Volatile Anesthetics Depress Glutamate Transmission Via Presynaptic Actions

M. Bruce MacIver, M.Sc.,† Anthony A. Mikulec, B.A., B.S.,† Shanti M. Amagasu, B.Sc., M.S.,‡† Frances A. Monroe, B.A.§

Background: Recent evidence for a presynaptic depression of glutamate release produced by volatile anesthetics prompted the current study of isoflurane and halothane effects on glutamate-mediated transmission in the mammalian central nervous system.

Methods: Electrophysiologic recordings from CA1 neurons in rat hippocampal brain slices were used to measure anesthetic effects on glutamate-mediated excitatory postsynaptic potential (EPSP) amplitudes and paired pulse facilitation. Paired pulse facilitation is known to be altered when the calcium-dependent release of glutamate is depressed, but not when EPSP amplitudes are depressed by postsynaptic mechanisms.

Results: Isoflurane depressed EPSP amplitudes over a concentration range of 0.35–2.8 vol %, with a 50% depression (EC50) occurring at 1.0 vol % (0.71 rat minimum alveolar concentration). This depression was accompanied by an increase in paired-pulse facilitation of ~30% at 1.7 vol %, using interpulse intervals of 120 ms. Halothane depressed EPSP amplitudes in a concentration-dependent manner (0.3–2.4 vol %, EC50 = 1.1 minimum alveolar concentration; 1.3 vol %) and also increased facilitation by ~20% at 1.2 vol %. These effects persisted in the presence of 10 μM bicuculline, indicating that enhanced gamma-aminobutyric acid-mediated inhibition was not involved. The anesthetic-induced increase in facilitation and EPSP depression was mimicked by lowering extracellular calcium, which is known to depress glutamate release at these synapses. The postsynaptic glutamate receptor antagonist 6-cyano-7-nitroquinolxaline-2,3-dione depressed EPSP amplitudes with no change in facilitation.

Conclusions: Our results confirm earlier findings that clinically relevant concentrations of volatile anesthetics depress glutamate-mediated synaptic transmission. The observed increases in synaptic facilitation support recent findings from biochemical and electrophysiologic studies indicating presynaptic sites of action contribute to anesthetic-induced depression of excitatory transmission. This anesthetic-induced reduction in glutamate release would contribute to the central nervous system depression associated with anesthesia by adding to postsynaptic depressant actions on glutamate receptors. (Key words: Anesthetics, volatile; halothane; isoflurane; Brain; CA1 neuron; hippocampal slices; synapses; synaptic transmission. Measurement techniques, electrophysiologic recordings; EPSP facilitation. Neurotransmitters, glutamate; release; GABA.)

AMINO acid neurotransmitters, glutamate and gamma-aminobutyric acid (GABA), are used by a majority of synapses in higher brain centers. Thus, anesthetic actions at amino acid-mediated synapses would have important consequences for central nervous system (CNS) excitability. There is a growing consensus that general anesthetics enhance GABA-mediated synaptic inhibition,1,2 and depress excitatory glutamate-mediated transmission.3,4 Enhanced inhibition is thought to involve an anesthetic-induced prolongation of postsynaptic chloride currents.5,6 In contrast, depression of excitatory synaptic transmission could result from a decrease in transmitter release; this has been postulated for a number of years based on results from electrophysiologic studies using peripheral synapse preparations.7 Weakly8 was the first to demonstrate that anesthetics reduce the quantal content of motorneuron excitatory postsynaptic potentials (EPSP), but do not depress miniature EPSP amplitudes, suggesting that a reduction in evoked transmitter output could occur. In the CNS, clinical concentrations of pentobarbital reduced the amount of L-glutamate and L-aspartate released from lateral olfactory tract fibers.9 Propofol also depressed glutamate release from rat synaptosomes10. glutamate uptake and synaptosomal adenosine triphosphatase activity were also altered by low concentrations (2.5 to 35 μM) of propofol. Similar effects have been reported for several anesthetics on amino acid

†Assistant Professor of Neurophysiology, Stanford Anesthesia.
‡M.D. Candidate, Stanford University School of Medicine.
§M.S. Graduate Student, Stanford Biological Sciences.
¶Research Assistant, Stanford University School of Medicine.

