100 nM SP to 71.0 ± 12.3%, 73.6 ± 9.2%, and 56.7 ± 8.6% of the control at concentrations of 0.1, 1, and 10 μM, respectively (Figs. 1 and 2). After washout of the ODT, the size of SPR-induced currents was reversed to almost the same as the control levels.

We previously reported that treatment with the PKC inhibitor GF109203X (200 nM), which has a Ki value of 20 nM for the inhibition of PKC activity (10), produced the enhancement of the initial Cl^- currents activated by 100 nM SP (4, 5). The control currents before ODT treatment was 35.1 ± 27.6 nA. GF109203X enhanced the currents to 398 ± 86% of the control currents (119 ± 79.6 nA), which was similar to our previous report. The inhibitory effects of ODT on SP-induced currents were observed in the oocytes pretreated with GF109203X (Fig. 3). ODT (10 μM) inhibited the action of 100 nM SP to 52.0 ± 9.7%, while treatment of GF109203X resulted in the action of ODT (10 μM) to 45.9 ± 14.6% of control (Fig. 3), although the effect was not significantly different.

Tramadol undergoes biotransformation in the liver by two metabolic pathways to form five *N*- or *O*-desmethylated metabolites. ODT is one of the five main metabolites of tramadol; and the others are mono-*N*-desmethyl tramadol, di-*N*-desmethyl tramadol, tri-*N*,*O*-desmethyl tramadol, and di-*N*,*O*-desmethyl tramadol. We have previously reported that tramadol had little effect on SPR function (5). In another paper, we reported that a low concentration (under 0.1 μM) of ODT did not suppress SP-induced currents in oocytes expressing the SPR (11). Grond et al. (12) reported the mean ODT concentrations after a 200 mg bolus IV infusion of tramadol and those after patient controlled analgesia with demand doses of 20 mg for 24 h in 92 patients. In our study, the mean concentration of ODT was 84.0 ± 34 ng/mL (approximately 0.3 μM). Sindrup et al. (13) also reported mean ODT concentrations of 5.0 – 122 ng/mL (maximally 0.4 μM) in patients who received 200 – 400 mg of tramadol. In the present study, 0.1 μM and higher concentrations of ODT actually inhibited SP-induced Ca^{2+}-activated Cl^- currents. From the present results, ODT at higher levels,

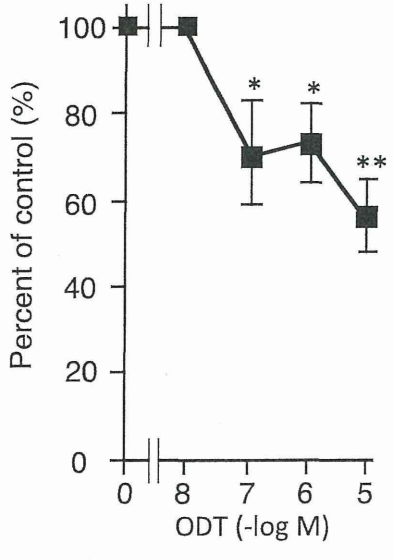

Fig. 2. Concentration–response relationship of *O*-desmethyl tramadol (ODT) on substance P (SP)-induced currents. ODT (10 μM – 10 nM) was applied to the oocytes for 2 min, and then 100 nM of SP was applied for 20 s. Data represent the mean ± S.E.M. of 40 oocytes. $*P < 0.05$ and $**P < 0.01$, compared with the control response using analysis of variance.

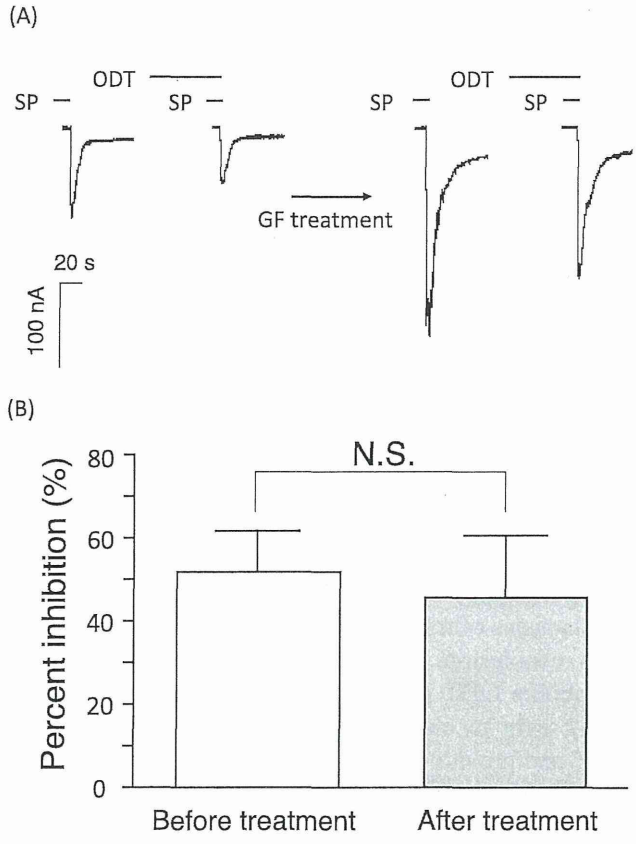

Fig. 3. The effects of GF109203X (protein kinase C inhibitor: PKCI) on inhibition by *O*-desmethyl tramadol (ODT) on substance P (SP)-stimulated currents in *Xenopus* oocytes expressing SP receptors (SPR). A) Tracings obtained from a single oocyte expressing SPR show the effect of ODT on SP (100 nM)-induced currents before and after treatment of PKCI. SP was applied for 20 s with or without 2-min ODT treatment. PKCI was treated for 120 min. B) Comparison of the effects of PKCI on the inhibitory effects of ODT. Oocytes were incubated with 200 nM GF109203X (PKCI) for 120 min. ODT (10 μM) shown was preapplied for 2 min before being co-applied with SP (100 nM) for 20s. "Before treatment" indicates the effects of ODT before application of bisindolylmaleimide. Data represent the mean ± S.E.M. for 10 separate determinations. A paired Student's *t*-test was used for the statistical analysis.

