Effects of Halothane and Propofol on Excitatory and Inhibitory Synaptic Transmission in Rat Cortical Neurons

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ABSTRACT
General anesthetics are thought to act on both excitatory and inhibitory neuronal pathways at both post- and presynaptic sites. However, the literature in these regards is somewhat controversial. The aim of the present study was to reassess the relative importance of the various anesthetic actions using a common preparation. Rat cortical neurons in primary culture were used to record spontaneous miniature postsynaptic currents by the whole-cell patch-clamp technique. Halothane at clinically relevant concentrations prolonged the decay phase of spontaneous miniature inhibitory postsynaptic currents (mIPSCs) recorded in the presence of tetrodotoxin and at higher concentrations decreased the frequency of mIPSCs. The mIPSC amplitudes underwent little change. Spontaneous action potential-dependent IPSCs recorded in the absence of tetrodotoxin were similarly affected by halothane. Halothane also decreased the frequency of spontaneous miniature non-N-methyl-D-aspartate (NMDA) excitatory postsynaptic currents (mEPSCs) as well as spontaneous action potential-dependent NMDA EPSCs and non-NMDA EPSCs without affecting their decay phase. The halothane effect on mIPSC and mEPSC frequency was dependent on the external calcium concentration. In contrast to halothane, the only effect of propofol was the prolongation of the decay phase of mIPSCs and IPSCs. The prolongation of mIPSCs and IPSCs by halothane and propofol coupled with the ineffectiveness on mEPSCs and EPSCs suggests a selective postsynaptic modulation of GABA_A receptors. The additional calcium-dependent inhibition of mIPSC and mEPSC frequency by halothane (but not propofol) suggests a more general mechanism by this anesthetic on presynaptic transmitter release.

It is reasonable to hold that anesthesia can be achieved by enhancing neuronal inhibition, by decreasing neuronal excitation, or by a combination of both. There is ample evidence indicating that most general anesthetics potentiate GABAergic inhibitory synaptic responses (Franks and Lieb, 1994; Mody et al., 1994) and potentiate GABA-activated Cl^- currents (Nakahiro et al., 1989; Jones and Harrison, 1993; Inoue et al., 1999). Studies with site-directed mutagenesis of the glycine 819 in transmembrane region 4 was shown to be important for halothane-induced potentiation of kainate-inhibited currents (Dildy-Mayfield et al., 1996). General anesthetics are thought to act on both excitatory and inhibitory neuronal pathways at both post- and presynaptic sites. However, the literature in these regards is somewhat controversial. The aim of the present study was to reassess the relative importance of the various anesthetic actions using a common preparation. Rat cortical neurons in primary culture were used to record spontaneous miniature postsynaptic currents by the whole-cell patch-clamp technique. Halothane at clinically relevant concentrations prolonged the decay phase of spontaneous miniature inhibitory postsynaptic currents (mIPSCs) recorded in the presence of tetrodotoxin and at higher concentrations decreased the frequency of mIPSCs. The mIPSC amplitudes underwent little change. Spontaneous action potential-dependent IPSCs recorded in the absence of tetrodotoxin were similarly affected by halothane. Halothane also decreased the frequency of spontaneous miniature non-N-methyl-D-aspartate (NMDA) excitatory postsynaptic currents (mEPSCs) as well as spontaneous action potential-dependent NMDA EPSCs and non-NMDA EPSCs without affecting their decay phase. The halothane effect on mIPSC and mEPSC frequency was dependent on the external calcium concentration. In contrast to halothane, the only effect of propofol was the prolongation of the decay phase of mIPSCs and IPSCs. The prolongation of mIPSCs and IPSCs by halothane and propofol coupled with the ineffectiveness on mEPSCs and EPSCs suggests a selective postsynaptic modulation of GABA_A receptors. The additional calcium-dependent inhibition of mIPSC and mEPSC frequency by halothane (but not propofol) suggests a more general mechanism by this anesthetic on presynaptic transmitter release.

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The excitatory synaptic system is also a potential target of general anesthetics (Peoples and Weight, 1997). Excitatory postsynaptic potentials (EPSPs) and orthodromic population spikes in hippocampus were depressed by isoflurane and halothane (MacIver et al., 1996; Wakasugi et al., 1999). Excitatory postsynaptic currents (EPSCs) induced by NMDA and AMPA were suppressed by isoflurane and halothane (Wakamori et al., 1991; de Sousa et al., 2000).

