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Widespread Inhibition of Sodium Channel–dependent Glutamate Release from Isolated Nerve Terminals by Isoflurane and Propofol

Ratnakumari Lingamaneni, Ph.D., Martin L. Birch, B.S.,† Hugh C. Hemmings, Jr., M.D., Ph.D.‡*

Background: **Controversy persists concerning the mechanisms and role of general anesthetic inhibition of glutamate release from nerve endings. To determine the generality of this effect and to control for methodologic differences between previous studies, the authors analyzed the presynaptic effects of isoflurane and propofol on glutamate release from nerve terminals isolated from several species and brain regions.**

Methods: **Synaptosomes were prepared from rat, mouse, or guinea pig cerebral cortex and also from rat striatum and hippocampus. Release of endogenous glutamate evoked by depolarization with 20 μM veratridine (which opens voltage-dependent Na^+ channels by preventing inactivation) or by 30 mM KCl (which activates voltage-gated Ca^{2+} channels by membrane depolarization) was monitored using an on-line enzyme-linked fluorometric assay.**

Results: **Glutamate release evoked by depolarization with increased extracellular KCl was not significantly inhibited by isoflurane up to 0.7 mM (~2 minimum alveolar concentration; drug concentration for half-maximal inhibition > 1.5 mM) or propofol up to 40 μM in synaptosomes prepared from rat, mouse, or guinea pig cerebral cortex, rat hippocampus, or rat striatum. Lower concentrations of isoflurane or propofol significantly inhibited veratridine-evoked glutamate release in all three species (isoflurane IC_{50} = 0.41–0.50 mM; propofol IC_{50} = 11–18 μM) and rat brain regions. Inhibition of veratridine-evoked release was insensitive to the γ-aminobutyric acid receptor type A antagonist bicuculline (100 μM) in rat cortical synaptosomes.**

Conclusions: **Isoflurane and propofol inhibited Na^+ channel–mediated glutamate release evoked by veratridine with greater potency than release evoked by increased KCl in synaptosomes prepared from three mammalian species and three rat brain regions. These findings are consistent with a greater sensitivity to anesthetics of presynaptic Na^+ channels than of Ca^{2+} channels coupled to glutamate release. This widespread presynaptic action of general anesthetics is not mediated by potentiation of γ-aminobutyric acid type A receptors, though additional mechanisms may be involved.**

GENERAL anesthetics enhance inhibitory synaptic transmission and depress excitatory synaptic transmission in the central nervous system.[1] These actions involve both postsynaptic and presynaptic mechanisms. Prolongation of synaptic inhibition by positive modulation of postsynaptic γ-aminobutyric acid type A ($GABA_A$) receptor function at γ-aminobutyric acid–mediated synapses is an important component of the depressant effects of volatile anesthetics and of several chemically distinct intravenous anesthetics at clinical concentrations.[2,3] Depression of excitatory transmission is also observed at clinically relevant concentrations of many general anesthetics,[4–8] although the mechanisms involved in this action are less clear. Actions that may contribute to the depressant effects of general anesthetics on excitatory transmission include depression of presynaptic action potential conduction, inhibition of transmitter release, and blockade of postsynaptic receptors.[1]

Inhibition of the release of glutamate, the principal excitatory neurotransmitter in mammalian brain,[9] seems to have an important role in the depressant effects of general anesthetics. At central glutamatergic synapses, a variety of evidence indicates that inhibition of transmitter release is more sensitive to the actions of most agents than depression of action potential conduction or postsynaptic receptor blockade. Halothane or isoflurane at clinical concentrations depresses excitatory transmission by a presynaptic mechanism,[7,10] and halothane depresses excitatory postsynaptic currents mediated by non–*N*-methyl-D-aspartate and *N*-methyl-D-aspartate receptors equipotently in rat hippocampal CA1 pyramidal neurons.[6] Postsynaptic glutamate receptors are relatively insensitive to most agents.[6,10–12]

