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Contrasting Synaptic Actions of the Inhalational General Anesthetics Isoflurane and Xenon

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Background: **The mechanisms by which the inhalational general anesthetics isoflurane and xenon exert their effects are unknown. Moreover, there have been surprisingly few quantitative studies of the effects of these agents on central synapses, with virtually no information available regarding the actions of xenon.**

Methods: **The actions of isoflurane and xenon on γ-aminobutyric acid–mediated (GABAergic) and glutamatergic synapses were investigated using voltage-clamp techniques on autaptic cultures of rat hippocampal neurons, a preparation that avoids the confounding effects of complex neuronal networks.**

Results: **Isoflurane exerts its greatest effects on GABAergic synapses, causing a marked increase in total charge transfer (by approximately 70% at minimum alveolar concentration) through the inhibitory postsynaptic current. This effect is entirely mediated by an increase in the slow component of the inhibitory postsynaptic current. At glutamatergic synapses, isoflurane has smaller effects, but it nonetheless significantly reduces the total charge transfer (by approximately 30% at minimum alveolar concentration) through the excitatory postsynaptic current, with the *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor–mediated components being roughly equally sensitive. Xenon has no measurable effect on GABAergic inhibitory postsynaptic currents or on currents evoked by exogenous application of GABA, but it substantially inhibits total charge transfer (by approximately 60% at minimum alveolar concentration) through the excitatory postsynaptic current. Xenon selectively inhibits the NMDA receptor–mediated component of the current but has little effect on the AMPA/kainate receptor–mediated component.**

Conclusions: **For both isoflurane and xenon, the most important targets appear to be postsynaptic. The authors' results show that isoflurane and xenon have very different effects on GABAergic and glutamatergic synaptic transmission, and this may account for their differing pharmacologic profiles. (Key words: Analgesia; anesthesia; autapses; ligand-gated channels; noble gases.)**

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ALTHOUGH it has been widely accepted for many years that inhalational general anesthetics most probably act at synapses,[1] there have been surprisingly few quantitative studies of the effects of these drugs on central synaptic transmission (for a review see Pocock and Richards[2]). What has emerged from the work performed so far is a picture of some complexity, with contradictory conclusions drawn regarding both the extent to which synaptic transmission is affected[3,4] and, in some cases, whether particular classes of synapse are inhibited or potentiated.[5-9] Nonetheless, a consensus is developing that inhalational anesthetics generally depress excitatory synapses[10,11] and potentiate inhibitory synapses,[5,12] but there is little agreement about either the molecular targets involved or which of these effects is the most important for any given anesthetic. This is true even for the most widely used agents, such as isoflurane, and for a number of recently introduced anesthetics (*e.g.*, desflurane and sevoflurane). For the anesthetic gas xenon, which is being evaluated for routine surgical use, there is, other than a preliminary report from our laboratory,[13] essentially no information regarding molecular mechanisms.

For much of the previous work regarding the synaptic effects of inhalational anesthetics, brain slices have been used. These preparations maintain some of the native circuitry intact but have the concomitant difficulty of disentangling direct effects on particular synapses from indirect effects mediated by complex neuronal path-

ways. In addition, the problems encountered by several workers in handling highly volatile general anesthetics, in terms of evaporative losses and proper accounting for the temperature dependence in animal potencies,[14] has led to considerable uncertainties about whether the various synaptic effects described occur at clinically relevant concentrations.

These problems can be largely circumvented using the "microisland" culture technique, in which phenotypically identical populations of excitatory and inhibitory synapses can be studied in isolated neurons,[15-17] with volatile or gaseous anesthetics being rapidly applied at defined concentrations in aqueous solution. This approach has been used to elucidate the effects of nitrous oxide on glutamatergic and γ-aminobutyric acid–mediated (GABAergic) synapses in hippocampal neurons.[18] In the study reported herein, we followed similar protocols to investigate the actions of the widely used volatile anesthetic isoflurane and the "inert" gas xenon. These two inhalational agents have very different pharmacologic profiles (*e.g.*, isoflurane causes a substantial degree of cardiovascular depression with little analgesia; xenon has little or no effect on the cardiovascular system but confers profound analgesia), and it is possible that these differences originate from differential effects on synaptic transmission.

Materials and Methods

This study conforms to the United Kingdom Animals (Scientific Procedures) Act of 1986.

Culturing Hippocampal Neurons

Hippocampal neurons were grown in culture using the methods described previously.[15-17] Briefly, hippocampi from Sprague-Dawley rats (postnatal days 1–3) were dissected, roughly sliced, and agitated in a papain-containing solution (20 U/ml) for 30 min at 37°C. After washing with enzyme-free solution, the tissue was gently triturated with a fire-polished Pasteur pipette, and the cells were plated out at a density of $8-10 \times 10^4$ cells/ml and cultured (95% air–5% CO_2) at 37°C. Glass coverslips used for culturing the cells were first coated with agarose (0.15% wt/vol) and then sprayed with a fine mist of poly-D-lysine (0.1 mg/ml) and rat-tail collagen (0.5 mg/ml) from a glass microatomizer and sterilized by ultraviolet exposure. This produced microislands of permissive substrate with diameters of between 100 and 1000 μm. At 3 or 4 days after plating, when the glial cell layer was approximately 80% confluent, an antimitotic agent (cytosine β-D-arabinofuranoside, 5 μM) was added to arrest glial cell proliferation. Neuronal cultures were then allowed to mature for another 4–9 days. We used microislands that contained single, isolated neurons in which axonal processes and dendritic trees formed multiple self-synapses (autapses). This procedure provided a large population of either excitatory or inhibitory monosynaptic connections.

Electrophysiology

The neurons were voltage clamped using the whole cell recording technique (Axopatch 200 amplifier; Axon Instruments, Foster City, CA). Electrodes were fabricated from borosilicate glass and typically had resistances between 3 and 5 MΩ. Series resistance was compensated by 75–90%. Neurons were voltage clamped at −60 mV, and synaptic responses were stimulated by a 2-ms depolarizing pulse to +20 mV. Shortly after the restoration of the membrane potential to −60 mV, a large (1- to 20-nA) postsynaptic current was observed and recorded. For the synaptic measurements, data were sampled at 50 kHz, filtered at 20 kHz (−3 dB, eight-pole Bessel), and stored on a computer. The extracellular recording solution was 137 mM NaCl, 5 mM KCl, 3 mM $CaCl_2$, 5 mM HEPES, 10 mM glucose, 0.001 mM glycine, and 0.0001 mM strychnine-HCl, titrated to pH 7.3 with NaOH; and the intracellular (pipette) solution was 140 mM KCl, 4 mM NaCl, 0.5 mM EGTA, 2 mM MgATP, and 10 mM HEPES, titrated to pH 7.25 with KOH.

