Anesthesiology 2004; 100:347–58

Determinants of the Sensitivity of AMPA Receptors to Xenon

Andrew J. R. Plested, Ph.D., Scott S. Wildman, Ph.D.,† William R. Lieb, Ph.D.,‡ Nicholas P. Franks, Ph.D.§*

Background: **There is substantial and growing literature on the actions of general anesthetics on a variety of neurotransmitter-gated ion channels, with the greatest attention being focused on inhibitory γ-amino butyric acid type A receptors. In contrast, glutamate receptors, the most important class of fast excitatory neurotransmitter-gated receptor channels, have received much less attention, and their role in the production of the anesthetic state remains controversial.**

Methods: **α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors formed from a variety of different subunits were expressed in *Xenopus* oocytes and HEK-293 cells, and their sensitivities to the inhalational general anesthetics xenon, isoflurane, and halothane were determined using two-electrode voltage clamp and patch clamp techniques. The effects of desensitization on anesthetic sensitivity were investigated using cyclothiazide and site-directed mutagenesis. An ultrarapid application system was also used to mimic rapid high-concentration glutamate release at synapses.**

Results: **The authors show that xenon can potently inhibit AMPA receptors when assayed using bath application of kainate. However, when the natural neurotransmitter L-glutamate is used under conditions in which the receptor desensitization is blocked and the peak of the glutamate-activated response can be accurately measured, the pattern of inhibition changes markedly. When desensitization is abolished by a single-point mutation (L497Y in GluR1 and the equivalent mutation L505Y in GluR4), the xenon inhibition is eliminated. When AMPA receptors are activated by glutamate using an ultrarapid application system that mimics synaptic conditions, sensitivity to xenon, halothane, and isoflurane is negligible.**

Conclusions: **AMPA receptors, when assayed in heterologous expression systems, showed a sensitivity to inhalational anesthetics that was minimal when glutamate was applied rapidly at high concentrations. Because these are the conditions that are most relevant to synaptic transmission, the authors conclude that AMPA receptors are unlikely to play a major role in the production of the anesthetic state by inhalational agents.**

IONOTROPIC glutamate receptors underlie the majority of fast excitatory synaptic transmission in the mammalian central nervous system. It has long been thought that the excitatory synaptic conduction mediated by these glutamate receptors may be an important target for general anesthetics.[1] The profound reduction in whole-animal anesthetic requirement that is observed[2,3] when anesthetics are coadministered with glutamate receptor antagonists has been used as support for this view. Moreover, there is clear evidence that excitatory synaptic transmission can be substantially affected by clinically relevant concentrations of anesthetics.[4–8] However, even for closely related anesthetics, different responses have been reported, with some investigators showing[4,9] that pentobarbital strongly inhibits excitatory synaptic transmission, whereas others report[10] that thiopental causes no significant inhibition. Similarly disparate results have been obtained with inhalational anesthetics. For example, some studies have found no effects on stimulus-evoked excitatory responses[11,12] in rat hippocampus, whereas others[6,8] have found that excitatory postsynaptic potential amplitudes are significantly affected. These disparate findings may be a consequence of both different experimental techniques and preparations, but whatever the reason, the sensitivity of excitatory transmission to anesthetics remains uncertain.

Even in cases in which anesthetic inhibition of excitatory synaptic conduction seems unambiguous, it has generally not been possible to determine whether the primary targets are presynaptic, postsynaptic, or both. One potentially direct approach would be to determine the effects of anesthetics on defined glutamate receptor subtypes expressed in *in vitro* expression systems. Indeed, there have been several articles[13–18] reporting the results of such studies, but, as with the synaptic systems, a complex picture emerges. Some studies report significant inhibition, whereas others report insensitivity; however, this depends on both the general anesthetic studied and the receptor subunit composition. Importantly, it has also been shown that anesthetic sensitivity can depend greatly on the neurotransmitter agonist used.[4,19] While glutamate is the major neurotransmitter released at excitatory synapses, postsynaptic glutamate receptors are categorized according to both sequence homology and their sensitivity to artificial agonists: *N*-methyl-D-aspartate (NMDA), kainic acid, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA).

