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Effects of Intravenous General Anesthetics on [^{3}H]GABA Release from Rat Cortical Synaptosomes

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Background: **Potentiation by general anesthetics of γ-aminobutyric acid (GABA)-mediated inhibitory transmission in the central nervous system is attributed to $GABA_A$ receptor-mediated postsynaptic effects. However, the role of presynaptic mechanisms in general anesthetic action is not well characterized, and evidence for anesthetic effects on GABA release is controversial. The effects of several intravenous general anesthetics on [^{3}H]GABA release from rat cerebrocortical synaptosomes (isolated nerve terminals) were investigated.**

Methods: **Purified synaptosomes were preloaded with [^{3}H]GABA and superfused with buffer containing aminooxyacetic acid and nipecotic acid to inhibit GABA metabolism and reuptake, respectively. Spontaneous and elevated potassium chloride depolarization–evoked [^{3}H]GABA release were evaluated in the superfusate in the absence or presence of various anesthetics, extracellular Ca^{2+}, GABA receptor agonists and antagonists, and 2,4-diaminobutyric acid.**

Results: **Propofol, etomidate, pentobarbital, and alphaxalone, but not ketamine, potentiated potassium chloride–evoked [^{3}H]GABA release (by 1.3 to 2.9 times) in a concentration-dependent manner, with median effective concentration values of 5.4 ± 2.8 μM (mean ± SEM), 10.1 ± 2.1 μM, 18.8 ± 5.8 μM, and 4.4 ± 2.0 μM. Propofol also increased spontaneous [^{3}H]GABA release by 1.7 times (median effective concentration = 7.1 ± 3.4 μM). Propofol facilitation of [^{3}H]GABA release was Ca^{2+} dependent and inhibited by bicuculline and picrotoxin, but was insensitive to pretreatment with 2,4-diaminobutyric acid, which depletes cytoplasmic GABA pools.**

Conclusions: **Low concentrations of propofol, etomidate, pentobarbital, and alphaxalone facilitated [^{3}H]GABA release from cortical nerve terminals. General anesthetics may facilitate inhibitory GABA-ergic synaptic transmission by a presynaptic mechanism in addition to their well-known postsynaptic actions. (Key words: Mechanisms of anesthesia; neurotransmitter release; neuroprotection.)**

GENERAL anesthetics enhance inhibitory synaptic transmission[1] and inhibit excitatory synaptic transmission in the central nervous system.[2,3] Electrophysiologic and neurochemical studies indicate that most general anesthetics potentiate inhibitory transmission mediated by γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in mammals. For example, clinically relevant concentrations of volatile anesthetics[4] and propofol[5] potentiate GABA-activated Cl^- currents in rat hippocampal pyramidal neurons. These actions suggest that postsynaptic $GABA_A$ receptors are important targets for general anesthetic action.[6]

In contrast to the considerable evidence supporting postsynaptic effects of general anesthetics on GABA-ergic transmission, evidence for presynaptic anesthetic actions on GABA release is controversial. This study evaluates the effects of five structurally distinct intravenous anesthetics (propofol, etomidate, pentobarbital, alphaxalone, and ketamine), all of which enhance postsynaptic $GABA_A$-mediated effects,[6] on spontaneous (basal) and potassium chloride–evoked [^{3}H]GABA release from superfused rat cerebrocortical synaptosomes. Synaptosomes (a subcellular fraction containing pinched-off nerve endings) provide an unexcelled model to study neurotransmitter release because all the presynaptic components necessary for neurotransmitter synthesis, storage, release, and reuptake are present in the absence of intact neuronal circuits and glia, which can complicate experiments with brain slices.[7,8] Superfusion is used widely to study GABA release because it minimizes problems with transmitter reuptake and enables parallel analysis of multiple drug treatments under identical conditions. Our results indicate that certain intravenous general anesthetics potentiate presumptive vesicular GABA release by a presynaptic mechanism.

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Materials and Methods

Materials

Propofol was purchased from Aldrich Chemicals (Milwaukee, WI) or was a gift from Zeneca Pharmaceuticals (Wilmington, DE). Etomidate was from Janssen Biotech n.v. (Olen, Belgium). Percoll density gradient medium was from Pharmacia AB (Uppsala, Sweden). Alphaxalone (5α-pregnane-3α-hydroxy-11,20-dione), ketamine, pentobarbital, and picrotoxin were from Sigma Chemical Company (St. Louis, MO). (−)-Baclofen, (+)-bicuculline, and muscimol hydrobromide were from Research Biochemicals International (Natick, MA). [^{3}H]GABA (specific activity, 60–86 Curie/mmol) was from DuPont/New England Nuclear (Boston, MA). All other chemicals were of reagent grade.

