

Enflurane Directly Depresses Glutamate AMPA and NMDA Currents in Mouse Spinal Cord Motor Neurons Independent of Actions on GABA_A or Glycine Receptors

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Background: The spinal cord is an important anatomic site at which volatile agents act to prevent movement in response to a noxious stimulus. This study was designed to test the hypothesis that enflurane acts directly on motor neurons to inhibit excitatory synaptic transmission at glutamate receptors.

Methods: Whole-cell recordings were made in visually identified motor neurons in spinal cord slices from 1- to 4-day-old mice. Excitatory postsynaptic currents (EPSCs) or potentials (EPSPs) were evoked by electrical stimulation of the dorsal root entry area or dorsal horn. The EPSCs were isolated pharmacologically into glutamate N-methyl-D-aspartate (NMDA) receptor- and non-NMDA receptor-mediated components by using selective antagonists. Currents also were evoked by brief pulse pressure ejection of glutamate under various conditions of pharmacologic blockade. Enflurane was made up as a saturated stock solution and diluted in the superfusate; concentrations were measured using gas chromatography.

Results: Excitatory postsynaptic currents and EPSPs recorded from motor neurons by stimulation in the dorsal horn were mediated by glutamate receptors of both non-NMDA and NMDA subtypes. Enflurane at a general anesthetic concentration (one minimum alveolar anesthetic concentration) reversibly depressed EPSCs and EPSPs. Enflurane also depressed glutamate-evoked currents in the presence of tetrodotoxin (300 nM), showing that its actions are postsynaptic. Block of inhibitory γ -aminobutyric acid A and glycine receptors by bicuculline (20 μ M) or strychnine (2 μ M) or both did not significantly reduce the effects of enflurane on glutamate-evoked currents. Enflurane also depressed glutamate-evoked currents if the inhibitory receptors were blocked and if either D,L-2-amino-5-phosphonopentanoic acid (50 μ M) or 6-cyano-7-nitroquinoxaline-2,3-dione disodium (10 μ M) was applied to block NMDA or α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-kainate receptors respectively.

Conclusions: Enflurane exerts direct depressant effects on both α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and NMDA glutamate currents in motor neurons. Enhancement of γ -aminobutyric acid A and glycine inhibition is not needed for this effect. Direct depression of glutamatergic excitatory transmission by a postsynaptic action on motor neurons thus may contribute to general anesthesia as defined by immobility in response to a noxious stimulus. (Key words: AMPA receptor; anesthetic mechanisms; NMDA receptor; volatile anesthetic agent.)

GENERAL anesthetics and alcohol are known to act on multiple target sites. Enhancement of γ -aminobutyric

acid A (GABA_A) inhibition is considered to be an important common factor in general anesthesia produced by a variety of agents.^{1,2} Both volatile anesthetic agents and ethanol enhance currents at both glycine and GABA_A receptors.³⁻⁸ However, the effects of volatile anesthetics on glutamate excitatory transmission are less well understood.

We previously showed that isoflurane⁹ and ethanol¹¹ depress synaptic transmission to motor neurons in intact spinal cord *in vitro*. However, the previous studies could not discriminate between postsynaptic depression of responses to transmitter and presynaptic depression of transmitter release. Postsynaptic actions might be mediated *via* enhancement of inhibition rather than depression of response to excitatory transmitter. We have shown that ethanol directly depresses excitatory postsynaptic currents (EPSCs) in rat spinal cord.¹¹ In hippocampus, halothane did not appear to depress excitatory synaptic responses by a postsynaptic mechanism,¹² suggesting that its actions were entirely presynaptic. However, more recent studies by the same group showed that halothane can depress both α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- and N-methyl-D-aspartate (NMDA)-evoked currents and suggested that both pre- and postsynaptic actions contribute to halothane's depressant effects.^{13,14} It is unknown whether volatile anesthetic agents at clinically relevant concentrations directly depress glutamatergic synaptic excitation.

The current study explored the actions of enflurane at one minimum alveolar anesthetic concentration (MAC) on synaptic transmission to motor neurons in a mouse spinal cord slice preparation. Figure 1A is a diagrammatic representation of this transmission pathway and relevant receptors. MAC is the anesthetic concentration that just prevents movement in response to a noxious stimulus¹⁵ and is determined by anesthetic actions in the spinal cord.¹⁶⁻¹⁹ Prevention of nocifensive movement is the most common endpoint for comparing potencies among volatile anesthetic agents. The current studies were designed to test the following hypotheses: that enflurane at 1 MAC acts postsynaptically on motor neurons to depress synaptic transmission; that enflurane directly depresses glutamate-evoked responses independent of actions on inhibitory chloride channels; and that both AMPA and NMDA glutamate currents are sensitive to enflurane.

Methods

Experiments were carried out in spinal cord slices from postnatal mice 1-4 days of age. These mice are

