Modulation of NMDA Receptor Function by Ketamine and Magnesium. Part II: Interactions with Volatile Anesthetics

Markus W. Hollmann, MD†§, Hong-Tao Liu, MD†, Christian W. Hoenemann, MD†‡, Wei-Hua Liu, MD†, and Marcel E. Durieux, MD, PhD*†

*Department of Anesthesiology and Pain Management, University Hospital Maastricht, Maastricht, The Netherlands; †Department of Anesthesiology, University of Virginia, Charlottesville, Virginia; ‡Department of Anesthesiology and Intensive Care, University of Muenster, Muenster; and §Department of Anesthesiology, University of Heidelberg, Heidelberg, Germany

Mg^{2+} and ketamine interact superadditively at *N*-methyl-D-aspartate (NMDA) receptors, which may explain the clinical efficacy of the combination. Because patients are usually exposed concomitantly to volatile anesthetics, we tested the hypothesis that volatile anesthetics interact with ketamine and/or Mg^{2+} at recombinantly expressed NMDA receptors. NR1/NR2A or NR1/NR2B receptors were expressed in *Xenopus* oocytes. We determined the effects of isoflurane, sevoflurane, and desflurane on NMDA receptor signaling, alone and in combination with S(+)-ketamine (4.1 μM on NR1/NR2A, 3.0 μM on NR2/NR2B) and/or Mg^{2+} (416 μM on NR1/NR2A, 629 μM on NR1/NR2B). Volatile anesthetics inhibited NR1/NR2A and NR1/NR2B glutamate receptor function in a reversible, concentration-dependent, voltage-insensitive and noncompetitive manner (half-maximal inhibitory concentration at NR1/NR2A receptors: 1.30 ± 0.02 minimum alveolar anesthetic concentration [MAC] for isoflurane, 1.18 ± 0.03 MAC for desflurane, 1.24 ± 0.06 MAC for sevoflurane; at NR1/NR2B receptors: 1.33 ± 0.12 MAC for isoflurane, 1.22 ± 0.08 MAC for desflurane, and 1.28 ± 0.08 MAC for sevoflurane). On both NR1/NR2A and NR1/NR2B receptors, 50% inhibitory concentration for volatile anesthetics was reduced approximately 20% by Mg^{2+}, approximately 30% by S(+)-ketamine, and approximately 50% by the compounds in combination. Volatile anesthetic effects on NMDA receptors can be potentiated significantly by Mg^{2+}, S(+)-ketamine, or—most profoundly—both. Therefore, the analgesic effects of ketamine and Mg^{2+} are likely to be enhanced in the presence of volatile anesthetics.

(Anesth Analg 2001;92:1182–91)

In Part I of this investigation, we described the interactions between ketamine and Mg^{2+} at *N*-methyl-D-aspartate (NMDA) glutamate receptors. Mg^{2+} and ketamine interacted in a super-additive manner at both NR1/NR2A and NR1/NR2B receptors. The impetus for this study were clinical findings indicating that ketamine and Mg^{2+} have significant analgesic properties, and that the combination is more effective than either drug alone (1). Thus, by combining the compounds, additional benefit for the patient may be obtained without an increase in side effects. This issue may be relevant not only to the analgesic effects of NMDA receptor blockade, but also to its neuroprotective actions (2,3).

However, clinical preemptive analgesia with ketamine and Mg^{2+} takes place in the setting of surgery and anesthesia, and, therefore, the patient is usually exposed concomitantly to volatile anesthetics. Our experimental model as described in Part I is therefore not fully comparable with the clinical situation. Various lines of evidence suggest interactions between volatile anesthetics and NMDA receptor signaling. In addition, the potency of volatile anesthetics is increased by noncompetitive (4,5) and competitive (6) NMDA antagonists. This, in turn, suggests that at the NMDA receptor, volatile anesthetics might interact with ketamine and/or Mg^{2+}, administered as analgesics. As a result, they may modulate the effect of these compounds. Inasmuch as it is not known whether

H-TL and W-HL are supported by a grant from the Rockefeller Foundation. MWH is supported in part by the Department of Anesthesiology (Direktor: Univ.-Prof. Dr. E. Martin), University of Heidelberg, Heidelberg, Germany, and by a grant of the German Research Society (DFG HO 2199/1-1), Bonn, Germany. Supported in part by the "Innovative Medizinische Forschung" fund, Münster, Germany, Grants Hö-1-6-II/96-8 and 1-6-II/97-27 to CWH, and by an American Heart Association grant, Mid-Atlantic Affiliation (VHA 9920345 U), Baltimore, MD, and National Institutes of Health Grant GMS 52387, Bethesda, MD.

Accepted for publication January 3, 2001.

Address correspondence and reprint requests to Marcel E. Durieux, MD, PhD, Department of Anesthesiology and Pain Management, University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, The Netherlands. Address e-mail to mdu@sane.azm.nl.

0003-2999/01

volatile anesthetics selectively influence NMDA receptors of various subunit compositions, it is conceivable that interactions among these drugs may depend on the specific NMDA receptor present in relevant neurons.

Therefore, in this part of our investigation, we tested the hypothesis that volatile anesthetics, at clinically relevant concentrations, interact with ketamine and/or Mg^{2+} at recombinantly expressed NMDA receptors.

Methods

The study protocol was approved by the Animal Care and Use Committee at the University of Virginia.

Our methodology for receptor expression and study was as described in Part I of the investigation. Briefly, oocytes were obtained from *Xenopus laevis* frogs, defolliculated, and injected with cDNA encoding the appropriate NMDA receptor subunits. After allowing appropriate time for receptor expression, Ba^{2+} currents in response to glutamate were measured by using a two-electrode voltage clamp.

Isoflurane, sevoflurane, and desflurane were selected for the study because they are the compounds most frequently used in clinical practice in the United States. Anesthetic was bubbled for at least 10 min through a reservoir filled with 40 mL of Tyrode's solution. Air, at a flow rate of 500 mL/min, was used as the carrier gas. After equilibration, the solution was perfused through the recording chamber, superfusing the oocyte at a flow rate of approximately 2 mL/min; measurements were obtained after 10 bath volumes had been exchanged (approximately 3 min). Anesthetic concentrations in the recording chamber were quantified by gas chromatography. To allow comparisons among the anesthetics, partial pressures were expressed as minimum alveolar anesthetic concentration (MAC) fractions, where aqueous concentrations equivalent to one MAC anesthetic in air were 0.23 mM for isoflurane, 0.14 mM for sevoflurane, and 0.26 mM for desflurane (7). For multiple experiments in the same oocyte, we superfused the cell with anesthetic-free Tyrode's solution for at least 10 min, at which time current had returned completely to baseline.

