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Sevoflurane inhibits presynaptic calcium influx without affecting presynaptic action potentials in hippocampal CA1 region

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ABSTRACT

Although diverse effects of volatile anesthetics have been investigated in various studies, the mechanisms of action of such anesthetics, especially sevoflurane, remain elusive. In contrast to their potent modulation of inhibitory synaptic transmission there is little information about their effects on excitatory transmission in the brain. In this study, we examined the effect of sevoflurane on the excitatory synaptic transmission at CA1 synapses in hippocampal slices of mice. Sevoflurane at 5% was mixed with 95% O₂ and 5% CO₂ and bubbled in artificial cerebral spinal fluid (0.69 mM). Extracellular recordings of the field excitatory postsynaptic potential (fEPSP) and presynaptic fiber volley (FV) were made at physiological temperature. In addition, fluorescent measurements of presynaptic Ca²⁺ transients were performed while simultaneously recording fEPSP. Application of sevoflurane reduced the amplitude of fEPSP (45 ± 8%, n = 5). This effect was accompanied by concurrent enhancement of the paired-pulse facilitation of fEPSP (127 ± 5%, n = 12), suggesting a possible presynaptic site of action of sevoflurane. The amplitude of FV was not significantly affected (102 ± 5%, n = 5). In contrast, fluorescent measurements revealed that presynaptic Ca²⁺ influx was suppressed by sevoflurane (69 ± 5%, n = 7), as was simultaneously recorded fEPSP (44 ± 5%, n = 7). Our results suggest that sevoflurane potently suppresses excitatory synaptic transmission via inhibition of presynaptic Ca²⁺ influx without affecting presynaptic action potentials.

Although countless patients have undergone surgery under general anesthesia, the mechanisms of anesthetics, especially volatile anesthetics, have not been well clarified. Until the 1980s, nonspecific, that is “lipid-based” anesthetic theory was based on the Meyer-Overton correlation (22). This theory holds that the lipids of nerve membranes are the principal anesthetic target sites. After the 1980s, a new theory has become popular based on some examples that did not obey Meyer-Overton correlation (5, 13). This theory suggests that signaling proteins are the target sites of anesthetics. However, signaling proteins in-

clude ion channels, receptors, intracellular pathways and so on. Accordingly, the exact action sites of anesthetics are still controversial. With regard to the ion channels, there is little information about the effect on excitatory transmission such as that through voltage-gated channels, although the contribution of GABA_A receptors has been explored (4, 7).

Sevoflurane, one of the recent representative volatile anesthetics, was developed in Japan and has been available worldwide since the 1990s (3). The safety and efficacy of this anesthetic are well established and many studies have been conducted to define its effects precisely in different patient populations and organ systems (2). In the central nervous system, sevoflurane is a cerebral vasodilator and has shown anesthetic preconditioning potential in animals, which suggests that it may have neuroprotective properties (21). On the other hand, neonatal exposure to sevo-

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flurane at a higher concentration causes alterations in the hippocampal synaptic plasticity that persists into adulthood in rats (12). This indicates neurotoxicity for the developing brain. However, there have been few studies on its mechanism of anesthesia (20).

Accordingly, in this study, we aimed to clarify the effect of sevoflurane on the excitatory transmission in the brain by using mouse hippocampal slices. First, the effect of the amplitude of the field excitatory postsynaptic potential (fEPSP) was evaluated. Second, variations of the paired-pulse ratio (PPR) were studied. Third, the amplitude of the presynaptic fiber volley (FV) was measured. Finally, fluorescent measurements of presynaptic Ca^{2+} transients were performed while simultaneously recording fEPSP.

MATERIALS AND METHODS

Animals and slice preparations. C57BL/6J mice of both sexes were used in the present study. They were treated according to the guidelines for the care and use of laboratory animals of Hokkaido University. Transverse hippocampal slices were prepared from 9- to 37-day-old mice as described previously (11, 23). Animals were anesthetized with diethyl ether and the brain was dissected out in an ice-cold sucrose solution containing the following (in mM): 40 NaCl, 25 NaHCO₃, 10 glucose, 150 sucrose, 4 KCl, 1.25 Na₂PO₄, 0.5 CaCl₂, and 7 MgCl₂. The bilateral hippocampi were cut into transverse slices 400 μm thick by using a Super Microslicer Zero 1 (D. S. K; Dosaka-EM, Tokyo). Then the sucrose-containing solution was replaced with artificial cerebrospinal fluid (ACSF) containing the following (in mM): 127 NaCl, 1.5 KCl, 1.2 KH₂PO₄, 26 NaHCO₃, 10 glucose, 2.4 CaCl₂, and 1.3 MgCl₂. The hippocampal slices were incubated for 1 h in the above solution at 30°C, and were kept in an interface-type chamber saturated with 95% O₂ and 5% CO₂ at room temperature.