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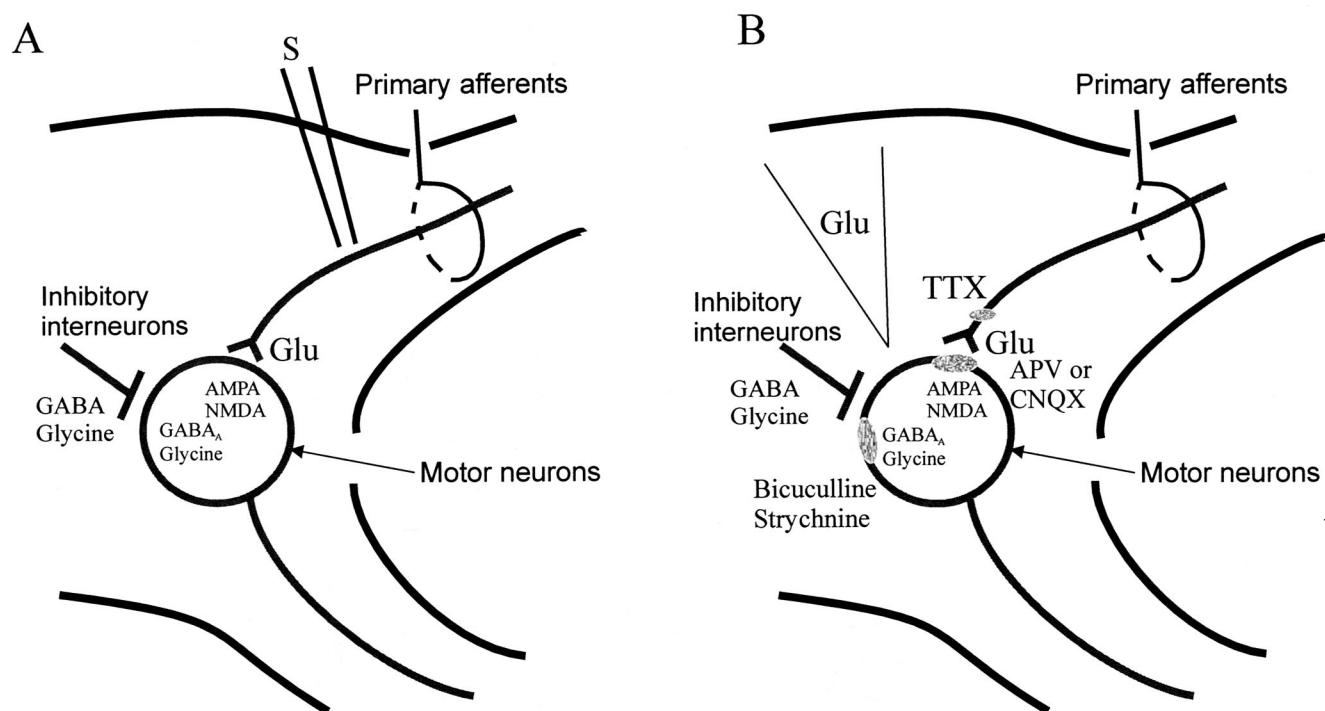


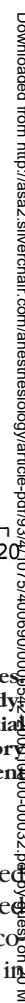
Fig. 1. Normal pathways in spinal cord and the isolation of postsynaptic receptors. (A) Under normal conditions and if electrical stimulation is used in the dorsal root area (S), action potentials reach the terminals of primary afferents and release glutamate (Glu) onto α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors on motor neurons. γ -Aminobutyric acid and glycine released from inhibitory interneurons activate the respective receptors on motor neurons. (B) Stippled areas indicate pharmacologic block. Tetrodotoxin (TTX) blocks generation of action potentials, and presynaptic input is bypassed by applying glutamate directly to the cell from a pressure-operated pipette. In various experiments, γ -aminobutyric acid or glycine receptors are blocked by their respective antagonists bicuculline and strychnine, and either AMPA or NMDA receptors by their antagonists 6-cyano-7-nitroquinoxaline-2,3-dione disodium or D,L-2-amino-5-phosphonopentanoic acid to isolate NMDA and AMPA currents respectively.

offspring of breeding pairs derived from C57GL/6J \times 129/Sv/SvJ and were wild-type offspring of animals heterozygous for a genetically engineered mutation. Wild type for the mutation present in the colony was verified by genotypic analysis using Southern blot techniques. Neonatal mice and rats mount a recognizable withdrawal reflex in response to tail or paw pinch; MAC for rats and mice of this age is approximately 20% higher than for adult animals.^{20,21} In a protocol approved by Stanford's panel on laboratory animal care and use, the animals were anesthetized with halothane and decapitated, and spinal cords quickly removed and placed in a cold oxygenated artificial cerebrospinal fluid (ACSF). The ACSF was composed as follows: NaCl: 123 mM; KCl: 4 mM; NaH₂PO₄: 1.2 mM; MgSO₄: 1.3 mM; NaHCO₃: 26 mM; d-glucose: 10 mM; and CaCl₂: 2 mM. Slices 350 μ m thick were prepared as previously described.¹¹ Briefly, slices were sectioned from the lumbar region on a Vibratome (Technical Products International, St. Louis, MO), and removed to oxygenated ACSF at room temperature for a 1-h recovery period. Individual slices were transferred to a chamber constantly superfused with oxygenated ACSF. All experiments were carried out at room temperature.

Cells in the spinal cord slice were visualized on a

closed-circuit television monitor using infrared illumination and a 40 \times water immersion objective. Studies were carried out in the large cell bodies in the ventral horn most commonly seen in the ventral lateral or ventral medial area (figs. 2A and 2B). In separate studies these cells were identified as motor neurons by fluorescent labeling with Evans blue dye injected into the hind limb the day before sacrifice, as previously described.¹¹

Patch pipettes were pulled on a Flaming-Brown pipette puller (Sutter Instruments, Novato, CA) and filled with solution of the following composition: NaCl: 15 mM; K⁺ gluconate: 110 mM; HEPES: 10 mM; MgCl₂: 2 mM; EGTA: 11 mM; CaCl₂: 1 mM; and MgATP: 2 mM, with pH adjusted with KOH to 7.3. Pipettes typically had a tip resistance of 3–8 M Ω . The patch pipette was directed toward a motor neuron cell body under visual control. After establishment of a Gigaohm seal, the patch was ruptured by brief negative pressure and subsequent measurements made in the whole-cell ruptured patch configuration in either current clamp or voltage clamp mode using an Axopatch 200B patch clamp amplifier (Axon Instruments, Foster City, CA). Motor neuron responses were evoked by electrical stimulation of the dorsal horn by a concentric bipolar platinum electrode with tip diameter of 0.025 mm, using square