Synaptosomes, a subcellular fraction containing pinched-off nerve terminals, provide a useful system for analyzing the presynaptic actions of general anesthetics on glutamatergic transmission. Synaptosomes, which are capable of accumulating, storing, and releasing neurotransmitters, do not contain intact neuronal circuits and thus are free of indirect effects mediated by postsynaptic receptors, as occur in more intact preparations, such as brain slices.[13] Pharmacologic effects on transmitter release observed in synaptosomes indicate a presynaptic site of action, in contrast to results obtained using brain slices, in which anesthetics can also reduce glutamate release indirectly through postsynaptic $GABA_A$ receptor activation and possibly facilitation of neuronal or glial glutamate uptake or both. Despite consistent observations of inhibition by general anesthetics of glutamate release from synaptosomes, the underlying mechanisms are controversial. The presynaptic effects of general anesthetics

* Instructor, † Medical Student, ‡ Professor and Vice Chair of Research and Associate Professor of Pharmacology.

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Address reprint requests to Dr. Hemmings: Box 50, LC-203A, Weill Medical College of Cornell University, 525 East 68th Street, New York, New York 10021. Address electronic mail to: hchemmi@med.cornell.edu.

have been attributed variously to blockade of presynaptic Na^+ channels[14–16] or Ca^{2+} channels,[17] increased glutamate uptake,[18] or to actions on other steps involved in neurotransmitter release.[19–21] Variable sensitivities to anesthetics could result from regional differences, species differences, or both in presynaptic Ca^{2+} channel subtypes[22–25] or other components of the release machinery. The current study was undertaken to determine whether anesthetics have similar effects in several different species and brain regions and to clarify some conflicting results from previous studies by analyzing the effects of isoflurane and propofol, representative volatile and intravenous anesthetics, on glutamate release from synaptosomes (isolated nerve terminals). Comparable inhibition of glutamate release in multiple species and brain regions would confirm this as a fundamental neurochemical property of central mammalian glutamatergic nerve terminals relevant to anesthetic action.

Materials and Methods

Experiments were done in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals as approved by the Weill Medical College of Cornell University Institutional Animal Care and Use Committee (New York, NY).

Materials

$NADP^+$, L-glutamate dehydrogenase (*Proteus* species), bovine serum albumin (essentially fatty acid–free), veratridine, bicuculline, and dimethyl sulfoxide were obtained from Sigma Chemical Co. (St. Louis, MO); HEPES was obtained from Calbiochem (San Diego, CA). Propofol was from Aldrich Chemical Co. (St. Louis, MO) or was a gift from Zeneca Pharmaceuticals (now AstraZeneca Pharmaceuticals LP; Wilmington, DE). Isoflurane USP was from Abbott Laboratories (North Chicago, IL). All other chemicals were of analytical grade.

Glutamate Release

Crude synaptosomes (P2 fraction) were prepared from striatum, hippocampus, or cerebral cortex of young adult male Sprague-Dawley rats (150–200 g), from cerebral cortex of adult male C57BL/6 mice (25–30 g), or from cerebral cortex of adult male guinea pigs (400–500 g) as described.[26] Synaptosomes were suspended in HEPES buffered medium (composition: 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.2 mM Na_2HPO_4, 5 mM $NaHCO_3$, 10 mM D-glucose, and 20 mM HEPES/NaOH, pH 7.4), collected by centrifugation at 8,000*g* for 10 min, and stored on ice for up to 5 h until use. Release of endogenous glutamate was measured by an enzyme-linked fluorometric method.[27] Synaptosomal pellets (0.5 mg protein, determined by the method of Bradford[28]) were resuspended in HEPES buffered medium plus 16 μM bovine serum albumin, 1 mM $NADP^+$, 100 U L-glutamate dehydrogenase, and 1.3 mM $CaCl_2$ (1.5 ml final volume). Stirred samples were equilibrated at 37°C for 4 min in a spectrofluorometer cuvette, and data acquisition was initiated (excitation wavelength = 340 nm; emission wavelength = 460 nm). After recording basal fluorescence change (ΔF) for 60 s, HEPES buffered medium saturated with isoflurane at 22°C or propofol in dimethyl sulfoxide (< 0.02% [vol/vol] final concentration) was added, and ΔF was measured from 0–60 s. At 200 s after the addition of anesthetic when baseline release had stabilized, secretogogue (3 μl veratridine, 10 mM, or 15 μl KCl, 3 M) was added, and the initial (0–60 s) ΔF was measured. Longer preincubation with anesthetics did not produce greater effects (data not shown). The fluorescence signal was calibrated by adding 5 nmol L-glutamate to the cuvette at the end of each experiment. Addition of up to 0.05% (vol/vol) dimethyl sulfoxide did not affect basal or evoked glutamate release. Veratridine was used to activate Na^+ channels, and increased KCl was used to activate Ca^{2+} channels to compare their roles in anesthetic inhibition of glutamate release.[13] This distinct ion channel dependence was confirmed in rat cortical synaptosomes.[29–31] Veratridine-evoked release in the presence of 1.3 mM $CaCl_2$ was inhibited 82% by 1 μM tetrodotoxin (a specific Na^+ channel blocker) and 64% by 100 μM $CdCl_2$ (an inorganic Ca^{2+} channel blocker), consistent with a role for both Na^+ and Ca^{2+} channel activation. Release evoked by 30 mM KCl in the presence of 1.3 mM $CaCl_2$ was inhibited 7% by 1 μM tetrodotoxin and 75% by 100 μM $CdCl_2$, consistent with a role for Ca^{2+} channel but not Na^+ channel activation (n = 2–4).

