British Journal of Pharmacology (2002) 136, 673–684

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Pre- and postsynaptic volatile anaesthetic actions on glycinergic transmission to spinal cord motor neurons

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1 A common anaesthetic endpoint, prevention of withdrawal from a noxious stimulus, is determined primarily in spinal cord, where glycine is an important inhibitory transmitter. To define pre- and postsynaptic anaesthetic actions at glycinergic snyapses, the effects of volatile anaesthetic agents on spontaneous and evoked glycinergic currents in spinal cord motor neurons from 6–14-day old rats was investigated.

2 The volatile anaesthetic agents enflurane, isoflurane and halothane significantly increased the frequency of glycinergic mIPSCs, enflurane to 190.4% of control ± 22.0 (mean ± s.e.m., $n=7$, $P<0.01$), isoflurane to 199.0% ± 28.8 ($n=7$, $P<0.05$) and halothane to 198.2% ± 19.5 ($n=7$, $P<0.01$). However without TTX, isoflurane and halothane had no significant effect and enflurane decreased sIPSC frequency to 42.5% of control ± 12.4 ($n=6$, $P<0.01$). All the anaesthetics prolonged the decay time constant (τ) of both spontaneous and glycine-evoked currents without increasing amplitude. With TTX total charge transfer was increased; without TTX charge transfer was unchanged (isoflurane and halothane) or decreased (enflurane).

3 Enflurane-induced mIPSC frequency increases were not significantly affected by Cd^{2+} (50 μM), thapsigargin (1–5 μM), or KB-R7943 (5 μM). KB-R7943 and thapsigargin together abolished the enflurane-induced increase in mIPSC frequency.

4 There are opposing facilitatory and inhibitory actions of volatile anaesthetics on glycine release dependent on calcium homeostatic mechanisms and sodium channels respectively. Under normal conditions (no TTX) the absolute amount of glycinergic inhibition does not increase. The contribution of glycinergic inhibition to anaesthesia may depend on its duration rather than its absolute magnitude.

British Journal of Pharmacology (2002) **136,** 673–684

Keywords: Enflurane; isoflurane; halothane; miniature inhibitory postsynaptic currents; glycine; motor neurons; spinal cord; transmitter release

Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP-5, D,L-2-amino-5-phosphonopentanoic acid; CNQX, 6-Cyano-7-nitroquinoxaline-2,3-dione disodium; $GABA_A$, γ-aminobutyric acid A; KB-R7943, 2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiorea; mIPSCs, (miniature inhibitory synaptic currents); NMDA, N-methyl-D-aspartate; sIPSCs, spontaneous inhibitory synaptic currents; TTX, tetrodotoxin

Introduction

Prevention of movement in response to a noxious stimulus is a common anaesthetic endpoint for testing hypotheses concerning anaesthetic mechanisms. The minimum volatile anaesthetic partial pressure (MAC) which just prevents movement in response to a noxious stimulus (Eger *et al*., 1965) is a standard for comparing potencies among anaesthetic agents. Block of withdrawal responses in response to a noxious stimulus is determined by anaesthetic actions in the spinal cord (Antognini, 1997; Antognini & Schwartz, 1993; Borges & Antognini, 1994; King & Rampil, 1994; Rampil, 1994).

Enhancement of inhibitory transmission at γ-aminobutyric acid A ($GABA_A$) receptors is widely considered to be an important mechanism of anaesthesia (Franks & Lieb, 1993; 1994; Franks, 1998). In spinal cord, glycine as well as $GABA_A$ reeptors play important roles in inhibition. Miniature inhibitory postsynaptic currents (mIPSCs) are mediated by both glycine and $GABA_A$ receptors in spinal motor neurons of immature rats (Gao & Ziskind-Conhaim, 1995; 1998). Anaesthetic agents and ethanol enhance currents at glycine as well as $GABA_A$ receptors (Harrison *et al*., 1993; Mihic *et al*., 1997).

Both pre- and postsynaptic actions of volatile anaesthetics on $GABA_A$ receptors have been characterized in supraspinal brain regions. In hippocampal pyramidal cells halothane prolongs spontaneous inhibitory postsynaptic currents (Gage & Hamill, 1981; Mody *et al*., 1991); volatile anaesthetics exert dual blocking and prolonging actions on postsynaptic $GABA_A$ receptors in hippocampus (Banks & Pearce, 1999). Also in hippocampus volatile anaesthetics increase miniature IPSC frequencies but depress both spontaneous and evoked IPSC amplitudes (Nishikawa & MacIver, 2000; 2001).

Comparatively little is known about anaesthetic actions on glycinergic inhibition in spinal cord. In spinal motor neurons halothane has been reported to decrease both amplitude and frequency of spontaneous inhibitory postsynaptic potentials,

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and to decrease amplitudes of evoked inhibitory postsynaptic potentials (Takenoshita & Takahashi, 1987). The present study was designed to test whether volatile anaesthetics act presynaptically as well as postsynaptically to increase glycinergic transmission to motor neurons in rat spinal cord. Experiments were carried out on spontaneous glycinergic IPSCs in motor neurons in rat spinal cord slices under two conditions, with sodium channels blocked by tetrodotoxin (TTX) (miniature inhibitory postsynaptic currents, mIPSCs) and with sodium channels unblocked (spontaneous inhibitory postsynaptic currents, sIPSCs). Currents evoked by glycine application were also examined.

Methods

Spinal cord preparation

Spinal cord slices from 6–14 day old Sprague–Dawley rats were prepared as previously described (Cheng & Kendig, 2000). In a protocol approved by Stanford's panel on laboratory animal care and use, the animals were anaesthetized with halothane, decapitated, and spinal cords quickly removed and placed in cold oxygenated artificial cerebrospinal fluid (ACSF). The ACSF was composed as follows (mM): NaCl 123, KCl 4, NaH_2PO_4 1.2, $MgSO_4$ 1.3, $NaHCO_3$ 26, d-glucose 10, $CaCl_2$ 2. Slices 350 μm thick were sectioned from the lumbar region on a vibratome (Technical Products International, Inc.), and removed to oxygenated ACSF at room temperature for a 1 h recovery period. Individual slices were transferred to a recording chamber constantly superfused with oxygenated ACSF at a rate of 3 ml $min.^{-1}$ All experiments were carried out at room temperature.

Whole-cell recordings

Whole-cell patch clamp recordings were made from visually identified motor neurons in the spinal cord slice using infrared DIC videomicroscopy as previously described (Cheng & Kendig, 2000). The largest multipolar or round cells (15–25 μm in diameter) in the ventral horn, most commonly seen in the ventral lateral or ventral medial area, were identified as motor neurons in separate studies by fluorescent labelling (Wang *et al*., 1999; Wang & Kendig, 2000). Patch pipettes were pulled on a Flaming–Brown pipette puller (Sutter Instruments, Novato, CA, U.S.A.) and filled with a solution of the following composition (mM): CsCl 120, NaCl 10, HEPES 10, $MgCl_2$ 2, EGTA 10, $CaCl_2$ 1, MgATP 4, TEA-Cl 10, pH adjusted with CsOH to 7.3. Pipettes typically had a tip resistance of 2–8 MΩ. The patch pipette was directed toward a motor neuron cell body under visual control. Following establishment of a Gigohm seal the patch was ruptured by brief negative pressure and subsequent measurements made in the whole cell ruptured patch configuration in voltage clamp mode using an Axopatch 200B patch clamp amplifier (Axon Instruments, Foster City, CA, U.S.A.). Series resistance of 10–15 MΩ was compensated by 60%. All cells accepted for data analysis displayed stable resistance values throughout the experiment. Capacitance was also compensated. Holding potential was −60 mV. The membrane potential value was not corrected for junction potential, which was −11 mV. Miniature inhibitory postsynaptic currents (mIPSCs) were recorded in the presence of tetrodotoxin (TTX, 0.3 μM) in the bath solution to block Na^+-dependent action potentials. Spontaneous inhibitory synaptic currents (sIPSCs) were recorded without TTX. To ascertain postsynaptic effects on glycine receptors, responses were evoked by direct pressure application of glycine (Picospritzer, General Valve Division of Parker Hannefin Corporation, Fairfield, NJ, U.S.A.) from a pipette positioned near the cell body. Pressure pulse was 10 p.s.i., the duration of the pulse was 2.5 to 200 ms. Glycine concentration in the pipette was varied from 100 μM to 1 mM. Glycine applications at 1-min intervals produced stable inward currents over the course of each experiment.

Drugs

Glycinergic currents were pharmacologically isolated by blocking excitatory α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl D-aspartate (NMDA) and inhibitory $GABA_A$ receptors with 6-Cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX) (10 μM), D,L-2-amino-5-phosphonopentanoic acid (AP-5) (50 μM), and bicuculline (5 μM) respectively. Remaining miniature currents were identified as glycinergic by strychnine sensitivity (400 nM). In some experiments tetrodotoxin (TTX) (0.3 μM) was used to block sodium channels.