wave stimuli 0.1 ms in duration, 1–20 V nominal intensity, and frequency of 0.03–0.10 Hz. Excitatory postsynaptic potentials (EPSPs) or EPSCs were measured individually or analyzed statistically by averaging groups of 5–10. In addition to synaptic currents evoked by dorsal root stimulation, responses were evoked by direct pressure application of glutamate from a pipette positioned near the cell body (Picospritzer, General Valve Division of Parker Hannafin, Fairfield, NJ). Pressure pulse was 9 psi; the duration of the pulse was 200 ms. Glutamate concentration in the pipette was 5 mM. We found that these parameters consistently gave a reproducible inward current of good amplitude. Glutamate applications at 1-min intervals produced stable responses over the course of each experiment; receptor desensitization was not observed at this rate of application. In voltage clamp studies, holding potential was usually -60 mV. The membrane potential value was not corrected for junction potential, which was -13 mV. A software

Pharmacologic agents tetrodotoxin, bicuculline methiodide, strychnine hydrochloride, 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX), D,L-2-amino-5-phosphonopentanoic acid (AP-5), and enflurane were made up as stock solutions, dissolved in ACSF at the desired concentration, and applied in the superfusate. Concentrations expressed as MAC refer to MAC determinations in adult animals and thus are lower than MAC for animals of this age.^{20,21} Enflurane was applied for 10 min. Enflurane effects were measured by taking the average of responses during the last 5 min after application compared with the average of responses in the 5 min immediately before the start of application. Enflurane ef-

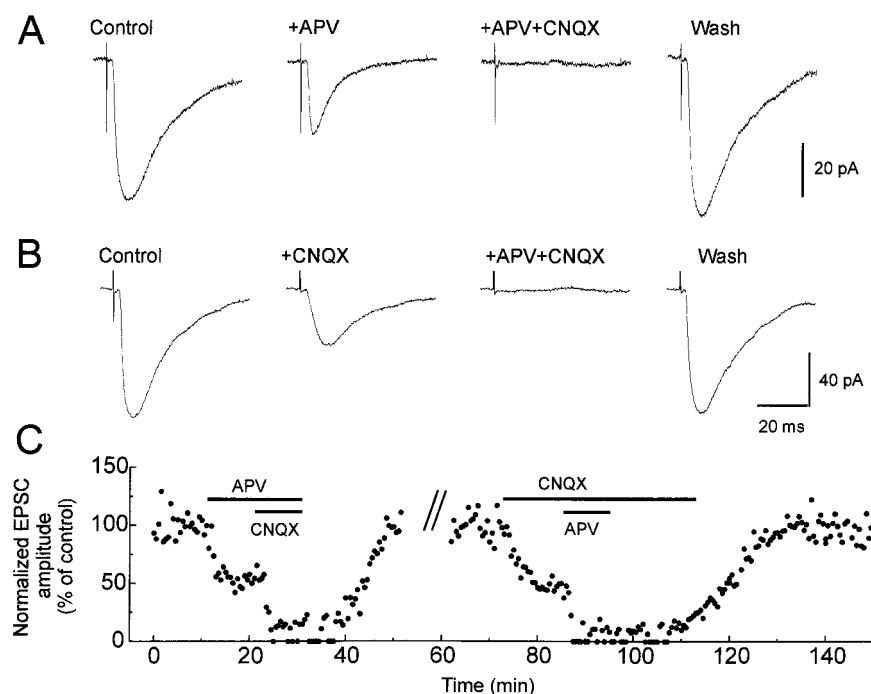


Fig. 3. As in rat spinal cord, both α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors contribute to excitatory postsynaptic currents (EPSCs) in mouse motor neurons. (A) The glutamate NMDA antagonist D,L-2-amino-5-phosphonopentanoic acid ($50 \mu\text{M}$) reduced the size of the synaptic currents, which were blocked completely by addition of the AMPA-kainate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione disodium ($10 \mu\text{M}$), indicating that the remaining fast-decaying EPSC was mediated by AMPA-kainate receptors. The blockade was reversible after washout. Each EPSC trace shown here is an average of five consecutive responses. (B) In the same cell, the sequence of application of glutamate antagonists was reversed. The slow-rising and slow-decaying EPSC in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione disodium was blocked further by addition of D,L-2-amino-5-phosphonopentanoic acid indicating the slow-decaying EPSC was mediated by NMDA receptors. (C) The amplitude of EPSCs recorded from this motor neuron versus time. Block by each antagonist was completely reversible in the presence of the other.

effects were expressed as mean \pm SEM, and statistical significance was tested with the Student *t* test or one-way analysis of variance and the Dunnett multiple comparison test.

Results

Effects of Enflurane on Synaptically Evoked Currents and Potentials

The pathway underlying synaptic transmission to motor neurons if an electrical stimulus is applied to the dorsal root is diagrammed in figure 1A, and the pharmacologic strategies used in the study are shown in figure 1B. To examine the effects of enflurane on synaptically evoked potentials and currents recorded from motor neurons, a stimulus intensity that evoked a response at a half-maximal amplitude was chosen from the stimulus intensity-response curve of each cell (figs. 2C and 2D). The receptors responsible for generation of the synaptic currents were determined by applying AP-5, a glutamate NMDA receptor antagonist, and CNQX, a non-NMDA receptor antagonist. An example of a typical experiment is shown in figure 3. Application of $50 \mu\text{M}$ AP-5 and $10 \mu\text{M}$ CNQX together completely abolished synaptic currents. Reduction of synaptic currents by either AP-5 or CNQX applied alone is unrelated to the application sequence. Washout of antagonists returned currents to their control levels. The data clearly show that EPSCs recorded from mouse motor neurons are mediated by both NMDA and non-NMDA receptors, results very similar to what we have observed in rat motor neurons.¹¹

