Presynaptic Actions of General Anesthetics Are Responsible for Frequency-Dependent Modification of Synaptic Transmission in the Rat Hippocampal CA1

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BACKGROUND: In clinical anesthesia, robust surgical stress occasionally causes unintended light anesthesia during operation. To test the hypothesis that neural input condition could modify actions of general anesthetics as a result of presynaptic alteration in the central nervous system, we investigated the mechanisms by which the stimulus frequency modifies synaptic transmission of the rat hippocampus in the presence of general anesthetics.

METHODS: Field population spikes (PSs) of CA1 pyramidal neurons were elicited using orthodromic stimulation of Schaffer collateral-commissural fibers (test-pulse). A second stimulating electrode was placed in the region of the alveus hippocampi to activate recurrent inhibition of area CA1 (pre-pulse). The pre-pulses were applied as train stimuli (100–200 Hz) to activate release and then deplete the neurotransmitter (γ-aminobutyric acid [GABA]) at presynaptic terminals of inhibitory interneurons.

RESULTS: After the activation of inhibitory interneurons with pre-pulses, both IV (thiopental and pentobarbital) and volatile (sevoflurane and isoflurane) anesthetics attenuated the PS amplitudes elicited with test-pulses (test-PS). The IV anesthetics, but not the volatile drugs, produced stimulus frequency- and use-dependent recurrent inhibition of test-PSs. Neither a GABA type A agonist nor a GABA uptake inhibitor produced frequency-dependent modification. The pre-pulse train protocol revealed that IV anesthetics, but not volatile drugs, can enhance GABA release from presynaptic terminals.

CONCLUSIONS: IV anesthetics, but not volatile drugs, enhance the discharge of a readily releasable pool of GABA vesicles from presynaptic terminals. Depletion of an active pool of GABA after high-frequency stimuli would produce frequency- and use-dependent recurrent inhibition in the presence of IV anesthetics. The stimulus frequency-dependent modification of synaptic transmission might be responsible for the unsuccessful immobilization or hypnosis during general anesthesia after IV anesthetic administration. (Anesth Analg 2010;110:1607–13)

It has been proposed that the actions of general anesthetics occur via specific and multiple sites and that the effects seem to be both pathway and agent specific.[1–3] We have shown that volatile anesthetics mainly inhibit glutamate-mediated orthodromic pathways, whereas IV anesthetics primarily enhance the γ-aminobutyric acid type A ($GABA_A$) receptor–related recurrent inhibitory pathways.[4–7] We speculated that the observed different sensitivities to specific pathways may be due to the anesthetic actions on the neurotransmitter kinetics at synapses.[5]

If general anesthetics have direct actions on the presynaptic functions, the synaptic transmission would be modified in the neural input-dependent manner, because the concentration and clearance of neurotransmitters at synapses modulate synaptic transmission. In clinical practice, anesthesiologists often experience unexpected patient movements or awareness that takes place occasionally after surgical stress during operation, even when a clinically relevant concentration of general anesthetic is administered. The observations are compatible with the idea that general anesthetics can modify presynaptic functions.

The studies of presynaptic effects of general anesthetics, however, have been inconsistent. The IV but not volatile anesthetics inhibit GABA uptake in brain synaptosomes,[8] whereas general anesthetics have also been reported to have no significant effect on clearance of GABA at the synaptic cleft.[9] To test the hypotheses (a) that neural input properties modify actions of general anesthetics in the central nervous systems, and (b) that the modification is due to the presynaptic actions of general anesthetics, we have studied the effects of neural input frequency and neurotransmitter discharges from presynaptic terminals in the presence of general anesthetics.

METHODS

Preparation

Ethical approval was obtained from the Animal Research Committee of the University of Toyama. The methods for preparation of rat hippocampal slices and electrophysiological experiments were described by Hirota and Roth[10] and Asahi et al.[5] In brief, male Wistar rats (50–100 g) were deeply anesthetized with sevoflurane and then decapitated. The brain was rapidly removed, and 400-μm transverse