Pharmacologic agents were made up as stock solutions, dissolved in ACSF at the desired concentration, and applied in the superfusate. Anaesthetic actions were examined at the concentrations associated with abolition of movement (MAC). This was 0.6 mM for enflurane, 0.3 mM for isoflurane, and 0.3 mM for halothane. The concentrations of volatile anaesthetics in the bath chamber were measured by gas chromatography. Volatile anaesthetics were applied for 15 min. For analysis of anaesthetic effects on sIPSCs and mIPSCs, the controls were events in the 5 min immediately before the start of anaesthetic application. Volatile anaesthetic effects on mIPSC and sIPSC frequencies were measured over the entire period of application. Effects on kinetic properties and on total charge transfer were measured over the last 5 min of drug application. All anaesthetic actions were reversible on washing.

Data analysis

Experiments were carried out on a single cell in each slice. A software package (pClamp version 8, Axon Instruments, Foster City, CA, U.S.A.) was used to acquire data. The spontaneous synaptic currents were digitized at 5 kHz, stored in computer and analysed off-line using Mini Analysis Program 4.3 (Synaptosoft Inc.). The amplitude threshold for detection of spontaneous miniature synaptic currents was set above the noise level and events were subsequently verified visually. The analysis of mIPSC and sIPSC properties included: frequency, mean peak amplitude, 10–90% rise time, and decay time constant (τ_{decay}). Decay time constants were obtained by fitting from averaged events without overlapping. In cases in which τ_{decay} was best fit by more than a single exponential, overall decay time constant was quantified by computing weighed time constant $\tau_{WT} = (A_1 \cdot \tau_1 + A_2\tau_2)/(A_1 + A_2)$, where A$i$ is the amplitude of the ith component. The calculated τ_{WT} provides a good estimate of overall decay τ (Banks & Pearce, 1999).

Effects were expressed as mean ± standard error of the mean (s.e.mean). Statistical significance of anaesthetic effects was assessed by student's *t*-test for paired data. Differences between anaesthetics were tested with one-way ANOVA and Dunnet's multiple comparison test.

Results

The majority of spontaneous inhibitory postsynaptic currents in motor neurons are mediated by glycine receptors

Under the conditions of the present experiments, inhibitory currents are inward. When glutamatergic NMDA and non-NMDA excitatory receptors are blocked with 50 μM AP-5 and 10 μM CNQX respectively, all remaining events are abolished by a combination of the $GABA_A$ receptor antagonist bicuculline (5 μM) and the glycine receptor antagonist strychnine (400 nM) (Figure 1A3, B3). Application of bicuculline alone slightly reduced the frequency and amplitude of these inhibitory postsynaptic currents, but the majority of events remained (Figure 1A1, A2). Cumulative amplitude and inter-event interval histograms confirmed that the bicuculline-induced changes in frequency and amplitude of spontaneous IPSCs were small (Figure 1C). On the other hand strychnine (400 nM) markedly reduced the frequency and amplitude of inhibitory currents (Figure 1B1, B2). The remaining currents, which had a much smaller amplitude and slower decay, were completely blocked by 5 μM bicuculline. Strychnine produced a clear rightward shift in the cumulative inter-event interval and leftward shift in amplitude distributions (Figure 1C). The comparison between the pronounced actions of strychnine and the smaller actions of bicuculline suggests most of the spontaneous inhibitory input to motor neurons is glycinergic.

The glycinergic mIPSC vs *sIPSC in rat motor neurons*

In all subsequent experiments $GABA_A$ receptors were blocked by bicuculline. The frequency of glycinergic sIPSCs recorded in motor neurons varied among cells. The mean

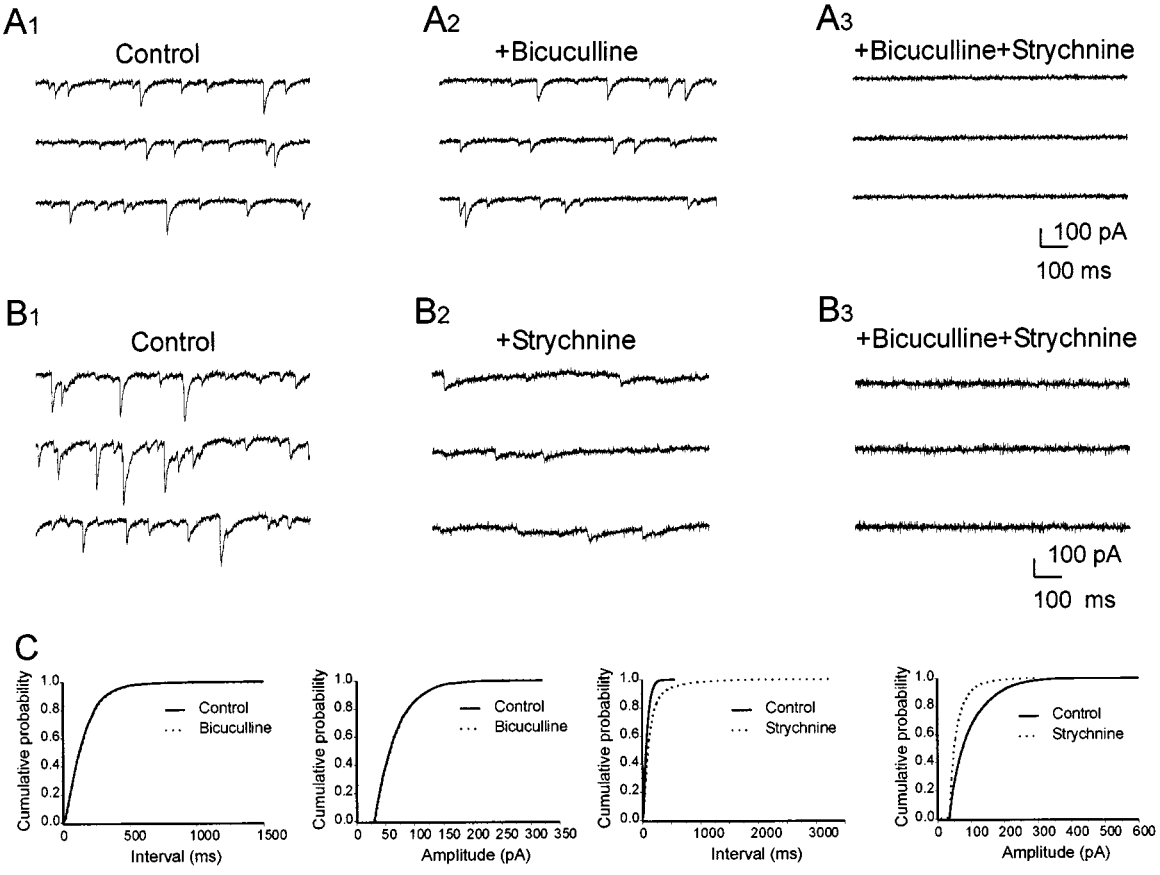

Figure 1 In spinal cord motor neurons sIPSCs are predominantly glycinergic. Excitatory glutamate receptors were blocked by AP-5 and CNQX. Holding potentials were −60 mV for this and all other experiments. The currents were inward (downward direction) due to the high Cl^- concentration in the patch pipette. (A) The $GABA_A$ receptor antagonist bicuculline (5 μM) had little effect on sIPSC frequency or amplitudes. The remaining currents were completely blocked by the glycine antagonist strychnine (400 nM). (B) In another cell, the sequence of application of inhibitory receptor antagonists was reversed. Application of strychnine 400 nM removed the majority of events, leaving a population of sIPSCs that were smaller and had slower kinetics. These events were blocked by bicuculline 5 μM. (C) Cumulative histograms of interval and amplitude in the presence of bicuculline (left two graphs in C) and in the presence of strychnine (right two graphs in C). Bicuculline was not associated with change in frequency or amplitude distribution, whereas strychnine produced a rightward shift in interevent interval (decrease in frequency) and a leftward shift in amplitude distribution.

frequency was 4.83±0.96 Hz ($n=18$) when sodium channels were not blocked by tetrodotoxin (TTX). The mean amplitude was 134.3±18.4 pA. TTX (0.3 μM) significantly decreased the mean frequency of mIPSCs to 0.41±0.08 Hz ($n=21$, $P<0.001$), about one-tenth of the frequency of sIPSCs without TTX (Figure 2). The mean amplitude of mIPSCs was also significantly lower than that of sIPSCs, a mean of 58.9±11.9 pA ($n=21$, $P<0.05$) (Figure 2). TTX thus selectively blocked larger amplitude events.

