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Sevoflurane Inhibits Angiotensin II–induced, Protein Kinase C–mediated but Not Ca^{2+}-elicited Contraction of Rat Aortic Smooth Muscle

Jingui Yu, M.D., Yasuyuki Tokinaga, M.D.,† Koji Ogawa, M.D.,‡ Shizue Iwahashi, M.D.,† Yoshio Hatano, M.D.§*

Background: **Whether volatile anesthetics attenuate angiotensin II–mediated vascular tone has not been determined. The current study was designed to investigate the effects of sevoflurane on the angiotensin II–stimulated, Ca^{2+}- and protein kinase C (PKC)–mediated contraction of rat aortic smooth muscle.**

Methods: **The dose-dependent effects of sevoflurane on angiotensin II (10^{-7} M)–induced contraction, the increase in intracellular Ca^{2+} concentration, and PKC phosphorylation of rat aortic smooth muscle were measured using an isometric force transducer, a fluorometer, and Western blotting, respectively.**

Results: **Angiotensin II induced a transient increase in intracellular Ca^{2+} concentration, phosphorylation of Ca^{2+}-dependent PKC (cPKC)-α, and consequently, a transient contraction of rat aortic smooth muscle. Phosphorylation of the Ca^{2+}-independent PKC-ϵ was not detected. The angiotensin II–induced contraction was almost completely abolished by removing extracellular Ca^{2+} and was significantly inhibited by the selective cPKC inhibitor Gö 6976 (10^{-5} M) but was not inhibited by the nonselective PKC inhibitor Ro 31-8425 (10^{-5} M). Sevoflurane dose-dependently inhibited the angiotensin II–induced contraction, with reductions of 14.2 ± 5.2% ($P > 0.05$), 26.7 ± 8.9% ($P < 0.05$), and 38.5 ± 12.8% ($P < 0.01$) (n = 10) in response to 1.7, 3.4, and 5.1% sevoflurane, respectively. The angiotensin II–elicited increase in intracellular Ca^{2+} concentration was not significantly influenced by 3.4, 5.1, or 8.5% sevoflurane. However, cPKC-α phosphorylation induced by angiotensin II was inhibited dose dependently by 1.7, 3.4, and 5.1% sevoflurane, with depressions of 20.5 ± 14.2% ($P > 0.05$), 37.0 ± 17.8% ($P < 0.05$), and 62.5 ± 12.2% ($P < 0.01$) (n = 4), respectively.**

Conclusion: **The current study indicates that Ca^{2+} and cPKC-α are involved in angiotensin II–induced vascular contraction. Sevoflurane dose-dependently inhibited the angiotensin II–stimulated, cPKC-mediated but not Ca^{2+}-elicited contraction of rat aortic smooth muscle.**

VOLATILE anesthetics decrease blood pressure in a concentration-dependent manner, at least in part, by direct dilation of the blood vessels. Angiotensin II, the active component of the renin–angiotensin system, plays an important role in regulating vascular tone. Angiotensin II–induced vasoconstriction is mediated by changes in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and myofilament Ca^{2+} sensitivity.

Ca^{2+}-elicited phosphorylation of the myosin light chain and protein kinase C (PKC)–mediated Ca^{2+} sensitization of contraction-associated proteins are the most important mechanisms involved in angiotensin II–induced contraction.[1] Although sevoflurane is known to inhibit the vasoconstriction in response to various neurohumoral agonists, no information is available regarding the effect of sevoflurane on angiotensin II–induced vascular contraction. If the anesthetic affects angiotensin II–mediated contraction, intracellular Ca^{2+} signaling pathway and PKC-mediated Ca^{2+} sensitization are possible targets for anesthetic.

When PKC is activated, it translocates to the plasma membrane and is autophosphorylated. Autophosphorylation increases PKC activity and affinity to substrates.[2,3] The aim of the current study was to investigate the effects of sevoflurane on the angiotensin II–stimulated, Ca^{2+}- and PKC-mediated contraction of rat aortic smooth muscle by measurement of isometric force, $[Ca^{2+}]_i$, and phosphorylation of PKC isoenzymes.