Application of sevoflurane and measurement of concentration in ACSF. Sevoflurane was vaporized through a calibrated commercial vaporizer (Savotec 3; Ohmeda, BOC Health Care). Sevoflurane at 5% was mixed with a carrier gas containing 95% O₂ and 5% CO₂, and was bubbled in ACSF for at least 20 min. The gas concentration of sevoflurane was continuously measured with a multi-gas monitor (Narcotica; Fukuda Denshi, Tokyo). ACSF with or without sevoflurane was delivered to the recording chamber using a gravity-feed and vacuum system (Perista Pump SJ-1211; Bioinstrument ATTA). Poly-

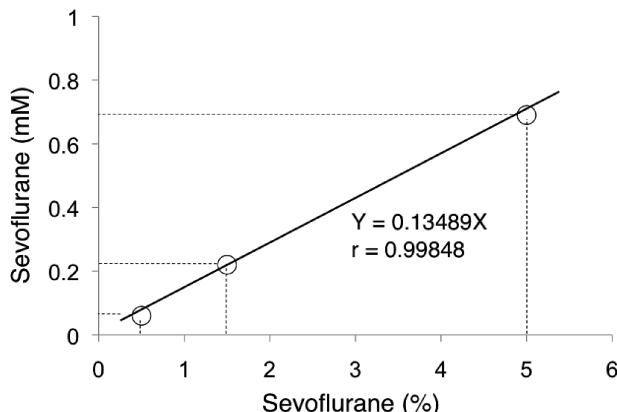


Fig. 1 The aqueous concentrations of sevoflurane in ACSF. ACSF containing sevoflurane at concentrations of 0.5%, 1.5%, and 5% was sampled from the recording chamber, and the aqueous concentrations of sevoflurane were measured using gas chromatography ($n = 5$ each). The aqueous concentrations were calculated by comparison to that of standard solution containing sevoflurane at 1.0 mM. A linear curve fit was applied.

ethylene tubes were used as circuits for bubbling sevoflurane in ACSF and for delivering ACSF to minimize the loss of volatile anesthetics. To determine the actual aqueous concentrations of sevoflurane in the submerged recording chamber, samples of ACSF containing sevoflurane were taken from the recording chamber with airtight syringes, and the concentrations of sevoflurane were measured using gas chromatography. Fig. 1 shows the relationship between aqueous and gas concentrations of sevoflurane in our experimental conditions. Sevoflurane at 0.5, 1.5, and 5.0% was equivalent to 0.06, 0.22, and 0.69 mM ($n = 5$ for each), respectively, and this relationship had a positive correlation calculated ($r = 0.99848$).

Electrophysiology. Hippocampal slices in the recording chamber were continuously perfused with ACSF saturated by the carrier gas at 2 mL/min with the above delivery system. The glass recording electrodes were pulled with a micropipette puller (Sutter Instruments P-97/IVF), and were shaped with a tip diameter of 10 μm . For extracellular recording in CA1 synapses, the tips of both stimulating and recording electrodes were placed on the middle of the stratum radiatum in the CA 1 region, and were submerged at a depth of 200 μm from the surface of the slice. Electrical stimuli of 100 μs duration were given every 10 s. All field recordings were made at $35 \pm 1^\circ\text{C}$ to examine the effect of sevoflurane under physical conditions. Most previous studies examining

the mechanisms of anesthetics were conducted at room temperature. Therefore, we decided to examine the effects of sevoflurane on neurotransmission at a higher temperature to confirm them in the physiological condition of a living body. Only fluorescent measurements of presynaptic calcium influx were tested at $25 \pm 1^\circ\text{C}$ because fluorescent signals hardly stabilized at the higher temperature while recording. ACSF was perfused through a warmer (T-324B; Warner Instruments Corporation) set near the recording chamber and the desired temperature was maintained automatically.

Measurement of fEPSP. At the beginning of this study, we examined suppression of fEPSP by sevoflurane at a level similar to that used under our experimental conditions. We tested fEPSP with a single subthreshold stimulation so that it did not merge with the population spike, an electrical activity of pyramidal cells in the CA1 region. After the baseline setting of fEPSP stabilized, sevoflurane was applied to the hippocampal slice for 20 min, and the change of the amplitude of fEPSP was recorded through the washout phase. The amplitude of fEPSP of the control was aligned at around 1 mV.

Measurements of paired-pulse ratio (PPR) of fEPSP. Depression of fEPSP could involve both presynaptic and postsynaptic mechanisms such as a decrease of neurotransmitter release or downregulation of postsynaptic receptors. The variations of PPR after application of sevoflurane were studied to confirm whether the inhibition of excitatory neurotransmission by sevoflurane involved presynaptic actions. We tested PPR with paired electrical stimulations with a 50 ms interval. PPR was calculated by dividing second fEPSP amplitudes by first fEPSP amplitudes.