The subject of this article is the anesthetic sensitivity of AMPA receptors (AMPARs). These receptors are probably tetramers[20] formed from combinations of four subunits (GluR1–4) and give rise to the fast current component at glutamatergic synapses. Functional heterogeneity of native AMPARs is generated not only by variable subunit stoichiometry, but also by the existence of splice variants and posttranscriptional RNA editing.[21]

Although AMPARs can, by definition, be activated selectively by AMPA, they can also be activated by kainate (which is a partial agonist[22]) as well as, of course, glutamate. The application of glutamate to isolated receptors leads to an extremely rapidly desensitizing response,[23] with

* Research Student. Current position: Department of Pharmacology, University College London, London, United Kingdom. † Research Associate. Current position: Department of Physiology, Royal Free and University College Medical School, London, United Kingdom. ‡ Professorial Research Fellow, § Professor of Biophysics and Anesthetics.

Received from the Biophysics Section, The Blackett Laboratory, Imperial College London, London, United Kingdom. Submitted for publication August 4, 2003. Accepted for publication October 6, 2003. Supported by grants from the Medical Research Council, London, United Kingdom, and Carburos Metálicos, Madrid, Spain. Dr. Plested received a Medical Research Council Studentship.

Address reprint requests to Dr. Franks: Biophysics Section, The Blackett Laboratory, Imperial College London, London SW7 2AZ, United Kingdom. Address electronic mail to: n.franks@ic.ac.uk. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

a time course of the order of milliseconds. It has been common practice, when assessing the pharmacologic sensitivity of glutamate receptors, to use artificial agonists such as kainate, which elicit much slower, nondesensitizing responses that are experimentally more convenient to measure and easier to record. The use of artificial agonists that give responses with kinetics that are very different from those of the natural neurotransmitter, although undoubtedly convenient, runs the risk that their inhibition by anesthetics, or any other drug for that matter, may not accurately reflect their sensitivity when glutamate is used as the agonist.

In previous work from this laboratory,[24,25] we have shown that the "inert" gas xenon has little or no effect on γ-amino butyric acid type A receptors (which are sensitive[26] to most other general anesthetics) but is an apparently selective blocker of the NMDA subtype of glutamate receptors. At a concentration that causes general anesthesia in humans (approximately 70% of 1 atmosphere [atm]), xenon inhibited the slow component of the excitatory postsynaptic currents, which can be identified as being mediated by NMDA receptors, while having no effect on the fast component of the response, which can be identified as being due to AMPARs. Subsequently, during experiments that initiated the current study, we found that AMPARs were potently blocked when expressed in *Xenopus* oocytes with kainate used as an agonist, a result that apparently contradicted our experiments using the synaptic preparation.[24] Because of the importance of the possibility that the pharmacologic sensitivity of glutamate receptors might depend critically on the nature of the agonist and/or on the expression system, we explored this further and have investigated the effects of inhalational anesthetics (with emphasis on xenon) on AMPARs using *in vitro* expression systems.

Materials and Methods

Preparation of RNA and cDNA

Plasmid DNA constructs encoding the four rat AMPAR subunit complementary DNAs (cDNAs) (GluR1–4) were gifts from Peter H. Seeburg, Ph.D. (Professor, Max Planck Institute for Medical Research, Heidelberg, Germany). The complementary RNA (cRNA) was synthesized from linearized plasmid DNA constructs. Transcription of capped RNA transcripts was performed using the RiboMAX kit with SP6 RNA Polymerase and Ribo m^7G Cap Analog (Promega, Southampton, United Kingdom). The quality of the RNA was assessed by gel electrophoresis and spectrophotometry. The RNA was stored in Tris-EDTA buffer (1 mM Tris-HCl, 0.1 mM EDTA, titrated to pH 8.0 with NaOH) at −80°C in single-use aliquots.

To generate the nondesensitizing mutant AMPARs,[27] point mutations were introduced into AMPAR clones using the Quikchange kit (Stratagene, Amsterdam, The Netherlands). A pair of short (25–45 bases) complementary oligonucleotide primers, incorporating the intended mutation, was synthesized (MWG-Biotech, Ebersberg, Germany). To aid identification of successful mutants, a silent restriction site was included in the primer sequence. Mutant DNA constructs were sequenced (MWG-Biotech) to confirm the introduction of the correct mutated bases.