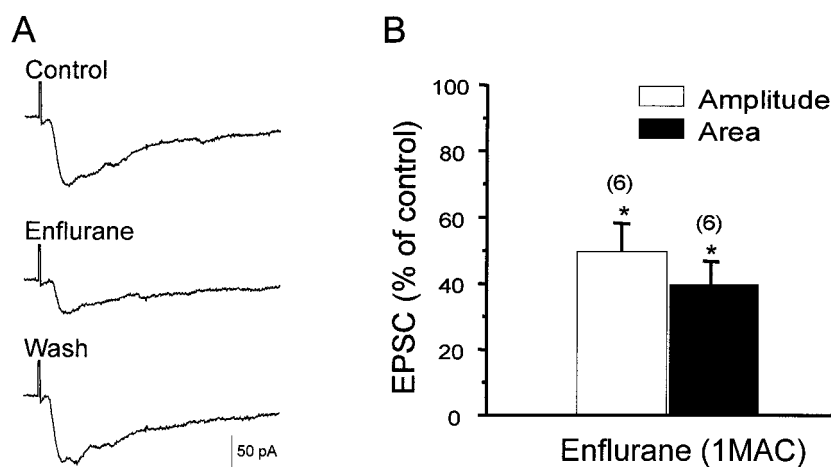
Stimuli to the mouse dorsal horn were given at a constant frequency of 0.03 Hz during the control period, enflurane application, and washout. Under either voltage

clamp or current clamp conditions, enflurane at concentrations equivalent to 1 MAC (0.6 mM) reduced EPSCs and EPSPs in all cells tested. An example of enflurane's effects is shown in figure 4A. The depressant effect of enflurane is reversible after washout. Figure 4B summarizes the data from six motor neurons exposed to enflurane for 10 min at a concentration of 1 MAC under voltage clamp conditions. Peak EPSC amplitudes were significantly reduced to a mean of $49.7 \pm 8.38\%$ of control (mean \pm SEM, $P < 0.05$); the area under the curve of the response to a mean of $39.9 \pm 6.85\%$ of control ($P < 0.01$). In another six cells, we examined enflurane depressant effects under current clamp conditions. Enflurane at 1 MAC significantly depressed EPSP amplitude to a mean of $49.6 \pm 10.89\%$ of control ($P < 0.05$) and EPSP area to a mean of $49.9 \pm 12.83\%$ of control ($P < 0.05$). A typical example is shown in figure 5. Enflurane at 1 MAC reversibly depressed EPSP amplitude and area with minimal change of membrane resistance. The extent of enflurane depression of EPSPs in the current clamp condition is not significantly different from that of EPSCs in the voltage clamp condition.

Effects of Enflurane on Glutamate-evoked Responses

The experimental arrangement for glutamate application is diagrammed in figure 1B. Pulses of pressure applied to the glutamate-containing pipette produced inward currents in the motor neurons (fig. 6A). In the presence of 0.3 mM tetrodotoxin, enflurane at a concentration of 1 MAC for 10 min reversibly depressed glutamate-induced inward currents in nine cells tested (fig. 6B), indicating a direct postsynaptic effect of enflurane.

Fig. 4. Enflurane had a significant depressant effect on synaptic currents in motor neurons evoked by dorsal horn stimulation under voltage clamp at a holding potential of -60 mV. (A) Sample records from a motor neuron in a slice from a 1-day-old mouse. Enflurane at one minimum alveolar anesthetic concentration for 10 min reversibly decreased both amplitude and area of the inward currents evoked by stimulation in the dorsal horn. The effect was fully reversible on washing for 20 min. (B) Histogram summarizing the depressant effect of enflurane on excitatory postsynaptic currents in six cells tested. The synaptic responses were depressed to about half of their size at an enflurane concentration of one minimum alveolar anesthetic concentration. Data are means of six cells, each from a different slice; error bars are SEMs.



Peak amplitude and area underneath the curve of glutamate-evoked currents at 5–10 min after exposure to enflurane were decreased significantly, to means of $67.0 \pm 3.97\%$ ($P < 0.01$) and $71.7 \pm 6.04\%$ ($P < 0.01$) of control, respectively. In four of the nine cells, recovery on enflurane washout was complete, and all cells met the criterion of at least partial recovery.

Postsynaptic Depressant Effects of Enflurane Did Not Require Action on Inhibitory Chloride Channels

To exclude the possibility that inhibitory channels were involved in the depressant effect of enflurane, bicuculline ($20 \mu\text{M}$) and strychnine ($2 \mu\text{M}$) were used to block GABA_A and glycine receptors, respectively, as illustrated in figure 1B. Enflurane at 1 MAC depressed glutamate-evoked responses if GABA_A receptors were blocked by bicuculline (fig. 7A). Peak amplitude and area of glutamate-induced inward currents were reduced to means of $81.4 \pm 2.82\%$ ($n = 5$, $P < 0.01$) and $79.3 \pm 6.46\%$ ($n = 5$, $P < 0.05$) of control, respectively. Enflurane also depressed inward currents if glycine receptors were blocked by strychnine (fig. 7B). The peak amplitude and area of glutamate-induced currents were significantly depressed, to means of $61.2 \pm 8.24\%$ ($n = 4$, $P < 0.01$) and $63.3 \pm 10.08\%$ ($n = 4$, $P < 0.05$) of control, respectively. If both inhibitory receptors were blocked (fig. 7C), enflurane similarly depressed glutamate-evoked currents, and the effect was reversible. In six cells treated with a combination of bicuculline ($20 \mu\text{M}$) and strychnine ($2 \mu\text{M}$), enflurane significantly depressed peak glutamate-evoked currents to a mean of $67.2\% \pm 4.60\%$ of control ($P < 0.01$) and area to $64.6 \pm 4.18\%$ of control ($P < 0.01$).