Measurements of presynaptic FV. We measured presynaptic FV by loading Ca^{2+} -free ACSF containing the following (in mM): 127 NaCl, 1.5 KCl, 1.2 KH_2PO_4 , 26 NaHCO_3 , 10 glucose, and 3.7 MgCl_2 . Using Ca^{2+} -free ACSF can easily isolate FV from the fEPSP without applying any glutamate receptor blocker such as CNQX or AP5. The amplitude of FV was measured as the difference between the initial positive peak and the following negative peak.

Field potential was filtered at 2 kHz and digitized at 20–40 kHz for measurement of FV. To confirm that the precise measurement of the spikes reflected nothing but FV, 1 μM tetrodotoxin (TTX), a sodium channel blocker, was applied at the end of the ex-

periment in a control study.

Fluorescence measurements of presynaptic Ca^{2+} influx. Fluorescence recordings of presynaptic Ca^{2+} influx at the presynaptic terminals of the Schaffer collaterals in the hippocampal CA1 region were made as described previously (9). A membrane-permeable fluorescent calcium indicator, Oregon Green 488 BAPTA 1 AM (OGB-1 AM), was focally injected near the stimulating electrode in the stratum radiatum layer of hippocampal area CA1. Then, OGB-1 was loaded to presynaptic terminals via axonal transport by Schaffer collaterals, resulting in selective labeling of the axons and presynaptic terminals (injection loading technique). The fluorescence intensity (excitation at 510–560 nm and monitoring above 580 nm) from the area (100 μm diameter) containing the labeled terminals was measured as a voltage transient with a single photodiode (S2281-01; Hamamatsu Photonics, Hamamatsu, Japan), and the fEPSPs from the same area were monitored simultaneously. The $\Delta\text{F/F}$ value evoked by a single electrical stimulus was used as a measure of the $[\text{Ca}^{2+}]_{\text{i}}$ increase during an action potential. Subtraction of the background fluorescence was not performed because the fluorescence of the unlabeled regions of the slices at this wavelength was negligible. No attempt was made to relate the $\Delta\text{F/F}$ value to peak $[\text{Ca}^{2+}]_{\text{i}}$ because our methods detected the fluorescence signals not only from the active zone of the presynaptic terminals but also from the axons of Schaffer collaterals, and we could not estimate the relative contribution to the total signals. Accordingly, we measured the volume-averaged intensity of fluorescence loaded in the labeled area containing presynaptic terminals and axons in the stratum radiatum layer. The output of the photodiode was I–V converted, amplified, and filtered at 500 Hz with an eight-pole Bessel filter (FLA-1; Cygnus Technology, Delaware Water Gap, PA). The signal was then digitized with a 12 bit analog-to-digital converter (Digidata 1200A; Axon Instruments, Foster City, CA) and acquired at 10 kHz using pClamp 10 software (Molecular Devices).

Data analysis. All data were acquired and analyzed using pClamp 10 software (Molecular Devices). Data in the text and figures are expressed as mean \pm SEM. Statistical analysis was performed using the paired t test unless otherwise noted, and $P < 0.05$ was considered statistically significant.