Currents evoked in NMDA, AMPA, and kainate receptors expressed in Xenopus oocytes were all inhibited by enflurane with approximately the same potency (Lin et al., 1993). Although isoflurane, enflurane, and halothane inhibited kainate responses in Xenopus oocytes expressing GluR1, GluR3, or GluR2 + 3 receptors, they potentiated kainate-inhibited currents in GluR6 receptors (Dildy-Mayfield et al., 1996). Glycine 819 in transmembrane region 4 was shown to be important for halothane-induced potentiation of kainate-inhibited currents (Minami et al., 1998).

In addition to postsynaptic receptor modulation, general anesthetics have been shown to act on presynaptic sites. Whereas halothane inhibited NMDA and non-NMDA receptor-mediated EPSCs, currents induced by direct application of NMDA or AMPA were less sensitive to halothane, suggest-
Halothane and Propofol on Synaptic Transmission

Materials and Methods

Cell Preparation. Rat cortical neurons in primary culture were used as a source of functional synapses. In this preparation, neurons reform a synaptic network expressing GABAergic, NMDA glutamatergic, and non-NMDA ( AMPA/kainate) glutamatergic postsynaptic receptors (Marszalec et al., 1996). Their synaptic activities could be observed in the absence of TTX by recording spontaneous compound EPSCs and IPSCs, which depend in part on the propagation of action potentials throughout the neuronal network. However, in the presence of TTX to block action potential activity spontaneous GABAergic and glutamatergic postsynaptic currents continued to be observed as smaller unitary mEPSCs and mIPSCs. EPSCs, IPSCS, mEPSCs, and mIPSCs could all be isolated pharmacologically by adding appropriate blockers. NMDA-EPSCs and non-NMDA-EPSCs could also be isolated by adding selective blocking agents.

Cortical neurons were cultured by the method previously used for hippocampal cells (Marszalec and Narahashi, 1993). In brief, 17-day embryonic rat pups were removed from a pregnant Sprague-Dawley rat under methoxyflurane anesthesia. Small wedges of frontal cortex were excised and incubated in a phosphate-buffered saline solution containing 0.25% (w/v) trypsin (type XI, Sigma-Aldrich, St. Louis, MO) for 25 min at 37°C. After mechanical trituration by repeated passages through a Pasteur pipette, the dissociated cells were suspended in Dulbecco’s modified Eagle’s medium with 10% (v/v) Ham’s F-12 supplement, 2 mM glutamine, and 20 U penicillin/20 ng of streptomycin per milliliter. The cells were plated into 25-mm culture wells at a concentration of 200,000 cells/ml. Each well contained five 12-mm glass coverslips with a confluent layer of glia that were probably related to synaptic redevelopment (Muramoto et al., 1983). Cells used in these experiments were cultured for 2 to 4 weeks. All current recordings were made from pyramidal-shaped neurons, 30 to 50 μm in diameter. Smaller oval bipolar cells were also present but were not used for experimentation.

Electrophysiological Methods. Spontaneous postsynaptic currents were recorded by the standard whole-cell patch-clamp technique using an Axopatch 1B amplifier (Axon Instruments, Union City, CA) at room temperature (22–25°C). Recording electrodes were pulled from borosilicate glass (Kimble, Vineland, NJ) on a vertical puller to a final resistance of 1.5 to 2.5 MΩ when filled with internal solution.

The external solution used for recording spontaneous synaptic
figures showing the glutamate release from presynaptic terminals is inhibited by this anesthetic (Kirson et al., 1998). Presynaptic modulation was also suggested by the observation of paired pulse facilitation of EPSPs caused by halothane and isoflu-
currents consisted of 150 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂, 5.5 mM HEPES-acid, 4.5 mM HEPES-Na⁺, and 10 mM glucose, and pH was adjusted to 7.3 with NaOH. To observe the NMDA-mediated component of EPSCs, Mg²⁺ was omitted from the external solution to avoid voltage-dependent Mg²⁺ block. The internal pipette solution contained 140 mM KCl, 2.0 mM MgCl₂, 1.0 mM CaCl₂, 11 mM EGTA, 10 mM HEPES-acid, 2 mM Mg²⁺ATP, and 0.2 mM Na⁺-GTP, and pH was adjusted to 7.3 with KOH.