Materials and Methods

Isometric Force Measurement

With the approval of the Wakayama Medical University Animal Care and Use Committee (Wakayama City, Japan), male Wistar rats (weight, 250–350 g) were anesthetized with halothane and euthanized by exsanguination from the common carotid artery. The descending thoracic aorta was dissected carefully, and adherent fat and connective tissue was removed. The prepared aorta was cut into rings 3–4 mm in length, and the endothelium was denuded by gentle rubbing of the internal surface with a stainless steel needle to examine the contraction of rat aortic smooth muscle. The prepared rings were mounted vertically on force-displacement transducers (Nihondenki-sanei Co., Tokyo, Japan) and were bathed in 10-ml organ chambers containing Krebs bicarbonate solution (KBS) with the following composition: 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 10.0 mM dextrose. KBS in the chamber was gassed with a mixture of 95% O_2–5% CO_2 to keep the pH value within the range 7.35–7.45 and was maintained at 36.5°–37.5°C. The rings were maintained at a resting tension of 3.0 g and were equilibrated for 60 min in the control KBS, which was replaced every 20 min.

* Research Fellow, † Graduate Student, § Professor and Chairman, Department of Anesthesiology, Wakayama Medical University. ‡ Assistant Professor, Surgical Center Division, Wakayama Medical University.

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Address reprint requests to Dr. Hatano: Department of Anesthesiology, Wakayama Medical University, 811-1 Kimiidera, Wakayama City, 641-0012, Japan. Address electronic mail to: yhatano@wakayama-med.ac.jp.

After equilibration, all the aortic rings were exposed to KBS containing KCl (3×10^{-2} M) to assess the integrity of smooth muscle cells and their overall contractile responsiveness. Removal of the endothelium was confirmed in phenylephrine (3×10^{-7} M)–precontracted rings by the lack of relaxation to 10^{-5} M acetylcholine.

A submaximal concentration, 10^{-7} M, of angiotensin II was used to induce contraction in the study. In a preliminary experiment, tachyphylaxis of the rings to angiotensin II was demonstrated (*i.e.*, the contractile response to the first application of angiotensin II was higher than that to the second and the third applications, and the responses to the last two applications were almost same [data not shown]). Therefore, the contractile response to the second application of angiotensin II was used as control. The response to the third application was used to test the effects of agents. The rings were incubated in normal KBS, Ca^{2+}-free KBS, KBS with the selective Ca^{2+}-dependent PKC (cPKC) inhibitor Gö 6976 (10^{-5} M), the nonselective PKC-inhibitory Ro 31-8425 (10^{-5} M), or sevoflurane (1.7, 3.4, and 5.1%) for 15 min. Angiotensin II was then applied, and resultant tension was measured.

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

Endothelium-denuded aortic strips approximately 5 mm long and 3.5 mm wide were prepared from the isolated rat descending thoracic aorta and were treated with 10^{-5} M acetoxymethyl ester of fura-2 solution for 6–9 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.

The determination of $[Ca^{2+}]_i$ was performed using a fluorometer designed to measure the surface fluorescence of living tissues (CAF-110; Japan Spectroscopic, Tokyo, Japan). The muscle strip was held horizontally in a temperature-controlled organ bath. One end of the strip was connected to an isometric force transducer to measure muscle tension. A passive tension of 3.0 g was applied, and the muscle strip was allowed to equilibrate for 60 min before the start of the experiment. The intimal surface of the muscle strip was illuminated at 50 Hz, alternately at excitation wavelengths of 340 and 380 nm, and the amount of fluorescence at 510 nm induced by 340 nm excitation (F340) and that induced by 380 nm excitation (F380) was measured. The ratio of F340 to F380 (F340:F380) was used to indicate the $[Ca^{2+}]_i$.

Isometric contraction and F340:F380 induced by 3×10^{-2} M KCl were measured, and the values were taken as the reference (100%). Sevoflurane (0 [control], 3.4, 5.1, or 8.5%) was introduced into the 95% O_2–5% CO_2 mixture for 15 min. Thereafter, angiotensin II (10^{-7} M) was administered when F340:F380 returned to the resting level. The changes in muscle tension and F340:F380 were recorded simultaneously and were expressed as a percentage of the reference values.

