Anesthesiology 2008; 108:447–56

Amnestic Concentrations of Sevoflurane Inhibit Synaptic Plasticity of Hippocampal CA1 Neurons through γ-Aminobutyric Acid–mediated Mechanisms

Junko Ishizeki, M.D., Koichi Nishikawa, M.D., Ph.D.,† Kazuhiro Kubo, M.D.,‡ Shigeru Saito, M.D., Ph.D.,§ Fumio Goto, M.D., Ph.D.‖*

Background: **The cellular mechanisms of anesthetic-induced amnesia are still poorly understood. The current study examined sevoflurane at various concentrations in the CA1 region of rat hippocampal slices for effects on excitatory synaptic transmission and on long-term potentiation (LTP), as a possible mechanism contributing to anesthetic-induced loss of recall.**

Methods: **Population spikes and field excitatory postsynaptic potentials were recorded using extracellular electrodes after electrical stimulation of Schaffer-collateral-commissural fiber inputs. Paired pulse facilitation was used as a measure of presynaptic effects of the anesthetic. LTP was induced using tetanic stimulation (100 Hz, 1 s). Sevoflurane at concentrations from amnestic (0.04 mM) to clinical concentrations (0.23–0.41 mM) were added to the perfusion solution.**

Results: **In the presence of 0.04 mM sevoflurane, the amplitude of population spikes was significantly depressed, and tetanic stimulation induced only posttetanic potentiation and then failure of LTP. These inhibitory effects were antagonized by bicuculline (10 μM), a γ-aminobutyric acid type A receptor antagonist. Sevoflurane at 0.23–0.41 mM further depressed the amplitude of field excitatory postsynaptic potentials in a dose-dependent manner and completely blocked LTP. Bicuculline only partially antagonized 0.41 mM sevoflurane–induced profound inhibition of LTP. Sevoflurane at 0.23–0.41 mM, but not at 0.04 mM, significantly increased paired pulse facilitation, suggesting that sevoflurane has presynaptic actions to reduce glutamate release from nerve terminals.**

Conclusions: **The current study provides evidence that amnestic concentrations of sevoflurane inhibit LTP of hippocampal CA1 neurons through γ-aminobutyric acid–mediated mechanisms, and these actions seem to account for the effects of amnestic sevoflurane on synaptic plasticity.**

MECHANISMS of anesthetic-induced amnesia are of great clinical importance because failure of intraoperative amnesia results in cases of recall. In clinical settings, most general anesthetic agents, even at low doses, are thought to prevent conscious recall of intraoperative events. For example, some patients who are woken intraoperatively for surgical procedures do not have recollection of actually being awake despite being awake and cooperative during the procedure.[1] Galinkin *et al.*[2] compared subjective, psychomotor, cognitive, and analgesic effects of sevoflurane (0.3% and 0.6%) with those of nitrous oxide at equal minimum alveolar concentrations (MACs) in healthy volunteers. They found that sevoflurane produced a greater degree of amnesia and psychomotor impairment than did an equal MAC of nitrous oxide but had no analgesic actions. However, similar doses of sevoflurane (0.2–0.7%) do not affect the Bispectral Index in humans.[3] Animal studies[4,5] have demonstrated that a significant reduction in memory retention latency occurred with 0.3–0.4% sevoflurane. Together, available data suggest that sevoflurane (0.3–0.6%) may have amnestic actions and that higher doses of sevoflurane have both amnestic and sedative/hypnotic actions.

However, the cellular and circuit-level mechanisms of sevoflurane-induced amnesia are still poorly understood. The cellular mechanisms involved in memory formation are complex; long-lasting changes in the synaptic efficacy of signaling between neurons in the central nervous system are widely believed to be involved in memory consolidation and recall. For example, trains of high-frequency stimulation to the hippocampus induce an activity-dependent increase in the amplitude of excitatory postsynaptic potentials, lasting for at least a period of hours. This phenomenon is known as *long-term potentiation* (LTP) and is believed to represent a mechanism involved in information storage during learning and memory.[6,7] Because clinically relevant concentrations of volatile anesthetics seem to modify ligand-gated ion channels such as glutamate receptors[8] and γ-aminobutyric acid type A ($GABA_A$) receptors,[9–12] sevoflurane may affect the induction and the maintenance of LTP. Clarifying the effects of sevoflurane on LTP will be useful for assessing the mechanisms by which general anesthetics produce amnesia.

As a first step, we examined the effects of sevoflurane on excitatory synaptic transmission mediated by glutamate receptors in the CA1 area of rat hippocampal slices. Although halothane and isoflurane seemed to depress glutamate receptor–mediated excitatory synaptic transmission in a dose-dependent manner,[13–15] details of sevoflurane actions on excitatory synaptic transmission remain lacking. In particular, we focused on the influence of amnestic concentrations of sevoflurane, which produced amnestic actions but no hypnotic actions, on

* Assistant Professor, † Associate Professor, ‡ Research Fellowship for Young Scientists in Japan Society for the Promotion of Science, § Professor and Chair, ‖ Special Duty Professor.

Received from the Department of Anesthesiology, Gunma University Graduate School of Medicine, Maebashi City, Japan. Submitted for publication July 9, 2007. Accepted for publication November 2, 2007. Supported by Grants-in-Aid for Scientific Research Nos. 17390425 and 17659483 (to Dr. Nishikawa) and No. 187866 (to Dr. Kubo) from the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan; in part by Initiatives for Attractive Education in Graduate Schools (to Dr. Ishizeki) from the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan; and by a Grant-in-Aid (to Dr. Nishikawa) from the Japan Medical Association, Tokyo, Japan (Clinical Research 2005).

Address correspondence to Dr. Nishikawa: Department of Anesthesiology, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi City 371-8511, Japan. nishikaw@med.gunma-u.ac.jp.

population spikes (PSs) and field excitatory postsynaptic potentials (fEPSPs). Second, we studied the effects of sevoflurane on the induction and maintenance of LTP as a possible mechanism contributing to anesthetic-induced loss of recall. Although clinically relevant concentrations of isoflurane and propofol have been shown to eliminate LTP[16,17]; little is known about the effects of sevoflurane on long-term changes of synaptic efficacy.

Materials and Methods

Brain Slice Preparation

As approved by the Institutional Animal Care and Use Committee (Gunma University Graduate School of Medicine, Maebashi City, Japan), Sprague-Dawley rats at 30–35 days old (mean body weight, 100 g) were decapitated during anesthesia, and the head was immediately immersed in cold (1–4°C) modified Ringer's solution, comprising 234 mM sucrose, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 10 mM $MgSO_4$, 0.5 mM $CaCl_2$, 26 mM $NaHCO_3$, and 11 mM glucose saturated with 95% O_2 and 5% CO_2. A block of tissue containing the hippocampus was dissected out quickly and glued to a DTK-1000 vibratome tray (Dosaka EM, Tokyo, Japan) using oxygenated cold modified Ringer's solution. Hippocampal slices (thickness, 500 μm) were cut from the brain and then kept in the prechamber (Brain Slice Chamber System; Harvard Apparatus, Holliston, MA) filled with artificial cerebrospinal fluid consisting of 125 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, and 11 mM glucose, bubbled with 95% O_2 and 5% CO_2 at room temperature (22°–24°C). Slices were allowed at least 1 h for recovery in the prechamber, which was designed to keep 8–12 slices viable for several hours.