For the experiments in which GABA or glutamate were exogenously applied, the neurons were grown in mass culture and used 3–11 days after plating. Data were sampled at 200 Hz and filtered at 100 Hz (−3 dB, eight-pole Bessel). The extracellular recording solution for glutamate-evoked responses was 150 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 10 mM HEPES, 10 mM glucose, 0.0002 mM tetrodotoxin citrate (Tocris Cookson, Bristol, UK), 0.1 mM picrotoxin, 0.0001 mM strychnine-HCl, and 0.001 mM glycine, titrated to pH 7.40 with NaOH; the extracellular recording solution for GABA-evoked responses was 150 mM NaCl, 4 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, and 0.0002 mM tetrodotoxin citrate, titrated to pH 7.40 with NaOH; the intracellular (pipette) solution for GABA- and glutamate-evoked responses was 140 mM CsCl, 3 mM NaCl, 11 mM EGTA, 2 mM MgATP, 10 mM HEPES, titrated to pH 7.20 with CsOH. Unless otherwise stated, all chemicals were obtained from Sigma Chemical (Poole, Dorset, UK). Test solutions were applied to the cells using a rapid-switch-

ing perfusion system.[19] All electrophysiologic measurements were carried out at room temperature (20–23°C).

Preparation of Anesthetic Solutions

Isoflurane solutions were prepared as fractions of an aqueous saturated solution at room temperature. The concentration of a saturated solution was taken to be 15.3 mM.[20] Reservoirs containing the anesthetic solutions were sealed with rigid plastic floats, and all tubing and valves were made of polytetrafluoroethylene. With these precautions, losses of anesthetic from the perfusion system were found to be negligible, as measured by gas chromatography.[21] Isoflurane was obtained from Abbott Laboratories (Queenborough, Kent, UK). Solutions for the xenon experiments were prepared by first bubbling pure gases (oxygen, nitrogen, or xenon) through fine sintered-glass bubblers in 250- or 500-ml Drechsel bottles filled with extracellular recording saline. Solutions were bubbled for 1.5–2.0 h, although equilibrium was reached within 45 min. (To minimize oxidation, the neurotoxins and neurotransmitters were absent in the fully oxygenated saline but present at the appropriate concentrations in the xenon and nitrogen solutions.) During bubbling, the solutions were continually stirred at room temperature. These solutions were then mixed to achieve the desired final concentrations of the gases. Control solutions usually contained 80% of the nitrogen solution and 20% of the oxygen solution; test solutions usually contained 80% of the xenon solution and 20% of the oxygen solution. Using a Bunsen water–gas partition coefficient[22] of 0.0965 we calculated that the standard test solution contained 3.4 mM xenon. Xenon (research grade, 99.993% pure) was supplied by BOC Gases (Guildford, Surrey, UK). In all cases, xenon and isoflurane were preapplied to the neurons for at least 30 s before the initiation of synaptic currents.

Integration of the Synaptic Responses

To obtain an estimate for the total charge transfer, the excitatory postsynaptic currents (EPSCs) or inhibitory postsynaptic currents (IPSCs) were integrated numerically. However, because in some cases the currents had not decayed to baseline by the end of the recording period, a correction (which was invariably less than 5% of the total charge transfer) was applied by extrapolating the observed current to the baseline using a biexponential fit to the decay phase of the response (see Results).

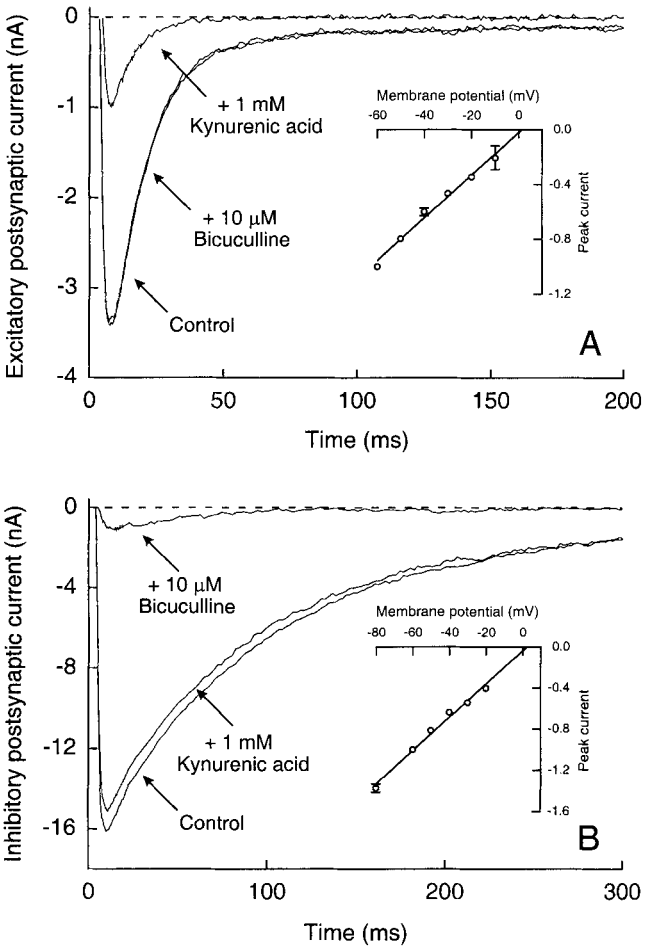

Fig. 1. Control excitatory and inhibitory postsynaptic responses. (*A*) A representative excitatory postsynaptic current (EPSC) and its sensitivity to 1 mM kynurenic acid and insensitivity to 10 μM bicuculline. (*Inset*) The linear current–voltage relation for the peak of the EPSC, normalized to the current measured at −60 mV; the data points represent mean values (for an average of five cells). (*B*) A representative inhibitory postsynaptic current (IPSC) and its sensitivity to 10 μM bicuculline and insensitivity to 1 mM kynurenic acid. (*Inset*) The linear current–voltage relation for the peak of the IPSC, normalized to the current measured at −60 mV; the data points represent mean values (for an average of six cells). Errors bars are SEM but are not shown if smaller than the size of the symbol. For excitatory and inhibitory responses, inward currents are evoked because the intracellular and extracellular ionic concentrations in the experiments were such that, for inhibitory chloride currents and excitatory cation currents, the reversal potentials were close to zero.