Measurement of PKC Phosphorylation

Isolated endothelium-denuded aortas were bathed in 95% O_2–5% CO_2 KBS and equilibrated for 60 min. To examine the time course of the angiotensin II–induced phosphorylation of classic PKC-α (cPKC-α) and novel PKC-ϵ (nPKC-ϵ), aortas were exposed to angiotensin II (10^{-7} M) for 0, 2, 4, 6, 8, or 10 min and then rapidly frozen with dry ice. The time course study showed that phosphorylation of cPKC-α reached a peak level 4 min after angiotensin II application, and a phosphorylated nPKC-ϵ band was not detected. Therefore, the dose effect of sevoflurane on angiotensin II–stimulated phosphorylation of only cPKC-α was examined 4 min after angiotensin II application in the following experiment. Aortas were incubated with 0 (control), 1.7, 3.4, or 5.1% sevoflurane for 15 min and were quickly frozen 4 min after exposure to angiotensin II.

The frozen tissues were homogenized in ice-cold lysis buffer with 0.2% Triton X-100.[4] Homogenates were centrifuged at 1,000*g* for 30 min at 4°C, and the supernatant was collected and centrifuged at 10,000*g* for 30 min at 4°C. The supernatants, containing both the cytosolic and membrane fractions, were assayed for protein concentration using the bicinchoninic acid method and were used for subsequent detection of PKC phosphorylation. The lysis buffer contained 250 mM sucrose, 5 mM EDTA, 10 μg/ml aprotinin, 20 μg/ml soybean tyrosine inhibitor, 100 μg/ml phenylmethanesulfonyl fluoride, 100 μg/ml leupeptin, 10 mM HEPES (pH 7.4), and 5 μM each of phosphoserine, phosphothreonine, phosphotyrosine, β-glycerophosphate, p-nitrophenylphosphate, and sodium orthovanadate.[4]

In each experiment, samples were used at equal content of total protein. Proteins were separated by sodium-dodecyl-sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Membranes were treated with anti–cPKC-α, anti–nPKC-ϵ (1:10,000 and 1:2,000, respectively), anti–phosphorylated cPKC-α, or anti–phosphorylated nPKC-ϵ antibodies (1:1,000) for 4 h, followed by incubation with horseradish peroxidase–conjugated antibody (1:2,000) for 1.5 h. The densities of immunoreactive bands were detected using chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ) and were assessed with image analysis software (NIH Image 1.62, Bethesda, MD). The levels of phosphorylated cPKC-α were expressed as a percentage of the cPKC-α density.

Administration of Sevoflurane

Sevoflurane was introduced into the gas mixture through an agent-specific vaporizer (Penlon Limited, Abingdon, Oxon, United Kingdom) as needed. The concentration in the resulting gas mixture was monitored and adjusted using an Atom 303 anesthetic agent monitor (Atom, Tokyo, Japan). The concentrations of sevoflu-

rane in KBS were measured by gas chromatography (Shimazu Seisakasho, Kyoto, Japan).

Because the same organ-bathing chambers were used to incubate the tissues for the tension measurement experiment and for PKC detection experiment, sevoflurane concentrations in KBS were considered to be the same if equal concentrations of sevoflurane were added to the gas mixture. The equilibrated concentrations of sevoflurane in the KBS were 0.17 ± 0.05, 0.35 ± 0.02, and 0.50 ± 0.03 mM at concentrations of 1.7, 3.4, and 5.1% in the gas mixture, respectively (n = 8–12). For the $[Ca^{2+}]_i$ measurement experiment, the surface area of the organ-bathing chamber in which the aortic strips were incubated was larger; therefore, the vaporizing amount of sevoflurane from the KBS was greater, and the actual concentration of sevoflurane in KBS was lower than that for the tension measurement and PKC detection experiments, although an equal concentration of sevoflurane was added to the gas mixture. The concentrations of sevoflurane in the KBS for the $[Ca^{2+}]_i$ measurement experiment were 0.14 ± 0.01, 0.27 ± 0.02, 0.38 ± 0.06, and 0.62 ± 0.08 mM at sevoflurane concentrations of 1.7, 3.4, 5.1, and 8.5% in the gas mixture, respectively (n = 3). Because it was difficult to adjust the concentration of sevoflurane in the KBS to equivalent levels for the experiments of tension measurement, PKC detection, and the experiment of $[Ca^{2+}]_i$ measurement and because sevoflurane did not significantly influence the angiotensin II–induced increase in $[Ca^{2+}]_i$ at concentrations of 3.4 and 5.1% in the current study, we attempted an even higher concentration (8.5%).