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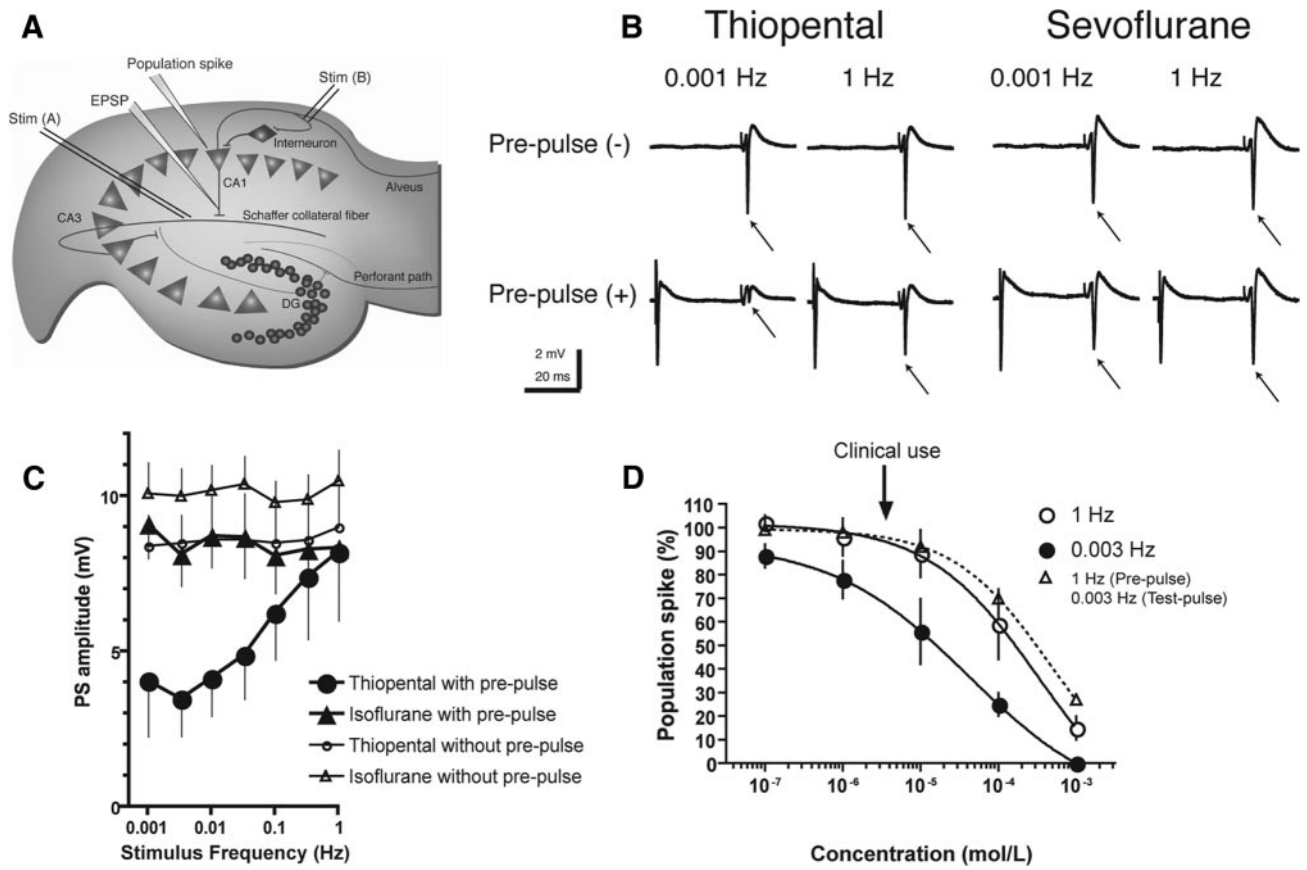

Figure 1. A, Two sets of glass extracellular recording microelectrodes (population spike and excitatory postsynaptic potentials) were placed in the cell body and dendrite region of CA1 pyramidal neurons, respectively. One bipolar stimulating electrode (A) was placed in the region of Schaffer collateral-commissural fibers to stimulate the input to CA1 neurons, and the second electrode (B) was placed in the region of alveus hippocampi to activate inhibitory interneurons of the CA1. B, The stimulus frequency-dependent modification of thiopental (2×10^{-5} mol/L) and sevoflurane (3.0 vol%) on the inhibitory synaptic transmission. Pre-pulse (−): recurrent inhibition is inactive. Pre-pulse (+): recurrent inhibition is active. Arrows indicate population spikes (PSs) elicited by test-pulses. C, Relations between stimulus frequency and effects of thiopental (2×10^{-5} mol/L, $n = 7$) and isoflurane (2.0 vol%, $n = 6$) on the test-PS amplitudes. Data represent mean ± SD. Closed circles (thiopental with pre-pulse) were significantly different ($P < 0.0001$) from closed triangles (isoflurane with pre-pulse). D, The relations between the pentobarbital concentrations and the test-PS amplitude at stimulus frequency of 1 and 0.003 Hz. Open circles: 1 Hz ($n = 5$). Closed circles: 0.003 Hz ($n = 5$). Open triangles: Pre- and test-pulses were applied on 1 and 0.003 Hz, respectively ($n = 1$). Arrow indicates the clinically relevant concentration of pentobarbital. Data represent mean ± SD. Closed circles (0.003 Hz) were significantly different ($P < 0.0001$) from open circles (1 Hz).

slices were prepared from the dissected hippocampus in cold, oxygenated artificial cerebrospinal fluid (ACSF) using a Rotorslicer DTY-7700 (DSK, Osaka, Japan). The slices were placed on a nylon mesh screen at the interface of ACSF liquid (90 mL/h) and humidified 95% O_2/5% CO_2 gas (1 L/min) phases in a recording chamber. Slices were warmed to 37°C slowly and then allowed to equilibrate for 90 to 120 minutes without electrical stimulation.

Anatomy and Electrophysiology

As summarized in Figure 1A, the hippocampal CA1 pyramidal neurons are monosynaptically connected with Schaffer collateral fibers (Sch, axons of CA3 pyramidal neurons). The CA1 neurons are also controlled by GABAergic inhibition through the inhibitory interneurons. The recurrent inhibition can be selectively activated by antidromic stimulation of CA1 pyramidal cell axons in alveus hippocampi (Alv). In this study, 2 sets of glass extracellular recording microelectrodes (3–5 MΩ filled with 2 mol/L NaCl) were placed in the cell body and dendrite region of CA1 pyramidal neurons to record population spikes (PSs) and excitatory postsynaptic potentials (EPSPs), respectively. Two sets of bipolar stimulating electrodes were placed in Sch and Alv to stimulate the input to CA1 neurons and to activate inhibitory interneurons, respectively.[5,11]

Square-wave stimuli (5–10 V, 50 μs), generated with a SEN-3301 stimulator (Nihon Kohden, Tokyo, Japan), were delivered to both pathways simultaneously. The minimal stimulus intensity that elicited the maximal amplitude (maximal stimulus) was used. Field potentials were amplified with an MEZ-8301 amplifier (Nihon Kohden) and filtered 1 Hz to 10 kHz. Analog-digital conversions of data were made at a rate of 100 kHz using InstruNet (GW Instruments, Somerville, MA). The results were stored on the hard drive of a Macintosh computer (Apple, Cupertino, CA) and analyzed using SuperScope software (GW Instruments).

