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Effects of Halothane on Glutamate Receptor-mediated Excitatory Postsynaptic Currents

A Patch-Clamp Study in Adult Mouse Hippocampal Slices

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Background: **The effects of halothane on excitatory synaptic transmission in the central nervous system of mammals have been studied *in vivo* and *in vitro* in several investigations with partially contradicting results. Direct measurements of the effects of halothane on isolated glutamate receptor–mediated (glutamatergic) excitatory postsynaptic currents (EPSCs), however, have not been reported to date.**

Methods: **The effects of halothane on glutamatergic EPSCs were studied *in vitro* by using tight-seal, whole-cell recordings from CA1 pyramidal cells in thin slices from the adult mouse hippocampus. The EPSCs were pharmacologically isolated into their non–N-methyl-D-aspartate (non-NMDA) and NMDA receptor–mediated components by using selective antagonists. The effects of halothane on EPSC amplitude and kinetics were analyzed at various membrane potentials and were compared with its effects on currents evoked by exogenously applied glutamatergic agonists.**

Results: **Halothane (0.2–5.1%; 0.37–2.78 mM) reversibly blocked non-NMDA and NMDA EPSCs. This effect was voltage independent; concentrations producing 50% inhibition were 0.87% (0.66 mM) and 0.69% (0.57 mM), respectively. Currents induced by bath-applied glutamatergic agonists were not affected even by the high concentrations of halothane.**

Conclusions: **Halothane depresses glutamatergic EPSCs irrespective of receptor subtype, most likely by inhibition of glutamate release. (Key words: Anesthetics, volatile: halothane. Animals: mouse. Brain: hippocampus. Central nervous system: excitatory postsynaptic currents. Central nervous system, receptors: glutamate; N-methyl-D-aspartate; non–N-methyl-D-aspartate. Measurement techniques: hippocampal slice; patch-clamp.)**

GENERAL anesthetics affect synaptic transmission at concentrations likely to be found in the brain during surgical anesthesia.[1] This interaction may take place at multiple sites and may differ from one neurotransmitter system to another.[2,3] The precise sites and mechanisms of this interaction as well as their relative importance to the anesthetic state are not yet understood.

Halothane affects the inhibitory γ-aminobutyric acid (GABA)–mediated transmitter system at various sites. First, in cortical slices, it reduces the breakdown of GABA, thus potentially increasing its concentration in the synaptic cleft.[4] Second, it prolongs the time constant of decay of spontaneous, $GABA_A$ receptor–mediated postsynaptic currents in hippocampal slices through release of intracellular calcium.[4] These effects may contribute to the depressant effect of halothane on the central nervous system (CNS).

Several studies have investigated the effects of halothane on excitatory synaptic transmission in a variety of mammalian CNS preparations. The results of experiments conducted *in vivo* have been contradictory.[5] In the cat, halothane was found to facilitate excitatory synaptic transmission in the cuneate nucleus[6] and to depress it in the spinal cord[7] but in the rat hippocampus was found to leave it unaffected.[8] More consistent results were obtained in experiments in CNS preparations *in vitro*. Halothane depressed excitatory synaptic transmission in the guinea pig olfactory cortex[9] and dentate gyrus[10] as well as in the spinal cord of newborn rats.[11] Contradictory results, however, were obtained in the rabbit olfactory bulb[12] and in some pathways of

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the rat hippocampus.[13,14] This inconsistency may reflect the variety of preparations and recording techniques or may result from multiple indirect CNS effects of halothane, which may mask its direct action on excitatory synaptic transmission.

In most if not all of the above preparations, as in many other mammalian CNS synapses, the neurotransmitter mediating fast excitatory synaptic transmission is thought to be glutamate.[15] Synaptically released glutamate has been shown to activate two main subtypes of postsynaptic receptors coupled to ionic channels, namely the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)–kainate receptor and the n-methyl-D-aspartate (NMDA) receptor, as well as a G-protein–coupled (metabotropic) receptor.[16] Whether halothane depresses excitatory synaptic transmission by interacting directly with any of these receptors and whether it affects them equally or differentially are not known.

The purposes of this study were to investigate the effects of halothane on glutamatergic synaptic transmission and to gain insight into its mechanism of action by comparing its effects on AMPA–kainate (*i.e.*, non-NMDA) and on NMDA receptor–mediated responses in a central mammalian neuron. We obtained tight-seal, whole-cell recordings of excitatory postsynaptic currents (EPSCs) and agonist-induced currents from adult mouse CA1 hippocampal pyramidal cells in thin slices.[17,18]

Materials and Methods

Preparation of Slices

Institutional approval for the performance of the experiments was obtained. Experiments were performed on thin hippocampal slices from adult mice (49–90 days old). Slices were prepared by the same technique used to prepare thin slices from juvenile rat brain.[17,18] In brief, mice were anesthetized with ether and decapitated with a guillotine. The brain was quickly removed and cooled in ice-cold saline. A block of tissue containing one hippocampus was glued with cyanoacrylate to the stage of a vibratome. Transverse slices 150 μm thick (fig. 1A) were cut from the hemispheric region containing the hippocampus, transferred to an incubation chamber containing oxygenated (95% O_2–5% CO_2) saline at 34°C, and used one at a time after 1 h of incubation. In the recording chamber the slice was continuously perfused (2.5 ml · min^{-1}) with oxygenated saline at room temperature (21–23°C) (fig. 1B).