although within the clinically relevant concentrations, would inhibit SPR functions clinically.

The present study raises the question of how ODT inhibits SPR-mediated responses. We have reported that ODT had little effect on the function of muscarinic M_3R, which share the same downstream signaling steps as the SPR following Gq protein activation, expressed in *Xenopus* oocytes. These findings suggest that the inhibitory effect of ODT on the SP-induced Cl^- current is likely due to the inhibition of the SPR before activation of Gq proteins. There is considerable evidence that PKC plays an important role in regulating the function of GPCRs (14) and the functions of some GPCRs are inhibited by PKC activation. We reported that the inhibitory effects of halothane, isoflurane, enflurane, diethyl ether, and ethanol on SP-induced currents were suppressed in oocytes treated with the PKC inhibitor, suggesting that these anesthetics and ethanol inhibit SPR function via activation of PKC. However, in our present experiments, GF109203X did not alter the inhibitory effects of ODT on SPR function, suggesting that PKC may not be involved in the cases of the inhibitory effects of ODT on SPR.

Although much attention has been paid to the μ-opioid receptor and monoamine uptake in the central nervous system as targets for tramadol and ODT, several studies have shown that some GPCRs and ligand-gated ion channels are also targets for tramadol (15). In our present results, the inhibitory effects of ODT seem to be weaker than that on μ-opioid receptors and transporters. Nonetheless, SPR might also be one of the targets for ODT. The inhibitory effects of ODT on SPR might also contribute to the side effects of tramadol. More information about SPR may help to elucidate the role of SPR in the mechanisms of tramadol activity.

In conclusion, we demonstrated that the tramadol metabolite ODT inhibited SPR function. Our findings might help to elucidate the pharmacological basis of ODT and provide a better understanding of its neuronal action and the antinociceptive effects of tramadol. More definitive studies, such as the use of the SPR knockout mouse model, would be required.

Acknowledgments

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SHORT COMMUNICATION

Effects of sevoflurane on voltage-gated sodium channel $Na_v1.8$, $Na_v1.7$, and $Na_v1.4$ expressed in *Xenopus* oocytes

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Abstract Sevoflurane is widely used as a volatile anesthetic in clinical practice. However, its mechanism is still unclear. Recently, it has been reported that voltage-gated sodium channels have important roles in anesthetic mechanisms. Much attention has been paid to the effects of sevoflurane on voltage-dependent sodium channels. To elucidate this, we examined the effects of sevoflurane on Na_v 1.8, Na_v 1.4, and Na_v 1.7 expressed in *Xenopus* oocytes. The effects of sevoflurane on Na_v 1.8, Na_v 1.4, and Na_v 1.7 sodium channels were studied by an electrophysiology method using whole-cell, two-electrode voltage-clamp techniques in *Xenopus* oocytes. Sevoflurane at 1.0 mM inhibited the voltage-gated sodium channels $Na_v1.8$, $Na_v1.4$, and $Na_v1.7$, but sevoflurane (0.5 mM) had little effect. This inhibitory effect of 1 mM sevoflurane was completely abolished by pretreatment with protein kinase C (PKC) inhibitor, bisindolylmaleimide I. Sevoflurane appears to have inhibitory effects on $Na_v1.8$, $Na_v1.4$, and Na_v 1.7 by PKC pathways. However, these sodium channels might not be related to the clinical anesthetic effects of sevoflurane.

Keywords Sevoflurane · Voltage-gated sodium channel · *Xenopus* oocytes

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Sevoflurane has commonly been used as an anesthetic in clinical practice. Until now, previous studies have examined the mechanisms of sevoflurane [1–4], but many aspects of the mechanism have remained unclear. Voltage-gated sodium channels play important roles in the action of potential initiation and propagation in excitable cells of nerve and muscle [5]. Recent reports have shown a relationship between volatile anesthetics and sodium channels [6–12], suggesting voltage-dependent sodium channels as a target of anesthetics. However, so far as sevoflurane is concerned, there has been little information on the functions of voltage-gated sodium channels.