Electrophysiologic Recordings

The methods of brain slice electrophysiology have been described previously.[15,18,19] Briefly, slices were transferred to a submerged recording chamber (1 ml in volume, Brain Slice Chamber System) and perfused at a rate of approximately 3 ml/min with oxygenated artificial cerebrospinal fluid. PSs and fEPSPs were evoked using bipolar tungsten stimulating electrodes (1–2 MΩ; World Precision Instruments, Sarasota, FL) placed in the stratum radiatum of CA1 regions to activate Schaffer-collateral-commissural fibers under a dissection microscope (SD30 and SZ-STU1 base unit; Olympus, Tokyo, Japan). Electrical stimuli comprised square wave paired pulses (duration, 0.35 ms; interpulse interval, 60 ms) delivered by a SEN 3301 stimulus generator (Nihon Kohden, Tokyo, Japan) connected through an SS-202 J stimulation isolation unit (Nihon Kohden) to electrodes every 15 s, to minimize frequency-dependent changes in synaptic transmission. Stimulus intensity was set to evoke 50–70% of the maximal amplitude of responses. In the LTP study, the intensity of stimulation used was 40–50% of maximum amplitude to avoid a ceiling effect. LTP was induced using a tetanic stimulation (100 Hz, 1 s). In all experiments, baseline recordings were monitored for at least 20 min before drug application or delivery of tetanic stimulation.

All fEPSPs and PSs were recorded using a glass microelectrode filled with artificial cerebrospinal fluid (GDC-1.5; OD, 1.5 mm; ID, 1.0 mm; Narishige, Tokyo, Japan). Recording electrodes were placed directly in line with stimulating electrodes in striatum radiatum using an MO-150 micromanipulator (Narishige), but separated from stimulating electrodes by 1.0–1.5 mm, or placed in stratum pyramidale to record PS field potentials. Signals were recorded using a DAM-80 AC differential amplifier (World Precision Instruments), amplified at least 1,000-fold, filtered at 2–5 kHz, and digitized at 10 kHz using a Digidata 1322A system (Axon Instruments, Union City, CA). All data were stored on a Pentium-based PC for later analysis. All experiments were performed at room temperature (21°–24°C).

Application of Sevoflurane and Concentration Measurement

Artificial cerebrospinal fluid solution at room temperature was bubbled with a carrier gas (95% O_2, 5% CO_2) passing through a calibrated commercial vaporizer (Sevotec 5; Ohmeda, BOC Health Care, West Yorkshire, United Kingdom) at the designated concentration and was applied to the recording chamber using a gravity-feed and vacuum system. High-quality polytetrafluoroethylene was used for tubing and valves to minimize loss of volatile anesthetic and drug binding. Sevoflurane (Maruishi Pharmaceutical, Osaka, Japan) concentrations used for this study were 0.5–5.0%. To determine the actual aqueous concentrations of sevoflurane in the submerged recording chamber for each concentration used, aliquots of the solution were taken from the recording chamber and filled into airtight glass containers for gas chromatographic measurements as described previously.[20] Figure 1 shows the final aqueous sevoflurane concentrations in our recording condition (n = 5, each). A strong positive correlation (r = 0.99971) was observed; for example, 0.5, 2.8, and 5.0% of sevoflurane were actually 0.04, 0.23, and 0.41 mM, respectively. Therefore, 0.04 mM was used as an amnestic dose, and 0.23–0.41 mM was used as clinically relevant concentrations. Drugs were purchased from Sigma-Aldrich Chemicals (Tokyo, Japan).

Data Analysis

Data acquisition and analysis were performed with pCLAMP version 8.1 software (Axon Instruments) and IGOR Pro version 5.0 software (WaveMetrics, Lake Oswego, OR). Amplitudes of PSs were measured from peak positive to peak negative voltages. Fiber volley ampli-

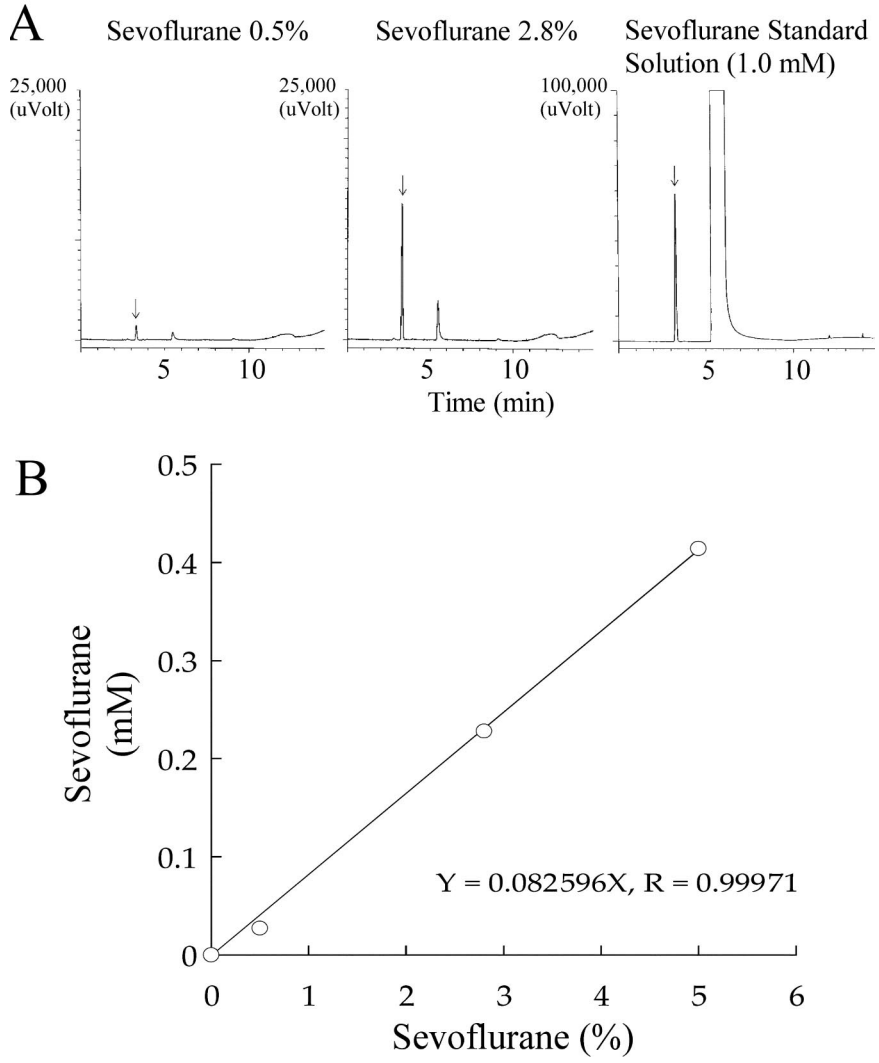

Fig. 1. The aqueous sevoflurane concentrations in the recording chamber. The aqueous sevoflurane concentrations at room temperature (22°–24°C) were measured using gas chromatography. Aliquots of the solution were directly taken from the recording chamber for gas chromatographic measurements. Sample traces in 0.5%, 2.8%, and standard solution (1 mM) are shown in *A*. The peak, which was indicated by an *arrow*, was observed 3 min after injection in each trace. The area under the curve was measured, and the aqueous sevoflurane concentration was calculated by comparing to that of sevoflurane standard solution (1.0 mM), in which 20 μl sevoflurane was dissolved in ethanol (100 ml). (*B*) A linear curve fit was applied. Data are expressed as mean ± SD (n = 5 each). In some cases, error bars are within the symbol.

tudes were measured from baseline to peak negative voltage. Rise times and decay times of fEPSPs were measured as time intervals between 10% and 90% of peak amplitude and as the decay time from peak amplitude to half amplitude, respectively. In PS and fEPSP recordings, responses were normalized as a percent of control, based on mean amplitude during a 10-min recording immediately before the perfusion of anesthetic. In the LTP study, after 10 min of control recordings, sevoflurane was administered for 20 min before providing an LTP-inducing stimulus, in the continued presence of the anesthetic.