Analysis of Recurrent Inhibition

Initially, the region of Alv was stimulated with pre-pulses to activate the recurrent inhibition. Because pre-pulses

activate both inhibitory interneurons orthodromically and CA1 pyramidal cells antidromically, field PSs (pre-PS) were elicited (Fig. 1A). Next, stimulation to Sch was applied as a test-pulse, and field PS (test-PS) was recorded to examine the enhancement of recurrent inhibition. The interval between the pre- and test-pulses was set at 40 milliseconds. Because test-PSs were not influenced by physiological recurrent inhibition under the 40-millisecond interval, the enhancement (prolongation) of inhibition by drugs can be detected as a depression of test-PS amplitude.[5] EPSPs were simultaneously recorded to monitor excitatory synaptic transmission.

Analysis of Presynaptic GABA Release

To focus on the effects of general anesthetics on presynaptic neurotransmitter release of inhibitory interneurons, we developed a new pre-pulse train protocol for the analysis of GABA discharge from presynaptic terminals. The pre-pulse train (100 Hz) protocol enhanced release of GABA from presynaptic terminals and then induced depletion of neurotransmitter (see Results section).

Drug Application

Volatile anesthetics, sevoflurane and isoflurane, were administered as vapors in the prewarmed carrier gas (95% O_2/5% CO_2) above the slices using calibrated commercial vaporizers such as Tec 3 (Omeda, Steeton, West Yorkshire, UK) and Forawick (Murako, Tokyo, Japan), respectively. Concentrations, expressed as volume percent (vol%), refer to the dial settings on the vaporizers. Concentrations of volatile anesthetics in the perfusate of the recording chamber were determined using gas chromatography (Shimazu, Kyoto, Japan): a linear relationship (0.64 and 0.55 mmol/L per 1.0 vol% of sevoflurane and isoflurane, respectively) up to 5.0 vol%. Thiopental, pentobarbital, and nipecotic acid were dissolved in ACSF at required concentrations before use. All anesthetics and drugs were administered for a minimum of 20 minutes to reach equilibrium, as previously demonstrated.[4]

The composition of the ACSF was (mmol/L): NaCl 124, KCl 5, $CaCl_2$ 2, NaH_2PO_4 1.25, $MgSO_4$ 2, $NaHCO_3$ 26, AP5 0.1, and glucose 10, prepared with purified water. The ACSF was precooled (8°C–10°C) and kept saturated with a 95% O_2/5% CO_2 gas mixture before use (pH 7.1–7.3). DL-2-Amino-5-phosphonovaleric acid (AP5, 100 μmol/L) was used to prevent *N*-methyl-D-aspartate receptor–related synaptic plasticity.[12] Sevoflurane and isoflurane were purchased from Dinabot (Osaka, Japan) and Maruishi Pharmaceutical Co. (Osaka, Japan), respectively. Thiopental and pentobarbital were purchased from Tanabe Pharmaceutical Co. (Osaka, Japan) and Dinabot. All other chemicals used were purchased from Sigma (St. Louis, MO).

Drug Doses

The dose of thiopental and pentobarbital required to anesthetize experimental animals is approximately 25 mg/kg.[13] Because the IV anesthetics will be diluted by the extracellular fluid (20%–30% of total body weight), the maximal concentration will be 3 to 5 $\times$ 10^{-4} mol/L, and this concentration will progressively decrease. The minimal alveolar concentrations of sevoflurane and isoflurane in experimental animals are 2.4 and 1.4 vol%.[14] On the basis of these reports, the concentration-response relations were generated in preliminary experiments, and the effective concentrations of each anesthetic were then determined for this study: thiopental 2 $\times$ 10^{-5} mol/L, pentobarbital 10^{-5} mol/L, sevoflurane 3.0 vol%, and isoflurane 2.0 vol%.

Data Analysis

PS amplitudes were determined from peak positive to peak negative of the waveform.[5] All preparations used in this study showed control variability <5% during the initial data acquisition period and after washout of anesthetic drugs. Recovery responses were recorded at least 30 minutes after washout of anesthetic-equilibrated ACSF from the chamber. Statistical differences were tested by repeated-measures analysis of variance, and differences between paired sets of data were compared by the Bonferroni/Dunn test. A P value <0.05 was considered significantly different. Statistical analysis and curve fitting were performed using Prism software (GraphPad, San Diego, CA).