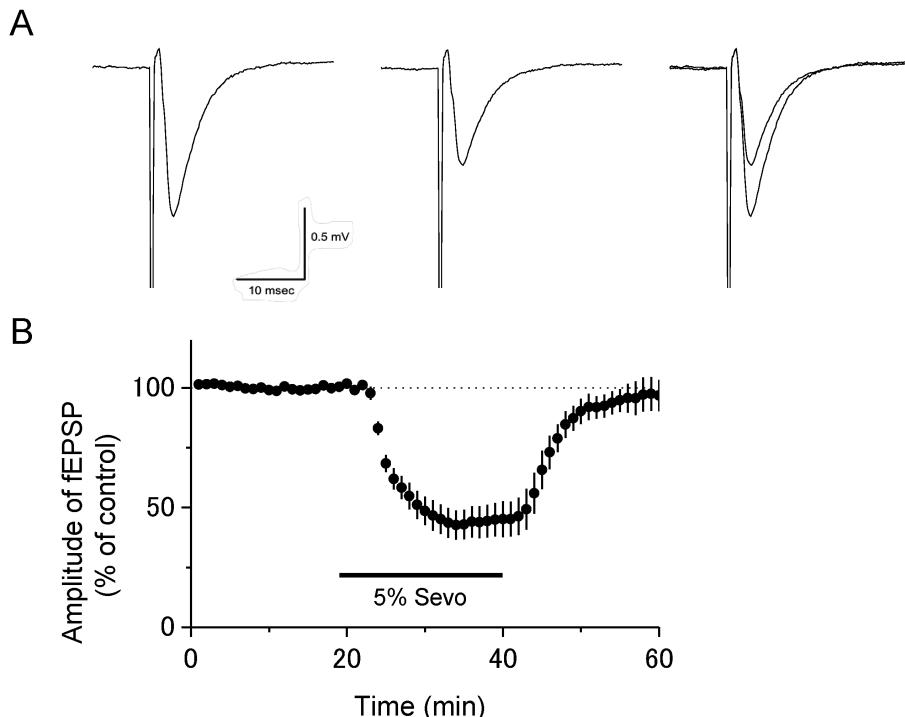


Fig. 2 The reversible suppression of fEPSP by sevoflurane. (A) Sample traces of fEPSP of the control (Left), at 20 min after application of 0.69 mM sevoflurane (Middle), and Left + Middle (Right). fEPSPs were recorded from the middle of the stratum radiatum in the CA 1 region in response to electrical stimulation of Schaffer collateral commissural fibers. (B) Time course of the amplitude of fEPSP. Sevoflurane (Sevo) at 0.69 mM (5%) reversibly suppressed the amplitude of fEPSP to $45 \pm 8\%$ ($P < 0.01$, $n = 5$).

RESULTS

Sevoflurane at 5% reversibly inhibited field EPSP with an increase in PPR

Application of sevoflurane at 5% (0.69 mM) significantly suppressed the amplitude of fEPSP to $45 \pm 8\%$ of the control level (Fig. 2). Suppression of fEPSP reached a balanced value in about 10 min, and was entirely recovered 20 min after the washout of sevoflurane. PPR was recorded with a pair of electrical stimulations with a 50 ms interval, and sevoflurane increased paired-pulse facilitation (PPF) to $127 \pm 5\%$ of the control level (Fig. 3). This effect was also reversible, the same as the suppression of the amplitudes of fEPSPs.

Presynaptic fiber volleys were not changed by application of 5% sevoflurane

We tested the effect of sevoflurane at 5% on presynaptic Na^+ channels in the hippocampal CA1 region. In our study, the amplitude of the presynaptic fiber volley was not significantly affected by application of 5% sevoflurane (to $102 \pm 5\%$ of the control value) (Fig. 4). The latency of the fiber volley was also un-

changed.

Five percent sevoflurane inhibited presynaptic Ca^{2+} influx while suppressing simultaneously recorded fEPSP

Five percent sevoflurane suppressed presynaptic Ca^{2+} influx to $69 \pm 6\%$ of the baseline level and fEPSP to $44 \pm 2\%$ of the control level (Fig. 5). The relationship between the amplitude of fEPSP and the Ca^{2+} concentration was nonlinear and could be fitted by the equation:

$$Y = X^m \quad (10, 27)$$

Our data showed that m was 2.3.

DISCUSSION

Our findings in this study can be summarized as follows. First, sevoflurane reversibly suppressed the amplitude of fEPSP. Second, this effect was accompanied by an increase in PPF. Third, the amplitude of presynaptic FV was not significantly affected by sevoflurane. Fourth, fluorescent measurements revealed that Ca^{2+} influx into the presynaptic terminals was strongly suppressed by sevoflurane, with simultaneous suppression of fEPSP.