Preparation of Synaptosomes

Synaptosomes were prepared as described by Dunkley *et al.*[7] Adult male Sprague Dawley rats (150–200 g) were anesthetized with 20% oxygen and 80% carbon dioxide and killed by decapitation. Brains were removed and placed in ice-cold 0.32 M sucrose. The cerebral cortex was removed and homogenized in 10 ml/g ice-cold 0.32 M sucrose using a Teflon (Potter-Elvehjem) homogenizer (Thomas Scientific, Swedesboro, NJ) at 900 rpm (eight strokes). The homogenate was centrifuged at 3,020*g* for 2 min at 4°C. The supernatant (S1 fraction) was transferred to a 50-ml polycarbonate tube and centrifuged at 14,600*g* for 12 min at 4°C. The pellet (P2 fraction; "crude synaptosomes") was resuspended in 8 ml of 0.32 M sucrose/brain; 2-ml aliquots of this suspension were transferred onto a discontinuous gradient consisting of 2.5 ml each of filtered (through a 0.46-μm filter) 23%, 10%, or 3% (vol/vol) Percoll density gradient medium in 0.32 M sucrose, 1 mM dithioethrietol, and 0.2 mM ethylenediaminetetraacetic acid at *p*H 7.4. The gradients were centrifuged at 35,100*g* for 6.5 min at 4°C. The fractions at the 23–10% interface were transferred to 27 ml aerated (95% oxygen, 5% carbon dioxide) HEPES-buffered medium (HBM) composed of 140 mM NaCl, 5 mM potassium chloride, 5 mM $NaHCO_3$, 1 mM $MgCl_2$, 20 mM HEPES, 1.2 mM Na_2HPO_4, 10 mM D-glucose, *p*H 7.4; the suspension was centrifuged at 23,000*g* for 10 min at 4°C. The pellet was resuspended in ice-cold HBM and stored on ice until needed for superfusion (within 1 h). The protein concentration of the synaptosomal preparation was determined with bovine serum albumin as a standard.[9]

Determination of [^{3}H]GABA Release

Synaptosomes (1 mg/ml in HBM) were equilibrated at 37°C for 15 min in the presence of 1.3 mM $CaCl_2$ and incubated with [^{3}H]GABA (final concentration, 0.04 μM) at 37°C for an additional 15 min. Aliquots of synaptosomes (100 μg protein) were transferred to individual superfusion chambers maintained at 37°C containing a GF/B filter (1 μm; Brandel, Gaithersburg, MD) and a GF/F filter (0.7 μm; Brandel) layer at the inflow port and a GF/F filter at the outflow port. Synaptosomes were superfused with HBM containing 50 μM aminooxyacetic acid, a GABA transaminase inhibitor,[10] and 10 μM nipecotic acid, a GABA reuptake inhibitor,[11] at 0.5 ml/min. Superfusion solutions were aerated continuously with 95% oxygen and 5% carbon dioxide. The superfusate collected during a 30-min prewash period was discarded. Thereafter, fractions were collected at 1-min intervals into scintillation vials using an automated fraction collector for 15 or 20 min, including a 5- or 10-min baseline, a 5-min control or drug treatment period, and a 5-min posttreatment washout. Propofol was prepared from a 1-M stock solution in dimethyl sulphoxide and diluted in HBM (final dimethyl sulphoxide concentration ≤ 0.01%, vol/vol). When elevated potassium chloride was used, the NaCl concentration in HBM was reduced accordingly to maintain isoosmolarity. At the end of the experiment, filters (with synaptosomes) were removed and solubilized in 0.4 ml of 1 N NaOH to determine the amount of [^{3}H]GABA present in the synaptosomes. Radioactivity in samples and filters was determined using liquid scintillation spectrometry after 3.5 ml of a Bio-Safe II scintillation cocktail (Research Products International, Mount Prospect, IL) was added.

Analysis of [^{3}H]GABA Release

The [^{3}H]GABA released (radioactivity) during each 1-min collection period was expressed as fractional release, that is, the radioactivity in that fraction divided by the total amount of radioactivity present in the synaptosomes when that collection period began. Total radioactivity present in the synaptosomes at each collection period was determined by back-calculation. Thus, the total radioactivity in synaptosomes at the end of the experiment plus the radioactivity in the last fraction equals the total radioactivity in the synaptosomes present before the last fraction was collected. Fractional release of [^{3}H]GABA was calculated for each fraction at each time point and expressed as a percentage of total radioactivity in the synaptosomes at the time of collection (percentage fractional release). Baseline [^{3}H]GABA

Table 1. Propofol Concentrations during Superfusion

Nominal μM	Prechamber μM
5	1.6 ± 0.2
10	4.7 ± 0.5
20	10.6 ± 1.7
50	31.2 ± 2.1
100	44.5 ± 3.3

Propofol concentrations were determined by HPLC analysis of fractions collected immediately prior to entry of the drug solution into the superfusion chamber containing synaptosomes (flow rate = 0.5 ml/min). Values are the mean ± SEM of four consecutive 1 min fractions (n = 2).

release was defined as the percentage fractional release in the fraction immediately before the addition of control or drug solution. Data obtained for control and drug-treated groups were converted to percentages of baseline release for each group (*i.e.*, the percentage fractional release in experimental fraction divided by baseline percentage fractional release). Stimulation-evoked [^{3}H]GABA release was determined from the peak percentage fractional release observed during superfusion with drugs in HBM and compared with simultaneous release in synaptosomes superfused with HBM alone (control).

Analysis of Propofol Concentrations

Propofol concentrations were determined in the superfusate as it entered the synaptosome chamber by high-performance liquid chromatography (HPLC).[12] Appropriate dilutions (5–100 μM) of propofol were made into HBM. The propofol solutions were pumped through the superfusion apparatus at 0.5 ml/min, and 1-min fractions were collected and stored at −20°C until analysis. To the thawed samples was added 250 μl acetonitrile: 69%–71% perchloric acid (67:33; vol/vol) containing 1 μg dibutylpthalate as an internal standard. An aliquot (200 μl) of this solution was injected directly onto a C_{18} column (ODS Hypersil column, 5-μm particle size, 100 × 4.6 mm; The Nest Group, Southboro, MA). Propofol was eluted using a mobile phase of 67% acetonitrile (vol/vol) in water adjusted to *p*H 4.0 with glacial acetic acid at a flow rate of 1.5 ml/min. Propofol was detected at 270 nm and quantified by peak area using propofol standard calibration curves. Propofol concentrations entering the chamber were approximately 50% of the nominal concentrations (table 1). The concentrations of propofol in the text and the figures refer to the measured concentrations.

