Isoflurane Blocks Synaptic Plasticity in the Mouse Hippocampus

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Background: The volatile anesthetic isoflurane depresses glutamatergic transmission. In this study, the authors investigated the effects of isoflurane on the induction of long-term potentiation (LTP) and long-term depression (LTD) in slices from the juvenile and adult mouse hippocampus. Both forms of synaptic plasticity involve the activation of glutamate receptors.

Methods: Field excitatory postsynaptic potentials and excitatory postsynaptic currents from neurons in the CA1 area were evoked by stimulation of the Schaffer collateral–commissural pathway. Two independent synaptic inputs were stimulated. Clinically relevant concentrations (0.2–0.3 mM) of isoflurane were added to the perfusion solution.

Results: Field excitatory postsynaptic potentials from slices of juvenile and adult mice were depressed to 37.3 ± 6.1% and 58.3 ± 7.4%, respectively, and excitatory postsynaptic currents were reduced to 36.7 ± 5.4% by isoflurane. A brief tetanic stimulation (100 Hz, 1 s) induced stable LTP of field excitatory postsynaptic potentials. In the presence of isoflurane, tetanization failed to induce LTP. The effect of isoflurane on LTP induction was reversible and could be prevented by antagonizing γ-aminobutyric acid type A receptors (GABA_A). Low-frequency stimulation (1 Hz/900 pulses) induced LTD. In the presence of isoflurane, low-frequency stimulation failed to induce LTD.

Conclusions: The prevention of the isoflurane-induced depression of LTP by the GABA_A antagonist picrotoxin suggests an involvement of GABA_A receptors. An enhancement of the efficacy of GABA-mediated inhibitory synaptic transmission prevents the depolarization of the postsynaptic membrane during tetanus, necessary for the induction of use-dependent alteration of synaptic strength. An impairment of these processes may be a cause for the transient loss of recall and cognitive impairment after anesthesia in juvenile and adult brains.

GLUTAMATE is probably the major excitatory neurotransmitter mediating fast synaptic transmission in the mammalian central nervous system.1 Long-term potentiation (LTP) and long-term depression (LTD), alterations in neuronal excitability widely assumed to be correlates for learning and memory,2,3 critically involve two classes of ionotropic glutamate receptors: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)–kainate receptors1 and N-methyl-D-aspartate (NMDA) receptors.2,5 Volatile anesthetics such as isoflurane and halothane both reduce NMDA and AMPA–kainate-mediated excitatory synaptic transmission.6–8 Recent studies suggest that isoflurane and halothane block central glutamatergic transmission irrespective of receptor subtype via pre-synaptic9,10 and postsynaptic sites of actions.9 Isoflurane also reduces postsynaptic Ca^{2+} currents of L-, N-, P-, and T-type Ca^{2+} channels.11

A transient lowering of the excitability of central neurons is probably crucial for loss of recall and cognitive impairment.12 As such, after general anesthesia it seems pertinent to know whether volatile anesthetics affect the induction of LTP and LTD.

In this study, we investigated the effects of a clinically relevant concentration of isoflurane on synaptic transmission, LTP, and LTD in the mouse hippocampal slice preparation. LTP and LTD are reflected as a persistent increase or decrease in synaptic response of the activated synapses after a certain pattern of activation of a set of afferents. Because clinical experience suggests age dependency, we studied the effects of isoflurane on synaptic transmission in juvenile and adult animals.

Materials and Methods

Brain Slice Preparation

Transverse hippocampal slices (300 μm thick) were obtained from juvenile (14 days) and adult (2 months) mice that were ether-anesthetized and decapitated. The experimental protocols were approved by the Ethical Committee on Animal Care and Use of the Government of Bavaria, Germany. The brain was rapidly removed, and slices were prepared in ice-cold Ringer solution using a vibroslicer. All slices were placed in a holding chamber for at least 60 min and were then transferred to a superfusing chamber for extracellular or whole-cell recordings. The flow rate of the solution through the chamber was 1.5 ml/min. The composition of the solution was 124 mM NaCl, 3 mM KCl, 26 mM NaHCO_3, 1 mM CaCl_2, 1 mM MgSO_4, 10 mM D-glucose, and 1.25 mM Na_2HPO_4, bubbled with a 95% O_2–5% CO_2 mixture, and had a final pH of 7.3. All experiments were performed at room temperature.

Electrophysiologic Recording

Extracellular recordings of field excitatory postsynaptic potentials (fEPSPs) were obtained from the dendritic region of the CA1 region of the hippocampus (fig. 1) using glass micropipettes (1–2 MΩ) filled with superfusion solution. For LTP induction, high-frequency stimulation conditioning pulses (100 Hz/1 s) were applied to the Schaffer collateral–commissural pathway. For LTD

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induction, low-frequency stimulation (1 Hz/900 pulses) was applied. Measurements of the slope of the fEPSP were taken between 20 and 80% of the peak amplitude. Slopes of fEPSPs were normalized with respect to the 30-min control period before tetanic stimulation or low-frequency stimulation.

