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Presynaptic and postsynaptic actions of halothane at glutamatergic synapses in the mouse hippocampus

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1 Whole-cell patch-clamp recordings in adult mouse hippocampal slices were used to test the mechanism by which the volatile anesthetic halothane inhibits glutamate receptor-mediated synaptic transmission. Non-*N*-methyl-D-aspartate (nonNMDA) and NMDA receptor-mediated currents in CA1 pyramidal cells were pharmacologically isolated by bath application of D,L-2-amino-5-phosphonovaleric acid (APV; 100 μM) or 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX; 5 μM), respectively.

2 Halothane blocked both nonNMDA and NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) to a similar extent (IC_{50} values of 0.66 and 0.57 mM, respectively).

3 Partial blockade of the EPSCs by lowering the extracellular concentration of calcium ($[Ca^{2+}]_o$), but not by application of CNQX (1 μM), was accompanied by an increase in paired-pulse facilitation (PPF). Halothane-induced blockade of the EPSCs also was associated with an increase in PPF.

4 The effects of halothane on α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and NMDA receptor-mediated currents induced by agonist iontophoresis, were compared. AMPA-induced currents were blocked with an IC_{50} of 1.7 mM. NMDA-induced currents were significantly less sensitive to halothane (IC_{50} of 5.9 mM).

5 The effect of halothane on iontophoretic AMPA dose-response curves was tested. Halothane suppressed the maximal response to AMPA without affecting its EC_{50}, suggesting a noncompetitive mechanism of inhibition.

6 All effects of halothane were reversible upon termination of the exposure to the drug.

7 These data suggest that halothane blocks central glutamatergic synaptic transmission by presynaptically inhibiting glutamate release and postsynaptically blocking the AMPA subtype of glutamate receptors.

Keywords: Halothane; volatile anesthetic; AMPA; paired pulse facilitation; hippocampus; mouse

Introduction

General anesthetics depress glutamate receptor-mediated excitatory synaptic transmission in the mammalian central nervous system, but it is not yet clear which components of this process are affected (Richards & White, 1975; for review see Pocock & Richards, 1993). It was formerly thought that anesthetic compounds interact with the lipid bilayer of neuronal membranes, thereby affecting nonspecifically the operation of membrane ion channels (Kaufman, 1977). Contrary to this suggestion, it has been shown more recently that the effects of volatile anesthetics may be agent-specific and involve specific interactions with receptors and channels implicated in central synaptic transmission (MacIver & Roth, 1988; Forman *et al*., 1995; Harris *et al*., 1995). For example, halothane may increase γ-aminobutyric acid (GABA)-mediated synaptic transmission by prolonging the time course of $GABA_A$ mediated postsynaptic currents (Tanelian *et al*., 1993). In addition, halothane blocks voltage-activated Ca^{2+} and Na^+ channels and may potentiate voltage-activated K^+ channels in central neurons (Krnjevic, 1992).

We have recently shown that glutamate receptor-mediated excitatory postsynaptic currents (EPSCs) in hippocampal CA1 neurones are reversibly blocked by halothane in a dose-dependent manner (Perouansky *et al*., 1995; 1996). Several lines of evidence suggested that this effect is due predominantly to presynaptic inhibition of glutamate release. First, halothane depressed the EPSCs without affecting their time course. Secondly, halothane similarly depressed non-*N*-methyl-D-aspartate (nonNMDA) and NMDA EPSCs. Finally, agonist-induced NMDA receptor-mediated currents were resistant to halothane at doses which markedly suppressed the EPSCs. However, the interaction of halothane with α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, the major nonNMDA receptor type in CA1 (Greenamyre *et al*., 1985), was not examined, though these receptors generate most of the EPSC in normal conditions (Davies & Collingridge, 1989).

In this study, we compared the effects of halothane on AMPA versus NMDA receptor-mediated currents in CA1 pyramidal cells *in situ*. The drugs were applied iontophoretically onto the pyramidal cell dendrites, rather than bath-applied, to minimize receptor desensitization (Trussell *et al*., 1988; Trussell & Fischbach, 1989). Our data suggest that halothane preferentially blocks AMPA receptors in a noncompetitive manner. This postsynaptic action of halothane may complement the presynaptic inhibition of glutamate release (MacIver *et al*., 1996) in exerting central anesthesia.

Methods

Slice preparation

Experiments were performed on thin hippocampal slices obtained from adult (>8 week-old) Sabra mice. Methods for preparation of thin slices were similar to those described

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previously (Edwards *et al*., 1989; Perouansky *et al*., 1995). Briefly, mice were anesthetized with ether and decapitated with a guillotine. The brain was removed and immediately immersed in ice cold oxygenated (95% O_2; 5% CO_2) dissection saline. The caudal two thirds of one hemisphere (containing one hippocampus) were glued to the stage of a vibratome (FTB, Bensheim, Germany). Transverse slices, 170 μM thick, were cut from the region of the hemisphere containing the anterior hippocampus. The hippocampal portion was dissected out of each slice and transferred to an incubation chamber containing the oxygenated incubation solution held at 28°C. After an incubation period of at least 1 h, slices were transferred, one at a time, to a recording chamber where they were continuously perfused (2.5 ml min^{-1}) with oxygenated experimental saline at room temperature (21–24°C).

Solutions

The dissection saline consisted of (mM): NaCl, 125; KCl, 2.5; $NaHCO_3$, 26.7; N-[2-Hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES), 13; NaH_2PO_4, 1.25; glucose, 6.3; $CaCl_2$, 0.5; and $MgSO_4$, 4; pH 7.3. The incubation saline was identical except for $NaHCO_3$, 22.5 mM. The standard experimental saline consisted of (mM): NaCl, 125; KCl, 2.5; $NaHCO_3$, 26.7; $CaCl_2$, 2.5; $MgCl_2$, 1; Hepes, 13; glucose, 12.5; pH 7.3; osmolarity 300 mOsm. In Mg^{2+}-free saline, $MgCl_2$ was omitted. All salines also contained bicuculline methiodide (10 μM) to block GABA-mediated chloride currents and glycine (5 μM) to saturate the glycine binding sites in NMDA receptors. To block NMDA or nonNMDA receptors, 100 μM D,L-2-aminophosphonovaleric acid (APV) or 5 μM 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX) were added to the saline, respectively. The intracellular (pipette) solution in all experiments consisted of (mM): CsF, 130; NaCl, 10; EGTA, 10; HEPES, 10; $MgCl_2$, 2; $CaCl_2$ 1; pH 7.2; osmolarity 270–280 mOsm. In experiments where AMPA currents were recorded, 4 mM Mg-ATP, 0.3 mM Na-GTP and 10 mM creatine-phosphate were added routinely to the pipette solution.