Statistical Analysis

Differences between control and drug treatments were analyzed by analysis of variance using the Newman-Keuls multiple-range test. Differences between mean control and drug treatment values were analyzed using unpaired two-tailed Student's *t* test. The drug concentration that produced 50% of the maximal response (EC_{50}) were obtained by graphical determination.

Results

Method Validation

Elevated extracellular potassium chloride, which directly depolarizes the plasma membrane and is used widely as a secretogogue to study GABA release, increased [^{3}H]GABA release from rat cerebrocortical synaptosomes in a concentration-dependent manner (table 2). Omission of extracellular Ca^{2+} markedly diminished [^{3}H]GABA release. Pretreatment of synaptosomes with 2,4-diaminobutyric acid, a GABA analog that selectively depletes cytosolic GABA,[13] did not significantly affect baseline [^{3}H]GABA release or 15-mM potassium chloride-evoked [^{3}H]GABA release (table 2). 4-Aminopyridine, a K^+ channel antagonist that evokes neurotransmitter release by repetitive Na^+ channel-dependent, tetrodotox-

Table 2. Characterization of KC1-evoked [^{3}H]GABA Release

Treatment	[^{3}H]GABA Release (% control) + Ca^{2+}	[^{3}H]GABA Release (% control) − Ca^{2+}	n
15 mM KCl	214 ± 11*	114 ± 6†	6
30 mM KCl	313 ± 21*	123 ± 14†	6
200 μM 4-aminopyridine	170 ± 24*		3
+ 11 μM propofol	252 ± 21*†		3
100 μM DABA	93 ± 8		4
15 mM KCl	235 ± 28		4
+ 100 μM DABA	214 ± 40		4
15 mM KCl	233 ± 11		6
+100 μM (−)-baclofen	179 ± 14†		6
+100 nM muscimol	383 ± 36†		4
+100 μM muscimol	160 ± 15†		4
50 nM muscimol	149 ± 17‡		4

Synaptosomes were superfused with HBM (control) or with 15 or 30 mM KCl in the presence (+ Ca^{2+}; 1.3 mM $CaCl_2$) or absence (− Ca^{2+}; +1 mM EGTA) of extracellular Ca^{2+} during 11–15 min of superfusion. EGTA was present in the superfusion medium for 10 min before addition of KCl. To test the releasable GABA pool involved in the KCl effect, synaptosomes were superfused with 100 μM 2,4-diaminobutyric acid (DABA) for 30 min prior to KCl. Results show peak [^{3}H]GABA release (mean ± SEM) as % of control. Control fractional release values were 1.06–1.17%.

* $P < 0.01$ *versus* control (no addition).

† $P < 0.01$ *versus* 15 mM KCl + Ca^{2+} or 200 μM 4-aminopyridine values.

‡ $P < 0.05$ *versus* control (no addition).

in-sensitive depolarizations,[8] also increased [^{3}H]GABA release (table 2) with an EC_{50} = 225 ± 31 μM.

Effects of GABA Receptor Agonists on [^{3}H]GABA Release

(−)-Baclofen, a $GABA_B$ receptor agonist that reduces GABA release by a presynaptic mechanism,[14] partially inhibited potassium chloride-evoked release of [^{3}H]GABA. Muscimol, a $GABA_A$ receptor agonist, had a biphasic effect on potassium chloride-evoked [^{3}H]GABA release: low concentrations (10-100 nM) stimulated and high concentrations (1-100 μM) inhibited 15 mM potassium chloride-evoked [^{3}H]GABA release (table 2).[15] Muscimol increased basal [^{3}H]GABA release at 50 nM (table 2) but had no significant effect at 0.01, 0.1, 1, 10, or 100 μM.

Effects of Intravenous Anesthetics on [^{3}H]GABA Release

Propofol, etomidate, pentobarbital, and alphaxalone enhanced 15-mM potassium chloride-evoked [^{3}H]GABA release. Propofol had a biphasic effect (fig. 1): release was enhanced up to 31 μM but returned to control values at 45 μM. The EC_{50} for the stimulatory effect of propofol on potassium chloride-evoked [^{3}H]GABA release was 5.4 ± 2.8 μM, with a maximal effect at 11 μM (188 ± 18% of control). Propofol also facilitated spontaneous [^{3}H]GABA release from unstimulated synaptosomes (fig. 1). Superfusion of synaptosomes for 5 min with propofol alone (2-45 μM) increased [^{3}H]GABA release in a concentration-dependent manner (EC_{50} = 7.1 ± 3.4 μM). Propofol-induced [^{3}H]GABA release was maximal at 11 μM propofol (173 ± 14% of control).

Etomidate (5-50 μM) increased potassium chloride-evoked [^{3}H]GABA release in a concentration-dependent manner (EC_{50} = 10.1 ± 2.1 μM; fig. 2). This effect was maximal at 20 μM (289 ± 27% of control). The peak effect of etomidate on spontaneous [^{3}H]GABA release was 120 ± 12% of control at 20 μM etomidate (P = 0.13).

The effect of pentobarbital on 15 mM potassium chloride-evoked [^{3}H]GABA release was biphasic (fig. 2): stimulation (EC_{50} = 18.8 ± 5.7 μM; peak response of 133 ± 9% of control at 50 μM) was followed by inhibition at higher concentrations. The peak effect of pentobarbital on spontaneous [^{3}H]GABA release was 114 ± 15% of control at 50 μM pentobarbital (P = 0.46, n = 6).

Alphaxalone enhanced potassium chloride-evoked [^{3}H]GABA release in a concentration-dependent manner (EC_{50} = 4.4 ± 2.0 μM; fig. 2). The effect of alphaxalone on potassium chloride-evoked [^{3}H]GABA release reached a plateau at 5-20 μM (148 ± 9% of control at 5 μM). Alphaxalone did not affect spontaneous [^{3}H]GABA release (102 ± 6% of control at 5 μM alphaxalone; P = 0.47, n = 4).