Received from the Department of Anesthesia, Stanford University School of Medicine, Stanford, California. Submitted for publication January 22, 1996. Accepted for publication May 25, 1996. Supported by National Institutes of Health grant GM49811. Presented in part at the annual meeting of the American Society of Anesthesiologists, San Francisco, California, October 15–19, 1994.

Address reprint requests to Dr. MacIver: Department of Anesthesia, Stanford, California 94305-5117. Address electronic mail to: bruce.maciver@forsythe.stanford.edu.

Anesthesiology. V 85, No 4, Oct 1996
release from in vitro slices of thalamus. Thiopental, ketamine, and halothane have been shown to decrease glutamate concentrations in rat brain stem and cortex, in vivo, but this could reflect an overall depression of neuronal activity associated with anesthesia. In the current study, volatile anesthetic actions at Schaffer-collateral fiber glutamate synapses on CA1 pyramidal neurons were studied using paired-pulse facilitation of EPSP responses as a measure of presynaptic actions. Paired-pulse facilitation is known to increase after manipulations that reduce calcium-mediated glutamate release from Schaffer-collateral fibers. For example, lowering the external calcium concentration produces a depression of CA1 neuron EPSP amplitudes that results from a decrease in calcium influx through voltage-gated channels in presynaptic nerve terminals, secondary to a reduction in the driving force for calcium. This EPSP depression was inversely correlated with paired-pulse facilitation. Similarly, adenosine and N-methyl-D-aspartate are known to depress CA1 neuron EPSP amplitudes by reducing a G-protein-linked calcium current in Schaffer-collateral nerve terminals, which also is accompanied by an increase in paired-pulse facilitation. In contrast, manipulations which alter CA1 neuron EPSPs via postsynaptic actions do not appear to alter paired-pulse facilitation. Recent studies showing anesthetic-induced depression of voltage-activated calcium currents (particularly Q- and N-type channels involved in transmitter release) provide one possible mechanism for anesthetic-induced depression of transmitter release.

The extent to which volatile anesthetic-induced CNS depression results from reduced transmission at excitatory glutamate-mediated synapses remains controversial. In vivo studies of anesthetic effects on Schaffer-collateral to CA1 neuron synapses in rats have reported either marked depression or little observable effect. Several in vitro studies have demonstrated anesthetic-induced depression of glutamate synapses, with both presynaptic and postsynaptic actions appearing to contribute to the depression. Some differences in results could be explained by the different temperatures used by various groups investigating anesthetic effects on glutamate synapses. The current study investigated the effects produced by halothane and isoflurane on excitatory postsynaptic potentials at 22°C, to allow comparison with our earlier studies at physiologic temperatures (35°C). Materials and Methods

Experiments were conducted on brain slices isolated from adult male Sprague-Dawley rats (weighing 90-110 g). Animals were housed with a 12-h light cycle with food and water provided ad libitum, and were anesthetized with halothane or diethyl ether before use. While deeply anesthetized, rats were killed by a blow to the back of the thorax to stop the heart and reduce bleeding as the brain was removed. At all stages of handling, the research protocol was approved by the Stanford University Animal Research Committee, sanctioned by the Council of Laboratory Animal Medicine (USA), and adhered to National Institutes of Health guidelines. For experiments conducted at 35°C an interface brain slice chamber was used, as previously described. Room temperature (22 ± 1.7°C, mean ± SD, n = 30) experiments were conducted using a submission chamber.