For transient transfection into mammalian cells, AMPAR cDNAs encoding GluR1 (flip) and GluR4 (flip) were subcloned into a vector containing a cytomegalovirus promoter (pcDNA 3.1; Invitrogen, Paisley, United Kingdom). The start codon was placed in strong context by substituting an optimal Kozak sequence.[28] Constructs were sequenced to ensure the correct orientation and identity of the insert in each case.

Preparation of Xenopus *Oocytes*

Adult female *Xenopus laevis* frogs (Blades Biological, Cowden, Kent, United Kingdom), maintained in freshwater holding tanks at 20°–22°C, were anesthetized by immersion in a 0.2% (weight/volume) solution of tricaine (3-aminobenzoic acid ethyl ester, methanesulfonate salt). Portions of the ovaries were surgically removed and teased apart with forceps and stored in Barth's medium (composition: 88 mM NaCl, 1 mM KCl, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 2.4 mM $NaHCO_3$, 0.41 mM $CaCl_2$, 10 mM HEPES, 0.1 mg/ml gentamicin, pH 7.4). Oocytes at stage 5 or 6 of development were selected by visual inspection and incubated for 1 h in Ca^{2+}-free modified Ringer's solution (composition: 110 mM NaCl, 1 mM KCl, 1.8 mM $BaCl_2$, 5 mM HEPES, pH 7.45) containing collagenase (type 1A, 2 mg/ml) at room temperature (22°C). After careful rinsing to remove collagenase, oocytes were placed in a hypertonic modified Ringer's solution (composition: 220 mM NaCl, 2 mM KCl, 10 mM HEPES, pH 7.45) to facilitate mechanical removal of the follicular layer with fine forceps. Selected oocytes were injected with 50 nl Tris-EDTA buffer containing 10–50 ng AMPAR cRNA, using a calibrated micropipette (10- to 16-μm tip diameter) and a Drummond automated pipetter. When combinations of subunits were injected in mass ratios, the Tris-EDTA buffer containing the relevant subunits was mixed in the appropriate ratio before injection. Injected oocytes were maintained in a cooled incubator (BDH, Poole, Dorset, United Kingdom) at 18°C in Barth's medium for 1–7 days before use. All chemicals, unless otherwise stated, were obtained from Sigma (Poole, Dorset, United Kingdom).

Solution Preparation

Cyclothiazide (Tocris Cookson, Avonmouth, United Kingdom) was prepared in ethanol. The maximum ethanol concentration in solutions containing cyclothiazide was 25.6 mM. Ethanol was added at this concentration to

all control solutions, and its inclusion had no effect on AMPAR currents or resting membrane currents.

Solutions for xenon experiments were prepared by bubbling pure gases (oxygen, nitrogen, or xenon) through fine sintered-glass bubblers in Dreschel bottles filled with extracellular saline. During bubbling, the solutions were continuously stirred at room temperature. The solutions were bubbled at a rate of 30 ml/min for 20 min. Gas chromatography measurements showed that xenon would saturate a solution bubbled in this way within 10 min. Drugs and agonists were not included in oxygenated saline to minimize oxidation but were included in the nitrogen and xenon solutions at the appropriate concentrations. Generally, two mixtures of saline saturated with gas at 1 atm were formed. Mixing nitrogen- and oxygen-saturated solutions in the ratio 4:1 formed a control saline solution, with the partial pressures of the dissolved gases equivalent to 80% atm nitrogen and 20% atm oxygen. The xenon test solution was prepared in a similar manner and contained the equivalent of 80% atm xenon and 20% atm oxygen. The concentration of xenon in such a solution at room temperature is 3.44 mM.[24] Xenon (99.9%; research grade) was obtained from Air Products (Basingstoke, United Kingdom), and nitrogen and oxygen were obtained from BOC (Guildford, Surrey, United Kingdom). Solutions containing the volatile anesthetics isoflurane (Aerrane; Baxter Healthcare, Newbury, Berkshire, United Kingdom) or halothane were prepared as volume fractions of saturated aqueous solutions. The concentration of a saturated aqueous solution of isoflurane at 20°C is 15.3 mM[29] and of halothane is 17.5 mM.[30] Anesthetic solutions were stored in sealed volumetric flasks until experiments began and thereafter in glass syringe barrels containing a polypropylene float. Gas chromatography measurements showed that losses from such devices are negligible over periods as long as 12 h. Teflon tubing and nylon valves were used throughout the experimental setups to minimize nonspecific losses of anesthetic. Anesthetics were preapplied for 90–120 s to allow equilibration to occur.