Statistics

The statistical significance of data were determined using a Student *t* test or one-way analysis of variance with a Tukey *post hoc* test to compare the differences from three or more groups and to determine anesthetic effects with time-matched control responses. Data are expressed as mean ± SD. *P* values less than 0.05 were considered statistically significant.

Results

Amnestic Concentrations of Sevoflurane Depress Synaptically Evoked Discharge of CA1 Neurons in Part by Enhancing $GABA_A$ Receptor–medicated Inhibition

Sevoflurane (0.04–0.23 mM) depressed synaptically evoked discharge of CA1 neurons, measured as a block of PS responses (figs. 2A and B). The following study focused on two concentrations: amnestic dose (0.04 mM) and clinically relevant concentrations of sevoflurane (0.23 mM). Sevoflurane produced a dose-dependent depression of PS amplitude (57.3 ± 15.0% of control at 0.04 mM and 26.9 ± 6.19% of control at 0.23 mM, respectively; $P < 0.001$, n = 5 slices from individual rats, compared with preanesthetic control responses, using analysis of variance–Tukey). These effects were reversible on washout of the agent with drug-free artificial cerebrospinal fluid. PS responses completely disappeared when a higher dose (0.41 mM) of sevoflurane was applied (n = 5).

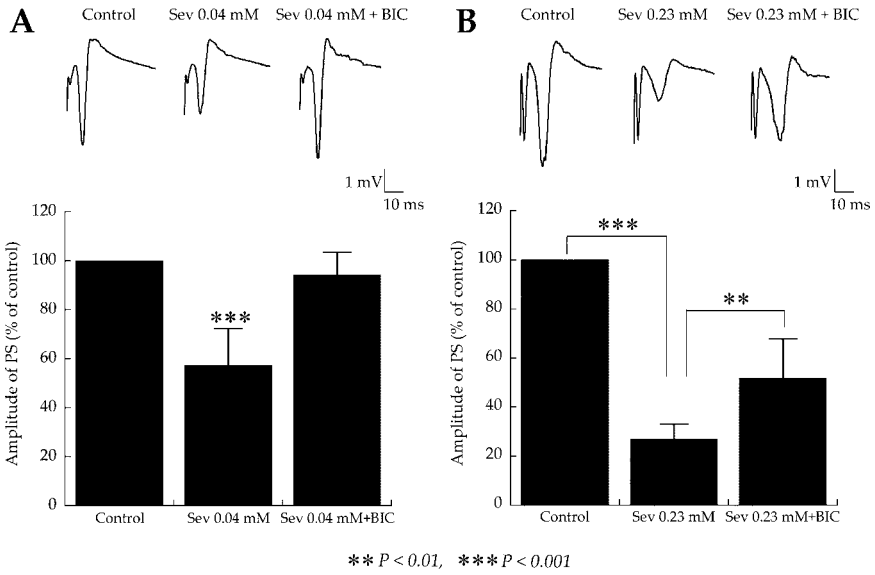

Fig. 2. The effects of sevoflurane on population spikes (PSs). (*A*) PS responses (*upper*) were recorded from CA1 neurons in response to electrical stimulation of Schaffer-collateral-commissural fibers. Bar graphs (*lower*) summarize the effect of sevoflurane (Sev) on PS amplitude. With 0.04 mM sevoflurane, PS amplitude was significantly depressed (n = 5, *** $P < 0.001$). However, when 10 μM bicuculline (BIC) was applied, PS amplitude recovered completely. (*B*) With 0.23 mM sevoflurane, PS amplitude was depressed more markedly than with 0.04 mM sevoflurane (n = 5, $P < 0.05$). PS amplitude recovered partially but significantly with 10 μM bicuculline ($P < 0.01$). Data are expressed as mean ± SD.**

To determine whether the effects of sevoflurane on PSs involve $GABA_A$ receptors, we examined the effects of sevoflurane on PS responses in the presence of bicuculline, a $GABA_A$ receptor antagonist. The effects of bicuculline alone on PS responses were initially tested in the absence of sevoflurane. Although bicuculline promoted epileptoid activity in PS responses and made it difficult to measure the amplitude correctly, bicuculline (10 μM) increased PS amplitude to 127.2 ± 8.1% of control (n = 5, $P < 0.01$). Next, bicuculline (10 μM) was applied in the continued presence of sevoflurane to attempt reverse depression of PS amplitude. Bicuculline (10 μM) reinstated 0.04 mM sevoflurane–induced PS depression to 94.3 ± 9.2% of control (n = 5; fig. 2A). However, bicuculline (10 μM) only partially reinstated 0.23 mM sevoflurane–induced depression to 51.8 ± 16.0% of control (n = 5; fig. 2B). Taken together, γ-aminobutyric acid–mediated (GABAergic) inhibition seemed to play an important role, at least in part, in PS depression with amnestic doses of sevoflurane.

Sevoflurane Depresses Glutamate-mediated Excitatory Synapses in a Dose-dependent Manner

To determine whether sevoflurane-induced PS depression resulted from depressed glutamate-mediated excitatory synaptic inputs to CA1 neurons, fEPSPs were recorded from dendritic regions in the stratum radiatum. Sevoflurane depressed the amplitude of fEPSP responses in a dose-dependent manner (figs. 3A and B), with 0.23 mM sevoflurane to 70.7 ± 11.7% of control ($P < 0.05$, n = 10) and 0.41 mM sevoflurane to 41.9 ± 19.2% ($P < 0.001$, n = 10). Bicuculline (10 μM) did not reverse fEPSP depression produced by sevoflurane at any concentrations tested (up to 0.41 mM; fig. 3C). Depressed glutamate-mediated synaptic excitation thus seemed to play an important role in PS depression produced by clinical concentrations of sevoflurane. To determine the effects of sevoflurane on nerve conduction velocity, the duration from electrical stimulation to fiber volley response was measured. Sevoflurane up to 0.41 mM did not change the duration from electrical stimulus to fiber volley amplitude throughout the experiments (figs. 3A and B). Bicuculline alone had no effect on the amplitude of fiber volley or fEPSP.

Clinical, but Not Amnestic, Concentrations of Sevoflurane Increase Paired-pulse Facilitation

To determine whether presynaptic actions also contribute to anesthetic effects at glutamatergic synapses, paired-pulse facilitation (PPF) of Schaffer-collateral-commissural fiber evoked fEPSPs (60-ms intervals) were studied. In the presence of 0.41 mM sevoflurane, no apparent change in fEPSP rise time or decay kinetics was observed (fig. 4A), but PPF was transiently increased to approximately 130–150% of control during sevoflurane application and returned to baseline after washout (fig. 4B). This effect was observed in a dose-dependent manner (fig. 4C) and was independent of $GABA_A$ receptor–mediated actions because bicuculline (10 μM) did not affect sevoflurane-induced changes in PPF. This increase in facilitation is consistent with a presynaptic depression of glutamate release from nerve terminals, perhaps *via* depressant actions on voltage-activated calcium or sodium channels that couple axon spike depolarization to transmitter release.