Brain Slice Preparation

Coronal brain slices (450 µm) were prepared on a vibratome (Vibraslice, Boston, MA), hemisected and placed on filter papers at the interface of a humidified oxygen/carbon dioxide (95%/5%) carbogen gas phase and artificial cerebrospinal fluid (ACSF; see Materials) liquid phase. Slices were allowed at least 1 h for recovery before being submerged in ACSF in a recording chamber. The ACSF was saturated with carbogen gas and perfused at a rate of 2.5 ml/min, at room temperature. Rapid and accurate solution changes were made using an AutoMate (Oakland, CA) computerized perfusion system, and high-quality polytetrafluoroethylene was used for reservoirs, valves, and tubing to minimize volatile anesthetic loss and drug binding. Volatile agent concentrations were adjusted using calibrated commercial vaporizers (Fraser Harlake, Orchard Park, NY) and a Puritan-Bennett UV anesthetic agent monitor (Model 222, Wilmington, MA) to measure concentrations. Anesthetic concentrations in the ACSF were measured using calcium-sensitive electrodes to ensure that steady-state concentrations were achieved.

Electrophysiology

Bipolar tungsten microelectrodes (0.5 MΩ, Frederick Haer, Brunswick, ME) were placed in stratum radiatum


Anesthesiology, V 85, No 4, Oct 1996.
to electrically activate Schaffer-collateral fibers (fig. 1). Electrical stimuli consisted of square wave paired pulses (0.25 ms duration; 5–8 V) delivered every 10 s, to minimize frequency-dependent changes in transmission, from digitally timed and controlled isolation units (Grass Instruments, Quincy, MA). An interpulse interval of 120 ms was used to study paired pulse facilitation and was varied between 10 and 1000 ms to determine the time course of facilitation. Glass microelectrodes filled with ACSF (0.5–1.0 MΩ, Garner Glass Company, Claremont, CA) were placed in stratum radiatum to record EPSPs, or at the stratum pyramidale/oriens border to record evoked population spikes (fig. 1). Recorded signals were amplified (10,000×), filtered (1.0 Hz to 50 kHz, bandpass) and digitized at 50 kHz for computer storage and analysis (Data Wave Technologies, Longmont, CO). Response measures were plotted on-line during experiments to ensure stable baseline activity (≤5% variation for 20 min) was achieved from each preparation before the administration of anesthetics.
Data Analysis

Excitatory postsynaptic potential amplitudes were measured as peak negativity from baseline. Population spike amplitudes were measured from peak negativity to peak positivity. All measurements were made using Data Wave Technologies software. Anesthetic effects were expressed as a percent of control (effect measure/control measure × 100) and the mean ± SD was calculated based on measures from at least eight preparations. Each preparation was exposed to only a single concentration of a given anesthetic, to avoid possible residual drug effects. Statistical significance was determined using analysis of variance (ANOVA; AXUM, Tri-Metrix, Seattle, WA) to compare time-matched control responses with those produced by the anesthetics.

Materials

The ACSF had the following ionic composition (in mm): Na⁺ - 151, K⁺ - 3.5, Ca²⁺ - 2.0, Mg²⁺ - 2.0, Cl⁻ - 131, HCO₃⁻ - 26, SO₄²⁻ - 2.0, PO₄³⁻ - 2.0, PO₃⁻ - 1.25, glucose - 10. All chemicals were reagent grade or better, obtained from J.T. Baker Chemical Company (Phillipsburg, NJ). Water for solutions was spectrophotometric and high-perfusion liquid chromatographic grade, obtained from EM Sciences (Gibbstown, NJ). 6-Cyano-7-nitroquinolin-2,3-dione and 2-hydroxypropyl-β-cyclodextrin (CNQX:HBC complex) and APV (±)-2-amino-5-phosphonopentanoic acid) and bicuculline methiodide were obtained from RBI (Natick, MA). Halothane was obtained from Halocarbon Laboratories (North Augusta, SC). Isoflurane was obtained from Abbott Laboratories (North Chicago, IL). All solutions, including concentrated drug stocks, were made fresh before each experiment. Rats were obtained from Simonsen Laboratories (Gilroy, CA) and housed under the continuous care of technologists and veterinarians in the Stanford University Department of Laboratory Animal Medicine facilities.