Volatile anaesthetics increase mIPSC frequency

In 21 cells we tested volatile anaesthetic effects on the frequency of mIPSCs in the presence of TTX (0.3 μM). Under these conditions the volatile anaesthetic enflurane (0.6 mM), isoflurane (0.3 mM) and halothane (0.3 mM) all significantly increased the frequency of mIPSCs by about 2 fold, and the effects were reversible. These concentrations are equivalent to those which block movement in response to a noxious stimulus (MAC). Although all agents increased baseline noise level in some cells, the mean increase in noise measured as standard deviation of the baseline was not significant for any agent. Figure 3 shows sample traces of glycinergic mIPSCs before and during exposure to each of the volatile anaesthetic agents, and the time course of anaesthetic actions on frequency in individual cells. The persistence of frequency enhancement varied among the cells. Some showed a continuous enhancement in frequency during anaesthetic exposure, as shown in Figure 3A. However, the majority of the cells responded with initial maximal enhancement for a period of 5–10 min, followed by a decline to a lower level but still well above the baseline level, as shown in Figure 3B,C.

Anaesthetic actions on mIPSC frequency are shown graphically in Figure 4A and summarized as per cent of control in Table 1. Frequency increases were significant for all agents and the extent of frequency enhancement did not differ among agents.

Anaesthetic actions on mIPSC amplitude, kinetics and total charge transfer

None of the anaesthetics significantly changed mean mIPSC amplitude (Figure 4A, Table 1) or rise time (Table 1). There were no significant shifts in mean cumulative amplitude distribution, although some individual cells showed a small leftward shift (data not shown). All significantly increased the decay time constant (Figure 4, Table 1). Effects on decay time constant were reversible. There were no differences among the anaesthetics in actions on decay time constants.

The decay times of naïve glycinergic mIPSCs were best fitted by a single exponential function in all cells tested. After exposure to the volatile anaesthetic enflurane, isoflurane and halothane for 15 min the decay times were best fitted by a single exponential function in the majority of cells tested, and in 6 out of 39 cells by two exponential functions. Sample traces of averaged mIPSCs from a single cell for each anaesthetic in control and anaesthetic periods illustrate the increase in decay time (Figure 4B).

Increased frequency combined with prolonged duration led to a significant increase in total charge transfer during the last 5 min of anaesthetic exposure compared to the 5 min control period (Table 1) for all agents. The anaesthetic agents did not significantly differ from each other. Therefore in the presence of TTX, all the volatile agents increased inhibition in motor neurons by both presynaptically mediated increases in frequency and postsynaptic kinetic changes which prolonged the duration of each event.

Mechanism of enflurane-induced mIPSC frequency increase

Studies were carried out to test the hypothesis that enflurane increases mIPSC frequency by increasing the concentration of calcium in the nerve terminal. Three possibilities were examined: increase in calcium influx *via* voltage-dependent calcium channels, increase in release from endoplasmic reticulum, and alteration in the Na-Ca exchanger. Cadmium (50 μM), which blocks both calcium channels and the operation of the Na-Ca exchanger (Iwamoto & Shigekawa, 1998; Shigekawa & Iwamoto, 2001) had no significant effect on the frequency of mIPSCs by itself; frequency in a group of cells treated with cadmium was 0.3565±0.23 s^{-1}, mean± s.e.mean, $n=5$, *versus* 0.3685±0.12 s^{-1} in untreated cells ($n=7$). These were unpaired data, and it is possible paired data from individual cells before and during Cd treatment might have shown an effect. Cadmium also did not block the enflurane-induced mIPSC frequency increase (Figures 5A, 6). Thapsigargin (1–5 μM), which depletes calcium from intracellular stores by blocking its uptake (Treiman *et al*., 1998), increased the variability in the response to enflurane so that the increase was no longer significant (Figures 5B, 6). The compound KB-R7943 (2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiorea), which selectively blocks the Na-Ca exchanger operating in the reverse direction (Iwamoto & Shigekawa, 1998; Shigekawa & Iwamoto, 2001) did not block enflurane-induced increases in mIPSC frequency (Figures 5C, 6). The combination of thapsigargin 1 μM and KB-R7943 5 μM completely blocked enflurane-induced increases in mIPSC frequency (Figures 5D, 6).

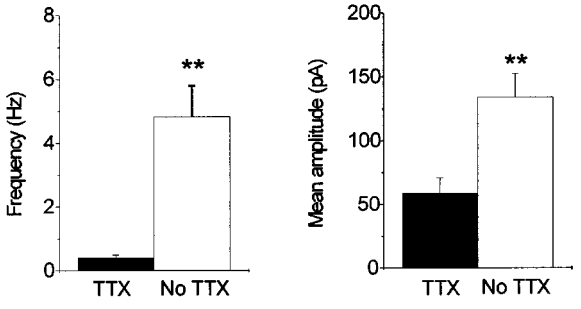

Figure 2 Histograms comparing glycinergic mIPSC and sIPSC frequency and amplitude. TTX reduced both frequency and amplitude of mIPSCs compared to sIPSCs. The results suggest that the majority of sIPSCs are TTX-sensitive Na^+ channel-dependent events, and that their amplitudes are larger than those of sodium channel-independent events. $n=18-21$, **, significantly different from mIPSCs, $P<0.01$.

Sodium channels modulate the effects of anaesthetics on glycinergic transmission

In 18 cells we tested volatile anaesthetic effects on the frequency of glycinergic sIPSCs in the absence of TTX.

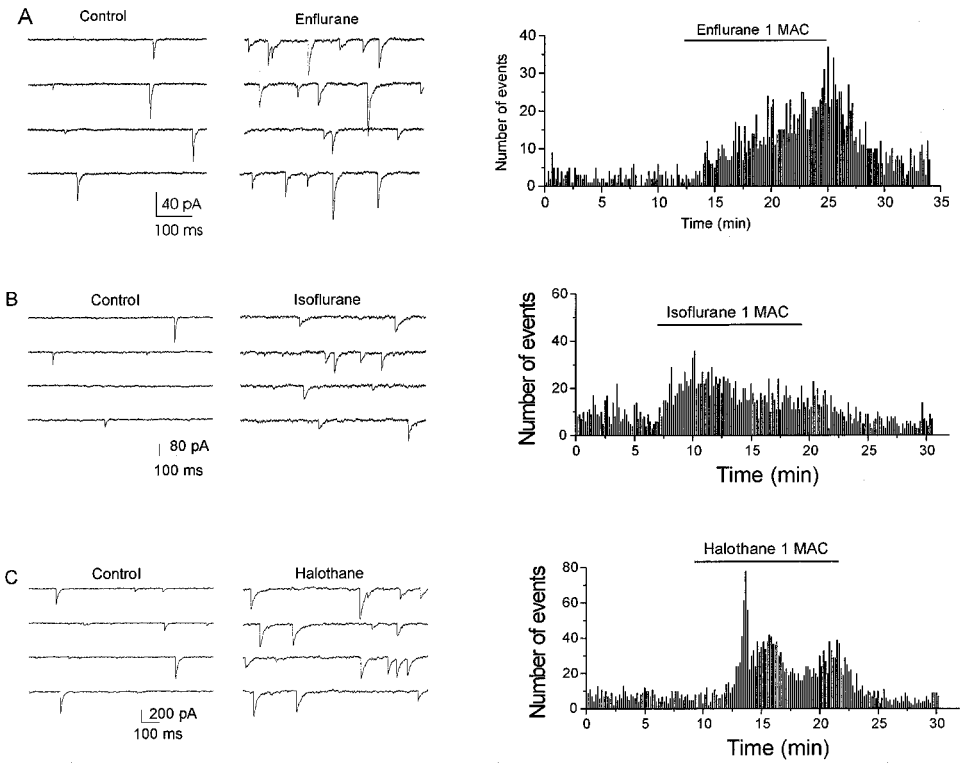

Figure 3 Volatile anaesthetics increased the frequency of miniature glycinergic IPSCs in the presence of TTX. A–C, mIPSCs in control conditions and during exposure to each of the three anaesthetics. Graphs to the right of each set of traces show the time course of the frequency changes during and after anaesthetic exposure in the same neurons. Bin width = 10 s.

Figure 7 shows sample traces of glycinergic sIPSCs before and during exposure to each of the agents, and a record of the time course of anaesthetic actions on sIPSC frequency. When sodium channels were not blocked, halothane and isoflurane decreased sIPSC frequency in some cells as shown in Figure 7 but the effect was not significant over the entire sample. Enflurane, however, significantly decreased sIPSC frequency over the entire sample (Table 1). Some cells displayed increases in baseline noise but this was not statistically significant for any agent.

Effects of volatile anaesthetic on sIPSC amplitude and kinetics

When sodium channels were not blocked enflurane significantly reduced sIPSC mean amplitude; the other agents had no effect on amplitude. The results are tabulated as per cent of control in Table 1. Differences among the agents were not statistically significant.

Effects on sIPSC kinetics were similar to those on mIPSC kinetics. None of the agents caused significant changes in rise time (Table 1). There was an obvious and consistent prolongation of decay time course as measured by sIPSC decay time constant for all volatile anaesthetics (Table 1).

