Volatile Anesthetic Effects on Glutamate versus GABA Release from Isolated Rat Cortical Nerve Terminals: Basal Release

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ABSTRACT

The effects of three volatile anesthetics (isoflurane, enflurane, and halothane) on basal release of glutamate and GABA from isolated rat cerebrocortical nerve terminals (synaptosomes) were compared using a dual isotope superfusion method. Concentration-dependent effects on basal release differed between anesthetics and transmitters. Over a range of clinical concentrations (0.5–2 minimum alveolar concentration), basal glutamate release was inhibited by all three anesthetics, whereas basal GABA release was unaffected (enflurane) or unaffected (halothane). These effects may represent a balance of stimulatory and inhibitory mechanisms between transmitters and anesthetics. There were no significant differences between anesthetic effects on basal release in the absence or presence of external Ca2+, whereas intracellular Ca2+ buffering limited volatile anesthetic inhibition of basal glutamate release. Although these results demonstrate fundamental differences in anesthetic effects on basal release between glutamatergic and GABAergic nerve terminals, all three volatile anesthetics at clinical concentrations consistently reduced the ratio of basal glutamate to GABA release. These actions may contribute to the net depression of glutamatergic excitation and potentiation of GABAergic inhibition characteristic of general anesthesia.

Depression of neuronal activity by general anesthetics involves reduced excitatory glutamatergic neurotransmission and enhanced inhibitory GABAergic neurotransmission involving a number of potential molecular targets (Hemmings et al., 2005). Volatile anesthetics modulate synaptic transmission by both presynaptic and postsynaptic mechanisms (MacIver, 1997). Anesthetic effects on basal and evoked glutamate versus GABA release together with potentiation of postsynaptic GABA receptors (Jones et al., 1992; MacIver, 1997) likely converge to produce CNS depression by reducing excitatory and enhancing inhibitory transmission.

Presynaptic action potentials trigger neurotransmitter release via activation of voltage-gated Ca2+ channels coupled to Ca2+-dependent exocytotic mechanisms (Südhof, 2004). Spontaneous vesicular fusion and nonvesicular reverse transporter operation can also release neurotransmitters in the absence of depolarization-evoked Ca2+ influx (Südhof, 2004; Cavelier et al., 2005). These modes of action potential-independent basal release employ distinct presynaptic mechanisms and are thought to be differentially regulated (Engelman and MacDermott, 2004). Action potential-independent vesicular transmitter release occurs in a random, unsynchronized manner by the spontaneous fusion of vesicles to the presynaptic membrane (Engelman and MacDermott, 2004). This spontaneous quantal release of glutamate and GABA produces miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs), respectively (Haseneder et al., 2004) and is subject to modulation by various Ca2+-dependent and Ca2+-independent mechanisms (Bouron, 2001). Volatile anesthetic-induced increases in the frequency of mIPSCs in rat hippocampal slices have been attributed to anesthetic-induced increases in spontaneous GABA release (Nishikawa and MacIver, 2000; Nishikawa and MacIver, 2001). In contrast, mEPSC frequency is reduced by volatile anesthetics in rat hippocampal (Nishikawa and MacIver, 2000) and spinal cord slices (Cheng and Kendig, 2003; Haseneder et al., 2004). Halothane decreases both mEPSC and mIPSC frequency in cultured rat cortical neurons (Kitamura et al., 2003), suggesting regional heterogeneity in presynaptic effects. Thus, volatile anesthetics can modulate the spontaneous release of the primary excitatory and inhibitory neurotransmitters, although the effects of specific agents and their mechanisms are unclear.

Volatile general anesthetics have been reported previously to have small effects on basal release of glutamate from

ABBREVIATIONS: CNS, central nervous system; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetrakis(acetoxymethyl) ester; 4AP, 4-aminoypyridine; MAC, minimum alveolar concentration; KHB, Krebs-HEPES buffer; mEPSCs, miniature excitatory postsynaptic currents; mIPSCs, miniature inhibitory postsynaptic currents; LDH, lactate dehydrogenase; FR, fractional release.
isolated nerve terminals (synaptosomes) (Hirose et al., 1992; Miao et al., 1995; Schlame and Hemmings, 1995; Westphalen and Hemmings, 2003a) and tissue slices (Bickler et al., 1995; Toner et al., 2001) and of GABA from synaptosomes (Hirose et al., 1992; Mantz et al., 1995; Westphalen and Hemmings, 2003a), which were considered as insignificant. However, even small changes in basal transmitter release may have significant effects on CNS function (Cavelier et al., 2005). Therefore, we attempted to better define these presynaptic effects by comparing the actions of several volatile anesthetics on basal (spontaneous) glutamate and GABA release from the same isolated rat cortical nerve terminal preparations, the results from which revealed agent- and transmitter-specific effects.