Results were reported as mean ± SEM. Because variability between batches of oocytes is common, responses were at times normalized to control responses from the same batch. Differences among treatment groups were analyzed by using Student's *t*-tests. If multiple comparisons were made, data were analyzed by using one-way analysis of variance followed by Dunnett's *post hoc* test, if necessary. $P < 0.05$ was considered significant. Concentration-response curves were fit to the following logistic function, derived from the Hill equation: $y = y_{min} + (y_{max} - y_{min})\ (1 - x^n/[x_{50}{}^n + x^n])$ where y_{max} and y_{min} are the maximum and minimum response obtained, n is the Hill coefficient, and x_{50} is the half-maximal effect concentration (EC_{50} for agonist) or the half-maximal inhibitory effect concentration (IC_{50} for antagonist).

Molecular biology reagents were obtained from Promega (Madison, WI). Isoflurane and desflurane were from Ohmeda (Liberty Corner, NJ), sevoflurane was from Abbott International (Abbott Park, IL), and other chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Results

Volatile Anesthetics Inhibit NMDA Receptor Signaling

To determine the effects of isoflurane, sevoflurane, and desflurane on NMDA receptor signaling, we activated recombinantly expressed NR1/NR2A and NR1/NR2B receptors by co-application of glutamate and glycine in the presence of three different concentrations (1, 2, and 3 MAC) of the volatile anesthetics. Glutamate and glycine were administered at EC_{50}, which on NR1/NR2A and NR1/NR2B receptors were 3.2 μM and 4.9 μM, respectively, for glutamate, and 150 nM and 78 nM, respectively, for glycine, as determined in Part I of this research. All three anesthetics inhibited NMDA receptor signaling reversibly, dose-dependently, and equipotently.

At NR1/NR2A receptors (Fig. 1A), IC_{50}, calculated from the Hill equation, were remarkably similar when expressed as MAC (Table 1). The largest anesthetic concentration tested corresponded to 3 MAC. Even larger concentrations would be outside the clinical range, and might yield confusing results because of nonspecific actions. At 3 MAC, NMDA receptor signaling was suppressed 84% ± 3% by isoflurane, 87% ± 8% by desflurane, and 69% ± 10% by sevoflurane. The anesthetic effects at NR1/NR2B receptors (Fig. 1B, Table 1) were similar to those obtained at NR1/NR2A receptors. Inhibition at 3 MAC was 85% ± 3% for isoflurane, 76% ± 7% for desflurane, and 73% ± 3% for sevoflurane. These results suggest that the NR2 subunit does not greatly modulate anesthetic effects on the NMDA receptor, and that volatile anesthetics have their primary site of action on the NR1 subunit.

The effects of volatile anesthetics on NMDA receptor signaling were fully reversible and not dependent on holding potential (data not shown).

Volatile Anesthetics Inhibit Glutamate Signaling in a Noncompetitive Manner

We determined the concentration-response relationship for glutamate (in the presence of glycine at EC_{50}) on NR1/NR2A and NR1/NR2B receptors alone and

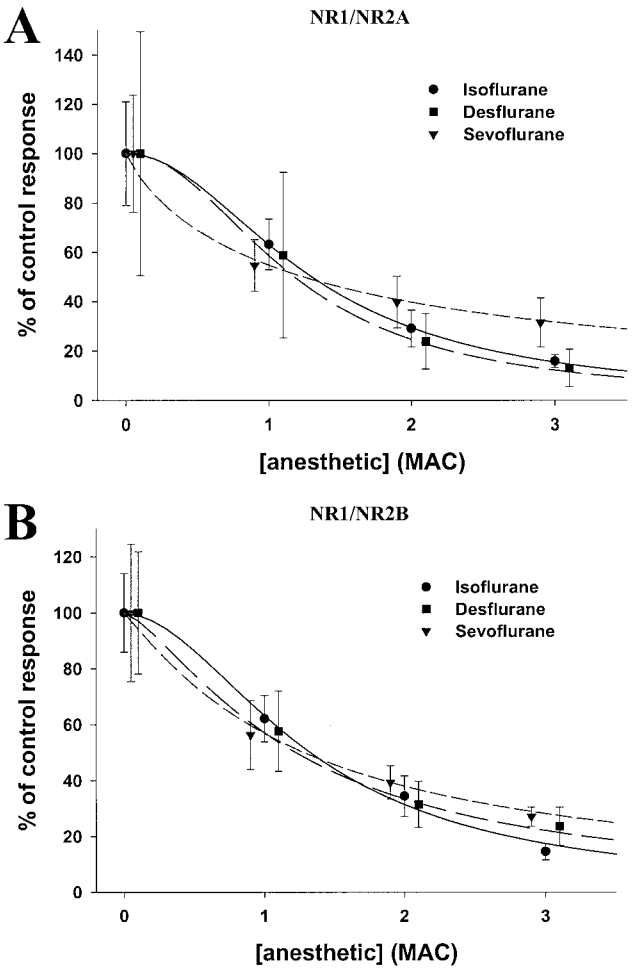

Figure 1. Volatile anesthetic inhibition of *N*-methyl-D-aspartate receptor signaling. A, Volatile anesthetic inhibition of NR1/NR2A receptor functioning. Half-maximal inhibitory concentration is 1.30 ± 0.02 minimum alveolar anesthetic concentration (MAC) for isoflurane, 1.18 ± 0.03 MAC for desflurane, and 1.24 ± 0.06 MAC for sevoflurane. Mean control peak current size is 2.5 ± 0.6 μA. B, Inhibition of NR1/NR2B receptors by volatile anesthetics. Calculated 50% inhibitory concentrations are 1.33 ± 0.12 MAC for isoflurane, 1.22 ± 0.08 MAC for desflurane and 1.28 ± 0.08 MAC for sevoflurane. Mean control peak current size is 2.38 ± 0.6 μA.

in the presence of each of the three anesthetics (administered at IC_{50}). As shown in Figure 2A and B, and Table 1, each of the anesthetics decreased significantly ($P < 0.001$) the maximal glutamate effect (E_{max}). Thus, the inhibitory action of the anesthetics could not be overcome by large agonist concentrations. Even at millimolar concentrations of agonist, response sizes were reduced by >50%.

These results indicate that volatile anesthetics interact with glutamate binding in a noncompetitive manner.

