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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

Toru Yokoyama · Kouichiro Minami · Yuka Sudo · Takafumi Horishita · Junichi Ogata · Toshihiko Yanagita · Yasuhito Uezono

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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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T. Yokoyama · K. Minami (✉) · J. Ogata
Department of Anesthesiology and Critical Care Medicine, Jichi Medical University, Tochigi 329-0483, Japan
e-mail: kminami@med.uoeh-u.ac.jp

T. Yokoyama · K. Minami · Y. Sudo · Y. Uezono
Cancer Pathophysiology Division, National Cancer Center Research Institute, Tokyo, Japan

Y. Sudo
Department of Molecular and Cellular Biology, Nagasaki University School of Biomedical Sciences, Nagasaki, Japan

T. Horishita
Department of Anesthesiology, University of Occupational and Environmental Health, Kitakyushu, Japan

T. Yanagita
Department of Pharmacology, University of Miyazaki, School of Medicine, Miyazaki, Japan

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

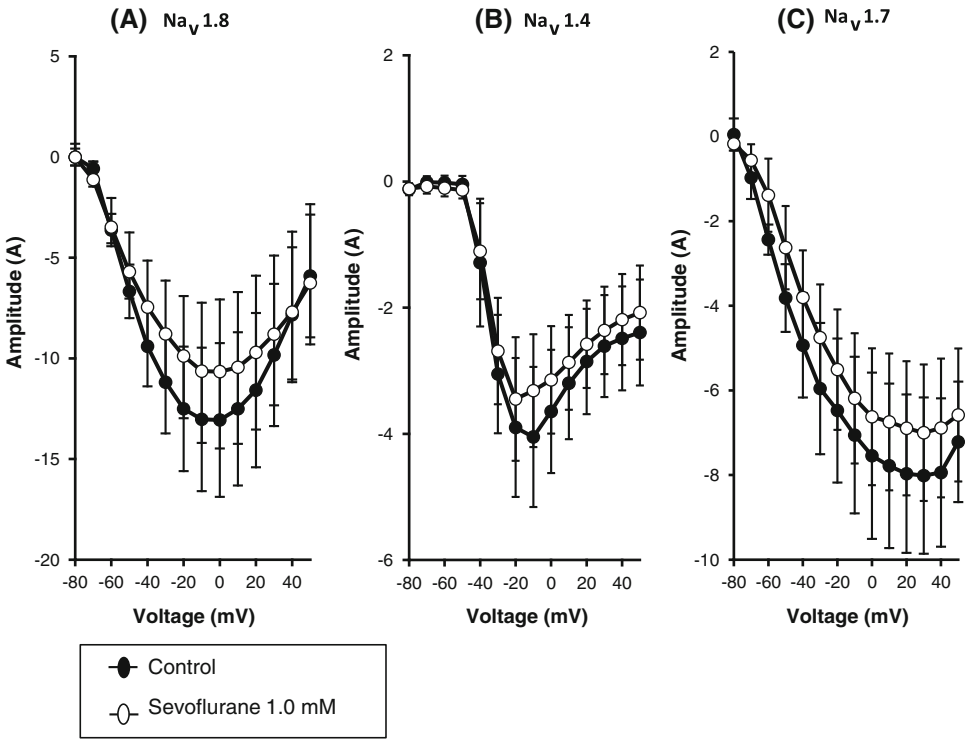

Fig. 1 Effects of sevoflurane on I–V relationship of sodium currents at holding potential of −70 mV: Na_v 1.8 (**a**), Na_v 1.4 (**b**), Na_v 1.7 (**c**). The peak currents were normalized to the maximal currents that were observed at −10 mV (Na_v 1.8 and Na_v1.4) and 30 mV (Na_v 1.7). *Closed circles*,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