Materials

Angiotensin II, Gö 6976, and Ro 31-8425 were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO). Sevoflurane was purchased from Dainabot Company Limited (Osaka, Japan). Polyclonal anti–cPKC-α, anti–nPKC-ε, anti–phosphorylated cPKC-α, and anti–phosphorylated nPKC-ε antibodies and the secondary antibody labeled with horseradish peroxidase were supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Other reagents for the mechanical experiment, Ca^{2+} measurement, and Western blotting were all of analytical grade. Angiotensin II was dissolved using distilled water, and Gö 6976 and Ro 31-8425 were dissolved using dimethyl sulfoxide.

Statistical Analysis

The data are presented as mean ± SD. The sample size (n values) represents the number of rats from which aortic rings (for tension measurement), aortic strips (for $[Ca^{2+}]_i$ measurement), or aortas (for Western blotting) were taken. Two-factorial analysis of variance was used to compare the effects of the different sevoflurane concentrations on the angiotensin II–induced contraction, $[Ca^{2+}]_i$, and phosphorylation of cPKC-α, using the software program StatView (SAS Institute Inc., Cary, NC). *P* values less than 0.05 were considered statistically significant.

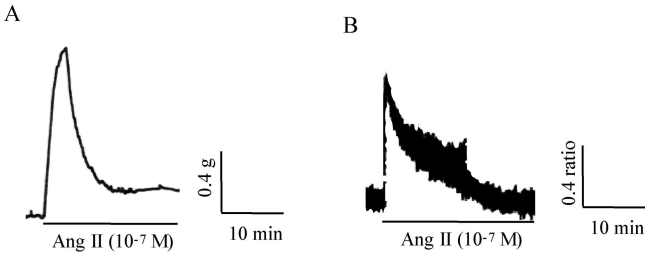

Fig. 1. The actual recordings of the angiotensin (Ang) II–induced contraction (*A*) and change in intracellular Ca^{2+} concentration (*B*) of endothelium-denuded rat aortic strips. The aortic strips were treated with the fura-2 solution for 6–9 h at room temperature. The amount of fluorescence in smooth muscle cells was measured in the presence of angiotensin II (10^{-7} M), using a fluorometer. Intracellular Ca^{2+} concentration was indicated as F340:F380.

Results

Angiotensin II–induced Contraction, Increase in $[Ca^{2+}]_i$, and Phosphorylation of cPKC-α and nPKC-ε

Angiotensin II (10^{-7} M) induced a transient contraction of rat aortic endothelium–denuded rings, reaching the maximal level of 69.0 ± 3.1% (n = 10) of the 3×10^{-2} M KCl–induced contraction 3.6 ± 0.2 min after application (fig. 1A). Angiotensin II (10^{-7} M) also elicited a transient increase in $[Ca^{2+}]_i$, immediately reaching a peak level of 78.6 ± 15.5% (n = 7) of that induced by 3×10^{-2} M KCl (fig. 1B). The absence of extracellular Ca^{2+} almost completely abolished the angiotensin II (10^{-7} M)–induced contraction (12.2 ± 5.3% of control, $P < 0.001$, n = 6; fig. 2). The selective cPKC inhibitor Gö 6976 (10^{-5} M) significantly attenuated (63.3 ± 7.9% of control, $P < 0.01$, n = 6), but the nonselective PKC inhibitor Ro 31-8425 (10^{-5} M) did not significantly inhibit the angiotensin II–induced contractile response (94.5 ± 7.6% of control, $P > 0.05$, n = 6; fig. 2).

The bands with molecular weights of approximately 80 and 90 kDa were identified as cPKC-α and nPKC-ε, respectively. In the same experiment, the densities of the cPKC-α band or the nPKC-ε band were almost identical for each sample. Similar to the contraction time course, angiotensin II (10^{-7} M) transiently increased the phosphorylation of cPKC-α, with the maximal level achieved 4 min after application (fig. 3A). A phosphorylated nPKC-ε band was not detected in the presence of angiotensin II (fig. 3B).