Enflurane Depresses Currents at Both AMPA and NMDA Receptors

To test whether enflurane depressed currents mediated by glutamate AMPA or NMDA receptors, the NMDA

receptor antagonist AP-5 or the non-NMDA receptor antagonist CNQX was applied as shown in figure 1B. Coapplication of both antagonists almost completely abolishes glutamate-evoked currents. A very small residual current (less than 3% of the original amplitude and area) may result from displacement of the competitive antagonists from the receptors by prolonged exposure to exogenous glutamate. Enflurane depressed glutamate-evoked currents in the presence of $50 \mu\text{M}$ AP-5 (fig. 8A), indicating an action on currents mediated by non-NMDA receptors. If NMDA receptors were blocked with AP-5, enflurane at 1 MAC significantly depressed the peak amplitude and area under the curve of the residual glutamate currents, to means of $66.3 \pm 4.95\%$ ($n = 5$, $P < 0.01$) and $66.1 \pm 3.60\%$ ($n = 5$, $P < 0.01$) of control. Enflurane also depressed inward currents in the presence of CNQX ($10 \mu\text{M}$), indicating an action on currents mediated by NMDA receptors (fig. 8B). If CNQX was used to block AMPA-kainate receptors, 1 MAC enflurane significantly depressed the remaining NMDA current peak amplitude, to a mean of $68.4 \pm 8.33\%$ of control ($n = 4$, $P < 0.05$), and area, to a mean of $71.8 \pm 10.82\%$ of control ($n = 4$, $P < 0.05$). These results are not different from the effects of enflurane in untreated preparations. Figure 9 summarizes the effects of enflurane on glutamate-evoked currents under various pharmacologic conditions. Analysis of variance of six groups revealed statistically significant difference in the peak amplitudes of glutamate-evoked currents ($n = 33$, $P < 0.05$) but no statistically significant difference in the areas of glutamate-evoked currents ($P > 0.05$). Comparing each of the groups treated with various combinations of receptor antagonists to the tetrodotoxin control group, the effects of enflurane on neither peak amplitudes nor areas were significantly different ($P > 0.05$ by the Dunnett multiple comparison test). Enflurane thus acts to depress currents mediated by both major subtypes of glutamate receptors in motor neurons, NMDA and AMPA-kainate.

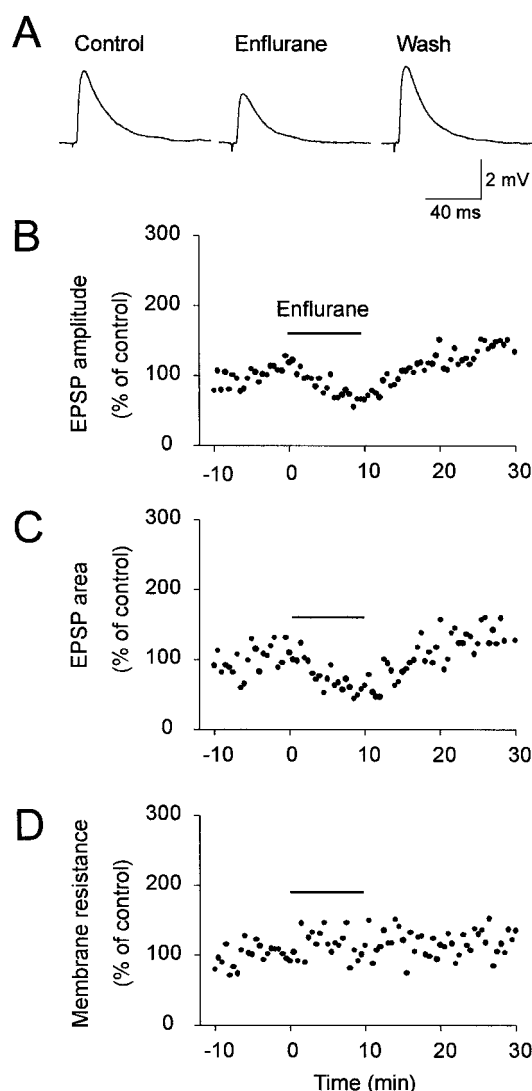


Fig. 5. Enflurane depressed synaptic potentials in mouse motor neurons under current clamp. (A) Sample records of excitatory postsynaptic potentials (EPSPs) that were evoked by stimulation in the dorsal horn area. Exposure to one minimum alveolar anesthetic concentration of enflurane for 10 min decreased EPSP size, and the depressant effect was reversible after washout. EPSPs were an average of five consecutive responses. (B,C) Time courses of EPSP amplitude and area, respectively, recorded from the same cell as in (A). (D) Enflurane decreased the amplitude and area of evoked EPSPs, with insignificant changes of membrane resistance.

When currents are evoked by glutamate application, block of inhibitory chloride channels does not significantly attenuate the depressant effects of enflurane.