RESULTS

Frequency-Dependent Recurrent Inhibition

The stimulus frequency-dependent modification of thiopental (2 $\times$ 10^{-5} mol/L) and sevoflurane (3.0 vol%) on the inhibitory synaptic transmission is shown in Figure 1B. In the absence of pre-pulse, recurrent inhibition is not activated (upper trace). Alternation of stimulus frequency (0.001–1 Hz) had no consistent effect on the PS amplitudes elicited with test-pulses (test-PSs). In the presence of pre-pulse, recurrent inhibition is activated (lower trace). Thiopental markedly depressed the test-PSs at 0.001 Hz. The depression, however, was attenuated at 1 Hz. The frequency-dependent recurrent inhibition was less prominent in the presence of sevoflurane. The frequency-dependent modification was not observed in EPSP recordings (data not shown).

The frequency-dependent effects of general anesthetics are summarized in Figure 1C. Barbiturates (thiopental and pentobarbital), but not volatile anesthetics (sevoflurane and isoflurane), depressed the test-PSs in a frequency-dependent manner (between 0.03 and 1 Hz) in the presence of pre-pulse. The relationship between pentobarbital concentrations and the test-PS amplitudes at a stimulus frequency of 1 and 0.003 Hz is shown in Figure 1D. The 50% effective concentration value, following curve-fit analysis, was determined to be 3.2 $\times$ 10^{-4} mol/L at 1 Hz. Low-frequency stimulation (0.003 Hz) shifted the concentration-response curve to the left and decreased the 50% effective concentration to 4.2 $\times$ 10^{-5} mol/L. When pre- and test-pulses were applied at 1 and 0.003 Hz, respectively, the inhibitory actions of IV anesthetics were the same as both pre- and test-pulses applied at 1 Hz. Thus, frequency-dependent modification is dependent on the pre-pulse frequency but not on the test-pulse frequency.

The use-dependent recurrent inhibition of the test-PS in the presence of thiopental is shown in Figure 2 (protocol A). After 60 seconds of control stimuli (1 Hz), stimuli were terminated for 5 minutes (repriming period, see Discussion section), and then 10 pairs of pre- and test-pulses were applied at 1 Hz. Although the first test-PS was markedly depressed, subsequent test-PSs gradually disinhibited in