Chemicals

Drugs were purchased from Sigma Chemical Co. (Rehovot, Israel) with the exception of CNQX (Tocris Neuramin, England) and halothane (Trofield Surgicals, Zug, Switzerland).

Application and measurement of halothane

The O_2/CO_2 mixture was directed *via* a flowmeter through an Enfluratec vaporizer containing halothane. The vaporizer was calibrated for halothane with an IRIS Gas Analyzer (Draeger, Germany). The gas mixture of O_2/CO_2/halothane was then used to bubble the experimental saline for at least 15 min before it was applied to the slice with a peristaltic pump. Halothane concentrations in the recording chamber were determined as described previously, using a Tracor 540 gas chromatograph (Perouansky *et al*., 1995). A modified aquarium pump was used for scavenging of waste gases (Perouansky & Kirson, 1996).

Whole-cell recordings

Cells in the CA1 field were visualized at 400× magnification with Nomarski optics using an upright Zeiss standard 18 microscope. Pyramidal cells were identified by their position in the pyramidal layer, the pyramidal shape of their somata and their prominent apical dendrite.

Recording pipettes were pulled from borosillicate glass on a vertical puller (List-medical, Germany) and coated with Sylgard resin (Dow Corning Chemical Co., U.S.A.). Pipette resistances were 3 to 10 MΩ when filled with CsF-based intracellular solution. After establishing whole-cell recording configuration, series resistance was compensated for by setting the series resistance compensation control of the amplifier (Axopatch 200A) to 70–90%. Experiments in which the series resistance exceeded 20 MΩ were discarded. Cleaning/stimulating pipettes were pulled from Boralex disposable micropipettes (Rochester Scientific, U.S.A.) to a tip diameter of 5–10 μM and filled with saline. When necessary, these pipettes were first used to clear the surface of the cell somata from debris and then placed in stratum radiatum for stimulation of afferent fibers.

Iontophoresis

A three-barrel electrode was used for agonist iontophoresis. One barrel (~100 MΩ) contained 100 mM NMDA and 50 mM NaCl, the second barrel (~100 MΩ) contained 100 mM AMPA and 50 mM NaCl and the third barrel (~20 MΩ) contained 150 mM NaCl. The tip of the electrode was placed about 100 μM apical to the soma of the patched cell. Agonists were ejected by passing 1 nA to 1 μA for 0.1 to 1 s. A retaining current of up to 10 nA was used to avoid leak of the agonists. At the beginning of each experiment, ejection and retaining currents were adjusted until the responses to stimuli delivered at 0.02 Hz were stable for at least 5 min.

Paired pulse facilitation

For paired-pulse facilitation (PPF) experiments, the cell was clamped at −60 mV. Pairs of nonNMDA EPSCs were evoked at a frequency of 0.08 Hz and an interval of 40 ms. PPF was assessed by calculating the percent in amplitude of the second (test) EPSC relative to the first (conditioning) EPSC. The amplitude of the test EPSC was measured after subtracting the residual decay of the conditioning EPSC. Because of trial to trial fluctuations 50 to 100 pairs of EPSCs were averaged in each experimental condition.

Analysis

Currents recorded were filtered on-line at 0.1–5 kHz, digitized at a sampling rate of 3–20 kHz and analysed off-line using a 486DX personal computer and software from Axon Instruments. Analysis of evoked EPSCs was performed on averages of 5–10 consecutive traces.

The dose-response curves for halothane were fitted with a one-site binding scheme using the following function:

$$\frac{I}{I_{\max}} \quad \frac{1}{1 \quad EC_{50}^{n} \quad hal^{n}}$$

where I is the measured current, I_{max} is the current amplitude before adding halothane, EC_{50} is the concentration of halothane which blocks half of the control current, [*hal*] is the concentration of halothane added and n is the Hill coefficient.

The iontophoretic AMPA activation curves were fitted with the following equation, assuming that for a given pipette and a constant ejection time the concentration of AMPA is almost linearly related to the ejection current (i.e. to the electric charge transferred by the pipette; Stone, 1985):

$$\frac{I}{I_{\max}} \quad \frac{1}{1 \quad EC_{50}^{n} \; I^{n_{eject}}}$$

where I is the measured current, I_{max} is the current amplitude at maximal ejection, EC_{50} is the iontophoretic current which evokes half of the maximal AMPA current, I_{eject} is the ejection current passed through the iontophoretic electrode, and n is the Hill coefficient.

Statistics

Data are presented as mean ± standard deviation (s.d.) unless stated otherwise. Significant differences between pairs of samples were tested with either a paired or a non-paired *t*-test. A significance level of $\alpha = 0.05$ was used in all tests. Fitting procedures used the Marquardt-Levenberg algorithm to seek parameter values that minimize the sum of the squared differences between the observed and predicted values of the dependent variables. Residuals of the best fit were tested with a runs-test to assure random distribution. Parameters of fitted functions were compared on a one-tailed normal curve (Z-table).

Results

Halothane depresses glutamatergic EPSCs

Glutamatergic excitatory postsynaptic currents (EPSCs) evoked in CA1 pyramidal cells at holding potentials negative to −60 mV comprise a large nonNMDA receptor-mediated fast component (nonNMDA EPSC) and a small NMDA receptor-mediated slow component (NMDA EPSC; Hestrin *et al*., 1990; Perouansky *et al*., 1995). Both EPSC components were similarly blocked by 0.64 mM halothane. In the example shown in Figure 1A, the fast (measured at the peak) and the slow (measured 20 ms after the peak) EPSC components were blocked to 51% and 48% of control, respectively. The pharmacologically isolated nonNMDA (Figure 1B) and NMDA EPSCs (Figure 1C) also were blocked by halothane to a similar extent. We have previously shown that the IC_{50} values for halothane blockade of nonNMDA and NMDA EPSCs are 0.66 and 0.57 mM, respectively (Perouansky *et al*., 1995). The halothane-induced blockade of the EPSCs was readily reversible upon termination of the exposure to the drug.