Materials and Methods

Materials. Isoflurane was obtained from Abbott Laboratories (Abbott Park, IL), thymol-free halothane was from Halocarbon Laboratories (River Edge, NJ), and enflurane was from BOC Health Care (Murray Hill, NJ). Tetrodotoxin, BAPTA-AM, and 4AP were obtained from Sigma-Aldrich (St. Louis, MO); L-[^3H]glutamate (42 Ci/mmol) was from GE Healthcare (Little Chalfont, Buckinghamshire, UK); and [14C]GABA (0.24 Ci/mmol) was from PerkinElmer Life and Analytical Sciences (Boston, MA).

Synaptosome Preparation and Loading. Experiments were performed 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. Synaptosomes (isolated nerve terminals) were prepared as described previously (Westphalen and Hemmings, 2003a), with the exception that reduction of cytoplasmic L-[^3H]glutamate with d-aspartate was not performed. In brief, the cerebral cortex of adult male Sprague-Dawley rats (200–280 g) was homogenized in ice-cold 0.32 M sucrose with a motor-driven (500 rpm) Teflon glass homogenizer and centrifuged for 2 min at 4000g. The resulting supernatant was demyelinated by centrifugation though a sucrose gradient. The final pellet containing isolated cerebrocortical nerve terminals was resuspended in ice-cold 0.32 M sucrose and incubated with 8 mM L-[^3H]glutamate and 440 nM [14C]GABA for 15 min at 30°C in Krebs-HEPES buffer (KHB; composition consists of 140 mM NaCl, 5 mM KCl, 20 mM HEPES, 1 mM MgCl₂, 1.2 mM Na₃HPO₄, 5 mM NaHCO₃, 0.1 mM EDTA, and 10 mM t-glucose, pH 7.4 with NaOH). Incubation was terminated by centrifugation for 10 min at 20,000g at 4°C, and prelabeled synaptosomes were resuspended in ice-cold 0.32 M sucrose.

Basal Glutamate and GABA Release. Prelabeled synaptosomes (0.3–0.8 mg of protein) were confined between Whatman GF/B glass fiber filter disks (Maidstone, UK) and superfused at 0.5 ml/min with KHB at 37°C using a customized (Scheme 1) SF12 superfusion apparatus (Brandel Inc., Gaithersburg, MD) set to collect 1-min fractions. Unless otherwise stated, anesthetics were applied for 6 min in the absence or presence of Ca²⁺ (final free [Ca²⁺] of 1.9 mM). Each experiment included the determination of evoked release (2 min pulse of 4AP in the presence of 1.9 mM Ca²⁺) for interassay data normalization to control for variations in synaptosome preparation. At the end of each experiment, synaptosomes were lysed with 0.2 M perchloric acid, and residual radioactivity in the synaptosomes and each fraction was quantified by liquid scintillation spectrometry with dual isotope quench correction (LS 6000IC; Beckman Coulter, Fullerton, CA) using BioSafe II scintillation cocktail (RPI, Mt. Prospect, IL). The accuracy of the dual radio-label assay has been verified previously in detecting changes in the release of each amino acid independently of the other (Westphalen and Hemmings, 2003b).

Application of Anesthetics. Anesthetics were prepared by dilution of saturated solutions (isoflurane, 11.5 mM; enflurane, 11.0 mM; and halothane, 16.5 mM) in KHB and introduced through Teflon tubing from glass syringes (closed system; see Scheme 1) as a 6-min pulse. In experiments that included BAPTA-AM, preloaded synaptosomes were perfused with 50 μM BAPTA-AM in KHB for 12 min at 37°C before anesthetic application. After each experiment, remaining solutions from each syringe containing anesthetic were drawn through the perfusion system and sampled upon immediate exit from the synaptosome chamber into a gas tight glass syringe and then extracted into n-heptane (1:10, v/v) for analysis by gas chromatography (Ratnakumari and Hemmings, 1998). Solution anesthetic concentrations were compared with their potencies as defined by minimum alveolar concentration (MAC) in rat: 0.35 mM for isoflurane, 0.75 mM enflurane, and 0.35 mM halothane (Taheri et al., 1991).