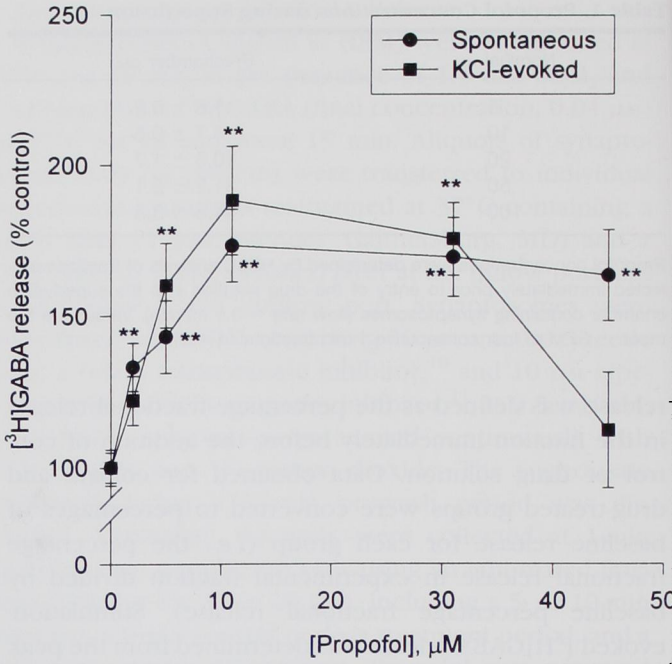

Fig. 1. Propofol enhancement of spontaneous and potassium chloride–evoked [^{3}H]γ-aminobutyric acid (GABA) release. Synaptosomes were superfused with HEPES-buffered medium (HBM) or 15 mM potassium chloride ± propofol (5–100 μM, nominal concentration) during 6–10 min of superfusion. Curves show peak [^{3}H] GABA release (mean ± SEM) in propofol-treated synaptosomes as a percentage of HBM or potassium chloride control. Propofol values plotted were measured by high-pressure liquid chromatography. The median effective values of propofol to enhance spontaneous and potassium chloride–evoked [^{3}H]GABA release were 7.1 ± 3.4 μM and 5.4 ± 2.8 μM, respectively. **P < 0.01 *versus* HBM or potassium chloride value (n = 6).

Ketamine (10-500 μM) did not significantly affect potassium chloride-evoked [^{3}H]GABA release (85 ± 10%, 98 ± 18%, 86 ± 13%, and 121 ± 15% [all P > 0.2] of control at 10, 50, 100, and 500 μM ketamine, respectively [n = 4]) or spontaneous [^{3}H]GABA release (92 ± 10%, 93 ± 16%, 88 ± 12%, and 102 ± 14% [all P > 0.5] of control at 10, 50, 100 and 500 μm ketamine, respectively [n = 4]).

Characterization of Propofol's Effect on Spontaneous [^{3}H]GABA Release

Time Course. [^{3}H]GABA release increased during the first 2 min of superfusion with propofol, with a peak [^{3}H]GABA release of 168 ± 14% of control at 7 min (fig. 3). This increase was followed by a gradual decrease in

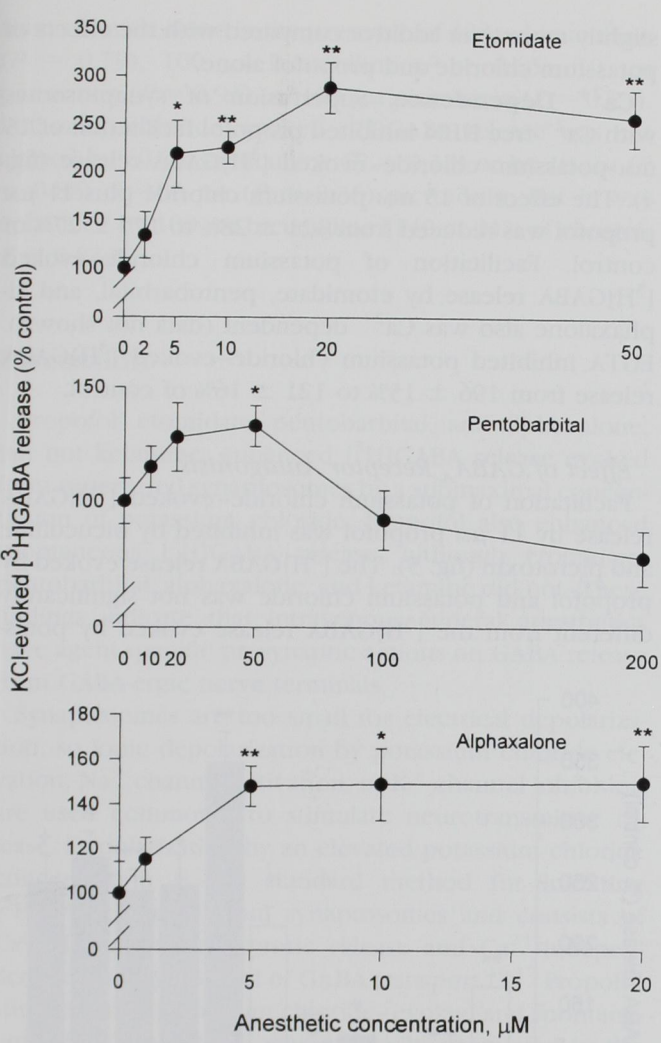

Fig. 2. Etomidate, pentobarbital, or alphaxalone enhancement of potassium chloride–evoked [^{3}H]γ-aminobutyric acid (GABA) release. Synaptosomes were superfused with 15 mM potassium chloride ± etomidate (2–50 μM), pentobarbital (10–200 μM), or alphaxalone (2–50 μM) during 6–10 min of superfusion. Curves show peak [^{3}H]GABA release (mean ± SEM) as a percentage of potassium chloride control (without anesthetic). The median effective concentration values of etomidate, pentobarbital, and alphaxalone to enhance potassium chloride–evoked [^{3}H]GABA release were 10.1 ± 2.1 μM, 18.8 ± 5.7 μM, and 4.4 ± 2.0 μM, respectively. *P < 0.05, **P < 0.01 *versus* potassium chloride alone (n = 4–6).