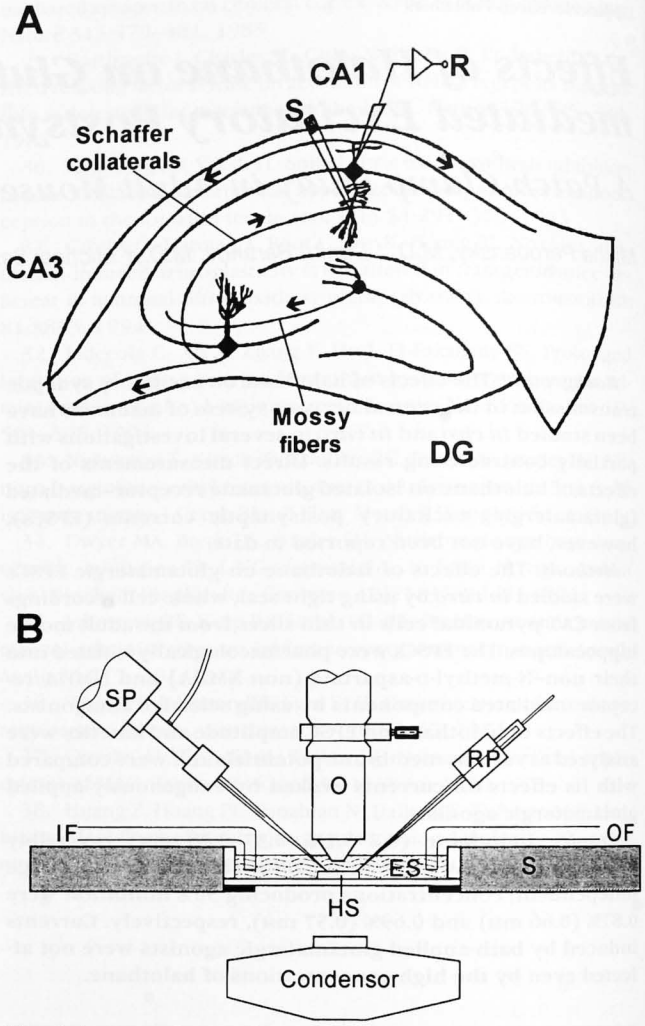

Fig. 1. Preparation and experimental setup. (*A*) Transverse hippocampal slice. The Schaffer collaterals were stimulated with an electrode (S) close to the pyramidal cell clamped by the recording electrode (R). Arrows = direction of action potential propagation within the hippocampal circuitry: from the dentate gyrus (DG) (filled circle = granule cell) *via* the mossy fibers to CA3 pyramidal cell (filled rhomboid) and further on *via* the Schaffer collaterals to CA1 pyramidal cells (filled rhomboid). Interneurons are not depicted. (*B*) System for stimulation and recording in slices. A chamber containing the hippocampal slice (HS) was mounted on the stage (S) of an upright microscope. Stimulating (SP) and recording (RP) pipettes were placed with visual control through a ×400 magnifying immersion objective (O). Inflow (IF) and outflow (OF) tracts for the experimental solutions were situated at opposite sides of the chamber.

Solutions and Drugs

The standard saline used for dissection and maintenance of slices consisted of (millimolar): NaCl 125, KCl 2.5, hydroxyethylpiperazineethane sulfonic acid

(HEPES) 13, NaH_2PO_4 1.25, glucose 12.5, $CaCl_2$ 1.5, and $MgSO_4$ 4 at *p*H 7.3. The concentration of $NaHCO_3$ was 26.7 mM in the dissection and experimental solutions and 13 mM in the incubation solution. All experimental salines also contained bicuculline methiodide (10 μM) to block $GABA_A$ receptor–mediated chloride currents, and glycine (5 μM) to ensure saturation of the glycine binding sites of NMDA receptors[19] and to increase the selectivity of 6-nitro-7-cyano-quinoxaline-2,3-dion (CNQX) to non-NMDA receptors.[20] In the experimental salines the concentration of $CaCl_2$ was increased to 2.5 mM, whereas $MgCl_2$ was either omitted completely or added at the specified concentrations. Saline without added $MgCl_2$ (Mg^{2+}-free saline) contained approximately 2 μM Mg^{2+}, as measured with atomic absorption spectroscopy. DL-2-Aminophosphonovaleric acid (APV) or CNQX were added to block NMDA or non-NMDA receptor–mediated currents, respectively.[21,22] In one series of experiments NMDA (5 μM) or glutamate (10 and 20 μM) was applied by bath perfusion. In this series all salines contained also tetrodotoxin (1 μM) to block neurally evoked transmitter release.

The intracellular (pipette) solution consisted of (millimolar): CsF 130, NaCl 10, hydroxyethylpiperazineethane sulfonic acid 10, ethyleneglycol-bis-(β-aminoethyl ether) tetraacetic acid (EGTA) 10, $MgCl_2$, $CaCl_2$ 1, tetraethyl ammonium 10. The *p*H was adjusted to 7.2–7.3. The osmolarity was approximately 10% lower than the measured osmolarity of the extracellular solution (300–310 mOsm). The use of cesium and tetraethyl ammonium to block K^+ conductances and of fluoride to reduce voltage-dependent Ca^{2+} currents, improved the space clamp. Drugs were purchased from Sigma Chemical (St. Louis, MO), with the exception of CNQX (Tocris Neuramin, Bristol, United Kingdom) and halothane (Trofield Surgicals, Zug, Switzerland).

Application and Measurement of Halothane

The fresh O_2–CO_2 mixture was directed by a flowmeter through a vaporizer (Enfluratec) containing halothane. The vaporizer was calibrated for halothane with an IRIS Gas Analyzer (Draeger, Lübeck, 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 measured with a gas chromatograph (Tracor 540) equipped with a 1.83-m × 0.3-cm glass column (packed with Gaschrom 220, 80/100 mesh, Alltech), flame ionization detector, and nitrogen as the carrier gas. The temperatures of the injector, detector, and oven were 165°C, 165°C, and 155°C, respectively. Aqueous test samples (10 μl) were withdrawn from the recording chamber with a gas-tight syringe (Hamilton) and injected directly into the column. Peak area values from the test samples were compared with those of known concentrations of halothane standards in methanol. Column retention times for halothane and methanol were approximately 3 min and 30 s, respectively. The results of the concentration measurements are illustrated in figure 2. Standard halothane concentrations of 0.2%, 0.4%, 0.9%, 2.5%, and 5.1%, as measured with a gas analyzer (IRIS) at the exit from the vaporizer, corresponded to concentrations of 0.37 ± 0.01, 0.43 ± 0.06, 0.64 ± 0.04, 1.49 ± 0.12, and 2.79 ± 0.24 mM in the slice-bathing solution (mean ± SD from two or three measurements).

Stimulation and Recording

Pyramidal cells in the CA1 field of the hippocampal slice were visualized at ×400 magnification with a wa-

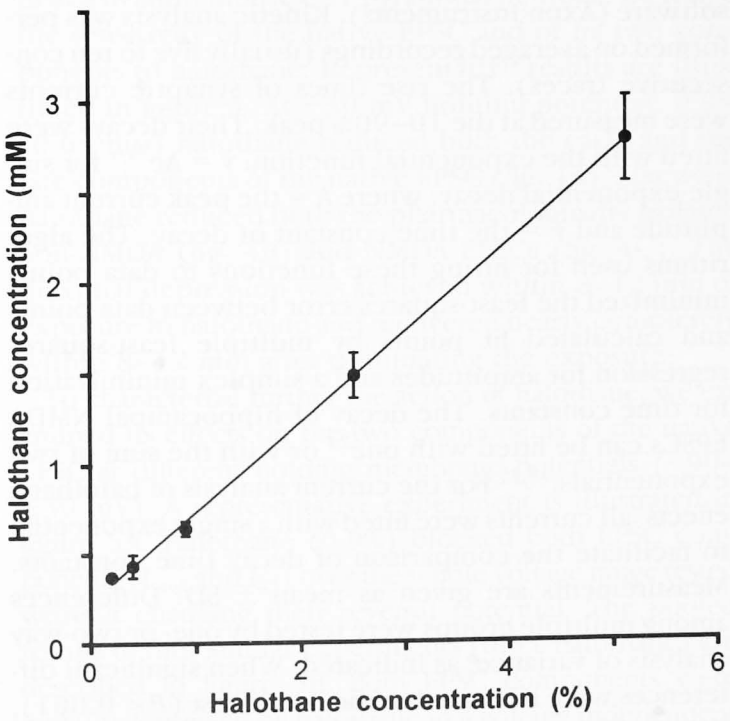

Fig. 2. Relation between the concentrations of halothane in the saline perfusing the slice chamber (millimolar) and in the gas mixture applied to the saline (percentage). Data points are mean ± SD of measured halothane concentrations in the saline bathing the slice at each vaporizer setting. The relation between the two variables in the tested range was linear.