To check the stability of recordings, access resistance was monitored as a current response to a small hyperpolarizing voltage command. Changes should be no more than 20%.

Drug Application. The neuron-containing glass coverslip was placed in a microscope-mounted recording chamber (0.5-ml volume) into which control and drug-containing solutions were perfused at a rate of 1 to 2 ml/min. Saturated halothane (Ayerst Laboratories, New York, NY) solutions were made by stirring halothane in external solution over 8 h in a sealed, glass container with very little air space. Halothane test solutions were prepared by diluting the saturated solution with external solution immediately before the experiment using sealed glass containers and glass pipettes. Using 19F-NMR spectroscopy (GE-NMR Instruments, Fremont, CA), the saturated solution was found to contain 18.0 mM halothane, a value identical to that determined previously (Seto et al., 1992). The solution diluted 80 times from the saturated solution was found to contain 0.23 mM halothane.

The mIPSCs were isolated by bath application of 1 μM 6-cyano-7-nitroquinoxaline (CNQX) (Tocris Cookson, St. Louis, MO) and 30 μM 2-amino-5-phosphonvaleric acid (APV) (Sigma/RBI, Natick, MA) to block AMPA- and NMDA-mediated currents, respectively. In a similar manner, mEPSCs were isolated in the presence of 20 μM bicuculline (Sigma-Aldrich) to block GABAergic currents. Action potential-dependent synaptic events were abolished by adding 0.1 μM TTX to the external solution.

Use of Miniature IPSCs and EPSCs to Distinguish Pre- and Postsynaptic Effects. The frequency of mIPSCs/mEPSCs was taken as a measure of the presynaptic effects of experimental manipulation, whereas the amplitudes of these events were thought to reflect postsynaptic processes (Banks and Pearce, 1999). According to the quantum theory of vesicular release, miniature postsynaptic currents are assumed to represent the spontaneous release of individual vesicles or quanta of neurotransmitter from the presynaptic membrane. Thus, drug-induced changes in the frequency of mIPSCs...
or mEPSCs are indicative of presynaptic actions. However, if a drug blocks postsynaptic receptors sufficiently, the frequency of events would tend to decrease as well.

**Data Acquisition and Analysis.** Miniature postsynaptic currents were continuously recorded at a holding potential of −70 mV for 3 min. Signals were filtered at 2 kHz and digitized at 200-μs sample interval. The algorithm for synaptic event detection started with an estimation of baseline signal variance using a user-specified segment of digitized data free of events. Synaptic currents were analyzed using a software package (Mini Analysis Program; Synaptosoft, Inc., Decatur, GA). The amplitude threshold was set as 3 × \( \sigma_{\text{noise}} \), where \( \sigma_{\text{noise}} \) was measured during periods when no visually detectable events occurred. Under control conditions, \( \sigma_{\text{noise}} \) was typically <4 pA, and the detection algorithm successfully detected more than 98% of miniature currents. When anesthetic application caused an increase in \( \sigma_{\text{noise}} \), the final 3-min records obtained during the application of the highest concentration of anesthetic were analyzed to determine the event threshold and were used to analyze all the data obtained at lower concentrations from that particular cell. From this baseline, the peak current was determined and the event was followed until the current declined to 5% of the baseline mean. A single exponential function was used to fit selected events using a nonlinear curve fitting routine to estimate the time constant. The current amplitude (in picoamperes), the time constant of current decay (in milliseconds), and the total charge integrated between the peak and the 5% baseline value were tabulated for statistical comparisons between control and halothane- or propofol-treated cells.

**Statistical Analysis.** All analysis, including curve fitting was performed using pClamp software and Mini Analysis Program (see above). Amplitudes and inter-event intervals of spontaneous synaptic currents in control versus test conditions were compared with the use of the Kolmogorov-Smirnov test with the criterion of \( P < 0.05 \). The results were analyzed for significant differences by two-tailed paired Student's \( t \) tests. Due to the large variability observed from cell to cell, the values of amplitude, frequency, and decay time of the currents are expressed as the values relative to the control values. Unless otherwise stated, data are presented as the mean ± S.D.