Results

Comparison of Anesthetic Effects and Synaptic Facilitation at 22°C and 35°C

Synaptically evoked population spike (PS) responses recorded from CA1 neurons were similar in appearance at the two temperatures, although response amplitude was smaller and the time course was prolonged at 22°C. After Schaffer-collateral stimulation, a single negative

Fig. 2. Volatile anesthetics produced a marked depression of excitatory postsynaptic potential amplitudes at clinically effective concentrations. Each panel shows Schaffer-collateral evoked excitatory postsynaptic potentials recorded in stratum radiatum of the CA1 area (top traces), together with a graph showing the time course of excitatory postsynaptic potential amplitude depression (expressed as a percent of control). Isoflurane- (A) and halothane-induced (B) depression of responses were mimicked by reducing extracellular calcium concentrations from 2.0 mM control levels to 1.5 mM (C).
PRESYNAPTIC ACTIONS OF ANESTHETICS

Fig. 3. Isoflurane and halothane produced a concentration-dependent depression of excitatory postsynaptic potential amplitudes. The concentration-response curves did not superimpose when plotted on a log minimum alveolar concentration axis, indicating that the two anesthetics were not equipotent at depressing glutamate-mediated excitatory synaptic transmission. Isoflurane (EC\textsubscript{50} = 0.71 minimum alveolar concentration) was ~1.5 times more potent than halothane (EC\textsubscript{50} = 1.1 minimum alveolar concentration), but both agents produced comparable depression of excitatory postsynaptic potentials over the clinically relevant range (0.5–2.0 minimum alveolar concentration). Each point represents the mean ± SD from at least eight determinations.

Compound action potential (PS) was superimposed on a slower positive potential (EPSP) seen at both temperatures (fig. 1). Population spike half decay constants were 1.5 times slower at 22°C (3.9 ± 0.19 ms; mean ± SD; n = 8) compared with 35°C (2.57 ± 0.35 ms; n = 5). Excitatory postsynaptic potential decay times were slowed by 1.4, from 9.7 ± 0.9 ms at 35°C to 13.2 ± 0.8 ms at 22°C. Decay time constants for both PS and EPSP responses were significantly (P < 0.005, ANOVA) slowed at 22°C.

Population spike responses were nearly abolished in the presence of 1 minimum alveolar concentration (MAC) of halothane (1.2 vol %, fig. 1) or isoflurane (1.4 vol %), regardless of temperature. The profiles of anesthetic effects were also similar at both temperatures, with an increase in PS latency accompanying depression of responses, although discharge thresholds were not altered. Halothane produced a 98.2 ± 3.1% depression of PS amplitude at 22°C and a 97.4 ± 4.2% depression at 35°C. Isoflurane produced a 98.7 ± 3.5% depression at 22°C and a 98.5 ± 4.2% depression at 35°C. There were no significant differences observed between halothane and isoflurane effects (P > 0.1, ANOVA, n = 11) or anesthetic effects at either temperature (P > 0.1). Anesthetic effects were comparable at both temperatures despite the observed slowing of PS voltages observed at 22°C and the expected increase in agent/ACSF solubility. Concentration-dependent depression of PS amplitudes was also observed at 0.25 and 0.5 MAC concentrations for both anesthetics at 22°C and 35°C. To further characterize temperature effects on CA1 circuitry, latency profiles for EPSP and PS facilitation were studied.

The time course of EPSP facilitation reflects presynaptic, calcium-dependent, vesicle release processes in Schaffer-collateral nerve terminals (see Discussion). When two identical stimuli were used to generate action potentials in these presynaptic fibers, the second EPSP amplitude was larger than the first for interstimulus intervals between 10 and ~500 ms. Peak facilitation occurred at 20–30 ms intervals (~40 Hz), and facilitation slowly declined during the next 300–600 ms. Facilitation remained intact at 22°C, and latency profiles were similar to those at 35°C (fig. 1), especially for responses at longer interstimulus intervals (>100 ms). Lowering the temperature did not appear to slow calcium-dependent release mechanisms in these nerve terminals, and comparable levels of facilitation (120–150%) were observed at both 22°C and 35°C.