Volatile Anesthetics Inhibit Glycine Signaling in a Noncompetitive Manner

Because glycine is an obligatory co-agonist at the NMDA receptor, its binding site could be a target for volatile anesthetic action. We therefore tested the effects of isoflurane, desflurane, and sevoflurane (at IC_{50}) on glycine signaling (in the presence of glutamate at EC_{50}). As indicated in Figure 2C and D, and Table 1, the anesthetics decreased E_{max} ($P < 0.05$) without affecting EC_{50}. Therefore, volatile anesthetics are unlikely to act on the agonist binding pocket for glycine, but inhibit glycine signaling by an allosteric antagonism.

Mg^{2+} Potentiates the Inhibitory Effect of Volatile Anesthetics on NMDA Receptor Signaling

Next we assessed whether Mg^{2+} enhances the inhibitory effect of volatile anesthetics on NMDA receptor signaling. On both NR1/NR2A and NR1/NR2B receptors, we determined the IC_{50} for each volatile anesthetic alone, and in the presence of Mg^{2+} at IC_{50} (416 μM at NR1/NR2A and 629 μM at NR1/NR2B) (Fig. 3, Table 1). IC_{50} was shifted significantly to the left in the presence of Mg^{2+}. On NR1/NR2A receptors, the reduction in IC_{50} was 28% ($P = 0.02$, *t*-test) for isoflurane, 16% ($P = 0.038$, *t*-test) for sevoflurane, and 24% ($P = 0.035$, *t*-test) for desflurane. On NR1/NR2B receptors, the shift was 30% ($P < 0.001$, *t*-test) for isoflurane, 12% ($P = 0.028$, *t*-test) for sevoflurane, and 14% ($P = 0.024$, *t*-test) for desflurane. There were no significant differences among the three anesthetics for NR1/NR2A receptors ($P = 0.624$), whereas for NR1/NR2B receptors, inhibition by isoflurane was significantly more ($P < 0.05$, analysis of variance, Student-Newman-Keuls) than by the other two anesthetics. Hill coefficients were similar in the presence and absence of Mg^{2+}.

Hence, the presence of Mg^{2+} enhances the inhibitory action of volatile anesthetics on NMDA receptor functioning.

S(+)-Ketamine Potentiates Inhibition of NMDA Receptor Signaling by Volatile Anesthetics

To determine whether S(+)-ketamine also enhances volatile anesthetic inhibition of NMDA receptor functioning, we applied several concentrations of each anesthetic in the presence of S(+)-ketamine at IC_{50} (4.0 μM [NR1/NR2A] and 2.9 μM [NR1/NR2B]), and determined the anesthetic IC_{50} (Fig. 4, Table 1). As did Mg^{2+}, S(+)-ketamine attenuated the anesthetic concentration required to achieve half-maximal inhibition. On NR1/NR2A receptors, calculated IC_{50} was reduced by 30% ($P < 0.001$, *t*-test) for isoflurane, 33% ($P = 0.004$, *t*-test) for sevoflurane, and 24% ($P = 0.004$, *t*-test) for desflurane. On NR1/NR2B receptors, IC_{50} was reduced by 38% ($P = 0.001$, *t*-test) for isoflurane, 33% ($P < 0.001$, *t*-test) for sevoflurane, and 24% ($P =$

Table 1. Pharmacologic Variables Describing Interactions Between Ketamine, Mg^{2+}, and Volatile Anesthetics

	NR1/NR2A				NR1/NR2B			
	EC_{50} (μM/MAC)	E_{max}	Hill coefficient	R	EC_{50} (μM/MAC)	E_{max}	Hill coefficient	R
CI ISO	1.3 (0.01)	—	2.0 (0.04)	1.00	1.3 (0.1)	—	1.9 (0.3)	1.00
CI SEV	1.2 (0.01)	—	0.9 (0.1)	1.00	1.3 (0.07)	—	1.1 (0.1)	1.00
CI DES	1.2 (0.02)	—	2.1 (0.01)	1.00	1.2 (0.08)	—	1.4 (0.2)	1.00
Comp Glu-ISO	2.8 (0.0)	2.1 (0.0)	0.3 (0.0)	1.00	4.9 (1.1)	1.1 (0.04)	0.5 (0.04)	1.00
Comp Glu-SEV	0.71 (0.16)	2.1 (0.1)	0.5 (0.04)	1.00	2.5 (1.1)	1.0 (0.06)	0.5 (0.06)	0.99
Comp Glu-DES	5.8 (0.0)	1.7 (0.0)	0.5 (0.0)	1.00	0.92 (0.0)	1.2 (0.0)	0.4 (0.0)	1.00
Comp Gly-ISO	0.14 (0.05)	1.9 (0.08)	0.5 (0.07)	0.99	0.18 (0.04)	1.5 (0.05)	0.5 (0.04)	1.00
Comp Gly-SEV	0.31 (0.21)	1.6 (0.1)	0.4 (0.07)	0.99	0.14 (0.06)	1.6 (0.09)	0.5 (0.07)	0.99
Comp Gly-DES	0.15 (0.05)	1.8 (0.08)	0.5 (0.07)	0.99	0.21 (0.12)	1.4 (0.1)	0.4 (0.08)	1.00
CI ISO + Mg	0.9 (0.1)	—	1.1 (0.13)	0.99	0.9 (0.04)	—	1.6 (0.1)	1.00
CI SEV + Mg	1.0 (0.07)	—	1.7 (0.17)	0.99	1.1 (0.02)	—	1.2 (0.23)	0.97
CI DES + Mg	0.9 (0.1)	—	1.1 (0.16)	0.99	1.0 (0.03)	—	1.0 (0.18)	0.98
CI ISO+Ket	0.9 (0.08)	—	3.8 (0.63)	0.96	0.8 (0.03)	—	3.9 (0.46)	0.99
CI SEV+Ket	0.8 (0.1)	—	2.6 (0.74)	0.96	0.9 (0.05)	—	3.5 (0.6)	0.98
CI DES+Ket	0.9 (0.08)	—	3.8 (0.49)	0.96	0.9 (0.03)	—	5.1 (0.7)	0.99
CI ISO+Mg+Ket	0.6 (0.05)	—	2.6 (0.05)	0.98	0.6 (0.06)	—	2.8 (0.05)	0.97
CI SEV+Mg+Ket	0.6 (0.07)	—	2.4 (0.58)	0.96	0.6 (0.08)	—	2.3 (0.29)	0.96
CI DES+Mg+Ket	0.7 (0.1)	—	2.8 (0.1)	0.96	0.7 (0.08)	—	2.9 (0.09)	0.96

Data are presented as mean (SEM).
EC_{50} = 50% effective concentration, MAC = minimum alveolar anesthetic concentration, CI = concentration-inhibition-relationship, Glu = glutamate, Gly = glycine, Ket = ketamine, Comp = competition assay, ISO = isoflurane, SEV = sevoflurane, DES = desflurance, R = regression coefficient.