ter immersion objective using an upright microscope equipped with Nomarski optics (standard 14, Zeiss, Germany) (fig. 1B). Tight-seal whole-cell recordings[23] were obtained with recording pipettes pulled from borosilicate glass (Hilgenberg, Maisfeld, Germany) on a vertical puller (List-medical, Germany) and coated with Sylgard resin (Dow Corning Chemical, Midland, MI). The series resistance (5–10 MΩ) was compensated to 50–70% by the patch-clamp amplifier (LM-EPC 7, List-Electronic, Darmstadt, Germany). The pipettes used for cleaning and stimulating were pulled from disposable micropipettes (Boralex, Rochester Scientific) and filled with saline. The EPSCs were evoked by stimulating close (20–70 μM) to the patched cell at a frequency of 0.1 Hz, using a bipolar electrode consisting of the aforementioned cleaning pipette and a remote Ag–AgCl pellet connected by a 100-μm–thick platinum wire to an isolation unit (World Precision Instruments, Aston, England). Stimulus strength was adjusted to evoke maximal stable EPSCs.

Data Analysis

All currents recorded were filtered at 3 kHz, digitized on-line at sampling rates between 0.7 and 2 kHz and analyzed off-line with a personal computer and pClamp software (Axon Instruments). Kinetic analysis was performed on averaged recordings (usually five to ten consecutive traces). The rise times of synaptic currents were measured at the 10–90% peak. Their decays were fitted with the exponential function: $y = Ae^{-t/\tau}$ for single-exponential decay, where A = the peak current amplitude and τ = the time constant of decay. The algorithms used for fitting these functions to data points minimized the least-squares error between data points and calculated fit points by multiple least-squares regression for amplitudes and a simplex minimization for time constants. The decay of hippocampal NMDA EPSCs can be fitted with one[24] or with the sum of two exponentials.[18,25] For the current analysis of halothane effects, all currents were fitted with a single exponential to facilitate the comparison of decay time constants. Measurements are given as mean ± SD. Differences among multiple groups were tested by one- or two-way analysis of variance, as indicated. When significant differences were indicated in the F ratio test ($P < 0.001$), the significance of differences between means of any two of these groups was determined by the modified Tukey method for multiple comparisons with an α of 0.05. Differences between paired sets of data were compared by using Student's *t* test.

The solid lines through the data points in current–voltage plots were fitted as described previously.[18] The dose–response curve for halothane was obtained by fitting experimental results with the Hill equation

$$I = I_m/(1 + (IC_{50}/[\text{halothane}])^n)$$

in which I_m = maximal inhibition; IC_{50} = the concentration producing 50% inhibition; and n = the Hill coefficient.

Results

Excitatory Postsynaptic Currents in Adult CA1 Neurons

The current results are based on recordings from 22 CA1 pyramidal cells from adult mouse hippocampi. Stimulation of afferent fibers in the vicinity of patched neurons reliably evoked stimulus-graded EPSCs. Peak amplitudes of EPSCs (I_{peak}) evoked by supramaximal stimuli at holding voltage of −60 mV varied from tens to hundreds of picoamperes in different cells. A representative example is shown in figure 3A. When evoked in standard saline (*i.e.*, containing 1 mM Mg^{2+}) and at holding potentials negative to −40 mV, the EPSCs had a relatively fast time course. The EPSC decay became progressively slower with depolarization (fig. 3A, left). Changing to Mg^{2+}-free saline also prolonged the decay of EPSCs evoked at negative holding potentials and augmented their amplitude (fig. 3A, right). This dependence on voltage and Mg^{2+} is characteristic of EPSCs mediated by admixed non-NMDA and NMDA receptors.[26]

To characterize more precisely the possible contribution of non-NMDA and NMDA receptors to the native EPSC, we determined the current–voltage relations of I_{peak} and of the EPSC measured 25 ms after the peak (I_{25}) (fig. 3B). Both I_{peak} and I_{25} reversed at holding potentials close to 0 mV, as expected for glutamate receptor–mediated currents in our experimental conditions.[18,24,26] The relation of I_{peak} *versus* holding potential in standard saline was nearly linear over a large voltage range, consistent with its mediation by non-NMDA receptors. By contrast, the corresponding relation of I_{25} *versus* holding potential displayed a region of negative slope conductance at voltages negative to −30 mV, indicating the presence of a slower NMDA receptor–mediated EPSC component. As expected, this region in the current–voltage relation was shifted to much more negative voltage upon perfusion with Mg^{2+}-