Analysis of Lactate Dehydrogenase Release. Prelabeled synaptosomes were exposed to 2-min pulses of high [K⁺] (20 mM KCl replacing equivalent NaCl), a K⁺ channel blocker (1 mM 4AP), or to H₂O for 1 min using the perfusion technique described above at a flow rate of 0.2 ml/min. Anesthetic effects were examined using 6-min pulses of 2, 4, and 8× MAC of isoflurane, enflurane, or halothane (Taheri et al., 1991). Perfusion was collected in one 20-min fraction (encompassing the entire release pulse) followed by a 20-min fraction collecting remaining synaptosomal contents after lysis with H₂O. Radioactivity was quantified as above, and lactate dehydrogenase (LDH) activity was determined by spectrophotometric measurement at 340 nm of NADH (0.5 mM) oxidation in the presence of excess pyruvate (50 mM) at 37°C for 60 min in 96-well microtiter plates using a plate reader (SoftMax Pro version 4.0; Molecular Devices, Sunnyvale CA).

Data Analysis. Release in each fraction was expressed as a fraction of synaptosomal content of labeled transmitter prior to each fraction collected. The magnitude of the release pulse was determined by subtracting baseline release (average of basal release be-
fore and after pulse) from cumulative fractional release values of the release pulse (sum \( \Delta F R \)).

Concentration-effect data for anesthetic (0.1–8 \times MAC) effects on basal L-[\(^{3}\)H]glutamate and [\(^{14}\)C]GABA release were fitted to a rational function (eq. 1, as determined by the equation fitting program TableCurve 2D v5.01; SYSTAT Software Inc., Richmond, CA). An equation was derived to represent the most complex x−y data, the effect of isoflurane on basal L-[\(^{3}\)H]glutamate release. The equation chosen was the most suitable approximating function with the highest degree-of-freedom adjusted coefficient of determination. This equation was then applied to data for all anesthetic effects on basal L-[\(^{3}\)H]glutamate and [\(^{14}\)C]GABA release (Prism software, version 4.0; GraphPad Software, San Diego CA).

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y = \frac{a + cx + ex^2}{1 + bx + dx^2 + fx^3 + gx^4}
\]

(1)

Release from single fraction experiments (transmitter versus LDH) was calculated as more (enhanced) or less (inhibited) than control basal release. The relationship between transmitter release and LDH release was determined by paired Student's t test after effective pairing was tested by the Pearson correlation coefficient (r). Significant correlations between anesthetic concentration and basal transmitter release were determined by linear regression analysis after establishing linearity using a Runs test. Statistical comparisons were made using Prism software, version 4.0.

**Results**

**Basal Release.** Isoflurane, enflurane, and halothane produced concentration-dependent and Ca\(^{2+}\)-independent effects on basal release of both L-[\(^{3}\)H]glutamate and [\(^{14}\)C]GABA (Fig. 1). Effects were either above (+ sum \( \Delta F \)R; stimulation) or below (− sum \( \Delta F \)R; inhibition) control baseline release. Anesthetic effects on basal glutamate release were biphasic (inhibition at clinical concentrations and enhancement at higher supraclinical concentrations). Basal [\(^{14}\)C]GABA release was enhanced by isoflurane at clinical concentrations and by all three volatile anesthetics at supraclinical concentrations (\( \geq 2\times \) MAC).

Isoflurane (Fig. 1A) had biphasic effects on basal L-[\(^{3}\)H]glutamate release; clinical concentrations (0.5–2× MAC) inhibited linearly \((P = 0.96; \text{Fig. 1A, right panel})\), whereas concentrations above the clinical range stimulated release exponentially. The effects of isoflurane on basal [\(^{14}\)C]GABA release increased linearly \((P = 0.36; \text{Fig. 1A, right panel})\) over the clinical concentration range. Regression analysis showed a significant negative correlation between isoflurane concentration and L-[\(^{3}\)H]glutamate release \((P = 0.004)\) and positive correlation with [\(^{14}\)C]GABA release \((P = 0.026)\), indicating concentration-dependent inhibition of L-[\(^{3}\)H]glutamate release and stimulation of [\(^{14}\)C]GABA release.