0.006, *t*-test) for desflurane. In contrast to the findings with Mg^{2+}, Hill coefficients were increased significantly in the presence of S(+)-ketamine. Although in the presence of multiple binding sites Hill coefficients are difficult to interpret (8), this difference between Mg^{2+} and ketamine suggests different modes of interaction among these drugs and volatile anesthetics.

Combined Application of Mg^{2+} and S(+)-Ketamine Profoundly Potentiates Inhibition of NMDA Receptor Functioning by Volatile Anesthetics

Results from Part I of this investigation suggest that combined administration of Mg^{2+} and S(+)-ketamine might further potentiate the inhibition of NMDA receptor signaling by volatile anesthetics. We therefore tested the inhibitory action of isoflurane, sevoflurane, and desflurane on glutamate/glycine-induced NMDA receptor currents in the presence and absence of Mg^{2+} and S(+)-ketamine (both at IC_{50}, for Mg^{2+} 416 μM [NR1/NR2A] and 629 μM [NR1/NR2B], and for S(+)-ketamine 4.0 μM [NR1/NR2A] and 2.9 μM [NR1/NR2B]) (Fig. 5, Table 1). IC_{50} for each of the anesthetics was decreased by approximately 50% in the presence of Mg^{2+} and ketamine ($P < 0.001$, *t*-test). Furthermore, the steepness of the inhibition curve (Hill coefficient) for volatile anesthetics was increased significantly in the presence of Mg^{2+} and S(+)-ketamine. As a result, whereas in the absence of Mg^{2+} and ketamine, 1.22 to 1.33 MAC of each volatile anesthetic inhibited NMDA receptors by approximately 50%, in the presence of Mg^{2+} and ketamine, this anesthetic concentration reduced signaling to 5% ± 2.4% of control. These findings indicate that volatile anesthetics, ketamine, and Mg^{2+} interact profoundly at NMDA glutamate receptors, resulting in virtual elimination of receptor signaling at clinically relevant concentrations of all three compounds.

Discussion

Our findings show that clinically relevant concentrations of isoflurane, sevoflurane, or desflurane inhibit functioning of NR1/NR2A and NR1/NR2B glutamate receptors expressed recombinantly in *Xenopus* oocytes. This inhibition is reversible, concentration-dependent, and voltage-insensitive, and results from noncompetitive antagonism of glutamate and glycine signaling, most likely by anesthetic interactions with the NR1 subunit of the receptor molecule. In addition, these effects can be potentiated significantly by co-application of either Mg^{2+}, S(+)-ketamine, or—most profoundly—both. Therefore, the analgesic effects of ketamine and Mg^{2+} are likely to be enhanced in the presence of volatile anesthetics; the cerebral protective effects of the compounds may be potentiated in a similar manner.

Our results provide additional evidence for functional effects of clinical concentrations of volatile anesthetics on NMDA receptors. Such interactions are reported variably in the literature. Kirson et al. (9) found NMDA receptors to be relatively insensitive to