Loss of presynaptically mediated frequency increases abolished the anaesthetic-mediated increases in total charge transfer, in the case of enflurane converting the increase to a significant decrease (Table 1).

Enflurane effects on glycine-evoked currents

To isolate postsynaptic enflurane actions we investigated currents evoked by pressure ejection of glycine. Pulses (200 ms) of pressure applied to the glycine-containing pipette produced inward currents in the motor neurons; the shape of currents varied among neurons (Figure 8A,B). The variation is probably due to differences in spread of glycine caused by variations in the physical relationships among pipette tip, cell body and any intervening cellular processes. In the presence of 0.3 μM TTX to block sodium channels, 50 μM AP-5 and 10 μM CNQX to block glutamate receptors, and bicuculline 10 μM to block $GABA_A$ receptors, bath application of enflurane at 0.6 mM (1 MAC) for 10–15 min produced variable effects on the amplitude of evoked currents but consistent effects on decay time. Enflurane reduced the amplitude of glycine-induced inward currents (Figure 8B) in most but not all cells (Figure 8A); mean amplitudes were not significantly changed. The mean peak amplitude of glycine-evoked currents in the presence of enflurane was $81.3.0 \pm 7.6\%$ of control value (averages 'f control and wash; $n = 15$, $P > 0.05$). The most consistent effect of enflurane was a reversible prolongation of decay time of the glycine-evoked currents. The decay time was significantly increased to $139.4 \pm 8.8\%$ of control ($n = 15$, $P < 0.01$) (Figure 8C). Prolongation of decay time was observed regardless of whether or not there was a change in amplitude (Figure 8A,B). The combination of variable decrease in amplitude with prolongation led to variable effects on total currents. The average area during enflurane exposure did not

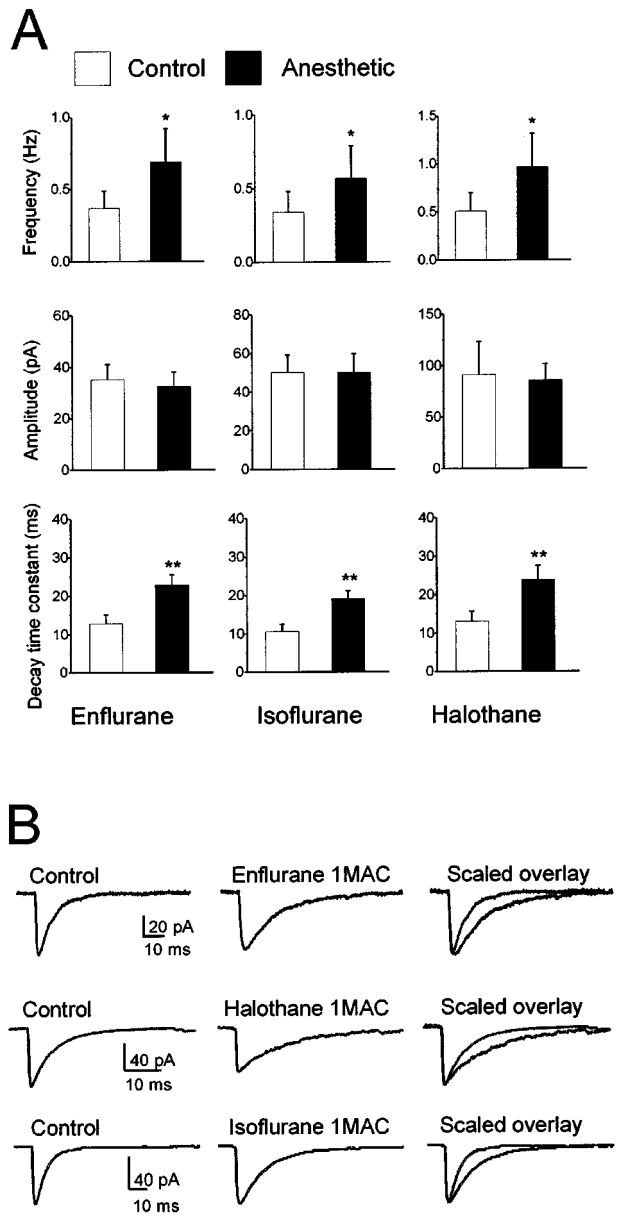

Figure 4 (A) Histograms showing actions of each anaesthetic on mIPSC frequency, amplitude and decay time constant. $n=7$ for each anaesthetic, error bars are s.e.m. *, ** significantly different from control at $P\times0.05$ and $P<0.01$, respectively. (B) Illustrations of the prolongation induced by anaesthetic on mIPSCs. Each trace is the mean IPSC in control and anaesthetic periods from a single cell. Scaled overlay shows anaesthetic current amplitude scaled to match control; the slower-decaying current is in the presence of the anaesthetic. The number of mIPSCs in each mean for enflurane is 166, halothane 133, and isoflurane 299.

significantly change (114.4%±21.5% of control) ($n=15$, $P>0.05$) (Figure 8C).

Since many reports describe an anaesthetic-induced increase in $GABA_A$ and glycine current amplitude as well as prolongation of currents, we were concerned that the amount of glycine ejected saturated and/or desensitized the glycine receptors and thus did not permit an amplitude increase. Therefore we reduced the amount of glycine ejected by decreasing the duration of the pressure pulse or by decreasing the concentration of glycine in the pipette. When ejection duration was varied from 2.5 to 200 ms, ejection of glycine evoked graded duration-dependent current responses. An example of a series of graded glycine-evoked currents with increasing ejection duration is shown in Figure 8D. Enflurane did not increase the amplitude of currents evoked by any duration of glycine application in any of five cells tested. The smallest detectible current that could be evoked by glycine ejection at 2.5 ms duration did not show any increase in amplitude of currents during enflurane exposure (Figure 8D). There was a prominent increase in decay time over the range of ejection durations. In separate experiments the concentration of glycine in the ejection pipette was reduced from 1 mM to 100 μM. Under these conditions enflurane again prolonged the current but did not change amplitude (Figure 8E).

Discussion

Spontaneous inhibition

Glycinergic currents are dominant contributors to spontaneous inhibition in spinal cord motor neurons, that is ongoing inhibition occurring without the experimenter's intervention. Strychnine markedly reduced the frequency of larger amplitude fast decaying spontaneous IPSCs, leaving a small number of bicuculline-sensitive currents with much smaller amplitudes and slower decay times. Both antagonists at high enough concentrations will cross-react with the other receptor. However the concentrations used to isolate mIPSCs and sIPSCs in the present study are below those at which appreciable crossover block occurs (Jonas *et al*., 1998). In that study glycine was the dominant contributor to evoked IPSCs, with faster kinetics than GABAergic currents.

Anaesthetic actions: presynaptic

When sodium channels are blocked, volatile anaesthetics markedly increase glycine release by a presynaptic mechanism, in addition to prolonging decay time postsynaptically. The combination of these actions leads to a significant increase in total chloride charge transfer. An apparent increase in frequency could result from amplitude increases bringing more events above the detection threshold. However, volatile anaesthetics did not increase amplitudes of either spontaneous or glycine-evoked currents. Others have reported that ethanol and volatile anaesthetics increase spontaneous inhibitory transmitter release (Banks & Pearce, 1999; Cheng *et al*., 1999; Mody *et al*., 1991; Nishikawa & MacIver, 2000; 2001). However, other studies at supraspinal CNS sites report that volatile anaesthetics do not increase $GABA_A$ release (Antkowiak & Heck, 1997) but do inhibit glutamate release from excitatory synapses (Kirson *et al*., 1998). Ethanol decreases glutamatergic miniature current frequency while increasing inhibitory miniature current frequency in motor neurons (Cheng *et al*., 1999). Thus volatile anaesthetics may have different effects on excitatory and inhibitory terminals.

Mechanisms of anaesthetic increase in mIPSC frequency

Spontaneous transmitter release, like evoked release, is calcium dependent (Emptage *et al*., 2001). Anaesthetics may

Table 1 Effects of the volative anaesthetics enflurane (0.6 mM), isoflurane (0.3 mM) and halothane (0.3 mM) on normalized frequency, mean peak amplitude, rise time, T_{decay} and total negative charge transfer of glycinergic mIPSCs and sIPSCs

	n	*Frequency* (%)	*Amplitude* (%)	*Rise time* (ms) *Control*	*Rise time* (ms) *Anaesthetics*	T_{decay} (ms) *Control*	T_{decay} (ms) *Anaesthetics*	*Charge transfer* (%)
Enflurane								
mIPSC	7	190.4±22.0**	92.6±7.0	1.46±0.14	1.60±0.16	12.9±2.30	22.9±2.51**	246.5±42.2*
sIPSC	6	42.5±12.4**	69.2±6.4**	1.03±0.12	1.13±0.12	17.9±2.29	28.1±2.86**	52.6±18.3*
Isoflurane								
mIPSC	7	199.0±28.8*	101.6±7.5	1.06±0.09	1.29±0.19	10.7±1.76	19.2±1.99**	242.1±33.0**
sIPSC	6	78.6±15.9	76.6±14.2	1.10±0.15	1.37±0.25	9.1±0.61	16.9±1.79**	107.3±41.4
Halothane								
mIPSC	7	198.2±19.5**	131.5±25.3	1.49±0.37	1.37±0.24	13.1±2.57	24.0±3.57**	401.9±73.7*
sIPSC	6	74.8±21.9	102.5±13.8	1.23±0.22	1.37±0.22	9.6±0.94	18.7±2.03**	142.5±60.5

Values are means±s.e.m. *n*, number of cells; mIPSC, miniature inhibitory postsynaptic current in presence of TTX; sIPSC, spontaneous inhibitory postsynaptic current in absence of TTX. * and **, statistically significant difference between control and anaesthetic treatments, $P<0.05$ and $P<0.01$ respectively.