Results

Control Synaptic Currents

We first characterized the control synaptic currents, which, consistent with the findings of previous stud-

Table 1. Control Parameters for Synaptic Currents

Parameter	Excitatory Currents	n‡	Inhibitory Currents	n‡
Time to peak (ms)	4.9 ± 0.6	12	5.0 ± 0.3	14
Decay half-time (ms)	7.8 ± 0.5	13	38.5 ± 3.5	16
τ_{fast} (ms)	9.0 ± 0.7	13	38.1 ± 3.9	16
τ_{slow} (ms)	210 ± 28	13	229 ± 44	16
I_{fast}/I_{total}*	0.92 ± 0.01	13	0.70 ± 0.04	17
Q_{slow}/Q_{total}†	0.61 ± 0.04	13	0.63 ± 0.04	16

* $I_{total} \equiv I_{fast} + I_{slow}$.

† $Q_{total} \equiv Q_{fast} + Q_{slow}$.

‡ Number of cells.

ies,[15,17] almost invariably fell into one of two populations. Approximately half the cells exhibited postsynaptic currents that decayed relatively rapidly (~8-ms half-time); the other half exhibited currents that were markedly slower (~40-ms half-time; fig. 1; table 1). The more rapid responses were recorded from cells with a rounded appearance and complex dendritic trees. Their sensitivity to kynurenic acid (80 ± 3% inhibition of the peak current with 1 mM kynurenic acid; n = 7 cells) and insensitivity to bicuculline (0.4 ± 1.3% inhibition of the peak current with 10 μM bicuculline; n = 13 cells) identifies these cells as excitatory glutamatergic neurons. In contrast, the slower synaptic currents were almost completely blocked by bicuculline (94 ± 1% inhibition of the peak current with 10 μM bicuculline; n = 8 cells) and unaffected by kynurenic acid (6 ± 4% inhibition of the peak current with 1 mM kynurenic acid; n = 5 cells), thus identifying these responses as GABAergic. These inhibitory neurons tended to be flatter, with simpler dendritic trees. Because of the recent finding that GABA and glycine can be coreleased by spinal cord interneurons,[23] we considered the possibility that the inhibitory responses we were recording were mediated, in part, by glycine receptors. However, the control current was barely affected (4 ± 2% inhibition; n = 6 cells) by 100 nM strychnine, confirming that the inhibitory currents were entirely GABAergic.

The excitatory and inhibitory currents had essentially identical rise times (table 1), and the peaks of the currents changed linearly with test potential (insets to fig. 1). The decay phase of the synaptic current I(t), in which t is the time measured from the peak of the current, was fit by a biexponential equation of the form

$$I(t) = I_{fast}e^{-t/\tau_{fast}} + I_{slow}e^{-t/\tau_{slow}}$$

where I_{fast} and I_{slow} are the amplitudes and τ_{fast} and τ_{slow} are the time constants of the fast and slow components, respectively. The values for these decay-time constants measured from control excitatory and inhibitory responses are given in table 1. In each case, approximately two thirds of the total charge transfer was carried by the slow component. For the excitatory glutamatergic responses, this slow component can be readily identified as mediated by NMDA receptors because it is completely (99 ± 1%; n = 10 cells) blocked by 200 μM DL-2-amino-5-phosphonopentanoate (AP5), a highly selective NMDA receptor antagonist.[24] In the presence of this concentration of AP5, the decay phase of the synaptic current could be fitted well by a single exponential with a magnitude and time course little different from those of the control fast component. This fast component, which very largely determines the magnitude of the peak excitatory current (I_{fast}/I_{total} = 92 ± 1%; n = 13 cells), can be attributed to currents mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors.[15,17]

Effects of Isoflurane on Excitatory Currents

We next looked at the effects of the inhalational general anesthetic isoflurane on excitatory glutamatergic currents. Isoflurane had a qualitatively similar effect at all concentrations tested. Representative traces are shown in figure 2A. The peak of the synaptic current, and the total charge transfer, decreased monotonically with increasing concentrations of isoflurane (figs. 2B and C). At the highest concentration studied (1.22 mM), the peak was reduced by ~50% and the total charge transfer by ~70%. Underlying these gross changes, an analysis of the decay phase of the response revealed subtly different effects of isoflurane on the fast and slow components. For the slow (NMDA receptor) component, the time constant τ_{slow}, although rather variable, did not change significantly (fig. 3A). Similarly, the time constant for the fast (AMPA/kainate receptor) component τ_{fast} decreased only slightly with increasing concentrations of isoflurane (at 1.22 mM isoflurane, τ_{fast} was reduced by ~25%). The