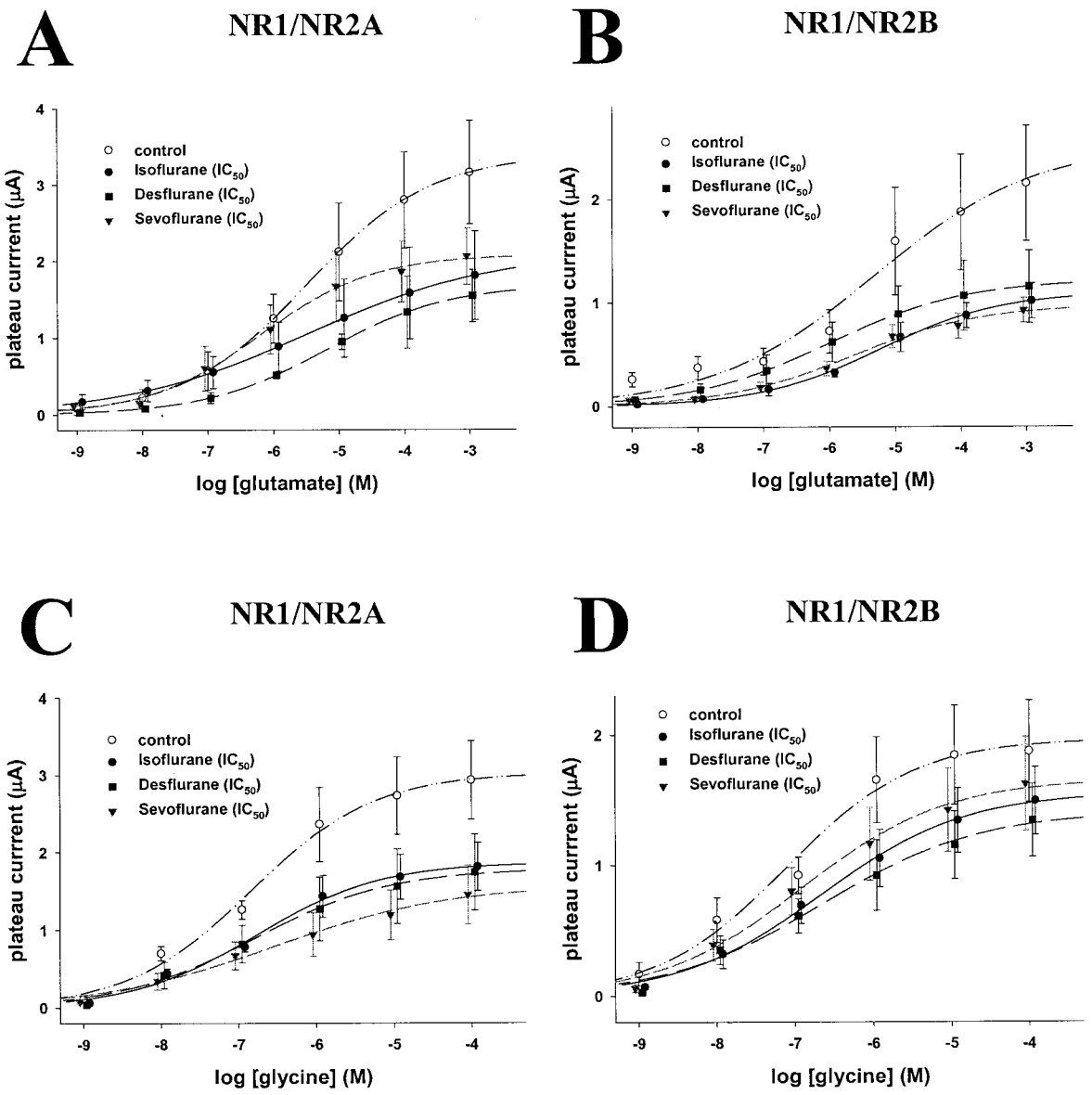

Figure 2. Volatile anesthetic interaction with glutamate and glycine signaling. A, Concentration-response relationship for glutamate on NR1/NR2A receptors in the absence (control) and presence of isoflurane, desflurane, or sevoflurane (at 50% inhibitory concentration [IC_{50}]). Each of the anesthetics decrease significantly ($P < 0.001$) the maximal glutamate effect (E_{max}), from 3.4 ± 0.01 μA under control conditions to 2.1 ± 0.01 μA (isoflurane), 1.7 ± 0.01 μA (desflurane), and 2.1 ± 0.06 μA (sevoflurane). B, Effects of the anesthetics (at IC_{50}) on glutamate signaling of NR1/NR2B receptors. E_{max} under control conditions is 2.5 ± 0.4 μA. This value decreases to 1.1 ± 0.03 μA with isoflurane, 1.2 ± 0.01 μA with desflurane, and 0.9 ± 0.05 μA with sevoflurane. C and D, Isoflurane, desflurane, and sevoflurane (at IC_{50}) effects on glutamate signaling (50% effective concentration for glutamate) in the presence of various concentrations of glycine. The anesthetics decreases E_{max} ($P < 0.05$) without affecting 50% effective concentration. At NR1/NR2A receptors (C), E_{max} is 3.0 ± 0.1 μA under control conditions, but decreases to 1.9 ± 0.09 μA with isoflurane, 1.7 ± 0.08 μA with desflurane, and 1.6 ± 0.1 μA with sevoflurane. At NR1/NR2B receptors, E_{max} is 2.0 ± 0.1 μA under control conditions, and decreases to 1.5 ± 0.05 μA with isoflurane, 1.4 ± 0.1 μA with desflurane, and 1.6 ± 0.08 μA with sevoflurane.

halothane in concentrations <0.64 mM. IC_{50} for halothane on NMDA receptors in these studies was approximately 5.9 mM. In agreement with our data, these investigators showed a voltage-independent mechanism for volatile anesthetic inhibition of NMDA receptors. Black (10) suggests that enflurane increases NMDA receptor activity (consistent with the epileptiform discharges observed during anesthesia with this agent). A study by Pearce et al. (11) showed that volatile anesthetics do not block NMDA receptors in rat hippocampus, because long-term potentiation (which is inhibited by NMDA receptor antagonists) still occurred in the presence of 1.5–2.1 MAC of volatile anesthetic. Many of these studies have limitations (receptor subunit composition not defined, or no functional measurements obtained), and the discrepancies

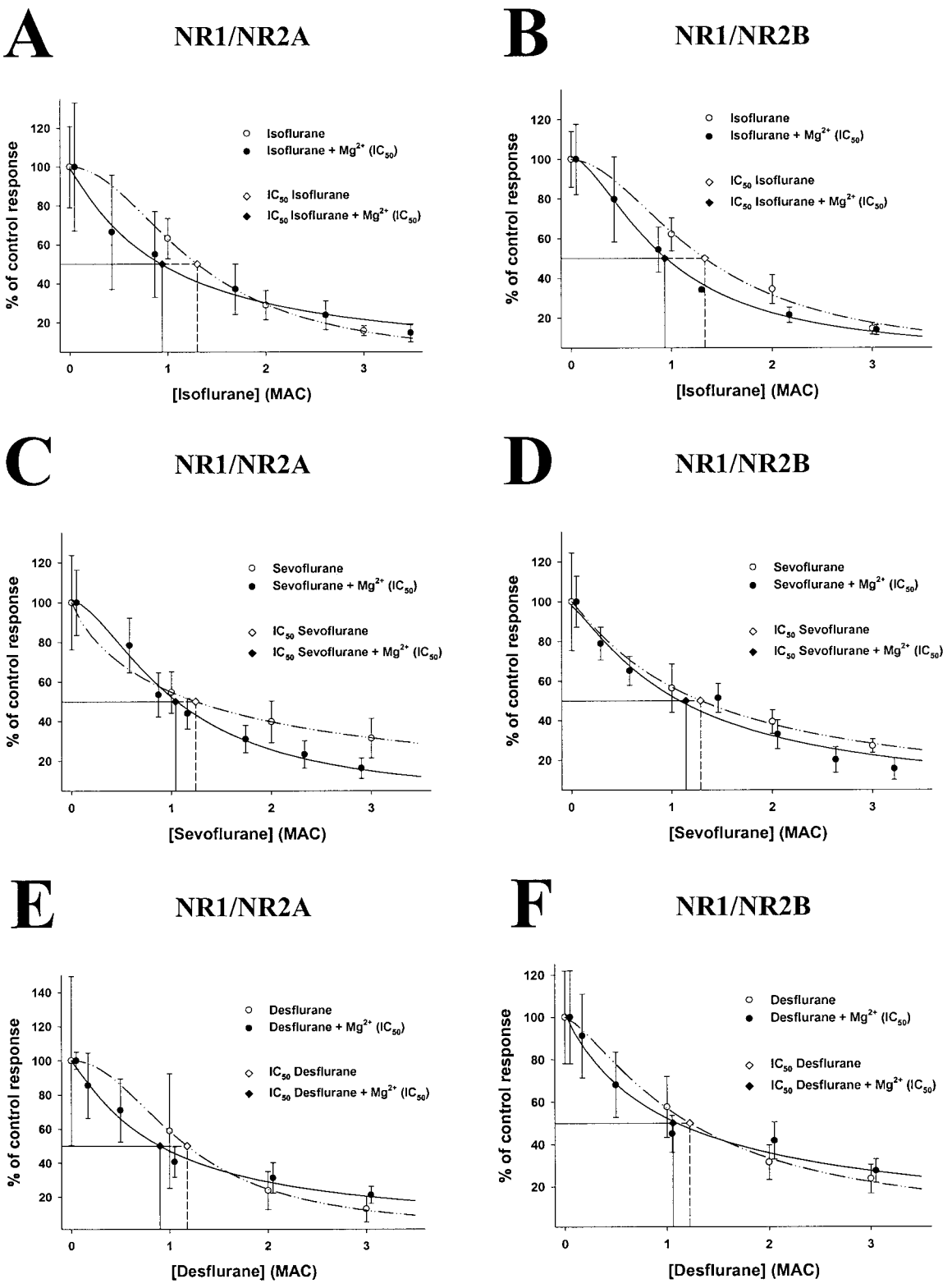

Figure 3. Mg^{2+} potentiates volatile anesthetic inhibition of *N*-methyl-D-aspartate receptor signaling. Concentration-inhibition curves for each volatile anesthetic alone and in the presence of Mg^{2+} at 50% inhibitory concentration (IC_{50}). *N*-methyl-D-aspartate receptors were stimulated by glutamate and glycine at 50% effective concentration. Half-maximal inhibition concentration is significantly shifted to the left in the presence of Mg^{2+}. At NR1/NR2A receptors, Mg^{2+} reduces IC_{50} for isoflurane by 28% (A), sevoflurane by 16% (C), and desflurane by 24% (E). For NR1/NR2B receptors, reduction in IC_{50} is 30% for isoflurane (B), 12% for sevoflurane (D), and 14% for desflurane (F). Mean control peak currents are comparable for each experiment (2.5 ± 0.7 μA). MAC = minimum alveolar anesthetic concentration.