propofol-evoked [^{3}H]GABA release during the ensuing 3 min (8–10 min) of exposure to propofol. The [^{3}H]GABA release returned to baseline levels during the washout period (11–15 min). Potassium chloride (15 mM) applied 5 min after superfusion with propofol for 5 min increased [^{3}H]GABA release to 229% of control, indicating that releasable [^{3}H]GABA was not depleted by the propofol treatment.

Ca^{2+} Dependence. The increase in [^{3}H]GABA release induced by 11 μM propofol was abolished in the absence of external Ca^{2+} (fig. 4). Superfusion of synaptosomes with Ca^{2+}-free HBM (plus 1 mM ethyleneglycol-bis-(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid [EGTA]) reduced [^{3}H]GABA release from 189 ± 16% to 104 ± 17% of control. The EGTA alone did not alter basal [^{3}H]GABA release (P = 0.48).

Effect of 2,4-diaminobutyric Acid. Propofol (11 μM)-evoked [^{3}H]GABA release was comparable in control (170 ± 13%) and 2,4-diaminobutyric acid–pretreated (163 ± 10%) synaptosomes (P = 0.46, n = 4).

Effect of Nipecotic Acid. The presence of 10 μM nipecotic acid in the superfusion medium to inhibit GABA reuptake did not alter the ability of propofol to enhance [^{3}H]GABA release. Peak [^{3}H]GABA release during superfusion with 11 μM propofol was 189 ± 22% of control in the absence and 193 ± 30% of control in the presence of 10 μM nipecotic acid (P = 0.88, n = 4).

Effect of $GABA_A$ Receptor Antagonists. The $GABA_A$ receptor antagonists bicuculline and picrotoxin inhibited 11 μM propofol enhancement of [^{3}H]GABA release

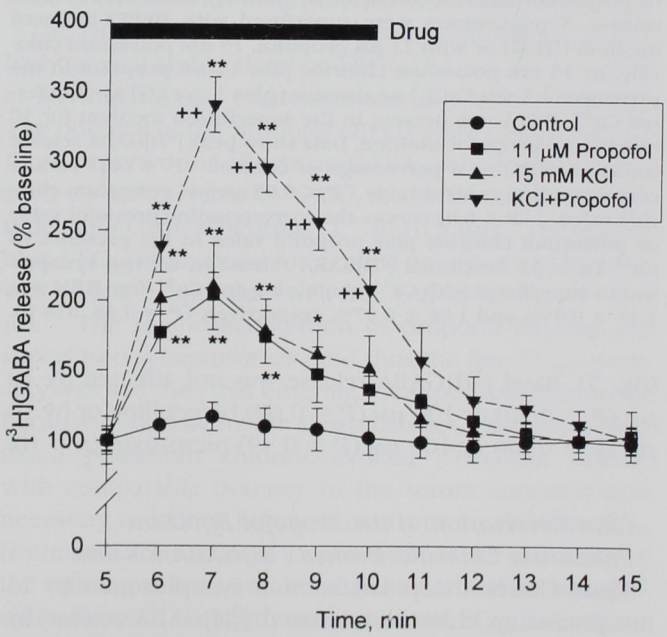

Fig. 3. Time course of the effect of propofol on spontaneous and potassium chloride–evoked [^{3}H]γ-aminobutyric acid (GABA) release. Shown is a typical time course observed for superfusion of synaptosomes during 6–10 min with HEPES-buffered medium (●), 11 μM propofol (■), 15 mM potassium chloride (▲), or 11 μM propofol plus 15 mM potassium chloride (▼). Curves show fractional [^{3}H]GABA release (mean ± SEM) as a percentage of baseline (fraction at 5 min). *P < 0.05, **P < 0.01 *versus* simultaneous control value; ^{++}P < 0.01 *versus* potassium chloride alone.