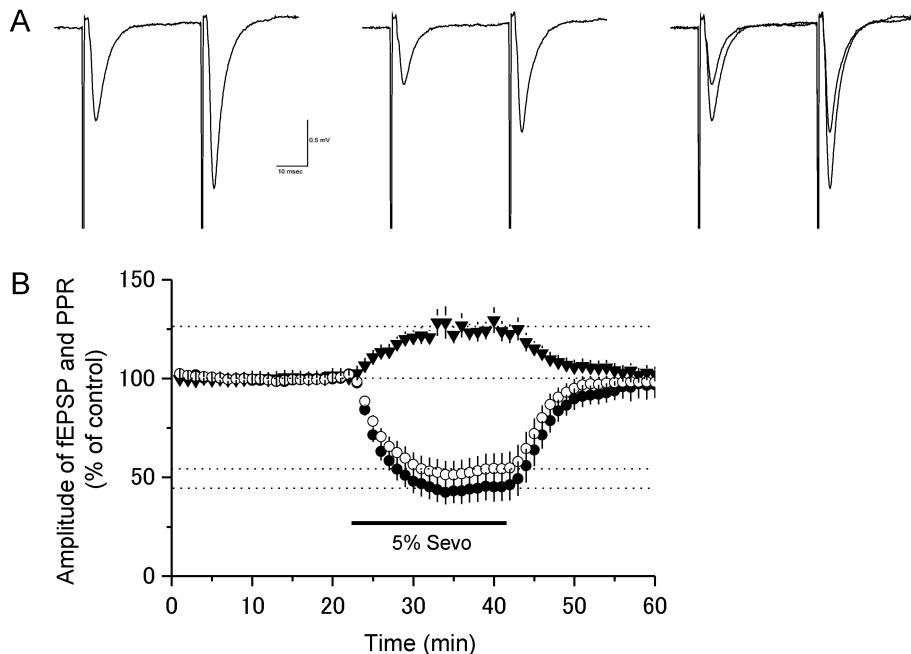


Fig. 3 Increase in PPR by sevoflurane. (A) Sample traces of pairs of fEPSPs of the control (Left), at 20 min after application of 0.69 mM sevoflurane (Middle), and Left + Middle (Right). A pair of electrical stimulations with a 50 ms interval was applied, and a pair of fEPSPs was recorded in the CA1 region. (B) Time courses of fEPSP1 (closed circle), fEPSP2 (open circle) and PPR (fEPSP2/fEPSP1) (triangle). Sevoflurane (Sevo) at 0.69 mM (5%) suppressed both fEPSP1 and fEPSP2. PPF was increased by sevoflurane (to $127 \pm 5\%$, $P = 0.02$, $n = 12$).

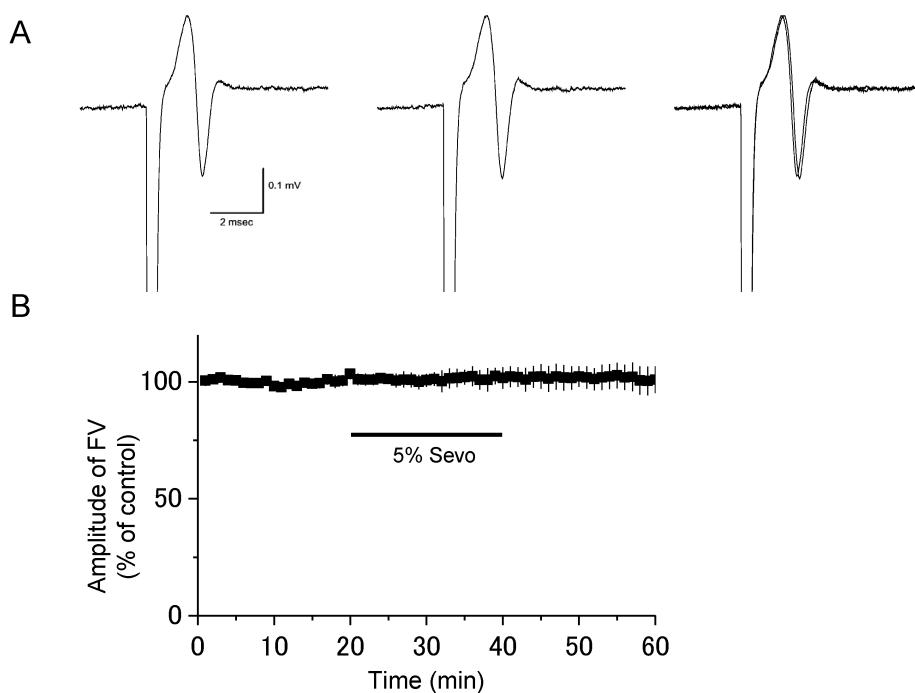


Fig. 4 No significant change in presynaptic fiber volley (FV). (A) Sample traces of FV of the control (Left), at 20 min after application of 0.69 mM sevoflurane (Middle), and Left + Middle (Right). FV was measured after perfusion of Ca^{2+} -free ACSF. The amplitude of the fiber volley was measured as the difference between the initial positive peak and the following negative peak. (B) Time course of FV. FV was not significantly affected by sevoflurane (Sevo) at 0.69 mM (5%) ($102 \pm 10\%$, $P < 0.01$, $n = 5$).

