Coadministered Nitrous Oxide Enhances the Effect of Isoflurane on GABAergic Transmission by an Increase in Open-Channel Block

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ABSTRACT

Clinically relevant concentrations of isoflurane (ISO) and nitrous oxide (N2O) enhance chloride currents induced by activating γ-aminobutyric acidA receptors (GABAAR). Channel blocking by ISO overcomes the enhancing effect at higher concentrations. In this study, the effect of coadministered ISO and N2O on responses evoked by GABA in transfected human embryonic kidney 293 cells carrying α2β2γ2L GABAAR was investigated. Patch-clamp recordings from these cells were performed in the whole cell mode. A piezo-driven “liquid filament” drug application system was used to apply solutions of GABA, ISO, and N2O. Increasing the concentration of ISO in steps from 0.15 to 1.2 mM resulted in a bell-shaped concentration-response curve for GABA-induced currents. The maximum increase in current (1.51 ± 0.14-fold) was seen at 0.45 mM ISO (about 1 minimum alveolar concentration, EC50). N2O (29.2 mM) increased GABA-evoked currents 1.54 ± 0.10-fold. The enhancing effects of ISO and N2O on the GABAergic response were not additive. However, a transient current, associated with the rapid withdrawal of ISO from the receptor, was markedly increased by N2O. Such rebound currents probably reflect the transition from a “channel-blocked” to a “reopened” state. An open-channel block at ligand-gated receptors can prolong postsynaptic currents. Thus, we conclude that coadministered N2O could increase the enhancing effect of ISO on the GABAergic transmission by an increase in open-channel block at the GABAAR.

With a minimum alveolar concentration (MAC) of 104% in humans, nitrous oxide (N2O) would require hyperbaric conditions to act as an anesthetic gas (Gonsowski and Eger, 1994). Therefore, in clinical practice, N2O is usually combined with other anesthetics, such as isoflurane (ISO). N2O has been reported to affect various ligand-gated ion channels, e.g., glutamate receptors (Macdonald and Ramsey, 1995; Jevtovic-Todorovic et al., 1998) and nicotinic acetylcholine receptors (Wachtel, 1995). Currently used intravenous and volatile anesthetics affect the γ-aminobutyric acidA receptor (GABAAR) (Tanelian et al., 1993). The activation of this ligand-gated chloride channel is of pivotal importance for synaptic inhibition (Möller et al., 1996). A receptor assembly, consisting of 2α1, 2β2, and 1γ2 subunits, predominates in the mammalian brain (Chang et al., 1996; Möller et al., 1996). Several animal studies demonstrate the involvement of the GABAAR system in the effects of N2O on, e.g., visually evoked potentials (Dzoljic et al., 1996), analgesia (Emmanouil and Quock, 1989), and anxiolysis (Emmanouil et al., 1994). A direct enhancing effect of N2O on a GABA-evoked response has been shown in acutely dissociated hippocampal neurons (Dzoljic and van Duijn, 1998).

Most studies report a potentiating effect of volatile anesthetics on GABAAR channels, i.e., an increase in GABA-evoked chloride flux (Moody et al., 1988; Harrison et al., 1993; Zimmerman et al., 1999). Some studies also found an additional blocking effect of ISO on the GABA-evoked response (Edwards and Lees, 1997; Adelsberger et al., 1998; Neumahr et al., 2000). However, a block of GABAergic transmission is not easily reconciled with the apparent decrease of neuronal excitability during anesthesia. Various studies suggest that volatile anesthetics, such as ISO, halothane, and enfurane, lower the excitability of central neurons by prolonging the decay of GABA-mediated inhibitory postsynaptic currents (IPSCs) (Jones and Harrison, 1993). Other studies report that volatile anesthetics prolong the decay and reduce the amplitude of GABAergic IPSCs (Banks and Pearce, 1999). It has been suggested that a prolonged

ABBREVIATIONS: MAC, minimum alveolar concentration; ISO, isoflurane; GABAAR, A-type receptor for γ-aminobutyric acid; GABA, γ-aminobutyric acid; IPSC, inhibitory postsynaptic current; HEK, human embryonic kidney.
flickering of GABAAR channels, which is caused by the anesthetic blocking the channel pore, could be responsible for the increased duration of IPSCs (Jones and Harrison, 1993). Evidence in favor of this assumption comes from observations made at the nociceptin acetylcholine receptor. At this receptor, the single channel burst duration increases on application of channel-blocking compounds (Beam, 1976; Neher and Steinbach, 1978).