Drug Concentrations

Concentrations of isoflurane in stock solutions and in final assays were determined by gas chromatography as described.[15] Free concentrations of propofol were analyzed in the buffer phase after equilibrium dialysis to take into account high tissue binding. After extraction into acetonitrile, samples were analyzed by high-performance liquid chromatography using a Hewlett-Packard (Palo Alto, CA) 1090 device with detections at 270 nm. Isocratic elution in 67% (vol/vol) acetonitrile/0.04% (vol/vol) acetic acid, pH 4.0, was performed on a 100 × 4.6–mm C_{18} column (5-μm particle size) with dibutyl phthalate as internal standard.[32] Propofol concentrations were quantified using peak area determinations compared with external standards (ChemStation software, Hewlett-Packard).

Statistical Analysis

Concentration–response data were fitted by nonlinear regression to the Hill equation ($Y = [L]^h([L]^h - IC_{50}{}^h)$), where Y is the effect, [L] is the drug concentration, IC_{50} is the drug concentration for half maximal inhibition,

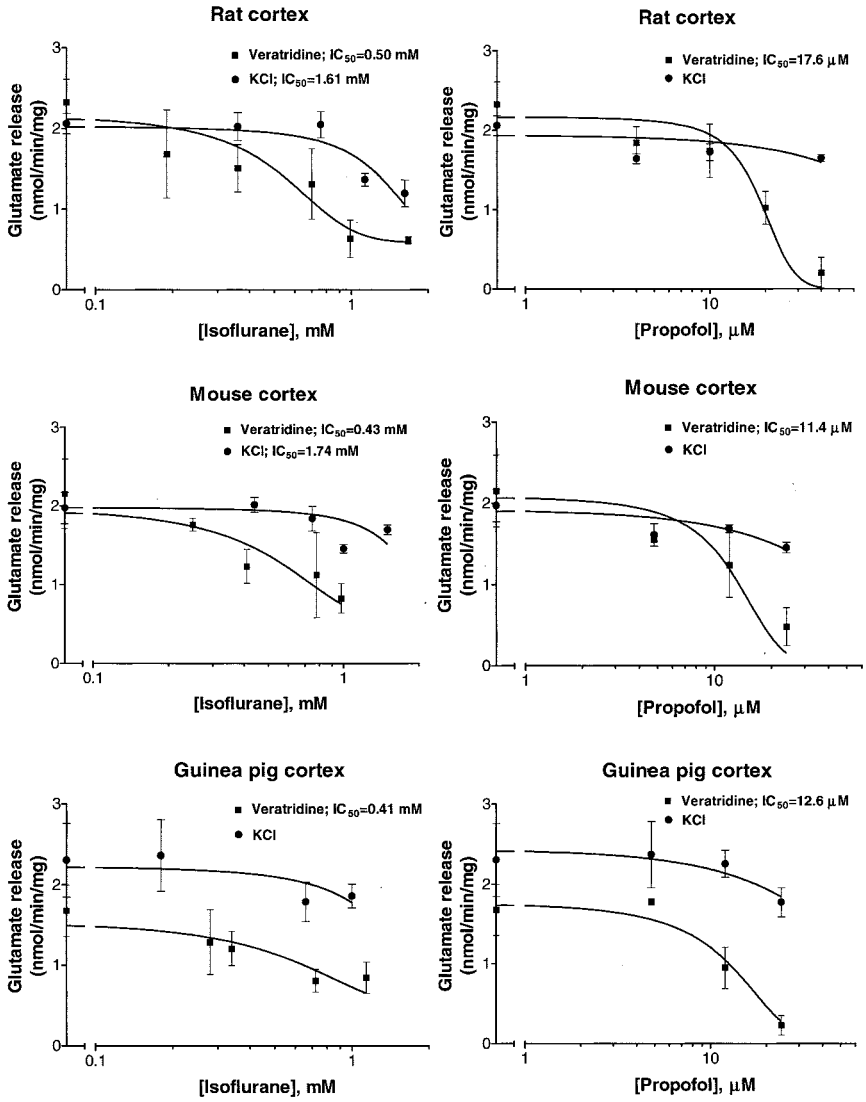

Fig. 1. Effects of isoflurane and propofol on glutamate release from rat, mouse, and guinea pig cerebrocortical synaptosomes. Concentration–response curves are shown for isoflurane (*left*) and propofol (*right*) actions on endogenous glutamate release evoked by 20 μM veratridine (■) or 35 mM KCl (●). Data are shown as mean ± SD (n = 3–4) and were fitted to the Hill equation by nonlinear regression analysis.

and h is the slope or Hill coefficient, using GraphPad Prism version 3.02 (GraphPad Software, San Diego CA).

Results

Effects on Glutamate Release in Rat, Mouse, and Guinea Pig Cerebral Cortex

The effects of isoflurane and propofol on endogenous glutamate release from synaptosomes prepared from three mammalian species were compared as a neurochemical assay of their presynaptic actions. These studies used a crude synaptosome fraction (P2) rather than purified synaptosomes to allow comparative analysis of endogenous glutamate release in the small tissue samples available; results with this preparation do not differ substantially from those obtained using purified rat cerebrocortical synaptosomes.