Enflurane (Fig. 1B) produced similar biphasic effects on basal L-[\(^{3}\)H]glutamate release and stimulated basal [\(^{14}\)C]GABA release at supraclinical concentrations. However, enflurane produced greater inhibition of basal L-[\(^{3}\)H]glutamate release without stimulating release at any concentration. Regression analysis of L-[\(^{3}\)H]glutamate or [\(^{14}\)C]GABA release versus clinical enflurane concentrations (0.5–2× MAC) showed linearity \((P = 0.74 \text{ and } P = 0.98, \text{respectively})\) with inhibition of L-[\(^{3}\)H]glutamate release \((P = 0.0004)\) and no effect on [\(^{14}\)C]GABA release \((P = 0.99; \text{ Fig. 1B, right panel })\).

Enflurane-evoked [\(^{14}\)C]GABA release (Fig. 1A) differed significantly from the concurrent release of LDH \((P < 0.005)\) and lacked correlation \((r^2 = 0.01, P = 0.95, \text{and } r^2 = 0.15, P = 0.3, \text{respectively})\). Similarly, K\(^{+}\)-evoked L-[\(^{3}\)H]glutamate \((P = 0.007)\) and [\(^{14}\)C]GABA release \((P = 0.001)\) differed from concurrent LDH release and lacked correlation \((r^2 = 0.03, P = 0.7 \text{ and } r^2 = 0.16, P = 0.4, \text{respectively})\). As a positive control for lytic release, a 1-min pulse of H\(_2\)O released LDH by hypotonic lysis in parallel with both L-[\(^{3}\)H]glutamate \((P = 0.7)\) and [\(^{14}\)C]GABA \((P = 0.6)\). The degree of LDH release correlated with the release of L-[\(^{3}\)H]glutamate \((r^2 = 0.59, P = 0.04)\) and [\(^{14}\)C]GABA \((r^2 = 0.85, P = 0.003)\), consistent with lytic release.

Because lytic release is not transmitter-selective (Fig. 2A), concurrent [\(^{14}\)C]GABA release and LDH release were compared to determine whether transmitter release by high anesthetic concentrations involved nonspecific nerve-terminal membrane disruption. Isoflurane-evoked [\(^{14}\)C]GABA and LDH release by high anesthetic concentrations was significantly different \((P < 0.001; \text{Fig. 2B})\), suggesting that stimulation of basal [\(^{14}\)C]GABA release by 4 to 8× MAC isoflurane was not due to membrane disruption. Release of [\(^{14}\)C]GABA and LDH by high concentrations \((\geq 8\times \text{MAC})\) of enflurane and halothane did not differ \((P = 0.8 \text{ and } 0.5, \text{respectively}; \text{Fig. 2, C and D})\), but [\(^{14}\)C]GABA versus LDH release did not correlate \((r^2 = 0.013, P = 0.8 \text{ and } r^2 = 0.097, P = 0.4, \text{respectively})\). These results suggest that loss of membrane integrity is not responsible for transmitter release in the presence of anesthetic concentrations up to 8× MAC.