Whole-cell recordings of excitatory postsynaptic currents (EPSCs) were obtained from visually identified somata of pyramidal neurons of the CA1 region by means of the infrared guided microscopy (for details, see Dodt et al.13). EPSCs and fEPSPs were evoked by stimuli (0.066Hz or 0.033Hz, 4–5 V, 20 μs), delivered via two bipolar tungsten electrodes insulated to the tip (5-μm tip diameter) and positioned in the Schaffer collateral–commissural pathway. The pipettes for whole-cell recording were filled with 130 mM CH4SO3, 130 mM CsOH, 0.05 mM EGTA, 5 mM HEPES, 1 mM MgCl2, 1 mM NaCl, and 5 mM QX314 and displayed resistances between 4.5 and 5 MΩ.

To isolate glutamatergic EPSCs, slices were perfused with picrotoxin (50 μM) and CGP 35348 (200 μM) to block γ-aminobutyric acid type A (GABA)A receptors and type B (GABAkB) receptors, respectively. Voltage-activated sodium and potassium channels were blocked by QX-314 and Cs+, applied intracellularly via the recording pipette. Currents were recorded with a switched voltage clamp amplifier with switching frequencies of 40–43 kHz (25% duty cycle). Series resistance was monitored and continuously compensated in bridge mode (for details see Misgeld et al.14). Neuronal input resistance was measured by injecting hyperpolarizing current pulses (300 ms, −10 mV). The mean input resistance of the neurons was 223 ± 64 MΩ (n = 93). All experiments were performed at room temperature at a holding potential of −70 mV. The recordings were amplified, filtered (3 kHz), and digitized (9 kHz). The digitized responses were stored to disk on a Macintosh computer using a data acquisition program.

Application and Concentration Measurement of Isoflurane

A saturated solution of isoflurane was prepared by adding a surplus of the anesthetic to extracellular solution in a glass bottle that was then stirred for at least 3 h under airtight conditions at room temperature. After this procedure, the saturated concentration of isoflurane in extracellular solution at room temperature was 15 mM, measured by gas chromatography.15 The minimum alveolar concentration equivalent of isoflurane was calculated to 0.5 mM using a Bunsen water–gas partition coefficient of 1.08 at 25°C.16 A more recent study17 that focused on the temperature dependence of the potency of volatile anesthetics, considers for isoflurane an aqueous-phase minimum alveolar concentration equivalent of 0.3 mM. For our experiments, three different dilutions (1:7.5, 1:15, or 1:30, resulting in 2 mM, 1 mM, or 0.5 mM isoflurane, respectively) of the saturated isoflurane solution (15 mM) were freshly prepared, oxygenated with carbogen (95% O2–5% CO₂), and applied to the perfusion system. High-quality polytetrafluorethylene was used for tubing to minimize loss of isoflurane and drug binding. To determine the final isoflurane concentrations, aliquots (1 ml) of the respective isoflurane dilution were then taken from the perfusion chamber and filled into airtight glass containers for gas chromatographic measurements. We found that all final isoflurane concentrations deriving from the different dilutions ranged from 0.2 to 0.3 mM throughout the study. Because these final isoflurane concentrations can be considered clinically
relevant, the 1:15 dilution of the stock solution of isoflurane was further used throughout this study.

Chemicals

The following compounds were used: all salts and picrotoxin (Sigma, Deisenhofen, Germany), QX-314 (lidocaine, N-ethyl bromide), and D-AP5 (D(-)-2-amino-5-phosphonopentanoic acid; RBI, Deisenhofen, Germany), CGP 35348 (3-amino-propyl(diethoxymethyl)-phosphonic acid; Ciba Geigy, Basel, Switzerland), GYKI52466 (gift from Dr. Tarnawa, Institute for Drug Research, Budapest, Hungary), and isoflurane (Furene, Abbott GmbH, Wiesbaden, Germany).

Because it has been reported that bicuculline is not a specific GABA<sub>α</sub> receptor antagonist,<sup>18</sup> we only used picrotoxin to block GABA<sub>α</sub> receptors.

Statistics

Data are expressed as mean ± SEM. Statistical significance was tested (Student t test, P < 0.05) 30 min after termination of stimulation.

Results

Isoflurane Reduced Glutamatergic Synaptic Transmission Age-dependently

We obtained both field and whole-cell recordings from neurons of the CA1 region of the hippocampus in brain slices of young (2 weeks) and adult mice (2 months). In young animals, isoflurane reduced the amplitude of EPSCs mediated by NMDA receptors and non-NMDA receptors and fEPSPs to 37.3 ± 6.1% and 36.7 ± 5.4%, respectively (n = 6; figs. 2A and 2B). In adult animals, the effect of isoflurane on fEPSP amplitude was less pronounced (reduction to 58.3 ± 7.4%, n = 9) and significantly different in comparison to young animals (P < 0.05, Student t test; fig. 2C).