Amnestic Concentrations of Sevoflurane Inhibit LTP through GABAergic Mechanisms

Tetanic stimulation (100 Hz, 1 s) induced a stable LTP (fEPSP slope after tetanization, 172.9 ± 15.1% of baseline, n = 9; fig. 5A), which was maintained for at least 1 h (fEPSP slope 30 min after tetanization, 154.8 ± 7.4% of baseline). First, we tested the effects of sevoflurane on the induction of LTP. Sevoflurane was administered 20

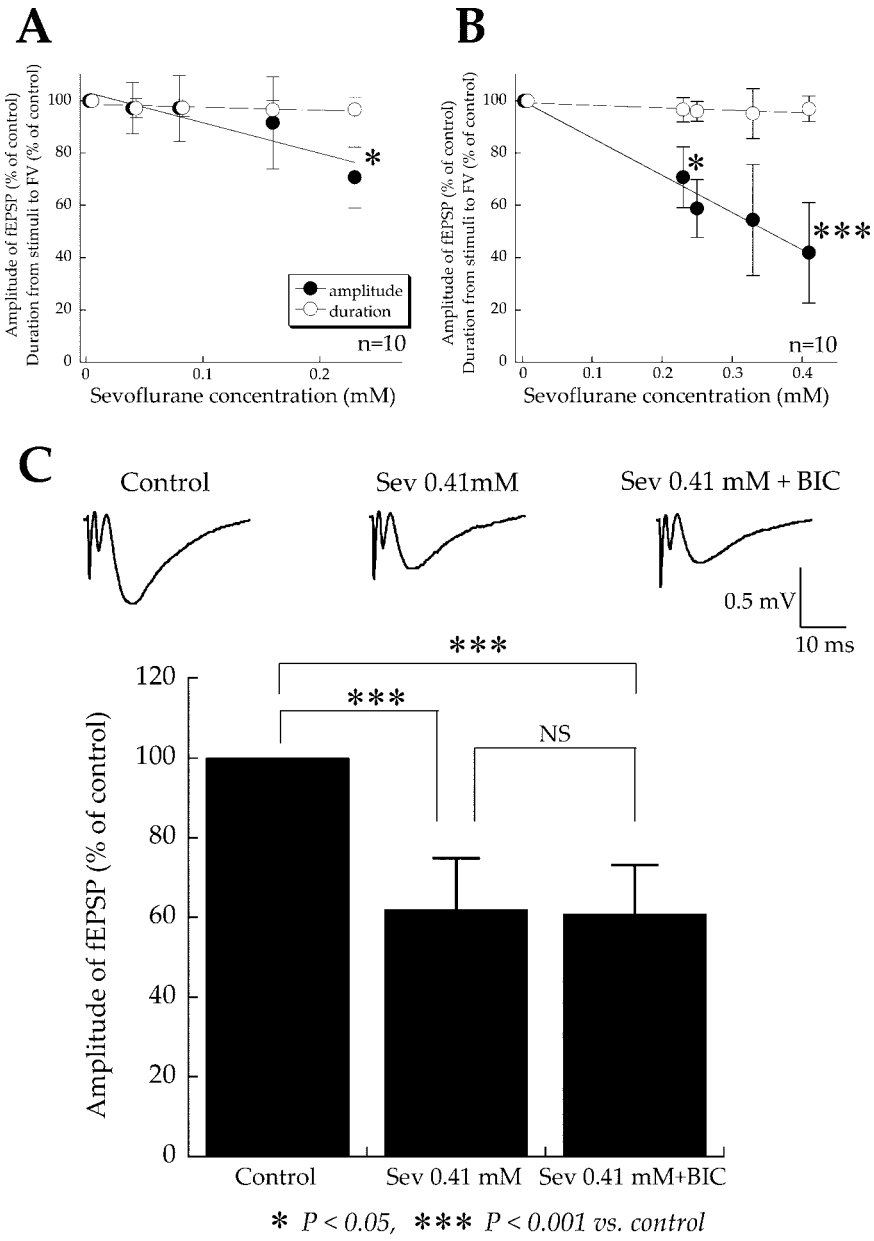

Fig. 3. The effects of sevoflurane (Sev) on field excitatory postsynaptic potentials (fEPSPs). (*A*) With amnestic concentrations of sevoflurane (<0.23 mM), there was no significant difference in fEPSP amplitude (*filled circle*, n = 10) and duration from stimuli to fiber volley (FV; *open circle*, n = 10). A linear curve fit was applied. (*B*) Clinical concentrations of sevoflurane (>0.23 mM) depressed fEPSP amplitude in a dose-dependent manner (*filled circle*, n = 10). However, duration from stimuli to FV (*open circle*, n = 10) did not change throughout the experiment. A linear curve fit was applied. (*C*) fEPSP responses (*upper*) were recorded from CA1 neurons. Bar graphs (*lower*) summarize the effects of sevoflurane on fEPSP amplitude. Sevoflurane (0.41 mM) depressed fEPSP amplitude markedly (n = 7, *** $P < 0.001$). Bicuculline (BIC; 10 μM) did not reverse sevoflurane-induced fEPSP depression. Data are expressed as mean ± SD. NS = not significant.

min before tetanic stimulation and delivered to the chamber throughout the experiment. In the presence of 0.04 mM sevoflurane, tetanic stimulation induced only posttetanic potentiation (141.8 ± 10.7% of baseline, n = 6; fig. 5A) and then failure of LTP. Posttetanic potentiation lasted only 5 min or less in all experiments (n = 6). To determine whether amnestic sevoflurane blocks the maintenance of LTP, 0.04 mM sevoflurane was applied after the LTP induction. As shown in figure 5B, administration of 0.04 mM sevoflurane 10 min after the LTP induction, however, had no influence on the maintenance of LTP (n = 7). The degree of LTP maintenance was similar to that of control LTP (fig. 5A). These findings indicate that amnestic sevoflurane inhibits the induction of LTP but has less effect on the maintenance. In the continued presence of higher concentrations of sevoflurane (0.41 mM), tetanic stimulation (100 Hz, 1 s) completely failed to induce LTP (fig. 6). Because 0.41 mM sevoflurane had inhibitory actions on fEPSP amplitude (fig. 3), baseline in the 0.41 mM sevoflurane group (n = 4) was decreased during anesthetic application (fig. 6).

To clarify concentration-dependent differential actions of sevoflurane on LTP, experiments were performed in the presence of bicuculline (10 μM). In this condition, tetanic stimulation could induce a stable LTP (fEPSP slope after tetanization 172.1 ± 10.5% of baseline; 30 min after tetanic stimulation, 134.2 ± 11.2%, n = 7; fig. 7A), indicating that bicuculline reinstates 0.04 mM sevoflurane–induced depression of LTP. The degree of LTP induction was similar to that of control LTP (fig. 5A), although the degree of maintenance was statistically less than control ($P < 0.05$). In the presence of 0.41 mM