There is evidence from studies on GABA receptors in insects that ISO apparently shares the binding site for picrotoxin (Edwards and Lees, 1997), a GABA antagonist that binds to the channel lumen of GABAAR (Gurley et al., 1995). An open-channel block by ISO was also suggested by studies performed at a GABAergic crayfish muscle synapse (Adelsberger et al., 1998). In addition to its effects at the GABAAR, ISO is an open-channel blocker at the nicotinic acetylcholine receptor (Scheller et al., 1997). At this site, ISO elicits a transient increase in the agonist-evoked current, when the agent is rapidly withdrawn from the receptor. Such rebound currents are established features of open-channel blockers (Dilger and Liu, 1992). They are considered to signal the unbinding of an open-channel blocker from its binding site in the channel pore (Scheller et al., 1997; Adelsberger et al., 1998; Neumahr et al., 2000).

In the present study, the effects of coadministered ISO and N2O were investigated on GABA channel activity to determine whether the additive effect observed clinically could be explained by interactions with the GABAAR.

Materials and Methods

Cell Preparation. Human embryonic kidney cells (HEK293; Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) were maintained in minimum essential medium, supplemented with 10% fetal calf serum, 4 mM l-glutamine, 100 U/ml of penicillin, and 100 U/ml of streptomycin, in an atmosphere of 5% CO2, 95% air, and 100% relative humidity at 37°C.

Transfection was performed, using an electroporation system (Biotechnologies and Experimental Research, Inc., San Diego, CA). The cells were cotransfected with plasmids containing cDNAs for rat α1, β2, and γ2 GABAAR receptor subunits, respectively. cDNA for green fluorescent protein as an expression marker was cotransfected. After harvesting, the cells were suspended in a buffer used for transfection (distilled H2O containing 50 mM K2HPO4·3H2O, 20 mM K+ -acetate, 25 mM MgSO4·7H2O, pH 7.35). Plasmids containing cDNAs for the GABAAR receptor subunits (5 μg for each subunit) and for green fluorescent protein (10 μg) were added to the cell suspension. Electroporation was performed at 290 V and 1 mF with a pulse time of 30 to 45 ms. Transfected cells were replaced in 10-× 35-mm culture dishes with supplemented medium and incubated (5% CO2, 95% air, and 100% relative humidity, 37°C) for 12 to 18 h before the experiments.

Electrophysiology. For the experiments, performed at 20–23°C, the medium was replaced by extracellular solution containing 162 mM NaCl, 5.3 mM KCl, 0.67 mM Na2HPO4, 0.22 mM KH2PO4, 2 mM CaCl2, 15 mM HEPES, 5.6 mM glucose, pH 7.4 adjusted with NaOH. The patch-clamp technique was used to measure GABA-evoked chloride currents under whole-cell voltage-clamp (−30 mV) conditions. Borosilicate glass pipettes (GC150TF-10; Clark Electromedical Instruments, Pangbourne Reading, UK) were pulled, using a two-step
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Fig. 2. A, within each experiment, GABA (5 × 10⁻⁶ M, control) was applied to the whole cell patches, with or without N₂O (29.2 mM), and with or without increasing concentrations of ISO. Solutions were prepared as described under Materials and Methods. The amplitude of the respective control current (GABA alone) was set for 1.0. N₂O alone increased the currents through α₁β₂γ₂ GABA₃R channels 1.54 ± 0.10-fold (n = 47) versus control (5 × 10⁻⁶ M GABA). GABA, combined with increasing concentrations of ISO, revealed a bell-shaped concentration-response curve. 0.15, 0.45, 0.75, 1.0, and 1.2 mM ISO resulted in 1.31 ± 0.14-, 1.49 ± 0.19-, 1.27 ± 0.17-, and 0.83 ± 0.12-fold current amplitudes, in combination with N₂O, 0.15, 0.45, 0.75, 1.0, and 1.2 mM ISO resulted in 1.57 ± 0.14-, 1.64 ± 0.16-, 1.63 ± 0.22-, 1.25 ± 0.16-, and 0.82 ± 0.10-fold current amplitudes (means ± S.E.M., current amplitudes were normalized to the control; *p < 0.05, **p < 0.01 versus control, n = 7–15 for each concentration of ISO). B, raw current traces are shown: N₂O and 0.6 mM ISO increased the GABA-induced current from −466 pA (control) to −686 pA and −679 pA, respectively. N₂O and 0.6 mM ISO combined were not worth mentioning as an additive in increasing the GABA-induced current (~719 pA). A rebound current, induced by the withdrawal of 0.6 mM ISO, was reversibly increased by N₂O. C, amplitudes of these rebound currents increased with increasing concentrations of ISO. N₂O further increased the rebound currents (means ± S.E.M., *p < 0.05 ISO + N₂O versus ISO, n = 5–13 for each concentration of ISO).