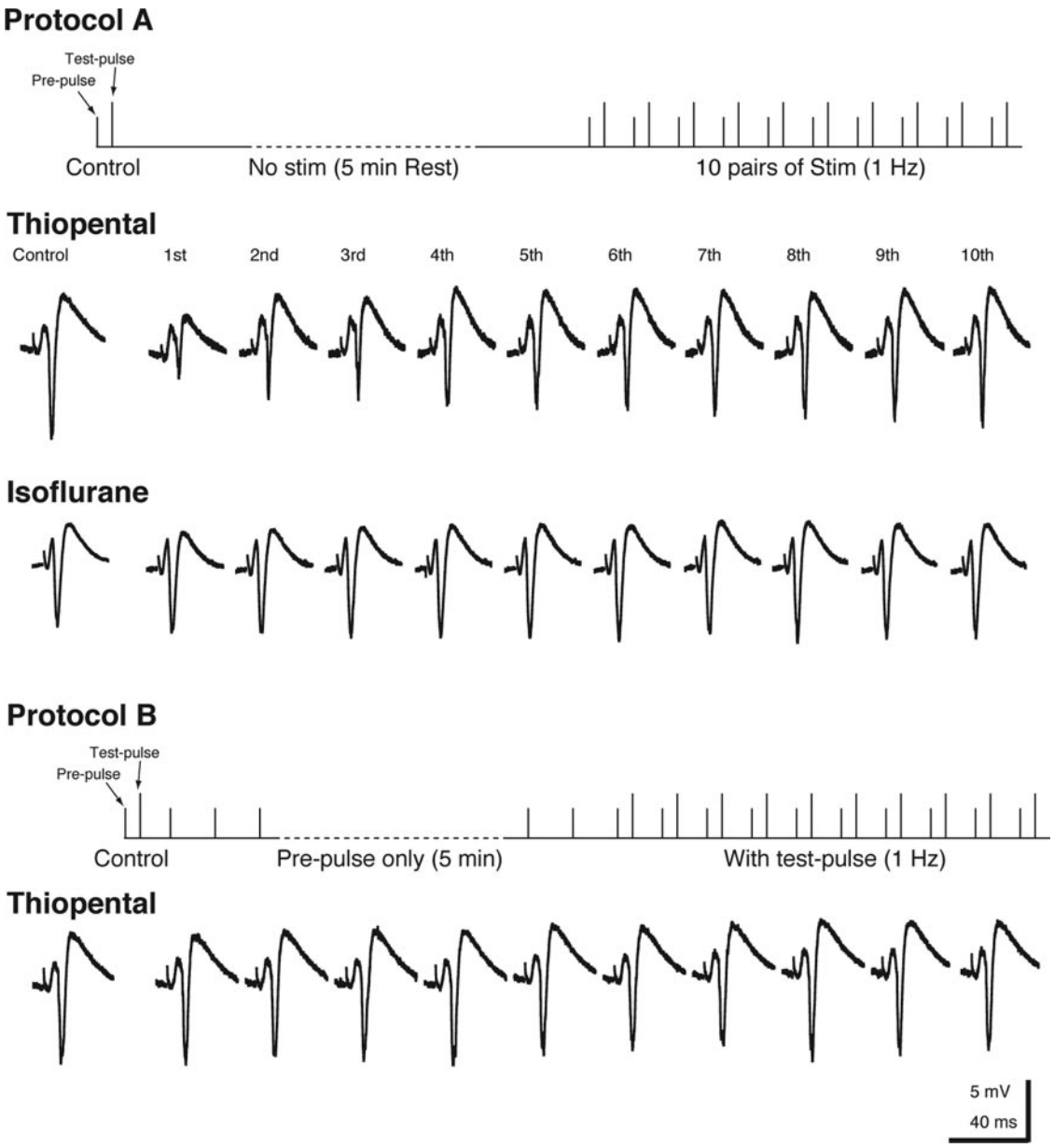

Figure 2. Protocol A, The use-dependent disinhibition of the test–population spike (PS) in the presence of thiopental (2×10^{-5} mol/L) and isoflurane (2.0 vol%). After 60 seconds of control recordings (1 Hz), the electric stimuli were terminated for 5 minutes, and then 10 pairs of pre-pulse and test-pulse were reapplied at 1 Hz. Protocol B, After 60 seconds of control stimuli (1 Hz), only the test-pulses were terminated for 5 minutes (the pre-pulses continued), and then 10 pairs of pre-pulse and test-pulse were applied at 1 Hz.

the presence of thiopental (2×10^{-5} mol/L). The use dependency was not observed in the presence of isoflurane (2.0 vol%). We also tested the actions of sevoflurane (3.0 vol%), muscimol ($GABA_A$ receptor agonist, 2×10^{-5} mol/L), baclofen ($GABA_B$ agonist, 10^{-5} mol/L), and nipecotic acid (GABA uptake inhibitor, 10^{-3} mol/L). None of these chemicals, however, produced frequency- and/or use-dependent modification.

Figure 2 (protocol B) demonstrates that the use-dependent modification is contingent on the pre-pulses but not on the test-pulses. After 60 seconds of control stimuli (1 Hz), only the test-pulses were terminated for 5 minutes (the pre-pulses continued), and then 10 pairs of pre- and test-pulses were applied at 1 Hz. Protocol B failed to produce the use-dependent changes.

Pre-Pulse Train Protocol Induces Release and Depletion of Neurotransmitter

To evaluate the mechanisms of frequency-dependent recurrent inhibition of barbiturates, we focused on the effects of general anesthetics on neurotransmitter release. An example of the pre-pulse train method used in this study to assess GABA release from presynaptic terminals is shown in Figure 3. When a single pre-pulse is applied ($n = 1$), the test-PS is slightly decreased because of enhancement of recurrent inhibition. A pre-pulse train (100 Hz, $n = 2$–10)

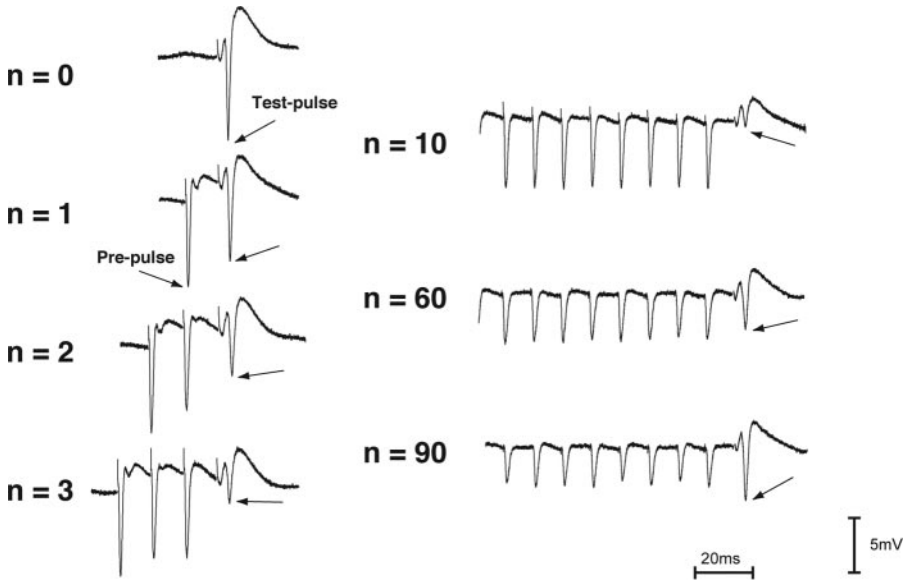

Figure 3. An example of the pre-pulse train method. Different number of pre-pulses (100 Hz, $n = 0–90$) were applied to assess the γ-aminobutyric acid release from presynaptic terminals. Arrows indicate the test population spikes.

accelerates the enhancement of inhibition. The degree of decrease of test-PS is dependent on the number of pre-pulses. Longer pre-pulse trains ($n = 60–90$) produce disinhibition of test-PSs, suggesting depletion of GABA at presynaptic terminals. The relationship between the number of pre-pulse trains (100 Hz) and test-PS amplitudes is shown in Figure 4A. In ACSF, short pre-pulse trains produced inhibition of test-PSs (phase I) and long trains induced disinhibition (phase II). Administration of a GABA uptake inhibitor, nipecotic acid (10^{-3} mol/L), accelerated the onset of phase II, indicating that the pre-pulse train protocol could enhance release of GABA from presynaptic terminals (phase I) and then induce depletion of the neurotransmitter (phase II). The pre-pulse train protocol did not have a consistent effect on EPSPs (data not shown).