Population spike facilitation provided a measure of postsynaptic feedforward and feedback inhibition. An early period of inhibition (0–20 ms intervals, fig. 1) results from the activation of voltage-dependent potassium channels (I\textsubscript{Ks}) and GABA\textsubscript{A}-gated chloride channels, which combine to limit CA1 neuron discharge. Maximal spike facilitation occurred with interstimulus intervals of 20–30 ms, similar to EPSP facilitation. With interstimulus intervals >40 ms, PS facilitation was markedly reduced, reflecting the activation of late inhibitory currents, including I\textsubscript{Ks}, I\textsubscript{KCl}, and GABA\textsubscript{A}. These late currents depressed spike discharge even though excitatory synaptic drive remained elevated (compare the time course of EPSP and PS facilitation, fig. 1). Lowering the temperature to 22°C did not significantly (P > 0.05 compared to 35°C) change the interstimulus latency profile for PS facilitation. Early inhibitory responses appeared to decay sooner at 35°C; late inhibition activated somewhat earlier and also appeared to decay sooner. These results, together with a similar EPSP facilitation profile, indicate that both excitatory and inhibitory synaptic circuits remained in-

Anesthesiology. V 85, No 4, Oct 1996
tact at 22°C. This temperature (22°C) was used for all subsequent experiments.

**Volatile Anesthetics Depressed Excitatory Synaptic Transmission**

Clinically effective concentrations (0.5–2.0 MAC) of both isoflurane and halothane produced a marked and consistent depression of Schaffer-collateral evoked EPSPs. The time course of anesthetic effects was slow at 22°C; 10–20 min was required to reach steady-state levels of depression and recovery required a similar amount of time (fig. 2). Onset and recovery kinetics followed a first-order exponential for both anesthetics, but isoflurane had a faster equilibration time (16.2 ± 0.6 min vs. 18.7 ± 1.4 min for halothane, mean ± SD, P < 0.01, n = 8 for each agent). Decreasing extracellular calcium concentrations from 2.0 to 1.5 mm produced a 70.4 ± 13.6% depression of EPSP amplitude with a faster equilibration time of 11 ± 0.7 min (n = 6).

**Anesthetic-induced Excitatory Postsynaptic Potential Depression was Concentration-Dependent**

Isoflurane produced a 75.8 ± 5.2% depression of EPSP amplitudes at a concentration of 1.7 vol% (1.2 rat MAC; mean ± SD, n = 11). Halothane was less potent and produced a 46 ± 9.4% depression at a concentration of 1.2 vol% (1.0 rat MAC; n = 8). Isoflurane was significantly more potent than halothane at low concentrations (0.5 MAC; P < 0.001, ANOVA, fig. 3), but the anesthetics were equipotent at higher concentrations (1.5–2.0 MAC). The concentration for 50% depression (EC50) of EPSP responses was 0.71 MAC for isoflurane and 1.1 MAC for halothane, determined from a least-squares fit of the concentration-response data (fig. 3).

**Gamma-aminobutyric Acid-mediated Inhibition and Anesthetic-Induced Excitatory Postsynaptic Potential Depression**

It was unlikely that enhanced recurrent GABA-mediated inhibition contributed to anesthetic-induced EPSP depression, because stimulus intensities were below threshold for producing CA1 discharge. Anesthetic-enhanced feedforward inhibition, however, could have contributed by shunting EPSP dendritic currents. To test whether feedforward inhibition was involved, the GABAA receptor antagonist bicuculline was studied. Bicuculline (10 μM) did not significantly alter EPSP amplitudes when tested alone (105 ± 3.7% of control; n = 5; P > 0.01, ANOVA) and produced only a small (<5%) reversal of responses to 1 MAC halothane (42.7 ± 9.9% depression, n = 5; P > 0.5 compared with halothane alone). A concentration of 10 μM bicuculline is known
PRESYNAPTIC ACTIONS OF ANESTHETICS

Fig. 5. Postsynaptic block of glutamate receptors with CNQX did not alter excitatory postsynaptic potential facilitation (A), but lowered calcium (B) and anesthetic-induced (C and D) depression of excitatory postsynaptic potential amplitudes was accompanied by an increased facilitation. Each panel shows paired pulse excitatory postsynaptic potential recordings (top) and enlarged overlays for comparing effects on fiber volley amplitudes and facilitation (bottom). For facilitation, depressed excitatory postsynaptic potential responses were rescaled to match control first excitatory postsynaptic potential amplitudes (bottom right; calibration bars are for control responses).

to depress electrically evoked monosynaptic and spontaneous GABA-mediated inhibitory postsynaptic currents by >90% at both 22°C and 35°C. The small effect of bicuculline to reverse anesthetic-induced EPSP depression was not accompanied by a change in facilitation, indicating that enhanced feedforward inhibition did not contribute to this presynaptic measure.