Paired-pulse facilitation

In the disinhibited hippocampus, application of two consecutive stimuli to afferent pathways results in facilitation of the second EPSC (Creager *et al*., 1980). This PPF presumably is inversely related to the magnitude of presynaptic Ca^{2+} entry (Manabe *et al*., 1993; for review see Zucker, 1989). Thus, reducing presynaptic Ca^{2+} entry decreases the EPSC while increasing PPF, whereas blocking postsynaptic glutamate receptors decreases the EPSC without affecting PPF (Manabe *et al*., 1993).

To further characterize the site of halothane's depressant action, we examined its effect on PPF of the nonNMDA EPSC and compared it to that of reducing presynaptic Ca^{2+} entry or blocking postsynaptic glutamate receptors. As illustrated in the control traces in Figure 2,

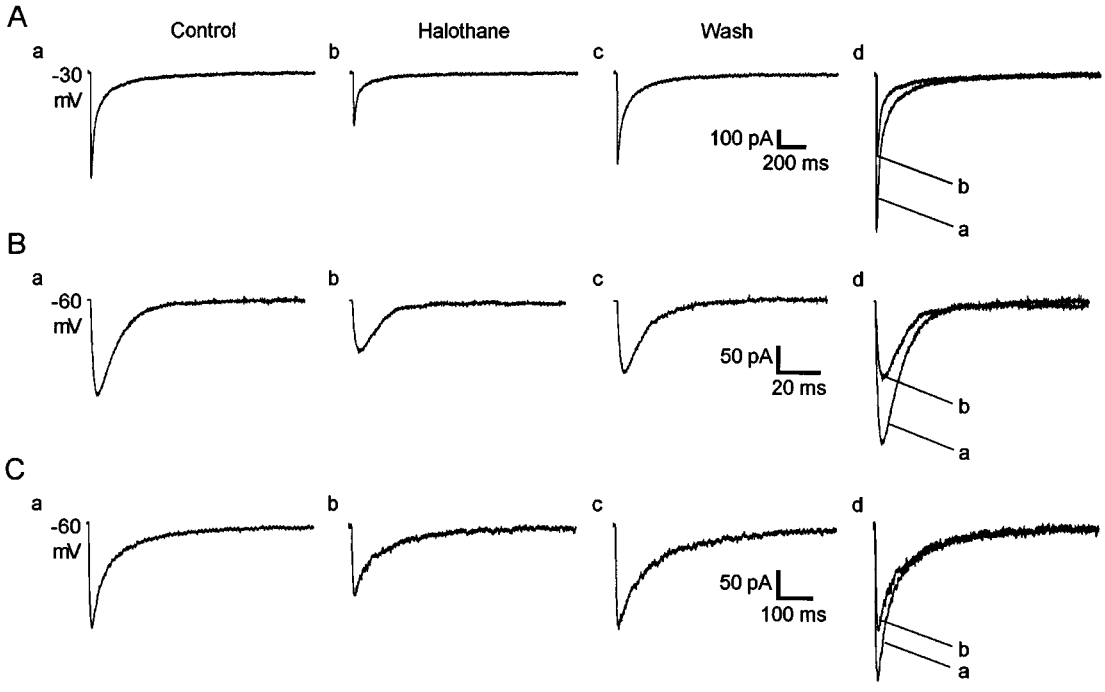

Figure 1 Halothane blocks both nonNMDA and NMDA receptor-mediated EPSCs in CA1 pyramidal cells. The effect of halothane was tested in three different conditions, namely, normal saline (A), saline containing 100 μM APV to isolate the nonNMDA EPSC (B), and Mg^{2+}-free saline containing 5 μM CNQX to isolate the NMDA EPSC (C). The control EPSCs are shown in panels (a) EPSCs were evoked at the indicated holding potentials by focal stimulation of afferent fibers. In all three conditions 0.64 mM halothane blocked the EPSCs to a similar extent (58 ± 9% of control; panels (b)). The EPSC blockade reversed upon wash (panels (c)). Traces of (a) and (b) are superimposed in panels (d). Each trace is an average of five consecutive records. Note the different calibration bars.

the second EPSC in a pair (test EPSC) was on average larger than the first (conditioning EPSC). Expectedly, reducing extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) to 0.5 mM depressed the conditioning EPSC more than the test EPSC, thus increasing PPF (Figure 2A,b, and A,c), whereas CNQX (1 μM) reduced both conditioning and test EPSCs to a similar degree without changing PPF (Figure 2B,b and B,c). Halothane (0.64 mM) mimicked low $[Ca^{2+}]_o$ in depressing the conditioning EPSC more than the test EPSC, causing PPF to increase (Figure 2C,b and C,c).