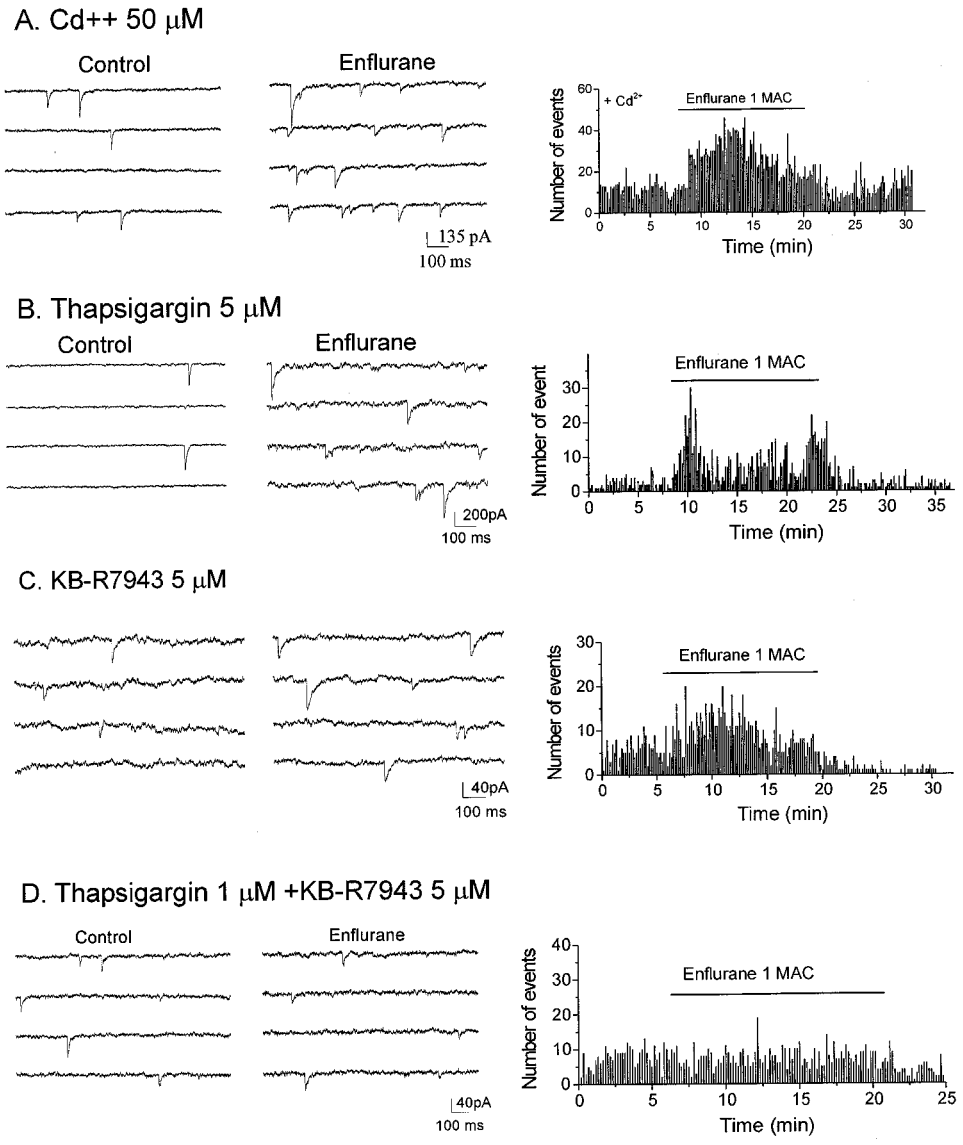

Figure 5 Examples of enflurane actions on mIPSC frequency in the presence of agents which block calcium influx and release from intraterminal stores. (A) Cadmium (50 μM), which blocks both entry through calcium channels and extrusion *via* the Na-Ca transporter; (B) Thapsigargin (5 μM), which blocks release from endoplasmic reticulum; (C) KB-R7943 (5 μM), which blocks influx *via* the Na-Ca transporter, and (D) the combination of Thapsigargin (1 μM) and KB-R7943 (5 μM). Only the combination prevented the enflurane-induced increase in mIPSC frequency.

act by increasing intracellular calcium (Kindler *et al*., 1999) or alternatively on steps in the release process itself. Enflurane-induced increases in mIPSC frequency were completely blocked by a combination of blocking calcium pumping into endoplasmic stores (thus depleting the stores) and inhibiting Ca influx *via* the Na-Ca exchanger operating in reverse (Pilitsis *et al*., 2001). This result suggests that enflurane increases in mIPSC frequency are due to increases in intraterminal calcium, and that both exchange and release are involved. Several studies suggest that volatile anaesthetics can increase intracellular calcium by increasing leakage from intracellular stores (Blanck *et al*., 1992; Hossain & Evers, 1994; Requena *et al*., 1985; Vassort *et al*., 1986).

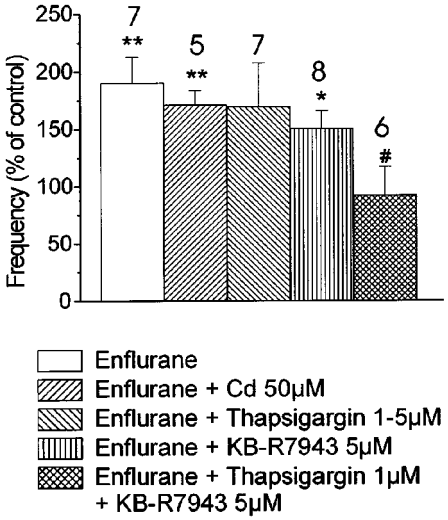

Figure 6 Quantitative effects of agents which block Ca influx and release on mIPSC frequency. Numbers over each bar are numbers of cells. **, *, significantly different from control, $P<0.01$ and $P<0.05$ respectively; #, significantly different from enflurane alone, $P<0.05$.

Role of Na channels in presynaptic anaesthetic actions

Anaesthetic-induced increase in spontaneous current frequency was only observed when sodium channels were blocked. When sodium channels were not blocked, anaesthetics either had no average effect or decreased frequency. Thus at glycinergic inhibitory synapses in spinal cord volatile anaesthetic agents exert two opposing actions on spontaneous transmitter release, one facilitatory and the other, dependent on active sodium channels, inhibitory. Presynaptic sodium channels are sensitive to volatile anaesthetic agents (Ratnakumari & Hemmings, 1998). Ethanol (Wu & Kendig, 1998) and halothane (Scholz *et al*., 1998) inhibit sodium currents in rat dorsal root ganglion cells. However direct action on sodium channels is not the only possible mechanism. Anaesthetics might decrease the spontaneous firing rate of inhibitory interneurons by increasing inhibition to them, or