Isoflurane Blocked the Induction of Long-term Potentiation

Long-term potentiation induction before and after isoflurane in the same slice, synaptic responses to a pair of stimuli (input 1–2) were recorded. After reaching a...
stable baseline for 20 min, a tetanus was delivered to input 1 that potentiated fEPSPs for 60 min, whereas fEPSPs evoked by input 2 did not undergo LTP (fig. 3). When isoflurane was applied, the amplitudes of the fEPSPs evoked \textit{via} both inputs were reduced, and tetanic stimulation of input 2 failed to induce LTP. Pooled data of all experiments are shown in figure 4. Tetanic stimulation of input 1 increased the fEPSPs amplitude to 158.9 ± 16.6% (fig. 4, left) for 60 min. After application of isoflurane, the same tetanus delivered to input 2 induced only short-term potentiation, and the amplitude of the fEPSP returned to control after 60 min (100.6 ± 2.7%; n = 9; \( P < 0.01 \), Student \( t \) test; fig. 4, right).

\textit{Block of GABA\textsubscript{A} Receptor Activation Prevented the Action of Isoflurane}

Isoflurane increases GABA\textsubscript{A} receptor currents\textsuperscript{19} and could thus reduce glutamatergic transmission during tetanization and consequently prevent LTP induction. To test this hypothesis, the GABA\textsubscript{A} receptor antagonist picrotoxin (50 \textmu M) was applied. As illustrated in figure 5, tetanic stimulation still induced a robust LTP (130.7 ± 12.9%; n = 8; left side of figure). When isoflurane was added during these conditions, tetanic stimulation was still able to evoke stable LTP and enhance fEPSPs to 132.5 ± 7.6% (fig. 5, right). In picrotoxin, the effect of isoflurane on EPSP amplitude reduction was less pronounced (isoflurane reduced EPSP amplitude to 72.1 ± 4.1% with picrotoxin and to 52.3 ± 1.8% without picrotoxin; \( P < 0.01 \), Student \( t \) test; data not shown). This indicates that GABA\textsubscript{A} receptors contribute to the reduction of the EPSP amplitude.

Since, in the presence of picrotoxin, lower levels of excitation may lead to LTP, we tested also whether picrotoxin can reverse impaired LTP produced by GYKI 52466 (10 \textmu M), a noncompetitive AMPA receptor antagonist\textsuperscript{20} that reduces the EPSP amplitude to a similar extent as isoflurane. GYKI 52466 reduced the EPSP amplitude to 46.2 ± 4.7% (n = 5) and blocked LTP. In the presence of picrotoxin, tetanization also failed to induce LTP (data not shown). These results are strong evidence in favor of a GABA-mediated (GABA\textsubscript{ergic}) involvement in the effect of isoflurane on LTP.
The Effect of Isoflurane on Long-term Potentiation Was Reversible

Isoflurane reduced fEPSP amplitude and totally blocked the induction of LTP. This effect was reversible. After the washout of isoflurane for 50 min, tetanization induced LTP again (fig. 6A). Pooled data from six experiments are shown in figure 6B. The amplitudes of fEPSPs in the presence of isoflurane have been normalized to baseline to emphasize that LTP did not occur. To ensure that a given slice is able to show LTP, at the beginning of each experiment another synaptic input was stimulated for control.

Isoflurane Blocked the Induction of Long-term Depression

After establishing a stable baseline for 20 min, low-frequency stimulation (1 Hz/900 pulses) delivered to input 1 induced LTD. fEPSPs were depressed to $83.0 \pm 6.3\% (n=6)$, whereas fEPSPs evoked by input 2 did not undergo LTD. Isoflurane reduced fEPSP amplitude...
The volatile anesthetic isoflurane reversibly depressed glutamatergic synaptic transmission and blocked the induction of LTP and LTD in the CA1 area of the hippocampus. In the hippocampus CA1 area, LTP induction after multiple trains of high-frequency stimulation and homosynaptic LTD generated by low-frequency stimulation require postsynaptic Ca$^{2+}$ influx$^{21,22}$ and the activation of glutamate receptors. In this area, NMDA receptor–dependent and –independent forms of LTP have been observed.$^2$ In the current study, only the homosynaptic NMDA receptor–dependent form of LTP and LTD was investigated.