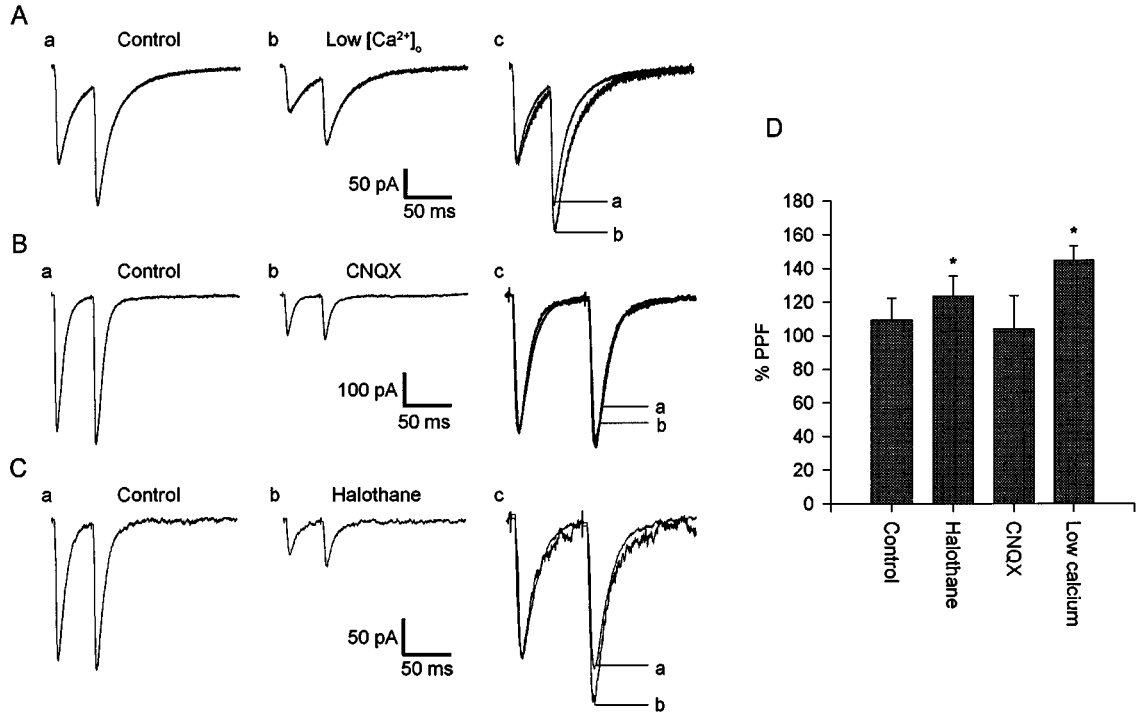

Figure 2 Halothane mimics low $[Ca^{2+}]_o$ in enhancing paired pulse facilitation. Paired nonNMDA EPSCs in saline containing 100 μM APV were evoked at -60 mV holding potential, by stimulation of afferent fiber at a 40 ms inter-stimulus interval. Paired pulse facilitation (PPF) was measured as the ratio between the peak amplitude of the second (test) EPSC (after subtracting the residual decay of the first EPSC) and the peak amplitude of the first (conditioning) EPSC. (A) Lowering $[Ca^{2+}]_o$ from 2.5 to 0.5 mM decreased the conditioning EPSC more than the test EPSC and thus increased PPF. (B) CNQX (1 μM) blocked both EPSCs equally without affecting PPF. (C) Halothane (0.64 mM) blocked the conditioning EPSC more than the test EPSC and thus increased PPF. (D) Bar histogram of pooled data. Asterisks represent significant increases compared to control ($P<0.05$). Traces are averages of 50–100 consecutive EPSCs.

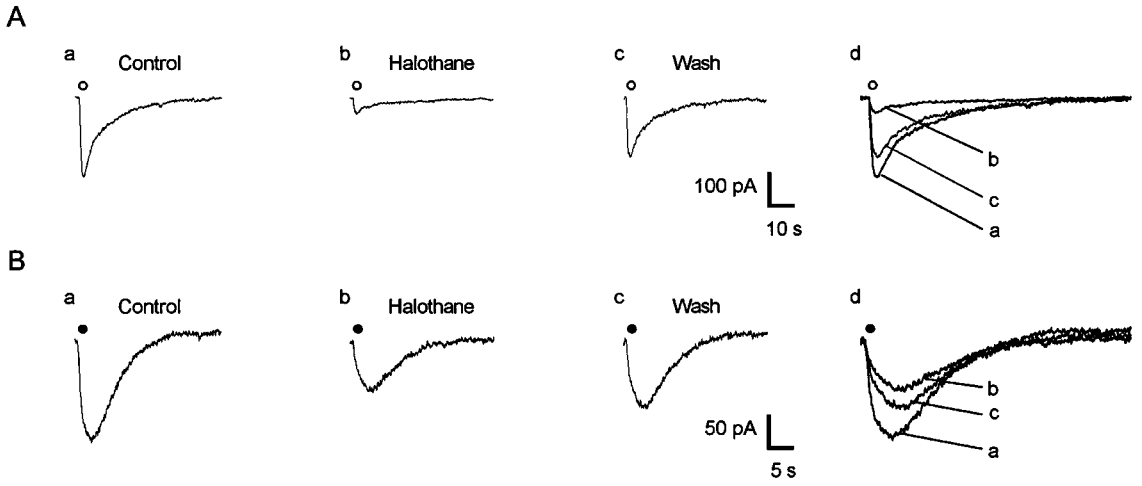

Figure 3 Halothane preferentially blocks currents evoked by AMPA iontophoresis. Whole-cell currents were evoked at -60 mV holding potential by iontophoretic application of either AMPA (100 mM; ○) or NMDA (100 mM; ●). The effect of halothane was tested in two different conditions, namely, saline containing 100 μM APV to isolate the AMPA-receptor mediated response (A), and Mg^{2+}-free saline containing 5 μM CNQX to isolate and maximize the NMDA-receptor mediated response (B). Representative traces of both types of experiments are shown in control conditions (panels (a)), after 15 min in the presence of 3.85 mM halothane (panels (b)), and after recovery from halothane (panels (c)).

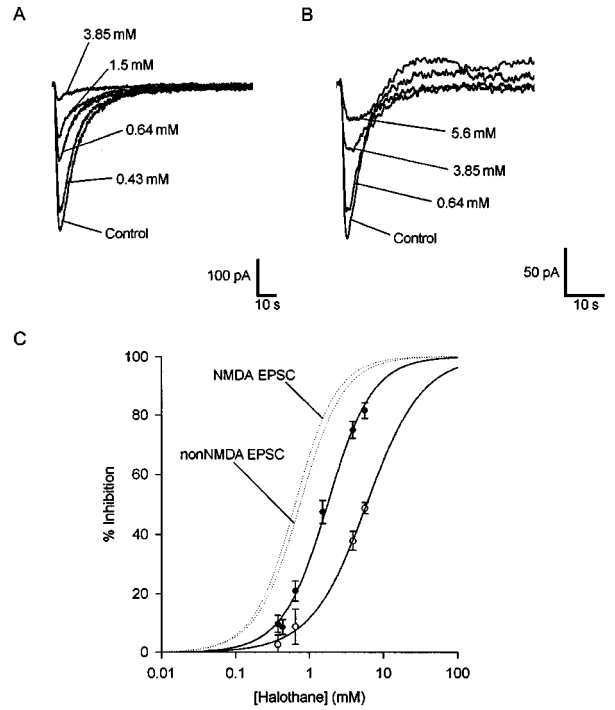

Figure 4 Dose-response relationship of halothane inhibition of AMPA versus NMDA currents. Whole-cell currents were evoked at −60 mV holding potential by iontophoretic application of either AMPA (100 mM; in control saline containing 100 μM APV) or NMDA (100 mM; in Mg^{2+}-free saline containing 5 μM CNQX) in different concentrations of halothane. (A, B) The effect of halothane on currents induced by AMPA (A) and NMDA (B). Each of the superimposed records was obtained after 8–12 min exposure to halothane at the indicated dose. (C) Dose-response relationship of halothane inhibition of AMPA- (●; $n=7$) and NMDA-induced currents (○; $n=7$). Solid lines represent fitted dose-response curves (see Methods). Bars represent standard error of the mean. Dotted lines represent the dose-response curves for halothane inhibition of NMDA and non-NMDA EPSCs published previously (Perouansky *et al*., 1995).