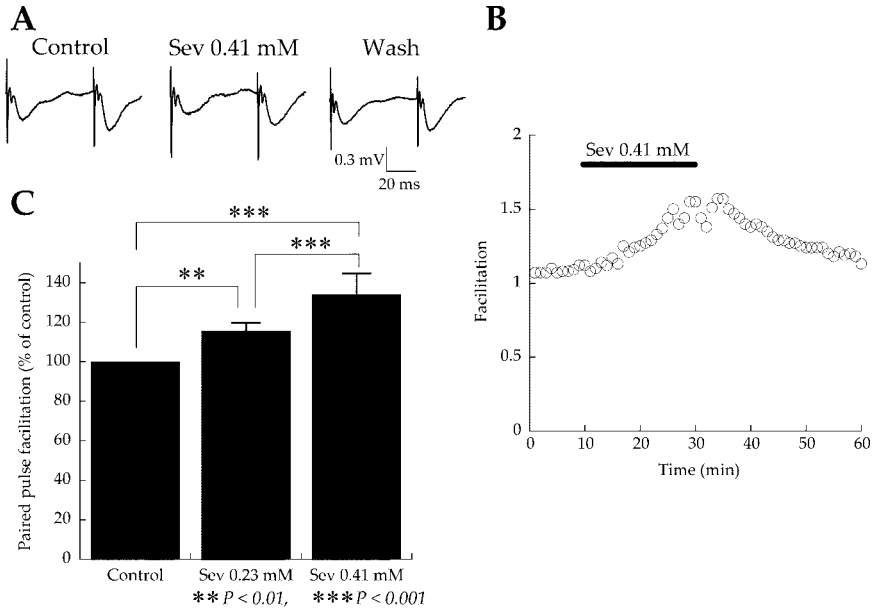

Fig. 4. The effects of sevoflurane on paired pulse facilitation (PPF). (*A*) Sample recordings obtained at the control period, during the maximal effect of 0.41 mM sevoflurane (Sev), and after washout. (*B*) The graph shows the time course of the effects of sevoflurane on PPF. PPF (pulse interval 60 ms) was recorded in the presence of 0.41 mM sevoflurane (*solid bar*) in normal artificial cerebrospinal fluid. Sevoflurane increased PPF gradually, and PPF returned to the control after the washout within 30 min. (*C*) A bar graph summarizes the effect of sevoflurane on PPF. Sevoflurane increased PPF of field excitatory postsynaptic potentials amplitude in a concentration-dependent manner ($P < 0.01$ *vs.* 0.23 mM sevoflurane, *** $P < 0.001$ *vs.* 0.41 mM sevoflurane; n = 6). Data are expressed as mean ± SD.**

sevoflurane, bicuculline reinstated only partially sevoflurane-induced depression of LTP (fig. 7B). Only posttetanic potentiation was observed after tetanic stimulation (151.1 ± 21.7% of baseline, n = 9). The effects of bicuculline alone on LTP were also examined in the absence of sevoflurane. Bicuculline (10 μM) alone had no effect on the induction and maintenance of LTP (fEPSP slope after tetanization 185.0 ± 19.2% of baseline; 30 min after tetanic stimulation, 150.2 ± 7.5%, n = 6; fig. 8). The degree of LTP was statistically similar to that of control LTP (fig. 5A) in the absence of bicuculline. Although bicuculline (10 μM) promoted epileptiform activity in PS responses, such a waveform was not significant in fEPSP recordings. This difference is probably due to relatively smaller stimulus intensity to evoke fEPSP.

Discussion

Major findings of the current study are summarized as follows. First, amnestic concentrations of sevoflurane inhibited PS amplitude and the induction of LTP with less effect on the maintenance through a GABAergic mechanism. Second, clinically relevant concentrations of sevoflurane dose-dependently depressed fEPSP amplitude and completely blocked LTP, in part by decreasing glutamate release as well as by enhancing GABAergic inhibitory tone. These data suggest that amnestic concentrations of sevoflurane inhibit synaptic plasticity of hippocampal CA1 neurons through GABAergic mechanisms and may thereby impair memory function.

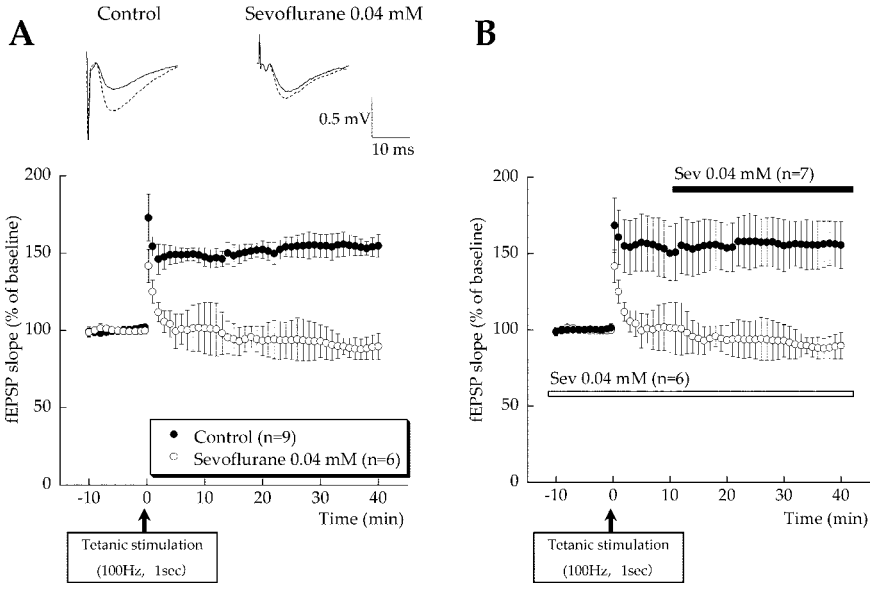

Fig. 5. The effects of amnestic concentrations of sevoflurane (Sev; 0.04 mM) on long-term potentiation. (*A*) Representative traces of field excitatory postsynaptic potentials (fEPSPs) recorded before (*solid trace*) and after (*dashed trace*) the delivery of tetanus stimulation (100 Hz, 1 s). The tetanus stimulation was delivered at time 0 (*arrow*). In control (*filled circle*), the tetanus stimulation (100 Hz, 1 s) consistently induced stable long-term potentiation. Continuous administration of 0.04 mM sevoflurane (*open circle*) throughout the experiments blocked long-term potentiation induction. The tetanus stimulation (100 Hz, 1 s) induced only posttetanic potentiation. (*B*) When sevoflurane at 0.04 mM was applied 10 min after the tetanus stimulation (n = 7), sevoflurane did not affect the maintenance of long-term potentiation (*filled circle*). Data are expressed as mean ± SD. BIC = bicuculline.

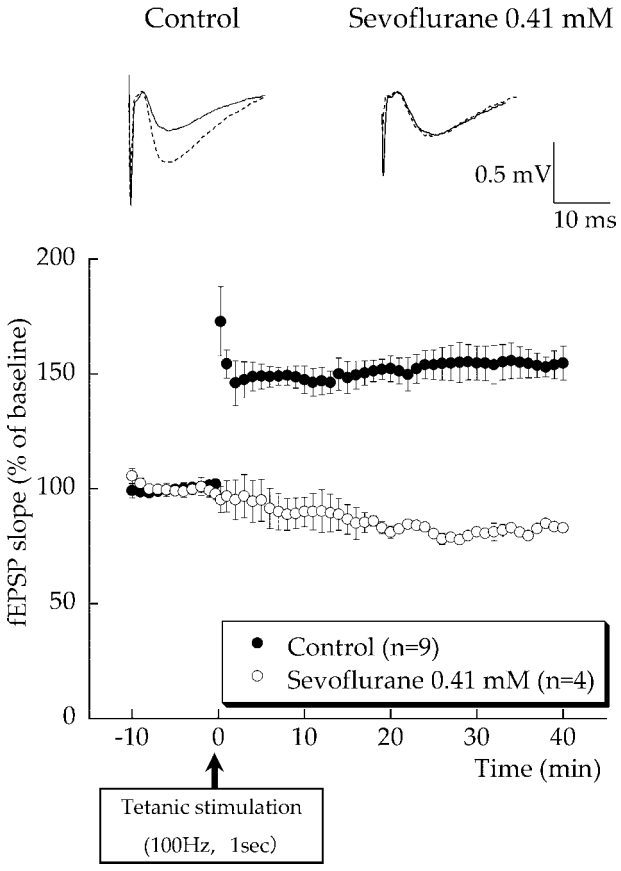

Fig. 6. The effects of clinical concentrations of sevoflurane (0.41 mM) on long-term potentiation. In the presence of sevoflurane at 0.41 mM (*open circle*), the tetanus stimulation (100 Hz, 1 s) did not induce long-term potentiation (n = 4). Posttetanic potentiation, which was observed in the presence of 0.04 mM sevoflurane (fig. 5A), was not observed in this condition. Data are expressed as mean ± SD. fEPSP = field excitatory postsynaptic potential.