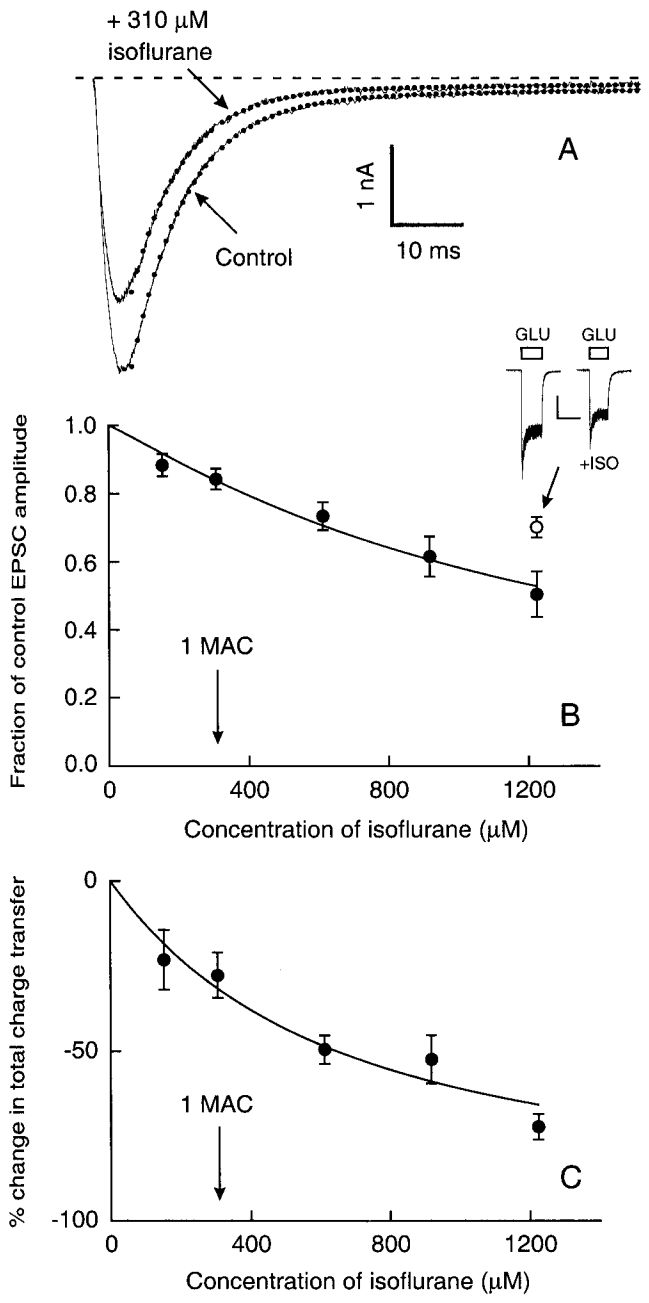

Fig. 2. The effects of isoflurane on excitatory postsynaptic currents. (*A*) Representative traces showing the effects of 310 μM (~ 1 minimum alveolar concentration [MAC]) isoflurane on glutamatergic synaptic currents. The principal effect is a reduction in peak height (in this example by approximately 24%; the average inhibition at 1 MAC was 16 ± 3%; n = 12 cells), with little change in time course. Dots represent biexponential fits to the measured currents. (*B*) The amplitude of the excitatory postsynaptic current decreases monotonically with increasing concentrations of isoflurane. The points represent mean values (for an average of 12 cells). The open circle (○) represents the degree of inhibition of the current evoked by the exogenous application of 100 μM glutamate (n = 13 cells). Representative traces are shown for a control response to glutamate and a response in the presence of 1.22 mM isoflurane (preapplied for 60 s). Calibration bars in the inset correspond to 200 nA and 20 s. (*C*) The decrease in total charge transfer (the total area under the excitatory postsynaptic current) is plotted as a percentage change from the control value. The points represent mean values (for an average of 12 cells). In both cases, the lines are drawn by eye and have no theoretic significance. The arrow marked "1 MAC" indicates a value for the isoflurane MAC for the rat of 310 μM.[14]

amplitudes of the two components, however, were rather more sensitive to isoflurane, and both decreased by approximately the same percentage (fig. 3B). Overall, the reduction in charge transfer by isoflurane was comparable for the NMDA and AMPA/kainate receptor-mediated components (fig. 3C); this was caused predominantly by reductions in peak amplitude.

To determine the extent to which this inhibition in the EPSC amplitude (fig. 2B) could be accounted for by an effect on the postsynaptic receptors, we measured the inhibition by isoflurane of the current induced by exogenously applied glutamate (100 μM). We found that, at the highest isoflurane concentration used (1.22 mM), the peak of the glutamate-evoked current was reduced by 30 ± 3% (n = 13 cells; inset to fig. 2B).

To assess the possible importance of these changes to the maintenance of the anesthetic state, it is crucial to quantify the changes in the control synaptic parameters (table 1) at isoflurane concentrations that are of clinical relevance. It has long been recognized that the most appropriate surgical benchmark is the concentration of a general anesthetic that prevents a purposeful response to a painful stimulus in 50% of a population of patients (or animals). For inhalational agents, this concentration is the minimum alveolar concentration (MAC).[25] For inhalational anesthetics, these gas concentrations can be converted to aqueous concentrations using Ostwald or Bunsen water-gas partition coefficients.[26] For isoflurane acting on humans, the MAC in aqueous solution is 270 μM; for the rat it is 310 μM.[14] The percentage changes in the various excitatory synaptic parameters for an isoflurane concentration of ~1 MAC are given in table 2.

Effects of Isoflurane on Inhibitory Currents

The effects of isoflurane on inhibitory synaptic currents were qualitatively different from those observed on excitatory currents. Representative traces are shown in

