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Effects of sevoflurane and desflurane on the nociceptive responses of substantia gelatinosa neurons in the rat spinal cord dorsal horn: An in vivo patch-clamp analysis

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Abstract

Background: Volatile anesthetics suppress noxiously evoked activity in the spinal dorsal horn, which could contribute in part to analgesia, immobility. Modulation of excitatory and inhibitory synaptic transmission in substantia gelatinosa neurons could lead to the suppression of dorsal horn activity; however, this phenomenon has not yet been investigated fully.

Methods: In urethane-anesthetized rats, extracellular activity of dorsal horn neurons (action potentials) and excitatory/inhibitory postsynaptic currents in substantia gelatinosa neurons were recorded using extracellular and in vivo patch-clamp techniques, respectively, to assess the spontaneous and the noxious-evoked activity. Sevoflurane or desflurane at concentrations ranging from 0.1 to 2 minimum alveolar concentration was administered by inhalation. Hot- and cold-plate tests were performed to assess nociceptive responses during the inhalation of volatile anesthetics at lower anesthetic doses (0.1–0.5 minimum alveolar concentration).

Results: At anesthetic doses (1 and 2 minimum alveolar concentration), both sevoflurane and desflurane decreased the frequency of action potentials in the dorsal horn and the activities of excitatory postsynaptic currents in substantia gelatinosa neurons during pinch stimulation and decreased the activities of spontaneous and miniature excitatory postsynaptic currents. Inhibition of the frequencies was more prominent than that of amplitudes in spontaneous and miniature excitatory postsynaptic currents at these anesthetic doses. However, at subanesthetic doses (0.1 and 0.2 minimum alveolar concentration), desflurane facilitated action potentials and excitatory postsynaptic currents. Inhibitory postsynaptic currents were inhibited by both anesthetics at anesthetic doses (1 and 2 minimum alveolar concentration). Hot- or cold-plate tests showed hyperalgesic effects of desflurane at subanesthetic doses (0.1 and 0.2 minimum alveolar concentration) and a dose-dependent analgesic effect of sevoflurane.

Conclusions: Sevoflurane and desflurane at anesthetic doses suppressed dorsal horn activity mainly via inhibition of excitatory postsynaptic currents in substantia gelatinosa neurons, which would contribute to their analgesic properties. Presynaptic mechanisms were likely in excitatory postsynaptic currents inhibition. Desflurane but not sevoflurane may have a hyperalgesic effect at subanesthetic doses.

Keywords

Sevoflurane, desflurane, analgesic effect, hyperalgesic effect, in vivo patch-clamp recording

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Introduction

Volatile anesthetics are known to induce powerful hypnosis through actions on a wide variety of receptors (e.g., Gamma Amino Butyric Acid $[GABA]_A$, glycine, nicotinic acetylcholine, and glutamate receptors) and ion channels (e.g., sodium, potassium, and calcium

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channels) in the central nervous system (CNS).[1–3] Reduction in spinal neuronal responses to noxious stimuli contributes in part to general anesthesia.[4–8] A previous in vivo electrophysiological study in rats showed that halothane depressed the nociceptive responses of spinal dorsal horn neurons partly via interactions with $GABA_A$ and glycine receptors.[9] In vitro studies using rat spinal cord slices demonstrated that isoflurane (1 minimum alveolar concentration (MAC)) postsynaptically augmented GABAergic inhibitory postsynaptic currents (IPSCs) and decreased glutamatergic excitatory postsynaptic currents (EPSCs) of substantia gelatinosa (SG) neurons in the spinal dorsal horn.[10,11] However, precise actions of volatile anesthetics inhalation on nociceptive transmission in spinal cord neurons have not yet been fully elucidated. However, evidence suggested that volatile anesthetics, including desflurane, induced hyperalgesia at subanesthetic doses ($\leq$0.1 MAC).[12–15] These observations suggest that volatile anesthetics may produce biphasic modulation of nociceptive responses in the spinal cord dorsal horn, depending on the dose.

The SG neurons in the superficial dorsal horn (laminar II) play an important role in nociceptive transmission.[16,17] These SG neurons are interneurons that receive both excitatory (nociceptive) signals from peripheral afferent fibers[18–21] and inhibitory (antinociceptive) signals from descending inhibitory fibers and GABAergic or glycinergic interneurons.[22–24] The SG neurons depolarize and produce action potentials (APs) when the summation of EPSCs exceed the threshold and transfer pain signals to ascending afferent neurons. Extracellular recordings can only detect the APs in the dorsal horn, but a recently developed in vivo patch-clamp recording technique from an SG neuron allowed understanding of the detailed synaptic transmission via recording the EPSCs or IPSCs.[18] This technique would also be advantageous for evaluating nociceptive responses in SG neurons under clinically relevant condition (e.g., painful stimuli, drug administration).

In this study, we performed in vivo extracellular recordings and in vivo patch-clamp analysis to examine the effect of sevoflurane and desflurane on nociceptive transmission in the spinal cord. The hot- and cold-plate tests were also performed to confirm the nociceptive behavioral responses under inhalation of both volatile anesthetics. We found that sevoflurane and anesthetic doses of desflurane dose dependently suppressed nociceptive responses in the spinal dorsal horn, mainly via inhibition of excitatory neurotransmission. We also found that desflurane, but not sevoflurane, induced hyperalgesic responses at subanesthetic doses (0.1 and 0.2 MAC).

Materials and methods

Animals

This study was reviewed and approved by the Institutional Animal Care and Use Committees of Osaka City University (approval number: 13044) and was performed according to the International Association for the Study of Pain guidelines for the use of the animals in research.

Male Sprague-Dawley rats (Kiwa Laboratory Animals Co., Wakayama, Japan) were housed in a temperature-controlled room ($21 \pm 1^{\circ}C$) with a 12-h light/dark cycle and given free access to food and water. A total of 115 rats aged 6 to 10 weeks and weighing 180 to 350 g were used. We made maximum efforts to reduce the number of animals required for this study. Therefore, we sometimes undertook experiments in the same rat if the general condition allowed. In cases of sequential experiments, we set the interval period at least 120 min between each experiment based on previous studies. [25,26] At the end of the experiments, the rats were euthanized with an additional injection of urethane (4 g/kg, i.p.).

Preparations for electrophysiological recordings

The experimental preparations and methods used for the in vivo electrophysiological recordings were performed using previously described procedures.[27–30] Rats were anesthetized with intraperitoneally administered urethane (1.2–1.5 g/kg, i.p.). After a tracheostomy, a silicon tube (outer diameter; 2.3 mm) was inserted into the trachea. Thoracolumbar laminectomy was performed at the level of Th12 to L2 to expose the lumbar enlargement of the spinal cord. Rats were mechanically ventilated (tidal volume; 0.02 mL/g, respiration rate; 100 bpm) using an Apta Ventilator 27050 (Ugo Basile, Gemonio, Italy) and placed in a stereotaxic apparatus (Model ST-7, Narishige, Tokyo, Japan). After the dura was opened, the dorsal root above the recording site was lifted, and the pia-arachnoid membrane was removed to allow insertion of the electrode into the SG. The surface of the spinal cord was irrigated with 95% O_2- and 5% CO_2-equilibrated Krebs solution (in mmol/L: 117 NaCl, 3.6 KCL, 2.5 $CaCl_2$, 1.2 Mg Cl_2, 1.2 NaH_2PO_4, 11 glucose, and 25 $NaHCO_3$) at a flow rate of 10 to 15 mL/min at 38.0°C.