Effects of Sevoflurane on Angiotensin II–induced Contraction, Increase in $[Ca^{2+}]_i$, and Phosphorylation of cPKC-α

Sevoflurane concentration-dependently inhibited the angiotensin II (10^{-7} M)–induced contraction, with reduc-

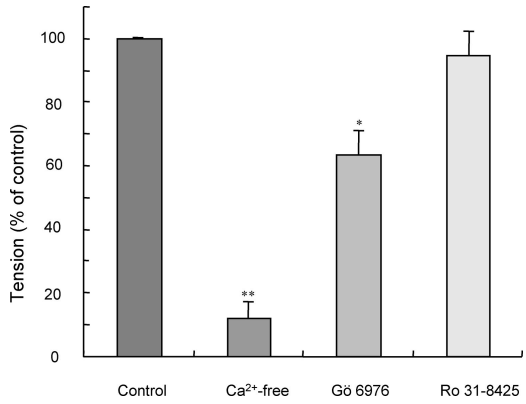

Fig. 2. The effects of extracellular Ca^{2+}-free Krebs bicarbonate solution (KBS) and protein kinase C inhibitors on angiotensin II–induced contraction of endothelium-denuded rat aortic rings. The endothelium-denuded rat aortic rings were incubated with normal KBS, Ca^{2+}-free KBS, and KBS with the selective classic protein kinase C inhibitor Gö 6976 (10^{-5} M) or with the nonselective protein kinase C inhibitor Ro 31-8425 (10^{-5} M) for 15 min and were then exposed to angiotensin II (10^{-7} M). The tension was measured using a force-displacement transducer. The angiotensin II–induced increase in tension in normal KBS (control) was considered 100%. * $P < 0.01$, ** $P < 0.001$ *versus* control (n = 6).

tions of 14.2 ± 5.2% ($P > 0.05$), 26.7 ± 8.9% ($P < 0.05$), and 38.5 ± 12.8% ($P < 0.01$) in response to 1.7, 3.4, and 5.1% sevoflurane, respectively (n = 8, fig. 4).

The presence of sevoflurane did not significantly affect the angiotensin II (10^{-7} M)–elicited increase in $[Ca^{2+}]_i$, with levels of 78.6 ± 15.5, 63.1 ± 12.8, 68.5 ± 15.0, and 64.1 ± 10.5% of that induced by 3×10^{-2} M KCl in response to 0, 3.4, 5.1, and 8.5% sevoflurane ($P > 0.05$, n = 6–9; fig. 5), respectively, but were accompanied with a dose-dependent attenuation in contraction,

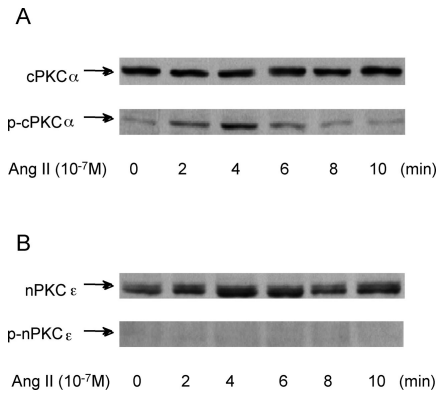

Fig. 3. The time course of angiotensin (Ang) II–induced Ca^{2+}-dependent protein kinase C α (cPKC-α) and Ca^{2+}-independent protein kinase C ε (nPKC-ε) phosphorylation in rat aortic smooth muscle. Endothelium-denuded rat aortas were exposed to angiotensin II (10^{-7} M) for 0, 2, 4, 6, 8, or 10 min and were frozen immediately. The immunoreactive bands of cPKC-α and phosphorylated cPKC-α (*A*) and nPKC-ε and phosphorylated nPKC-ε (*B*) were detected with Western blotting using specific antibodies. The image is representative of four independent experiments. p-cPKC-α = phosphorylated cPKC-α; p-nPKC-ε = phosphorylated nPKC-ε.