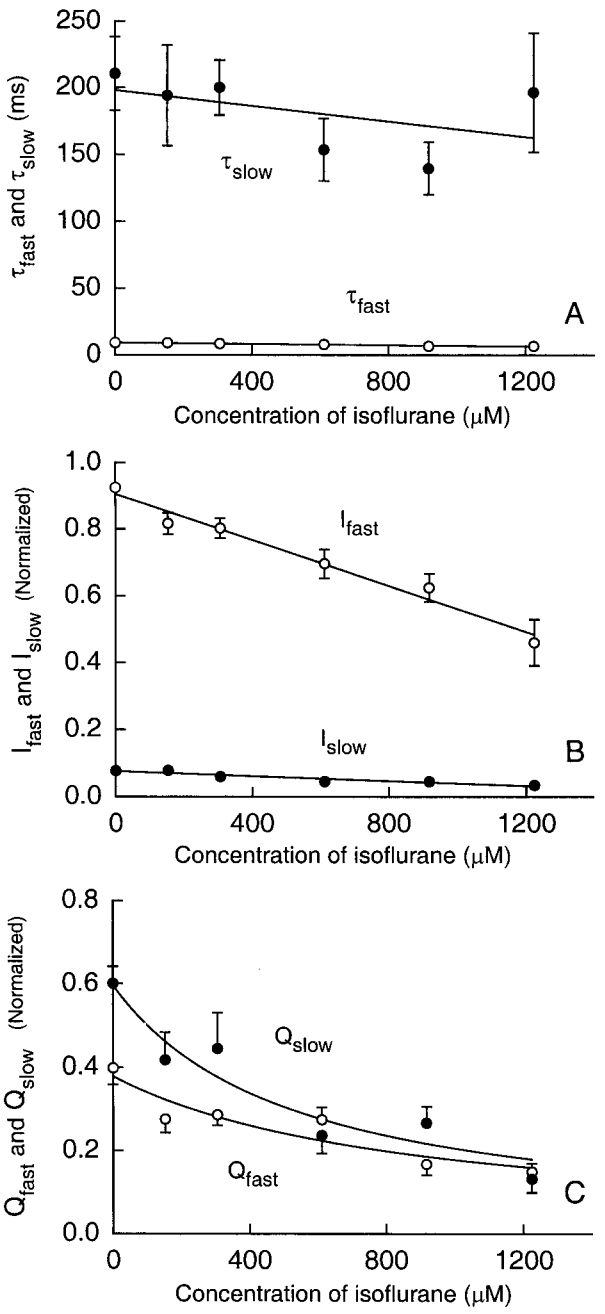

Fig. 3. The effects of isoflurane on the fast and slow components of the excitatory postsynaptic currents (EPSCs) derived from biexponential fits. (*A*) Changes in the fast (○) and slow (●) time constants. (*B*) Changes in the amplitudes of the fast and slow components, normalized such that the sum of the fast and slow components is equal to 1 in the absence of isoflurane. (*C*) Changes in the charge transfer for the fast and slow components, normalized such that the total charge transfer for the control equals unity. The points represent mean values (for an average of 12 cells). In all cases the lines are drawn by eye and have no theoretic significance.

figure 4A. Although the peak of the synaptic current was inhibited at high isoflurane concentrations (reaching approximately 40% inhibition at the highest concentration tested, 1.22 mM), the inhibition was very modest over the clinically relevant range (fig. 4B). At each concentration of isoflurane, however, there was a significant increase in the total charge transfer (fig. 4C), which reached an apparent maximum of approximately 130% (*i.e.*, a 2.3-fold increase). An analysis of the kinetics of the decay phase revealed significantly different sensitivities for the fast and slow components. Although the time constants τ_{fast} and τ_{slow} increased with increasing concentrations of isoflurane (fig. 5A) by roughly the same extent (~1.8-fold at 1.22 mM), the effects on the amplitudes were qualitatively different, with I_{slow} increasing somewhat and I_{fast} being strongly inhibited (fig. 5B). The net result is that the total charge carried by the fast component of the inhibitory current decreased modestly, and the charge transferred by the slow component increased markedly (fig. 5C).

The percentage changes in the various inhibitory synaptic parameters at about 1 MAC isoflurane are given in table 2.

Effects of Xenon on Synaptic Currents

The gaseous concentration of xenon that prevents a response to a painful stimulus (*i.e.*, MAC) appears to vary among species, being 71% atm in humans,[27] 98% atm in rhesus monkeys,[28] and 161% atm in rats.[29] If these values are converted to free aqueous concentrations at 37°C,[14,26] using an Ostwald water-gas partition coefficient of 0.0887,[30] we obtain values of 2.5 mM, 3.4 mM, and 5.6 mM for humans, monkeys, and rats, respectively, with the average value being 3.8 mM. For our experiments, performed at room temperature, the concentration of xenon in the standard test solution was 3.4 mM. At this concentration, xenon had negligible effects on the inhibitory synaptic currents, but strongly depressed the excitatory currents. This is illustrated with representative traces in figure 6.

For the GABAergic synaptic currents, 3.4 mM xenon affected neither the peak value nor the time course of the postsynaptic currents. Percentage changes in the various inhibitory synaptic parameters are listed in table 3, in which it can be seen that none were significantly changed. We also looked for effects of xenon on currents evoked by a low (3 μM) concentration of exogenously applied GABA. Here, 4.3 mM xenon had no significant effect on the GABA-induced current (2 ± 3% potentiation; n = 4 cells). A representative pair of traces

Table 2. The Effects of 310 μM (~1 MAC) Isoflurane on Synaptic Currents

	Excitatory Currents			Inhibitory Currents		
Parameter	% Change (mean ± SEM)	↑, ↓ or NS*	n†	% Change (mean ± SEM)	↑, ↓ or NS*	n†
I_{peak}	−15.7 ± 3.0	↓	12	−2.8 ± 2.8	NS	12
Decay half-time	−10.2 ± 1.9	↓	13	72.7 ± 8.1	↑	12
Total charge transfer	−27.7 ± 6.7	↓	12	68.7 ± 10.7	↑	12
I_{fast}	−12.6 ± 3.4	↓	12	−19.6 ± 5.4	↓	12
I_{slow}	−25.8 ± 6.4	↓	12	45.9 ± 18.6	↑	12
τ_{fast}	−8.5 ± 1.7	↓	12	45.2 ± 13.5	↑	12
τ_{slow}	−6.3 ± 8.6	NS	12	60.1 ± 11.4	↑	12
Q_{fast}	−19.1 ± 4.7	↓	12	26.7 ± 17.1	NS	12
Q_{slow}	−33.0 ± 10.5	↓	12	115.2 ± 30.1	↑	12

Percent effect on a parameter $P \equiv \left(\frac{P_{isoflurane} - P_{control}}{P_{control}}\right) \times 100\%$. To minimize the effects of cell-to-cell variability, the percentage changes in the various parameters have been obtained by first calculating the changes from individual measurements and then pooling these values to obtain means and standard errors.

* Not significant at the 95% confidence level (Student *t* test).

† Number of cells.

is shown in the inset to figure 6A. In contrast, 3.4 mM xenon greatly depressed the glutamatergic synaptic current, with the effect being confined, almost exclusively, to the slow NMDA receptor-mediated component of the current (fig. 6B). This is evident in the percentage changes in the various excitatory synaptic parameters, which are listed in table 3. Here it can be seen that the qualitative effects of xenon are remarkably closely mimicked by the effects of AP5. At the concentration of AP5 used (200 μM), the NMDA receptor component would be expected to be almost completely blocked[24,31]; this is consistent with the 99% block of I_{slow} (table 3). This is accompanied by a 75% reduction in total charge transfer, close to the 61% of the total charge that we estimate to be carried by the slow NMDA receptor-mediated component (table 1). The difference may be accounted for by the small but significant reduction of the fast time constant τ_{fast} by AP5 (table 3). Likewise, xenon causes a large inhibition (70%) of I_{slow} and a large inhibition (56%) of the total charge transfer, with only a small effect on the fast AMPA/kainate receptor-mediated component.