In vivo extracellular recording from dorsal horn neurons

To confirm and quantitatively evaluate the nociceptive response of dorsal horn neurons, the frequency of APs during pinch stimulation was analyzed by extracellular recordings. A tungsten electrode (impedance, 1.3 MΩ)

(Unique Medical, Osaka, Japan) was used for this recording. The electrode was slowly advanced into the spinal cord using a micromanipulator (Model MHW-4, Narisige, Tokyo, Japan) toward the SG at a depth of 30 to 250 μm from the surface. APs were obtained by applying the pinch stimuli for the right hind paw using specially manufactured forceps (Unique Medical, Osaka, Japan) before and during the inhalation of sevoflurane or desflurane at concentrations ranging from 0.1 to 2 MAC. The forceps can convert the pinch force into an electric signal (0.1 V/kg) and enabled us to monitor the constant stimulus application. We used track mode and the signals were amplified (Axopatch 200B; Molecular Devices, San Joes, CA, USA), digitized (Digidata 1321 A, Molecular Devices), and stored on a personal computer using a data acquisition program (Clampex version 10.2, Molecular Devices). The data were sampled at 20 kHz and low-pass filtered at 2 kHz. We took at least 20 s to confirm stable signals and then recorded APs. The pinch stimuli (2 kg force, 3 s) were applied three times with 5 s intervals before and during the inhalation of sevoflurane or desflurane at doses ranged from 0.1 to 2 MAC. During 5 s intervals between pinch stimuli, the activity of APs recovered to the baseline level. Because extracellular recordings might include multiunit signals from not only SG neurons but also axons or dendrites of other layer neurons in dorsal horn, recorded signals were spike sorted with Offline Sorter software (version 4.4.2.0; Plexon, Dallas, TX, USA) and used as dorsal horn neuron's firings. After the spike sorting, the frequency of each neuron during the pinch stimuli was assessed, and the three data sets were averaged.

In vivo patch-clamp recording from SG neurons

In vivo whole-cell patch-clamp recordings from SG neurons were performed using previously described procedures[18,29,30] to elucidate the detailed synaptic mechanism of nociceptive responses during inhalation of volatile anesthetics. Recording pipettes fabricated from thin-walled borosilicate glass capillaries (Ken Enterprise, Kanagawa, Japan) were used and pulled in two stages on a vertical pipette puller (Model PC-10, Narisige, Tokyo, Japan). The pipette resistance was approximately 8 to 15 MΩ when filled with a pipette solution (in mmol/L: 110 Cs_2SO_4, 5 TEA-Cl, 0.5 $CaCl_2$, 2 $MgCl_2$, 5 Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 5 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), and 5 Mg-ATP, pH 7.2 adjusted with CsOH). The patch electrode was slowly advanced into the spinal cord using a micromanipulator (Model MHW-4, Narisige, Tokyo, Japan) toward the SG neurons existing at a depth of 30 to 250 μm from the surface using a blind approach. We identified the SG neurons on the basis of their depth reported in previous studies and response of EPSCs or IPSCs to pinch stimuli.[18,29,30] Whole-cell configurations were established after the formation of a gigaseal. Voltage-clamp recording of EPSCs and IPSCs were made at a holding potential of −70 mV and 0 mV, respectively, using a patch-clamp amplifier (Axopatch 200B; Molecular Devices, San Joes, CA, USA). Spontaneous EPSCs and IPSCs (sEPSCs and sIPSCs) were recorded in the absence of noxious stimuli. The EPSCs and IPSCs during pinch stimulation were recorded using the same pinch stimuli protocol as APs recordings. The pinch stimuli (2 kg force, 3 s) were applied to a right hind paw using forceps three times with 5 s intervals. During the 5 s intervals after the pinch stimulus, the activity of EPSCs and IPSCs recovered to the control level. For recordings of miniature EPSCs and IPSCs (mEPSCs and mIPSCs), tetrodotoxin (1 μM) (Wako, Osaka, Japan) was added in the Krebs solution and perfused on the surface of the spinal cord to inhibit all neuronal firing, which activates presynaptic terminals. Miniature EPSCs and IPSCs reflect the occasional neurotransmitter release of the presynaptic terminals connected to SG neurons.

The electrical signals were amplified and digitized on a personal computer using the same system described in the section of extracellular recordings. The EPSCs and IPSCs were recorded before and during the inhalation of sevoflurane or desflurane at doses ranging from 0.1 to 2 MAC. The currents were analyzed using Minianalysis software (Synaptosoft, Fort Lee, NJ, USA) to assess frequencies and amplitudes. The decay time and synaptic charge area of IPSCs were also analyzed via Minianalysis software.

Measurements of sensory thresholds for hot/cold pain

To assess the consistency between electrophysiological findings and actual behavioral findings, hot- and cold-pain thresholds were measured using the hot- and cold-plate tests in the absence and the presence of volatile anesthetics. Sensory thresholds for hot or cold pain were measured by using an NG 35150 hot/cold plate (Ugo Basile, Gemonio, Italy) which can provide hot or cold stimuli through the metal plate. The behavioral tests were performed in a soundproof room away from the colony room during daylight hours and at standard temperature (25 ± 1°C). Twenty-four male rats, aged 7 weeks, were included in this experiment, with 12 rats each in the sevoflurane group and the desflurane group. For administration of volatile anesthetics, the hot/cold plate was placed in a sealed cylindrical chamber (20 cm in diameter, 25 cm height). The chamber was equipped with a port which enabled the exchange of the inner air with anesthetic-containing gas. Before initiating experiments, rats placed on the metal plate were allowed to acclimatize

to the environment for 10 min. Sevoflurane or desflurane (doses corresponding to 0.1, 0.2, and 0.5 MAC) mixed with oxygen was delivered in a stepwise manner into this sealed chamber. The concentrations of volatile anesthetics inside the chamber were monitored from a drainage tube inserted in the chamber and maintained a target concentration for at least 10 min. Higher concentrations than 0.5 MAC were not tested because the hot/cold stimuli can damage the paw with deep sedation. The sedation rating scale developed by Chuck et al.[31] were evaluated at the same time before measuring the thermal thresholds. The sedation rating scales were as follows: 5 denoted awake, active: engaged in locomotion, rearing, head movements, or grooming; 4 denoted awake, inactive: eyes fully open, head up, little to no locomotion, rearing or grooming, and normal posture; 3 denoted mild sedation: eyes partly closed, head somewhat down, and impaired locomotion including abnormal posture, use of only some limbs, dragging, and stumbling; 2 denoted moderate sedation: head mostly or completely down, eyes partly closed, flattened posture, and no spontaneous movement; 1 denoted heavy sedation: eyes mostly closed and loss of righting reflex; 0 denoted asleep: eyes fully closed, body relaxed, and asleep. Thermal thresholds to hot/cold pain were measured using the ramp mode in which the plate temperature was increased or decreased constantly within a fixed interval with reference to Hargreaves' assay.[32] The stimuli were promptly halted upon detection of nociceptive responses (licking, paw flinching, paw lifting, biting, and jumping), and the threshold temperatures were recorded. The ramp settings were as follows: baseline 36°C, target 52°C, ramp time 300 s for hot stimuli and baseline 24°C, target 8°C, ramp time 320 s for cold stimuli.

Drug administration

For administration of volatile anesthetics, sevoflurane (Maruishi Pharmaceutical Co., Osaka, Japan) was vaporized using a Sevoflurane Vapor 19.2 device (Drägerwerk AG, Lübeck, Germany), and desflurane (Baxter, Deerfield, IL, USA) was delivered using a Tec 6 plus apparatus (Datex-Ohmeda, Steeton, West Yorkshire, UK). Concentrations of 1 MAC of sevoflurane and desflurane for rats were defined as 2.7% and 5.7%, respectively, according to the literature.[33,34] In electrophysiological experiments, sevoflurane or desflurane mixed with oxygen (1 L/min) was administered by inhalation via a tracheostomy tube during mechanical ventilation, and the doses corresponding to 0.1, 0.2, 0.5, 1, and 2 MAC were delivered in a stepwise manner. The inhalation of each target dose was maintained for at least 5 min to confirm stable end-tidal anesthetic concentrations and stable neural signals before electrophysiological recordings. End-tidal anesthetic concentrations were monitored from a port close to the tracheostomy tube. Recovery of the neural response was assessed 10 min after discontinuation of drug administration (expressed as "washout" in each figure). In behavioral tests, volatile anesthetics mixed with oxygen 3 L/min were delivered at doses corresponding to 0.1, 0.2, and 0.5 MAC in a stepwise manner into the cylindrical chamber for at least more than 10 min in each concentration. The end-tidal or intrachamber anesthetic concentration was continuously monitored using a Capnomac Ultima ULT-1-27-07 Anesthesia Monitor (Datex, Helsinki, Finland).