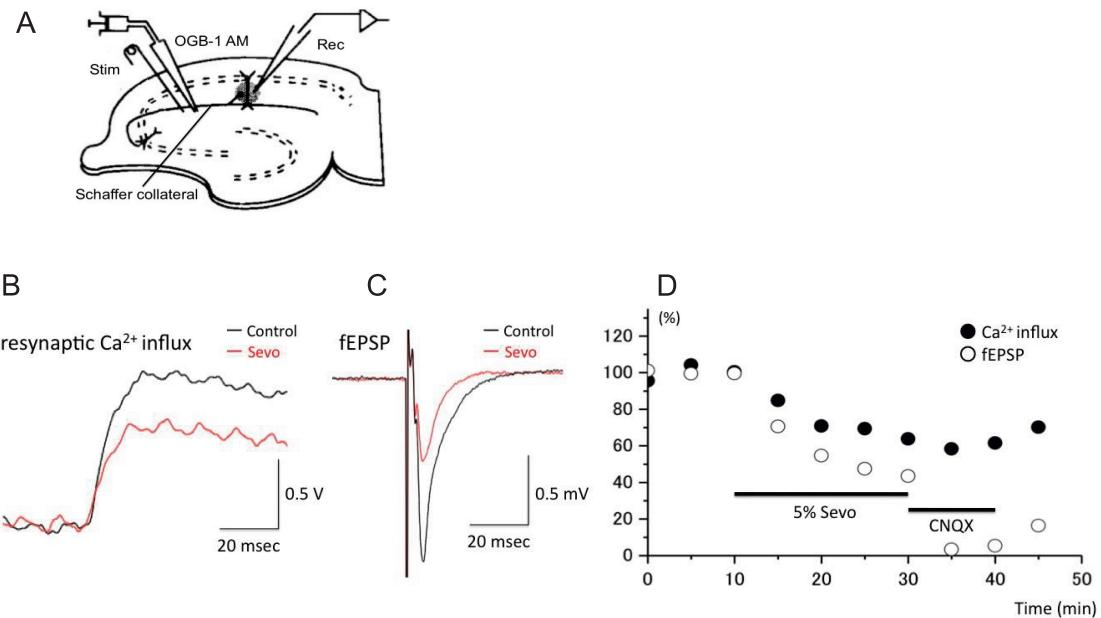


Fig. 5 Fluorescence measurements of presynaptic Ca^{2+} influx. (A) Schematic injection loading technique for fluorescence Ca^{2+} measurements. OGB-1 AM was focally injected near the stimulating electrode, and was loaded to presynaptic terminals by axonal transport. (B) Sample traces of presynaptic Ca^{2+} influx of the control and at 20 min after application of sevoflurane (Sevo). Relative changes of fluorescence intensity ($\Delta F/F$) were used as a measurement of the amount of Ca^{2+} influx, and fluorescence intensity was converted into voltage and recorded as the peak amplitude. (C) Sample traces of simultaneously recorded fEPSPs of the control and at 20 min after application of sevoflurane (Sevo). (D) Time courses of presynaptic Ca^{2+} influx and fEPSP. Sevoflurane at 0.69 mM inhibited presynaptic Ca^{2+} influx (to $69\% \pm 6\%$, $P < 0.02$, $n = 7$), and simultaneously recorded fEPSP (to $4\% \pm 2\%$, $P < 0.01$, $n = 7$). CNQX, a non-NMDA glutamate antagonist was applied to rule out nonspecific labeling of the postsynaptic component.

Few studies have evaluated the effect of sevoflurane on fEPSP and PPF. One reported that sevoflurane dose-dependently diminished fEPSP in the CA1 stratum radiatum of the mouse hippocampus and that the calculated IC_{50} value was 0.57 mM (6). In our study, application of sevoflurane at 0.69 mM significantly suppressed the amplitude of fEPSP to $45 \pm 8\%$ of the control level. Accordingly, this result is compatible with the previous study. In another study, the effect of sevoflurane on population spikes and PPF in two synaptic pathways (Schaffer collateral fibers–CA1, and perforant path–dentate gyrus [DG]) were evaluated in rat hippocampal slices (8). In that study, sevoflurane (0.4–5.0 vol%) significantly decreased the amplitudes of population spikes of CA1 and DG in a dose-dependent manner. At 2.0 vol% it significantly enhanced PPF from 127% and 263% to 153% and 494% in CA1 and DG, respectively. In another study, halothane and isoflurane depressed fEPSP accompanied by an increase in PPF (15), which is known to increase after manipulations that reduce calcium-mediated glutamate release from Schaffer-collateral fibers (15). Accordingly, it is suggested that volatile anesthetics depress excitatory

transmission by acting at presynaptic sites regardless of their kinds.

FV in Schaffer collateral fibers represents a summation of closely timed multiple action potentials (26). In our study, the amplitude of FV was not significantly affected by application of 5% sevoflurane. As far as we know, there seem to be no studies evaluating the effect of sevoflurane on FV. However, one study reported that isoflurane dose-dependently depressed FV in small unmyelinated fibers in the hippocampal CA1 region, whereas the effect on myelinated fibers was small (1). Another study evaluated the relation between the excitatory postsynaptic current (EPSC) induced by whole-cell voltage clamp and presynaptic action potential at a calyx-type synapse in the rat brainstem (28). In that study, isoflurane at 0.35–1.05 mM reduced the EPSC and the inhibition of the presynaptic action potential contributed to 62–78% of its inhibition (28). Moreover, in a study using rat hippocampal slices, halothane (1.2 vol%) produced an 18% depression of FV amplitudes in Schaffer-collateral fibers (19). On the other hand, isoflurane at 0.35 mM depressed EPSP by ~60% and increased PPF by ~20% without de-

pression of FV amplitudes in rat hippocampal slices in response to Schaffer-collateral fiber stimulation (26). Thus, whether volatile anesthetics inhibit FV, that is axon conductance, seems to still be controversial. However, our finding that FV was not significantly affected by sevoflurane suggested that sevoflurane may suppress neurotransmission by affecting downstream steps of depolarization in axons.