Paired Pulse Facilitation of Excitatory Postsynaptic Potential Responses Did Not Depend on Amplitude

An interstimulus interval of 120 ms was used to study paired pulse facilitation of Schaffer-collateral EPSP responses, because this interval produced a comparable degree of facilitation at both 22°C and 35°C (fig. 1). In control conditions using a stimulus intensity of 75% of maximal response, EPSP facilitation was 1.22 ± 0.07 (n = 15, ~20% increase in amplitude of the second EPSP compared to the first). When stimulus intensity was decreased to produce smaller EPSP responses, facilitation did not change even for responses that were <10% of control amplitudes (fig. 4). A change in facilitation should not have occurred because decreasing the stimulus intensity reduced the number of Schaffer-collateral fibers activated, but would not change the characteristics of synaptic transmission. Each nerve ending should still be depolarized by the same amount because the action potential in activated fibers would not change. Similarly, reducing EPSP amplitudes by blocking postsynaptic glutamate receptors with a competitive antagonist (CNQX 10 μM) did not change paired pulse facilitation (fig. 5A). CNQX produced a 73.4 ± 1.9% depression of EPSP amplitudes (n = 6), with no apparent change in presynaptic action potential amplitude or conduction velocity, since Schaffer-collateral fiber volley responses superimposed before and after CNQX application (fig. 5A).

Decreased Calcium Influx and Anesthetics Increased Synaptic Facilitation

When EPSP amplitudes were reduced by decreasing extracellular calcium concentrations (control = 2.0 mM), facilitation was increased to 131.2 ± 7.6% (1.5

Anesthesiology, V 85, No 4, Oct 1996
depression and increased facilitation produced by volatile anesthetics and lowered calcium. Best fit lines are shown for at least 20 data points at various anesthetic (0.25–2 minimum alveolar concentration) or calcium concentrations and error bars show SEM for five determinations at 50% depression of excitatory postsynaptic potential amplitudes. Differences were significant (P < 0.01 analysis of variance) for comparisons of facilitation at 50% excitatory postsynaptic potential depression produced by each anesthetic compared to the increase produced by lowered calcium.

Discussion

Isoflurane and halothane depressed glutamate-mediated excitatory synaptic transmission in a concentration-dependent manner. Significant depression of EPSP amplitudes occurred at 0.5 MAC for both anesthetics; half maximal depression occurred at 0.71 MAC for isoflurane and 1.1 MAC for halothane. These results confirm previous findings that volatile anesthetics24,30,39 and especially halogenated ethers31 depress glutamate-mediated transmission at clinically relevant concentrations. The increase in facilitation that accompanied EPSP depression is consistent with a presynaptic anesthetic action to reduce glutamate release from Schaffer-collateral terminals. These findings confirm earlier electrophysiologic results from brain slices at physiologic temperatures29,31 and agree with recent findings from biochemical studies of anesthetic-induced depression of glutamate release.29,39

Synaptic Responses Remained Intact at Lowered Temperatures

Decreasing the temperature had little effect on Schaffer-collateral evoked synaptic responses (fig. 1) or on second-order physiologic responses such as EPSP and PS facilitation. A 15°C temperature decrease (from 35°C...
PRESYNAPTIC ACTIONS OF ANESTHETICS