Discussion

Effects of Isoflurane on Excitatory Currents

Isoflurane, at clinically relevant concentrations, modestly but significantly depressed glutamatergic EPSCs (fig. 2; table 2). This finding is qualitatively consistent with previous observations of postsynaptic potentials in brain slice preparations,[9,10,32] and with some studies using other volatile agents.[11,33,34]

The principal effects we observed were a concentration-dependent reduction in the amplitude of the EPSC and, concomitantly, a reduction in the total charge transfer (fig. 2). The overall time course of the EPSC was affected less. Similarly, when the decay phase of the postsynaptic current was analyzed, the main effects were a reduction in the amplitudes of the fast and slow components, with their respective time constants being only slightly reduced (fig. 3); fast and slow components of charge transfer were affected equally. Hence the two major subclasses of glutamate receptor (NMDA and AMPA/kainate) mediate currents that are roughly equally sensitive to isoflurane.

The sensitivity to isoflurane of responses to exogenously applied glutamate (inset to fig. 2B) suggests that approximately half of the effect of isoflurane on the peak of the EPSC can be accounted for by an inhibition of postsynaptic receptors. Unfortunately, there are very few studies on the effects of volatile anesthetics on postsynaptic glutamate receptors. Because of the very fast decay kinetics of glutamate-activated currents,[35] results using either slow perfusion in brain slice preparations[11] or incubation with glutamate in binding experiments[36] are very difficult to extrapolate to functional synapses. However, in our experiments, the rate of agonist application was not ultrarapid, as is necessary to accurately measure peak responses. We were also limited to studying extrajunctional receptors in which subunit composition and pharmacology might differ from

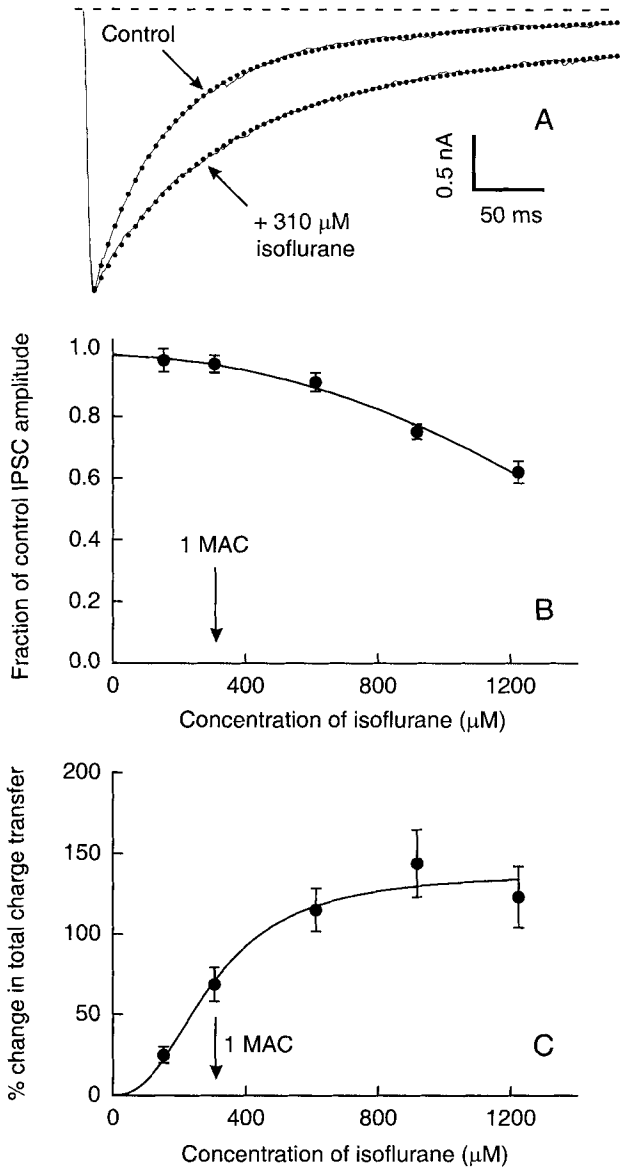

Fig. 4. The effects of isoflurane on inhibitory postsynaptic currents. (*A*) Representative traces showing the effects of 310 μM (~ 1 minimum alveolar concentration [MAC]) isoflurane on GABAergic synaptic currents. The principal effect is a prolongation in the current decay (in this example, the decay half-time is increased by approximately 100%), with little or no effect on peak height. Dots represent biexponential fits to the measured currents. (*B*) The amplitude of the inhibitory postsynaptic current decreases monotonically with increasing concentrations of isoflurane. The points represent mean values (for an average of 12 cells). (*C*) The increase in total charge transfer (the total area under the inhibitory postsynaptic current) is plotted as a percentage change from the control value. The points represent mean values (for an average of 12 cells). In each case, the line is drawn by eye and has no theoretic significance. The arrow marked "1 MAC" indicates a value for the isoflurane MAC for the rat of 310 μM.[14]