Previous studies have shown that clinically relevant concentrations of volatile anesthetic agents inhibit glutamate receptor–mediated responses.$^{7,8,12}$ It was suggested that this effect is mainly produced via a presynaptic site of action.$^{7,8}$ Both halothane and isoflurane depressed NMDA receptor–mediated and non-NMDA receptor–mediated responses.$^9$ Most recently it has been reported that isoflurane preferentially depresses NMDA receptor–mediated synaptic transmission.$^8$ Compared with halothane, isoflurane reduced the amplitude of NMDA receptor–mediated responses more effectively. These findings were interpreted as evidence in favor of an additional postsynaptic site of action.$^7,8,12$ In the CA1 region of rats in vitro, halothane but not methoxyflurane reduced the probability of LTP induction.$^{23}$ These data may suggest that volatile anesthetics differ in their effects on LTP.

The present study provides strong evidence for GABA$_A$ receptors to be crucially involved in the blocking effect of isoflurane on the induction of LTP. A blockade of GABA$_A$ receptors prevented the effect of isoflurane but...
not the effect of an AMPA receptor antagonist on LTP. Recent studies show that isoflurane potentiates GABA A-activated currents subunit-dependent in recombinant receptors in vitro. Therefore, it cannot be excluded that the enhanced blocking effect of isoflurane on LTP observed in slices obtained from juvenile animals is a result of interactions with different GABA A receptor assemblies present in different developmental stages (see also below). The role of different GABA A receptor assemblies for the actions of isoflurane have been investigated in a previous study in which HEK 293 cells were transfected with cDNA encoding for various subunits. In cells endowed with GABA A receptor assemblies containing α1β2γ2L, the most ubiquitous subunits in the mammalian central nervous system, isoflurane clearly enhanced Cl− currents evoked by GABA. An enhancement of GABA receptor–mediated hyperpolarizing responses will reduce the activation of the voltage-sensitive NMDA receptors, and it is therefore feasible to assume that the induction of LTD and LTP in the target neuron will also be attenuated. It has been reported that an enhancement of GABA A receptor function, e.g., by benzodiazepines, can disrupt memory formation and hippocampal synaptic plasticity. Isoflurane is not acting via the benzodiazepine binding site but shares the enhancing effect on hyperpolarizing neuronal responses that consequently should impair LTP induction.

In addition, the induction of LTD is NMDA receptor-dependent and can be modulated by GABA A receptor activation. It is conceivable that similar mechanisms mediate the isoflurane-induced inhibition of LTP and LTD. In a previous study, it was concluded that blocking LTD induction by enhancing GABA release is a result of a less effective depolarization, which entrains a reduced NMDA receptor activity.

Long-term potentiation can be enhanced by picrotoxin via a decrease in tonic inhibition. Thus, picrotoxin might reverse the isoflurane effect through an indirect or functional antagonism related primarily to excitatory synaptic transmission. However, in contrast to our findings with isoflurane, in the presence of an AMPA receptor antagonist, picrotoxin was not able to reinstate any LTD. These results clearly speak in favor of a crucial involvement of a GABAergic link in the isoflurane-mediated effect on LTP.

A previous study reported that isoflurane inhibits various types of voltage-gated calcium channels in hippocampal pyramidal neurons. In addition, shifting the membrane potential to more hyperpolarized levels and the associated decrease in membrane input resistance reduces the amplitude of action potentials back-propagating from the soma into the dendrites and also alters their shape. Action potentials back-propaga...
agating in dendrites that open voltage-gated calcium channels will probably fail to invade more remote dendritic areas during these changes. There is evidence from recent work that dendritic Ca$^{2+}$ influx, associated with back-propagating action potentials is crucial in the induction of synaptic plasticity.

It remains unclear whether isoflurane reduced the amplitude of fEPSPs more effectively in slices that were obtained from juvenile animals because of different expression profile of the NMDA, AMPA, and GABA$_A$ receptor subunits. There is evidence that central neurons in young animals show a lower initial probability of transmitter release, a lower responsiveness to NMDA receptor agonists, and slower kinetics of NMDA receptor-mediated EPSCs. It is feasible to assume that during these conditions, isoflurane could become more effective in suppressing NMDA receptor-mediated synaptic plasticity.

A synopsis of the presently available data suggests that at clinically relevant concentrations, volatile anesthetics interfere with synaptic transmission and inhibit the induction of long-term alterations in the excitability of central neurons. Cognitive impairment and loss of recall are important features of general anesthesia, and mechanisms related to LTP and LTD are assumed to be important steps in learning and memory formation. The reduction of glutamatergic transmission is probably induced by an increase in GABA$_A$ receptor-mediated inhibition, which reduces the activation of voltage-sensitive NMDA receptors and voltage-gated calcium channels, prerequisites for the induction of alterations in synaptic strength. These actions are probably also involved in what was proposed as the multisite agent-specific mechanism for the anesthetic action of isoflurane.

Most importantly, the blocking effects of the volatile anesthetic isoflurane on LTP and LTD is reversible, indicating that this agent does not induce persistent changes in neuronal excitability.

References

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