**Effects of Internal Calcium Chelation.** The membrane-permeable Ca\(^{2+}\)-chelator BAPTA-AM combined with external Ca\(^{2+}\)-chelation by EGTA significantly reduced preanesthetic baseline fractional release of L-[\(^{3}\)H]glutamate \((0.0230 \pm 0.0002 \text{ to } 0.0216 \pm 0.0003; P = 0.002)\) and [\(^{14}\)C]GABA \((0.0130 \pm 0.0003 \text{ to } 0.0095 \pm 0.0002; P < 0.0001)\),...
Fig. 1. Anesthetic effects on basal glutamate and GABA release. The effects of isoflurane (A), enflurane (B), and halothane (C) on basal release of L-[3H]glutamate and [14C]GABA from preloaded rat cortical synaptosomes were tested in the absence or presence of 1.9 mM Ca\(^{2+}\). Sum ΔFR values from 6-min pulses of anesthetic or tetrodotoxin, standardized to 1 mM 4AP-evoked release in the presence of 1.9 mM Ca\(^{2+}\), were fitted to a rational function (see Materials and Methods for eq. 1) and shown to be Ca\(^{2+}\)-independent. The dotted lines represent the fitted curve to combined data in the absence and presence of Ca\(^{2+}\). Shaded area highlights the clinical concentration range (0.5–2× MAC). Linear regression analyses of data between 0.5 and 2× MAC in the absence and presence of Ca\(^{2+}\) showed significant linearity (nonsignificant nonlinearity) for all groups (right panels). Isoflurane concentrations significantly correlated with basal L-[3H]glutamate and [14C]GABA release, indicating inhibition and stimulation, respectively. Enflurane and halothane concentrations significantly correlated with L-[3H]glutamate release but not with [14C]GABA release, indicating inhibition and no effect, respectively. For each volatile anesthetic tested, the difference between basal L-[3H]glutamate versus [14C]GABA release increased in magnitude as the concentration increased. See Results for statistical analysis.
suggesting a contribution of intracellular Ca$^{2+}$ to basal release rate. The greater reduction by BAPTA-AM in baseline $[^{14}\text{C}]$GABA release compared with L-[3H]glutamate release suggests that basal $[^{14}\text{C}]$GABA release has a greater sensitivity to internal Ca$^{2+}$.

The dependence on internal Ca$^{2+}$ for anesthetic effects on basal release was analyzed on maximal glutamate release inhibition (4× MAC enflurane and halothane; Fig. 1, B and C, left panels) or significant GABA release stimulation (4× MAC isoflurane; Fig. 1A, center panel) to maximize the “signal-to-noise” ratio. Internal Ca$^{2+}$ chelation by BAPTA-AM diminished inhibition of basal L-[3H]glutamate release by pulses of enflurane (control sum $\Delta FR = -0.030 \pm 0.004, n = 11$ versus +BAPTA-AM $= -0.012 \pm 0.007, n = 10; P = 0.03$) or halothane (control sum $\Delta FR = -0.048 \pm 0.003, n = 11$ versus +BAPTA-AM $= -0.031 \pm 0.005, n = 10; P = 0.03$). This suggests a decrease in the inhibitory component of anesthetic effects on basal L-[3H]glutamate release by BAPTA-AM across anesthetic type. The baseline release of L-[3H]glutamate after pulses of anesthetic was higher than the preanesthetic baseline for isoflurane ($P < 0.0001$), enflurane ($P = 0.0002$), and halothane ($P < 0.0001$). This difference between preanesthetic and postanesthetic baseline release was also evident in the presence of BAPTA-AM, although the magnitude was significantly less for isoflurane ($P = 0.014$), enflurane ($P = 0.028$), and halothane ($P = 0.031$). This suggests persistent internal Ca$^{2+}$-dependent effects on L-[3H]glutamate release by anesthetics. The effects of pulse application of all three anesthetics on basal $[^{14}\text{C}]$GABA release were unaffected by BAPTA-AM (Fig. 3). Internal Ca$^{2+}$ chelation by BAPTA-AM did not alter the overall profile of anesthetic effects on basal L-[3H]glutamate and $[^{14}\text{C}]$GABA release between pulse and continuous application (Fig. 3).

**Pulse versus Continuous Application.** Effects of pulse-applied volatile anesthetics at concentrations that produced maximal glutamate release inhibition and significant GABA release stimulation were compared with effects produced by continuously applied anesthetic. Enflurane (Fig. 3B) and halothane (Fig. 3C) produced inhibition followed by delayed increases in basal release of L-[3H]glutamate above control levels after discontinuation of the pulse. Continuous application of isoflurane produced transient stimulation of basal glutamate release (Fig. 3A), whereas continuous application of enflurane (Fig. 3B) or halothane (Fig. 3C) produced sustained inhibition. Stimulation of $[^{14}\text{C}]$GABA release by isoflurane returned toward control values after the pulse application. Equivalent MAC concentrations of enflurane applied as a pulse stimulated $[^{14}\text{C}]$GABA release but not to the extent as isoflurane, whereas halothane had no effect. When continuously applied, enflurane produced sustained stimulation of $[^{14}\text{C}]$GABA release, whereas halothane had no effect.