Discussion

Pharmacology of Mouse Spinal Cord

Because of the advantages offered by genetic engineering, mice rapidly are becoming a study animal of choice in investigations that formerly used rats. These studies are among the first to examine the physiology and phar-

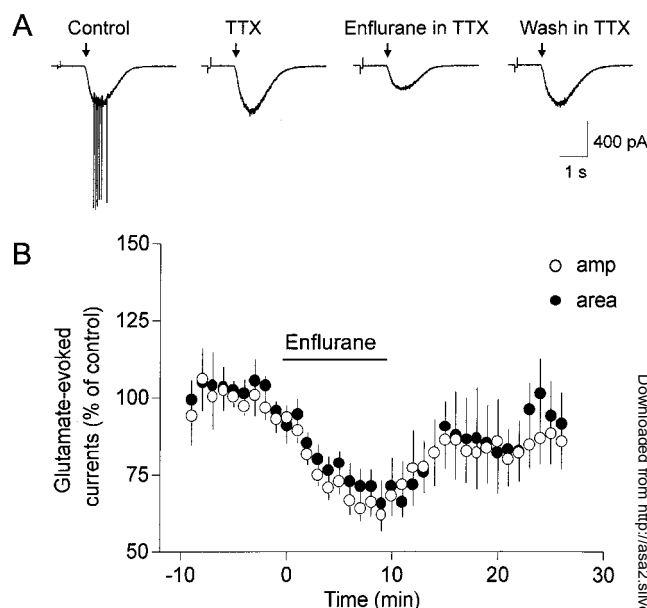
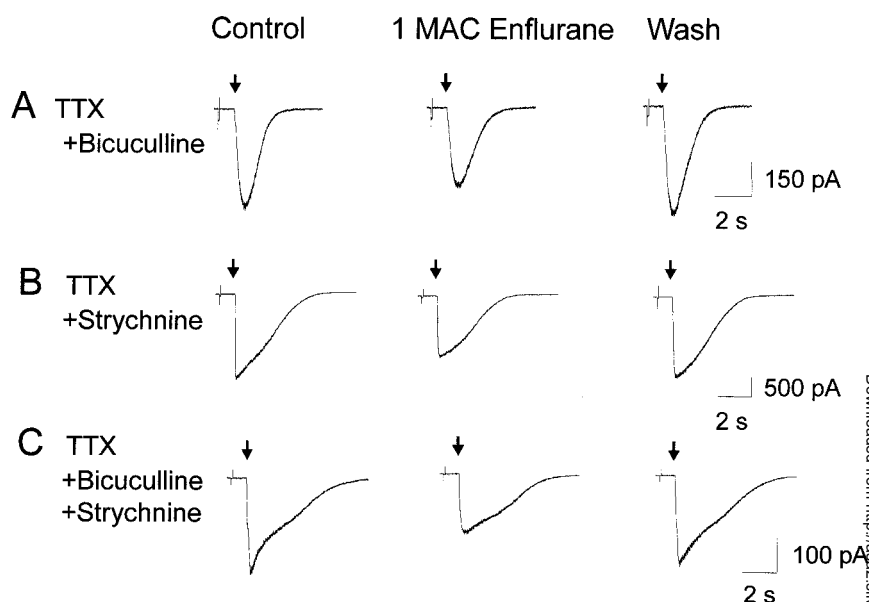


Fig. 6. Enflurane effects on glutamate-induced inward currents in motor neurons in mouse spinal cord slices. (A) Inward currents were evoked by pressure ejection of glutamate (5 mM) at the time indicated by the arrow. The pipette containing glutamate was placed close to the recording cell body. The duration of the pulse was 200 ms. Hyperpolarizing voltage steps of 10 mV before glutamate injection were imposed to measure changes in membrane conductance. Tetrodotoxin (0.3 mM) was used to block action potentials in all cells tested. Holding potentials were -60 mV. Enflurane at one minimum alveolar anesthetic concentration, applied for 10 min, reversibly depressed the inward currents evoked by glutamate. There was an insignificant change in membrane conductance and a slight decrease in holding current in this cell during enflurane application. (B) Time course showing enflurane effect on glutamate-evoked inward currents in motor neurons of slices treated with tetrodotoxin. Enflurane at one minimum alveolar anesthetic concentration was applied for 10 min. The peak amplitudes (open circles; $n = 9$) and areas (filled circles; $n = 9$) of glutamate-evoked currents were depressed by enflurane. The effect was reversible after washout, in four cells completely and in the others partially. Error bars are SEM.

macology of transmission to mouse spinal cord motor neurons. The glutamate receptor antagonists CNQX and AP-5 each reduced EPSC amplitude, and the combination almost completely blocked EPSCs evoked by dorsal root stimulation or glutamate application. In mice as in rats short-latency excitatory synaptic transmission to motor neurons is mediated by glutamate receptors of both NMDA and non-NMDA subtypes. Non-NMDA receptors are of two classes, AMPA and kainate. In intact mouse spinal cord a selective kainate antagonist does not depress ventral root population evoked responses (H. Haeberle, B.S., D. L. Tauck, Ph.D., J. J. K., unpublished data, 1998). Selective AMPA antagonists, on the other hand, almost completely block ventral root responses in rat if coapplied with an NMDA antagonist (J. Knape, M.S., J. J. K., unpublished data, 1999). These results suggest that the glutamate non-NMDA receptors that mediate excitation to rodent motor neurons under the

Fig. 7. Enflurane's depressant actions on glutamate-induced inward currents are not blocked by inhibitory chloride channel antagonists. Experiments were done in the presence of tetrodotoxin; control and washout were with antagonists present in the artificial cerebrospinal fluid. Glutamate 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. (A,B) Enflurane at a concentration of one minimum alveolar anesthetic concentration reversibly reduces the glutamate-evoked response in the presence of either the γ -aminobutyric acid A receptor antagonist bicuculline (20 μ M) or the glycine receptor antagonist strychnine (2 μ M), respectively. (C) A similar depressant effect on glutamate-induced currents by enflurane at one minimum alveolar anesthetic concentration was seen in the presence of both antagonists. Records in each part (A–C) are from a different cell.



current experimental conditions are predominantly of the AMPA class.