The pooled results from a series of similar experiments are summarized in Figure 2D. In control conditions PPF averaged 109±13% ($n=7$). In low $[Ca^{2+}]_o$ (0.5 mM) PPF significantly increased to 145±8.5% ($n=3$; $P<0.05$). In CNQX (1 μM) PPF did not change significantly (104±19%, $n=5$; $P>0.05$). Application of halothane (0.64 mM) significantly increased PPF to 123±12% ($n=7$; $P<0.05$). These data are consistent with the hypothesis that halothane inhibits glutamate release by blocking presynaptic Ca^{2+} influx.

Effect of halothane on iontophoretic AMPA and NMDA currents

We have previously shown that pyramidal cell responses to bath-applied glutamate are unaffected by halothane (Perouansky *et al*., 1995). However, due to rapid desensitization of AMPA receptors (Trussell *et al*., 1988; Trussell & Fischbach, 1989), the response to glutamate applied in the bath is mediated predominantly by NMDA receptors. Therefore, our previous results did not rule out an interaction of halothane with AMPA receptors. To test whether AMPA receptors are sensitive to halothane, we examined its effect on currents evoked by iontophoretic application of AMPA onto the proximal apical cell dendrites. The specificity of this effect was tested by examining the effect of halothane on currents similarly evoked by NMDA. Exemplary experiments are illustrated in Figure 3. Halothane (3.85 mM) markedly depressed the AMPA-induced current. The NMDA-induced current was reduced as well, albeit to a lesser extent, disclosing a marked difference in the sensitivity of AMPA and NMDA receptors to halothane. AMPA receptor-mediated currents were blocked to 25±6% of control by 3.85 mM halothane ($n=5$; Figure 3A), whereas isolated NMDA receptor-mediated currents were blocked only to 62±9% of control ($n=7$; Figure 3B).

Halothane blockade of isolated AMPA (Figure 4A) and NMDA (Figure 4B) receptor-mediated currents was dose-dependent over the tested range of 0.37 to 5.6 mM. The averaged dose-response relations were fitted by a simple one binding-site scheme (see Methods). The IC_{50} values for inhibition of AMPA and NMDA currents were 1.7 mM and 5.9 mM, respectively (Figure 4C), and were significantly different from each other ($z=19.9$; $P<0.0001$). The Hill coefficients for both curves were near unity, suggesting a lack of cooperativity in the blocking action of halothane.

Nature of antagonism of AMPA receptors by halothane

To distinguish between competitive versus noncompetitive inhibition of AMPA receptors by halothane, we examined the dose-response relation of AMPA in absence versus presence of halothane. Tissue concentrations of AMPA were increased by logarithmically increasing iontophoretic ejection currents while maintaining ejection time constant (Stone, 1985). For each experiment the AMPA-induced responses were normalized to the maximal response obtained in control saline. The iontophoretic dose-response relations to AMPA were fitted with a simple agonist-binding scheme (see Methods). Halothane (1.49 mM) significantly reduced the maximal AMPA-induced currents without affecting the AMPA dose required for a half maximal response (Figure 5A).

We compared these results to those obtained for the known competitive AMPA receptor antagonist CNQX (Honore *et al*., 1988). As expected, CNQX (1 μM) did not effect the maximal AMPA-induced current, but caused a rightward shift in the AMPA dose-response relation (Figure 5B).

Discussion

The main finding in this study is that halothane blocks glutamatergic EPSCs in hippocampal neurons by a dual presynaptic and postsynaptic mechanism of action. At low halothane concentrations (i.e. 0.64 mM), a presynaptic inhibition of glutamate release predominates. At higher halothane concentrations (1.5 to 5.6 mM) a selective, noncompetitive, postsynaptic inhibition of AMPA receptors also contributes to EPSC suppression by halothane.

Presynaptic action

We have previously suggested that low concentrations of halothane inhibit glutamate release from afferent fibers to CA1 pyramidal cells (Perouansky *et al*., 1995). This notion was tested further by examining the effects of halothane on PPF. Drugs that inhibit neurally evoked glutamate release generally cause an increase in PPF, whereas postsynaptic antagonists do not (e.g. Greager *et al*., 1980; Manabe *et al*., 1993; Andreasen & Hablitz, 1994). We found that already at low doses