**Discussion**

The halogenated ethers isoflurane and enflurane and the halogenated alkane halothane differentially affected basal glutamate and GABA release from isolated rat cortical nerve terminals. Effects on basal glutamate release were biphasic and agent-specific. Basal glutamate release was inhibited in the clinical concentration range (0.5–2× MAC) to a degree that varied between anesthetics. This inhibition was followed by enhancement (isoflurane) or reduced inhibition (enflurane and halothane) as anesthetic concentrations increased above clinical concentrations. In contrast, basal GABA release was enhanced by isoflurane at clinical concentrations and by enflurane and halothane at supraclinical concentrations. Despite these agent-specific effects, there was a consistent trend toward a concentration-dependent net decrease in basal glutamate relative to GABA release over clinical concentrations.

Concentrations of isoflurane (up to 8× MAC) and enflurane (up to 4× MAC) enhanced transmitter release but did not corelease LDH, an indicator of membrane lysis. This suggests that the divergent and biphasic effects of volatile anesthetics on basal glutamate versus GABA release are pharmacological effects rather than nonspecific toxic effects. The distinct effects of anesthetics on glutamate versus GABA release support transmitter-specific sites of action on presynaptic release mechanisms, whereas the biphasic nature of these effects indicates both inhibitory and stimulatory targets associated with each transmitter. Effects on nerve-terminal glutamate and GABA transporters resulting in reverse transport are probably not the anesthetic mechanism responsible, because maximal inhibition of synaptosomal GABA uptake is <20% (Westphalen and Hemmings, 2003b), whereas similar isoflurane concentrations produced expo-
nential release and carrier-mediated release is unaffected by 1 mM isoflurane (Westphalen and Hemmings, 2003b).

Preferential depression of basal glutamate over GABA release suggests fundamental physiological differences between glutamatergic and GABAergic nerve terminals. The physiological difference(s) between glutamatergic and GABAergic nerve terminals that underlie their distinct responses to volatile anesthetics remains to be determined. Although nerve-terminal Na\(^+\) channels are inhibited by volatile anesthetics (Ratnakumari et al., 2000) and Na\(^+\) channel block differentially affects evoked glutamate versus GABA release (Prakriya and Mennerick, 2000; Westphalen and

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**Fig. 3.** Effects of pulse versus continuous application of volatile anesthetics on transmitter release. A 6-min pulse (P) or continuous (C) application of isoflurane (P: 1.45 ± 0.03 mM, n = 13; C: 1.49 ± 0.03 mM, n = 7) (A), enflurane (P: 3.33 ± 0.08 mM, n = 11; C: 3.01 ± 0.15 mM, n = 8) (B), or halothane (P: 1.57 ± 0.04 mM, n = 11; C: 1.44 ± 0.07 mM, n = 7) (C) was tested on basal release of L-[\(^{3}\)H]glutamate and [\(^{14}\)C]GABA from rat cortical synaptosomes. Effects were compared with basal release from synaptosomes loaded with 50 μM BAPTA-AM using pulse and continuous perfusion of isoflurane (P: 1.45 ± 0.05 mM, n = 9; C: 1.48 ± 0.02 mM, n = 5) (A), enflurane (P: 3.27 ± 0.17 mM, n = 10; C: 2.95 ± 0.13 mM, n = 6) (B), or halothane (P: 1.58 ± 0.05 mM, n = 10; C: 1.47 ± 0.11 mM, n = 6) (C). Baseline release before pulse and continuous anesthetic application (first four to five fractions) was normalized.
Hemmings, 2003a, 2005), the selective Na⁺ channel blocker tetrodotoxin did not discriminate between basal release of both glutamate and GABA. The small effect of tetrodotoxin on basal release may reflect suppression of spontaneous action potential-evoked release or hyperpolarization of the isolated nerve terminals.