[14–16] Glutamate release was evoked by veratridine or increased KCl (from small to 35 mM) to assess the involvement of presynaptic ion channels as targets for drug actions.[13] Veratridine leads to voltage-gated Na^+ channel activation (by slowing inactivation) through a direct interaction with the channel, thereby depolarizing the plasma membrane, leading to Ca^{2+} entry and Na^+ channel–dependent (tetrodotoxin-insensitive) neurotransmitter release. Increased extracellular KCl concentrations depolarize the plasma membrane by shifting the K^+ equilibrium potential above the threshold potential for activation of voltage-gated Ca^{2+} channels, leading to Ca^{2+} entry and Na^+ channel–independent (tetrodotoxin-insensitive) neurotransmitter release, whereas Na^+ channels are inactivated. The concentrations of veratridine and KCl used as secretogogues to stimulate glutamate release were of comparable intensity because there were no significant differences in the initial rates of glutamate release induced in control incubations (fig. 1). Control values for veratridine- and KCl-evoked glutamate release (nmol/min/mg protein) were 2.32 ± 0.28 (n = 11) and 2.20 ± 0.35 (n = 12) for rat, 2.15 ± 0.44 (n = 4) and 1.97 ± 0.53 (n = 7) for mouse, and 1.60 ± 0.25 (n = 7) and 2.00 ± 0.62 (n = 6) for

Table 1. Glutamate Release from Rat, Mouse, and Guinea Pig Cerebrocortical Synaptosomes

	Veratridine-evoked		KCl-evoked
	IC_{50} (mM)*	Effect at ~1 MAC†	IC_{50} (mM)*
Isoflurane			
Rat	0.50 [0.31–0.82]	−35%§ @ 0.36 mM	1.61 [0.44–5.9]
Mouse	0.43 [0.29–0.64]	−43%§ @ 0.41 mM	1.74 [0.96–3.1]
Guinea pig	0.41 [0.29–0.59]	−25%§ @ 0.34 mM	NS
	IC_{50} (μM)*	Effect at 10–12 μM‡	IC_{50}
Propofol			
Rat	17.6 [9.6–32]	−25%§ @ 10 μM	NS
Mouse	11.4 [6.9–32]	−42%§ @ 12 μM	NS
Guinea pig	12.6 [7.5–21]	−43%§ @ 12 μM	NS