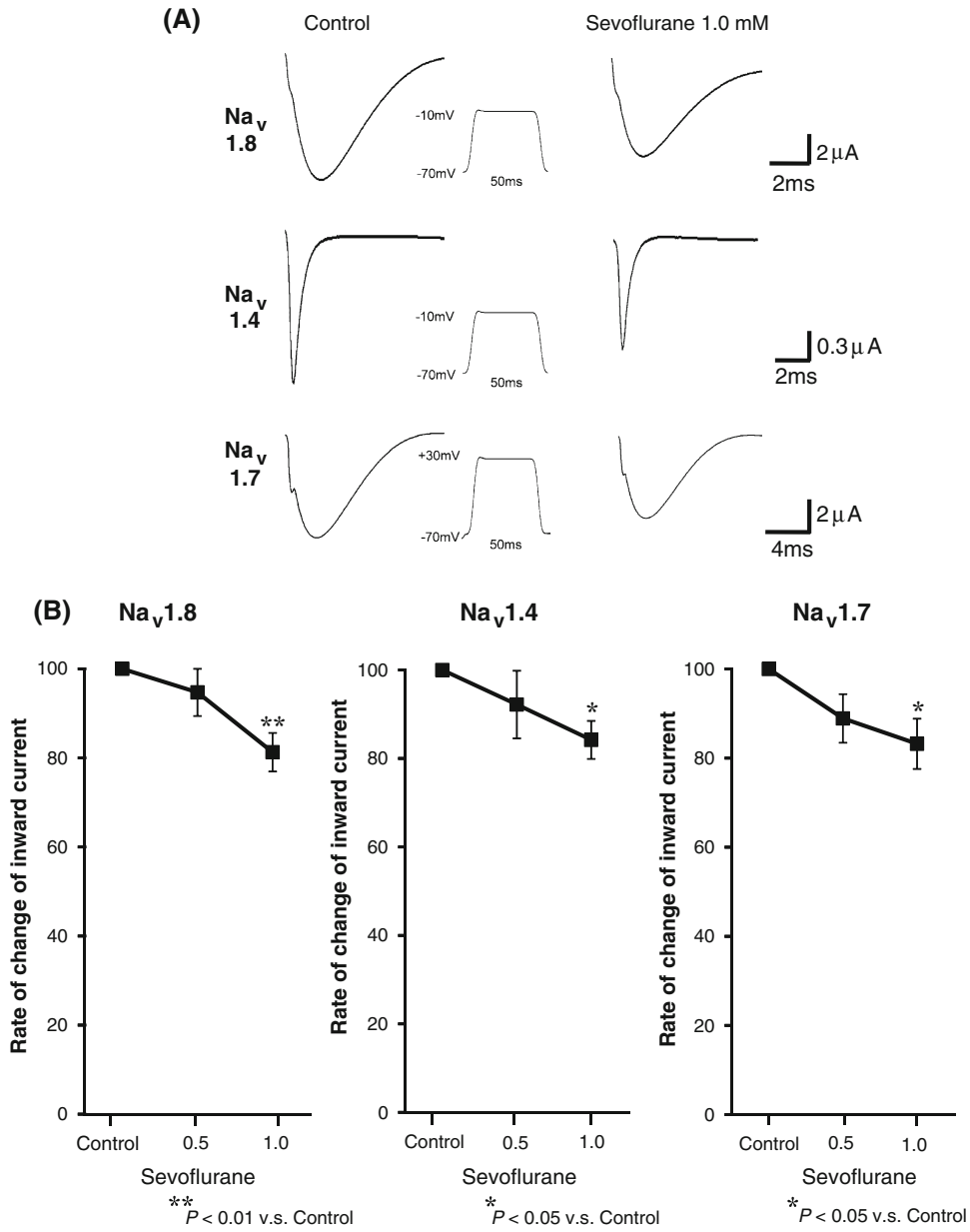

Fig. 2 Effects of sevoflurane on peak sodium currents in oocyte expressing Na_v 1.8, Na_v 1.4 and Na_v 1.7 expressed in *Xenopus* oocytes. **a** Representative I_{Na} traces in control and presence of sevoflurane in oocytes expressing Na_v1.8, Na_v 1.4, Na_v 1.7. **b** Concentration–response relationship 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