Tissue Culture

Modified HEK-293 cells (tsA 201) were maintained in 5% CO_2–95% air in a humidified incubator at 37°C in growth medium (composition: 90% Dulbecco's Modified Eagle's Medium; 10% fetal bovine serum; 100 units/ml penicillin; and 0.1 mg/ml streptomycin). When the HEK-293 cells were 80% confluent, they were split and plated for transfection onto glass coverslips coated with poly-D-lysine (1 mg/ml) to ensure good cell adhesion.

The HEK-293 cells were transiently transfected using the calcium phosphate method: 3 μg cDNA coding for GluR1 or GluR4 subunits were added to each 35-mm well, and 0.5 μg of a plasmid encoding the cDNA of green fluorescent protein was included to identify cells expressing AMPARs. After a 24-h incubation period at 3% CO_2, 97% air the cells were rinsed with saline, and fresh growth medium was added to the wells. The cells were incubated at 37°C with 5% CO_2–95% air for 12–72 h before electrophysiologic assay.

Electrophysiology

Ionic currents in *Xenopus* oocytes, evoked by bath application of glutamate or kainate, were recorded using the two-electrode voltage clamp technique with an Axoclamp 2A amplifier (Axon Instruments, Union City, CA). Recording pipettes were pulled using a PP-8 puller (Narishige, Tokyo, Japan) from thick-walled glass capillaries (GC150F-7.5; Harvard Apparatus, Edenbridge, United Kingdom). When back-filled with 3 M KCl, their resistance was approximately 1 MΩ. Currents were filtered at 5 Hz (−3 dB) before being digitized and stored on a computer. Oocytes were perfused at 1 ml/min with modified Ringer's solution (composition: 110 mM NaCl, 1 mM KCl, 1.8 mM $BaCl_2$, 5 mM HEPES, titrated to pH 7.45 with NaOH). Oocytes with resting membrane potentials greater than −30 mV were discarded. All experiments were performed at room temperature (21°–23°C).

When multiple AMPAR subunits are expressed in *Xenopus* oocytes, a heterogeneous population of receptors is formed, and the proportions of channels that contain a given subunit is determined by the mass ratio of injected cRNA.[31] To form heteromeric receptors of GluR2 with GluR 1, 3, or 4, cRNAs were injected in a mass ratio of 5:1 (in favor of GluR2). Current–voltage (I-V) relations were determined to check that the bulk of the current was passed by heteromeric receptors, incorporating the GluR2 subunit (as opposed to homomeric receptors not containing GluR2). The response to a voltage ramp (−80 mV to + 40 mV at 60 mV/s) during control conditions (*i.e.*, in the absence of agonist) was subtracted from the response during an application of a nondesensitizing agonist. Homomeric AMPARs exhibit strong inward rectification, but heteromeric receptors containing edited GluR2 subunits have a weakly outward-rectifying I-V response.[32] The extent of rectification was determined by calculating the ratio of the baseline-subtracted slope conductance between 35 and 45 mV and that between −75 and −65 mV. For oocytes expressing heteromeric receptors activated by kainate, a typical value for the rectification ratio was 2, in agreement with Verdoorn *et al.*[32] Cells that displayed a rectification ratio greater than 1.55 were taken to have expressed a broadly heteromeric population.[33] The rectification ratio was considerably reduced when determined in the presence of cyclothiazide because cyclothiazide blocks the weak outward rectification of heteromeric receptors[22] and also potentiates homomeric receptors more strongly. In this case, the reference rectification ratio was 0.55, according to the methods of Partin *et al.*[33]

Agonist solutions were applied until currents reached

a steady-state peak response. The order of applications was randomized, and at each concentration of agonist, control solutions were usually applied in pairs before and after paired applications of test solution to ensure reversibility. To monitor receptor rundown, saturating agonist concentrations were applied periodically throughout the record as a reference. Sets of control and test data points were pooled and fitted (unweighted least-squares method) to a Hill equation:

$$I = I_{MAX} \cdot \frac{[\text{agonist}]^{n_H}}{[\text{agonist}]^{n_H} + [EC_{50}]^{n_H}}$$

where *I* is the agonist-induced current, I_{MAX} is the maximum current, [agonist] is the concentration of agonist, $[EC_{50}]$ is the concentration of agonist that produces a half-maximal response, and n_H is the Hill coefficient.