**Results**

As reported previously (Marszalec et al., 1998), spontaneous IPSCs were observed when 1 μM CNQX and 30 μM APV...
were present in the external bathing solution to block non-NMDA and NMDA EPSCs, respectively. When TTX was added to the bath at a concentration of 0.1 mM, spontaneous mIPSCs with much smaller amplitudes were discernible (Fig. 1A, control). To observe spontaneous EPSCs, 20 μM bicuculline was added to the external solution to block GABAA receptors (Marszalec et al., 1998). The additional inclusion of 0.1 mM TTX allowed us to observe spontaneous mEPSCs. NMDA EPSCs and non-NMDA EPSCs could be separately recorded by using 1 μM CNQX and 30 μM APV, respectively. Miniature non-NMDA EPSCs were recorded by inclusion of 0.1 μM TTX as well (Fig. 1C, control). Miniature NMDA EPSCs of smaller amplitudes were recorded in the presence of 1 μM CNQX and 0.1 mM TTX (data not shown).

**Effects of Halothane on Miniature IPSCs and Miniature non-NMDA EPSCs.** Halothane at concentrations ranging from 0.15 to 1.2 mM had little or no effect on the amplitude of mIPSCs and non-NMDA mEPSCs observed in the presence of TTX. Only 1.2 mM halothane slightly but significantly decreased in the amplitude of mIPSCs detected (Fig. 2A). In contrast, the frequency of mIPSCs and non-NMDA mEPSCs was significantly decreased by halothane at 0.6 and 1.2 mM (Fig. 2B), reflecting inhibition of the presynaptic release mechanism. Marked differential actions of halothane were observed in the time constant of current decay. Although the decay time constant of mIPSCs was significantly increased by 0.3, 0.6, and 1.2 mM halothane (Fig. 2C), indicative of postsynaptic effects, that of non-NMDA mEPSCs was not affected at all (Fig. 2C). Reflecting the increase in decay time constant of mIPSCs, the total charge transfer per mIPSC was greatly increased by 0.3, 0.6, and 1.2 mM halothane (Fig. 2D). These results indicated that halothane at 0.6 and 1.2 mM inhibited spontaneous release of both inhibitory and excitatory transmitters while selectively prolonging mIPSCs even at a lower concentration of 0.3 mM.

**Effects of Halothane on IPSCs and EPSCs.** Spontaneous IPSCs and EPSCs were observed in the absence of TTX, and their activities reflected not only transmitter release and postsynaptic responses but also the conduction of impulses in the neuronal network formed in culture. The effects of halothane on IPSCs, non-NMDA EPSCs, and NMDA EPSCs were similar to those on mIPSCs and non-NMDA mEPSCs described in the preceding section. The amplitude of IPSCs and NMDA EPSCs was slightly but significantly suppressed at a high concentration (1.2 mM) of halothane, whereas that of non-NMDA EPSCs was not affected (Fig. 3A). The frequency of IPSCs, non-NMDA EPSCs, and NMDA EPSCs was markedly decreased by 0.6 and 1.2 mM halothane (Fig. 3B). Although the decay time constant and the total charge transfer per IPSC were increased by halothane at 0.3, 0.6, and 1.2 mM, those of non-NMDA and NMDA EPSCs were not affected (Fig. 3, C and D). The similarity of halothane action on mIPSCs/mEPSCs and on IPSCs/EPSCs led us to the conclusion that the involvement of impulse propagation in the

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**Fig. 4.** A, effects of halothane (0.6 and 1.2 mM) on the frequency of spontaneous miniature non-NMDA EPSCs (mNN-EPSCs) in the presence and absence of calcium in the external solution. The ordinate expresses the amplitudes and frequencies normalized to the control condition of 2.5 mM calcium and 0 mM halothane. The percentage of decrease in EPSC frequency observed with 0.6 mM halothane and 2.5 mM Ca<sup>2+</sup> was nearly identical to that of Ca<sup>2+</sup>-free solution and no halothane, or Ca<sup>2+</sup>-free solution and 0.6 mM halothane. The EPSC amplitude was not affected under any of these conditions.