Fig. 7. Volatile anesthetics reduced presynaptic transmitter release, possibly via a depression of calcium influx into nerve terminals, observed as an increase in exocytotic postsynaptic potential facilitation. Facilitation occurs after calcium entry into a nerve terminal, secondary to action potential-induced opening of voltage-operated calcium channels (VOCC; A). Calcium influx causes vesicle release and promotes vesicle docking. The calcium-induced docking after the first of a pair of action potentials (B) promotes increased transmitter release, because more vesicles dock and may be released after the second action potential. Facilitation increases with each stimulus evoked action potential until all docking/release sites become occupied, or until action potentials are separated by enough time to allow undocking (500–1,000 ms). Vesicle docking and release could be differentially controlled by calcium (C); with low calcium concentrations promoting vesicle docking whereas higher concentrations cause release. Facilitation increases, but release decreases when calcium influx is reduced (e.g., in low extracellular calcium). Anesthetics could act at any of a number of sites to depress transmitter release and increase facilitation, because the process of release is known to involve several proteins associated with vesicle and nerve terminal membranes (D). A simple explanation would be that anesthetics depress calcium channels in presynaptic terminals, but this could come about via actions on synaptotagamin, or syntaxin, which are known to bind to voltage-operated calcium channels and form a vesicle docking complex (together with SNAP, VAMP, synaptophysin, TAP, actin, and other proteins).  

to 22°C slowed synaptic and spike discharge responses by 1.5- to 2.0-fold, but a comparable level of EPSP facilitation was observed (~120% for interstimulus intervals of 100 to 200 ms). Similarly, the time course of PS facilitation did not change significantly, nor did the early and late stages of inhibition, which limit spike discharge. These results agree with previous findings from hippocampal brain slices 40,41 and from rats with chronically implanted hippocampal electrodes. 42,43 Hippocampal synaptic currents appear to slow with a Q10 of ~1.5. Excitable membranes and CNS function adapt well to decreasing temperatures 44,45 and rats remain capable of learning motor tasks at brain temperatures of 28–30°C. 46 Long-term potentiation of hippocampal synaptic responses remains functional at temperatures above 20°C. 46 Although cooling can produce anesthesia, rodents remain conscious at brain temperatures from ~20–40°C. 47

Anesthetic Effects Were Not Altered by Cooling Volatile anesthetic-induced depression of population spike responses were not significantly different at 22°C compared with effects at 35°C. The increase in volatile agent solubility that occurs at 22°C was offset by evaporative loss in the submersion chamber, whereas experiments conducted at 35°C were performed in a closed chamber where the ACSF concentrations were in equilibrium with gas phase concentrations. Preliminary experiments using an ion-sensitive electrode to measure aqueous halothane concentrations in both chambers (22°C and 35°C), indicated that similar concentrations of anesthetic were present (220–250 μM at 1.0 MAC, P > 0.05). Thus, comparable concentrations of halothane appeared to produce a similar degree of synaptic depression at 22°C compared with that at physiologic temperatures. This would argue against changes in bulk membrane fluidity contributing to the anesthetic effect, because fluidity would be reduced over two orders of magnitude by a 15°C temperature change compared to increases in fluidity produced by the anesthetics. 45

Anesthetics Depress Excitatory Postsynaptic Potentials Via a Presynaptic Mechanism Increased facilitation produced by halothane and isoflurane was consistent with a presynaptic site of

Anesthesiology, V 85, No 4, Oct 1996
action (fig. 6). Perouansky and coworkers also suggest a presynaptic action to account for the equieffective depression of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and N-methyl-D-aspartate (NMDA)-mediated EPSCs, and lack of halothane effect for exogenously applied glutamate responses, on Schaffer-collateral to CA1 neuron synapses in mouse hippocampal slices at 22°C. Similar conclusions were reached using rat hippocampal slices maintained at physiologic temperatures (35°C). These electrophysiologic results support the direct measures from biochemical studies showing volatile anesthetic-induced depression of glutamate release from brain slices and synaptosomes. This presynaptic action may not account for all of the anesthetic-induced depression of excitatory synaptic responses. There is growing evidence that alcohol, volatile agents, and barbiturates can interfere with postsynaptic glutamate receptor activation and depression of N-methyl-D-aspartate receptors appears to be the major effect produced by ketamine. Similar depressant actions on glutamate receptors also have been reported for propofol. Taken together, there is now good evidence for anesthetic effects at both presynaptic and postsynaptic sites of glutamate synapses. It remains to be determined which sites of action contribute most to depression of glutamate-mediated transmission. These effects at excitatory synapses, in combination with enhanced inhibition at GABA synapses, could account for most of the CNS depression produced by some anesthetics. It remains unknown the extent to which these individual presynaptic and postsynaptic effects contribute to the overall CNS depression, but depression of glutamate transmission accounts for ~50% of hippocampal PS response depression.