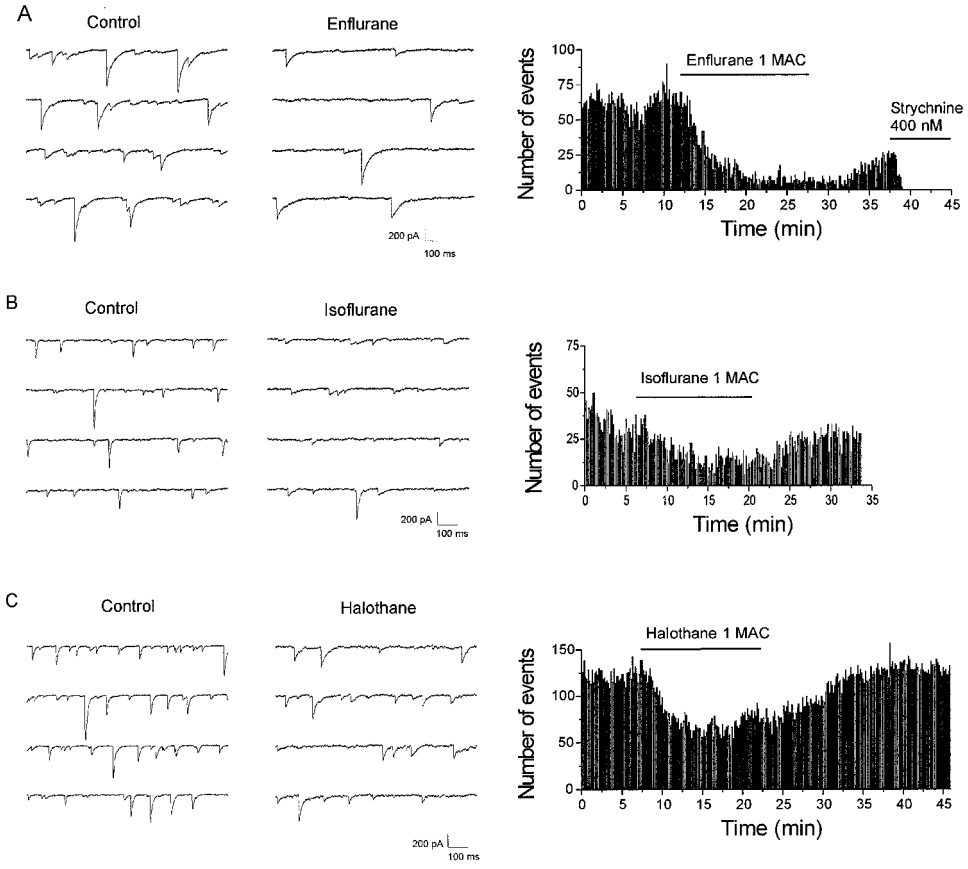

Figure 7 In some neurons volatile anaesthetic decreased the frequency of glycinergic sIPSCs in the absence of TTX; over the entire sample the decrease was significant only for enflurane. (A–C) Sample records of sIPSCs before and during exposure to each of the three anaesthetic agents. To the right of each set of traces are the time courses of sIPSC frequency from the same neuron during and after anaesthetic exposure.

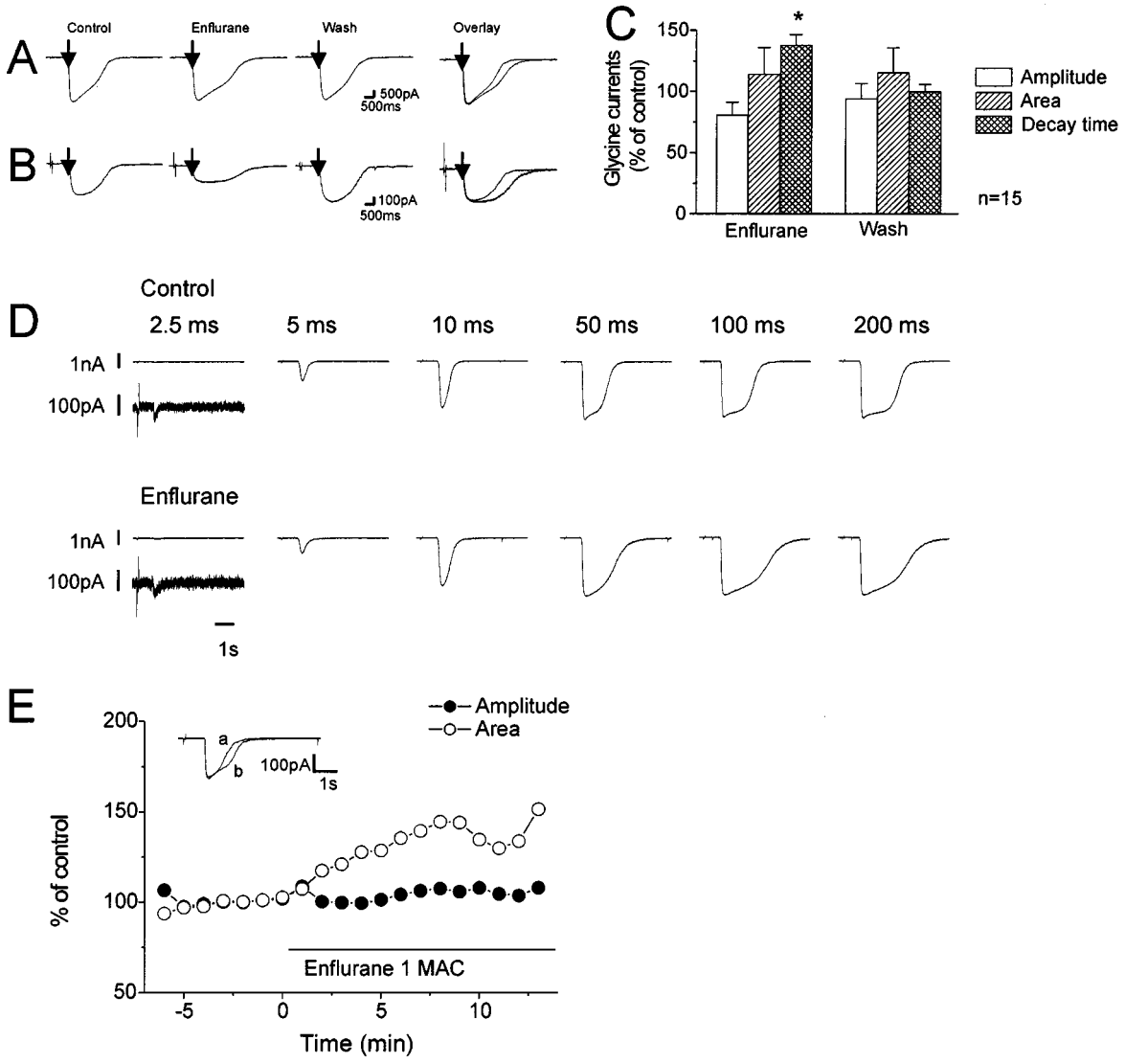

Figure 8 (A,B) Two examples of enflurane actions on glycine-evoked currents. Glycine pulses of 200 ms were given at the time indicated by the arrows. Sharp deflections are brief hyperpolarizing voltage steps of 10 mV to monitor input resistance. In the example shown in (A) enflurane did not appreciably affect amplitude but prolonged decay. Overlay shows control and enflurane-modified currents scaled to the same amplitude. In the cell shown in (B), enflurane depressed the amplitude of glycine-evoked currents but still prolonged the decay time constant. (C) Histogram summarizing enflurane effects on glycine-evoked currents in motor neurons ($n=15$). Error bars are s.e.m. Enfluorane significantly prolonged the decay time of the currents ($P<0.05$, compare to control), but did not significantly alter amplitude or area. (D) Ejection duration was reduced to test the possibility that prolonged glycine exposure altered enflurane actions by saturating receptors or promoting desensitization. Glycine currents were evoked by a graded series of increasing durations of 1 mM glycine application. As was the case with the long duration applications, enflurane (0.6 mM) prolonged the currents evoked by brief glycine applications but did not increase amplitudes in any of the five cells tested. Insets under the shortest duration (2.5 ms) show the small currents at enlarged amplification. (E) The concentration of glycine in the pipette was reduced to 100 μM. Inset shows the currents are prolonged but not increased in amplitude. Graph shows stable amplitude over the course of enflurane application and an increase in area.

might increase conductance across the presynaptic terminal membrane.

Anaesthetic actions: postsynaptic

The results of the current study show that all three volatile anaesthetics prolong the decay time of glycinergic mIPSCs and sIPSCs but do not increase amplitude. This result pertains only to the inhaled agents included in the present studies; other types of general anaesthetic agents may not modulate glycinergic function.

Other factors besides direct action on the receptors can produce a change in kinetics. Although motor neurons are electronically relatively compact, space clamp at synaptic sites distant from the cell body may not be complete. However anaesthetics would not be expected to make space clamp problems worse. That the apparent increase in decay time is not due to progressive loss of clamp control during the experiment is shown by the complete reversibility of kinetic effects. At some synapses variance in $GABA_A$ current decay time constant is a function of the spatiotemporal profile of $GABA_A$ concentrations in the synaptic cleft (Nusser *et al.*,

2001); however in the present study decay time constant increased whether or not transmitter release was increased. Increase in intracellular Ca^{2+} can induce prolongation of mIPSC decay (de Konninck & Mody, 1996). There are conflicting reports on whether volatile anaesthetics increase basal intracellular Ca^{2+} in hippocampal neurons (Bleakman *et al*., 1995; Kindler *et al*., 1999). Intracellular calcium was not found to be important in anaesthetic enhancement of $GABA_A$ currents in oocytes (Lin *et al*., 1992). Our results are consistent with the hypothesis that volatile anaesthetic prolong $GABA_A$ and glycine receptor-mediated currents by direct actions on the receptors (Banks & Pearce, 1999; Beckstead *et al*., 2000; Koltchine *et al*., 1999; Mihic *et al*., 1997). However since glycinergic currents are terminated by glycine uptake by glycine transporters (Lopez-Corcuera *et al*., 2001), anaesthetic action on transporters could also prolong currents.

