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 µM) or strychnine (2 µM) 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 D,L-2-amino-5-phosphonopentanoic acid (50 µM) or 6-cyano-7-nitroquinoxaline-2,3-dione disodium (10 µM) 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 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 ethanol10 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
offspring of breeding pairs derived from C57GL/6J × 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 mm 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× 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 a 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 MΩ. The patch pipette was directed toward a motor neuron cell body under visual control. After 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 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 Hannifin, 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 package (pClamp version 7, Axon Instruments) was used to acquire data, which were stored digitally and analyzed off-line, and to trigger an isolated stimulator or a Picospritzer. Experiments were carried out on a single cell in each slice.

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-

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Fig. 2. (A) Schematic representation of a lumbar spinal cord slice showing the position of recording and stimulating electrodes. (B) Infrared videomicroscopy of motor neuron in a spinal cord slice, with patch pipette electrode apposed to the cell body. (C) Excitatory postsynaptic currents recorded in a motor neuron from a 1-day-old mouse under voltage clamp at a holding potential of −60 mV. Currents were evoked by electrical stimulation in the dorsal horn area. Arrows indicate where peak excitatory postsynaptic current (EPSC) amplitudes were measured. (D) Current–stimulus intensity relation of the EPSCs from the experiment illustrated in (C). Peak EPSC amplitudes were plotted against stimulus strength.
Effects were expressed as mean ± SEM, and statistical significance was tested with the Student’s t test or one-way analysis of variance and the Dunnet 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 μM AP-5 and 10 μM 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 ± 8.38% of control (mean ± SEM, P < 0.05); the area under the curve of the response to a mean of 39.9 ± 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 ± 10.89% of control (P < 0.05) and EPSP area to a mean of 49.9 ± 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.
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 ± 3.97% (P < 0.01) and 71.7 ± 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 μM) and strychnine (2 μM) were used to block GABA<sub>A</sub> and glycine receptors, respectively, as illustrated in figure 1B. Enflurane at 1 MAC depressed glutamate-evoked responses if GABA<sub>A</sub> receptors were blocked by bicuculline (fig. 7A). Peak amplitude and area of glutamate-induced inward currents were reduced to means of 81.4 ± 2.82% (n = 5, P < 0.01) and 79.3 ± 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 ± 8.24% (n = 4, P < 0.01) and 63.3 ± 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 μM) and strychnine (2 μM), enflurane significantly depressed peak glutamate-evoked currents to a mean of 67.2% ± 4.60 of control (P < 0.01) and area to 64.6 ± 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 μM 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 ± 4.95% (n = 5, P < 0.01) and 66.1 ± 3.60% (n = 5, P < 0.01) of control. Enflurane also depressed inward currents in the presence of CNQX (10 μM), 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 ± 8.33% of control (n = 4, P < 0.05), and area, to a mean of 71.8 ± 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.
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 pharmacology 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. 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.

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.
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 μM) or the glycine receptor antagonist strychnine (2 μM), 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 actions are involved in hippocampus. 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. There are reports that presynaptic sodium channels are sensitive to volatile anesthetic agents, suggesting that this mechanism also may contribute to depression of synaptic transmission. We have observed inhibition of sodium currents by ethanol 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 transmis-

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 μM) and strychnine (2 μM); 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 μM), 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 μM). The depressant effect was reversible. Glutamate was applied at the time indicated by the arrows.
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Action Independent of Inhibitory Channels

It is a pervasive theory in the field of anesthesia that actions on GABA<sub>A</sub> receptors are the dominant factor in producing the anesthetic state. Ethanol and volatile general anesthetics enhance and prolong GABA<sub>A</sub> and glycine currents. In addition, both may increase tonic inhibition by increasing spontaneous inhibitory transmitter release. The results of the current study exclude both GABA<sub>A</sub> and glycine receptors as essential to anesthetic endpoints such as amnesia, 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. In addition, there are volatile anesthetics that, although immobilizing animals, do not enhance activity at GABA<sub>A</sub> receptors. With respect to abolition of nocifens...
sive movement as the definition of anesthesia, a case may be made that receptors other than GABA<sub>A</sub> 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<sub>A</sub> receptor and glycine currents have been well studied, there are comparatively few studies of volatile agents on excitatory currents. There is a broad consensus that NMDA receptors are sensitive to ethanol at both intoxicating and anesthetic concentrations. Pentobarbital and isoflurane also have been found to depress the function of native NMDA receptors. Both nitrous oxide and xenon have been reported to depress NMDA receptors. There has been debate about the sensitivity of glutamate non-NMDA receptors to anesthetics and ethanol. Halothane recently was found to depress AMPA and NMDA components of synaptic currents equally in mouse hippocampus. In locus coeruleus, ethanol (100 mM) equally inhibits NMDA- and AMPA-induced inward currents. A recent study showed that both isoflurane and xenon depress glutamate currents in hippocampal autaptic cultures, and that although isoflurane enhances GABA<sub>A</sub> currents, xenon does not. Whereas xenon is selective for NMDA currents, isoflurane depresses AMPA-kainate and NMDA currents equally.

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, certain 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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