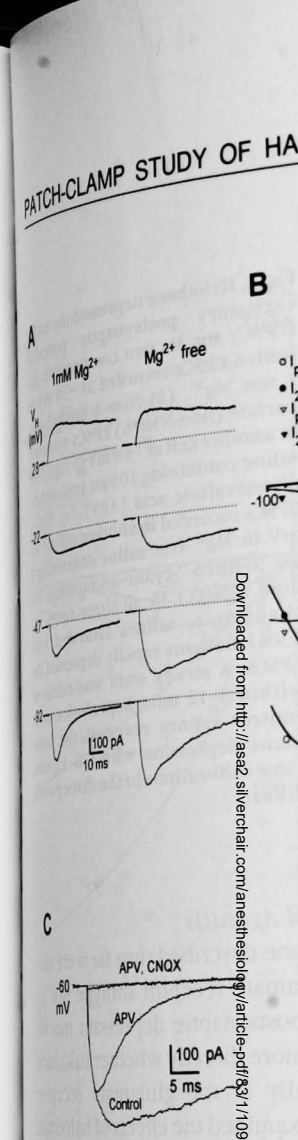

Fig. 3. Glutamatergic excitatory post
in adult CA1 pyramidal cells consis
methyl-D-aspartate) and NMDA re
nents. (*A*) Exemplary native EPSCs re
potentials (V_H) in standard saline (
Mg^{2+}-free saline (*A, right*). In standa
recruited a slow EPSC component. Th
at all V_Hs in Mg^{2+}-free saline. (*B*) C
lation of the EPSCs from the experi
peak EPSC amplitude (I_{peak}) and the
25 ms after the peak (I_{25}) were plo
Mg^{2+} saline (closed triangles) display
conductance typical for NMDA rec
The deviation from linearity of I_{peak}
less than −30 mV suggests that NMD
also in 1 mM Mg^{2+} saline (see below
aration of the non-NMDA and NM
left) EPSCs recorded at −60 mV in Mg
of 100 μM aminophosphonovaleric
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maining EPSC had a fast rise time an
within 25 ms after the peak. It was
6-nitro-7-cyano-quinoxaline-2,3-dio
hydroxy-5-methylisoxazole-4-propio
antagonist. (*C, right*) EPSCs from an
mV in 1 mM Mg^{2+} saline. Applicatio
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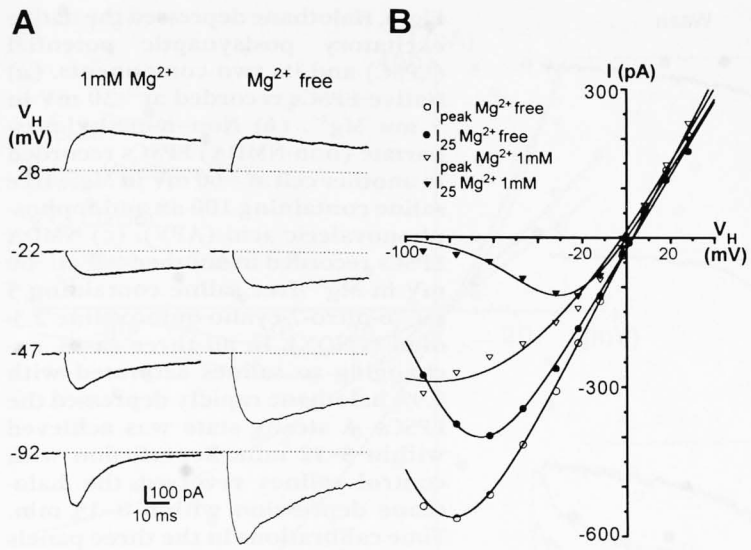

Fig. 3. Glutamatergic excitatory postsynaptic currents (EPSCs) in adult CA1 pyramidal cells consisted of non-NMDA (non-n-methyl-D-aspartate) and NMDA receptor–mediated components. (*A*) Exemplary native EPSCs recorded at various holding potentials (V_H) in standard saline (1 mM Mg^{2+}) (*A, left*) and in Mg^{2+}-free saline (*A, right*). In standard saline, depolarizing V_H recruited a slow EPSC component. This component was present at all V_Hs in Mg^{2+}-free saline. (*B*) Current–voltage ($I–V_H$) relation of the EPSCs from the experiment illustrated in *A*. The peak EPSC amplitude (I_{peak}) and the EPSC amplitude recorded 25 ms after the peak (I_{25}) were plotted against V_H. $I_{25}–V_H$ in Mg^{2+} saline (closed triangles) displays an area of negative slope conductance typical for NMDA receptor–mediated currents. The deviation from linearity of $I_{peak}–V_H$ (open triangles) at V_Hs less than −30 mV suggests that NMDA EPSCs contribute to I_{peak}, also in 1 mM Mg^{2+} saline (see below). (*C*) Pharmacologic separation of the non-NMDA and NMDA EPSC components. (*C, left*) EPSCs recorded at −60 mV in Mg^{2+}-free saline. Application of 100 μM aminophosphonovaleric acid (APV), an NMDA receptor antagonist, blocked a slow EPSC component. The remaining EPSC had a fast rise time and decayed to near baseline within 25 ms after the peak. It was blocked entirely by 5 μM 6-nitro-7-cyano-quinoxaline-2,3-dion (CNQX), an α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid–kainate receptor antagonist. (*C, right*) EPSCs from another cell, recorded at −45 mV in 1 mM Mg^{2+} saline. Application of 5 μM CNQX blocked a fast EPSC component. The remaining EPSC was almost completely suppressed by 100 μM APV. Although the rise phase of the NMDA EPSC component is slower than that of the native EPSC, it contributes substantially to I_{peak}. All sample traces are averages of five consecutive records. Calibration bars in the two panels differ.

free saline (fig. 3B). Similar dual component EPSCs were previously described in hippocampal slices from juvenile rats.[18,24,26]

The non-NMDA and the NMDA EPSC components could be separated pharmacologically by using receptor subtype–specific antagonists. Blocking NMDA receptors with 100 μM APV isolated the fast non-NMDA EPSC, which was suppressed completely by addition of 5 μM CNQX (fig. 3C, left). Conversely, adding 5 μM CNQX to block non-NMDA receptors isolated the slow NMDA EPSC, which was abolished completely by addition of 100 μM APV (Fig. 3C, right).

As previously shown in juvenile rat hippocampal neurons,[18,24,26] the isolated non-NMDA EPSCs in adult mouse pyramidal cells were much faster than the NMDA EPSCs. The rise time and decay time constant of the former at −60 mV holding potential were 1.0 ± 0.4 and 7.4 ± 0.9 ms (mean ± SD; n = 6), respectively. By contrast, the rise time and decay time constant of the latter were 11.3 ± 3.9 and 198 ± 37 ms (n = 5), respectively.

Effects of Halothane on Excitatory Postsynaptic Currents

Having characterized the receptor subtypes mediating EPSCs in adult mouse CA1 pyramidal neurons, we tested the sensitivity of the native EPSC and of its two components to halothane. Representative results are illustrated in figure 4. At −30 mV holding potential, 0.9% (0.64 mM) halothane reduced both the early and the late components of the native EPSC (fig. 4A). Likewise, halothane reduced both the pharmacologically isolated non-NMDA (fig. 4B) and NMDA EPSCs (fig. 4C). The maximal depression was achieved within 8–12 min of exposure to halothane and recovered nearly completely within 8–12 min after terminating the exposure.

To characterize further the action of halothane, we examined its effects on the two components of the native EPSC at different holding membrane potentials (−90–+30 mV). A representative experiment is illustrated in figure 5A. Halothane (0.9%) blocked both EPSC components (I_{peak} and I_{25}) over the whole voltage range tested without changing their reversal potential. The pooled results of four similar experiments (0.9% halothane) are described in figure 5B. Both EPSC components were equally depressed by halothane in a voltage independent manner across the entire voltage range tested.