$Na_v1.8$ is exclusively expressed in dorsal root ganglion (DRG) neurons that give rise to C- and Aδ-fibers [13, 14] and peripheral nerves [15], which play important roles in afferent pain pathways transmitting nociceptive signals to the spinal cord [14]. $Na_v1.7$ expresses in DRG, sympathetic nerves, and peripheral nerves and $Na_v1.4$ expresses in skeletal muscles and plays a role in action potential initiation and transmission in skeletal muscles [5]. Reflex of muscles and inhibition of sympathetic nerves are necessary during the operation. Thus, it would be interesting to study

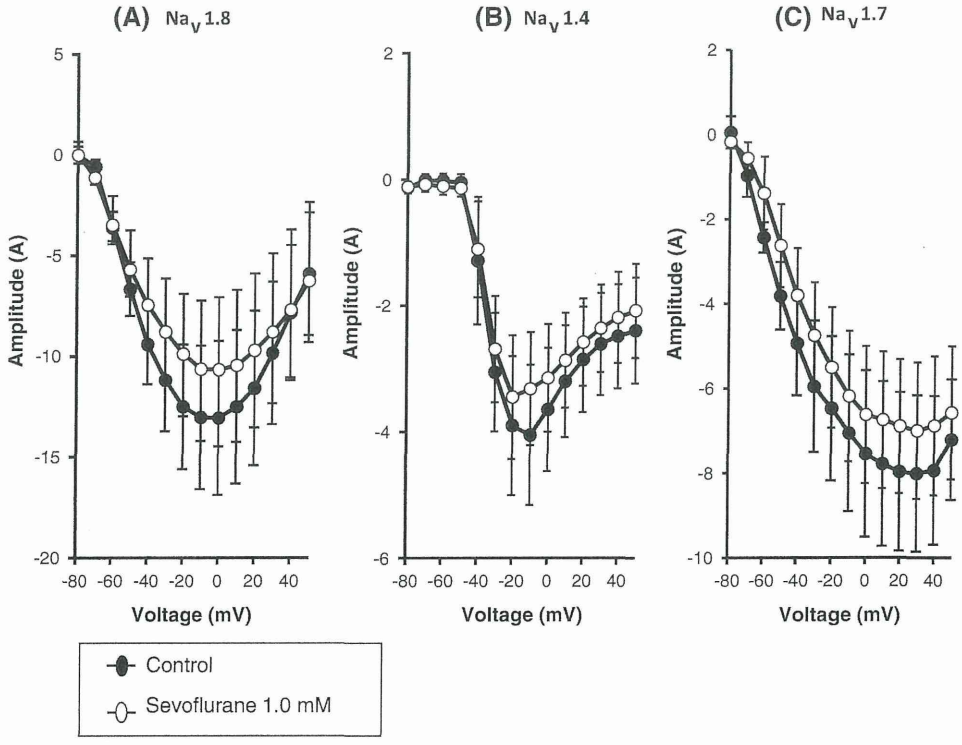

Fig. 1 Effects of sevoflurane on I–V relationship of sodium currents at holding potential of −70 mV: Na_v 1.8 (**a**), Na_v 1.4 (**b**), Na_v 1.7 (**c**). The peak currents were normalized to the maximal currents that were observed at −10 mV (Na_v 1.8 and Na_v1.4) and 30 mV (Na_v 1.7). *Closed circles*, control; *open circles*, sevoflurane

effects of sevoflurane on these voltage-dependent sodium channels.

The purpose of this study was to determine whether sevoflurane affects the functions of voltage-gated sodium channels. To this end, we examined the effects of sevoflurane on the function of $Na_v1.7$, $Na_v1.8$, and $Na_v1.4$ expressed in *Xenopus* oocytes using an electrophysiological method. Moreover, we investigated the mechanisms of the effects of sevoflurane on these channels.

Adult female *Xenopus laevis* frogs were purchased from Kato Kagaku (Tokyo, Japan), sevoflurane from Maruishi Pharmaceutical (Osaka, Japan), and bisindolylmaleimide I (GF109203X) from Calbiochem (La Jolla, CA, USA). Ultracomp *E. coli* Transformation Kit was purchased from Invitrogen (San Diego, CA, USA). Purification of cDNAs was performed with a Qiagen purification kit (Qiagen, Chatworth, CA, USA). Gentamicin, sodium pyruvate, cDNA for rat $Na_v1.6$ α-subunit (a gift from Dr. A.L. Goldin, University of California, Irvine, CA, USA), cDNA for rat $Na_v1.8$ α-subunit (a gift from Dr. A.N. Akopian, University of Texas Health Science Center, San Antonio, TX, USA), and cDNA for human $Na_v1.7$ α-subunit (a gift from Dr. F. Hofmann, Universität München, München, Germany) were prepared.

Each of the cRNAs ($Na_v1.7$, $Na_v1.8$, and $Na_v1.4$) was prepared using a mCAP mRNA Capping Kit and transcribed with a SP6 RNA Polymerase in vitro Transcription Kit (Ambion, Austin, TX, USA). cDNA was linearized with $Na_v1.4$, $Na_v1.8$, and Na_v 1.7. Preparation of *Xenopus laevis* oocytes and microinjection of the cRNA ($Na_v1.7$, $Na_v1.8$, and $Na_v1.4$) were performed as previously described by Horishita et al. [16, 17].