Ca^{2+} ions inflated through presynaptic voltage-gated Ca^{2+} channels (VGCCs) activate the presynaptic vesicles for exocytosis of neurotransmitters, leading to the occurrence of fEPSPs. It is extremely difficult to examine presynaptic Ca^{2+} influx into minute synaptic terminals including hippocampal synapses because of the requirement for an advanced technique like the presynaptic patch clamp. Wu *et al.* studied the presynaptic Ca^{2+} influx with the presynaptic patch clamp technique in a large, atypical calyx-type synapse in the medial nucleus of the trapezoid body in the rat brainstem (28). However, no studies have reported on presynaptic Ca^{2+} influx in hippocampal synapses. Accordingly, we studied the presynaptic Ca^{2+} influx in the hippocampal CA1 region by employing fluorescence measurements of presynaptic Ca^{2+} influx (11). This technique can measure not only Ca^{2+} influx, but also evaluate fEPSP simultaneously.

Our study demonstrated that Ca^{2+} influx into the presynaptic terminals was strongly suppressed by sevoflurane with simultaneous suppression of fEPSP and that the estimated value for the exponent m was 2.3. In a report that evaluated these relationships with the same technique using guinea-pig CA1 synapses, the exponent m was 3.5 when applying cadmium to block VGCC (27). The slight difference in the m value may be due to different affinities and concentrations of indicators within terminals as well as the different ages and species of the animals (11). We hypothesize that sevoflurane may inhibit VGCC based on our study. This accords with a previous report, using guinea pig cerebrocortical synaptosomes, demonstrating that isoflurane, enflurane and halothane depress K^+ -depolarization-induced increases in presynaptic $[\text{Ca}^{2+}]$ and glutamate release in a dose-dependent fashion (18). The study concluded that these anesthetics decreased presynaptic $[\text{Ca}^{2+}]$ in a manner consistent with Ca^{2+} entry, possibly via VGCCs. However, a later study using rat cerebrocortical synaptosomes reported that isoflurane reduced the calcium-dependent glutamate release evoked by membrane depolarization with 4-aminopyridine without a significant change in free cytosolic calcium (14). Moreover, another study using rat cerebrocortical synaptosomes demonstrated that sevoflurane re-

duced calcium-dependent glutamate release in a dose-dependent manner without a significant change in free cytosolic calcium (25). Accordingly, whether volatile anesthetics inhibit VGCCs also remains controversial, although our study using another methodology for mouse hippocampal slices indicated the contribution of VGCCs.

A limitation of this study is that we examined only one high concentration (5%) of sevoflurane. Accordingly, dose-dependent and low concentration effects should be evaluated as the next step. Second, we used hippocampal slices as a model of excitatory transmission. The hippocampus is known to be the main location of memory in the brain (17). It is important to inhibit memory to prevent intraoperative awareness. Accordingly, amnesia is one of indispensable components of anesthesia. However, memory and consciousness are different (17). Recently, it was reported that consciousness is related to the default mode network (24), which is one of 6 widely distributed resting state networks identified by electroencephalopathy and functional magnetic resonance imaging (16). The default mode network includes the posterior cingulate cortex/precuneus, medial prefrontal cortex, and so on (24). Accordingly, it may be more proper to examine such areas. Third, our and previous findings strongly suggest that volatile anesthetics inhibit fEPSP by targeting presynaptic parts of synapses. However, it is still controversial whether they inhibit axon conductance and VGCCs. We could not clarify the causes of the differences between our study and previous studies with opposite results. Additional studies will be necessary to evaluate these.

In conclusion, our study suggests that sevoflurane suppresses excitatory synaptic transmission in the presynaptic terminals, like other volatile anesthetics. This may be related to the inhibition of presynaptic calcium influx without modulating action potentials. However, further study will be necessary to confirm the mechanism.

REFERENCES

- Berg-Johnsen J and Langmoen IA (1986) The effect of isoflurane on unmyelinated and myelinated fibres in the rat brain. *Acta Physiol Scand* **127**, 87–93.
- Brioni JD, Varughese S, Ahmed R and Bein B (2017) A clinical review of inhalation anesthesia with sevoflurane: from early research to emerging topics. *J Anesth* **31**, 764–778.
- De Hert S and Moerman A (2015) Sevoflurane. *F1000Res* **4**, 626.
- Franks NP (2008) General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat Rev Neurosci* **9**, 370–386.