Dose–Response Relation

To test whether halothane preferentially affects one subtype of glutamate receptors, we compared the de-

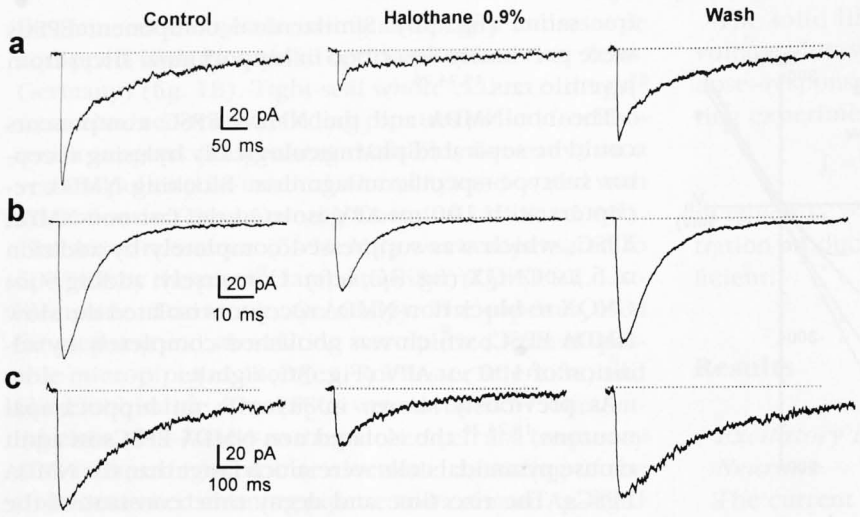

Fig. 4. Halothane depressed the native excitatory postsynaptic potential (EPSC) and its two components. (*a*) Native EPSCs recorded at −30 mV in 1 mM Mg^{2+}. (*b*) Non-n-methyl-D-aspartate (non-NMDA) EPSCs recorded in another cell at −60 mV in Mg^{2+}-free saline containing 100 μM aminophosphonovaleric acid (APV). (*c*) NMDA EPSCs recorded in another cell at −60 mV in Mg^{2+}-free saline containing 5 μM 6-nitro-7-cyano-quinoxaline-2,3-dion (CNQX). In all three cases, exchanging to salines saturated with 0.9% halothane rapidly depressed the EPSCs. A steady state was achieved within 8–12 min. Reperfusion with control salines reversed the halothane depression within 8–15 min. Time calibrations in the three panels differ.

pressant action of several halothane concentrations on pharmacologically isolated non-NMDA and NMDA EPSCs. The results obtained from 15 pyramidal cells are summarized in figure 6. We could not detect a significant difference in the depression of non-NMDA *versus* NMDA EPSCs (two-way analysis of variance). At the lowest halothane concentration applicable with our vaporizer (0.2%, 0.37 mM), the non-NMDA and NMDA EPSCs were depressed by 24 ± 8% and 18 ± 6%, respectively (n = 3 for for each EPSC type). Higher halothane doses caused more EPSC depression, with the highest concentration tested (5.1%, 2.79 mM) causing almost a complete suppression of both EPSCs (97 ± 4% and 97 ± 5%; n = 4 and n = 5, respectively). The IC_{50} values, interpolated from the fitted dose–response curves, were 0.69% (0.57 mM) and 0.87% (0.66 mM) for the NMDA and non-NMDA EPSCs, respectively (fig. 6).

Kinetics of Excitatory Postsynaptic Currents

We next examined the effects of halothane on the time course of isolated non-NMDA and NMDA EPSCs evoked at −60 mV holding potential. These measurements were not executed on data obtained with high halothane concentrations, because the marked depression of the EPSCs rendered them unreliable. The pooled results are shown in figure 7. At the three concentrations tested (0.4%, 0.9%, and 2.5%), halothane had no effect on the rise times, and halothane in concentrations to 0.9% did not significantly affect the decay time constants of isolated EPSCs.

Exogenously Applied Agonists

The effects of halothane described thus far were neither specific for a glutamate receptor subtype nor indicative of a specific postsynaptic depressant mechanism of action. To test more directly whether halothane may act postsynaptically at the glutamate receptor channel complex, we examined the effects of halothane on currents evoked by bath-applied glutamatergic agonists. Representative results from a series of six experiments are illustrated in figure 8. The salines contained 1 μM tetrodotoxin to block neurally evoked transmitter release from nerve terminals. At −60 mV holding potential, application of 5 μM NMDA induced a large inward current (fig. 8A). Halothane in concentrations to 5.1% (shown above to depress the EPSCs almost completely) did not depress the NMDA-induced currents (figs. 8B and 8C). These currents were entirely depressed by 400 μM APV (data not shown).

In two additional experiments we similarly tested the effects of halothane on currents evoked by bath-applied glutamate (10 and 20 μM). Halothane (5.1%) had no effects on these currents, which could be entirely abolished by the concomitant administration of APV and CNQX (400 and 10 μM, respectively; data not shown).

Discussion

The main finding of this study is that halothane suppresses glutamate receptor–mediated EPSCs in adult hippocampal pyramidal cells in a dose-dependent manner. Both non-NMDA and NMDA EPSCs are revers-

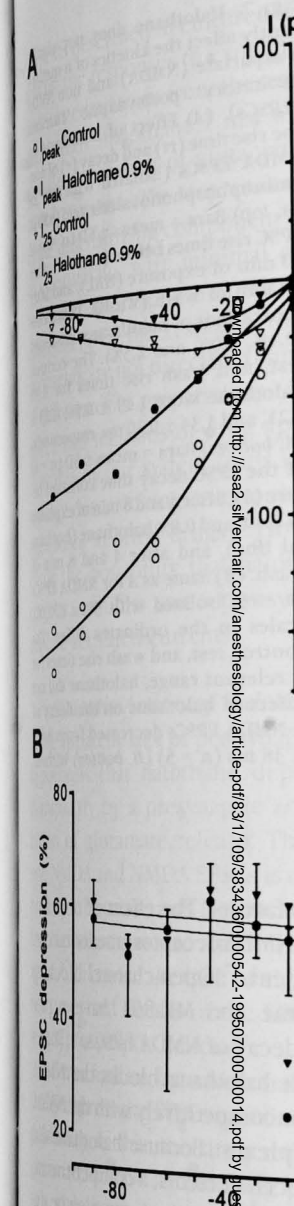

Fig. 5. Halothane-induced depressi aptic potentials (EPSCs) was indepe age. (*A*) Relations of peak EPSC amp plitude recorded 25 ms after the p versus holding potential (V_H) in 1 m after equilibration with 0.9% halo ponents were depressed across the e +30 mV). Voltage dependence of th (*A, Inset*) Exemplary EPSCs record control and in 0.9% halothane salin I_{peak} (circles; mostly non-n-methy component) and I_{25} (triangles; NM halothane at various V_Hs. Data poi results from four experiments (me the reversal potential were not analy amplitudes. The slopes of the line −0.03 for non-NMDA and NMDA con halothane blocks both EPSC comp pendent manner.