We studied the effects of IV anesthetics (thiopental [2×10^{-5} mol/L], pentobarbital [10^{-5} mol/L]) and volatile anesthetics (sevoflurane [3.0 vol%], isoflurane [2.0 vol%]) on GABA release and depletion using the pre-pulse train protocol (Fig. 4, B and C). Because barbiturates prolonged phase I and delayed the onset of phase II, this suggested that IV drugs enhanced GABA release. In contrast, volatile anesthetics failed to completely depress phase II, indicating that the actions of IV anesthetics on presynaptic GABA release would be greater than those of volatile anesthetics.

DISCUSSION

Our previous studies, using the same experimental model as this study,[4,5] demonstrated that IV anesthetics (thiopental and propofol) enhance inhibitory signaling to a greater extent than do volatile anesthetics (sevoflurane and isoflurane).We observed, for the first time, the frequency- and use-dependent modification of inhibitory synaptic transmission in the presence of IV anesthetics (thiopental and pentobarbital), whereas the volatile anesthetics (sevoflurane and isoflurane) were not effective. The specific effects of the IV drugs were only observed during excitation of inhibitory interneurons, because EPSP did not show frequency- or use-dependent changes, and the modification was dependent on the pre-pulse stimulation (activation of inhibitory interneurons). Our data suggest GABAergic mechanisms may be responsible for the modification. Because the $GABA_A$ agonist (muscimol), $GABA_B$ agonist (baclofen), or GABA uptake inhibitor (nipecotic acid) did not produce frequency- or use-dependent modification, the observed phenomenon is likely not due to actions at postsynaptic GABA receptors or GABA reuptake.

To assess the frequency- and use-dependent modification of IV anesthetics, we used the pre-pulse train protocol to accelerate neurotransmitter (GABA) release. In hippocampal neurons, a single action potential releases 0.5% of the neurotransmitter pool from presynaptic terminals.[15] Therefore, the pre-pulse train could first enhance GABA release and attenuate the PS amplitude (phase I). Subsequently, >200 pulses of a pre-pulse train could release all the active pool of GABA and temporally deplete readily releasable neurotransmitter (phase II). IV drugs depressed phase I and prolonged the onset of phase II, whereas volatile anesthetics had a minimal effect on phase I without changing in configuration of phase II. This suggests that the IV anesthetics enhance GABA release at presynaptic terminals.

Presynaptic neurotransmitter release occurs by a process of vesicle exocytosis.[16] Studies of cultured hippocampal cells found a very small active pool of neurotransmitter vesicles and a large number of resting vesicles at the presynaptic terminals.[17] After neurotransmitter release, the fusion pores seal rapidly, and the vesicles immediately refill with neurotransmitter. The entire process of neurotransmitter release, uptake, and repriming occurs within several seconds.[15]

Taken together with these data, we propose a mechanism for the frequency-dependent effect of IV anesthetics (Fig. 5). IV anesthetics enhance the release of the active pool of GABA vesicles from presynaptic terminals. In the presence of low-frequency stimuli, the enhanced GABA release produces depression of neural excitation. High-frequency stimulation depletes the active pool of vesicles from the presynapse, as a result of the resting vesicles not being

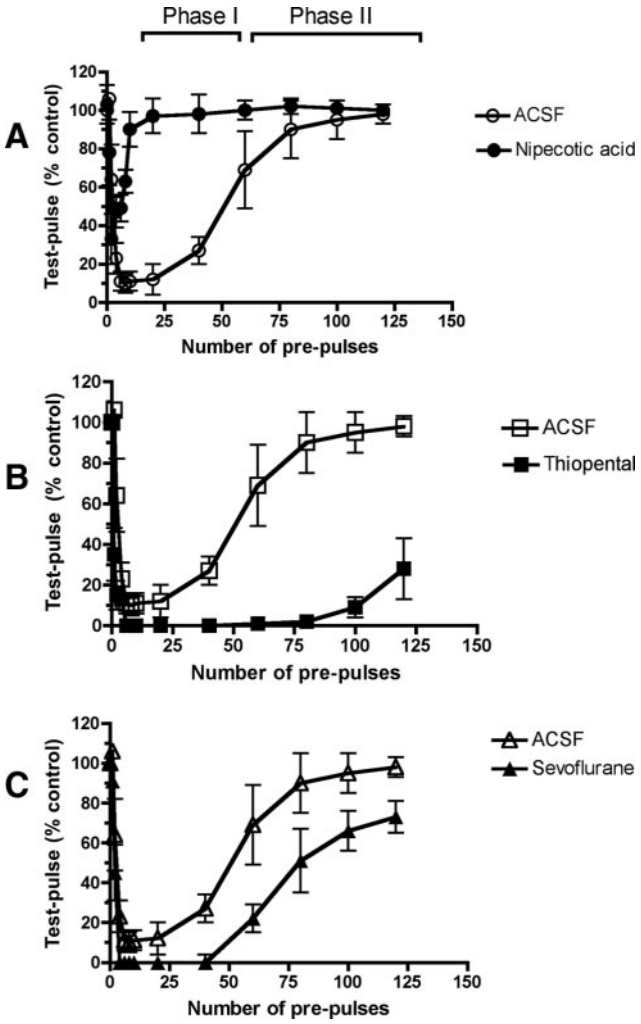

Figure 4. A, Relations between number of pre-pulses and the test–population spike (PS) amplitudes in the absence and presence of nipecotic acid (10^{-3} mol/L, $n = 5$). Phase I: Enhancement of γ-aminobutyric acid (GABA) release from presynaptic terminal. Phase II: Depletion of GABA at presynaptic terminal. Closed circles (nipecotic acid) were significantly different ($P < 0.0001$) from closed circles (artificial cerebrospinal fluid [ACSF]). B and C, Relations between number of pre-pulses and the test-PS amplitudes in the presence of thiopental (2×10^{-5} mol/L, $n = 5$, trace B) and sevoflurane (3.0 vol%, $n = 5$, trace C). Closed symbols (thiopental, sevoflurane) were significantly different ($P < 0.0001$) from closed symbols (ACSF). Data represent mean ± SD.

recruited to releasable vesicles within seconds, resulting in disinhibition of test-PS amplitudes.