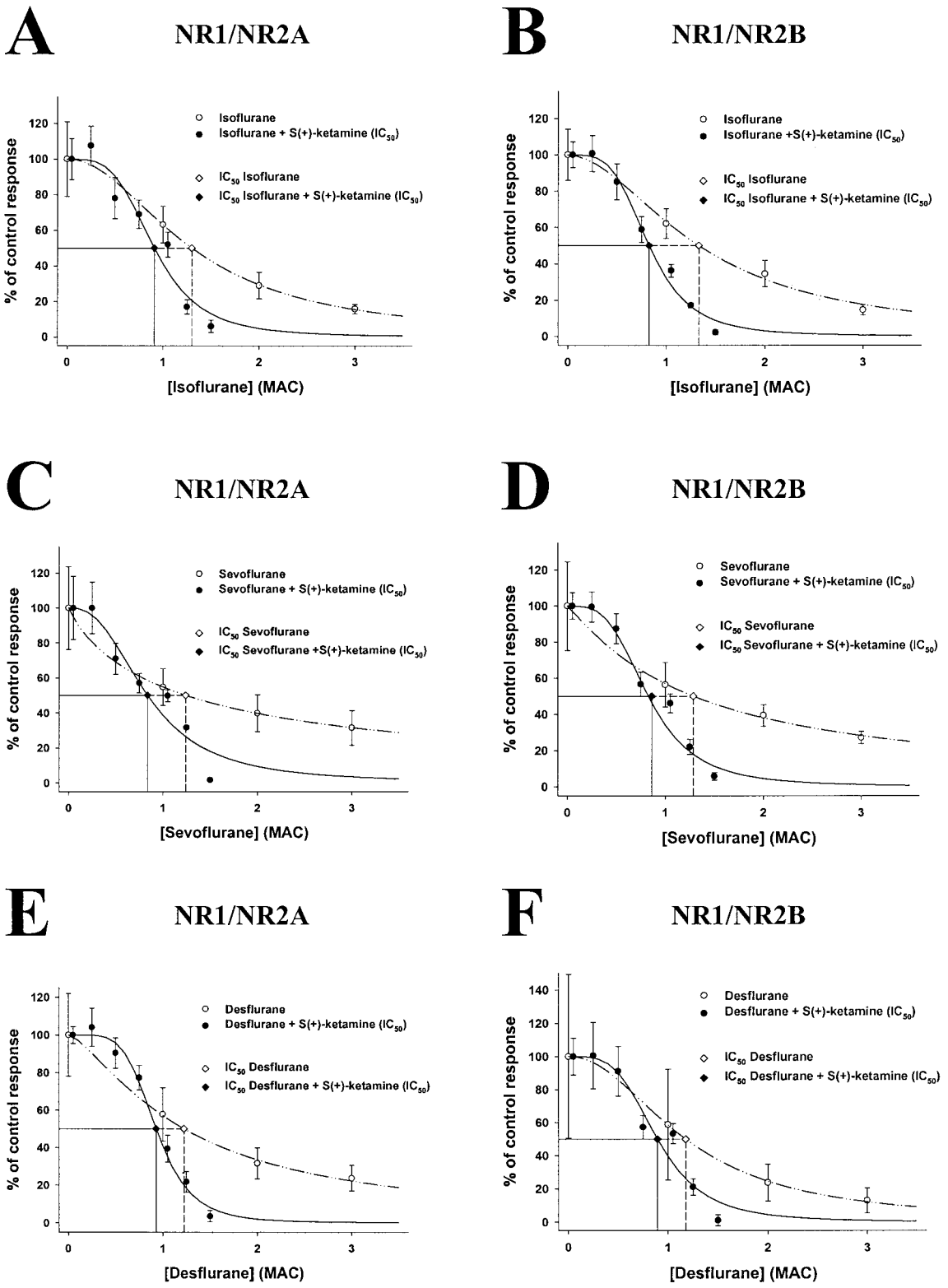

Figure 4. S(+)-ketamine potentiates volatile anesthetic inhibition of *N*-methyl-D-aspartate receptor signaling. Concentration-inhibition relationship for volatile anesthetics alone and while administering S(+)-ketamine at 50% inhibitory concentration (IC_{50}). *N*-methyl-D-aspartate receptors were stimulated by glutamate and glycine at 50% effective concentration. S(+)-ketamine decreases the minimum alveolar anesthetic concentration (MAC) required to achieve half-maximal inhibition on NR1/NR2A receptors for isoflurane (A) by 30%, for sevoflurane (C) by 33%, and for desflurane (E) by 24%. MAC-reducing effects on NR1/NR2B receptors are 38% for isoflurane (B), 33% for sevoflurane (D), and 24% for desflurane (F). Mean control peak currents are comparable for each experiment (2.7 ± 0.6 μA).