* Followed by 95% confidence interval. Obtained by fitting the data to a four-parameter logistic concentration–response function with maximal inhibition (E_{max}) of 75% for isoflurane and 100% for propofol. † Data from assays performed at concentrations close to the minimum alveolar concentration (MAC) of isoflurane in rat (0.35 mM[33]; n = 3) compared with control values in the absence of anesthetic (n = 11). ‡ Data from assays performed at 10–12 μM propofol (free concentration; ~5 times the EC_{50} in tadpoles[34]; n = 4) compared with control values in the absence of anesthetic (n = 11). § $P < 0.05$ *versus* control (no anesthetic) by analysis of variance with Newman-Keuls *post hoc* test.

NS = slope not significantly different from zero by linear regression.

guinea pig, respectively. Both veratridine-evoked and increased KCl-evoked release of endogenous glutamate were largely dependent on the presence of extracellular Ca^{2+} in the medium (data not shown).[16,25]

Isoflurane and propofol inhibited veratridine-evoked glutamate release more potently than KCl-evoked release from synaptosomes prepared from rat, mouse, or guinea pig cerebral cortex (fig. 1 and table 1). The isoflurane IC_{50} values for inhibition of veratridine-evoked glutamate release ranged from 0.41 to 0.50 mM compared with a minimum alveolar concentration (MAC) of 0.35 mM in rat.[33] Significant inhibition was observed in all three species at concentrations approximating 1 MAC (table 1). The propofol IC_{50} values for inhibition of veratridine-evoked glutamate release ranged from 11 to 18 μM (free concentrations) compared with an EC_{50} for loss of righting reflex in the tadpole of 2.2 μM.[34]

The maximal inhibition of veratridine-evoked release produced by propofol exceeded that of isoflurane at the concentrations examined in rat cerebral cortex (fig. 1). The effects of isoflurane reached a plateau at approximately 75% inhibition at concentrations of approximately 1 mM and greater, whereas the effects of high concentrations of propofol approached 100% inhibition, as determined by fitting the data to a logistic equation. Higher concentrations of isoflurane could not be achieved in this assay because of the limited volume and concentration of the saturated stock solution that could be added.

Glutamate release evoked by increased KCl was not inhibited significantly by isoflurane up to 0.7 mM (2 MAC)

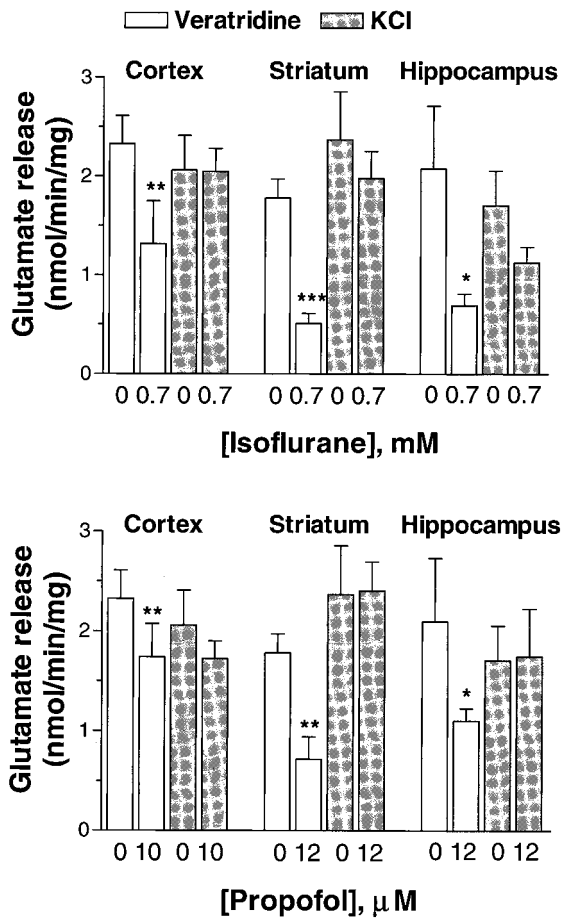

Fig. 2. Effects of isoflurane (*top*) and propofol (*bottom*) on glutamate release from rat cerebrocortical, striatal, and hippocampal synaptosomes. Release was evoked with 20 μM veratridine or 35 mM KCl. Data are shown as mean ± SD (n = 3–4; control, n = 11); *$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$ by Student unpaired two-tailed *t* test.

or by propofol up to 40 μM (free concentration). Significant inhibition by higher concentrations of isoflurane allowed determination of approximate IC_{50} values of 1.6–1.7 mM (> 4 MAC) in rat and mouse.