Generally, anesthetic inhibition was noncompetitive with only minor changes in the Hill coefficient, n_H, and EC_{50}. Hence, the percent inhibition was calculated from the reduction in the maximum current, I_{MAX}:

$$\text{Percent Inhibition} = \left(1 - \frac{I^A_{MAX}}{I^0_{MAX}}\right) \times 100\%$$

where I^0_{MAX} is the maximum current in the absence of anesthetic, and I^A_{MAX} is the maximum current in the presence of anesthetic.

Values throughout this article are given as mean ± SEM. In graphs where error bars are not shown, they are smaller than the size of the symbol. Statistical significance was assessed using the Student *t* test.

A rapid perfusion system driven by a piezoelectric actuator was used to apply brief pulses of glutamate to outside-out patches from HEK-293 cells containing AMPARs. Briefly, theta-section agonist application pipettes were fabricated from borosilicate theta glass capillaries with a thin septum (septum 0.117 mm, OD 2 mm; Hilgenberg, Malsfeld, Germany). The tubing was flame-pulled to a break, and the tip was cut back to a diameter of 150–200 μm using a diamond pencil. Fine Teflon tubing (OD 0.6 mm; Bohlender, Grünsfeld, Germany) was glued into each lumina using cyanoacrylate glue (type 702; Loctite, Welwyn Garden City, United Kingdom). A fine (approximately 1 μm) laminar interface was formed by the efflux of the control and agonist-containing solutions, and the rapid activation of the piezo-translator, under computer control, stepped this interface back and forth to form brief pulses of agonist at the tip of the patch pipette. The control solution was diluted 2% with water to allow measurement of solution exchange corresponding to a change in liquid junction potential. This was measured at the end of each recording by blowing out the patch and was typically 150 μs (10–90% rise time). The time to peak after glutamate application was typically approximately 400 μs (10–90% rise time), comparable with the time to peak in miniature excitatory postsynaptic currents.[34] The composition of the control extracellular solution was 145 mM NaCl, 1.7 mM $MgCl_2$, 1.7 mM $CaCl_2$, 5 mM D-glucose, 8.53 mM HEPES (titrated to pH 7.3 with NaOH), 300 mOsm. Glass electrode micropipettes were pulled from thick-walled borosilicate glass capillaries (GC150F-7.5; Harvard Apparatus) using a two-stage vertical puller (PP-