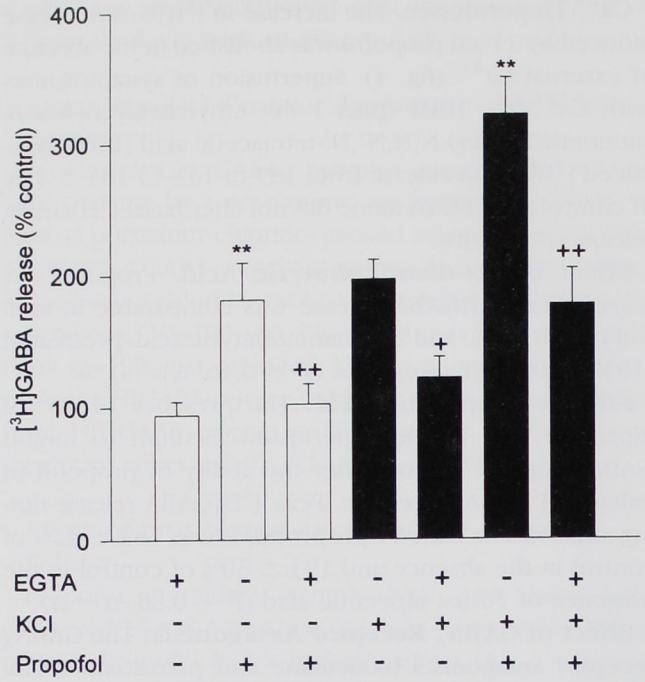

Fig. 4. Ca^{2+}-dependence of propofol facilitation of spontaneous or potassium chloride–evoked [^{3}H]γ-aminobutyric acid (GABA) release. Synaptosomes were superfused with HEPES-buffered medium (HBM) or with 11 μM propofol, 15 mM potassium chloride, or 15 mM potassium chloride plus 11 μM propofol in the presence (1.3 mM $CaCl_2$) or absence (plus 1 mM EGTA) of external Ca^{2+}. EGTA was present in the superfusion medium for 10 min before propofol addition. Data show peak [^{3}H]GABA release (mean ± SEM) as a percentage of control. $^{**}P < 0.01$ *versus* corresponding control value, $^{+}P < 0.05$ *versus* potassium chloride value, $^{++}P < 0.01$ *versus* the corresponding propofol value or potassium chloride plus propofol value in the presence of Ca^{2+} (n = 6). Fractional [^{3}H]GABA release in control synaptosomes superfused with Ca^{2+}-containing and Ca^{2+}-free HBM was 1.17 ± 0.05% and 1.06 ± 0.07%, respectively ($P = 0.48$, n = 6).

(fig. 5). Basal [^{3}H]GABA release was not affected by 10 μM ($P = 0.90$) or 100 μM ($P = 0.62$) bicuculline or by 10 μM ($P = 0.88$) or 100 μM ($P = 0.49$) picrotoxin (n = 6).

Characterization of the Propofol Effect on Potassium Chloride-Evoked [^{3}H]GABA Release

Time Course. Depolarization of synaptosomes by 15 mM potassium chloride increased [^{3}H]GABA release by approximately two times greater than the control values (table 2). Potassium chloride-evoked [^{3}H]GABA release increased during the first 2 min, with a peak increase of 196 ± 29% of control at 7 min (fig. 3). This was followed by a slow return to baseline from 11 to 15 min. Propofol-enhanced potassium chloride-evoked [^{3}H]GABA release peaked at 7 min (318 ± 19% of control) and gradually returned to baseline from 11 to 15 min. This effect was slightly more than additive compared with the effects of potassium chloride and propofol alone.

Ca^{2+} Dependence. Superfusion of synaptosomes with Ca^{2+}-free HBM inhibited propofol facilitation of 15 mM potassium chloride-evoked [^{3}H]GABA release (fig. 4). The effect of 15 mM potassium chloride plus 11 μM propofol was reduced from 321 ± 28% to 177 ± 27% of control. Facilitation of potassium chloride-evoked [^{3}H]GABA release by etomidate, pentobarbital, and alphaxalone also was Ca^{2+} dependent (data not shown). EGTA inhibited potassium chloride-evoked [^{3}H]GABA release from 196 ± 15% to 121 ± 16% of control.

Effect of $GABA_A$ Receptor Antagonists

Facilitation of potassium chloride-evoked [^{3}H]GABA release by 11 μM propofol was inhibited by bicuculline and picrotoxin (fig. 5). The [^{3}H]GABA release evoked by propofol and potassium chloride was not significantly different from the [^{3}H]GABA release evoked by potas-

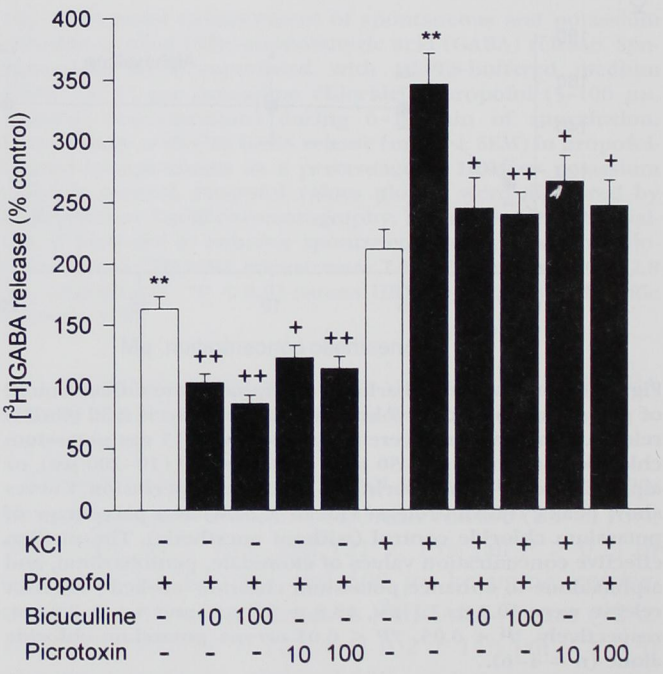

Fig. 5. Inhibition of propofol facilitation of spontaneous and potassium chloride–evoked [^{3}H]γ-aminobutyric acid (GABA) release by $GABA_A$ receptor antagonists. Synaptosomes were superfused with HEPES-buffered medium (HBM) or 11 μM propofol, 15 mM potassium chloride, or 15 mM potassium chloride plus 11 μM propofol. Bicuculline (10 and 100 μM) and picrotoxin (10 and 100 μM) were present in the superfusion medium for 10 min before propofol was added. Data show peak [^{3}H]GABA release (mean ± SEM) as a percentage of control. $^{**}P < 0.01$ *versus* corresponding control value, $^{+}P < 0.05$, $^{++}P < 0.01$ *versus* propofol or potassium chloride plus propofol value (n = 4–6).

sium chloride alone in the presence of 10 μM bicuculline ($P = 0.79$), 100 μM bicuculline ($P = 0.65$), 10 μM picrotoxin ($P = 0.94$), or 100 μM picrotoxin ($P = 0.74$). Potassium chloride–evoked [^{3}H]GABA release was not altered by 10 μM bicuculline ($P = 0.12$) or by 10 μM ($P = 0.88$) or 100 μM ($P = 0.36$) picrotoxin, but it was enhanced by 100 μM bicuculline (140 ± 21% of control, $P = 0.032$).