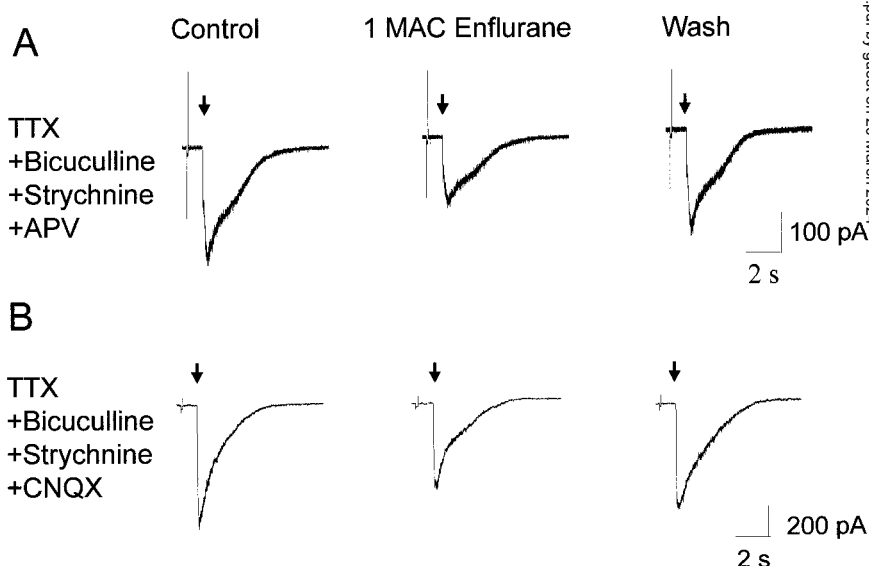
Postsynaptic Actions of Enflurane

When presynaptic impulse activity is blocked by tetrodotoxin, enflurane depresses currents evoked by direct glutamate application. This is a classic test for a postsynaptic action of a drug on excitatory synaptic transmission. It remains possible that glutamate acts on presynaptic receptors to release glutamate by a direct action not dependent on impulse activity.

A presynaptic mechanism for volatile anesthetic actions has been suggested based on evidence obtained at other central nervous system sites. There is contradictory evidence suggesting either that halothane acts only presynaptically or that both pre- and postsynaptic ac-

tions are involved in hippocampus.^{12–14} At other supraspinal central nervous system sites, volatile agents depress synaptically evoked glutamatergic transmission, but some do not alter responses to exogenous glutamate application, a result suggesting that their actions are pre- rather than postsynaptic.^{22–25} There are reports that presynaptic sodium channels are sensitive to volatile anesthetic agents,²⁶ suggesting that this mechanism also may contribute to depression of synaptic transmission. We have observed inhibition of sodium currents by ethanol²⁷ and halothane (J. V. Wu, Ph.D., J. J. K., unpublished data, 1998–1999) in rat dorsal root ganglion cells, suggesting the possibility of presynaptic inhibitory actions upstream from the calcium channels that mediate transmitter release. Volatile anesthetics also were found to depress hippocampal glutamatergic synaptic transmission.

Fig. 8. Enflurane depresses both *N*-methyl-D-aspartate (NMDA) receptor- and non-NMDA receptor-mediated inward currents evoked by glutamate application. Experiments were done in the presence of tetrodotoxin, with both γ -aminobutyric acid A and glycine inhibitory chloride channels blocked by their respective antagonists, bicuculline (20 μ M) and strychnine (2 μ M); control and washout were with antagonists present in the artificial cerebrospinal fluid. (A) In the presence of the NMDA receptor antagonist D,L-2-amino-5-phosphonopentanoic acid (50 μ M), glutamate evoked inward currents presumably through non-NMDA receptors. Enflurane reversibly depressed these currents. (B) Enflurane depressed inward currents if non-NMDA receptors were blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (10 μ M). The depressant effect was reversible. Glutamate was applied at the time indicated by the arrows.



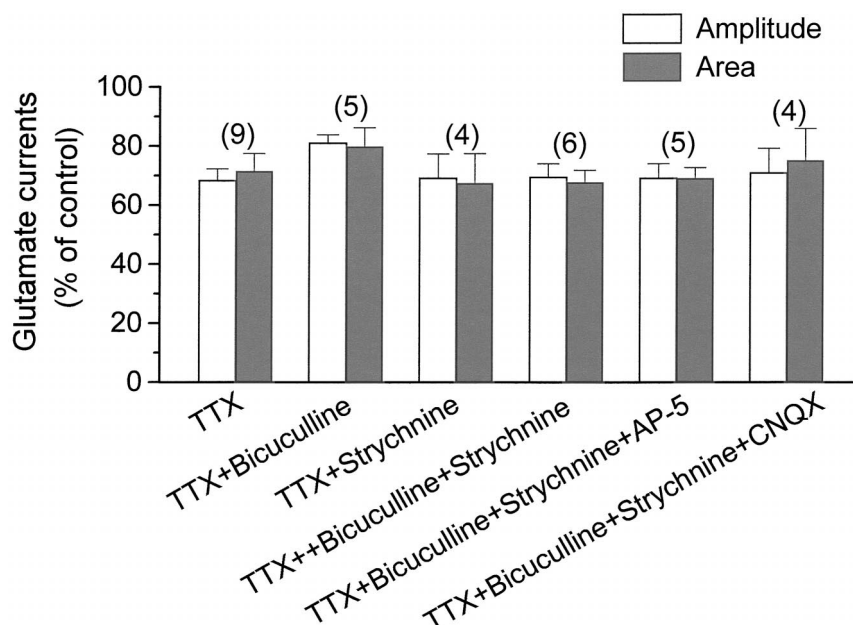


Fig. 9. Enflurane at one minimum alveolar anesthetic concentration significantly depresses glutamate-induced currents under all conditions of pharmacologic blockade. The peak amplitudes (open columns) and areas (filled columns) of glutamate-induced currents were depressed significantly, by about 20–30%, by enflurane at a concentration of one minimum alveolar anesthetic concentration. Effects of enflurane were measured by taking the average of the responses during the last 5 min of enflurane application, compared with the average of responses in the 5 min immediately before the start of enflurane application. Data are expressed as mean \pm SEM, and statistical significances were tested with analysis of variance and the Dunnet multiple comparison test ($n = 33$). Analysis of variance revealed statistically significant differences between all groups for peak amplitude but not area. Enflurane has similar depressant effects on glutamate-induced currents in each of the various treatment groups compared with the tetrodotoxin-treated group ($P > 0.05$ by the Dunnet multiple comparison test).