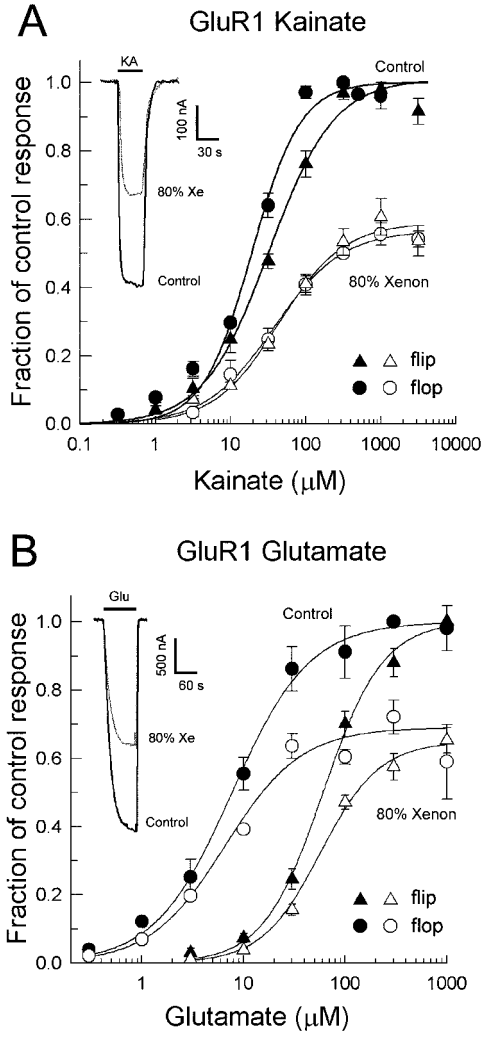

Fig. 1. Inhibition of homomeric α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors by 80% xenon. (*A*) GluR1 receptors expressed in *Xenopus* oocytes and activated by kainate are strongly inhibited by xenon. Equilibrium concentration–response data for GluR1 currents (flop isoform plotted as *circles*, flip isoform plotted as *triangles*) shows that AMPA receptors expressed in *Xenopus* oocytes are noncompetitively inhibited by a clinically relevant concentration of xenon. *Filled symbols* are control data and *open symbols* are responses in the presence of 80% xenon. The *inset* shows a GluR1 (flip) receptor response to 1 mM kainate in the presence (*gray trace*) and absence (*black trace*) of xenon. (*B*) GluR1 receptors are less strongly inhibited when activated by the endogenous agonist L-glutamate in the presence of 100 μM cyclothiazide. Xenon depressed currents due to GluR1 (flop) receptors (*circles*) by 31 ± 2% and those due to flip isoforms (*triangles*) by 35 ± 2%. The *inset* shows GluR1 (flip) control (*black trace*) and 80% xenon (*gray trace*) responses to 1 mM glutamate. The control data were recorded in the presence of 80% N_2.

Table 1. Inhibition by 80% Xenon of Homomeric AMPA Receptors Expressed in *Xenopus* Oocytes

AMPA Receptor Subunit/Splice Isoform	Kainate				Glutamate (+ 100 μM Cyclothiazide)			
	No. of Cells	Inhibition by 80% Xenon,* %	Control EC_{50}, μM	Test EC_{50}, μM	No. of Cells	Inhibition by 80% Xenon,* %	Control EC_{50}, μM	Test EC_{50}, μM
GluR1								
Flip	4	41.7 ± 2.3	32.3 ± 3.2	43.7 ± 7.8†	5	35.2 ± 1.8	60.7 ± 4.8	57.2 ± 8.2†
Flop	5	43.5 ± 2.3	19.1 ± 2.1	37.6 ± 7.2†	5	31.0 ± 2.2	7.7 ± 1.2	6.6 ± 1.6†
GluR3								
Flip	4	52.1 ± 3.6	98.0 ± 11.1	54.8 ± 14.0	6	19.8 ± 1.7	264.5 ± 50.3	352.7 ± 62.0†
Flop	4	50.6 ± 2.8	59.2 ± 6.1	100.6 ± 16.0†	5	39.0 ± 4.3	33.0 ± 3.6	33.7 ± 10.3†
GluR4								
Flip	5	49.2 ± 1.8	63.9 ± 4.8	25.1 ± 2.7	4	18.4 ± 0.9	34.7 ± 2.3	39.6 ± 5.5†
Flop	5	44.6 ± 2.6	46.6 ± 6.6	38.2 ± 6.4†	5	59.6 ± 2.7	55.9 ± 4.2	62.0 ± 7.0†

* Inhibition of I^0_{MAX}, calculated from fits to the Hill equation. † Not significantly different from control EC_{50} ($P > 0.05$, unpaired Student *t* test).
AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

830; Narishige). Fire-polished pipettes were back-filled with 0.2 μm–filtered intracellular solution (composition: 130 mM CsCl, 10 mM CsF, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM EGTA, 10 mM HEPES [titrated to pH 7.2 with CsOH], 300 mOsm). The pipette resistance was 4–7 MΩ. The output of the patch clamp amplifier (Axopatch 200; Axon Instruments) was filtered at 10 kHz using an eight-pole Bessel filter (−3 dB; Frequency Devices 902, Lyons Instruments, Waltham Cross, United Kingdom). The rapid rising and falling current phases were adequately described when digitized at 40 kHz (Digidata 1322; Axon Instruments). All recordings were stored on a computer hard disk. Agonist applications were made at intervals of 1–10 s.

Depending on the signal-to-noise ratio for the current records (which was assessed by eye), up to 15 records were averaged. Usually the traces were overlaid, and records that contained anomalous features (such as amplifier integrator resets or spurious noise) were excluded. The average of the preapplication holding current (typically $< |10$ pA$|$) was subtracted from the entire record to give a null baseline. The charge transfer was calculated to a point 80 ms after the peak. The current decay was fitted over the same range with a single exponential. Normalized parameters were calculated from the ratios of control and test values. The charge transfer, Q, of the pulse was calculated by integrating the area under the current trace. Unless stated otherwise, all voltage clamp experiments were performed at a standard holding potential of −60 mV.