The whole-cell sodium current from oocytes was measured using a two-microelectrode voltage clamp. An oocyte was placed in a 100-μl recording chamber and perfused with frog Ringer's solution at room temperature (22°–24°C), containing 115 mM NaCl, 2.5 mM KCl, 10 mM HEPES, and 1.8 mM $CaCl_2$ at pH 7.2, at a rate of 1.8 ml/min using a perfusion pump (MINIPLUS3; GILSON, Middleton, France). The electrodes were triple-pulled with a puller (P-97; Sutter Instrument, Novoto, CA, USA) from a glass capillary. Microelectrodes were filled with 3 M KCl/0.5% low-melting-point agarose, and they had a final resistance of 0.3–0.5 MΩ. The whole-cell voltage clamp was achieved through these two electrodes using a Warner Instrument model OC-725C (Hampden, CT, USA). Currents were recorded and analyzed using pCLAMP software (Axon Instruments, Foster City, CA, USA). The voltage dependence activation was determined by eliciting 50-ms depolarizing pulses from a holding potential of −70 mV to potential range from −90 mV to 50 mV in 10-mV increments. We analyzed the peak component of the transient inward currents with methodology described by Horishita et al. [17]. A solution of sevoflurane, freshly prepared immediately before use, was applied for 2 min. We calculated the final concentration of sevoflurane in the recording chamber using gas chromatography. To determine whether activation of protein kinase C (PKC) plays a

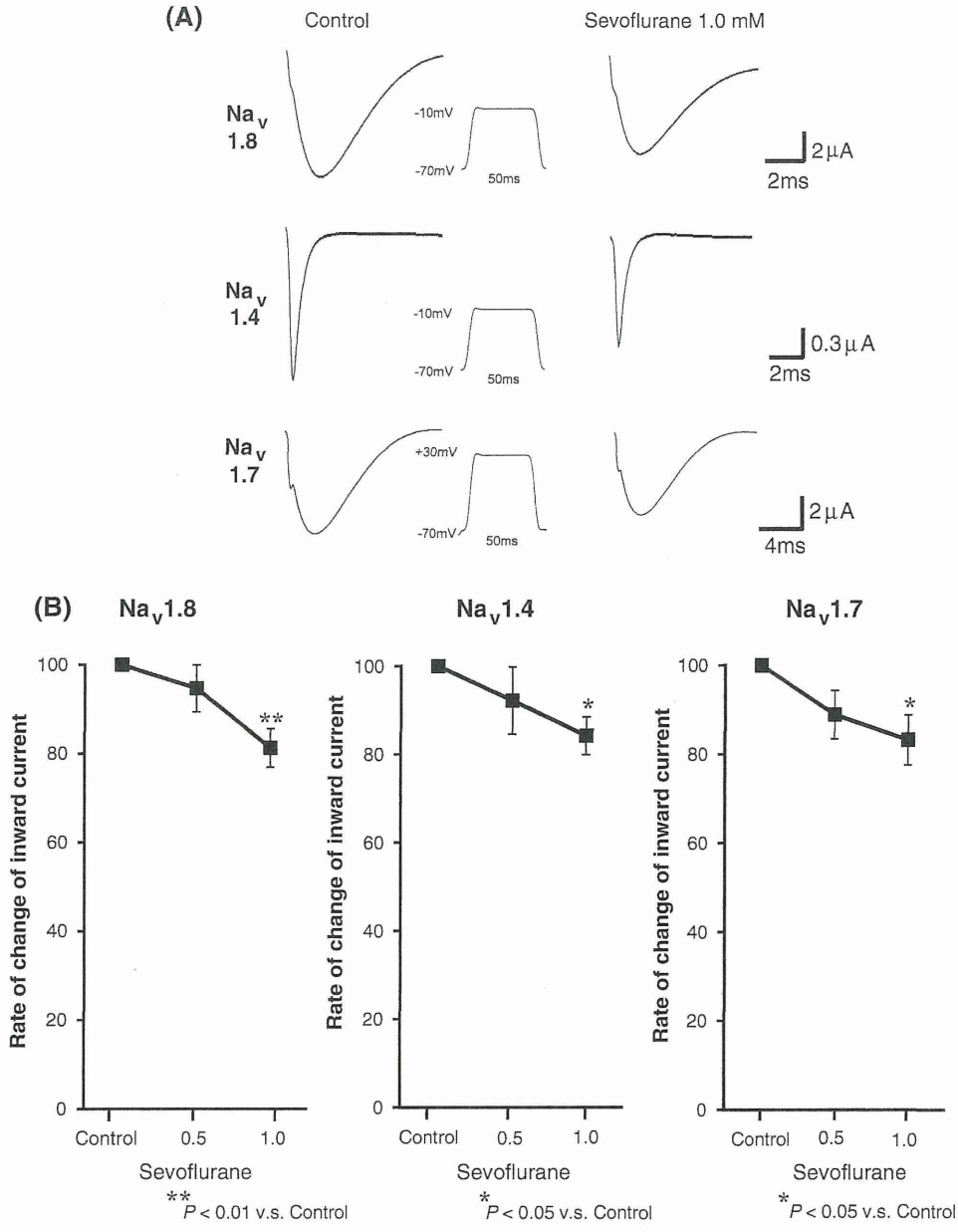

Fig. 2 Effects of sevoflurane on peak sodium currents in oocyte expressing Na_v 1.8, Na_v 1.4 and Na_v 1.7 expressed in *Xenopus* oocytes. **a** Representative I_{Na} traces in control and presence of sevoflurane in oocytes expressing Na_v1.8, Na_v 1.4, Na_v 1.7. **b** Concentration–response relationship of sevoflurane-induced inward current of voltage gated sodium channels. The effects were expressed as rate of change (± SEM). $^*P < 0.05$, $^{**}P < 0.01$ versus control

role in sevoflurane modulation on voltage-dependent sodium channels, oocytes were exposed to a PKC inhibitor, bisindolylmaleimide I (GF109203X)(200 nM) [18–20] in modified Barth's saline (MBS) for 120 min before recording. We compared the effects of sevoflurane on the peak component of the transient inward currents before and after the exposure to GF109203X.