Statistical analysis

All numerical data are expressed as the mean ± standard deviation. According to the power analysis, six cells were required for each concentration (alpha error = 0.05; beta error = 0.2). The variation of each parameter (frequency of APs, frequency, and amplitude of EPSCs and IPSCs, decay time, and synaptic charge area of IPSCs) were assessed using the Wilcoxon signed-rank test for absolute values. The Kruskal–Wallis test with Bonferroni's post hoc test was used in the behavioral tests. Statistical significance was determined at $P < 0.05$ in all cases.

Results

Effects of inhaled sevoflurane and desflurane on APs during pinch stimulation in dorsal horn neurons

Stable and reproducible recordings of APs were obtained from spinal dorsal horn ($n = 12$ for sevoflurane; $n = 11$ for desflurane) of 10 rats. The signal of APs was obtained at a depth of 137 ± 87.7 μm from the surface of the spinal cord.

Figure 1(a) and (b) shows the representative traces of APs during pinch stimulation recorded successively for each volatile anesthetic. Sevoflurane inhalation at a subanesthetic dose (0.2 MAC) slightly inhibited APs, but an anesthetic dose (2 MAC) strongly inhibited APs (Figure 1 (a)). Conversely, desflurane at a subanesthetic dose (0.2 MAC) enhanced APs, but an anesthetic dose (2 MAC) inhibited APs (Figure 1(b)). Sevoflurane significantly decreased the frequency of APs in a dose-dependent manner (Figure 1(a) and (c)). Sevoflurane at 1 and 2 MAC showed strong suppression of APs (25.1 ± 12.4% of control, $n = 12$, $P < 0.01$; 18.5 ± 12.3% of control, $n = 12$, $P < 0.01$). Conversely, desflurane at 0.1 and 0.2 MAC enhanced APs (151.0 ± 57.3% of control, $n = 11$, $P < 0.01$; 127.3 ± 33.8% of control, $n = 11$, $P = 0.042$) and inhibited at 1 and 2 MAC (76.1 ± 29.6% of control $n = 11$, $P = 0.03$; 48.7 ± 30.2% of control, $n = 11$,

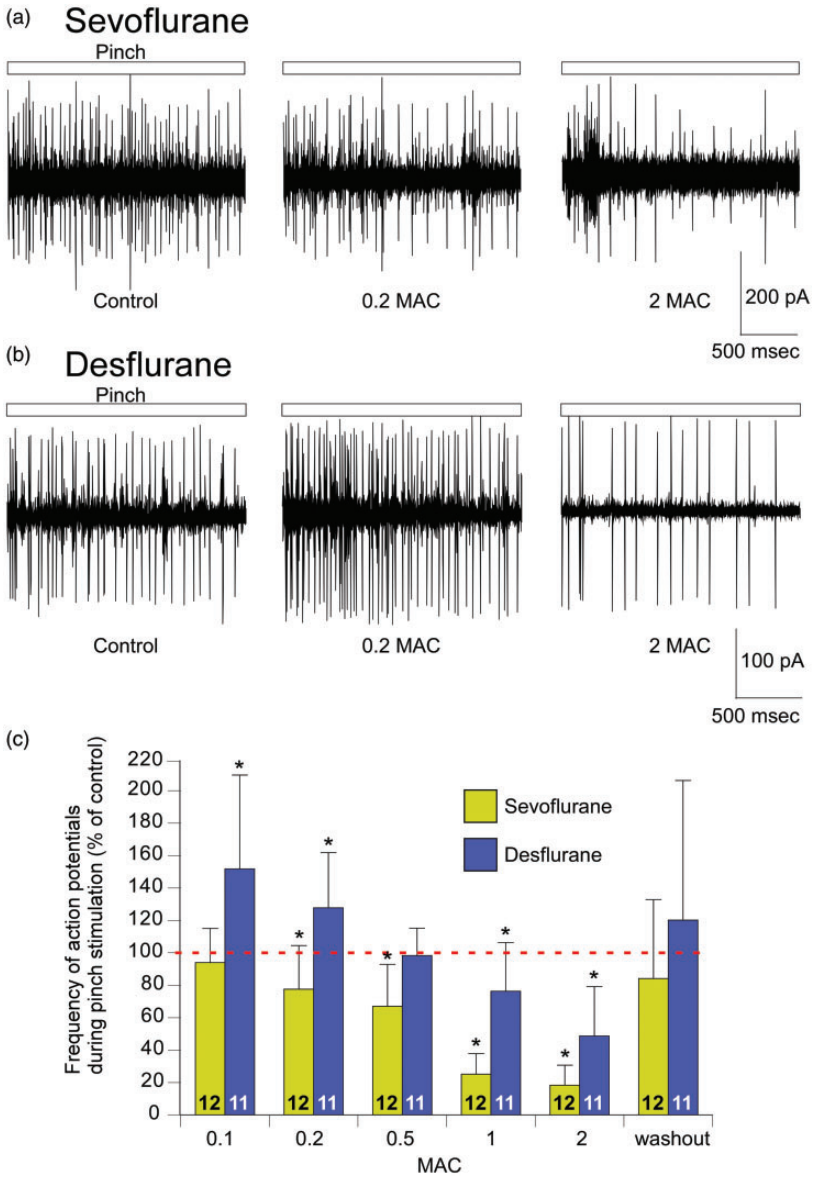

Figure 1. Effects of volatile anesthetics on action potentials (APs) during pinch stimulation from dorsal horn neurons. (a) The activity of APs slightly decreased under inhalation of sevoflurane at 0.2 MAC and remarkably decreased at 2 MAC. (b) The activity of APs increased under inhalation of desflurane at 0.2 MAC and decreased at 2 MAC. (c) A bar graph summarizing the effects of sevoflurane and desflurane on APs during pinch stimulation. The number in each bar indicates the studied cells. Responses 10 min after discontinuation of inhaled anesthetics were presented as "washout." Sevoflurane dose dependently suppressed the frequency of APs, whereas desflurane inhalation enhanced the frequency of APs at $\leq$0.2 MAC and decreased them at $\geq$1 MAC. The asterisks indicate a significant difference from the control (*$P < 0.05$, **$P < 0.01$). MAC: minimum alveolar concentration.

$P < 0.01$). Consequently, desflurane inhalation exhibited biphasic modulation of nociceptive AP responses (Figure 1(b) and (c)). The frequency of APs recovered to a value which was not significantly different from control values 10 min after discontinuation of sevoflurane inhalation ($83.7 \pm 48.2\%$ of control, $n = 12$, $P = 0.37$) and desflurane inhalation ($119.7 \pm 85.8\%$ of control, $n = 11$, $P = 0.52$) (Figure 1(c)).

In vivo whole-cell current recordings from SG neurons

Spontaneous EPSCs/IPSCs (sEPSCs/sIPSC), EPSCs/IPSCs during pinch stimulation, and miniature EPSCs/IPSCs (mEPSCs/mIPSC) were analyzed in the absence and the presence of volatile anesthetics. Stable whole-cell patch-clamp recordings were performed in 119 SG neurons from 81 rats in vivo. All recorded neurons had resting membrane potentials more negative than -50 mV.