Clinically Relevant Concentration of Xenon

The gaseous concentration of xenon that prevents a purposeful response to a painful stimulus (the minimum alveolar concentration [MAC]) varies between species, being 161% atm in rats[35] and 71% in humans.[36] Converting to free aqueous concentrations at 37°C using an Ostwald water–gas partition coefficient of 0.0887,[37] the equivalent EC_{50} is 5.61 mM for rats and 2.47 mM for humans. For our experiments, conducted at room temperature, we used 80% xenon, equivalent to 3.4 mM. This value, intermediate between the MAC values for humans and rats, is considered to be a pharmacologically relevant concentration.

Results

Kainate was applied to oocytes held under a two-electrode voltage clamp to determine the effects of 80% xenon on homomeric AMPARs. The three subunits that yield measurable currents in native form (GluR1, GluR3, and GluR4) were tested in both flip and flop splice variants. (The native form of GluR2 is RNA-edited at the Q/R site[32] and does not form homomeric receptors with an appreciable single-channel conductance.) Kainate evoked no current from blank (noninjected, or water injected; n = 6, data not shown) oocytes. Homomeric AMPARs were substantially inhibited by 80% xenon (fig. 1A and table 1). The presence of xenon reduced the peak current by approximately half for each subunit tested, without greatly altering the EC_{50} for kainate or the Hill coefficient for the fitted curves, indicating that the inhibition was noncompetitive in nature (table 1).

Although the inhibition of kainate-evoked responses suggested that xenon could have a strong inhibitory effect on AMPARs, kainate is not an endogenous agonist for AMPARs. Therefore, we tested the xenon sensitivity of homomeric receptors activated by the endogenous neurotransmitter glutamate to see if xenon inhibition was preserved (fig. 1B). However, glutamate-activated responses desensitize extremely rapidly (in a few milliseconds) so that it is practically impossible to measure a meaningful peak response in oocytes unless this rapid desensitization is prevented. To ensure that the peak of the glutamate response was not masked by receptor desensitization, we included cyclothiazide, which has been shown to block desensitization.[22] Homomeric receptors activated by glutamate in the presence of cyclothiazide showed a varied sensitivity to xenon. AMPARs formed from GluR4 (flop) subunits were inhib-

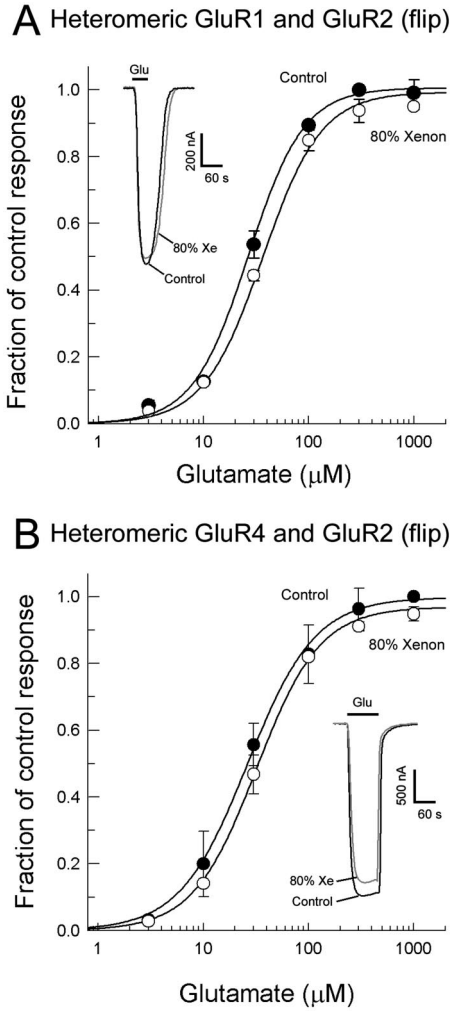

Fig. 2. The effects of xenon on heteromeric α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Coexpression of the GluR2 subunit with GluR1 or GluR4 in *Xenopus* oocytes greatly reduced the inhibitory effect of 80% xenon, compared with the corresponding homomeric receptors. The incorporation of the GluR2 subunit into functional receptors was confirmed by current–voltage plots (see Materials and Methods). (*A*) Inhibition of GluR1 + 2 (both flip) receptors by xenon is negligible (reduction in I_{MAX}: 1 ± 0.1%; n = 4 cells). (*B*) GluR4 + 2 (both flip) receptors were similarly insensitive to xenon with I_{MAX} depressed by 5 ± 0.3% (n = 5 cells). Representative traces of GluR1 + 2 currents (*A*, *inset*) and GluR4 + 2 (both flip) currents (*B*, *inset*) due to 1 mM glutamate are shown in the presence (*gray traces*) and absence (*black traces*) of xenon. Cyclothiazide, 100 μM, was included throughout.