Data are shown as the mean ± SEM. Results are expressed as percentages of control values obtained by peak current. The control responses were measured before sevoflurane application. Statistical analyses were performed using a one-way analysis of variance (ANOVA) and the Bonferroni correction using GraphPad Prism 4 (GraphPad Software, La Jolla, CA, USA). A P value <0.05 was considered significant.

Sevoflurane did not cause a shift in the current–voltage relationship (Fig. 1). Sevoflurane (1.0 mM) significantly inhibited the peak component of the transient inward currents of Na_v 1.8 (81.3% ± 4.32% of control, $P < 0.01$, $n = 8$), Na_v 1.4 (84.2% ± 4.35% of control, $P < 0.05$, $n = 12$), and Na_v 1.7 (83.2% ± 5.64% of control, $P < 0.05$, $n = 5$) (Figs. 1, 2). However, 0.5 mM sevoflurane had little effect on the peak component of the transient inward currents of these channels.

We next studied the effects of PKC on the inhibition of a high concentration of sevoflurane (1 mM) on Na_v 1.8, Na_v

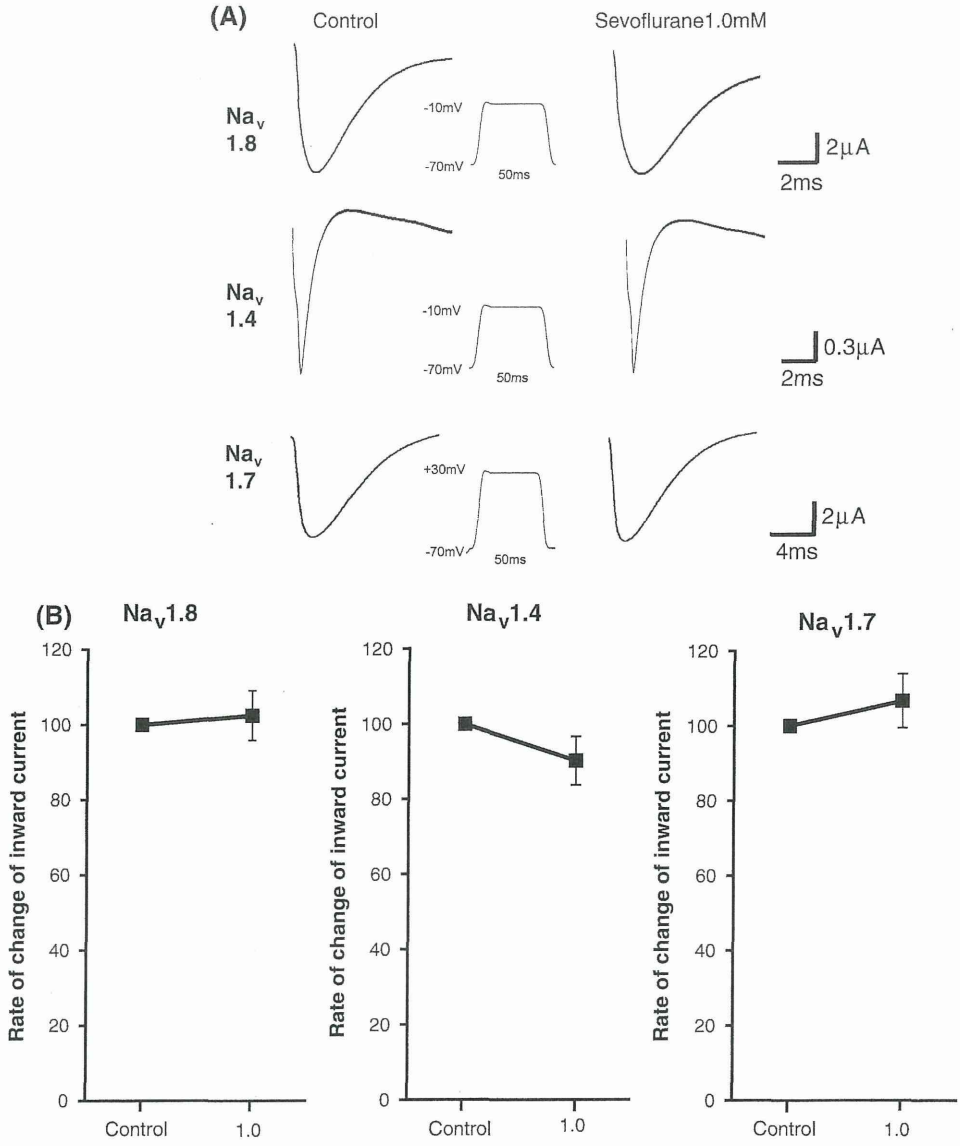

Fig. 3 Sevoflurane modulates voltage-gated sodium channels through the protein kinase C pathway. **a** Representative examples of the effect of bisindolylmaleimide I (GF109203X) on Na_v 1.8, Na_v 1.4 and Na_v 1.7. **b** Summary data for the effects of GF109203X on sevoflurane on peak inward current of voltage-gated sodium channels (Na_v 1.8, Na_v 1.4, and Na_v 1.7.). The effects were expressed as rate of change (± SEM)

1.4, and Na_v 1.7. In the control condition, the PKC inhibitor did not affect the voltage-gated inward currents. Pretreatment with GF109203X (200 nM) for 120 min abolished the sevoflurane-induced inhibition of voltage-evoked inward currents in *Xenopus* oocytes expressing $Na_v1.4$, $Na_v1.8$, and Na_v 1.7 ($Na_v1.4$, 90.2% ± 6.5% of control, $P > 0.05$, $n = 9$; $Na_v1.8$, 102% ± 6.6% of control, $P > 0.05$, $n = 11$; Na_v 1.7, 106% ± 7.2% of control, $P > 0.05$, $n = 9$) (Fig. 3a,b).