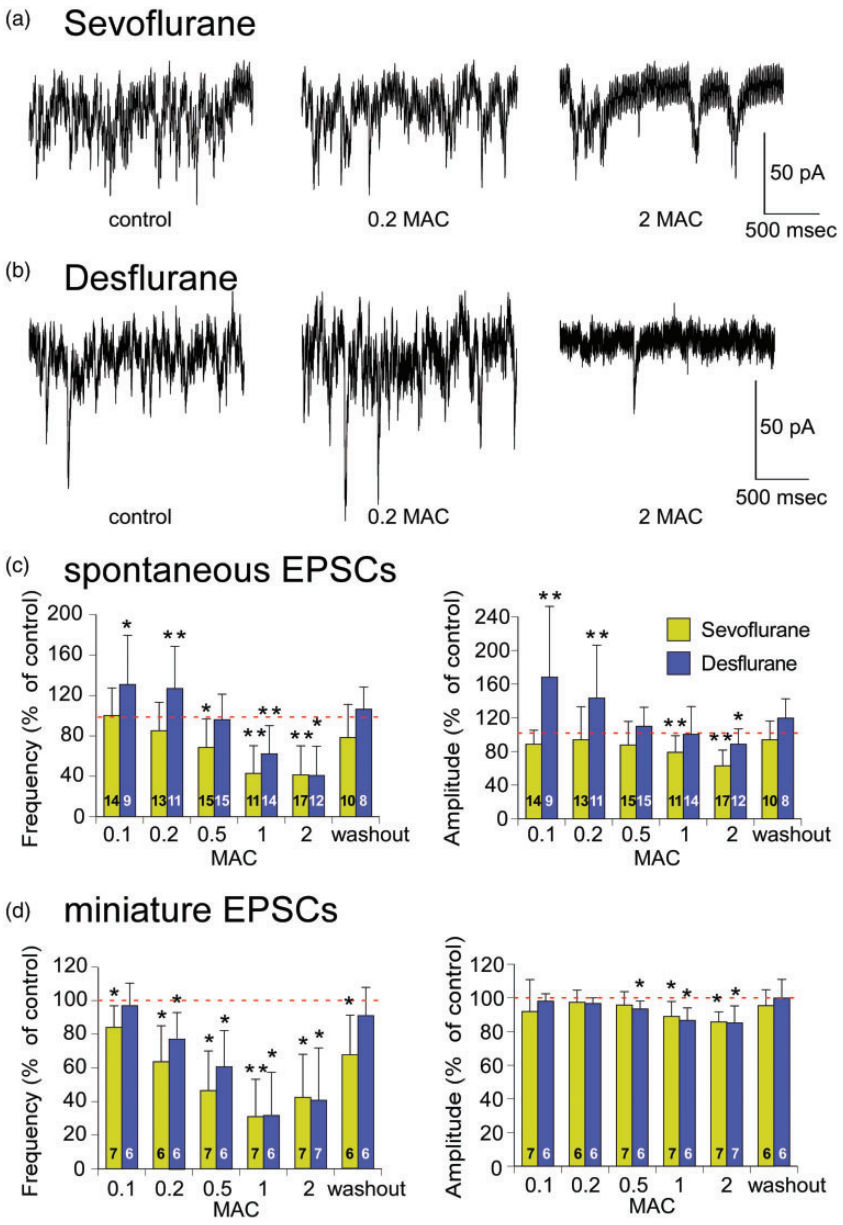

Figure 2. Effects of volatile anesthetics on spontaneous and miniature EPSCs (sEPSCs and mEPSCs) in SG neurons. (a) The activity of sEPSCs (inward currents) slightly decreased under inhalation of sevoflurane at 0.2 MAC and remarkably decreased at 2 MAC. (b) The activity of sEPSCs increased under inhalation of desflurane at 0.2 MAC and decreased at 2 MAC. (c) The bar graphs summarize the effects of sevoflurane and desflurane on frequency (left) and amplitude (right) of sEPSCs. The number in each bar indicates the studied cells. Sevoflurane and desflurane reduced the frequency and the amplitude at anesthetic doses ($\geq$1 MAC). Subanesthetic doses of desflurane (0.1 and 0.2 MAC) enhanced both frequency and amplitude. (d) The bar graphs summarize the effects of sevoflurane and desflurane on frequencies (left) and amplitudes (right) of mEPSCs. Both anesthetics significantly decreased only the frequency of mEPSCs. mEPSCs were not enhanced by subanesthetic doses of desflurane. The asterisks indicate a significant difference from the control treatment (*$P < 0.05$, **$P < 0.01$). EPSC: excitatory postsynaptic current; MAC: minimum alveolar concentration.

Effects of inhaled sevoflurane and desflurane on sEPSCs and mEPSCs in SG neurons

Sixty-seven neurons from 45 rats were included in this protocol. Figure 2(a) and (b) shows sEPSCs recorded successively for each volatile anesthetic. Sevoflurane inhalation of a subanesthetic dose (0.2 MAC) hardly affected sEPSCs, but an anesthetic dose (2 MAC) strongly inhibited sEPSCs (Figure 2(a)). Conversely, a subanesthetic dose of desflurane enhanced sEPSCs, but an anesthetic dose inhibited sEPSCs (Figure 2(b)). At anesthetic doses, sevoflurane (0.5, 1, and 2 MAC) and desflurane (1 and 2 MAC) significantly decreased the frequency and the amplitude of sEPSCs in a dose-