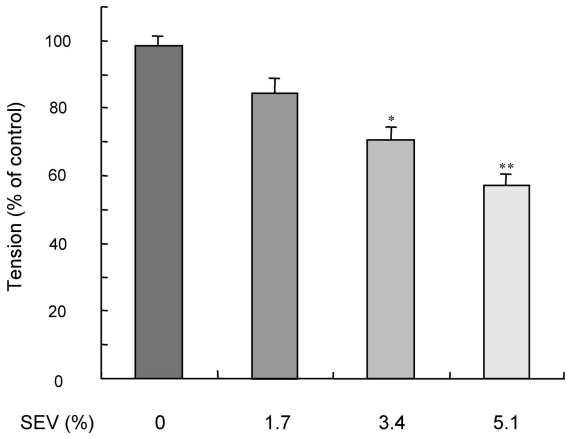

Fig. 4. The dose-dependent effects of sevoflurane (SEV) on the angiotensin II–induced contraction of endothelium-denuded rat aortic rings. The rings were incubated with 0 (control), 1.7, 3.4, or 5.1% sevoflurane for 15 min and were then challenged by angiotensin II (10^{-7} M). The tension produced by angiotensin II in the absence or presence of sevoflurane was measured with a force-displacement transducer. * $P < 0.05$, ** $P < 0.01$ *versus* control (n = 10).

which was measured spontaneously in the same experiments (data not shown).

Sevoflurane depressed the angiotensin II (10^{-7} M)–stimulated phosphorylation of cPKC-α in a concentration-dependent manner, with decreases of 20.5 ± 14.2% ($P > 0.05$), 37.0 ± 17.8% ($P < 0.05$), and 62.5 ± 12.2% ($P < 0.01$) in response to 1.7, 3.4, and 5.1% sevoflurane, respectively (n = 4; fig. 6).

Discussion

The main findings of this study are as follows: Angiotensin II (10^{-7} M) induced a rapid increase in $[Ca^{2+}]_i$, a

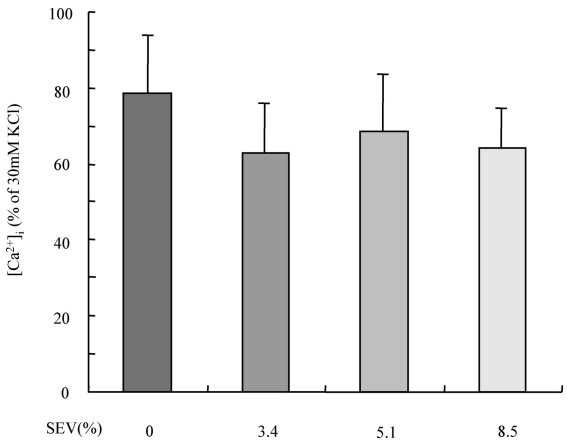

Fig. 5. The effects of sevoflurane (SEV) on the angiotensin II–elicited increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in rat aortic smooth muscle. The endothelium-denuded rat aortic strips were treated with the fura-2 solution and were then exposed to 0 (control), 3.4, 5.1, or 8.5% sevoflurane for 15 min. The amount of fluorescence in the presence of angiotensin II (10^{-7} M) was measured using a fluorometer. $[Ca^{2+}]_i$ was indicated as F340:F380 and was expressed as a percentage of the 30 mM KCl–elicited increase in $[Ca^{2+}]_i$ (n = 7–9; control).

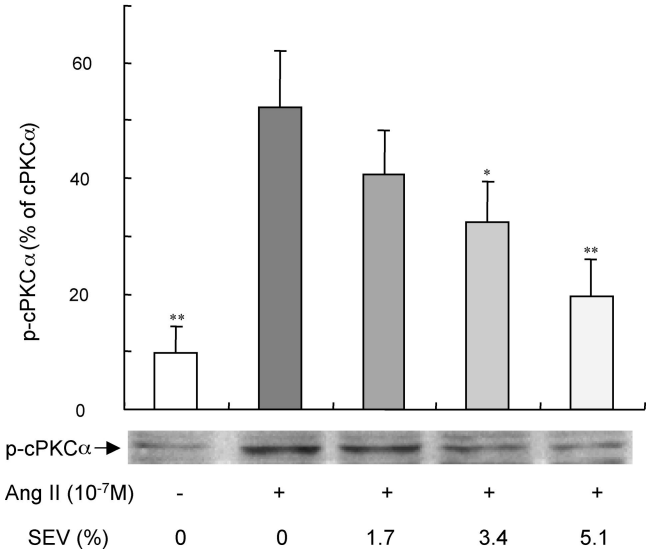

Fig. 6. The dose-dependent effects of sevoflurane (SEV) on angiotensin (Ang) II–stimulated phosphorylation of Ca^{2+}-dependent protein kinase C α (cPKC-α) in rat aortic smooth muscle. The rat endothelium-denuded aortas were incubated to 0, 1.7, 3.4, or 5.1% sevoflurane for 15 min and were frozen 4 min after application of angiotensin II (10^{-7} M). Phosphorylated cPKC-α band was detected using a specific antibody with Western blotting. The levels of phosphorylated cPKC-α were expressed as a percentage of cPKC-α band density. * $P < 0.05$, ** $P < 0.01$ *versus* the value in the presence of angiotensin II and 0% sevoflurane (n = 4). p-cPKC-α = phosphorylated cPKC-α.