Volatile anaesthetics did not increase peak amplitudes of either spontaneous or evoked glycinergic currents even when glycine was reduced to the minimum. In the glycine puff experiments, unlike the experiments on release, bicuculline concentrations were in the range in which some crossover block of glycine receptors is observed (Jonas *et al*., 1998); however this should not have affected anaesthetic actions on the remaining glycine currents. In some neurons amplitudes were decreased, although the decrease was significant only for sIPSCs in the presence of enflurane. This result is not in agreement with many studies in both oocytes and neurons showing that volatile agents increase amplitudes as well as prolonging $GABA_A$ and glycine currents (Banks & Pearce, 1999; Downie *et al*., 1996; Jones *et al*., 1992; Jones & Harrison, 1993; Lin *et al*., 1992; Mihic *et al*., 1994a, b; 1997; Wakamori *et al*., 1991). However in hippocampus enflurane and isoflurane decrease the amplitudes of GABAergic mIPSCs (Antkowiak & Heck, 1997; Banks & Pearce, 1999). Our results differ in not showing a decrease in mIPSC mean amplitude. Two possible explanations for inconsistent actions on amplitude are differences in subunit composition and in intracellular phosphorylation pathways. Alternatively, concentration-dependent dual actions of volatile anaesthetics have been reported on $GABA_A$ currents, potentiation at low concentrations being overridden by block at higher (Hapfelmeier *et al*., 2001). The anaesthetic concentrations used in the present studies may have been at the transition point between the two actions.

Intravenous anaesthetic agents increase a tonic GABAergic inhibition in hippocampal neurons pharmacologically distinct from mIPSCs (Bai *et al*., 2001). This inhibition is seen as baseline noise. However in the present study increases in baseline noise were not statistically significant.

The role of glycinergic inhibition in anaesthesia

In vivo, block of glycine receptors increases anaesthetic requirement to a maximum of approximately 30% (Zhang *et al*., 2001). *In vitro* in intact neonatal mouse spinal cord strychnine does not significantly attenuate enflurane actions (Wong *et al*., 2001). Both ethanol (Wang *et al*., 1999) and enflurane (Cheng & Kendig, 2000) can depress glutamate-evoked currents in spinal motor neurons independent of actions on glycine or $GABA_A$ receptors. In the present study all anaesthetics increased glycinergic inhibition by both pre- and postsynaptic actions, but only under the artificial condition of sodium channel block. When sodium channels were functioning, the presynaptically mediated increase in frequency was abolished, although the postsynaptic actions on kinetics remained. The result was to prevent any increase in total charge transfer and thus in the extent of spontaneous inhibition. Enflurane is of particular interest, since unlike the other agents it is associated with seizure-like EEG activity (Modica *et al*., 1990). Alone among the agents enflurane significantly depressed sIPSC frequency, amplitude and total charge transfer. It is possible that a more pronounced depressant action on inhibitory transmission contributes to enflurane's convulsant properties.

Quantification of inhibition as total charge transfer is a measure that ignores potentially important temporal factors. Withdrawal in response to a noxious stimulus, the behavioural measure of anaesthetic action, is a polysynaptic response requiring time to develop. Prolonging inhibition into the time period when the excitatory response is developing may have functional consequences beyond the total amount of inhibition.

Supported by NIH grants NS13108 and GM47818 to J.J. Kendig. We are indebted to the members of Program Project Group GM47818 for helpful discussion, and to Diane Gong and Michael Laster in E.I. Eger's laboratory at the University of California, San Francisco for measuring anaesthetic concentrations.

References

ANTKOWIAK, B. & HECK, D. (1997). Effects of the volatile anesthetic enflurane on spontaneous discharge rate and GABA(A)-mediated inhibition of Purkinje cells in rat cerebellar slices. *J. Neurophysiol.,* **77,** 2525 – 2538.

ANTOGNINI, J.F. (1997). The relationship among brain, spinal cord and anesthetic requirements. *Med. Hypotheses,* **48,** 83 – 87.

ANTOGNINI, J.F. & SCHWARTZ, K. (1993). Exaggerated anesthetic requirements in the preferentially anesthetized brain. *Anesthesiology,* **79,** 1244 – 1249.

BAI, D., ZHU, G., PENNEFATHER, P., JACKSON, M.F., MACDONALD, J.F. & ORSER, B.A. (2001). Distinct functional and pharmacological properties of tonic and quantal inhibitory postsynaptic currents mediated by gamma-aminobutyric acid(A) receptors in hippocampal neurons. *Mol. Pharmacol.,* **59,** 814 – 824.

BANKS, M.I. & PEARCE, R.A. (1999). Dual actions of volatile anesthetics on GABA(A) IPSCs: dissociation of blocking and prolonging effects. *Anesthesiology,* **90,** 124 – 134.

BECKSTEAD, M.J., WEINER, J.L., EGER, 2ND E.I., GONG, D.H. & MIHIC, S.J. (2000). Glycine and gamma-aminobutyric acid(A) receptor function is enhanced by inhaled drugs of abuse. *Mol. Pharmacol.,* **57,** 1199 – 1205.

BLANCK, T.J., PETERSON, C.V., BAROODY, B., TEGAZZIN, V. & LOU, J. (1992). Halothane, enflurane, and isoflurane stimulate calcium leakage from rabbit sarcoplasmic reticulum. *Anesthesiology,* **76,** 813 – 821.

BLEAKMAN, D., JONES, M.V. & HARRISON, N.L. (1995). The effects of four general anesthetics on intracellular [Ca2 +] in cultured rat hippocampal neurons. *Neuropharmacology,* **34,** 541 – 551.

BORGES, M. & ANTOGNINI, J.F. (1994). Does the brain influence somatic responses to noxious stimuli during isoflurane anesthesia? *Anesthesiology,* **81,** 1511 – 1515.

CHENG, G., GAO, B., VERBNY, Y. & ZISKIND-CONHAIM, L. (1999). Ethanol reduces neuronal excitability and excitatory synaptic transmission in the developing rat spinal cord. *Brain Res.,* **845,** 224 – 231.

CHENG, G. & KENDIG, J.J. (2000). Enflurane directly depresses glutamate AMPA and NMDA currents in mouse spinal cord motor neurons independent of actions on GABAA or glycine receptors. *Anesthesiology,* **93,** 1075 – 1084.

DE KONNINCK, Y. & MODY, I. (1996). The effects of raising intracellular calcium on synaptic GABAA receptor-channels. *Neuropharmacology,* **35,** 1365 – 1374.

DOWNIE, D.L., HALL, A.C., LIEB, W.R. & FRANKS, N.P. (1996). Effects of inhalational general anaesthetics on native glycine receptors in rat medullary neurones and recombinant glycine receptors in Xenopus oocytes. *Br. J. Pharmacol.,* **118,** 493 – 502.

EGER, E.I.D., SAIDMAN, L.J. & BRANDSTATER, B. (1965). Minimum alveolar anesthetic concentration: a standard of anesthetic potency. *Anesthesiology,* **26,** 756 – 763.

EMPTAGE, N.J., REID, C.A. & FINE, A. (2001). Calcium stores in hippocampal synaptic boutons mediate short-term plasticity, store-operated Ca2 + entry, and spontaneous transmitter release. *Neuron,* **29,** 197 – 208.

FRANKS, N.P. & LIEB, W.R. (1993). Selective actions of volatile general anaesthetics at molecular and cellular levels. *Br. J. Anaesth.,* **71,** 65 – 76.

FRANKS, N.P. & LIEB, W.R. (1994). Molecular and cellular mechanisms of general anaesthesia. *Nature,* **367,** 607 – 614.

FRANKS, N.P. & LIEB, W.R. (1998). Which molecular targets are most relevant to general anaesthesia? *Toxicol. Lett.,* **100 – 101,** 1 – 8.

GAGE, P.W. & HAMILL, O.P. (1981). Effects of anesthetics on ion channels in synapses. *Int. Rev. Physiol.,* **25,** 1 – 45.

GAO, B.X., CHENG, G. & ZISKIND-CONHAIM, L. (1998). Development of spontaneous synaptic transmission in the rat spinal cord. *J. Neurophysiol.,* **79,** 2277 – 2287.

GAO, B.X. & ZISKIND-CONHAIM, L. (1995). Development of glycine- and GABA-gated currents in rat spinal motoneurons. *J. Neurophysiol.,* **74,** 113 – 121.

HAPFELMEIER, G., SCHNECK, H. & KOCHS, E. (2001). Sevoflurane potentiates and blocks GABA-induced currents through recombinant alpha1beta2gamma2 GABAA receptors: implications for an enhanced GABAergic transmission. *Eur. J. Anaesthesiol.,* **18,** 377 – 383.