Sevoflurane Concentrations in Physiologic Experiments at Room Temperature

Volatile anesthetics are administered to mammals in the gas phase at body temperature (approximately 37°C), whereas most *in vitro* physiologic experiments are performed at room temperature. However, gas-phase potencies are basically temperature dependent, increasing markedly with decreasing temperatures.[21] Therefore, the common procedure of using gas-phase EC_{50} concentrations for room temperature experiments can result in overdosing the *in vitro* preparations. In this context, Franks and Lieb[22] have recommended mammalian MAC values for volatile anesthetics expressed as free aqueous concentration in saline, *e.g.*, sevoflurane was 0.33 mM. As shown in figure 1, we used 0.04 mM for amnestic concentrations and 0.23–0.41 mM for clinical doses. We believe that these values are within reasonable range as clinical relevant concentrations.

Our LTP experiments using 100 Hz stimulus were performed at room temperature. These techniques are widely used, but room temperature is at least 10°C below physiologic conditions for mammalian neurons. It has been reported that temperature has large effects on synaptic transmission, plasma membrane state, and enzyme kinetics.[23,24] For example, a form of presynaptic plasticity is more prominent at room temperature.[23] It should be noted that temperature is a crucial experimental factor in studies of mammalian synaptic function.

Bicuculline as a $GABA_A$ Receptor Antagonist

We found that bicuculline reinstated 0.04 mM sevoflurane–induced depression of LTP and that bicuculline alone had no effect on control LTP in the absence of sevoflurane. Bicuculline is one of widely used $GABA_A$ receptor antagonists. Several recent studies have reported that bicuculline also blocks small-conductance Ca^{2+} activated K^+ (SK) channels.[25,26] SK channels regulate membrane excitability in CA1 neurons and modulate hippocampal synaptic plasticity and learning. Stackman *et al.*[27] reported that blockade of SK channels by apamin, a selective SK channel antagonist, decreased the threshold for the induction of hippocampal synaptic plasticity. After 50-Hz stimulus, significantly more LTP was induced in SK channel blocked slice, although with 100-Hz stimulus, equal extents of LTP were observed in control and SK channel blocked slice. Moreover, Hammond *et al.*[28] showed that SK2 channels selectively regulated the hippocampal synaptic plasticity, and SK2 overexpression impaired the induction of hippocampal LTP in a frequency-dependent manner. Induction of LTP with 50-Hz stimulus was significantly reduced in SK2 overexpression, although with 100-Hz stimulus, induction of LTP was equivalent. In the current study, we used the stimulus of 100 Hz for the induction of LTP. Taken together, we think that the influence of the blockade of SK channels by bicuculline on LTP is small in our recording conditions, and that $GABA_A$ receptors are crucially involved in amnestic concentrations of sevoflurane induced inhibition of LTP.

Amnestic Concentrations of Sevoflurane Depress Synaptically Evoked Discharge of CA1 Neurons in Part by Enhancing $GABA_A$ Receptor–mediated Inhibition

We found that sevoflurane reversibly inhibited PS amplitude in a dose-dependent manner and that the inhibition produced by 0.04 mM sevoflurane was antagonized by bicuculline, but profound inhibition by 0.23 mM sevoflurane was only partially antagonized by bicuculline. However, we found that bicuculline alone slightly but significantly increased PS amplitude. Taking these factors into consideration, enhanced GABAergic inhibition plays a role for amnestic doses of sevoflurane-induced depression. In this regard, Pittson *et al.*[29] reported that halothane and isoflurane at 1.0 MAC produced nearly complete depression of PS responses, but reversal of anesthetic-induced depression after bicuculline administration was only 20% in each anesthetic. Based on

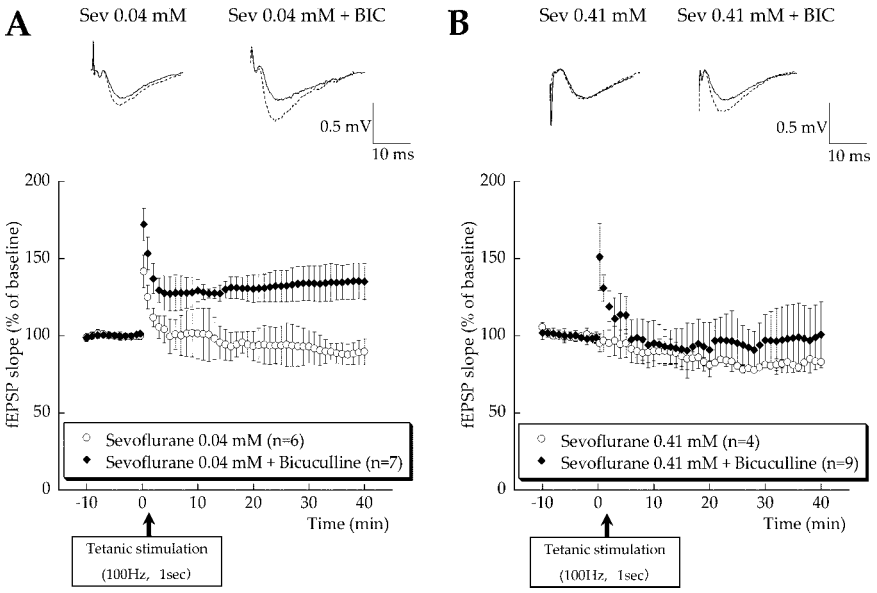

Fig. 7. Bicuculline reinstates amnestic sevoflurane (Sev)–induced long-term potentiation inhibition. Bicuculline was added 30 min before the tetanus stimulation (100 Hz, 1 s). (*A*) Bicuculline (10 μM) reinstated 0.04 mM sevoflurane–induced inhibition of long-term potentiation (n = 7, *filled rhombus*). (*B*) In the presence of 0.41 mM sevoflurane and 10 μM bicuculline, the tetanus stimulation (100 Hz, 1 s) induced only posttetanic potentiation (n = 9, *filled rhombus*) and failure of long-term potentiation. Data are expressed as mean ± SD. fEPSP = field excitatory postsynaptic potential.

these data, amnestic sevoflurane seemed to have similar properties in terms of anesthetic-induced depression of evoked discharge of CA1 neurons.