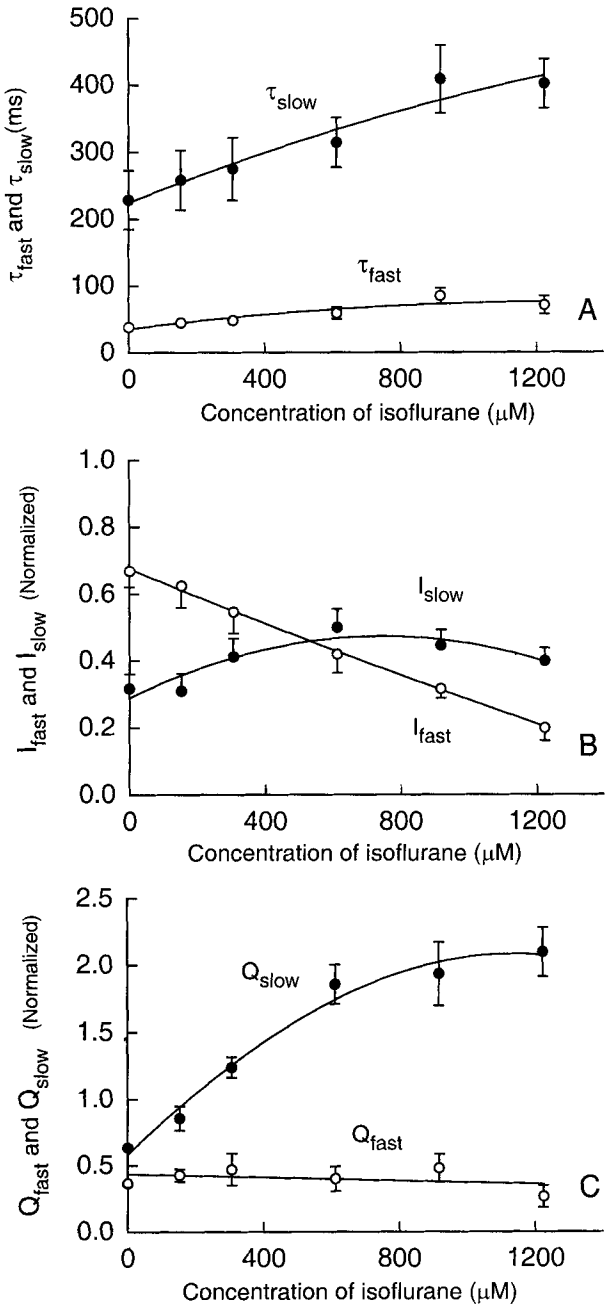

Fig. 5. The effects of isoflurane on the fast and slow components of the inhibitory postsynaptic currents derived from biexponential fits. (*A*) Changes in the fast (○) and slow (●) time constants. (*B*) Changes in the amplitudes of the fast and slow components, normalized such that the sum of the fast and slow components is equal to unity in the absence of isoflurane. (*C*) Changes in the charge transfer for the fast and slow components, normalized such that the total charge transfer for the control equals unity. The points represent mean values (for an average of 12 cells). In each case, the line is drawn by eye and has no theoretic significance.

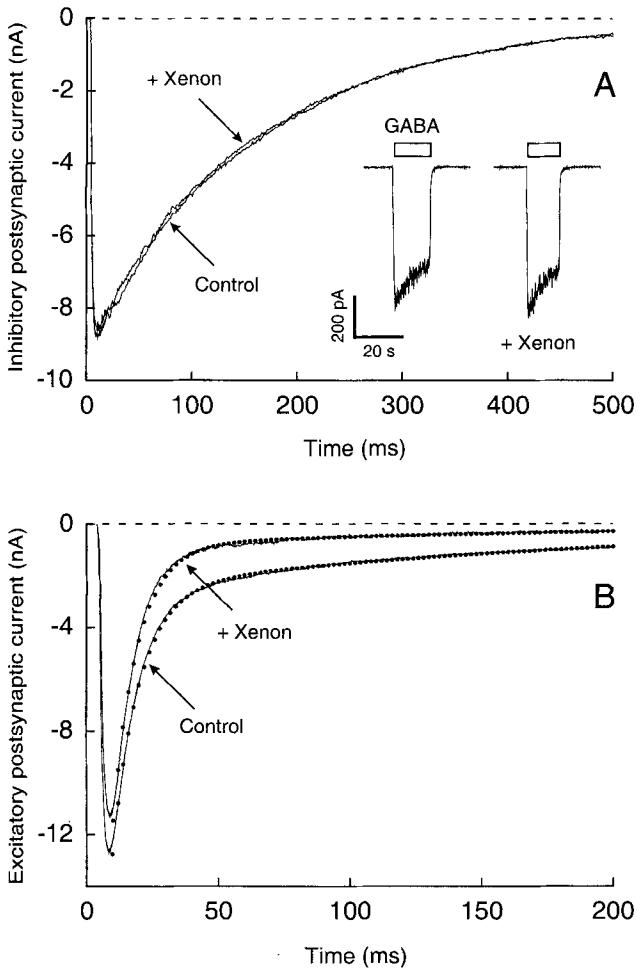

Fig. 6. Representative traces showing the effects of xenon on inhibitory and excitatory postsynaptic responses. (*A*) GABAergic postsynaptic currents in the presence and absence of 3.4 mM xenon. Xenon has no significant effect on the inhibitory postsynaptic current. (*Inset*) Representative traces illustrating the lack of an effect of 4.3 mM xenon on the current evoked by 3 μM GABA. (*B*) Glutamatergic postsynaptic currents in the presence and absence of 3.4 mM xenon. These traces (see also Franks *et al.*[13]) illustrate the quality of the biexponential fits, which are represented by dots. The principal effect is a reduction in the slow component of the current (in this example, by approximately 70%).

synaptic receptors.[37] Even so, from our results and the best available data,[38-40] it seems likely that much of the effect of isoflurane at glutamatergic synapses is postsynaptic in origin. Future work on miniature EPSCs may help to clarify this point.

This leaves a significant component of the inhibition of the EPSC, however, that must presumably be presynaptic.[10] The presynaptic targets may be voltage-gated calcium channels, although this is difficult to assess. The predominant calcium channel that mediates neurotransmitter release at hippocampal glutamatergic synapses, the P/Q-type channel,[41,42] is very insensitive to isoflurane.[43,44] Conversely, neurotransmitter release can be sensitive to small changes in presynaptic calcium entry[45]; therefore, a small inhibition of calcium channels might translate into larger changes in glutamate release.

Effects of Isoflurane on Inhibitory Currents

The effects of isoflurane on GABAergic IPSCs were, for the most part, qualitatively and quantitatively different from its effects on glutamatergic EPSCs. The one feature in common (figs. 2B and 4B) was a concentration-dependent decrease in the amplitude of the postsynaptic current, although there was no significant inhibition of the IPSC amplitude over the clinically relevant range of concentrations. The predominant effect of isoflurane was a marked prolongation in the time course of the IPSC, which, even with the reduction in the peak amplitude at higher concentrations, always translated into a substantial increase in the total charge transfer (fig. 4C). These findings are consistent with some previous voltage-clamp studies of volatile anesthetics acting at GABAergic synapses.[5,12,46]

An analysis of the kinetics of the IPSC showed that, although both time constants (τ_{fast} and τ_{slow}) increased monotonically with increasing anesthetic concentration (fig. 5A), the amplitude of the slow component increased slightly but the amplitude of the fast component was strongly inhibited (fig. 5B). These combined effects resulted in a large increase in the charge transferred by the slow component of the IPSC, with little effect—in fact, a small reduction—in the charge carried by the fast component (fig. 5C). Similar results with volatile anesthetics have been reported by Jones and Harrison[12] with cultured hippocampal neurons, and by Pearce[46] with hippocampal slices.