Discussion

Propofol, etomidate, pentobarbital, and alphaxalone, but not ketamine, enhanced [^{3}H]GABA release evoked from superfused synaptosomes by a submaximal concentration of potassium chloride. Propofol also enhanced spontaneous [^{3}H]GABA release, although etomidate, pentobarbital, alphaxalone, and ketamine did not. These findings indicate that intravenous general anesthetics have agent-specific presynaptic actions on GABA release from GABA-ergic nerve terminals.

Synaptosomes are too small for electrical depolarization, so ionic depolarization by potassium chloride elevation, Na^+ channel activation, or K^+ channel inhibition are used commonly to stimulate neurotransmitter release. Depolarization by an elevated potassium chloride concentration is the standard method for initiating [^{3}H]GABA release from synaptosomes and consists of Ca^{2+}-dependent exocytotic release and Ca^{2+}-independent release by reversal of GABA transport.[16,17] Propofol stimulation of potassium chloride–evoked and spontaneous [^{3}H]GABA release was markedly attenuated in the absence of extracellular Ca^{2+}, consistent with an effect of propofol on Ca^{2+}-dependent release from vesicular stores.[13] Propofol also stimulated [^{3}H]GABA release evoked by 4-aminopyridine, a K^+ channel inhibitor that induces vesicular neurotransmitter release by causing repetitive action potential–like depolarizations of the nerve terminal that more closely mimic physiologic terminal depolarization.[18] Insensitivity to 2,4-diaminobutyric acid pretreatment indicated that propofol-evoked [^{3}H]GABA release was from vesicular rather than cytoplasmic stores. Although propofol facilitation of spontaneous [^{3}H]GABA release was completely Ca^{2+} dependent, potassium chloride–evoked [^{3}H]GABA release exhibited a Ca^{2+}-independent component that was also potentiated by propofol. Propofol facilitation of [^{3}H]GABA release is not caused by inhibition of [^{3}H]GABA reuptake because facilitatory effects were observed in the presence of nipecotic acid and were Ca^{2+} dependent. Therefore, both effects of propofol principally involve potentiation of vesicular release rather than transporter-mediated effects, such as inhibition of reuptake or stimulation of reversed uptake.

The order of potency to facilitate potassium chloride–evoked [^{3}H]GABA release was alphaxalone > propofol > etomidate > pentobarbital. Etomidate was the most effective agent (2.9-fold stimulation), followed by propofol, alphaxalone, and pentobarbital. A similar order of potency for increased [^{3}H]GABA binding, reduced [^{35}S]*t*-butyl bicyclophosphorothionate binding, and potentiation of muscimol-induced $^{36}Cl^-$ uptake into cerebral cortical membrane vesicles mediated by $GABA_A$ receptors has been reported for alphaxalone, propofol, and pentobarbital.[19] Interactions of propofol, etomidate, alphaxalone, and pentobarbital with $GABA_A$ receptors are well documented.[6] Ketamine, which stereoselectively inhibits N-methyl-D-aspartate receptors at clinically relevant concentrations and interacts with $GABA_A$ receptors only at much higher concentrations (0.5 mM),[20] did not affect [^{3}H]GABA release. Therefore, the potencies of these agents in facilitating [^{3}H]GABA release correlate with their potencies at $GABA_A$ receptors.

The facilitatory effects of propofol, etomidate, pentobarbital, and alphaxalone on [^{3}H]GABA release occurred at low micromolar concentrations and were reversible. The potency of the propofol effects were comparable to those reported for effects on $GABA_A$ receptors *in vitro*, such as the increase in GABA-mediated current amplitude in rat cortical neurons (1.7 to 16.8 μM).[5] The whole-blood EC_{50} of propofol for general anesthesia in humans was 85 μM,[21] equivalent to a free concentration of ~1.3 μM.[22] The free concentration of propofol bathing the superfused synaptosomes (and thus the free EC_{50}) probably was less than that entering the superfusion chamber because of uptake by the synaptosomes. Etomidate facilitated potassium chloride–evoked [^{3}H]GABA release with comparable potency to the serum concentration necessary to induce hypnosis in rats (8.2 μM).[6] The EC_{50} (estimated from plasma concentrations for recovery of the righting reflex in mice) of pentobarbital for anesthesia (~50 μM)[23] is slightly more than the EC_{50} to facilitate potassium chloride-evoked [^{3}H]GABA release. The peak plasma alphaxalone concentration during anesthesia in humans (7.5 μM)[24] is comparable to the EC_{50} to facilitate potassium chloride–evoked [^{3}H]GABA release.

The time course for propofol facilitation of spontaneous [^{3}H]GABA release suggests desensitization of the effect. It is unlikely that the gradual inhibition of the propofol response resulted from depletion of radiola-

beled GABA because greater [^{3}H]GABA release was observed with 30 mM potassium chloride (table 2), and a subsequent stimulus with 15 mM potassium chloride evoked a twofold increase in [^{3}H]GABA release. The time course for potassium chloride-evoked [^{3}H]GABA release exhibited a similar decrement, suggesting that this effect may be an intrinsic property of stimulated GABA release from nerve terminals.