HARRISON, N.L., KUGLER, J.L., JONES, M.V., GREENBLATT, E.P. & PRITCHETT, D.B. (1993). Positive modulation of human gamma-aminobutyric acid type A and glycine receptors by the inhalation anesthetic isoflurane. *Mol. Pharmacol.,* **44,** 628 – 632.

HOSSAIN, M.D. & EVERS, A.S. (1994). Volatile anesthetic-induced efflux of calcium from IP3-gated stores in clonal (GH3) pituitary cells. *Anesthesiology,* **80,** 1379 – 1389; discussion 27A – 28A.

IWAMOTO, T. & SHIGEKAWA, M. (1998). Differential inhibition of Na + /Ca2 + exchanger isoforms by divalent cations and isothiourea derivative. *Am. J. Physiol.,* **275,** C423 – C430.

JONAS, P., BISCHOFBERGER, J. & SANDKUHLER, J. (1998). Corelease of two fast neurotransmitters at a central synapse. *Science,* **281,** 419 – 424.

JONES, M.V., BROOKS, P.A. & HARRISON, N.L. (1992). Enhancement of gamma-aminobutyric acid-activated Cl- currents in cultured rat hippocampal neurones by three volatile anaesthetics. *J. Physiol.,* **449,** 279 – 293.

JONES, M.V. & HARRISON, N.L. (1993). Effects of volatile anesthetics on the kinetics of inhibitory postsynaptic currents in cultured rat hippocampal neurons. *J. Neurophysiol.,* **70,** 1339 – 1349.

KINDLER, C.H., EILERS, H., DONOHOE, P., OZER, S. & BICKLER, P.E. (1999). Volatile anesthetics increase intracellular calcium in cerebrocortical and hipocampal neurons. *Anesthesiology,* **90,** 1137 – 1145.

KING, B.S. & RAMPIL, I.J. (1994). Anesthetic depression of spinal motor neurons may contribute to lack of movement in response to noxious stimuli. *Anesthesiology,* **81,** 1484 – 1492.

KIRSON, E.D., YAARI, Y. & PEROUANSKY, M. (1998). Presynaptic and postsynaptic actions of halothane at glutamatergic synapses in the mouse hippocampus. *Br. J. Pharmacol.,* **124,** 1607 – 1614.

KOLTCHINE, V.V., FINN, S.E., JENKINS, A., NIKOLAEVA, N., LIN, A. & HARRISON, N.L. (1999). Agonist gating and isoflurane potentiation in the human gamma-aminobutyric acid type A receptor determined by the volume of a second transmembrane domain residue. *Mol. Pharmacol.,* **56,** 1087 – 1093.

LIN, L.H., CHEN, L.L., ZIRROLLI, J.A. & HARRIS, R.A. (1992). General anesthetics potentiate gamma-aminobutyric acid actions on gamma-aminobutyric acidA receptors expressed by Xenopus oocytes: lack of involvement of intracellular calcium. *J. Pharmacol. Exp. Ther.,* **263,** 569 – 578.

LOPEZ-CORCUERA, B., GEERLINGS, A. & ARAGON, C. (2001). Glycine neurotransmitter transporters: an update. *Mol. Membr. Biol.,* **18,** 13 – 20.

MIHIC, S.J., MCQUILKIN, S.J., EGER, 2ND E.I., IONESCU, P. & HARRIS, R.A. (1994a). Potentiation of gamma-aminobutyric acid type A receptor-mediated chloride currents by novel halogenated compounds correlates with their abilities to induce general anesthesia. *Mol. Pharmacol.,* **46,** 851 – 857.

MIHIC, S.J., WHITING, P.J. & HARRIS, R.A. (1994b). Anaesthetic concentrations of alcohols potentiate GABAA receptor-mediated currents: lack of subunit specificity. *Eur. J. Pharmacol.,* **268,** 209 – 214.

MIHIC, S.J., YE, Q., WICK, M.J., KOLTCHINE, V.V., KRASOWSKI, M.D., FINN, S.E., MASCIA, M.P., VALENZUELA, C.F., HANSON, K.K., GREENBLATT, E.P., HARRIS, R.A. & HARRISON, N.L. (1997). Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. *Nature,* **389,** 385 – 389.

MODICA, P.A., TEMPELHOFF, R. & WHITE, P.F. (1990). Pro- and anticonvulsant effects of anesthetics (Part II). *Anesth. Analg.,* **70,** 433 – 444.

MODY, I., TANELIAN, D.L. & MACIVER, M.B. (1991). Halothane enhances tonic neuronal inhibition by elevating intracellular calcium. *Brain Res.,* **538,** 319 – 323.

NISHIKAWA, K. & MACIVER, M.B. (2000). Membrane and synaptic actions of halothane on rat hippocampal pyramidal neurons and inhibitory interneurons. *J. Neurosci.,* **20,** 5915 – 5923.

NISHIKAWA, K. & MACIVER, M.B. (2001). Agent-selective effects of volatile anesthetics on gabaa receptor-mediated synaptic inhibition in hippocampal interneurons. *Anesthesiology,* **94,** 340 – 347.

NUSSER, Z., NAYLOR, D. & MODY, I. (2001). Synapse-specific contribution of the variation of transmitter concentration to the decay of inhibitory postsynaptic currents. *Biophys. J.,* **80,** 1251 – 1261.

PILITSIS, J.G., DIAZ, F.G., O'REGAN, M.H. & PHILLIS, J.W. (2001). Inhibition of Na(+)/Ca(2 +) exchange by KB-R7943, a novel selective antagonist, attenuates phosphoethanolamine and free fatty acid efflux in rat cerebral cortex during ischemia-reperfusion injury. *Brain Res.,* **916,** 192 – 198.

RAMPIL, I.J. (1994). Anesthetic potency is not altered after hypothermic spinal cord transection in rats. *Anesthesiology,* **80,** 606 – 610.

RATNAKUMARI, L. & HEMMINGS, JR, H.C. (1998). Inhibition of presynaptic sodium channels by halothane. *Anesthesiology,* **88,** 1043 – 1054.

REQUENA, J., WHITTEMBURY, J., TIFFERT, T., EISNER, D.A. & MULLINS, L.J. (1985). The influence of chemical agents on the level of ionized [Ca2 +] in squid axons. *J. Gen. Physiol.,* **85,** 789 – 804.

SCHOLZ, A., APPEL, N. & VOGEL, W. (1998). Two types of TTX-resistant and one TTX-sensitive Na + channel in rat dorsal root ganglion neurons and their blockade by halothane. *Eur. J. Neurosci.,* **10,** 2547 – 2556.

SHIGEKAWA, M. & IWAMOTO, T. (2001). Cardiac Na(+)-Ca(2 +) exchange: molecular and pharmacological aspects. *Circ. Res.,* **88,** 864 – 876.

TAKENOSHITA, M. & TAKAHASHI, T. (1987). Mechanisms of halothane action on synaptic transmission in motoneurons of the newborn rat spinal cord in vitro. *Brain Res.,* **402,** 303 – 310.

TREIMAN, M., CASPERSEN, C. & CHRISTENSEN, S.B. (1998). A tool coming of age: thapsigargin as an inhibitor of sarco-endoplasmic reticulum Ca(2 +)-ATPases. *Trends Pharmacol. Sci.,* **19,** 131 – 135.

VASSORT, G., WHITTEMBURY, J. & MULLINS, L.J. (1986). Increases in internal Ca2+ and decreases in internal H+ are induced by general anesthetics in squid axons. *Biophys J.,* **50,** 11–19.

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.

WANG, M.Y. & KENDIG, J.J. (2000). Patch clamp studies of motor neurons in spinal cord slices: a tool for high-resolution analysis of drug actions. *Acta Pharmacologica Sinica,* **21,** 481–576.

WANG, M.Y., RAMPIL, I.J. & KENDIG, J.J. (1999). Ethanol directly depresses AMPA and NMDA glutamate currents in spinal cord motor neurons independent of actions on GABAA or glycine receptors. *J. Pharmacol. Exp. Ther.,* **290,** 362–367.

WONG, S.M.E., CHENG, G., HOMANICS, G. & KENDIG, J.J. (2001). Enflurane actions on spinal cords from mice that lack the beta3 subunit of the $GABA_A$ receptor. *Anesthesiology,* **195,** 154–164.

WU, J.V. & KENDIG, J.J. (1998). Differential sensitivities of TTX-resistant and TTX-sensitive sodium channels to anesthetic concentrations of ethanol in rat sensory neurons. *J. Neurosci. Res.,* **54,** 433–434.

ZHANG, Y., WU, S., EGER, E.I. & SONNER, J.M. (2001). Neither GABA(A) nor strychnine-sensitive glycine receptors are the sole mediators of MAC for isoflurane. *Anesth. Analg.,* **92,** 123–127.

(Received February 11, 2002
Revised April 4, 2002
Accepted April 15, 2002)