5. Franks NP and Lieb WR (2004) Seeing the light: protein theories of general anesthesia. 1984. *Anesthesiology* **101**, 235–237.
6. Haseneder R, Kratzer S, von Meyer L, Eder M, Kochs E, *et al.* (2009) Isoflurane and sevoflurane dose-dependently impair hippocampal long-term potentiation. *Eur J Pharmacol* **623**, 47–51.
7. Hemmings HC, Jr, Akabas MH, Goldstein PA, Trudell JR, Orser BA, *et al.* (2005) Emerging molecular mechanisms of general anesthetic action. *Trends Pharmacol Sci* **26**, 503–510.
8. Hirota K and Roth SH (1997) The effects of sevoflurane on population spikes in CA1 and dentate gyrus of the rat hippocampus *in vitro*. *Anesth Analg* **85**, 426–430.
9. Kamiya H and Ozawa S (1999) Dual mechanism for presynaptic modulation by axonal metabotropic glutamate receptor at the mouse mossy fibre-CA3 synapse. *J Physiol* **518** (Pt 2), 497–506.
10. Kamiya H and Ozawa S (1998) Kainate receptor-mediated inhibition of presynaptic Ca^{2+} influx and EPSP in area CA1 of the rat hippocampus. *J Physiol* **509** (Pt 3), 833–845.
11. Kamiya H, Umeda K, Ozawa S and Manabe T (2002) Presynaptic Ca^{2+} entry is unchanged during hippocampal mossy fiber long-term potentiation. *J Neurosci* **22**, 10524–10528.
12. Kato R, Tachibana K, Nishimoto N, Hashimoto T, Uchida Y, *et al.* (2013). Neonatal exposure to sevoflurane causes significant suppression of hippocampal long-term potentiation in postgrowth rats. *Anesth Analg* **117**, 1429–1435.
13. Koblin DD, Chortkoff BS, Lesser MJ, Eger EI, 2nd, Halsey MJ, *et al.* (1994) Polyhalogenated and perfluorinated compounds that disobey the Meyer-Overton hypothesis. *Anesth Analg* **79**, 1043–1048.
14. Larsen M, Valo ET, Berg-Johansen J and Langmoen IA (1998) Isoflurane reduces synaptic glutamate release without changing cytosolic free calcium in isolated nerve terminals. *Eur J Anaesth* **15**, 224–229.
15. MacIver MB, Mikulec AA, Amagasaki SM and Monroe FA (1996) Volatile anesthetics depress glutamate transmission via presynaptic actions. *Anesthesiology* **85**, 823–834.
16. Mantini D, Perrucci MG, Del Gratta C, Romani GL and Corbetta M (2007) Electrophysiological signatures of resting state networks in the human brain. *Proc Natl Acad Sci USA* **104**, 13170–13175.
17. Mashour GA and Pryor KA (2015) Consciousness, memory, and anesthesia. In: *Miller's Anesthesia*. (Miller RD, ed), pp282–303, Elsevier Saunders, Philadelphia.
18. Miao N, Frazer MJ and Lynch C, 3rd (1995) Volatile anesthetics depress Ca^{2+} transients and glutamate release in isolated cerebral synaptosomes. *Anesthesiology* **83**, 593–603.
19. Mikulec AA, Pittson S, Amagasaki SM, Monroe FA and MacIver MB (1998) Halothane depresses action potential conduction in hippocampal axons. *Brain Res* **796**, 231–238.
20. Nishikawa K and Harrison NL (2003) The actions of sevoflurane and desflurane on the gamma-aminobutyric acid receptor type A: effects of TM2 mutations in the alpha and beta subunits. *Anesthesiology* **99**, 678–684.
21. Payne RS, Akca O, Roewer N, Schurr A and Kehl F (2005) Sevoflurane-induced preconditioning protects against cerebral ischemic neuronal damage in rats. *Brain Res* **1034**, 147–152.
22. Perouansky M, Pearce RA and Hemmings HC, Jr (2015) Inhaled anesthetics: mechanisms of action. In: *Miller's Anesthesia*. (Miller RD, ed), pp614–637, Elsevier Saunders, Philadelphia.
23. Shimizu H, Fukaya M, Yamasaki M, Watanabe M, Manabe T, *et al.* (2008) Use-dependent amplification of presynaptic Ca^{2+} signaling by axonal ryanodine receptors at the hippocampal mossy fiber synapse. *Proc Natl Acad Sci USA* **105**, 11998–12003.
24. Vanhaudenhuyse A, Noirhomme Q, Tshibanda LJ, Bruno MA, Boveroux P, *et al.* (2010) Default network connectivity reflects the level of consciousness in non-communicative brain-damaged patients. *Brain* **133**, 161–171.
25. Vinje ML, Moe MC, Valo ET and Berg-Johansen J (2002) The effect of sevoflurane on glutamate release and uptake in rat cerebrocortical presynaptic terminals. *Acta Anaesthesiol Scand* **46**, 103–108.
26. Winegar BD and MacIver MB (2006) Isoflurane depresses hippocampal CA1 glutamate nerve terminals without inhibiting fiber volleys. *BMC Neurosci* **7**, 5.
27. Wu LG and Saggau P (1994) Presynaptic calcium is increased during normal synaptic transmission and paired-pulse facilitation, but not in long-term potentiation in area CA1 of hippocampus. *J Neurosci* **14**, 645–654.
28. Wu XS, Sun JY, Evers AS, Crowder M and Wu LG (2004) Isoflurane inhibits transmitter release and the presynaptic action potential. *Anesthesiology* **100**, 663–670.