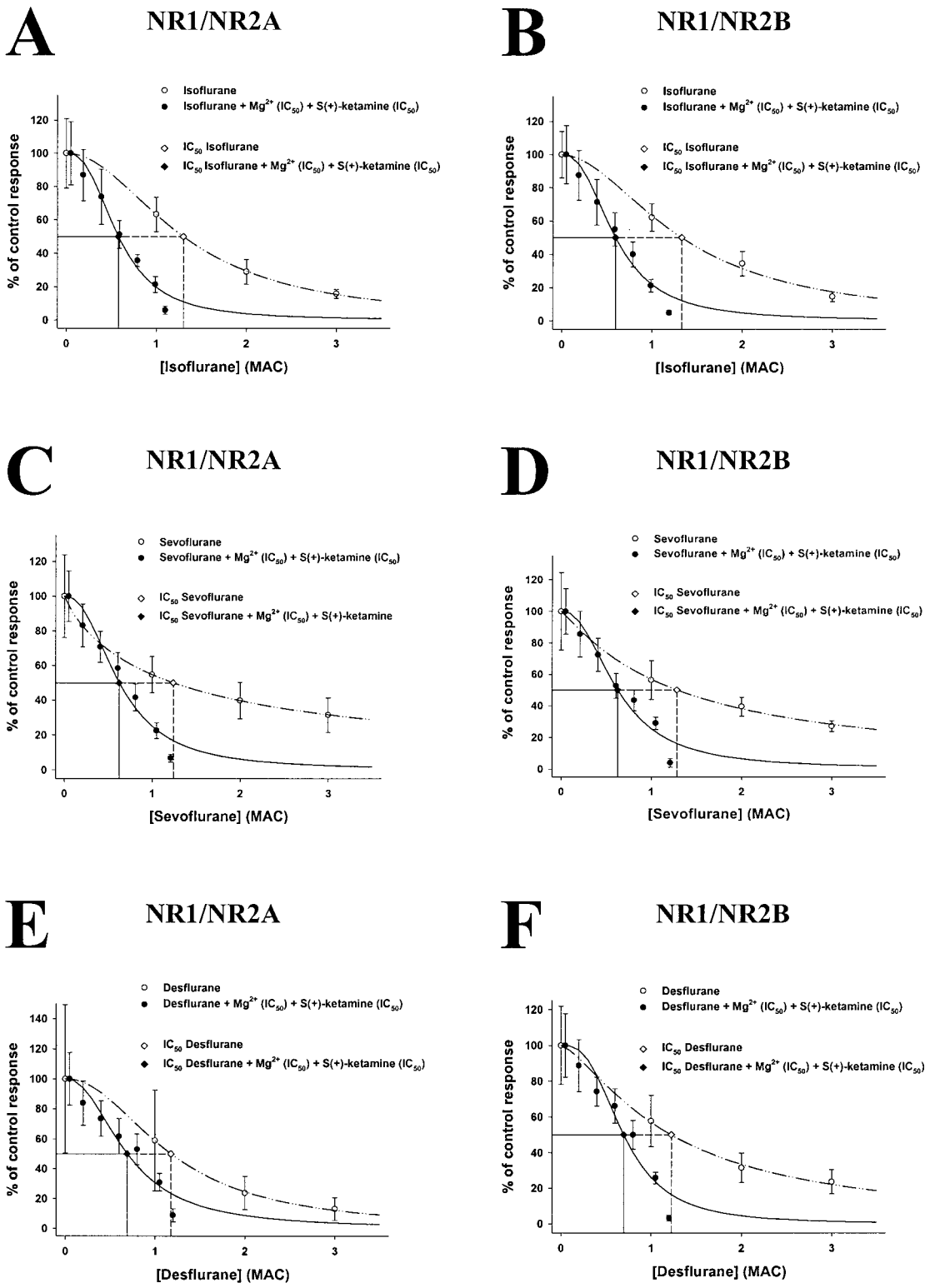

Figure 5. Combined administration of Mg^{2+} and S(+)-ketamine further potentiates inhibition of *N*-methyl-D-aspartate receptor functioning by volatile anesthetics. Inhibitory action of isoflurane, sevoflurane, and desflurane on glutamate/glycine (at 50% effective concentration)-induced *N*-methyl-D-aspartate receptor currents in the presence and absence of Mg^{2+} and S(+)-ketamine (both at 50% inhibitory concentration [IC_{50}]). The minimum alveolar anesthetic concentration (MAC) required for half-maximal inhibition of NR1/NR2A signaling is reduced for isoflurane (A) by 56%, for sevoflurane (C) by 50%, and for desflurane (E) by 42%. For NR1/NR2B receptors, the left shift of IC_{50} is 55% for isoflurane (B), 51% for sevoflurane (D), and 43% for desflurane (F). Mean control peak currents are comparable for each experiment (2.4 ± 0.4 μA).

among these studies and those mentioned below are likely attributable to the model systems used.

In contrast to these reports, our findings are in agreement with many studies supporting anesthetic interference with NMDA glutamate signaling. Isoflurane blocks NMDA-stimulated currents in cultured hippocampal neurons (12). Volatile anesthetics (IC_{50} for isoflurane 0.6–0.9 mM) depress glutamate-dependent intraneuronal translocation of Ca^{2+} (13), and halothane (0.8 mM) and enflurane (1 mM) (14) block NMDA receptor function (15). Glutamate (100 μM)-stimulated [^{3}H]MK-801 binding to the NMDA receptor was suppressed by halothane and enflurane (16,17). Eighty percent xenon reduced NMDA-activated currents by approximately 60% in a noncompetitive manner, suggesting that the NMDA receptor is instrumental in the anesthetic and analgesic effects of this compound (18). Nitrous oxide was suggested to be a mixed competitive/noncompetitive NMDA antagonist (19).

It is of interest that we found volatile anesthetics to be mainly equipotent in their effects on NMDA glutamate signaling despite differences in the corresponding millimolar concentrations. In addition, the measured IC_{50} values were very close to 1 MAC for each of the three compounds. This relationship between anesthetic potency and ability to inhibit NMDA receptors suggests that inhibition of NMDA glutamate signaling in the brain and spinal cord may contribute considerably to the anesthetic state. This hypothesis has been formulated previously (20), and our findings support it completely. If so, our results suggest that the administration of ketamine and Mg^{2+} should have a noticeable effect on volatile anesthetic requirements: in the presence of concentrations of ketamine and Mg^{2+} as used in this study, the IC_{50} of volatile anesthetics on NMDA receptors is shifted to approximately 0.5 MAC (Fig. 5). It would be of interest to test this hypothesis in a clinical study.

In addition, similar effects on NMDA signaling might help to explain why anesthetics with very different effects on cerebral metabolic rates have similar neuroprotective properties (21).

Both S(+)-ketamine and Mg^{2+} enhanced the inhibitory potency of volatile anesthetics on NMDA receptor signaling. These findings can explain results from animal studies showing a dose-dependent volatile anesthetic sparing effect of NMDA receptor antagonists. In rabbits, MK-801 (dizoclipine, plasma level 103 ± 28 ng/mL), a noncompetitive NMDA receptor antagonist, reduced MAC requirements for halothane and isoflurane by 46% and 67%, respectively (4). Whereas 0.3 μM MK-801 decreased isoflurane MAC by 67%, it required an approximately 10-fold larger concentration of S(+)-ketamine (4 μM) to increase isoflurane sensitivity by 30% in our model. This difference is probably attributable to an at least 10-fold more potency of MK-801 as compared with ketamine. Similar results have been reported for other noncompetitive NMDA receptor blockers, like phencyclidine (5), as well as for competitive NMDA receptor antagonists like $_D$-CPP-ene and CGS 19755 (Selfotel) (22). The ability of Mg^{2+} to decrease MAC has similarly been documented (23).