Effects on Glutamate Release in Rat Cerebral Cortex, Striatum, and Hippocampus

Brain region–specific presynaptic anesthetic actions possibly due to regional differences in presynaptic properties were assessed by comparing isoflurane and propofol effects on endogenous glutamate release from three rat brain regions with major glutamatergic inputs: cerebral cortex, striatum, and hippocampus. There were no significant differences between control values of glutamate release for each brain region by analysis of variance (fig. 2). Veratridine-evoked release was more sensitive than KCl-evoked release to 0.7 mM isoflurane (2 MAC) or 10–12 μM propofol (≈ 5 times the EC_{50}) in each brain region. Inhibition of veratridine-evoked release was significantly greater in striatum and hippocampus than in cerebral cortex for isoflurane ($P < 0.05$) and propofol

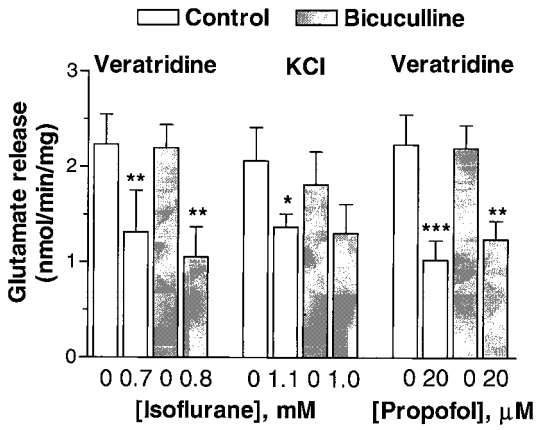

Fig. 3. Effects of bicuculline on inhibition of glutamate release. Rat cerebrocortical synaptosomes were incubated with 100 μM bicuculline for 200 s before addition of isoflurane or propofol. Data are shown as mean ± SD (n = 3); *$P < 0.05$, **$P < 0.01$, ***$P < 0.0005$ by Student unpaired two-tailed t test. Propofol (20 μM) did not inhibit KCl-evoked release (data not shown).

($P < 0.01$) at the concentrations tested. Inhibition of KCl-evoked release was observed only for isoflurane in the hippocampus, although this effect did not achieve statistical significance ($P = 0.06$).

Role of $GABA_A$ Receptors

Ligand-gated ion channels are among the most sensitive molecular targets for general anesthetic actions, of which $GABA_A$ receptors figure prominently in the neuronal depression produced by most agents, and by propofol in particular.[1] The role of $GABA_A$ receptor activation in the presynaptic actions of isoflurane and propofol to inhibit glutamate release was determined using the selective competitive $GABA_A$ receptor antagonist bicuculline. Glutamate release was insensitive to 100 μM bicuculline alone. Bicuculline did not significantly affect inhibition by isoflurane or propofol of glutamate release evoked by veratridine or increased KCl from rat cerebrocortical synaptosomes (fig. 3).

Discussion

This study represents the first systematic comparison of presynaptic anesthetic effects in multiple species and brain regions. Our results show consistent inhibitory effects of isoflurane and propofol on glutamate release from synaptosomes prepared from three mammalian species and three major brain regions. Thus, sensitivity to inhibition by general anesthetics is likely a conserved feature of central mammalian glutamatergic transmission.