In addition to their inhibitory effects on Na⁺ channels, volatile anesthetics can also affect K⁺ and Ca²⁺ channels (see Topf et al., 2003), ligand-gated ion channels (Franks and Lieb, 1994), cell signaling machinery, such as protein kinase C (Hemmings and Adamo, 1998) and G protein-coupled receptors (Yamakura et al., 2001), and synaptic vesicle docking machinery (Hawasaki et al., 2004), all of which can modulate spontaneous transmitter release (Engelmann and MacDermott, 2004). Distinct distributions of presynaptic ion channels occur within and between neurons. Nerve-terminal-specific differences in the expression of various subtypes of Na⁺ channels (Goldin, 2001), K⁺ channels (Veh et al., 1995), and Ca²⁺ channels (Reid et al., 2003) provide potential neurochemical substrates for differential pharmacological effects on glutamate versus GABA transmission through release modulation by a single agent. A major role for Na⁺ or Ca²⁺ channels in anesthetic effects on basal release is unlikely given the low sensitivity of basal release to tetrodotoxin or Ca²⁺ chelation. A role for the activation of K⁺ channels, including two-pore domain K⁺ channels, some of which are sensitive to volatile anesthetics (Franks and Honoré, 2004), is possible. Augmentation of Ca²⁺-independent basal GABA release by volatile anesthetics, despite concurrent inhibition of presynaptic Na⁺ channels and possibly K⁺ channel activation, suggests actions at additional presynaptic sites that are specific to GABA terminals and downstream or distinct from the Ca²⁺-dependent step(s) of vesicular transmitter exocytosis. The bidirectional nature of isoflurane effects on basal transmitter release may involve a cumulative balance between opposing stimulatory and inhibitory mechanisms that vary between transmitter type and anesthetic agents. The observed changes in basal release suggest a net increase in basal inhibitory versus excitatory transmitter release, which may represent a component of the mechanism(s) by which volatile anesthetics depress global CNS function.

Although spontaneous fusion of synaptic vesicles can occur in the absence of extracellular Ca²⁺ (Bouron, 2001), the resulting miniature postsynaptic potentials are sensitive to small changes in presynaptic Ca²⁺ concentrations by activation of voltage-gated Ca²⁺ channels, Ca²⁺-permeable ionicotropic receptors, and release of Ca²⁺ from intracellular stores (Angleson and Betz, 2001). Volatile anesthetics can inhibit multiple voltage-gated Ca²⁺ channels (Study, 1994) and N-methyl-D-aspartate receptors (Nishikawa and Maevler, 2000). However, the release of basal glutamate and GABA was Ca²⁺-independent, ruling out significant effects at these potential targets. Volatile anesthetics may also release Ca²⁺ from intracellular stores in neuronal cells (Modly et al., 1991), an effect that may also occur in specific nerve terminals. This could lead to preferential increase in basal GABA over glutamate release, as suggested by the greater sensitivity of GABA release to intracellular Ca²⁺ chelation compared with glutamate release. However, anesthetic effects on basal GABA release were essentially unchanged by external and internal calcium chelation with EGTA and BAPTA-AM, respectively. BAPTA-AM reduced the inhibition of basal glutamate release by enflurane or halothane, suggesting a role of intracellular Ca²⁺ in the depression of basal glutamate release. Enhanced stimulation of glutamate release by isoflurane in the presence of BAPTA-AM also supports this notion, with a resulting shift in the balance between stimulatory and inhibitory effects at the glutamatergic terminal.

Anesthetics applied continuously showed sustained effects on basal glutamate and GABA release, whereas pulse applications showed transient effects that included persistent increases in glutamate but not GABA release upon termination of the anesthetic pulse. This comparison again demonstrates transmitter-selective anesthetic effects, but with a caveat that supraclinical concentrations used here may recruit additional target sites. Anesthetic was not detectable within the synaptosome chamber >8 min after cessation of pulse application (Westphalen and Hemmings, 2003a) when basal glutamate release was significantly elevated. This indicates selective and persistent changes in the glutamate release machinery after a brief anesthetic exposure. This effect on basal glutamate release was partially dependent on internal Ca²⁺. Persistent and preferential effects that last beyond anesthetic exposure (this study; Kapinya et al., 2002; Culley et al., 2004) may underlie transient excitatory and/or sustained cognitive effects of anesthesia seen clinically (Monk et al., 2005).

In conclusion, volatile anesthetics differentially alter basal release of the major inhibitory (GABA) and excitatory (glutamate) amino acid neurotransmitters to increase the ratio of GABA to glutamate release. Preferential anesthetic inhibition of evoked (Westphalen and Hemmings, 2005) and basal (this study) glutamate release compared with GABA release combined with postsynaptic GABA(B) receptor potentiation (Jones et al., 1992) likely synergizes to reduce overall glutamatergic excitatory neurotransmission and potentiates GABAergic inhibitory transmission in the CNS.

References


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