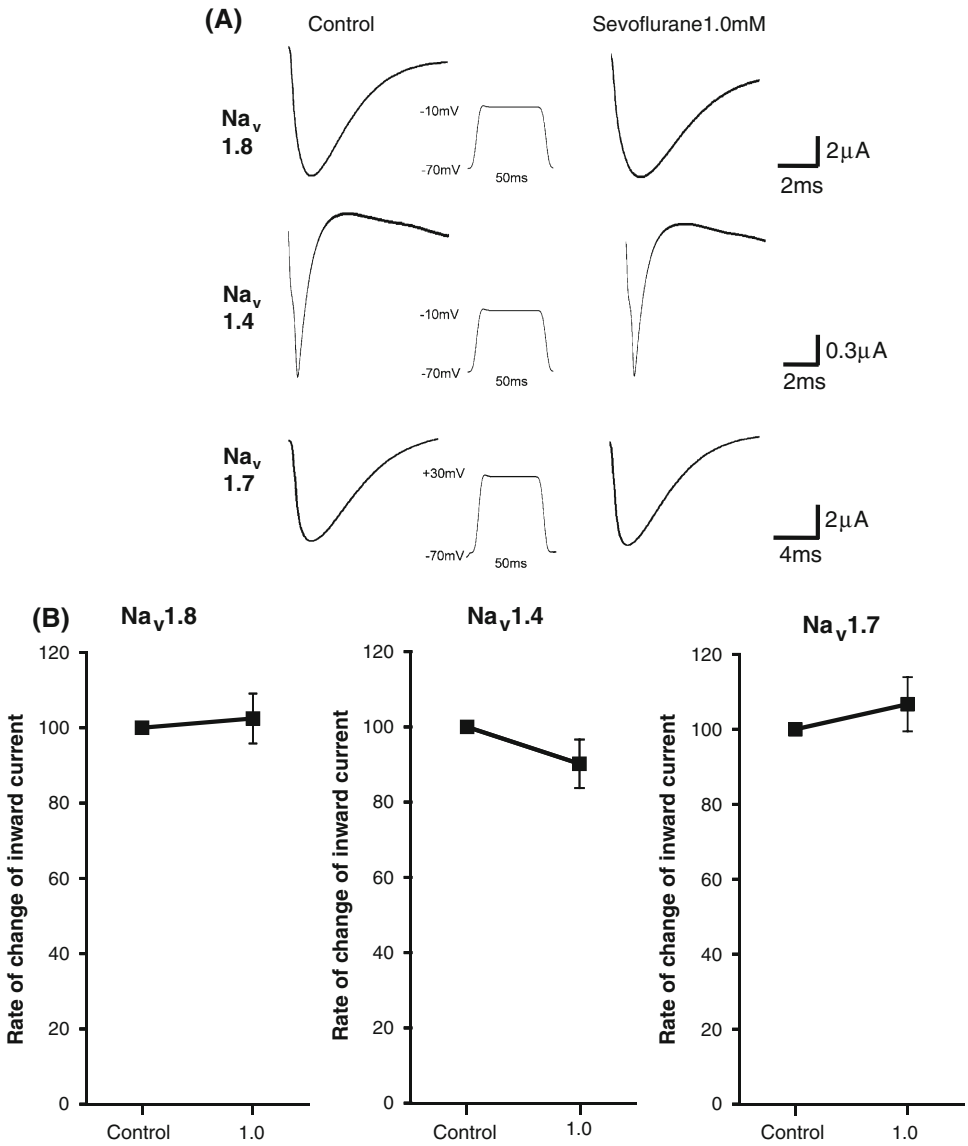

Fig. 3 Sevoflurane modulates voltage-gated sodium channels through the protein kinase C pathway. **a** Representative examples of the effect of bisindolylmaleimide I (GF109203X) on Na_v 1.8, Na_v 1.4 and Na_v 1.7. **b** Summary data for the effects of GF109203X on sevoflurane on peak inward current of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