The finding that isoflurane effectively only changes the charge transfer through the slow component of the IPSC poses an important question: Does this slow component reflect the existence of a population of $GABA_A$ receptors that is particularly sensitive to anesthetics, or does it simply reflect the complexity of channel kinetics? It has been suggested that kinetically distinct $GABA_A$ receptors may underlie the two components of GABAergic IPSCs,[47] and that these different receptor subtypes have very different anesthetic sensitivities.[46,48] However, this has yet to be shown in expression systems *in vitro*.[49] Moreover, it has been shown that a homogeneous population of $GABA_A$ receptors can display biexponential

Table 3. The Effects of 3.4 mM (~1 MAC) Xenon on Synaptic Currents and of 200 μM AP5 on Excitatory Synaptic Currents

	Inhibitory Currents			Excitatory Currents					
	+3.4 mM Xenon			+3.4 mM Xenon			+200 μM AP5		
Parameter	% Change (mean ± SEM)	↑, ↓ or NS*	n†	% Change (mean ± SEM)	↑, ↓ or NS*	n†	% Change (mean ± SEM)	↑, ↓ or NS*	n†
I_{peak}	−3.1 ± 2.2	NS	11	−13.5 ± 1.8	↓	10	−16.3 ± 2.1	↓	10
Decay half-time	−3.4 ± 2.4	NS	10	−22.7 ± 2.5	↓	11	−24.1 ± 3.3	↓	10
Total charge transfer	−2.3 ± 3.9	NS	10	−55.6 ± 2.7	↓	11	−75.2 ± 2.5	↓	10
I_{fast}	−4.3 ± 2.8	NS	11	−4.1 ± 5.2	NS	11	6.8 ± 6.2	NS	10
I_{slow}	18.8 ± 12.4	NS	11	−70.3 ± 2.4	↓	11	−98.8 ± 0.8	↓	10
τ_{fast}	−1.3 ± 3.5	NS	11	−12.3 ± 1.8	↓	11	−5.9 ± 2.3	↓	10
τ_{slow}	0.8 ± 6.0	NS	11	−6.7 ± 6.5	NS	11	—	—	—
Q_{fast}	1.5 ± 7.2	NS	12	−11.4 ± 5.6	NS	11	5.3 ± 9.6	NS	10
Q_{slow}	12.6 ± 8.4	NS	12	−68.2 ± 4.2	↓	11	−96.0 ± 3.4	↓	10

* Not significant at the 95% confidence level (Student *t* test).

† Number of cells.

decay kinetics,[50] indicating that the two decay components can simply result from different conducting states of a single $GABA_A$ receptor subtype.[51,52] Therefore, isoflurane could act by stabilizing one or more of the longer lived open states, or by affecting the kinetics of transitions between different states. Until more is known about the factors responsible for the biphasic decay of GABAergic IPSCs, however, this question remains difficult to answer.

In regard to the molecular site of action at inhibitory synapses, there can be little doubt that isoflurane exerts its principal effects postsynaptically; but, as with glutamatergic synapses, there may be small effects presynaptically that could be responsible for some of the depression in the amplitude of the IPSC (fig. 4B) at higher isoflurane concentrations. However, a thorough study of the effects of isoflurane on miniature IPSCs in hippocampal neurons[48] strongly suggests that much of this depression can be attributed to direct actions on the postsynaptic receptors.

Effects of Xenon on Synaptic Currents

The selectivity of action found with xenon was unexpected.[13] Because almost all general anesthetics potentiate the actions of GABA at $GABA_A$ receptors,[53,54] we anticipated that xenon would be no exception. However, the complete absence of an effect of xenon on $GABA_A$ receptors puts it into the same class of agents as ketamine, a so-called dissociative anesthetic, which is also ineffective at $GABA_A$ receptors[55] and is thought to act predominantly at NMDA receptors. Similarly, our results show that xenon selectively blocks NMDA receptors with little effect at AMPA/kainate receptors. This latter result strongly suggests that the actions of xenon are postsynaptic in origin. The lack of an effect of xenon on the decay time of the NMDA receptor component, however, rules out a simple open-channel block mechanism of inhibition, as do data[13] showing that xenon noncompetitively inhibits NMDA-evoked currents without affecting the NMDA EC_{50} (half-maximal) concentration. Whatever the exact molecular basis for the surprising selectivity of xenon for NMDA receptors, this selectivity simply accounts for many features of the unusual pharmacologic profile of xenon. NMDA receptor antagonists share a number of common features, including the ability to induce profound analgesia and psychotomimetic effects. A strong case has been made[56] that nitrous oxide (laughing gas) exerts many of its effects in this way. Likewise, we suggest that the action of xenon at NMDA receptors accounts for many of its analgesic and anesthetic properties, and its ability to induce a state of euphoria.[57] Consistent with this, the much smaller inhibition of the NMDA receptor by isoflurane correlates with its relative lack of analgesic potency.[58]

Significance for Anesthetic Mechanisms

We determined that isoflurane and xenon are surprisingly selective in their actions, having very different effects on excitatory and inhibitory synaptic transmission. At clinically relevant concentrations, isoflurane has its greatest effect at GABAergic synapses, causing a marked potentiation of total charge transfer. At glutamatergic synapses, the effects of isoflurane are smaller, but still significant. Although both of these effects may contribute to the anesthetic actions of isoflurane, additional criteria (*e.g.*, stereoselectivity) are needed before the

relative importance of these effects can be assessed.[59] Moreover, the relation between anesthetic-induced changes in charge transfer at individual synapses and changes in the firing patterns of neuronal networks involved in anesthesia are unknown. The actions of xenon may be accounted for solely in terms of effects at glutamatergic synapses, although other targets may well be identified in the future. Nonetheless, the insensitivity of GABAergic synapses to xenon indicates that its mechanisms of action are clearly different from those of most general anesthetics. At the mechanistic level, it is clear that, for both xenon and isoflurane, postsynaptic receptors are the most important molecular targets.

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