horizontal puller (Zeitz Instruments, Augsburg, Germany), and heat polished. The resulting tips had a series resistance of 4 to 9 MΩ. Pipettes were filled with intracellular solution containing 140 mM KCl, 11 mM EGTA, 10 mM HEPES, 10 mM glucose, 2 mM MgCl₂, 1 mM CaCl₂, pH 7.3 adjusted with KOH. GABA-induced currents were recorded with an Axopatch 200B patch-clamp amplifier, low-pass filtered at a cutoff frequency of 5 kHz, and then digitized at 10 kHz with a digidata 1200 A/D converter, performed with pClamp 6.0 software (all from Axon Instruments, Foster City, CA).

After formation of a giga seal, cell membrane rupture resulted in the whole-cell configuration, monitored by a voltage test pulse (5 mV for 100 ms). Cell membrane capacitance and serial resistance was compensated by the patch-clamp amplifier. Nonspecific linear leak current was negligible.

Agonist and Drug Application. To match the rapid kinetics of ligand-activated ion channels, a piezo-driven system for fast exchange of solutions was used (Franke et al., 1987). GABA was applied alone or combined with the drug under investigation to the whole cell patches. The drugs were administered to the cell via a “liquid filament”, i.e., a tiny jet of solution, discharged from a borosilicate glass tube (inner diameter 0.15 mm) inside the recording chamber, which was perfused by extracellular solution (Fig. 1A). This technique allows for a complete exchange of solutions in the vicinity of the cell, held in the whole cell mode, within 1 ms as measured by activation of voltage-operated calcium channels in separate experiments (data not shown). The liquid filament consisted of extracellular solution containing indicated concentrations of GABA alone (controls) or in combination with the respective agent (test solution). The test solution was applied to the whole cell patch in pulses of 1.5 s. An interval of 10 s between the pulses allowed full recovery of the GABA₃R channels from a desensitized state. Each current trace was averaged from at least three stable responses. N₂O was bubbled through a 10-ml vial, containing the GABA solution, with a continuous flow of 20 ml/min for at least 3.5 min. The container was sealed with a rubber top, punctured with a drain tube as an escape hole. A closed glass syringe served as reservoir. N₂O was studied at saturation. In control experiments, a saturated solution of ISO was prepared by adding a surplus of ISO₂ to the GABA solution. The test solution was applied to the whole cell patch in pulses of 1.5 s. An interval of 10 s between the pulses allowed full recovery of the GABA₃R channels from a desensitized state. Each current trace was averaged from at least three stable responses. N₂O was bubbled through a 10-ml vial, containing the GABA solution, with a continuous flow of 20 ml/min for at least 3.5 min. The container was sealed with a rubber top, punctured with a drain tube as an escape hole. A closed glass syringe served as reservoir. N₂O was studied at saturation. In control experiments, a saturated solution of oxygen or helium was used instead of N₂O. Oxygen or helium was bubbled through the 10-ml vial by the same procedure performed with N₂O. Thus, the GABA solution was either saturated with oxygen, or oxygen was indirectly removed from the solution via the gaseous phase of helium within the vial. Both oxygen, and replacing oxygen by helium, had no effect on the GABA-evoked currents.

A saturated solution of ISO was prepared by adding a surplus of the anesthetic to the extracellular solution, and by stirring in a closed glass bottle for at least 3 h under airtight conditions. The maximum solubility of ISO in extracellular solution at room temperature was 15 mM, measured by gas chromatography. Defined concentrations of ISO were prepared by diluting the saturated solution. To control the concentrations of ISO prepared and applied under our experimental conditions, the probes were passed through the appli-
cation system, collected, and analyzed by gas chromatography. The differences between the calculated and the measured concentrations were less than 15% (Scheller et al., 1997). The MAC equivalent for ISO was calculated to 0.5 mM, using a Bunsen water/gas partition coefficient of 1.08 at 25°C (Firestone et al., 1986), similar to the value of 0.51 mM reported by others (Jones and Harrison, 1993). The range of concentrations of ISO used in this study was 0.075 to 1.2 mM. To apply ISO, combined with N2O, via the liquid filament, aliquots of ISO solutions were added to a freshly prepared N2O solution in a tightly sealed glass syringe, to achieve final ISO concentrations of 0.075 to 1.2 mM. For each concentration of ISO (with and without N2O), one whole cell patch was used. All solutions were freshly prepared and used within 20 s. No change of pH was observed after addition of any agent to the extracellular solution.

