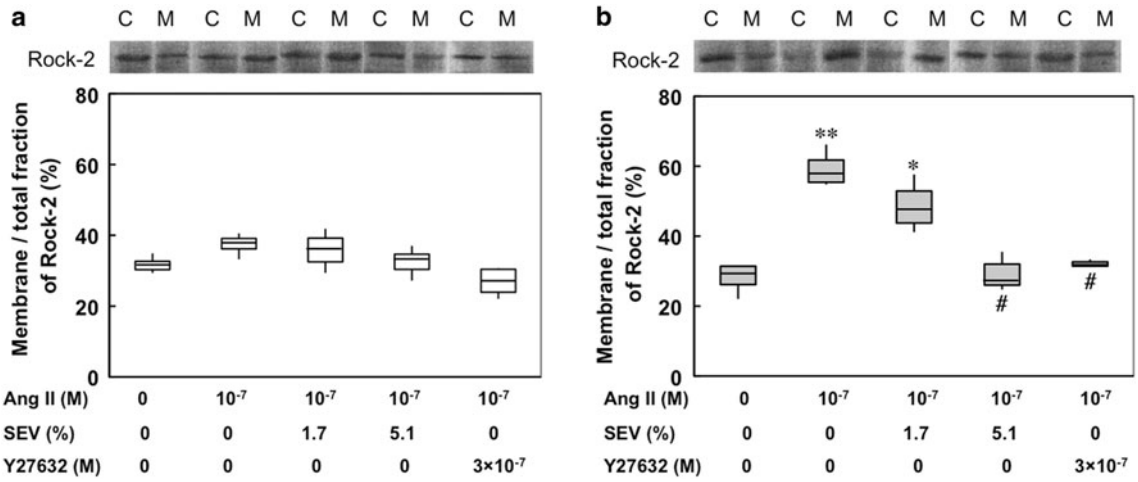

Fig. 4 Inhibitory effect of sevoflurane on Ang II (10^{-7} M)-stimulated membrane translocation of Rock-2 in rat aortic smooth muscle from WKY (**a**) and SHR (**b**). Endothelium-denuded rat aortas were incubated with 1.7 or 5.1% sevoflurane or Y27632 (3×10^{-7} M) for 15 min before exposure to Ang II, and were homogenized 4 min after the addition of Ang II. The immunoreactive bands of Rock-2 were detected with Western blotting. *Horizontal bars* represent medians, *boxes* represent 25th and 75th percentile ranges, and *vertical lines* above and below the *box* represent 5th and 95th percentile ranges. The amount of Rock-2 in the membrane fraction was expressed as a percentage of the total Rock-2 in both the cytosolic and the membrane fractions. *C* cytosolic fraction, *M* membrane fraction. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the value in the absence of Ang II (control), $^{\#}P < 0.05$ versus Ang II alone ($n = 7$, each)

the Ang II-activated translocation of Rock-2 in SHR (Fig. 4).

Discussion

Vascular smooth muscle contraction is regulated by both Ca^{2+}-dependent and Ca^{2+}-independent (or calcium sensitization) mechanisms. Receptor activation by Ang II increases $[Ca^{2+}]_i$ through the release of Ca^{2+} from the intracellular stores and the influx of Ca^{2+} from the extracellular space, which eventually facilitates MLC phosphorylation. Ang II also stimulates protein kinase C (PKC) and Rho kinase through G_q and $G_{12/13}$ protein activation, respectively [23]. Activation of these protein kinases can inhibit the dephosphorylation of phosphorylated MLC and thereby potentiate the contraction for a given $[Ca^{2+}]_i$ [24, 25].

Alteration of Ca^{2+} mobilization [5–9] and the promotion of activity in the Rho–Rho kinase signaling pathway [10–13] are reported to be involved in the pathogenesis and development of hypertension. In the current study, the increase in $[Ca^{2+}]_i$ in response to Ang II in SHR did not differ from that in WKY, while the inhibition of Ang II-induced vasoconstriction by Y27632, a Rho kinase inhibitor, was observed only in SHR. This finding is in accord with that from a previous study in which the relaxation response to Y27632 in mesenteric arteries preconstricted with norepinephrine was greater in SHR than in WKY [26]. In an in vivo animal experiment, Y27632 also decreased blood pressure in a hypertensive model, while it had little effect on the normotensive animal [12]. In addition, Western blotting analysis revealed that membrane translocation of Rock-2 in response to Ang II occurred in SHR, but not in WKY. These findings possibly account for the greater contribution of the Rho kinase-mediated signaling pathway to Ang II-induced vasoconstriction in SHR.