**Fig. 5.** Records of mIPSCs before (A, control) and during application of 3 μM propofol. Propofol slowed the decay phase of mIPSCs.
neuronal network in halothane modulation of the presynaptic and postsynaptic mechanisms was less likely.

Effects of Halothane on Presynaptic Elements. To elucidate the mechanism by which halothane might modulate transmitter release, experiments were performed to compare the previously observed effects of halothane with those recorded in calcium-free solution. Figure 4 illustrates the comparison of the effect of various combinations of halothane and calcium-free solution on non-NMDA mEPSCs. In agreement with the results presented in Fig. 2A, halothane at 0.6 and 1.2 mM in the presence of the normal Ca\(^{2+}\) concentration of 2.5 mM significantly decreased the frequency of non-NMDA mEPSCs while having no effect on their amplitudes. Removal of calcium from the media had virtually the same effect as that of halothane, decreasing the frequency while causing no effect on the amplitude of non-NMDA mEPSCs. Addition of 0.6 mM halothane to Ca\(^{2+}\)-free solution exerted no further effect beyond that observed with Ca\(^{2+}\)-free solution alone. These results suggest that halothane may inhibit calcium channels in the presynaptic terminals.

Effects of Propofol on Miniature IPSCs and Miniature non-NMDA EPSCs. Records of mIPSCs before and during application of 3 \(\mu\)M propofol are shown in Fig. 5. No marked changes in the mean amplitude and frequency of mIPSCs were noted. Propofol at concentrations ranging from 0.1 to 10 \(\mu\)M had no significant effect on the amplitude or frequency of mIPSCs and non-NMDA mEPSCs (Fig. 6, A and B). However, at 1 to 10 \(\mu\)M propofol did cause an increase in the current decay time constant and the total charge transfer per mIPSC without effect on the decay of non-NMDA mEP-
SCs (Fig. 6, C and D). The lack of the propofol action on the spontaneous transmitter release contrasts with the halothane action.

Effects of Propofol on IPSCs and EPSCs. Although the amplitude of IPSCs recorded in TTX-free solutions was not altered by propofol at 10 μM, that of non-NMDA and NMDA EPSCs was decreased somewhat at 3 to 10 μM and at 10 μM, respectively (Fig. 7A). The frequency of IPSCs, non-NMDA EPSCs, and NMDA EPSCs was decreased by propofol only at 10 μM (Fig. 7B).

Similar to the action of halothane, propofol at 1 to 10 μM slowed IPSC decay, resulting in an increase in the total charge transfer per IPSC (Fig. 7, C and D). The decay time constant of non-NMDA and NMDA EPSCs was not altered by propofol (Fig. 7C).

Discussion

Surgical anesthesia is associated with several behavioral phenomena, including hypnosis, amnesia, analgesia, and the loss of reflexive movement. These phenomena undoubtedly represent a complex integration of activity by neuronal networks within the brain and spinal cord. Our objective was to study the interaction of the anesthetics halothane and propofol with this neuronal system using a two-dimensional network of cortical neurons in culture. The underlying hypothesis of our study is that these anesthetics can alter the presynaptic release and/or the postsynaptic responses of the brain’s primary inhibitory and excitatory neurotransmitters, namely, GABA and glutamate.

The reestablishment of synaptic networks in these cultured cells is indicated by the spontaneous occurrence of postsynaptic currents generated by the release of vesicular GABA and glutamate, thus producing IPSCs and EPSCs, respectively (Marszalec et al., 1996, 1998). The spontaneous mIPSCs and mEPSCs observed in the presence of TTX arise from the fusion of single neurotransmitter vesicles to the presynaptic membrane and the release of their contents into the synaptic cleft. Although the perfusion of 0.3 mM halothane had no effect on the frequency of both mIPSCs and mEPSCs, halothane at 0.6 mM decreased the frequency of both mIPSCs and mEPSCs by approximately 20 and 30%, respectively. The halothane concentration of 0.6 mM is equivalent to approximately 2 MAC units, implying that this halothane effect may be attained only at higher concentrations of the anesthetic.