transient phosphorylation of cPKC-α, and a consequently transient contraction, which was almost completely abolished by removing extracellular Ca^{2+} and was significantly inhibited by the selective cPKC inhibitor Gö 6976 (10^{-5} M) but not by the nonselective PKC inhibitor Ro 31-8425 (10^{-5} M). Phosphorylation of nPKC-ϵ in the presence of angiotensin II was not detected in the current study. Sevoflurane dose-dependently attenuated the angiotensin II (10^{-7} M)–stimulated contraction and the phosphorylation of cPKC-α but not the angiotensin II–elicited increase in $[Ca^{2+}]_i$.

Angiotensin II–induced, Ca^{2+}- and PKC-mediated Vascular Contraction

The potent peptide pressor hormone angiotensin II is important in the maintenance of vascular tone. The contractile action of angiotensin II is initiated on binding to AT_1 receptors on the surface of vascular smooth muscle cells (VSMCs).[5] After activation of phospholipase C, phosphatidylinositol-4,5-bisphosphate is hydrolyzed, and inositol 1,4,5-trisphosphate and diacylglycerol are produced. Inositol 1,4,5-trisphosphate stimulates the release of Ca^{2+} from the sarcoplasmic reticulum. Angiotensin II also activates L-gated Ca^{2+} channels and enhances Ca^{2+} influx. Increased $[Ca^{2+}]_i$ elicits the rapid contraction of VSMCs. Diacylglycerol activates PKC, increasing the Ca^{2+} sensitivity of contraction-associated proteins.[6]

The key role of Ca^{2+} in the angiotensin II–induced vascular contraction was confirmed in our study, which showed that angiotensin II elicited a rapid increase in $[Ca^{2+}]_i$, faster than the resulting contraction, and that angiotensin II produced a lower contractile response in the absence of extracellular Ca^{2+}. The current study also verified the importance of PKC in the angiotensin II–mediated vascular contraction. Significant inhibition was demonstrable with the selective cPKC inhibitor Gö 6976 but not with the nonselective PKC inhibitor Ro 31-8425 (which probably primarily inhibits nPKC activity), suggesting that Ca^{2+}-dependent cPKC is involved in the angiotensin II–induced contraction. This correlates with the angiotensin II–stimulated transient increase in $[Ca^{2+}]_i$ and the consequently transient contraction. Ca^{2+}-independent nPKC is considered to primarily mediate the sustained contraction of smooth muscle.

Protein kinase C refers to a family of at least 11 different closely related serine–threonine kinases. Ca^{2+}-dependent (or classic) PKC (cPKC-α, β_I, β_{II}, and γ) requires Ca^{2+}, phospholipid, and diacylglycerol for activity. Ca^{2+}-independent (or novel) PKC (nPKC-δ, -ϵ, -η, and -θ) exhibits kinase activity, requiring only diacylglycerol and phospholipid. Atypical PKC (aPKC-τ, -λ, and -ζ) can be activated by diacylglycerol or phorbol ester in the absence of Ca^{2+} and phospholipid.[2]

The distribution of PKC isoenzymes varies and depends on the species, vascular domains, or both. cPKC-α, cPKC-β, nPKC-δ, nPKC-ϵ, and aPKC-ζ have been demonstrated in VSMCs, although not all of these seem to be expressed in all vascular smooth muscle tissues.[7–9] The most predominant isoenzymes, cPKC-α and nPKC-ϵ, were selected to be detected using specific antibodies with Western blotting in this study.

After stimulation of angiotensin II, PKC translocates to the membrane and is autophosphorylated. Autophosphorylation increases the enzyme's activity and affinity for substrates, effectors, or both.[3] The extent of PKC phosphorylation has been proposed to represent a useful indicator of its activation state.[10–13] Accordingly, the degree of phosphorylation of cPKC-α and nPKC-ϵ was used to represent the activation of both of them in the current study.