Anesthetic Mechanisms of Action at Glutamate Terminals

Although our understanding of the physiology underlying glutamate release from nerve terminals is incomplete, it is apparent that volatile anesthetics can act at several sites to depress transmitter release. Halothane has been shown to depress sodium channels and reduce action potential conduction in Schaffer-collateral fibers. In the current study, halothane depressed fiber volley amplitudes, indicative of a conduction failure in these axons, which could account for some of the EPSP depression observed. Anesthetic-induced depression of fiber conduction, however, would not contribute to the observed increase in EPSP facilitation (figs. 3, 5, and 6). Depression of calcium entry into nerve terminals would account for both the depression of EPSP amplitude and increase in facilitation. Volatile anesthetics have been shown to depress calcium channels in hippocampal neurons and this would provide a likely mechanism contributing to EPSP depression. Coupling among terminal depolarization, calcium entry, and glutamate release involves several proteins associated with both vesicle and nerve terminal membranes (fig. 7). These proteins come together via a calcium/actin-driven process to form a docking complex with a vesicle positioned next to a release site and presynaptic calcium channel. Only calcium channels with docked vesicles appear capable of opening to allow calcium entry during terminal depolarization. Anesthetics could depress glutamate release and alter facilitation by disrupting protein-protein interactions at vesicle docking sites (fig. 7), thus uncoupling calcium entry and vesicle release from nerve terminal depolarization. Such a mechanism, involving a site of action proximal to calcium influx has been proposed to account for volatile anesthetic-induced depression of glutamate release from synaptosomes. The presynaptic and postsynaptic depressant actions produced at glutamate synapses and opposite enhanced postsynaptic actions at GABA synapses provide further evidence that anesthetics act at multiple sites and via unique mechanisms. Differences in effect produced by halothane and isoflurane (e.g., on facilitation and fiber volley conduction) suggest that different mechanisms can contribute to CNS depression produced by halogenated alkanes versus others, in accordance with a multisite agent-specific hypothesis of anesthetic action.

The authors thank their Stanford colleagues Dr. Joan J. Kendig, Heath S. Lukatch, and Dr. Donald R. Stanski for helpful comments.

References


Anesthesiology. V 85, No 4, Oct 1996


29. Schlame M, Hemmings HC: Inhibition by volatile anesthetics of endogenous glutamate release from synaptosomes by a presynaptic mechanism. Anesthesiology 1995; 82:1406–16


32. MacIver MB, Kendig JJ: Enflurane-induced burst discharge of hippocampal CA1 neurons is blocked by the NMDA receptor antagonist APV. Br J Anaesth 1989; 63:296–305


41. Shen LF, Schwartzkroin PA: Effects of temperature alterations on population and cellular activities in hippocampal slices from mature and immature rabbit. Brain Res 1988; 475:305–16

42. Moser EI, Andersen P: Conserved spatial learning in cooled

Anesthesiology, V 85, No 4, Oct 1996


46. Krebstein MS, Thomas MP, Horowitz JM: Thermal effects on long-term potentiation in the hamster hippocampus. Brain Res 1990; 520:115–22


50. Mikulec AA, Amagasu SM, Monroe FA, MacIver MB: Three sites of action are necessary and sufficient for halothane-induced depression of hippocampal CA1 neurons (abstract). ANESTHESIOLOGY 1995; 83:A1266

51. Benno R, Xiao Y-H, Duch DS: Central nervous system sodium channels are significantly suppressed at clinical concentrations of volatile anesthetics. ANESTHESIOLOGY 1996; 84:1123–33

52. Scheller RH: Membrane trafficking in the presynaptic nerve terminal. Neuron 1995; 893–97