The combined application of ketamine and Mg^{2+} has not been studied. In our study, the combination profoundly enhanced inhibition of NMDA receptor functioning by volatile anesthetics, to the point in which clinically relevant concentrations of the compounds virtually eliminated NMDA receptor signaling. This has implications for neuroprotective and analgesic effects of these compounds. Selective noncompetitive and competitive NMDA receptor antagonists protect against focal cerebral ischemia (24). In agreement, ketamine is neuroprotective, with the S(+)-isomer being significantly more effective than the R(−) form (3). Mg^{2+} appears to have only a modest protective effect. Its benefit may be limited by the depolarization that takes place in damaged neurons, which in turn limits the (voltage-dependent) ability of Mg^{2+} to block NMDA receptors. The neuroprotective actions of volatile anesthetics are also thought to be mediated in part by inhibition of glutamate signaling (25). Volatile anesthetics with very different effects on cerebral metabolic rate show relatively similar neuroprotective potencies; this is in agreement with our findings of similar potency at the NMDA receptor. Our results suggest that combinations of these compounds might be significantly more effective than either compound used alone.

We thank Prof. Dr. med. Eike Martin (Ruprecht-Karls-Universität Heidelberg, Germany) and Prof. Dr. med. Hugo Van Aken (Westfälische-Wilhelms-Universität Muenster, Germany) for their support. Sincere thanks to Dagmar Westerling, MD, PhD, for a preliminary analysis of the results.

References

1. Lo B, Hoenemann CW, Durieux ME. Preemptive analgesia: ketamine and magnesium reduce postoperative morphine requirements after abdominal hysterectomy [abstract]. Anesthesiology 1998;89:A1163.
2. Tsuda T, Kogure K, Nishioka K, Watanabe T. Mg^{2+} administered up to twenty-four hours following reperfusion prevents ischemic damage of the CA1 neurons in the rat hippocampus. Neuroscience 1991;44:335–41.
3. Pfenninger E, Himmelseher S. Neuroprotection by ketamine at the cellular level. Anaesthesist 1997;46 Suppl 1:S47–54.
4. Scheller MS, Zornow MH, Fleischer JE, et al. The noncompetitive *N*-methyl-D-aspartate receptor antagonist, MK-801 profoundly reduces volatile anesthetic requirements in rabbits. Neuropharmacology 1989;28:677–81.
5. Daniell LC. The noncompetitive *N*-methyl-D-aspartate antagonists, MK-801, phencyclidine and ketamine, increase the potency of general anesthetics. Pharmacol Biochem Behav 1990;36:111–5.

6. Daniell LC. Effect of CGS 19755, a competitive *N*-methyl-D-aspartate antagonist, on general anesthetic potency. Pharmacol Biochem Behav 1991;40:767–9.
7. Franks NP, Lieb WR. Selective actions of volatile general anaesthetics at molecular and cellular levels. Br J Anaesth 1993;71:65–76.
8. Weiss JN. The Hill equation revisited: uses and misuses. FASEB J 1997;11:835–41.
9. Kirson ED, Yaari Y, Perouansky M. Presynaptic and postsynaptic actions of halothane at glutamatergic synapses in the mouse hippocampus. Br J Pharmacol 1998;124:1607–14.
10. Black GW. Enflurane. Br J Anaesth 1979;51:627–40.
11. Pearce RA, Stringer JL, Lothman EW. Effect of volatile anesthetics on synaptic transmission in the rat hippocampus. Anesthesiology 1989;71:591–8.
12. Yang J, Zorumski CF. Effects of isoflurane on *N*-methyl-D-aspartate gated ion channels in cultured rat hippocampal neurons. Ann NY Acad Sci 1991;625:287–9.
13. Puil E, El-Beheiry H, Baimbridge KG. Anesthetic effects on glutamate-stimulated increase in intraneuronal calcium. J Pharmacol Exp Ther 1990;255:955–61.
14. Aronstam RS, Martin DC, Dennison RL. Volatile anesthetics inhibit NMDA-stimulated 45Ca uptake by rat brain microvesicles. Neurochem Res 1994;19:1515–20.
15. Martin DC, Plagenhoef M, Abraham J, et al. Volatile anesthetics and glutamate activation of *N*-methyl-D-aspartate receptors. Biochem Pharmacol 1995;49:809–17.
16. Martin DC, Aronstam RS. Spermidine attenuation of volatile anesthetic inhibition of glutamate-stimulated [3H](5D,10S)-(+)-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine ([3H]MK-801) binding to *N*-methyl-D-aspartate (NMDA) receptors in rat brain. Biochem Pharmacol 1995;50:1373–7.
17. Martin DC, Abraham JE, Plagenhoef M, Aronstam RS. Volatile anesthetics and NMDA receptors: enflurane inhibition of glutamate-stimulated [3H]MK-801 binding and reversal by glycine. Neurosci Lett 1991;132:73–6.
18. Franks NP, Dickinson R, de Sousa SL, et al. How does xenon produce anaesthesia? Nature 1998;396:324.
19. Jevtovic-Todorovic V, Todorovic SM, Mennerick S, et al. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. Nat Med 1998;4:460–3.
20. Franks NP, Lieb WR. Molecular and cellular mechanisms of general anaesthesia. Nature 1994;367:607–14.
21. Warner DS, Zhou JG, Ramani R, Todd MM. Reversible focal ischemia in the rat: effects of halothane, isoflurane, and methohexital anesthesia. J Cereb Blood Flow Metab 1991;11:794–802.
22. Kuroda Y, Strebel S, Rafferty C, Bullock R. Neuroprotective doses of *N*-methyl-D-aspartate receptor antagonists profoundly reduce the minimum alveolar anesthetic concentration (MAC) for isoflurane in rats. Anesth Analg 1993;77:795–800.
23. Thompson SW, Moscicki JC, DiFazio CA. The anesthetic contribution of magnesium sulfate and ritodrine hydrochloride in rats. Anesth Analg 1988;67:31–4.
24. Ozyurt E, Graham DI, Woodruff GN, McCulloch J. Protective effect of the glutamate antagonist, MK-801 in focal cerebral ischemia in the cat. J Cereb Blood Flow Metab 1988;8:138–43.
25. Eilers H, Bickler PE. Hypothermia and isoflurane similarly inhibit glutamate release evoked by chemical anoxia in cortical brain slices. Anesthesiology 1996;85:600–7.