**MAC and Solubility of N2O.** The MAC value of N2O in humans is 1.04 atm (Hornbein et al., 1982). Based on the solubility coefficient for 37°C (Wilhelm et al., 1977), the MAC equivalent for dissolved N2O was calculated to 20.6 mM. The solubility of N2O in the extracellular solution, prepared for this study, was measured using a technique for a volumetric evaluation of the solubility of gases in fluids (Krauss and Gestrich, 1977). This technique was modified by Dr. Karl-Heinz Meister (Linde AG, Höllriegelskreuth, Germany). The measurements were performed at 20°C and 1 atm. N2O was applied to the extracellular solution under the same conditions as in the patch-clamp experiments. Saturation (>95%) was achieved within 2.5 ± 0.3 min. A calculated solubility coefficient of 0.654 ± 0.010 [published value for N2O in H2O (20°C, 1 atm) is 0.6788 (Wilhelm et al., 1977)], resulted in a concentration of N2O of 29.2 ± 0.4 mM in the extracellular solution at 20°C and 1 atm.

**Statistical Analysis.** Peak current and time to peak (10–90%) were measured using automated detection algorithms (AxoGraph software for MacOS). Data are presented as means ± S.E.M. with the number of experiments indicated. Statistical analysis was performed using Student’s paired t test (p < 0.05 was considered as significant).

**Sources of Anesthetics and Chemicals.** GABA was obtained from Sigma Chemical Co. (St. Louis, MO), N2O from Linde AG, and isoflurane (Forene) from Deutsche Abbott GmbH (Wiesbaden, Germany).

**Results**

**Agonist Application Using the Piezo-Driven Liquid Filament.** At recombinant α1β2γ2 GABA_A,R channels, transiently expressed in HEK293 cells, GABA (5 × 10^-6 M) elic-
ited 26 ± 3% (n = 28) of the maximum GABA response, evoked by a saturating concentration of GABA (10⁻³ M). GABA was applied by means of a piezo-driven liquid filament switch to cells recorded in the whole cell patch mode (Fig. 1A). The responses to GABA (5 × 10⁻⁶ M), which was used in the following experiments as a standard test, did not desensitize (Fig. 1B) and reversed at 0 mV, corresponding to the equilibrium potential for chloride ions under the chosen experimental conditions.

**Biphasic Effect of ISO on GABA-Induced Currents.**
Increasing the concentration of ISO in steps from 0.15 to 1.2 mM resulted in a bell-shaped concentration-response curve for GABA-induced currents. The maximum increase in current (1.51 ± 0.14-fold) was seen at 0.45 mM ISO (Fig. 2A). This finding suggests a dual effect of ISO on GABA-induced currents, i.e., an enhancing effect that predominates at lower ISO concentrations (<0.8 mM), and a prevailing blocking effect at higher ISO concentrations (>0.8 mM).

**N₂O Increased GABA-Induced Currents.**
The extracellular solution containing GABA (5 × 10⁻⁶ M) was saturated with N₂O and applied immediately. The concentration of N₂O was evaluated as described under Materials and Methods. N₂O (29.2 mM) significantly increased the response to GABA 1.54 ± 0.10-fold (n = 47, Fig. 2A). After washout, the effect of N₂O was reversible (data not shown; for details, see Hapfelmeier et al., 2000).

**Coapplication of ISO and N₂O.** ISO and N₂O were co-applied and the effect on GABA-induced currents was studied. On the one hand, the enhancing effect of N₂O on the GABA-evoked response was not significantly affected by the addition of ISO (Fig. 2A). On the other hand, there was no significant difference between the potentiating effect of ISO alone and ISO co-applied with N₂O (Fig. 2A).