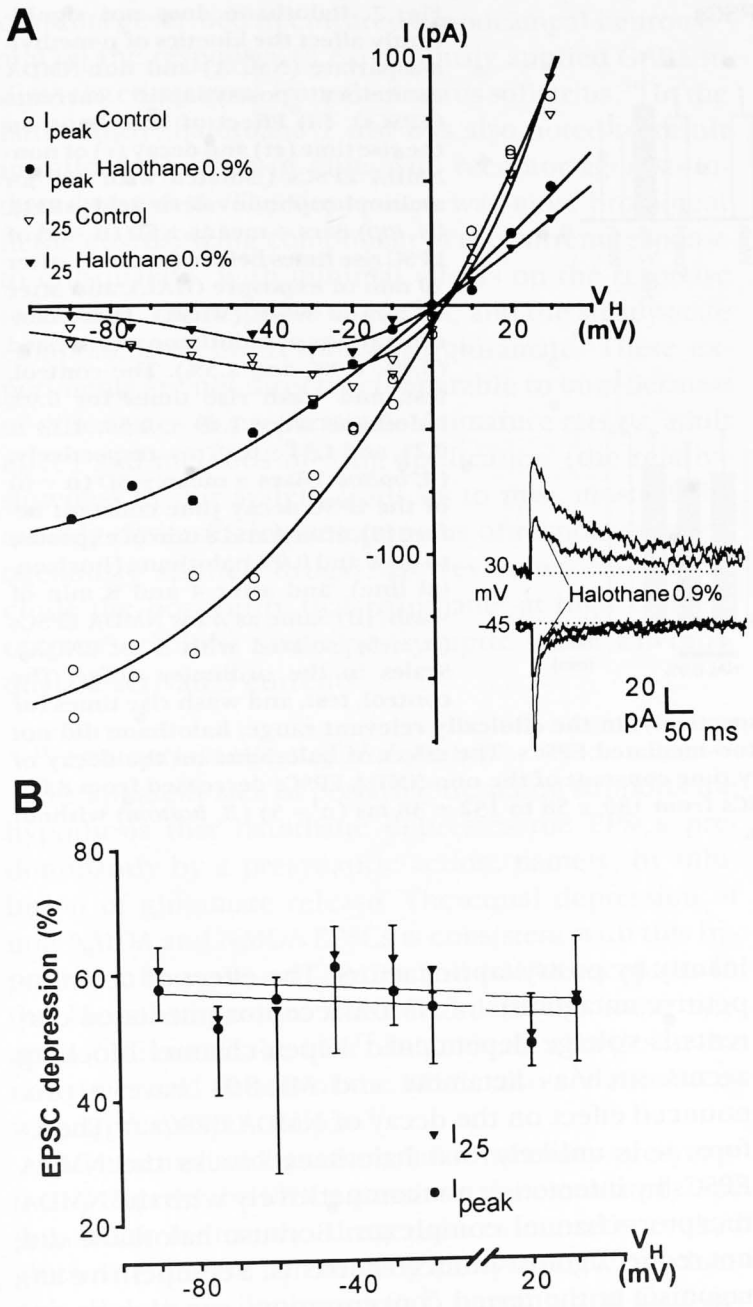

Fig. 5. Halothane-induced depression of excitatory postsynaptic potentials (EPSCs) was independent of membrane voltage. (*A*) Relations of peak EPSC amplitude (I_{peak}) and EPSC amplitude recorded 25 ms after the peak (I_{25}) of native EPSCs *versus* holding potential (V_H) in 1 mM Mg^{2+} saline before and after equilibration with 0.9% halothane. Both current components were depressed across the entire voltage range (−90–+30 mV). Voltage dependence of the block was not evident. (*A, Inset*) Exemplary EPSCs recorded at the indicated V_H in control and in 0.9% halothane saline. (*B*) Percentage block of I_{peak} (circles; mostly non-n-methyl-D-aspartate [non-NMDA] component) and I_{25} (triangles; NMDA component) by 0.9% halothane at various V_Hs. Data points represent the pooled results from four experiments (mean ± SD). EPSCs close to the reversal potential were not analyzed because of their small amplitudes. The slopes of the linear regression lines were −0.03 for non-NMDA and NMDA components, suggesting that halothane blocks both EPSC components in a voltage-independent manner.

ibly depressed by halothane in a voltage-independent manner, whereas agonist-induced currents are not affected.

Depression of Glutamatergic Excitatory Postsynaptic Currents

Several previous studies using extra- and intracellular recording techniques have demonstrated that anesthetic doses of halothane depress fast synaptic transmission in mammalian CNS preparations *in vitro*.[9–11,14] Our data suggest that this effect of halothane can be accounted for by a direct depression of the non-NMDA EPSC, which constitutes the predominant EPSC component during fast synaptic transmission in most CNS excitatory synapses.[15]

Halothane depressed the amplitudes of non-NMDA and NMDA EPSCs equally well. In this respect halothane

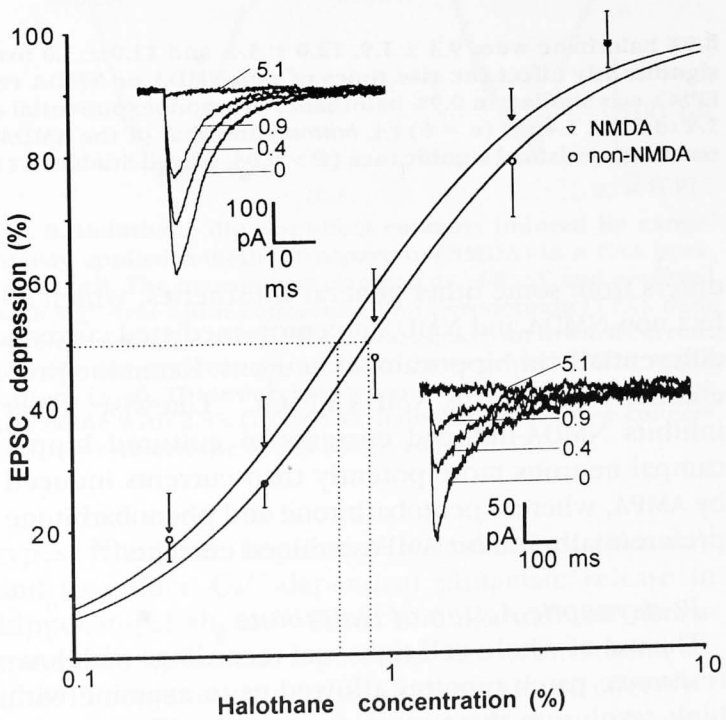

Fig. 6. Dose–response relation of halothane-induced depression of excitatory postsynaptic currents (EPSCs). The dose–response curves summarize the results from 15 pyramidal cells clamped at −60 mV in Mg^{2+}-free saline. Each cell was exposed to several concentrations of halothane. Non-n-methyl-D-aspartate (non-NMDA) and NMDA EPSCs were pharmacologically isolated with aminophosphonovaleric acid (APV) (100 μM) and 6-nitro-7-cyano-quinoxaline-2,3-dion (CNQX) (5 μM), respectively. Solid lines = lines fitted through the data points; dotted lines = concentrations producing 50% inhibition (IC_{50}): 0.87% (0.66 mM) for non-NMDA EPSCs and 0.69% (0.57 mM) for NMDA EPSCs. (*Insets*) Exemplary non-NMDA (*inset, top left*) and NMDA (*inset, bottom right*) EPSCs exposed to the indicated halothane concentrations. Sample records are averages of five consecutive traces.