The possibility that species and regional differences in ion channel isoforms, presynaptic receptor types, and other unspecified presynaptic properties coupled to glutamate release might result in differences in sensitivity to general anesthetics has not been investigated previously. We find that glutamate release evoked by veratridine is sensitive to isoflurane (IC_{50} = 0.41–0.50 mM) at clinically effective concentrations (rat MAC = 0.35 mM)[33] in synaptosomes prepared from three species and three brain regions, whereas glutamate release evoked by increased KCl is relatively insensitive at an equipotent concentration. Comparable results were obtained for propofol effects on veratridine-evoked glutamate release, but these effects are less potent (IC_{50} = 11.4–17.6 μM) compared with its clinically effective concentration (EC_{50} = 2.2 μM for loss of righting reflex in tadpoles[34]). Therefore, inhibition of Na^+ channel–dependent glutamate release occurred at clinically relevant concentrations of isoflurane, whereas small to minimal effects occurred at clinically relevant propofol concentrations. These findings are consistent with electrophysiologic evidence that depression of excitatory transmission[5–7] has a more important role in the neurodepressant actions of volatile than of intravenous anesthetics.[8] For example, depression by propofol of rat hippocampal CA1 output in response to field stimulation of the Schaffer collateral input was largely dependent on $GABA_A$ receptor–mediated inhibition (95% reversed by bicuculline treatment), whereas depression by isoflurane was insensitive to bicuculline (M. B. MacIver, Ph.D., Associate Professor, Stanford University, Palo Alto, CA, written communication, July 2000).

Our data suggest that glutamate release in the striatum and hippocampus is slightly more sensitive to anesthetics than in the cerebral cortex and that KCl-evoked release may be more sensitive to isoflurane in hippocampus than in striatum or cerebral cortex. The high tissue requirements for analyzing endogenous glutamate release (~0.5 mg protein/assay) coupled with the relatively small size of rat hippocampus and striatum impeded further characterization of these interesting observations. Detailed study of these differences will require more sensitive analytical methods for confirmation and mechanistic analysis.

General anesthetics inhibit glutamate release from both brain slices and synaptosomes as determined by neurochemical techniques. Effects observed in synaptosomes are indicative of a *direct* presynaptic site of action,[13] in contrast to results obtained using brain slices, in which anesthetics can also reduce glutamate release *indirectly* through postsynaptic $GABA_A$ receptor activation[35–37] and possibly by facilitation of neuronal or glial glutamate uptake or both.[38] Previous studies in synaptosomes prepared from rat cerebral cortex showed that volatile anesthetics and propofol inhibit Ca^{2+}-dependent glutamate release evoked by secretogogues that involve voltage-gated Na^+ channel activation (*e.g.*, veratridine, 4-aminopyridine) with greater potency than Na^+ channel-independent release (*i.e.*, evoked by increased KCl concentrations that bypass Na^+ channels).[15,16,39] Taken together with independent neurochemical[14,40] and electrophysiologic[41,42] evidence of anesthetic blockade of neuronal voltage-gated Na^+ channels, our findings suggest that anesthetics inhibit glutamate release by

blocking presynaptic Na^+ channels, thereby inhibiting nerve terminal depolarization. This conclusion is supported by recent observations that the model anesthetic 1-chloro-1,2,2-trifluorocyclobutane (F3) inhibits Na^+ channel–dependent glutamate release and blocks neuronal voltage-gated Na^+ channels, whereas the structurally similar non-anesthetic 1,2-dichlorohexafluorocyclobutane (F6) is ineffective at predicted anesthetic concentrations.[15] This is consistent with a greater anesthetic sensitivity of presynaptic Na^+ channels than of Ca^{2+} channels coupled to glutamate release or with a greater "safety factor" for Ca^{2+} channel blockade. Our findings do not rule out the possibility that this difference in anesthetic sensitivity between veratridine- and KCl-evoked glutamate release results from mechanistic differences between these two secretogogues other than or in addition to Na^+ channel involvement.[13]