Although the anesthetic-induced frequency-dependent modification was observed at an extremely low frequency range (0.001–0.1 Hz), it has been reported that synaptic modulation occurs on a slow timescale in the central nervous systems,[18] and that IV anesthetics prolong GABA-mediated inhibition more than seconds.[19,20] Therefore, it can take seconds to recruit GABA to a readily releasable pool at presynaptic terminals in the presence of IV anesthetics. Our data emphasize that the enhancement of GABA discharge at a very low input frequency might be one of the most important mechanisms for IV anesthetic actions in the central nervous system.

The use-dependent disinhibition could be based on the same mechanisms as the frequency-dependent modification. Five minutes of rest is sufficient time for vesicle repriming after depletion of the active pool of neurotransmitters with control stimulation (1 Hz).[17] The first stimulus enhances the neurotransmitter release in the presence of IV anesthetics, and the subsequent stimuli gradually deplete the active pool of vesicles, resulting in the use-dependent disinhibition of PS amplitudes.

Halothane was reported to reduce glutamatergic excitation of inhibitory interneurons in hippocampus,[21,22] and the hypnotic action of propofol was found to be closely related to presynaptic GABA content of the inhibitory interneurons.[23] However, the relationships between modification of inhibitory synaptic transmission and presynaptic function in the presence of various anesthetics were not known. This study has clearly demonstrated the difference between IV and volatile anesthetics with regard to presynaptic functions at interneurons.

Previous brain slice experiments revealed that relatively high concentrations of IV anesthetics were required to produce inhibitory actions on synaptic transmission. This differs from volatile anesthetics that were effective at concentrations (i.e., 50% effective dose values) close to the minimal alveolar concentration. The reasons for the paradox have been attributed to different diffusion characteristics between brain slices and intact brain tissues. These results, however, revealed that clinically relevant concentrations of IV anesthetics produce significant inhibitory actions at low-frequency stimulation. Because IV anesthetics enhance GABA release from presynaptic terminals, the

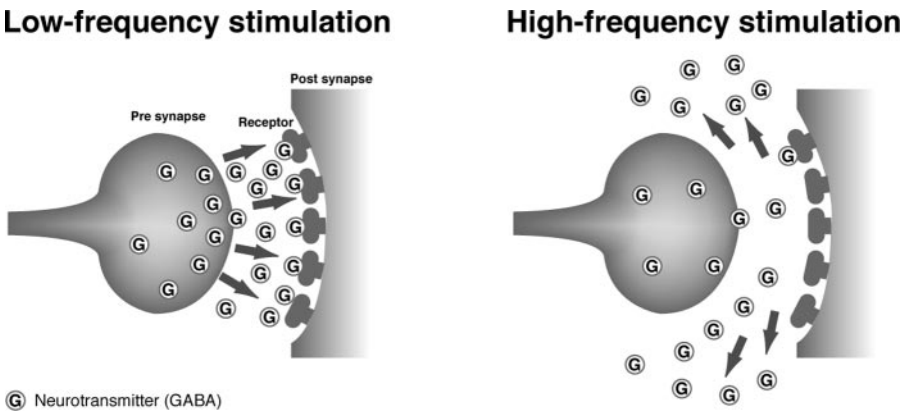

Figure 5. Suggested mechanisms of the frequency-dependent modification of inhibitory synaptic transmission by thiopental. Thiopental enhances γ-aminobutyric acid (GABA) release from the presynaptic terminal. The high-frequency stimuli gradually deplete the readily releasable GABA at presynapse, resulting in the disinhibition of test–population spike amplitudes.

conventional 0.1-Hz stimulation would deplete the readily releasable neurotransmitters, resulting in the necessity for higher concentrations.

Recent functional brain imaging studies revealed that anesthetic inhibition of hippocampal function could be responsible for amnesia rather than loss of consciousness.[24] The role of inhibitory (i.e., GABA) interneurons in the hippocampus as regulators of neuronal activity may be too simplistic.[25] A greater understanding of the complexity of their functions may provide a better understanding of the actions of general anesthetics. We previously reported the mechanisms by which extracellular magnesium ion modifies the actions of general anesthetics,[26] and the prophylactic effects of general anesthetics on anoxia using the same preparations.[27] Because the preparations used in this study can be obtained from different age, sex, or many kinds of disease model animals, this model is useful not only to investigate electrophysiological mechanisms of anesthetics but also to simulate the modification of anesthesia under various clinical conditions.