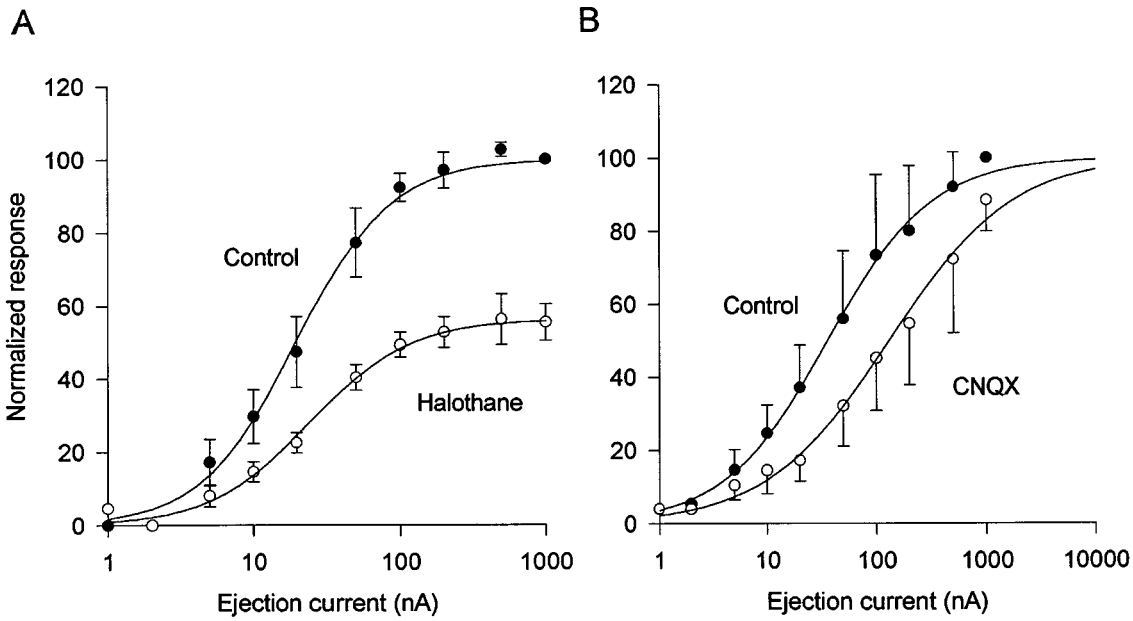

Figure 5 Halothane inhibits AMPA receptors in a noncompetitive manner. Whole-cell currents were evoked at −60 mV holding potential by iontophoretically applied AMPA. APV (100 μM) was included in the saline. The doses of applied AMPA were varied by changing the iontophoretic ejection currents. Qualitative dose-response relations for AMPA were obtained and fitted with a simple binding scheme (continuous line; see Methods). Bars represent standard error of the mean. (A) Dose-response relationship of AMPA in control saline (●; $n=5$) and in the presence of halothane (1.49 mM; ○; $n=5$). Halothane inhibited iontophoretic AMPA currents to a similar extent at all ejection currents employed. The half maximal response to AMPA was obtained at ejection currents of 19.6 nA in control saline and 24.4 nA in halothane containing saline. The maximal current evoked by AMPA application in the presence of halothane was 55.4% of control. (B) Dose-response relationship of AMPA in control saline (●; $n=4$) and in the presence of the competitive AMPA receptor antagonist CNQX (1 μM; ○; $n=4$). CNQX inhibited iontophoretic AMPA currents to a greater extent at lower ejection currents. The half maximal response to AMPA was obtained at ejection currents of 35.9 and 130.1 nA in control and CNQX, respectively. The maximal current evoked by AMPA application in the presence of 1 μM CNQX was 89% of control.

halothane augments PPF of monosynaptic non NMDA EPSCs. A recent study using extracellular recordings of compound excitatory postsynaptic potentials in rat hippocampal slices also showed that halothane enhances PPF (MacIver *et al*., 1996). Taken together, these findings provide further support to the presynaptic inhibition hypothesis of halothane action (Perouansky *et al*., 1995).

The inhibition of neurally evoked release of glutamate by halothane could be mediated by block of voltage-gated Na^+ channels, which would reduce presynaptic depolarization and Ca^{2+} entry through voltage-gated Ca^{2+} channels. Accordingly, recombinant central nervous system Na^+ channels were blocked by submillimolar concentrations of halothane (Rehberg *et al*., 1996). In contrast, Na^+-dependent spikes in hippocampal interneurones were not affected by 1.5 mM halothane (Perouansky *et al*., 1996). Thus, inhibition of glutamate release may result from a direct, rather than indirect, action of halothane on presynaptic Ca^{2+} channels. In line with the latter mechanism, halothane inhibited Ca^{2+} dependent glutamate release from cerebrocortical synaptosomes induced by KCl (Miao *et al*., 1995; Schlame & Hemmings, 1995).

Multiple types of Ca^{2+} channels are expressed in hippocampal neurones, of which the N- and P-types are thought to mediate neuronally evoked release of glutamate (Luebke *et al*., 1993; Takahashi & Momiyama, 1993). Whereas the sensitivity of the N-type current to halothane has not been tested to date, the P-type Ca^{2+} current in cerebellar Purkinje neurones was blocked by halothane with an IC_{50} of 1.17 mM (Hall *et al*., 1994). Halothane was shown to block more effectively T-type Ca^{2+} channels in rat sensory neurones ($IC_{50}=0.1$ mM; Takenoshita & Steinbach, 1991) and L-type Ca^{2+} channels in clonal (GH_3) pituitary cells ($IC_{50}=0.85$ mM; Herrington & Lingle, 1991). Interestingly, the volatile anesthetic isoflurane (1 mM) has been shown to significantly block isolated T-, L-, N-, and probably P-type Ca^{2+} channels in dissociated hippocampal neurones (Study, 1994). Though the sensitivity of presynaptic Ca^{2+} channels in hippocampal neurones to halothane is not yet known, even a partial block of these channels may cause a significant depression of glutamate release due to the large cooperativity in the Ca^{2+} dependence of transmitter release (Zucker, 1989).

Postsynaptic action

In line with previous studies (Crawford, 1970; Richards & Smaje, 1976), we found that glutamate receptors are relatively insensitive to halothane at concentrations lower than 0.64 mM. At higher concentrations AMPA receptors were significantly more sensitive to the blocking action of halothane than NMDA receptors (IC_{50} of 1.6 mM versus 5.9 mM, respectively). The interaction of halothane with AMPA receptors appeared to be noncompetitive in nature, suggesting a negative allosteric effect of halothane on the AMPA receptor protein.

Recent studies have shown that several volatile anesthetics, including halothane, have subunit specific effects on different recombinant glutamate receptors (Dildy Mayfield *et al*., 1996; for review see Harris *et al*., 1995). It was shown that currents mediated by AMPA-sensitive subunits (i.e. GluR3) are

inhibited, while those mediated by kainate-sensitive subunits (i.e. GluR6) are enhanced by halothane. These observations support a receptor-specific interaction between halothane and AMPA receptors.

Noncompetitive antagonism of AMPA receptors by a 2,3-benzodiazepine (Donevan & Rogawski, 1993; Zorumski *et al*., 1993) was described before. The lack of use-dependence in the action of this drug suggested the existence of a unique negative allosteric binding site on the AMPA receptor. Other studies have shown that pregnenolone-sulphate and pentobarbital also are noncompetitive antagonists of the AMPA receptor, but the use-dependence of their action suggested they act within the AMPA receptor channel (Marszalec & Narahashi, 1993; Wu & Chen, 1997). Whether halothane acts within or outside the AMPA receptor channel remains to be elucidated. Interestingly, halothane exerted a positive allosteric effect on the $GABA_A$ and glycine receptor proteins in rat tractus solitarius neurons (Wakamori *et al*., 1991).