Sevoflurane may have multiple sites of action to produce overall depression of the central nervous system.[30,31] Although there are many possible molecular targets, the sensitivity of each component to volatile anesthetic may be different. In fact, we demonstrated that the effects on GABA-mediated components contributed significantly to synaptically evoked discharge of CA1 neurons (fig. 2). We have previously studied the actions of sevoflurane on $GABA_A$ receptors expressed in human embryonic kidney 293 cells, particularly focusing on the effects of transmembrane 2 mutations in α and β subunits.[11] We found that low concentrations of sevoflurane (0.1–0.2 mM) significantly potentiated submaximal GABA-activated Cl^- currents in wild-type $GABA_A$ receptors, and that positions serine 270 of both the $GABA_A$ 1 and 2 subunits were identified as critical for regulation of the $GABA_A$ receptor by sevoflurane. Recently, Sebel *et al.*[12] also reported that subanesthetic sevoflurane and propofol modulated GABA receptor function in an additive manner and that these two drugs had separate binding sites and converging pathways of action on the $GABA_A$ receptor. Together, available data suggest that modulation of CA1 neurons by amnestic sevoflurane may be primarily due to activation of GABAergic inhibitory neurons and that clinical concentrations of sevoflurane depress evoked discharge of CA1 neurons by acting not only on GABAergic neurons, but also on other components such as excitatory synapses.

Clinically Relevant, but Not Amnestic, Concentrations of Sevoflurane Depress fEPSPs in Part by Decreasing Glutamate Release

Consistent with previous reports of other volatile anesthetics,[15,32] sevoflurane reversibly depressed fEPSP amplitude in a concentration-dependent manner. Differing from PS responses, 0.04 mM sevoflurane had no effect on fEPSPs, but higher concentrations of sevoflurane considerably inhibited the amplitude of fEPSPs. Bicuculline did not reverse sevoflurane (0.41 mM)-induced fEPSP inhibition. These findings support the idea that clinical concentrations of sevoflurane directly depress CA1 excitatory synapses.

Paired-pulse facilitation was used as a measure of presynaptic anesthetic actions at Schaffer-collateral-commissural fiber synapses on CA1 pyramidal neurons.[13,14,33] PPF has been shown to increase after manipulations that reduce calcium-mediated glutamate release from the Schaffer-collateral-commissural pathway.[33] In contrast, manipulations that depress CA1 neuron fEPSPs *via* postsynaptic actions do not change PPF.[33,34] We found that sevoflurane at 0.23–0.41 mM but not 0.04 mM significantly increased PPF, suggesting that anesthetic sevoflurane has presynaptic actions to reduce glutamate release from Schaffer-collateral terminals. These data confirm previous findings that clinical concentrations of halothane and isoflurane increased PPF,[15] suggesting that presynaptic sites are involved in fEPSP depression by volatile anesthetics.

Sevoflurane and Long-term Potentiation

In the presence of 0.04 mM sevoflurane, tetanic stimulation induced only posttetanic potentiation, which lasted for 5 min or less and was believed to involve only modifications at the presynaptic terminal.[35] Subsequent LTP is thought to involve an increase in the number of quanta released, or in the size of the response each quantum produces in the postsynaptic cells.[36] Therefore, amnestic sevoflurane appeared to have depressive synaptic actions on either presynaptic or postsynaptic mechanisms to induce LTP through GABAergic mechanisms.

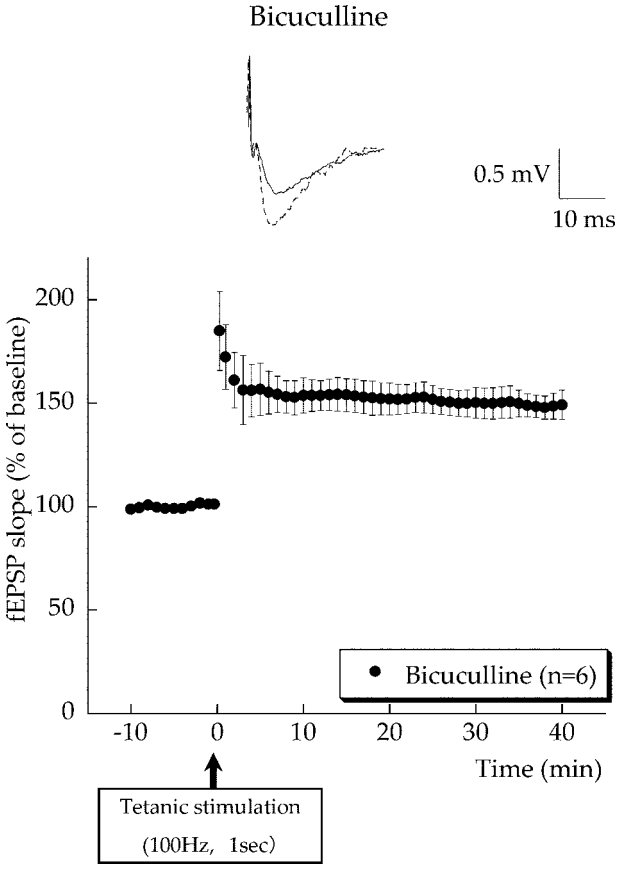

Fig. 8. The effects of bicuculline alone (10 μM) on long-term potentiation were examined in the absence of sevoflurane. Tetanic stimulation (100 Hz, 1 s) induced stable long-term potentiation (n = 6). The peak value of field excitatory postsynaptic potential (fEPSP) slope after tetanic stimulation was similar to that of control in the absence of bicuculline (fig. 5A). Data are expressed as mean ± SD.

There are several possible cellular targets in amnesic effects produced by general anesthetics; the GABAergic system may be one of the most important mechanisms.[16,17] Simon *et al.*[16] investigated the effects of clinically relevant concentrations of isoflurane (0.2–0.3 mM) on the induction of LTP and long-term depression in slices from the juvenile (14 days) and adult (2 months) mouse hippocampus. They found that isoflurane (0.2–0.3 mM) blocked the induction of LTP and that picrotoxin (50 μM) partially reversed isoflurane-induced LTP inhibition. Blocking effects of isoflurane on LTP and long-term depression were reversible, suggesting that this agent does not induce persistent change in neural excitability. Similarly, Nagashima *et al.*[17] reported that relatively higher concentrations of propofol (30 μM) inhibited LTP and that this inhibition was partially antagonized by picrotoxin (1 μM). These studies suggest that not only GABA mechanism but other factors may contribute to clinically relevant concentrations of anesthetic-induced LTP inhibition. Therefore, in the current study, we focused on amnestic concentrations of sevoflurane (0.04 mM), which produces amnesia but does not affect sedative/hypnotic actions. Our results offer new evidence that amnestic sevoflurane inhibits the induction of LTP with less effect on the maintenance of LTP, and that these actions alone seem to account fully for the effects of amnestic sevoflurane.

γ-Aminobutyric acid receptors have been previously reported to mediate sedative components of anesthesia[37] and amnesic properties of benzodiazepines.[38] Recently, Cheng *et al.*[39] studied the effects of etomidate on GABA α_5 subunit knockout mice. In the wild type, low-dose etomidate (0.1 M), which had no effect on the kinetics of GABA-mediated synaptic currents, significantly increased GABA tonic currents, impaired LTP, and impaired memory performance. However, α_5 null mutant mice showed similar hypnotic dose-responses to the wild type but had little etomidate-induced amnesia, LTP, and tonic current potentiation. These data support the idea that GABAergic mechanisms contribute significantly to anesthetic-induced amnesia.

In conclusion, the current study provides evidence for a $GABA_A$ receptor involvement contributing to effects of amnestic concentrations of sevoflurane on synaptic plasticity of hippocampal CA1 neurons.