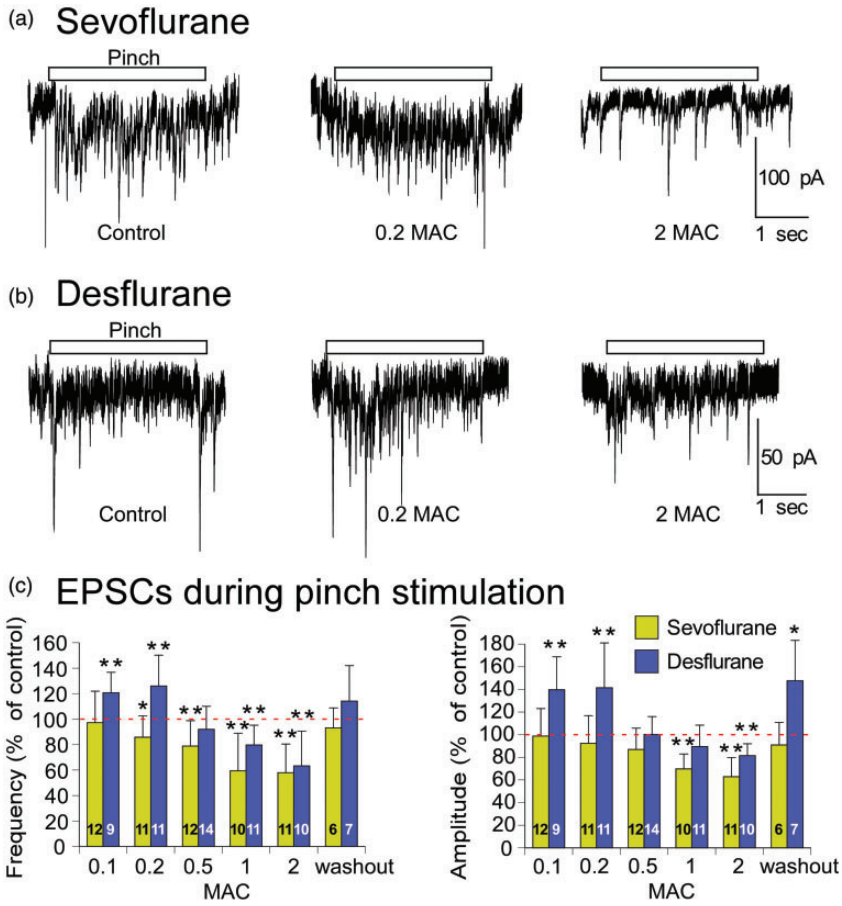

Figure 3. Effects of volatile anesthetics on EPSCs during pinch stimulation in SG neurons. (a) The activity of EPSCs slightly decreased under inhalation of sevoflurane at 0.2 MAC and remarkably decreased at 2 MAC. (b) The activity of EPSCs increased following inhalation of desflurane at 0.2 MAC and decreased at 2 MAC. (c) The bar graphs summarize the effects of sevoflurane and desflurane on the frequencies (left) and amplitudes (right) of EPSCs during pinch stimulation. The number in each bar indicates the studied cells. Sevoflurane dose dependently reduced the frequency and amplitude at anesthetic doses ($\geq$0.5 MAC). Subanesthetic doses of desflurane (0.1 and 0.2 MAC) enhanced both frequency and amplitude, but anesthetic doses of desflurane ($\geq$ 1 MAC) reversed this effect to reduce both frequency and amplitude. The asterisks indicate a significant difference (*$P < 0.05$, **$P < 0.01$). EPSC: excitatory postsynaptic current; MAC: minimum alveolar concentration.

dependent manner (Figure 2(c)). The inhibitory effects on the frequency and the amplitude as assessed by the percentage of control recordings were $41.4 \pm 28.8\%$ ($P < 0.01$) and $62.9 \pm 18.7\%$ ($P < 0.01$) for 2 MAC sevoflurane ($n = 17$), $40.8 \pm 28.9\%$ ($P = 0.03$) and $88.6 \pm 18.2\%$ ($P = 0.02$) for 2 MAC desflurane ($n = 12$) (Figure 2(c)). At 1 and 2 MAC, the frequencies were more prominently inhibited than the amplitudes by both anesthetics. At subanesthetic doses (0.1 and 0.2 MAC), desflurane but not sevoflurane significantly increased the frequency and the amplitude of sEPSCs. The frequency and the amplitude were enhanced to $130.9 \pm 48.8\%$ of control ($P < 0.01$) and $168.2 \pm 84.1\%$ of control ($P < 0.01$) at 0.1 MAC ($n = 9$), and to $126.9 \pm 41.7\%$ ($P < 0.01$) and $143.3 \pm 63.0\%$ ($P < 0.01$) of control at 0.2 MAC ($n = 11$) (Figure 2(b) and (c)). Thus, desflurane exhibited biphasic modulation of sEPSCs, which is similar to the AP recordings. These effects of sevoflurane and desflurane on sEPSCs were largely consistent with those of APs.

The effects of both anesthetics on mEPSCs in the presence of spinal application of tetrodotoxin (1 μM) are summarized in Figure 2(d). Sevoflurane and desflurane strongly inhibited the frequency but only slightly suppressed the amplitude of mEPSCs. Unlike the sEPSCs, the mEPSCs were not enhanced by subanesthetic desflurane (0.1 and 0.2 MAC).

Effects of inhaled sevoflurane and desflurane on EPSCs during pinch stimulation in SG neurons

Thirty-nine neurons from 28 rats were included in this protocol. Figure 3(a) and (b) shows EPSCs during pinch stimulation recorded successively for each volatile anesthetic. Sevoflurane inhalation at a subanesthetic dose (0.2 MAC) hardly affected EPSCs, but that of an

anesthetic dose (2 MAC) strongly inhibited EPSCs (Figure 3(a)). Conversely, a subanesthetic dose of desflurane enhanced EPSCs, but an anesthetic dose inhibited EPSCs (Figure 3(b)). Sevoflurane (0.2, 0.5, 1, and 2 MAC) and desflurane (1 and 2 MAC) dose dependently decreased the frequency and amplitude of EPSCs during pinch stimulation (Figure 3(c)). The inhibitory effects on the frequency and the amplitude of EPSCs as assessed by the percentage of control recordings were $58.0 \pm 22.5\%$ ($P < 0.01$) and $62.9 \pm 16.9\%$ ($P < 0.01$) for 2 MAC sevoflurane ($n = 11$), $63.3 \pm 27.3\%$ ($P < 0.01$) and $81.5 \pm 10.5\%$ ($P < 0.01$) for 2 MAC desflurane ($n = 10$). Similar to the effects on spontaneous EPSCs, the frequencies were more prominently inhibited than the amplitudes by both anesthetics at 1 and 2 MAC doses (Figure 3(c)). At subanesthetic doses (0.1 and 0.2 MAC), desflurane but not sevoflurane significantly facilitated the frequency and the amplitude of EPSCs. The frequency and the amplitude were enhanced to $120.7 \pm 16.1\%$ of control ($P < 0.01$) and $139.7 \pm 29.1\%$ of control ($P < 0.01$) at 0.1 MAC ($n = 9$) and to $126.0 \pm 24.2\%$ of control ($P < 0.01$) and $141.5 \pm 39.6\%$ of control ($P < 0.01$) at 0.2 MAC ($n = 11$). As a result, EPSCs were also biphasically modulated by desflurane. The effects of sevoflurane and desflurane on EPSCs during pinch stimulation were consistent with those on APs and sEPSCs.

Effects of sevoflurane and desflurane on sIPSCs and mIPSCs in SG neurons

Fifty-one neurons from 36 rats were included in this protocol. Figure 4(a) and (b) shows sIPSCs recorded successively for each volatile anesthetic. Sevoflurane inhalation of a subanesthetic dose (0.2 MAC) hardly affected sIPSCs, but that of an anesthetic dose (2 MAC) strongly inhibited sIPSCs (Figure 4(a)). On the other hand, desflurane at subanesthetic dose enhanced sIPSCs, but that at an anesthetic dose inhibited sIPSCs (Figure 4(b)). Sevoflurane (1 and 2 MAC) and desflurane (2 MAC) significantly decreased the frequency and amplitude of sIPSCs (Figure 4(c)). The inhibitory effects on the frequency and the amplitude as assessed by the percentage of control were $75.5 \pm 25.2\%$ ($P = 0.02$) and $62.7 \pm 21.5\%$ ($P < 0.01$) for 2 MAC sevoflurane ($n = 12$), and were $61.4 \pm 23.1\%$ ($P < 0.01$) and $78.0 \pm 14.2\%$ ($P = 0.02$) for 2 MAC desflurane ($n = 12$). Similar to the effects on EPSCs, the frequency and amplitude of sIPSCs were augmented by subanesthetic desflurane (0.1 and 0.2 MAC) but were inhibited at anesthetic doses (1, 2 MAC). Both anesthetics decreased the frequency of mIPSCs in a dose-dependent manner but had no significant effects on the amplitudes of mIPSCs (Figure 4(b)).

Effects of sevoflurane and desflurane on IPSCs during pinch stimulation in SG neurons

Twenty-eight neurons from 19 rats were included in this protocol. Figure 5(a) and (b) shows IPSCs during pinch stimulation recorded successively for each volatile anesthetic. Inhalation of a subanesthetic dose of sevoflurane (0.2 MAC) only mildly affected IPSCs, but that of an anesthetic dose (2 MAC) strongly inhibited IPSCs (Figure 5(a)). Similarly, inhalation of a subanesthetic dose of desflurane did not affect IPSCs, but an anesthetic dose inhibited IPSCs (Figure 5(b)). Sevoflurane (0.5, 1, and 2 MAC) and desflurane (1 and 2 MAC) significantly decreased the frequency and amplitude of IPSCs during pinch stimulation (Figure 5(c)). The inhibitory effects on the frequency and the amplitude as assessed by the percentage of control were $70.3 \pm 30.6\%$ ($P < 0.01$) and $56.0 \pm 26.4\%$ ($P < 0.01$) for 2 MAC sevoflurane ($n = 9$) and were $56.9 \pm 36.3\%$ ($P < 0.01$) and $62.4 \pm 28.1\%$ ($P < 0.01$) for 2 MAC desflurane ($n = 11$). Biphasic modulation by desflurane inhalation was not observed.