In contrast to our findings, two recent studies suggested that halothane may selectively block NMDA receptor channels. In the first study NMDA-stimulated $^{45}Ca^{2+}$ uptake by a microvesicle fraction of rat brain was highly sensitive to halothane (0.2–0.3 mM; Aronstam *et al*., 1994). However, blockage of voltage-gated Ca^{2+} channels by halothane in that study was not excluded. In the second study halothane blocked NMDA receptor-mediated field responses more effectively than those mediated by nonNMDA receptors (Narimatsu *et al*., 1996). However, the effect of halothane was tested in conditions which do not differentiate between pre- and postsynaptic sites of action, and the concentrations used were more indicative of a presynaptic effect.

Conclusions

Glutamate receptor antagonists have been shown to be compounds with anesthetic potential and to enhance the anesthetic potency of halothane *in vivo* (Scheller *et al*., 1989; Mantz *et al*., 1992; McFarlane *et al*., 1992). Our results indicate that the volatile anesthetic halothane blocks glutamatergic excitatory synaptic transmission. The mechanism of this blockage is complex, involving both pre- and postsynaptic sites of action. The more potent effect is inhibition of neurally evoked glutamate release, presumably due to reduction of presynaptic Ca^{2+} influx. This may result from a direct block of voltage-gated Ca^{2+} channels or from a block of voltage-gated Na^+ channels responsible for the presynaptic action potential. At higher concentrations, halothane blocks selectively and noncompetitively postsynaptic AMPA receptors. Because fast transmission across glutamatergic synapses in the hippocampus is mediated primarily by AMPA receptors (e.g. Davies & Collingridge, 1989; Herreras *et al*., 1989; Lambert *et al*., 1989), blocking these receptors may be involved in deeper states of halothane anesthesia. Notwithstanding, it is very likely that suppression of glutamatergic transmission is only one of several mechanisms that acting in concert are responsible for the high anesthetic potential of halothane.

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References

ANDREASEN, M. & HABLITZ, J.J. (1994). Paired-pulse facilitation in the dentate gyrus: a patch-clamp study in rat hippocampus in vitro. *J. Neurophysiol.,* **72,** 326–336.

ARONSTAM, R.S., MARTIN, D.C. & DENNISON, R.L. (1994). Volatile anesthetics inhibit NMDA-stimulated 45Ca uptake by rat brain microvesicles. *Neurochem. Res.,* **19,** 1515–1520.

CRAWFORD, J.M. (1970). Anaesthetic agents and the chemical sensitivity of cortical neurones. *Neuropharmacology,* **9,** 31–46.

CREAGER, R., DUNWIDDIE, T. & LYNCH, G. (1980). Paired-pulse and frequency facilitation in the CA1 region of the in vitro rat hippocampus. *J. Physiol. Lond.,* **299,** 409–424.

DAVIES, S.N. & COLLINGRIDGE, G.L. (1989). Role of excitatory amino acid receptors in synaptic transmission in area CA1 of rat hippocampus. *Proc. R. Soc. Lond. B. Biol. Sci.,* **236,** 373–384.

DILDY MAYFIELD, J.E., EGER, E.I. II & HARRIS, R.A. (1996). Anesthetics produce subunit-selective actions on glutamate receptors. *J. Pharmacol. Exp. Ther.,* **276,** 1058–1065.

DONEVAN, S.D. & ROGAWSKI, M.A. (1993). GYKI 52466, a 2,3-benzodiazepine, is a highly selective, noncompetitive antagonist of AMPA/kainate receptor responses. *Neuron,* **10,** 51–59.

EDWARDS, F.A., KONNERTH, A., SAKMANN, B. & TAKAHASHI, T. (1989). A thin slice preparation for patch clamp recordings from neurones of the mammalian central nervous system. *Pflugers Arch.,* **414,** 600–612.

FORMAN, S.A., MILLER, K.W. & YELLEN, G. (1995). A discrete site for general anesthetics on a postsynaptic receptor. *Mol. Pharmacol.,* **48,** 574–581.

GREENAMYRE, J.T., OLSON, J.M., PENNEY, J.B. JR. & YOUNG, A.B. (1985). Autoradiographic characterization of N-methyl-D-aspartate-, quisqualate- and kainate-sensitive glutamate binding sites. *J. Pharmacol. Exp. Ther.,* **233,** 254–263.

HALL, A.C., LIEB, W.R. & FRANKS, N.P. (1994). Insensitivity of P-type calcium channels to inhalational and intravenous general anesthetics. *Anesthesiology,* **81,** 117–123.

HARRIS, R.A., MIHIC, S.J., DILDY MAYFIELD, J.E. & MACHU, T.K. (1995). Actions of anesthetics on ligand-gated ion channels: role of receptor subunit composition. *FASEB J.,* **9,** 1454–1462.

HERRERAS, O., MENENDEZ, N., HERRANZ, A.S., SOLIS, J.M. & MARTIN DEL RIO, R. (1989). Synaptic transmission at the Schaffer-CA1 synapse is blocked by 6,7-dinitro-quinoxaline-2,3-dione. An in vivo brain dialysis study in the rat. *Neurosci. Lett.,* **99,** 119–124.

HERRINGTON, J. & LINGLE, C.J. (1991). Halothane reduces calcium currents in clonal (GH3) pituitary cells. *Ann. N.Y. Acad. Sci.,* **625,** 290–292.

HESTRIN, S., NICOLL, R.A., PERKEL, D.J. & SAH, P. (1990). Analysis of excitatory synaptic action in pyramidal cells using whole-cell recording from rat hippocampal slices. *J. Physiol. Lond.,* **422,** 203–225.

HONORE, T., DAVIES, S.N., DREJER, J., FLETCHER, E.J., JACOBSEN, P., LODGE, D. & NIELSEN, F.E. (1988). Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. *Science,* **241,** 701–703.

KAUFMAN, R.D. (1977). Biophysical mechanisms of anesthetic action: historical perspective and review of current concepts. *Anesthesiology,* **46,** 49–62.

KRNJEVIC, K. (1992). Cellular and synaptic actions of general anaesthetics. *Gen. Pharmacol.,* **23,** 965–975.

LAMBERT, J.D., JONES, R.S., ANDREASEN, M., JENSEN, M.S. & HEINEMANN, U. (1989). The role of excitatory amino acids in synaptic transmission in the hippocampus. *Comp. Biochem. Physiol. A.,* **93,** 195–201.