The effects of propofol and pentobarbital on potassium chloride-evoked [^{3}H]GABA release were biphasic, with facilitation at lower concentrations and a return to baseline or less at higher concentrations. These findings are consistent with previous reports of the biphasic effects of pentobarbital on GABA release[25–27] and emphasize the importance of anesthetic concentrations in studies of GABA release. The inhibitory effects of propofol and pentobarbital at higher concentrations may be caused by inhibition of voltage-dependent Ca^{2+} channels,[28,29] or by biphasic effects on $GABA_A$ receptors, as observed with muscimol.

Facilitatory effects have been reported in some studies of general anesthetic effects on GABA release,[25–27] but not in others.[30] Micromolar concentrations of pentobarbital increased, but millimolar concentrations inhibited, electric stimulation-evoked release of endogenous GABA from rat olfactory cortical slices[25] or spontaneous release of [^{3}H]GABA from rabbit retina.[26] Biphasic concentration–response curves also were reported for the effects of methohexital and thiopental on potassium chloride-evoked [^{14}C]GABA release from rat thalamic slices.[27] In contrast, pentobarbital and thiopental inhibited potassium chloride-evoked [^{14}C]GABA release from rat cerebrocortical slices with no enhancement at lower concentrations.[31] Pentobarbital inhibited potassium chloride-evoked [^{14}C]GABA release from mouse forebrain synaptosomes[32] but had no effect on [^{3}H]GABA release from whole mouse brain synaptosomes.[33] Pentobarbital enhanced electric stimulation-evoked,[34] but inhibited potassium chloride-evoked, [^{3}H]GABA release from rat cortical[35] and midbrain slices.[36] Several anesthetics, including thiopental, did not affect potassium chloride-evoked [^{3}H]GABA release from rat striatal synaptosomes,[30] although the same group reported that thiopental, but not halothane or isoflurane, inhibited potassium chloride-evoked [^{3}H]GABA release.[37] Differences in preparation (brain slice *vs.* synaptosomes), drug concentrations (biphasic effects), assay timing (desensitization), brain regions (cerebral cortex, striatum, or thalamus), ion concentrations, mode of stimulation (electrical *vs.* potassium chloride depolarization), and/or interactions with other neurotransmitter systems in slices, may have contributed to these disparate results.

Anesthetic facilitation of [^{3}H]GABA release may be mediated by presynaptic $GABA_A$ receptors. Bicuculline and picrotoxin inhibited propofol facilitation of spontaneous and potassium chloride-evoked [^{3}H]GABA release. The facilitatory effect of propofol on [^{3}H]GABA release was slightly more sensitive to bicuculline than to picrotoxin, perhaps because of involvement of subtly different mechanisms or receptor subtypes. Thiopental and methohexital facilitation of potassium chloride-evoked GABA release in thalamic slices was also sensitive to bicuculline.[27] Furthermore, muscimol at nanomolar concentrations stimulated, whereas micromolar concentrations inhibited, potassium chloride-evoked [^{3}H]GABA release. This biphasic response may explain previous reports of inhibition[15] or no effect[38] of muscimol on potassium chloride-evoked [^{3}H]GABA release in cortical synaptosomes. The anesthetic effects may result from more than one action on the nerve terminal. Indeed, the variable efficacy and biphasic effects of the anesthetics suggest that multiple agent-specific actions are involved.

The observation that propofol enhancement of GABA release is Ca^{2+}-dependent suggests the involvement of presynaptic membrane depolarization and Ca^{2+} channel activation. Pentobarbital has been shown to enhance potassium chloride-stimulated ^{45}Ca influx into cortical synaptosomes,[39] and halothane and isoflurane increased spontaneous miniature inhibitory postsynaptic current frequency in rat hippocampal slices.[40] The latter effect was associated with presynaptic neuron depolarization and appeared to require intracellular Ca^{2+} release. Other studies have shown that GABA can have excitatory effects in certain situations, such as in neonatal neurons, during high-frequency stimulation, or with high GABA concentrations.[41] Depolarizing effects of GABA followed by synaptic excitation have been described in several preparations, including adult rat hippocampal slices.[42] GABA-induced depolarization during conditions of increased intracellular Cl^- concentrations has been attributed to outward Cl^- or HCO_3^- flux through $GABA_A$ receptor channels as a result of the altered Cl^- equilibrium potential. Further analysis will be necessary to determine the mechanisms involved in the facilitatory presynaptic effects of anesthetics on GABA release.

In contrast to the facilitatory effects of intravenous anesthetics on [^{3}H]GABA release reported here, propofol[22] and volatile anesthetics[43,44] inhibited endogenous glutamate release from rat cortical synaptosomes, apparently by inhibiting Na^+ channels,[22,45] although Ca^{2+}

channel inhibition may also be involved.[44] These observations show transmitter-specific presynaptic actions of intravenous anesthetics and suggest the existence of distinct anesthetic-sensitive targets on GABA-ergic compared to glutamatergic nerve terminals.

Our results suggest that presynaptic enhancement of GABA release may be involved in the facilitation of inhibitory GABA-ergic transmission by certain intravenous anesthetics. This mechanism may be particularly relevant because of the evidence that postsynaptic $GABA_A$ receptors are nearly saturated by the GABA released from a single vesicle,[46] which would minimize the effect of postsynaptic $GABA_A$ receptor potentiation on the amplitude of inhibitory synaptic potentials. Potentiation of GABA release may also contribute to the neuroprotective effects of certain general anesthetics. An increase in the frequency of spontaneous (action potential independent) or stimulated (action potential dependent) quantal GABA release may be an important mechanism by which general anesthetics enhance inhibitory synaptic transmission.

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