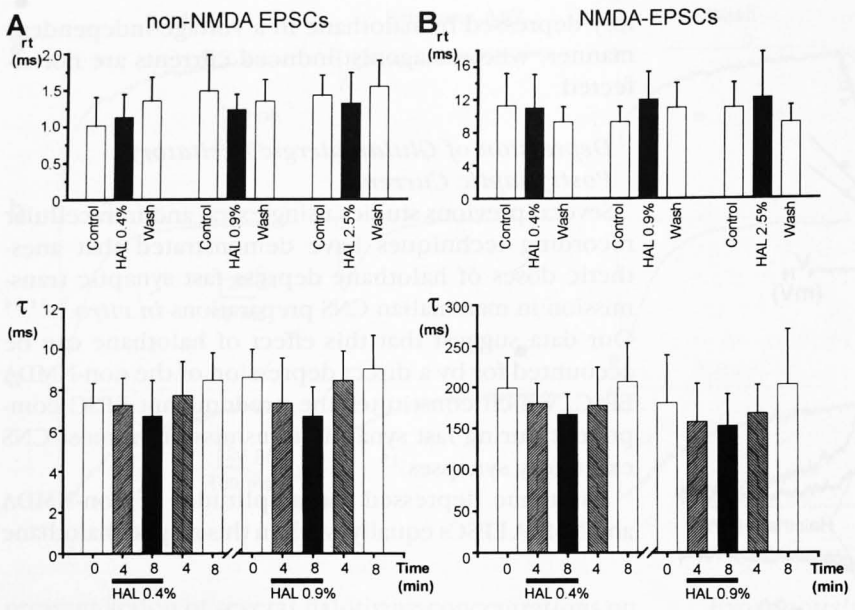

Fig. 7. Halothane does not significantly affect the kinetics of n-methyl-D-aspartate (NMDA) and non-NMDA excitatory postsynaptic currents (EPSCs). (*A*) Effect of halothane on the rise time (rt) and decay (τ) of non-NMDA EPSCs (isolated with 100 μM aminophosphonovaleric acid [APV]). (*A, top*) Bars = means ± SD (n = 6) of EPSC rise times before (control), after 10 min of exposure (HAL), and after 10 min of wash (Wash). Three concentrations of halothane were tested (0.4%, 0.9%, and 2.5%). The control, test, and wash rise times for 0.9% halothane were 1.49 ± 0.50, 1.23 ± 0.21, and 1.34 ± 0.30 ms, respectively. (*A, bottom*) Bars = mean ± SD (n = 6) of the EPSC decay time constant before (0), after 4 and 8 min of exposure to 0.4% and 0.9% halothane (horizontal line), and after 4 and 8 min of wash. (B) Same as *A* for NMDA EPSCs (n = 5; isolated with 5 μM CNQX). Scales in the ordinates differ. The control, test, and wash rise times for 0.9% halothane were 9.3 ± 1.9, 12.0 ± 3.4, and 11.0 ± 3.0 ms, respectively. In the clinically relevant range, halothane did not significantly affect the rise times of non-NMDA or NMDA receptor–mediated EPSCs. The effect of halothane on the decay of EPSCs was similar: in 0.9% halothane, the monoexponential decay time constant of the non-NMDA EPSCs decreased from 8.6 ± 1.4 to 6.7 ± 1.4 ms (n = 6) (*A, bottom*) and that of the NMDA EPSCs from 180 ± 58 to 152 ± 38 ms (n = 5) (*B, bottom*) without reaching statistical significance ($P > 0.05$, paired Student's *t* test).

differs from some other general anesthetics, which affect non-NMDA and NMDA receptor–mediated currents differentially in hippocampal neurons. Ketamine preferentially suppresses NMDA EPSCs.[27] Likewise, ether inhibits NMDA-induced currents in cultured hippocampal neurons more potently than currents induced by AMPA, whereas pentobarbitone and phenobarbitone preferentially inhibit AMPA-induced currents.[28]

Postsynaptic Action of Halothane

The use of whole-cell tight-seal recordings with low-resistance patch pipettes allowed us to examine with high resolution the time course of the EPSCs under reasonably good space-clamp conditions.[24] The rise times of non-NMDA and NMDA EPSCs were not affected by halothane in concentrations up to 2.5%. Similarly, the effect of halothane on the decays of both EPSCs did not reach statistical significance even at the highest concentration tested (0.9%). These results are in agreement with findings in spinal motoneurons of decerebrate cats, in which halothane depressed excitatory postsynaptic potentials without changing their decay.[7]

Several studies have shown that the decays of non-NMDA[29] and NMDA[30,31] EPSCs are determined predominantly by postsynaptic factors. The effect of noncompetitive antagonists of NMDA receptor–mediated currents is voltage dependent.[27] Open-channel blocking agents such as ketamine and MK-801 have a pronounced effect on the decay of NMDA EPSCs.[31] Therefore, it is unlikely that halothane blocks the NMDA EPSCs by interacting noncompetitively with the NMDA receptor–channel complexes. Because halothane did not reduce agonist-induced currents, a competitive antagonism at the tested concentrations can also be excluded. In a previous study in olfactory cortical slices, halothane in concentrations to 1% also did not affect the depolarizing response to exogenous glutamate in concentrations that suppressed excitatory synaptic transmission.[32] In contrast, pentobarbitone, ether, methoxyflurane, alphaxalone, and isoflurane were shown to reduce glutamate-induced currents at concentrations blocking excitatory synaptic transmission, thus indicating a postsynaptic site of action.[32,33] Isoflurane also was shown to decrease the frequency of opening and the mean open time of single NMDA receptor channels in cultured hippocampal neurons.[34]

These data stand in contrast to the marked effects of halothane on the kinetics of inhibitory postsynaptic

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Presynaptic Action of Halothe

The apparent lack of postsynap
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The mechanism by which hal
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currents described in cultured hippocampal neurons[35] and on the responses to exogenously applied GABA in dissociated neurons from the tractus solitarius.[36] In the latter study, halothane 1 mM was also noted to result in mild depression of glutamate receptor agonist–induced currents. The depression was most prominent in the desensitizing component of the current response to quisqualate, with minimal effects on the response to kainate, the response to NMDA, and the steady-state component of the response to glutamate. These experiments are not directly comparable to ours because of differences in preparations (immature rats *vs.* adult mice) and methods of drug application (the relative slowness of our system leads us to miss most of the quickly desensitizing components of responses to exogenously applied drugs). However, we cannot exclude the possibility that halothane, at high concentrations, exhibits some postsynaptic effect on quisqualate-activated currents.