Clinical anesthesiologists often experience unsuccessful immobilization or unexpected hypnosis occurs more frequently during IV anesthesia than inhaled anesthesia. Surgical stress increases the frequency of neural discharges in the central nervous system. These results indicate that the increased input frequency would produce the disinhibition of IV anesthetic actions. Frequency-dependent modification might explain, in part, the variability in anesthetic depth observed during clinical anesthesia.

AUTHOR CONTRIBUTIONS

KH helped to design the study, analyze the data, and write of manuscript. RS helped to analyze the data. SHR helped to design the study and write the manuscript. MY helped to conduct the study.

REFERENCES

1. MacIver MB, Roth SH. Inhalation anesthetics exhibit pathway-specific and differential actions on hippocampal synaptic responses in vitro. Br J Anaesth 1988;60:680–91
2. MacIver MB, Kendig JJ. Anesthetic effects on resting membrane potential are voltage-dependent and agent-specific. Anesthesiology 1991;74:83–8
3. Collins JG, Kendig JJ, Mason P. Anesthetic actions within the spinal cord: contributions to the state of general anesthesia. Trends Neurosci 1995;18:549–53
4. Wakasugi M, Hirota K, Roth SH, Ito Y. Effects of general anesthetics on excitatory and inhibitory synaptic transmission in area CA1 of the rat hippocampus in vitro. Anesth Analg 1999;88:676–80
5. Asahi T, Hirota K, Sasaki R, Roth SH, Yamazaki M. The intravenous anesthetics have more effect on the inhibitory pathways than volatile anesthetics in rat hippocampal CA1. Anesth Analg 2006;102:772–8
6. Hirota K, Roth SH. Sevoflurane modulates both GABAA and GABAB receptors in area CA1 of rat hippocampus. Br J Anaesth 1997;78:60–5
7. Hirota K, Roth SH, Fujimura J, Masuda A, Ito Y. GABAergic mechanisms in the action of general anesthetics. Toxicol Lett 1998;100:203–7
8. Mantz J, Lecharny JB, Laudenbach V, Henzel D, Peytavin G, Desmonts JM. Anesthetics affect the uptake but not the depolarization-evoked release of GABA in rat striatal synaptosomes. Anesthesiology 1995;82:502–11
9. Westphalen RI, Hemmings HC Jr. Effects of isoflurane and propofol on glutamate and GABA transporters in isolated cortical nerve terminals. Anesthesiology 2003;98:364–72
10. Hirota K, Roth SH. The effects of sevoflurane on population spikes in CA1 and dentate gyrus of the rat hippocampus in vitro. Anesth Analg 1997;85:426–30
11. Papatheodoropoulos C, Kostopoulos G. Development of a transient increase in recurrent inhibition and paired-pulse facilitation in hippocampal CA1 region. Dev Brain Res 1998;108:273–85
12. Winder DG, Sweatt D. Roles of serine/threonine phosphatases in hippocampal synaptic plasticity. Nat Rev Neurosci 2001;2:461–74
13. Richards CD. On the mechanism of barbiturate anaesthesia. J Physiol (Lond) 1972;227:749–67
14. Kazama T, Ikeda K. Comparison of MAC and the rate of rise of alveolar concentration of sevoflurane with halothane and isoflurane in the dog. Anesthesiology 1988;68:435–7
15. Ryan TA, Smith SJ. Vesicle pool mobilization during action potential firing at hippocampal synapses. Neuron 1995; 14:983–9
16. Stevens CF, Williams JH. "Kiss and run" exocytosis at hippocampal synapses. Proc Natl Acad Sci USA 2000;97:12828–33
17. Pyle JL, Kavalali ET, Piedras-Renteria ES, Tsien RW. Rapid reuse of readily releaseble pool vesicles at hippocampal synapses. Neuron 2000;28:221–31
18. Haydon PG. Glia: listening and talking to the synapse. Nat Rev Neurosci 2001;2:185–93
19. Albertson TE, Tseng C-C, Joy RM. Propofol modification of evoked hippocampal dentate inhibition in urethane-anesthetized rats. Anesthesiology 1991;75:82–90
20. Albertson TE, Walby WF, Joy RM. Modification of GABA-mediated inhibition by various injectable anesthetics. Anesthesiology 1992;77:488–99
21. Perouansky M, Kirson ED, Yaari Y. Halothane blocks synaptic excitation of inhibitory interneurons. Anesthesiology 1996; 85:1431–8
22. Nishikawa K, MacIver MB. Membrane and synaptic actions of halothane on rat hippocampal neurons and inhibitory interneurons. J Neurosci 2000;20:5915–23
23. Kubo K, Nishikawa K, Hardy-Yamada M, Ishizeki J, Yanagawa Y, Saito S. Altered response to propofol, but not ketamine, in mice deficient in the 65-kilodalton isoform of glutamate decarboxylase. J Pharmacol Exp Ther 2009;329:592–9
24. Franks P. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci 2008;9:370–86
25. McBain C, Fisahn A. Interneurons unbound. Nat Rev Neurosci 2001;2:11–23
26. Sasaki R, Hirota K, Roth SH, Yamazaki M. Extracellular magnesium ion modifies the actions of volatile anesthetics in area CA1 of rat hippocampus in vitro. Anesthesiology 2002;96:681–7
27. Sasaki R, Hirota K, Roth SH, Yamazaki M. Anoxic depolarization of rat hippocampal slices is prevented by thiopental but not by propofol or isoflurane. Br J Anaesth 2005;94:486–91