These are some discrepancies between the present results and those reported recently with respect to the effects of halothane on the frequency and amplitude of mIPSCs. The mIPSC frequency was not affected by 0.3 mM halothane and slightly (~20%) decreased by 0.6 mM halothane in our experiments using rat cortical neurons in culture. However, variable increases in the mIPSC frequency by 0.35 mM halothane in hippocampal pyramidal neurons and interneurons were reported by Nishikawa and Maclver (2000, 2001) and by Banks and Pearce (1999). The amplitudes of mIPSCs and mEPSCs were not affected by 0.3 and 0.6 mM halothane in the present study. In the studies of Nishikawa and Maclver (2000, 2001) using hippocampal neurons, the mIPSC amplitude was inhibited by approximately 20%, and the effect progressed slowly requiring 15 to 20 min to reach a steady state. However, Banks and Pearce (1999) did not see significant decreases in mIPSC amplitude in the presence of 0.6 mM halothane.

The reasons for the discrepancies are not completely clear, yet at least four possible factors are conceivable. One is the difference in material; we used cortical neurons and they used hippocampal neurons. Second is the difference in the methods used, i.e., long-term cultured neurons versus brain slices. Third is the time period of exposure to halothane; in our experiments, measurements were made 5 min after exposure to halothane, whereas in the studies of Nishikawa and MacIver (2000, 2001), the increase in mIPSC frequency and the decrease in mIPSC amplitude progressed slowly during halothane application, and the increase in frequency seemed to be small after ~5 min of exposure and became prominent only after 10 to 20 min of exposure.

Fourth is the effect via neuronal nicotinic acetylcholine (nACh) receptors. Rat cortex is reported to contain intrinsic cholinergic neurons (Cauli et al., 1997). Presynaptic or postsynaptic nACh receptors are known to modulate the release of various transmitters, including GABA, glutamate, dopamine, norepinephrine, and ACh itself (Role and Berg, 1996; Colquhoun and Patrick, 1997; Léna and Changeux, 1997; Lindstrom, 1997; Wonnacott, 1997; Alkondon et al., 2000), and halothane blocks e4β2-type nACh receptors with an IC50 value of 105 μM (Mori et al., 2001). Thus, the observed decrease in the frequency of mIPSCs and mEPSCs by halothane could be the result of inhibition of nACh receptors.

Halothane also reduced the frequency of spontaneous events in the absence of TTX. Although halothane had no significant effect on the frequency of IPSCs, non-NMDA EPSCs, and NMDA EPSCs at a concentration of 0.3 mM, it decreased their frequencies at 0.6 mM. The amplitude of these IPSCs and EPSCs was not affected at either 0.3 or 0.6 mM. These data on both frequency and amplitude agree with those of mIPSCs and miniature non-NMDA EPSCs, suggesting that propagation of action potentials in the synaptic network is not involved in the action of halothane. Such spontaneous bursts of currents resulting from action potential-dependent phasic transmitter release are observed either in culture as whole-cell currents (Marszalec et al., 1996, 1998) or in vivo as rhythmic EEG waves recorded in the resting brain (Weliky and Katz, 1999). In culture preparations, these synchronized bursts of activity spread through the culture network in a wave-like manner. Studies have found that the initiation of these action potentials is correlated with spontaneous mEPSCs that summate to depolarize a few random cells toward firing threshold and pacemaker status (Köller et al., 1993; Siebler et al., 1993). It is reasonable to expect that because halothane reduces the frequency of spontaneous mEPSCs, it also could reduce the initiation (and frequency) of the action potential-driven compound postsynaptic responses in culture.

Because halothane affected the frequency of both glutamate- and GABA-mediated quantal release in a similar manner, it might be hypothesized that the underlying mechanism involves one or more of the general processes leading to vesicular fusion to the presynaptic membrane. One element that participates in this process is the divalent cation calcium. The experiments summarized in Fig. 4 show that the frequency of spontaneous mEPSCs and mIPSCs is dependent in part on the extracellular level of calcium. Note that the frequency reductions observed with either 0.6 mM halothane...
or 0 mM external calcium are nearly identical. Furthermore, the effect resulting from the combination of both modifications is not additive. Taken together, this suggests that the halothane-induced decrease in mEPSC and mIPSC frequency may be the result of a reduction in presynaptic calcium channel activity or by an interference with the calcium-dependent proteins that mediate vesicular-membrane fusion.