Similar to the present study, we previously demonstrated that the inhibitory effect of sevoflurane on Ang-II induced vasoconstriction is enhanced in SHR [18]. However, the mechanism responsible for the greater inhibition of sevoflurane in SHR remained unclear. Vascular smooth muscle contraction is primarily mediated by changes in $[Ca^{2+}]_i$, and some anesthetics are known to inhibit the increase in $[Ca^{2+}]_i$ in response to Ang II. Samain et al. [27] demonstrated that isoflurane inhibited the Ang II-induced Ca^{2+} mobilization in both WKY and SHR. We have also shown in the normotensive rat that isoflurane attenuates an increase in $[Ca^{2+}]_i$ in response to Ang II [20], while sevoflurane does not have an inhibitory effect on $[Ca^{2+}]_i$ [15]. In the current study, sevoflurane did not alter the Ang II-induced increase in $[Ca^{2+}]_i$ up to 5.1% in both strains, suggesting that the enhanced inhibitory effect of sevoflurane on Ang II-induced vasoconstriction seen in SHR may not be mediated by the Ca^{2+}-dependent pathway. Rather, the Ca^{2+} sensitization mechanism might be involved in the different effect that sevoflurane had on SHR compared with WKY. The lack of inhibition of the Ang II-induced increase in $[Ca^{2+}]_i$ observed in the current study does not mean that sevoflurane has no inhibitory effect on $[Ca^{2+}]_i$ in response to various stimuli. Indeed, our recent study [28] indicated that sevoflurane decreased the arginine

vasopressin-stimulated increase in $[Ca^{2+}]_i$. The inhibitory effect of anesthetics on $[Ca^{2+}]_i$ may depend on the agonists and the mechanisms that mediate Ca^{2+} mobilization.

Next, we investigated the effect of sevoflurane on the Rho kinase activity. We hypothesized that the inhibition of the Ca^{2+} sensitization mechanism by sevoflurane might be a reason for the greater inhibition of the contraction in SHR, since our previous study demonstrated that sevoflurane attenuates GTPγS-stimulated Rho kinase activation, which regulates myofilament Ca^{2+} sensitivity [16]. As a result, sevoflurane at high concentration inhibited the membrane translocation of Rock-2 in SHR, but failed to do so in WKY up to a concentration of 5.1%. This finding is consistent with the results of the isometric force experiment in which the greatest inhibition of the vasoconstriction in SHR was observed at 5.1% sevoflurane, suggesting that Rho kinase inhibition by sevoflurane is involved in the enhanced inhibition of Ang II-induced vasoconstriction in SHR, at least at high concentrations.

PKC also plays a role in the regulation of the enhanced contraction in SHR [29, 30]. However, the contribution of PKC to agonist-induced contractions in the aortas of SHR has been reported to be equal to the equivalent contribution in WKY [31]. Some investigations have suggested that there is greater myogenic tone with increased calcium sensitization in SHR, largely because of Rho kinase activation, with a minor or no contribution from PKC activation [32, 33]. Although we have previously demonstrated that sevoflurane attenuates PKC phosphorylation in response to Ang II in the normotensive rat [15], the effect of sevoflurane on PKC activity in the hypertensive model has never been determined. Further study may be needed to clarify the issue.

The limitation of this in vitro study is that the present findings were obtained from endothelium-denuded aortic preparations. Removal of endothelium may alter the vascular responsiveness to various stimuli. However, the primary goal of the present study was to elucidate the mechanisms by which sevoflurane inhibits Ang II-induced contraction of vascular smooth muscle in hypertension. Because sevoflurane also has an inhibitory effect on endothelium function [34] which may interfere with the present findings, we used endothelium-denuded aortic preparation. Vascular responsiveness in large conduit arteries, including the aorta, may be different from that in small resistance arteries that mainly regulate systemic vascular resistance. However, it has been demonstrated that Rho kinase inhibition by Y27632 results in greater relaxation of norepinephrine-contracted arterial preparation from SHR compared with that from WKY, even in small mesenteric arteries [26], suggesting the possibility of enhanced Rho kinase activity in response to agonist stimuli in small resistance arteries. Another limitation of the present study is that the inhibition of muscle tension and Rho kinase activity induced by sevoflurane was only significant at supra-clinical concentrations. Because sevoflurane has very low water solubility, it easily vaporizes from the surface area of the bathing solution in the organ chamber in which the aortic preparations were incubated. In our previous study, the concentration of sevoflurane in KBS measured by gas chromatography was 0.50 ± 0.03 mM when 5.1% sevoflurane was added to the gas mixture [18]. An in vivo study has demonstrated that the arterial blood sevoflurane concentration in rats inspiring 2.5% sevoflurane reaches 0.5 mM [35]. Thus, the actual concentrations of sevoflurane used in the present study might be lower than those expressed. However, the present findings obtained from an in vitro study using a high concentration of sevoflurane cannot be directly extrapolated to the clinical situation.

In conclusion, sevoflurane inhibits the Ang II-induced contraction of aortic smooth muscle from SHR to a greater extent than that from WKY. Sevoflurane did not alter the $[Ca^{2+}]_i$ in response to Ang II in both strains. Ang II stimulates Rho kinase activity in SHR, which is significantly inhibited by sevoflurane. These findings suggest that the suppression of Rho kinase activity may be involved in the greater inhibition of the vasoconstriction by sevoflurane in SHR.

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