References

1. Whittle IR, Midgley S, Georges H, Pringle AM, Taylor R: Patient perceptions of "awake" brain tumour surgery. Acta Neurochir (Wien) 2005; 147:275–7
2. Galinkin JL, Janiszewski D, Young CJ, Klafta JM, Klock PA, Coalson DW, Apfelbaum JL, Zacny JP: Subjective, psychomotor, cognitive, and analgesic effects of subanesthetic concentrations of sevoflurane and nitrous oxide. Anesthesiology 1997; 87:1082–8
3. Hall DL, Weaver J, Ganzberg S, Rashid R, Wilson S: Bispectral EEG index monitoring of high-dose nitrous oxide and low-dose sevoflurane sedation. Anesth Prog 2002; 49:56–62
4. Alkire MT, Gorski LA: Relative amnesic potency of five inhalational anesthetics follows the Meyer-Overton rule. Anesthesiology 2004; 101:417–29
5. Alkire MT, Nathan SV: Does the amygdala mediate anesthetic-induced amnesia? Basolateral amygdala lesions block sevoflurane-induced amnesia. Anesthesiology 2005; 102:754–60
6. Bear MF, Malenka RC: Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol 1994; 4:389–99
7. Larkman AU, Jack JJ: Synaptic plasticity: Hippocampal LTP. Curr Opin Neurobiol 1995; 5:324–34
8. Yamakura T, Bertaccini E, Trudell JR, Harris RA: Anesthetics and ion channels: Molecular models and sites of action. Annu Rev Pharmacol Toxicol 2001; 41:23–51
9. Jenkins A, Franks NP, Lieb WR: Effects of temperature and volatile anesthetics on $GABA_A$ receptors. Anesthesiology 1999; 90:484–91
10. Nishikawa K, Jenkins A, Paraskevakis I, Harrison NL: Volatile anesthetic actions on the $GABA_A$ receptors: Contrasting effects of alpha 1(S270) and beta 2(N265) point mutations. Neuropharmacology 2002; 42:337–45
11. Nishikawa K, Harrison NL: The actions of sevoflurane and desflurane on the γ-aminobutyric acid receptor type A: Effects of TM2 mutations in the α and β subunits. Anesthesiology 2003; 99:678–84
12. Sebel LE, Richardson JE, Singh SP, Bell SV, Jenkins A: Additive effects of sevoflurane and propofol on γ-aminobutyric acid receptor function. Anesthesiology 2006; 104:1176–83
13. MacIver MB, Mikulec AA, Amagasu SM, Monroe FA: Volatile anesthetics depress glutamate transmission *via* presynaptic actions. Anesthesiology 1996; 85:823–34
14. Perouansky M, Baranov D, Salman M, Yaari Y: Effects of halothane on glutamate receptor–mediated excitatory postsynaptic currents: A patch-clamp study in adult mouse hippocampal slices. Anesthesiology 1995; 83:109–19
15. Nishikawa K, MacIver MB: Excitatory synaptic transmission mediated by NMDA receptors is more sensitive to isoflurane than are non–NMDA receptor-mediated responses. Anesthesiology 2000; 92:228–36
16. Simon W, Hapfelmeier G, Kochs E, Zieglgansberger W, Rammes G: Isoflurane blocks synaptic plasticity in the mouse hippocampus. Anesthesiology 2001; 94:1058–65

17. Nagashima K, Zorumski CF, Izumi Y: Propofol inhibits long-term potentiation but not long-term depression in rat hippocampal slices. ANESTHESIOLOGY 2005; 103:318–26

18. Nishikawa K, MacIver MB: Membrane and synaptic actions of halothane on rat hippocampal pyramidal neurons and inhibitory interneurons. J Neurosci 2000; 20:5915–23

19. Nishikawa K, Kubo K, Ishizeki J, Takazawa T, Saito S, Goto F: The interaction of noradrenaline with sevoflurane on GABA (A) receptor-mediated inhibitory postsynaptic currents in the rat hippocampus. Brain Res 2005; 1039: 153–61

20. Kuroda M, Yoshikawa D, Nishikawa K, Saito S, Goto F: Volatile anesthetics inhibit calcitonin gene-related peptide receptor-mediated responses in pithed rats and human neuroblastoma cells. J Pharmacol Exp Ther 2004; 311:1016–22

21. Franks NP, Lieb WR: Which molecular targets are most relevant to general anaesthesia? Toxicol Lett 1998; 100–101:1–8

22. Franks NP, Lieb WR: Temperature dependence of the potency of volatile general anesthetics: Implications for *in vitro* experiments. ANESTHESIOLOGY 1996; 84:716–20

23. Micheva KD, Smith SJ: Strong effects of subphysiological temperature on the function and plasticity of mammalian presynaptic terminals. J Neurosci 2005; 25:7481–8

24. Klyachko VA, Stevens CF: Temperature-dependent shift of balance among the components of short-term plasticity in hippocampal synapses. J Neurosci 2006; 26:6945–57

25. Khawaled R, Bruening-Wright A, Adelman JP, Maylie J: Bicuculline block of small-conductance calcium-activated potassium channels. Pflugers Arch 1999; 438:314–21

26. Debarbieux F, Brunton J, Charpak S: Effect of bicuculline on thalamic activity: A direct blockade of IAHP in reticularis neurons. J Neurophysiol 1998; 79:2911–8

27. Stackman RW, Hammond RS, Linardatos E, Gerlach A, Maylie J, Adelman JP, Tzounopoulos T: Small conductance Ca2+-activated K+ channels modulate synaptic plasticity and memory encoding. J Neurosci 2002; 22:10163–71

28. Hammond RS, Bond CT, Strassmaier T, Ngo-Anh TJ, Adelman JP, Maylie J, Stackman RW: Small-conductance Ca2+-activated K+ channel type 2 (SK2) modulates hippocampal learning, memory, and synaptic plasticity. J Neurosci 2006; 26:1844–53

29. Pittson S, Himmel AM, MacIver MB: Multiple synaptic and membrane sites of anesthetic action in the CA1 region of rat hippocampal slices. BMC Neurosci 2004; 5:52

30. Hollmann M, Heinemann S: Cloned glutamate receptors. Annu Rev Neurosci 1994; 17:31–108

31. Franks NP, Lieb WR: Molecular and cellular mechanisms of general anaesthesia. Nature 1994; 367:607–14

32. MacIver MB, Roth SH: Inhalation anaesthetics exhibit pathway-specific and differential actions on hippocampal synaptic responses *in vitro*. Br J Anaesth 1998; 60:680–91

33. Manabe T, Wyllie DJ, Perkel DJ, Nicoll RA: Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. J Neurophysiol 1993; 70:1451–9

34. Zucker RS: Short-term synaptic plasticity. Annu Rev Neurosci 1989; 12: 13–31

35. Hannay T, Larkman A, Stratford K, Jack J: A common rule governs the synaptic locus of both short-term and long-term potentiation. Curr Biol 1993; 3:832–41

36. Manabe T, Nicoll RA: Long-term potentiation: Evidence against an increase in transmitter release probability in the CA1 region of the hippocampus. Science 1994; 265:1888–92

37. Nelson LE, Guo TZ, Lu J, Saper CB, Franks NP, Maze M: The sedative component of anesthesia is mediated by GABA (A) receptors in an endogenous sleep pathway. Nat Neurosci 2002; 5:979–84

38. Ghoneim MM, Mewaldt SP: Benzodiazepines and human memory: A review. ANESTHESIOLOGY 1990; 72:926–38

39. Cheng VY, Martin LJ, Elliott EM, Kim JH, Mount HT, Taverna FA, Roder JC, Macdonald JF, Bhambri A, Collinson N, Wafford KA, Orser BA: Alpha5GABA$_A$ receptors mediate the amnestic but not sedative-hypnotic effects of the general anesthetic etomidate. J Neurosci 2006; 26:3713–20