It was indeed shown that various types of voltage-gated calcium channels were inhibited by volatile anesthetics such as halothane and isoflurane and intravenous anesthetics such as propofol (Asahina et al., 1998; Nikonorov et al., 1998; Hirota et al., 1999; Kamatchi et al., 1999, 2001; Kameyama et al., 1999; McDowell et al., 1999; Camara et al., 2001; Hu et al., 2001; Yamakage and Namiki, 2002).

Aside from the presynaptic modulation of mEPSC and mIPSC frequencies, halothane at 0.3, 0.6, and 1.2 mM increased the duration and charge transfer of mIPSCs, presumably by a postsynaptic effect. This effect was also observed with propofol at 1, 3, and 10 μM. Neither agent, however, affected the duration of the mEPSCs. This halothane action on mIPSCs was observed at a concentration of 0.3 mM (~1 MAC), suggesting that it may play a more important role in the early induction of anesthesia than the decreased mEPSC frequency seen at higher concentrations of 0.6 and 1.2 mM. It should be noted that halothane at 0.3 mM significantly increased the charge transfer associated with mIPSCs without effect on the frequency of mIPSCs. Thus, the mIPSC charge transfer per unit time was also increased by halothane at 0.3 mM. In a previously reported experiment using human embryonic kidney cells transfected to express the α1β2γ2S GABA subunit combination, halothane was found to increase the duration of GABA responses by reducing $k_{on}$, the rate constant for agonist-receptor unbinding (Li and Pearce, 2000). A similar effect may have occurred in the present study in the cortical cell GABA$_A$ receptors in culture.

The effect of halothane and propofol in increasing net IPSC
charge transfer occurred in both tonic spontaneous mIPSCs (in the presence of TTX) and in action potential-driven phasic IPSCs recorded in the absence of TTX. This implies that halothane and propofol can enhance the tonic inhibitory neuronal background activity, as well as the inhibition produced by action potential-driven phasic GABA release. Note, however, that neither anesthetic increased the average peak mIPSC amplitude. This suggests that in the cortical neuron culture the release of even a single GABA-filled vesicle nearly saturates the postsynaptic GABA_A receptors because the maximum GABA-induced current is not potentiated by halothane or propofol.

It should be noted that unlike halothane, the only effect observed with propofol is an increase in charge transfer associated with mIPSCs and IPSCs. This is in keeping with a recent thiopental study that showed an enhancement of GABAergic transmission without effect on glutamatergic transmission (Dickinson et al., 2002).

Higher concentrations of halothane (1.2 mM), however, reduced the amplitude of spontaneous GABAergic mIPSCs. This was also reported by Banks and Pearce (1999) for IPSCs in hippocampal slices in the presence of the anesthetics enfurane and isoflurane. They concluded that anesthetic binding to a secondary, lower affinity site produced this inhibitory effect distinct from the binding site that leads to IPSC prolongation. A similar two-site mode of action may also hold for the dual effects of halothane observed in cultured cortical neurons. A similar propofol-induced inhibition of the amplitude was observed only on EPSCs in the TTX-free condition. This propofol effect may stem from the anesthetic enhancement of GABAergic transmission that, in turn, reduces the spontaneous occurrence of action potential-driven EPSCs.

Overall, the present experiments indicate that the primary effect of halothane is the increase in charge transfer during both spontaneous mIPSCs and action potential-driven IPSCs. This action is observed at halothane concentrations equivalent to 1 MAC, and seems to be responsible for causing anesthesia. Propofol produces a similar effect. However, at concentrations just over 2 MAC, halothane has the additional effect of reducing the frequency of spontaneous mEPSCs and mIPSCs. The former effect of halothane is expected to enhance the degree of anesthesia by reducing an endogenous excitatory tone to the cells. The latter effect on IPSC frequency could paradoxically oppose the augmentation of IPSC charge transfer. Yet, it is clear that the prolongation and increase in charge transfer of IPSCs caused by halothane at 1 MAC must prevail over any decrease in the spontaneous mIPSC frequency observed at higher concentrations.

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