**ISO Additionally Induced an Open-Channel Block.** The rapid withdrawal of ISO from the whole cell patch induced a transient increase in the current response (Fig. 2B). This rebound current increased with increasing ISO concentrations (Fig. 2C). The rebound currents suggest that ISO evokes a dose-dependent open-channel block at the α₁β₂γ₂ GABAAR. Since the blocking effect of ISO is more prominent at high concentrations, the phenomena of rebound currents was also studied applying 1.5 and 15 mM ISO combined with a range of GABA concentrations (10⁻⁹–10⁻³ M). GABA was applied alone (Fig. 3A) and combined with 15 mM ISO (Fig. 3, B and C) or 1.5 mM ISO (Fig. 3C). An increasing number of blocked GABAAR, prior to the unbinding of ISO, is probably the reason for the clear dose-response relationship between the amplitude of the rebound current and the concentration of ISO and GABA, respectively (Fig. 3, B and C). It is a widely held belief (Scheller et al., 1997; Adelsberger et al., 1998; Neumahr et al., 2000) that these rebound currents reflect the transition from a channel-blocked to a channel...
reopened state. Compatible with this view, similar rebound currents were observed, when the GABA<sub>R</sub> channel blocker picrotoxin or the volatile anesthetic sevoflurane was applied. In contrast, the rapid withdrawal of the competitive antagonist bicuculline did not induce any rebound currents (Fig. 4).

**N<sub>2</sub>O Increased the Rebound Currents.** The rebound currents induced by the withdrawal of ISO were significantly increased by N<sub>2</sub>O (29.2 mM) (Fig. 2, B and C). N<sub>2</sub>O (29.2 mM), which increased the response to GABA, neither enhanced the potentiating effect of ISO, nor did it induce any rebound current (Fig. 2B). These findings suggest that, in contrast to ISO, N<sub>2</sub>O exhibited no channel block at the GABA<sub>R</sub>.

**Modeling of the Effects of N<sub>2</sub>O and ISO.** Our experimental data (Fig. 5) can be well described by a modified kinetic model (Fig. 6) for the GABA<sub>R</sub> (Jones and Westbrook, 1995; Haas and Macdonald, 1999). In respect of the theories of kinetic modeling (Colquhoun and Hawkes, 1981; Colquhoun and Sakmann, 1985), the rate constants were chosen based on our experimental data and kinetic studies on the GABA<sub>R</sub> used in this study. Macroscopic current modeling was performed using BIOQ-Biochemical Equations software (Parnas & Parnas Neurobiology Lab, Hebrew University, Jerusalem, Israel). The simulated currents, which fit well to the experimental data, are shown in Fig. 7.

The GABA-induced current that was increased by N<sub>2</sub>O (ISO) had a decreased (increased) time to peak (Fig. 5, B and C). Using the model, we explained these findings by an N<sub>2</sub>O-induced (ISO-induced) increase in <i>K<sub>on</sub></i> (decrease in <i>K<sub>off</sub></i>) of GABA (Fig. 6, B and C). The rebound current following the rapid withdrawal of ISO (Fig. 5C) was explained by the recovery from an open-channel block by ISO (Fig. 6C). The effect of coadministered N<sub>2</sub>O and ISO on current amplitude, time to peak, and rebound current (Fig. 5D) was well predicted by the combination of the models B and C from Fig. 6, resulting in Fig. 6D.

**Discussion**

In this study, we investigated the effects of ISO and N<sub>2</sub>O alone or in combination on GABA-induced currents recorded from HEK293 cells expressing a rat recombinant α<sub>1</sub>β<sub>2</sub>γ<sub>2L</sub> GABA<sub>R</sub>. We used a piezo-driven application system for fast exchange of solutions (Fig. 1).

**Effects of N<sub>2</sub>O and ISO on GABA-Induced Currents.** The present experiments show that both N<sub>2</sub>O and ISO at clinically relevant concentrations enhanced GABA-induced currents in cells carrying the most ubiquitous GABA<sub>R</sub> assembly (α<sub>1</sub>β<sub>2</sub>γ<sub>2L</sub>) present in the mammalian central nervous system. However, N<sub>2</sub>O and ISO are different in affecting the activation kinetics of the currents. According to the presented model, it is possible that N<sub>2</sub>O and ISO independently interact with the GABA<sub>R</sub>. Furthermore, in contrast to N<sub>2</sub>O, ISO additionally induces a blocking effect that becomes more prominent at higher concentrations.