The detectable cPKC-α and nPKC-ϵ bands with Western blotting in this study indicate that cPKC-α and nPKC-ϵ exist in rat aortic smooth muscle. The failure to detect phosphorylated nPKC-ϵ in the presence of angiotensin II and a similar time course for both cPKC-α phosphorylation and the contraction induced by angiotensin II further verified that a Ca^{2+}-dependent cPKC but not (or at least unpredominantly) Ca^{2+}-independent nPKC is involved in the angiotensin II–induced contraction of rat aortic smooth muscle.

Effects of Sevoflurane on Angiotensin II–stimulated, Ca^{2+}- and PKC-mediated Vascular Contraction

The effects of anesthetics on the contractile response of VSMCs vary, depending on anesthetics, agonists, spe-

cies, and vascular domains. A large body of evidence has revealed that among the complex mechanisms of contraction, Ca^{2+} and PKC are the major targets of anesthetics. Isoflurane has been demonstrated to inhibit serotonin- and endothelin-induced Ca^{2+} mobilization, without inhibition of the phorbol ester–activated PKC in cultured rat aortic smooth muscle cells,[14] but to enhance the phorbol ester-stimulated cytosolic PKC-mediated constriction of rat coronary artery smooth muscle.[15] In skinned pulmonary arteries, nPKC primarily mediates the isoflurane-induced contraction, but both cPKC and nPKC take part in the regulation of isoflurane-induced relaxation.[16] Halothane dose-dependently decreases Ca^{2+} accumulation in the sarcoplasmic reticulum and increases Ca^{2+} release from the sarcoplasmic reticulum in rabbit aortic smooth muscle cells[17] and attenuates the phorbol ester–stimulated cytosolic and membrane PKC-mediated contraction of rat coronary smooth muscle.[15] In skinned pulmonary arteries, the halothane-induced contraction is predominantly mediated *via* nPKC, but the relaxation is predominantly regulated *via* the Ca^{2+}-calmodulin–dependent protein kinase II.[18]

The results of the current study have shown, for the first time, that sevoflurane dose-dependently inhibited the angiotensin II–induced phosphorylation of cPKC-α but not the increase in $[Ca^{2+}]_i$, suggesting that sevoflurane depresses the angiotensin II–induced, Ca^{2+}-dependent cPKC-mediated but not Ca^{2+}-elicited contraction of rat aortic smooth muscle, even at clinically relevant concentrations (1.7–3.4%). Also using Western blotting, Zhong *et al.*[4] have shown that isoflurane increases the PKC activator phorbol-12-myristate-13-acetate–induced membrane translocation of nPKC-ϵ but not cPKC-α in cultured rat aortic smooth muscle cells, suggesting that isoflurane activates phorbol-12-myristate-13-acetate–stimulated nPKC but not cPKC. Differing from the mechanisms of the effects of sevoflurane on angiotensin II–induced contraction in this study, Akata *et al.*[19] have demonstrated that sevoflurane inhibits norepinephrine-induced contraction by both reducing Ca^{2+} influx and depressing the sensitivity of smooth muscle cells to Ca^{2+}, without significant attenuation of Ca^{2+} release from the sarcoplasmic reticulum in smooth muscle cells of mesenteric resistance arteries. Inconsistent with the current finding that sevoflurane did not affect the angiotensin II–stimulated increase in $[Ca^{2+}]_i$, isoflurane has been shown to dose-dependently decrease the angiotensin II–induced intracellular mobilization of Ca^{2+} by inhibiting Ca^{2+} release from internal stores and Ca^{2+} influx through nifedipine-insensitive Ca^{2+} channels in cultured rat aortic smooth muscle.[20] Therefore, the complex mechanisms of VSMC contraction suggests that anesthetics probably affect vascular contraction *via* the interruption of multiple signaling pathways. Whether sevoflurane affects other contraction-associated mechanisms remains to be clarified in future investigations.

In summary, the current study has demonstrated that Ca^{2+} and cPKC, especially cPKC-α, are involved in the angiotensin II–induced transient contraction of rat aortic smooth muscle and that sevoflurane dose-dependently inhibits the angiotensin II–induced, cPKC-α–mediated but not the Ca^{2+}-elicited contraction of rat aortic smooth muscle.

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