In our results, sevoflurane had little effects on the current–voltage relationship. However, sevoflurane (1.0 mM) significantly inhibited the peak component of the transient inward currents of Na_v 1.8, Na_v 1.4, and Na_v 1.7; 0.5 mM sevoflurane did not affect the peak component of the transient inward currents inward current of these three channels. In clinical situations, the free plasma concentration of sevoflurane is approximately 0.5 mM in humans [21, 22]. Ouyang et al. [12] reported that the function of $Na_v1.4$ was inhibited slightly by equipotent concentrations of sevoflurane (0.46 mM), consistent with our present results. From this evidence and our results, sevoflurane would have little effect on these channels, at least in a clinical situation.

In our present results, 1 mM sevoflurane inhibited the peak component of the transient inward currents of Na_v 1.8, Na_v 1.4, and Na_v 1.7. This finding raises the question of how sevoflurane inhibits these channel functions. Sodium channels are also rapidly phosphorylated by PKC [23], and recent reports have shown that the functions of Na_v 1.7 expressed in *Xenopus* oocytes are modulated by PKC [24]. Moreover, there are several lines of evidence revealing that sevoflurane activated PKC [2, 3]. Inhibition by sevoflurane on Na_v 1.8, Na_v 1.7, and Na_v 1.4 functions was abolished by pretreatment with the PKC inhibitor,

suggesting that sevoflurane would inhibit Na_v 1.8, Na_v 1.7, and Na_v 1.4 functions by PKC-mediated pathways.

In conclusion, we demonstrated inhibition by sevoflurane on the functions of Na_v 1.8, Na_v 1.7, and Na_v 1.4, and that the inhibition would be mediated by the PKC pathway. However, these sodium channels might not be related to the clinical anesthetic effects of sevoflurane.

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Sevoflurane Inhibits the μ-Opioid Receptor Function Expressed in *Xenopus* Oocytes

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Key Words
μ-Opioid receptor · $G_{i/o}$-protein-coupled receptors · Sevoflurane · *Xenopus* oocyte

Abstract
Sevoflurane is widely used for anesthesia, and is commonly used together with opioids in clinical practice. However, the effects of sevoflurane on μ-opioid receptor (μOR) functions is still unclear. In this study, the effects of sevoflurane on μOR functions were analyzed by using *Xenopus* oocytes expressing a μOR fused to chimeric Gα protein G_{qi5} (μOR-G_{qi5}). Sevoflurane by itself did not elicit any currents in oocytes expressing μOR-G_{qi5}, whereas sevoflurane inhibited the [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO)-induced Cl^- currents at clinically used concentrations. Sevoflurane did not affect the Cl^- currents induced by AlF_4^-, which directly led to activation of G proteins. The inhibitory effects of sevoflurane on the DAMGO-induced currents were not observed in oocytes pretreated with the protein kinase C (PKC) inhibitor GF109203X. These findings suggest that sevoflurane would inhibit μOR function. Further, the mechanism of inhibition by sevoflurane would be mediated by PKC.

Introduction

Sevoflurane is widely used as an inhalation general anesthetic agent due to its low solubility in blood. Opioids are commonly used with sevoflurane at the same time. There have been some reports that have pointed out interactions between sevoflurane and opioid receptors (ORs) in animal experiments; the potency of sevoflurane was modulated by the endogenous μ-opioid system, but not by the κ- and δ-opioid systems in experiments using mice lacking the ORs [1]. In animals, morphine decreases the minimal alveolar concentration (MAC) of sevoflurane [2, 3]. However, it has been reported that morphine does not affect MAC for sevoflurane in humans. More recently, it has been reported that μOR-knockout mice have no different MAC of sevoflurane [4]. The interaction between sevoflurane and OR function is still controversial.

The ORs belong to the G-protein-coupled receptor family and three types of receptors, μ, δ and κ, have been identified by molecular cloning [5]. Within the three subtypes of these receptors, μORs are the major receptors that mediate the analgesic effects of opioids [5]. On the basis of second-messenger signaling, μOR couples to $G\alpha_{i/o}$ protein to cause inhibition of adenylate cyclase, in-

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hibition of voltage-dependent Ca^{2+} channels, and activation of G-protein-coupled inwardly rectifying K^+ channels [5]. There have been many reports that revealed direct effects of general anesthetics on G_q-coupled receptors [6–11]. As far as the functions of $G_{i/o}$-coupled receptors including μOR are concerned, much less is known about the direct effects of volatile anesthetics. Moreover, we recently reported that the volatile anesthetic halothane inhibited μOR function at clinical concentrations [12]. It would be interesting to study whether sevoflurane affects μOR functions.