We also analyzed the decay time and integrated area of IPSCs during pinch stimulation, since volatile anesthetics can potentiate $GABA_A$ receptor activity by increased charge transfer.[10] In our in vivo recording, however, both anesthetics did not affect the decay phase durations of IPSCs at any doses, as shown in Figure 6 traces (numerical data are not shown).

Analgesic and sedative effects of inhaled anesthetics: sevoflurane and desflurane on thermal stimulation

Both anesthetics dose dependently decreased the sedation score (Figure 7(a)). The median sedation scores were as follows: 5 at 0 MAC, 3 at 0.1 MAC, 2 at 0.2 MAC, and 1 at 0.5 MAC for both anesthetics (Figure 7 (a)). Mean hot and cold thresholds in controls were as follows: $42.2 \pm 1.2°C$ and $11.3 \pm 1.3°C$ for the sevoflurane group ($n = 12$) and $43.6 \pm 0.8°C$ and $11.4 \pm 1.2°C$ for the desflurane group ($n = 12$). Sevoflurane dose dependently increased the hot thresholds and decreased the cold thresholds ($44.3 \pm 1.4°C$ and $9.3 \pm 0.8°C$, respectively, with 0.5 MAC, $P < 0.01$). Conversely, inhaled desflurane significantly decreased the hot threshold at 0.1 and 0.2 MAC ($41.3 \pm 1.1°C$, $41.3 \pm 1.2°C$, respectively, $P < 0.01$) and increased the cold threshold at 0.1 MAC ($14.3 \pm 1.7°C$, $P < 0.01$). Desflurane at 0.5 MAC increased the hot threshold and the cold threshold, which were comparable to the control values (hot: $44.5 \pm 1.6°C$, $P = 0.32$, cold: $10.9 \pm 3.3°C$, $P = 1$).

Discussion

This study investigated how inhaled sevoflurane or desflurane modulates the nociceptive synaptic transmission

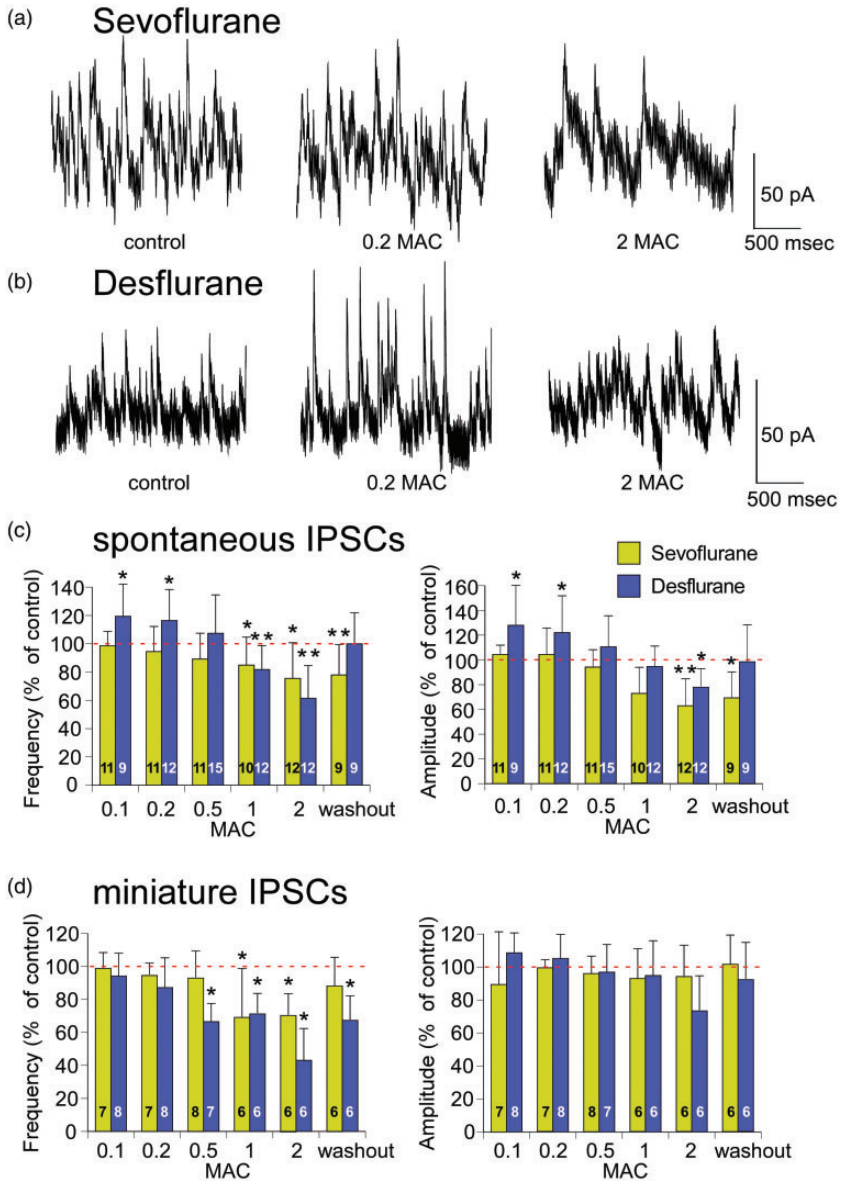

Figure 4. Effects of volatile anesthetics on spontaneous and miniature IPSCs (sIPSCs and mIPSCs) in SG neurons. (a) The activity of sIPSCs (outward currents) was not changed under inhalation of sevoflurane at 0.2 MAC and decreased at 2 MAC. (b) The activity of sIPSCs increased under inhalation of desflurane at 0.2 MAC and decreased at 2 MAC. (c) The bar graphs summarize the effects of sevoflurane and desflurane on the frequencies (left) and amplitudes (right) of sIPSCs. The number in each bar indicates the studied cells. Sevoflurane and desflurane reduced the frequency and the amplitude at anesthetic doses (1 and 2 MAC). Subanesthetic doses of desflurane (0.1 and 0.2 MAC) enhanced both the frequencies and amplitudes of sIPSCs. (d) The bar graphs summarize the effects of sevoflurane and desflurane on the frequencies (left) and amplitudes (right) of mIPSCs. Both anesthetics significantly decreased only the frequency of mIPSCs at anesthetic doses (1 and 2 MAC). Subanesthetic doses of desflurane did not enhance mIPSCs. The asterisks indicate a significant difference from the control group (*$P < 0.05$, **$P < 0.01$). IPSC: inhibitory postsynaptic current; MAC: minimum alveolar concentration.

of SG neurons in the spinal cord by using in vivo extracellular and patch-clamp analyses. Sevoflurane suppressed the APs and EPSCs during pinch stimulation in a dose-dependent manner. On the other hand, desflurane potentiated APs and EPSCs during pinch stimulation at lower doses (subanesthetic doses: 0.1 or 0.2 MAC) and suppressed at higher doses (anesthetic doses: > 0.5 MAC), consequently resulting in biphasic modulation. In behavioral experiments in which volatile anesthetic doses were tested at up to 0.5 MAC, sevoflurane reduced hot- and cold-pain sensations in a dose-dependent manner and desflurane induced hyperalgesia at subanesthetic doses (0.1 and 0.2 MAC). Unexpectedly, IPSCs were not potentiated but rather were suppressed by volatile anesthetics at anesthetic doses. The suppressions of APs and EPSCs would prove to be a substantial