Therefore, a major component of the presynaptic actions of general anesthetics in the inhibition of glutamate release seems to involve a step proximal to voltage-dependent Ca^{2+} channel activation, most likely inhibition of tetrodotoxin-sensitive voltage-dependent Na^+ channels. The lower efficacy of isoflurane compared with propofol suggests that the mechanisms underlying the presynaptic effects of these agents may differ, a question that requires further study for resolution. Direct evidence for anesthetic actions on presynaptic ion channels is lacking. Their small size (< 1 μm diameter) makes it difficult or impossible to identify the presynaptic ion channels that mediate neurotransmitter release using standard electrophysiologic techniques. Attempts to overcome this limitation by fusing synaptosomes to create larger structures have been unsuccessful (C. J. Tzan, M.D., Ph.D., Research Fellow, New York, NY, and H. C. H., unpublished data, July 1997). Alternate approaches, such as quantitative fluorescence confocal microscopy of cultured rat hippocampal neurons labeled with FM1–43 to monitor synaptic vesicle exocytosis may provide an independent method for probing the presynaptic actions of general anesthetics.[21] Despite the current lack of direct evidence, the indirect neurochemical evidence for anesthetic inhibition of presynaptic Na^+ channels is bolstered by electrophysiologic evidence that neuronal Na^+ channels are inhibited substantially at clinical concentrations of volatile anesthetics, including isoflurane (IC_{50} = 0.25 mM),[41] whereas higher concentrations of propofol relative to its clinical EC_{50} are required (IC_{50} = 10 μM).[42] These findings do not exclude the involvement of additional presynaptic targets in the depression of glutamate release by general anesthetics.

An effect of clinically relevant concentrations of general anesthetics on KCl-evoked glutamate release would have important implications for their presynaptic mechanisms of action. Depolarization of synaptosomes by increased concentrations of KCl increases the probability of Ca^{2+} channel opening, whereas Na^+ channels are inactivated.[13] This produces localized increases in intrasynaptosomal Ca^{2+} concentration ($[Ca^{2+}]_i$) at the pore of Ca^{2+} channels, some of which are clustered at active zones and closely coupled to the exocytosis of glutamate-containing synaptic vesicles.[43,44] Putative inhibitors of N-, P- and Q-type Ca^{2+} channels block rat cerebrocortical synaptosomal glutamate release evoked by increased KCl, veratridine, or 4-aminopyridine, indicating some contribution by each of these channel subtypes, as well as some contribution by a toxin resistant (R-type) Ca^{2+} channel.[45–47] Significant inhibition of the Ca^{2+} channel subtypes coupled to glutamate release should be reflected in comparable inhibition of Ca^{2+}-dependent glutamate release evoked by increased KCl, veratridine, or 4-aminopyridine because each of these secretogogues results in activation of Ca^{2+} channels as a final common pathway to transmitter release. Inhibition of KCl-evoked release at high concentrations of isoflurane but not of propofol is consistent with electrophysiologic data that indicate a greater sensitivity of Ca^{2+} channels to volatile anesthetics[48] than to propofol and other intravenous agents[49] relative to their clinical concentrations. This Ca^{2+} channel–mediated effect may become important at higher anesthetic concentrations.

The approximately twofold greater sensitivity of KCl-evoked synaptosomal glutamate release to volatile anesthetics reported previously in guinea pig (*e.g.*, $IC_{50} \approx 0.69$ mM for isoflurane)[17] compared with our results in rat (IC_{50} = 1.61 mM) cannot be explained by a species difference. We find that KCl-evoked glutamate release from guinea pig, rat, or mouse cortical synaptosomes is not affected significantly by isoflurane up to 0.7 mM (~2 MAC). This difference is also not due to the analytical methods used because both studies used enzyme-linked fluorometry. Different methods were used for synaptosome preparation, although synaptosomes prepared in our laboratory by the method used by Miao *et al.*[17] yielded results similar to those reported here (R. L. and H. C. H., unpublished data, July 2000). Inaccuracies in anesthetic concentration measurements could explain the absolute differences in the reported isoflurane IC_{50} values for KCl-evoked glutamate release. Nevertheless, the greater sensitivity of veratridine-evoked release relative to KCl-evoked release in our study (but not determined in other studies) remains independent of this variable.

Widespread inhibition of evoked glutamate release at clinical concentrations in multiple species and brain regions is consistent with an important role for depression of excitatory neurotransmission by a presynaptic mechanism in volatile anesthetic action. Whether this presynaptic action effect can be generalized to other neurotransmitter systems awaits further study. The available evidence supports the view that all general anesthetics depress nervous system function through facilitation of inhibitory transmission (usually by $GABA_A$ receptor modulation), depression of excitatory transmission (by presynaptic and postsynaptic mechanisms), or both. Agent-specific actions are thus determined by the

relative contributions of these (and other) general mechanisms in altering nervous system function to produce an anesthetic state.

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