It was shown previously that N<sub>2</sub>O increases the GABA-evoked response at this type of GABA<sub>Rs</sub> expressed in HEK293 cells (Hapfelmeier et al., 2000). This finding is also in line with other studies using cultured hippocampal neurons or transfected *Xenopus* oocytes (Dzoljic and van Duijn, 1998; Yamakura and Harris, 2000).

The observation that ISO enhanced GABA responses at low concentrations, and dose dependently reduced the response to GABA at higher concentrations, is reflected by the bell-shaped concentration-response curve (Fig. 2A). This dual effect of ISO on GABA-evoked currents was also reported previously (Neumahr et al., 2000). In the present study, the maximum increase in GABA-induced currents by ISO was seen in the millimolar concentration range, which corresponds to the MAC equivalent of ISO (Firestone et al., 1986; Jones and Harrison, 1993).

**ISO Induces an Open-Channel Block at the GABA<sub>R</sub>.**

Conspicuously, the rapid withdrawal of ISO induced a marked dose-dependent transient increase in the response to GABA. The most parsimonious explanation for this observation is that ISO evokes a dose-dependent open-channel block...
at the $\alpha_1\beta_2\gamma_2$ GABA$_A$R (Adelsberger et al., 1998; Neumahr et al., 2000). Compatible with our view, rebound currents were observed, when the GABA$_A$R channel blocker picrotoxin (see Introduction) was applied (Fig. 4). Both ISO and picrotoxin bind to a site inside the channel lumen (Edwards and Lees, 1997). Similar rebound currents were seen applying channel blockers at the nicotinic acetylcholine receptor (Dilger and Liu, 1992; Scheller et al., 1997).

The dose-dependence of this effect of ISO suggests that an increasing number of channels is blocked when the concentration of the anesthetic is increased. In line with this assumption is the observation that increasing the GABA concentration, which provokes the opening of more GABA$_A$R channels, also leads to an increase in the rebound current (Fig. 3).

An alternative theory for GABA$_A$R channel reopenings is given by others (Jones and Westbrook, 1995). The authors postulated that channel reopenings are caused by the recovery from desensitized states of the receptors. Desensitization is defined as a conformational change without any binding steps (Jones and Westbrook, 1995; Adelsberger et al., 1998). A desensitized receptor is inactive and insensitive for the agonist. The time required for resensitization of the GABA$_A$R studied is several seconds (Jahn et al., 1997). In our opinion, this is too long to elicit rebound currents with a time to peak of 10 to 50 ms.

**Effects of Coadministered N$_2$O and ISO.** When coadministered, the enhancing effects of ISO and N$_2$O on the fast GABA-induced response were not additive. However, the rebound currents elicited by the rapid withdrawal of ISO were clearly enhanced by N$_2$O. This suggests that the ISO-induced open-channel block at the $\alpha_1\beta_2\gamma_2$ GABA$_A$R was enhanced by N$_2$O. An additional enhancement of GABA$_A$R activation by N$_2$O (our data; Dzoljic and van Duijn, 1998; Yamakura and Harris, 2000), independent from its interaction with ISO, might even provide more open channels to be blocked by ISO. Based on a modified model of the GABA$_A$R (Haas and MacDonald, 1999), the suggested mechanism is depicted in Fig. 6.
Impact of Open-Channel Block for Synaptic Transmission. It is a widely held assumption that an open-channel block delays the transition from an "open" to a "closed" state of a channel. This delay induces a channel flickering with prolonged single channel burst durations (Neher and Steinbach, 1978), which may result in prolonged GABA-IPSCs (Jones and Harrison, 1993) or in prolonged cholinergic neuromuscular transmission (Legendre et al., 2000). A prolongation of GABAAR-mediated IPSCs by volatile anesthetics was observed at the ISO concentration of 0.6 mM (Banks and Pearce, 1999). The prolongation of these IPSCs will result in a net enhancement of GABAergic synaptic transmission, despite the decrease in IPSC amplitude observed in this study (Banks and Pearce, 1999). In our experiments, ISO (0.6 mM) induced a significant rebound current (Fig. 2C), indicating the open-channel block of GABAAR channels. The present data suggest that N2O could enhance the prolonging effect of ISO on GABAAR-mediated IPSCs by an increase in open-channel block. Thus, the effect of coadministered N2O and ISO on GABA-IPSCs and miniature IPSCs recorded from, e.g., hippocampal slices, could be a major point of further investigations. Taken together, the findings may provide an explanation how N2O enhances ISO actions under clinical conditions.

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