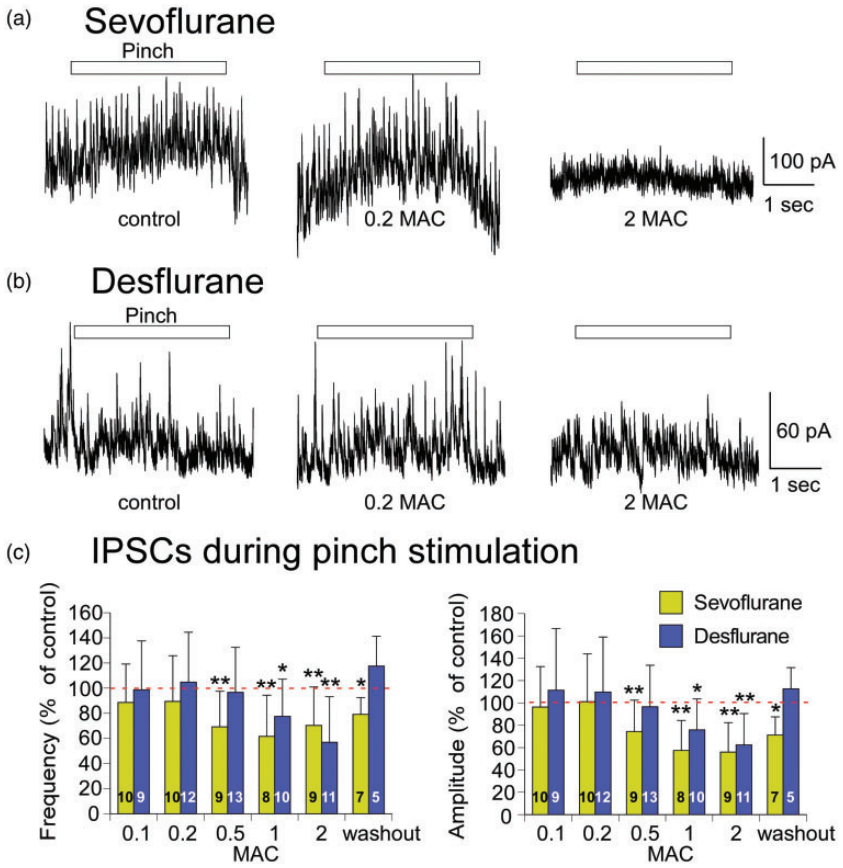

Figure 5. The effects of volatile anesthetics on IPSCs during pinch stimulation in SG neurons. (a) The activity of IPSCs was not changed following inhalation of sevoflurane at 0.2 MAC and remarkably decreased at 2 MAC. (b) The activity of IPSCs increased under inhalation of desflurane at 0.2 MAC and decreased at 2 MAC. (c) The bar graphs summarize the effects of sevoflurane and desflurane on the frequencies (left) and amplitudes (right) of IPSCs during pinch stimulation. The number in each bar indicates the studied cells. Sevoflurane reduced the frequencies and the amplitudes of IPSCs more than 0.5 MAC. Subanesthetic doses of desflurane (0.1 and 0.2 MAC) tended to increase IPSCs but were not significant. The asterisks indicate a significant difference (*$P < 0.05$, **$P < 0.01$). IPSC: inhibitory postsynaptic current; MAC: minimum alveolar concentration.

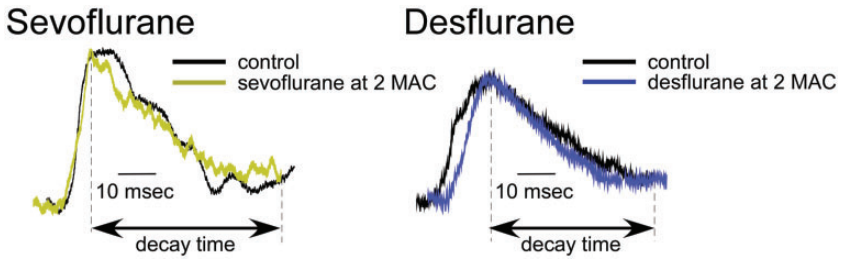

Figure 6. The peak amplitudes of single IPSC during pinch stimulation in the absence and the presence of each volatile anesthetic were normalized and superimposed. Sevoflurane (left) and desflurane (right) at 2 MAC had no significant effect on the decay time of IPSCs. MAC: minimum alveolar concentration.

antinociceptive property of the volatile anesthetics at anesthetic doses. The enhancement of APs and EPSCs by desflurane may indicate its potential hyperalgesic property at subanesthetic doses.

A previous study by Haseneder et al. examined the effects of volatile anesthetics on EPSCs in spinal SG neurons using patch-clamp analysis in spinal cord slices[11] in which isoflurane (equivalent to 1 MAC) reduced the amplitude of evoked EPSCs stimulated by the dorsal root ganglion and the frequency of sEPSCs and mEPSCs in SG neurons. That study indicated that the suppression of excitatory synaptic neurotransmission

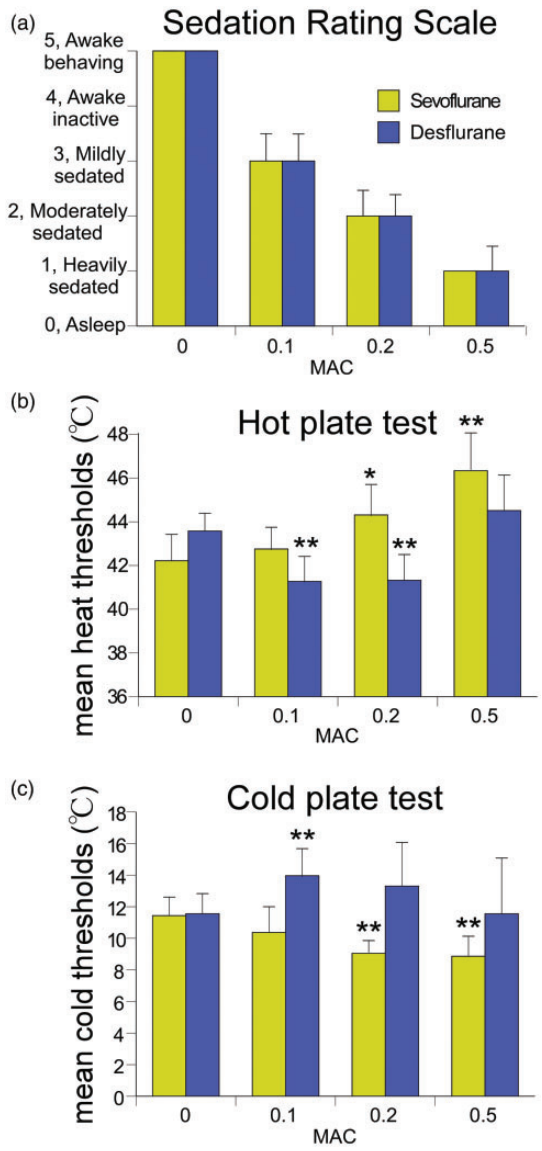

Figure 7. The effects of sevoflurane and desflurane on the sedation and pain thresholds for thermal stimuli. (a) Sevoflurane and desflurane exerted dose-dependent sedative effects (n = 12 for each anesthetic group). The sedation rating scores of the sevoflurane group and the desflurane group were the same at equivalent doses. Hot-plate (b) and cold-plate (c) tests were performed under inhalation of sevoflurane and desflurane at 0 (control), 0.1, 0.2, and 0.5 MAC (n = 12 for each anesthetic group). Sevoflurane dose dependently increased the heat threshold and decreased the cold threshold. Meanwhile, desflurane decreased the heat threshold and increased the cold threshold at subanesthetic doses (0.1 and 0.2 MAC). Thermal thresholds under inhalation of 0.5 MAC were similar to the control group. The asterisks indicate a significant difference from the control (*$P < 0.05$, **$P < 0.01$). MAC: minimum alveolar concentration.