The *Xenopus* oocyte expression system has widely been employed to study the functions of a number of G-protein-coupled receptors [13, 14]. In the case of G_q-coupled receptors, receptor stimulation results in activation of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes by G_q-mediated phospholipase C activation and subsequent formation of IP_3 and diacylglycerol [14]. The IP_3 formed causes release of Ca^{2+} from the endoplasmic reticulum, which in turn triggers the opening of Ca^{2+}-activated Cl^- channels endogenously expressed in the oocytes [14]. However, in case of $G_{i/o}$-coupled receptors, analysis has been difficult, due to lack of appropriate analytical output in oocytes. We have established an assay method for $G_{i/o}$ PCRs by using a μOR fused to G_{qi5} (μOR-G_{qi5}) in *Xenopus* oocytes [12, 15].

We examined the effects of sevoflurane on the function of μOR using this assay system. Moreover, we investigated the mechanisms of the effects of sevoflurane on μOR.

Materials and Methods

Materials

Adult *Xenopus laevis* female frogs were purchased from Kato Kagaku (Tokyo, Japan). The Ultracomp *E. coli* Transformation Kit was from Invitrogen (San Diego, Calif., USA). Sevoflurane was purchased from Maruishi Pharmaceutical (Osaka, Japan). Purification of cDNAs was performed with a Qiagen purification kit (Qiagen, Chatworth, Calif., USA). Gentamicin, sodium pyruvate, [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO) and other chemicals were from Nacalai Tesque (Kyoto, Japan). The rat μOR was provided by Dr. N. Dascal (Tel Aviv University, Ramat Aviv, Israel). The chimeric $G\alpha_{qi5}$ was a kind gift from Dr. B.R. Conklin (UCSF, San Fransico, Calif., USA). Each of the cRNAs was prepared using a mCAP mRNA Capping Kit, and transcribed with a T7 RNA polymerase in vitro transcription kit (Stratagene, La Jolla, Calif., USA).

Preparation of Chimeric μOR-G_{qi5}

The tandem cDNAs of chimeric μOR-G_{qi5} were created by ligating the receptor cDNA sequences into the *Nhe*I site of G_{qi5} cDNAs. The sequences of all PCR products were confirmed by sequencing with ABI3100 (Applied BioSystems, Tokyo, Japan). All cDNAs for the synthesis of cRNAs were subcloned into the pGEMHJ vector, which provides the 5′- and 3′-untranslated regions of the *Xenopus* β-globin RNA [16], ensuring a high level of protein expression in the oocytes. Each of the cRNAs was synthesized using the mCAP mRNA Capping Kit, with the T7 RNA polymerase in vitro transcription kit (Ambion, Austin, Tex., USA) from the respective linearized cDNAs.

Recording and Data Analysis

Isolation and microinjection of *Xenopus* oocytes were performed as previously described [7, 9–12, 17–19]. *Xenopus* oocytes were injected with appropriate amounts of cRNAs (in 50 ng; μOR-G_{qi5}) and incubated with ND 96 medium composed of (in mmol/l); NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5 (pH 7.4, adjusted with NaOH), supplemented with 2.5 mmol/l sodium pyruvate and 50 μg/ml gentamicin for 3–7 days until recording. Oocytes were placed in a 100-μl recording chamber and perfused with modified Barth's saline (MBS) composed of (in mmol/l): NaCl 88, KCl 1, $NaHCO_3$ 2.4, HEPES 10, $MgSO_4$ 0.82, $Ca(NO_3)_2$ 0.33, and $CaCl_2$ 0.91, (pH 7.4 adjusted with NaOH) at a rate of 1.8 ml/min at room temperature. Recording and clamping electrodes (1–2 MΩ) were pulled from 1.2-mm outside-diameter capillary tubing and filled with 3 mol/l KCl. A recording electrode was imbedded in the animal's pole of oocytes, and once the resting membrane potential stabilized, a clamping electrode was inserted and the resting membrane potential was allowed to restabilize. A Warner OC 725-B oocyte clamp (Hampden, Conn., USA) was used to voltage clamp each oocyte at –70 mV. We analyzed the peak component of the transient inward currents induced by receptor agonists because this component is dependent on the concentrations of the receptor agonist applied, and is quite reproducible, as described by Minami et al. [10]. Sevoflurane was applied for 2 min before and during the application of DAMGO (1 μmol/l) to allow complete equilibration in the bath. The solutions of sevoflurane were freshly prepared immediately before use. We calculated the final concentration of sevoflurane in the recording chamber using a gas chromatography method, and accordingly the concentrations of sevoflurane represent the bath concentrations.

AlF_4^- was used as a direct activator of G proteins, and with this system we could bypass the signal to G proteins from activated receptors. Under a two-electrode voltage clamp, we injected 30 nl of solution containing NaF and $AlCl_3$ into the oocyte by using a pressure injector (PM2000B; MicroData Instruments, South Plainfield, N.J., USA). The concentrations of NaF and $AlCl_3$ used in this study were 20 mmol/l and 60 μmol/l, respectively.

To determine whether activation of protein kinase C (PKC) plays a role in anesthetic modulation of μOR-mediated events, oocytes were exposed to a PKC inhibitor, bisindolylmaleimide I (GF109203X; 200 nmol/l) [20], in MBS for 120 min. We compared the effects of anesthetics on DAMGO (1 μmol/l)-induced Ca^{2+}-activated Cl^- currents in *Xenopus* oocytes expressing μOR-G_{qi5} before and after the exposure to GF109203X.