LUEBKE, J.I., DUNLAP, K. & TURNER, T.J. (1993). Multiple calcium channel types control glutamatergic synaptic transmission in the hippocampus. *Neuron,* **11,** 895–902.

MACIVER, M.B. & ROTH, S.H (1988). Inhalation anaesthetics exhibit pathway-specific and differential actions on hippocampal synaptic responses in vitro. *Br. J. Anaesthesiol.,* **60,** 680–691.

MACIVER, M.B., MIKULEC, A.A., AMAGASU, S.M. & MONROE, F.A. (1996). Volatile anesthetics depress glutamate transmission via presynaptic actions. *Anesthesiology,* **85,** 823–834.

MANABE, T., WYLLIE, D.J., PERKEL, D.J. & NICOLL, R.A. (1993). Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. *J. Neurophysiol.,* **70,** 1451–1459.

MANTZ, J., CHERAMY, A., THIERRY, A.M., GLOWINSKI, J. & DESMONTS, J.M. (1992). Anesthetic properties of riluzole (54274 RP), a new inhibitor of glutamate neurotransmission. *Anesthesiology,* **76,** 844–848.

MARSZALEC, W. & NARAHASHI, T. (1993). Use-dependent pentobarbital block of kainate and quisqualate currents. *Brain Res.,* **608,** 7–15.

MCFARLANE, C., WARNER, D.S., TODD, M.M. & NORDHOLM, L. (1992). AMPA receptor competitive antagonism reduces halothane MAC in rats. *Anesthesiology,* **77,** 1165–1170.

MIAO, N., FRAZER, M.J. & LYNCH, C. III. (1995). Volatile anesthetics depress Ca2+ transients and glutamate release in isolated cerebral synaptosomes. *Anesthesiology,* **83,** 593–603.

NARIMATSU, E., TSAI, Y.C., GERHOLD, T.D., KAMATH, S.H., DAVIES, L.R. & SOKOLL, M.D. (1996). A comparison of the effect of halothane on N-methyl-D-aspartate and non-N-methyl-D-aspartate receptor-mediated excitatory synaptic transmission in the hippocampus. *Anesth. Analg.,* **82,** 843–847.

PEROUANSKY, M., BARANOV, D., SALMAN, M. & YAARI, Y. (1995). Effects of halothane on glutamate receptor-mediated excitatory postsynaptic currents. A patch-clamp study in adult mouse hippocampal slices. *Anesthesiology,* **83,** 109–119.

PEROUANSKY, M. & KIRSON, E.D. (1996). Anesthetic gas-scavenging in the laboratory. *Anesthesiology,* **84,** 751.

PEROUANSKY, M., KIRSON, E.D. & YAARI, Y. (1996). Halothane blocks synaptic excitation of inhibitory interneurons. *Anesthesiology,* **85,** 1431–1438; discussion 1429A.

POCOCK, G. & RICHARDS, C.D. (1993). Excitatory and inhibitory synaptic mechanisms in anaesthesia. *Br. J. Anaesth.,* **71,** 134–147.

REHBERG, B., XIAO, Y.H. & DUCH, D.S. (1996). Central nervous system sodium channels are significantly suppressed at clinical concentrations of volatile anesthetics. *Anesthesiology,* **84,** 1223–1233; discussion 1227A.

RICHARDS, C.D. & SMAJE, J.C. (1976). Anaesthetics depress the sensitivity of cortical neurones to L-glutamate. *Br. J. Pharmacol.,* **58,** 347–357.

RICHARDS, C.D. & WHITE, A.E. (1975). The actions of volatile anaesthetics on synaptic transmission in the dentate gyrus. *J. Physiol. Lond.,* **252,** 241–257.

SCHELLER, M.S., ZORNOW, M.H., FLEISCHER, J.E., SHEARMAN, G.T. & GREBER, T.F. (1989). The noncompetitive N-methyl-D-aspartate receptor antagonist, MK-801 profoundly reduces volatile anesthetic requirements in rabbits. *Neuropharmacology,* **28,** 677–681.

SCHLAME, M. & HEMMINGS, H.C. JR (1995). Inhibition by volatile anesthetics of endogenous glutamate release from synaptosomes by a presynaptic mechanism. *Anesthesiology,* **82,** 1406–1416.

STONE, T.W. (1985). *Microiontophoresis and pressure ejection*. Chichester: John Wiley & Sons.

STUDY, R.E. (1994). Isoflurane inhibits multiple voltage-gated calcium currents in hippocampal pyramidal neurones [see comments]. *Anesthesiology,* **81,** 104–116.

TAKAHASHI, T. & MOMIYAMA, A. (1993). Different types of calcium channels mediate central synaptic transmission. *Nature,* **366,** 156–158.

TAKENOSHITA, M. & STEINBACH, J.H. (1991). Halothane blocks low-voltage-activated calcium current in rat sensory neurons. *J. Neurosci.,* **11,** 1404–1412.

TANELIAN, D.L., KOSEK, P., MODY, I. & MACIVER, M.B. (1993). The role of the GABAA receptor/chloride channel complex in anesthesia. *Anesthesiology,* **78,** 757–776.

TRUSSELL, L.O. & FISCHBACH, G.D. (1989). Glutamate receptor desensitization and its role in synaptic transmission. *Neuron,* **3,** 209–218.

TRUSSELL, L.O., THIO, L.L., ZORUMSKI, C.F. & FISCHBACH, G.D. (1988). Rapid desensitization of glutamate receptors in vertebrate central neurons. *Proc. Natl. Acad. Sci. U.S.A.,* **85,** 4562–4566.

WAKAMORI, M., IKEMOTO, Y. & AKAIKE, N. (1991). Effects of two volatile anesthetics and a volatile convulsant on the excitatory and inhibitory amino acid responses in dissociated CNS neurons of the rat. *J. Neurophysiol.,* **66,** 2014–2021.

WU, F.S. & CHEN, S.C. (1997). Mechanism underlying the effect of pregnenolone sulfate on the kainate-induced current in cultured chick spinal cord neurons. *Neurosci. Lett.,* **222,** 79–82.

ZORUMSKI, C.F., YAMADA, K.A., PRICE, M.T. & OLNEY, J.W. (1993). A benzodiazepine recognition site associated with the non-NMDA glutamate receptor. *Neuron,* **10,** 61–67.

ZUCKER, R.S. (1989). Short-term synaptic plasticity. *Annu. Rev. Neurosci.,* **12,** 13–31.

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