sion accompanied by increased paired pulse facilitation,²⁸ suggesting an involvement of a presynaptic mechanism. In the current study, enflurane decreased the sensitivities of both synaptically evoked responses and responses to glutamate in spinal cord slices, suggesting that with this volatile anesthetic agent depression of excitatory synaptic transmission in spinal cord results at least in part from direct postsynaptic actions on motor neurons. Enflurane depression of glutamate-evoked currents was less than that of synaptically evoked currents, suggesting that presynaptic actions may play a role in the latter. The results with enflurane are consistent with the observation that ethanol, which has general anesthetic properties, also exerts a direct action on motor neurons in rat spinal cord.¹¹ *In vivo*, a study was carried out to determine the effects of a volatile anesthetic on the F wave, a reflex that reflects motor neuron excitability. These results also suggest that motor neuron excitability may be decreased by a volatile agent at MAC.²⁹

Action Independent of Inhibitory Channels

It is a pervasive theory in the field of anesthesia that actions on GABA_A receptors are the dominant factor in producing the anesthetic state.^{1,2} Ethanol and volatile general anesthetics enhance and prolong GABA_A and glycine currents.^{3–8} In addition, both may increase tonic inhibition by increasing spontaneous inhibitory transmitter release.^{30–32} The results of the current study exclude both GABA_A and glycine receptors as essential to anesthetic depression of glutamate-evoked currents in spinal cord motor neurons. The concentrations of strychnine and bicuculline used here were sufficient to block all the spinal cord glycine and GABA_A receptors, respectively.^{33–35} Enflurane significantly depressed inward cur-

rents evoked by glutamate in the presence of bicuculline or strychnine, suggesting that the depressant effect was not dependent on anesthetic action on GABA_A receptors or glycine receptors. That enflurane exerts equal depressant effects on glutamate-evoked currents whether or not GABA_A and glycine receptors are blocked suggests that, under the current experimental conditions, actions of enflurane on GABA_A and glycine receptors do not contribute to the depression. However, it is possible that anesthetics directly gate inhibitory channels, in addition to enhancing the effects of the inhibitory transmitter acting at a site not blocked by the competitive antagonists. Direct gating of GABA_A receptors is known to occur for some intravenous anesthetics, and there is one report of direct gating with volatile agents.³⁶

Minimum alveolar anesthetic concentration is determined by anesthetic actions in the spinal cord.^{16–18} Anesthetic actions on spinal cord thus are directly relevant to general anesthesia.³⁷ The results of the current study do not rule out a role for GABA_A and glycine receptors in contributing to MAC; in intact mouse spinal cords, bicuculline attenuates enflurane's actions significantly (S. M. E. Wong, M.S., J. J. K., unpublished data, 1998–1999). However, the results suggest that other receptors are important, as well. The results of other studies also suggest that these inhibitory receptors, although they may be of essential importance to anesthetic endpoints such as anesthesia, may not be as important in preventing movement in response to a noxious stimulus. A recent study showed that an agent that potentiates activity at benzodiazepine-sensitive receptors does not alter MAC of the inhalation agent desflurane.³⁸ In addition, there are volatile anesthetics that, although immobilizing animals, do not enhance activity at GABA_A receptors.³⁹ With respect to abolition of nocifen-

sive movement as the definition of anesthesia, a case may be made that receptors other than GABA_A and glycine are important targets for volatile anesthetic agents.

AMPA and NMDA Receptor-mediated Currents Appear Equally Sensitive to Enflurane

Although the actions of enflurane on inhibitory GABA_A receptor and glycine currents have been well studied,³⁻⁸ there are comparatively few studies of volatile agents on excitatory currents.^{40,41} The current study shows that enflurane directly depresses both AMPA and NMDA receptor-mediated responses. There is a broad consensus that NMDA receptors are sensitive to ethanol at both intoxicating and anesthetic concentrations.⁴²⁻⁴⁵ Pento-barbitone and isoflurane also have been found to depress the function of native NMDA receptors.^{46,47} Both nitrous oxide and xenon have been reported to depress NMDA receptors.^{48,49} There has been debate about the sensitivity of glutamate non-NMDA receptors to anesthetics and ethanol.^{50,51} Halothane recently was found to depress AMPA and NMDA components of synaptic currents equally in mouse hippocampus.¹⁴ In locus coeruleus, ethanol (100 mM) equally inhibits NMDA- and AMPA-induced inward currents.⁵² A recent study showed that both isoflurane and xenon depress glutamate currents in hippocampal autaptic cultures, and that although isoflurane enhances GABA_A currents, xenon does not. Whereas xenon is selective for NMDA currents, isoflurane depresses AMPA-kainate and NMDA currents equally.⁵³

The results of the present study suggest that in spinal cord motor neurons, currents mediated by both AMPA and NMDA glutamate receptors are sensitive to enflurane, certainly at the concentrations associated with general anesthesia as defined by immobilization. Although in the present study there is no doubt that the currents mediated by these receptors are depressed, the results do not force the conclusion that enflurane acts directly on the receptors. Because glutamate was used to evoke the currents, it is possible that an indirect action mediated by metabotropic glutamate receptors might have contributed. In addition, enflurane and ethanol may exert other actions, possibly on calcium-mediated processes, that could produce an indirect depression of AMPA and NMDA receptor-mediated currents.

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