Statistical Analysis

Results are expressed as percentages of control responses. The control responses were measured before and after each drug ap-

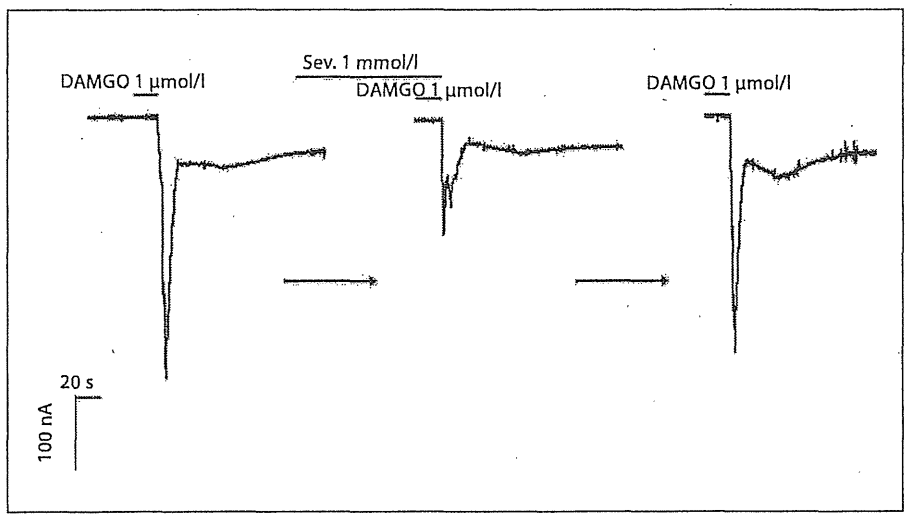

Fig. 1. Typical tracings of the effect of 1 mmol/l sevoflurane (Sev.) on the Cl^- current evoked by 1 μmol/l DAMGO in *Xenopus* oocytes expressing μOR-G_{qi5}.

plication, to take into account possible shifts in the control currents as recording proceeded. The 'n' values refer to the number of oocytes studied. Each experiment was carried out with oocytes from at least two different frogs. Statistical analyses were carried out by one-way ANOVA followed by Dunnett's correction and paired t test using GraphPad Prism 4 (GraphPad Softwear, Inc; La Jolla, Calif., USA). Values of $p < 0.05$ were considered to be significant.

Results

Pretreatment of sevoflurane by itself did not elicit any currents in oocytes expressing μOR-G_{qi5}, whereas the sevoflurane significantly inhibited DAMGO (1 μmol/l)-induced Ca^{2+}-activated Cl^- currents in a concentration-dependent manner (fig. 1); sevoflurane at 0.25, 0.5 and 1 mmol/l inhibited the DAMGO (1 μmol/l)-induced Cl^- currents to 84.7 ± 12.3, 50.6 ± 10.1 ($p < 0.01$), and 48.8 ± 8.2% ($p < 0.01$) of the control value, respectively (n = 8 each) (fig. 2).

AlF_4^- has been reported to bind to guanosine diphosphate on heterotrimeric G protein, and guanosine diphosphate-AlF_4^- complex promotes the dissociation of heterotrimeric G proteins into Gα and Gβγ subunits, which directly (without receptor stimulation) leads to the activation of G protein and subsequent G-protein-mediated pathways downstream [21]. The peak amplitude of AlF_4^--induced currents was 282 ± 121 nA (n = 6), and sevoflurane did not affect the AlF_4^--induced currents (418 ± 86.4 nA; n = 6) (fig. 3).

Treatment with a PKC inhibitor, GF109203X (200 nmol/l), which has a K_i value for inhibiting PKC activity of 20 nmol/l [20], produced enhancement of the initial Cl^- currents activated by DAMGO (1 μmol/l) (fig. 4a). After a 1-hour incubation of GF109203X, the response of DAMGO (1 μmol/l) increased to 2 times the initial currents (205 ± 27.6% of control), and this enhancement continued for 2 h (fig. 4b) (n = 6). Sevoflurane (1 mmol/l) inhibited DAMGO (1 μmol/l)-induced Ca^{2+}-activated Cl^- currents to 61.2 ± 15.8% of the control value (n = 6). However, the inhibitory effects of sevoflurane (1 mmol/l) on DAMGO (1 μmol/l)-induced currents were abolished after 2 h of pretreatment with GF109203X (115.8 ± 26.8% of control) (fig. 5).

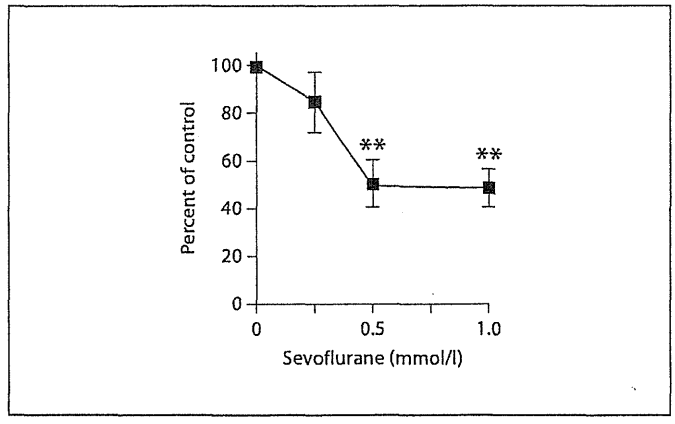

Fig. 2. Concentration-response curve for the inhibitory effects of sevoflurane on DAMGO (1 μmol/l)-induced Cl^- currents in oocytes expressing μOR-G_{qi5}. ** $p < 0.01$ vs. control. Values are expressed as means ± SEM.