Presynaptic Action of Halothane

The apparent lack of postsynaptic action supports the hypothesis that halothane depresses the EPSCs predominantly by a presynaptic action, namely, by inhibition of glutamate release. The equal depression of non-NMDA and NMDA EPSCs is consistent with this hypothesis, because non-NMDA and NMDA receptors in the hippocampus are colocalized to the same synapses.[37] Likewise the lack of significant changes in the rise times and decays of the EPSCs also are consistent with a presynaptic action.[31]

The mechanism by which halothane may presynaptically depress the EPSCs remains to be elucidated. One possibility is that the drug reduces neurally evoked glutamate release by blocking presynaptic Ca^{2+} channels. Halothane depressed Ca^{2+} currents and Ca^{2+}-dependent prolactin release in pituitary cells with an IC_{50} of 0.8 mM[38] and 0.4 mM,[39] respectively. Catecholamine release in pheochromocytoma cells also was inhibited by halothane with an IC_{50} of 0.7 mM.[40] These IC_{50} values, determined in different preparations under widely varying experimental conditions, are similar to the IC_{50} values reported here (0.57–0.66 mM) and may indicate common mechanisms of action. In support of this notion, halothane has been shown to block Ca^{2+} currents in cortical neurons.[41] However, the relevance of these currents to transmitter release is unclear. Isoflurane, on the other hand, has been shown to block multiple subtypes of voltage-gated Ca^{2+} currents in hippocampal pyramidal neurons,[42] including the N and the P subtypes, which are implicated in transmitter release,[43] and to reduce Ca^{2+}-dependent glutamate release in hippocampal slices.[44] These findings indicate neurotransmitter release as a potential site of anesthetic action. In conjunction with its aforementioned effects on NMDA receptor–operated channels,[34] these observations indicate that isoflurane may act by both pre- and postsynaptic mechanisms.

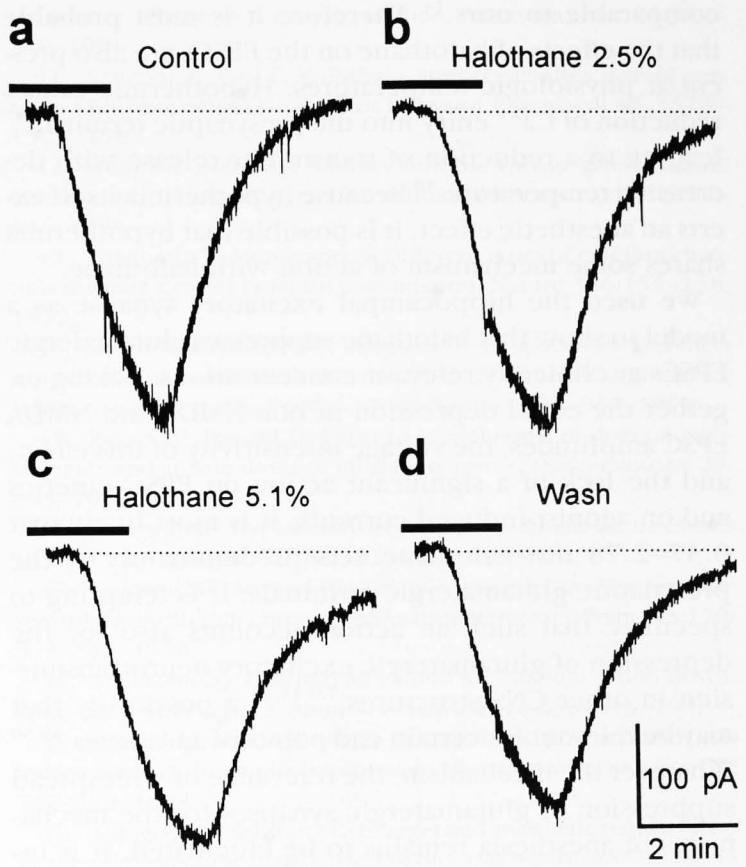

Fig. 8. Halothane did not affect currents induced by exogenously applied n-methyl-D-aspartate (NMDA) in a CA1 pyramidal cell. The neuron was clamped at −60 mV and perfused with Mg^{2+}-free saline containing 1 μM tetrodotoxin (TTX). Bath application of 5 μM NMDA for 2 min evoked an inward current (*a*). Similar currents were evoked by repeated NMDA applications (*b–d*). These currents were not reduced by saturating the saline with 2.5% (*b*) or 5.1% halothane (*c*). These concentrations reduced the EPSCs by 85–100%.

Relevance to Clinical Action of Halothane

We performed the experiments at hypothermic temperatures (21–23°C) only slightly greater than those that in higher mammals seem to reduce anesthetic requirements to zero (18–20°C).[45,46] However, other workers have shown that halothane inhibits excitatory neurotransmission in preparations kept close to normal body temperature[7,9,10,14] at free aqueous concentrations

comparable to ours.[14] Therefore it is most probable that the effects of halothane on the EPSCs are also present at physiologic temperatures. Hypothermia causes reduction of Ca^{2+} entry into the presynaptic terminal,[47] leading to a reduction of transmitter release with decreasing temperature.[48] Because hypothermia itself exerts an anesthetic effect, it is possible that hypothermia shares some mechanism of action with halothane.

We used the hippocampal excitatory synapse as a model to show that halothane suppresses glutamatergic EPSCs at clinically relevant concentrations. Taking together the equal depression of non-NMDA and NMDA EPSC amplitudes, the voltage insensitivity of this effect, and the lack of a significant action on EPSC kinetics and on agonist-induced currents, it is most likely that 0.37–2.78 mM halothane acts predominantly at the presynaptic glutamatergic terminals. It is tempting to speculate that such an action accounts also for the depression of glutamatergic excitatory neurotransmission in other CNS structures,[7,9,11,13] a possibility that may be relevant for certain end points of anesthesia.[49,50] Whatever the mechanism, the relevance of widespread suppression of glutamatergic synapses for the mechanisms of anesthesia remains to be elucidated. It is interesting, however, that the anesthetic effect of halothane *in vivo* is potentiated by drugs known to be antagonists of NMDA[51] and non-NMDA[52] receptors.

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