by volatile anesthetics could be induced by a reduction in excitatory neurotransmitter release and contribute to antinociception.[11] Comparable results were obtained in our in vivo preparations; sevoflurane and desflurane at anesthetic doses decreased the frequency more prominently than that of the amplitude of EPSCs. Presynaptic inhibition, rather than postsynaptic inhibition, was also observed in mEPSC recordings. These findings may suggest that both anesthetics at anesthetic doses suppressed the excitatory neurotransmission predominantly by inhibiting the presynaptic glutamate release of primary afferent nerves rather than inhibition of postsynaptic receptor activity in SG neurons. Some previous studies indicated that general anesthetics might dose dependently decrease the probability of neurotransmitter release at the neuromuscular junction and spinal cord via suppression of SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor) machinery.[35–38] Herring et al. proved that general anesthetics affected syntaxin1A, which is distributed in the CNS and impaired SNARE-mediated presynaptic neurotransmitter release.[35] Our study may corroborate this effect of general anesthesia on this presynaptic release machinery. Furthermore, the suppression of EPSCs by sevoflurane was more than that by desflurane at anesthetic doses, thus suggesting that sevoflurane could produce stronger analgesia than desflurane at equivalent doses.

Several previous studies have described the hyperalgesic properties of inhaled anesthetics such as isoflurane and desflurane at low doses.[12–15] In our study, at subanesthetic doses (0.1 and 0.2 MAC), desflurane inhalation, but not sevoflurane, induced hyperalgesic responses in electrophysiological and behavioral experiments. Desflurane at concentrations of 0.1 and 0.2 MAC enhanced APs and EPSCs during pinch stimulation; however, desflurane did not affect mEPSCs (see Figures 2 and 3). These results may suggest that subanesthetic doses of desflurane increased nociceptive inputs, facilitating primary afferent nerve conduction or peripheral nociceptor activation. Most previous studies indicate that the hyperalgesic properties of desflurane and isoflurane could be due to their irritant properties.[5,12–15] Several mechanisms have been proposed in the literature such as enhancement of excitatory neurotransmission by peripheral neuroinflammation,[13,39,40] or suppression of presynaptic nicotinic acetylcholine receptors in the descending inhibitory pathway.[15,39,40] Our study showed that desflurane at subanesthetic doses did not affect IPSCs of SG neurons during pinch stimulation, which indicated that its hyperalgesic property was not caused by the suppression of descending inhibition. The neuroinflammation produced by activation of transient receptor potential A1 (TRPA1) and V1 (TRPV1) channels on primary afferent fibers or peripheral nociceptors was thought to be involved in part in hyperalgesia induced by low doses of volatile irritant anesthetics such as isoflurane and desflurane.[13,41,42] The TRPA1 channel, a nonselective cation channel, is

known to be activated by cold pain (≤17°C), mustard oil, wasabi, and several volatile irritants including garlic.[43–45] The TRPV1 channel responds to hot pain (≥43°C), chemical irritants including capsaicin, protons, salt, and ethanol.[46] In our behavioral experiments, hyperalgesic responses to both cold and hot painful stimuli were observed during the inhalation of subanesthetic doses of desflurane. Further study will be needed to elucidate the involvement of TRPA1 and TRPV1 channels in the hyperalgesic properties of low-dose desflurane. It is possible that the neuronal response to thermal and mechanical stimulation are not exactly same. However, previous study showed that the APs were almost equally elicited by both thermal and mechanical stimuli in extracellular recordings from rat dorsal horn.[9] Then, we consider that our results of thermal behavioral tests almost correctly estimate the pain sensation.

GABAergic or glycinergic inhibitory systems in the CNS play an important roles in the actions of general anesthetics. The nociceptive pathways in the spinal cord are modulated by several inhibitory systems such as GABAergic interneurons and descending inhibitory pathways. The actions of volatile anesthetics on inhibitory systems may contribute to antinociception, immobility, and CNS depression. However, involvement of the GABAergic or glycinergic inhibitory system in antinociception induced by volatile anesthetics inhalation is still conflicting. Some studies indicate that $GABA_A$ receptors are not involved in the immobilizing effects of volatile anesthetics.[47,48] By contrast, Yamauchi et al. demonstrated that depression of spinal wide dynamic range neuronal responses by halothane (1.1%, 1 MAC for rats) was mediated in part via the $GABA_A$ and glycine systems in spinally transected rats.[9] In rat spinal cord slice preparations, isoflurane (0.37 mM, 1 MAC for rats) produced prolongation of the decay time constants of GABAergic currents, increase in the integrated area of monosynaptic GABAergic currents, and inhibition of dorsal root-evoked polysynaptic EPSCs, while dorsal root-evoked monosynaptic EPSCs, miniature EPSCs, and N-methyl-d-aspartate (NMDA) currents were unaffected.[10] In our in vivo experiments, the IPSCs were suppressed by both anesthetics at anesthetic doses, which might be attributed in part to the suppression of descending inhibitory systems. We could not find significant changes in decay time and synaptic charge area of IPSCs at any doses of both anesthetics. Our findings indicated that GABAergic or glycinergic inhibitory systems in SG neurons might not be involved in the antinociception induced by inhaled anesthetics under clinically relevant in vivo conditions. Similar to mEPSCs, both anesthetics suppressed the frequency but not the amplitude of mIPSCs at anesthetic doses, which probably indicated that presynaptic inhibition of neurotransmitter release was also involved in IPSC suppression.

As a limitation, the background anesthesia with urethane may modulate the nociceptive transmission in spinal SG neurons. Urethane is widely used in animal experiments as a useful anesthetic that can produce stable anesthesia. Previous studies have shown that urethane did not directly alter excitatory glutamate-mediated or inhibitory $GABA_A$-mediated synaptic transmission.[49–51] In patch-clamp experiments, urethane (14 μM) had little effect on EPSCs in spinal cord slices.[52] We could not completely exclude the modulation of urethane; however, stable anesthesia allowed us to evaluate the effects of volatile anesthetics on nociceptive synaptic transmission in spinal SG neuron under clinically relevant conditions.

In clinical situations, volatile anesthetics are commonly used in combination with analgesics such as opioids to produce general anesthesia. In the past, it was difficult to estimate the antinociceptive effect of volatile anesthetics as distinguished from their immobilization effect. However, our observation, together with the previous findings,[9–12] indicate that the substantial antinociceptive property of inhaled anesthetics. The combination of volatile anesthetics and opioids produces synergistic analgesia during general anesthesia. We further found a potentially nociceptive property of desflurane at subanesthetic doses. During emergence from general anesthesia, a blood desflurane concentration equivalent to 0.1 MAC may be maintained for more than 1 h even after restoring consciousness.[53,54] It should be noted that hyperalgesia can occur in early postoperative periods after desflurane anesthesia. Adequate treatments, including transitional analgesia, and multimodal analgesia, should be considered in such a case.

In conclusion, by using in vivo patch-clamp recordings, our study revealed that sevoflurane and desflurane differentially modulated the responses of SG neurons to noxious stimuli in the spinal dorsal horn. Sevoflurane dose dependently exhibited antinociceptive effects. Conversely, desflurane elicited biphasic effects: hyperalgesia at subanesthetic doses and analgesia at anesthetic doses. The suppression of EPSCs by sevoflurane and desflurane at anesthetic doses could contribute to antinociception and result from the inhibition of presynaptic glutamate release of primary afferent nerves. The enhancement of EPSCs by desflurane at subanesthetic doses implies that its hyperalgesic properties could be produced by the facilitation of primary afferent nerve activity.

Authors' Contributions

YI performed the experiments and analysis and wrote the manuscript. YF conceived and designed this study. HY supervised the design. TM revised the manuscript for important

intellectual content. KN revised and gave final approval of the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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