ited by 60 ± 5%; however, other homomeric forms were less strongly inhibited (table 1). Heteromeric receptors were almost completely insensitive to xenon (fig. 2 and table 2). The exceptions to this rule were receptors formed from GluR1 and GluR2 (both flop) that retained the sensitivity of the GluR1 (flop) homomeric receptor.

In general, the inhibition was greater for flop isoforms compared with flip isoforms, which correlates with the greater affinity of flip-containing receptors for cyclothiazide. For example, GluR4 (flip) receptors were relatively insensitive to 80% xenon when activated by glutamate in the presence of 100 μM cyclothiazide, in contrast with GluR4 (flop) homomeric receptors, and the apparent affinity of cyclothiazide is up to 10 times greater for flip isoforms than for flop.[33] The differences in the inhibition between the flip and flop isoforms are consistent with the differential block of desensitized states due to different affinities for cyclothiazide.

Therefore, we investigated the xenon sensitivity of the GluR4 (flip) isoform in the presence of submaximal concentrations of cyclothiazide to determine whether inhibition was increased. First, we constructed an affinity curve for CTZ block of GluR4 (flip) receptor desensitization.[33,38] The steady-state current, as a fraction of the peak response at high cyclothiazide, was taken as a measure of the extent that equilibrium desensitization was blocked. The IC_{50} for block of desensitization by cyclothiazide was 10.3 ± 0.1 μM (fig. 3A). In the presence of 100 μM cyclothiazide (where desensitization is almost completely blocked), GluR4 (flip) receptors were inhibited 18.4 ± 0.9% by 80% xenon (fig. 3B). In contrast, glutamate-evoked responses in the presence of 5 μM cyclothiazide (approximately IC_{10} for removal of desensitization) were much more strongly inhibited (fig. 3C) by 80% xenon with the maximum current (I_{MAX}) reduced by 51 ± 2% (n = 5 cells). Hence, the inhibition by xenon seems to be inversely proportional to the number of receptors in the open state during a slow application of agonist.

To test the relation between the mode of activating the AMPAR current and sensitivity of AMPARs to xenon, we investigated the effects of a point mutation in the extracellular glutamate binding domain (L497Y in GluR1) that is known to reduce AMPAR desensitization drastically.[27] Crystal structures of the GluR2 subunit show that this mutation has a similar effect to binding cyclothiazide.[39] Significantly, incorporating this mutation enabled us to test the xenon sensitivity of the peak of the receptor response, activated with glutamate, without extraneous pharmacologic manipulations. In contrast to wild-type receptors, xenon had virtually no effect (fig. 4 and table 2) on glutamate-evoked currents for homomeric receptors formed by mutated subunits GluR1-L497Y (flip), GluR1-L497Y (flop), and the equivalent mutated receptor GluR4-L505Y (flop).

To further explore AMPAR sensitivity to xenon, we used rapid agonist application techniques to apply brief pulses of glutamate to outside-out patches from HEK-293 cells containing AMPARs. We measured the effects of xenon, as well as two volatile agents, halothane and isoflurane, on the kinetics of these rapidly activated AMPAR currents. Xenon did not significantly inhibit GluR1 or GluR4 receptor patch responses to short (2-ms) or long (120-ms) pulses of glutamate (fig. 5). The peak current and charge transfer were marginally reduced for GluR1 receptors, but the effect was small compared with those observed for AMPARs expressed in *Xenopus*

Table 2. Inhibition of Heteromeric and a Mutant AMPA Receptor* by 80% Xenon

AMPA Receptor Subtype	Isoform	Inhibition by 80% Xenon, % (n = 5)	Reduction of Inhibition by 80% Xenon Compared with Homomer or Wild Type, %
GluR1 + 2	Flip	1.2 ± 0.1	33.9 ± 2.3
GluR3 + 2	Flip	1.0 ± 0.1	18.8 ± 2.1
GluR4 + 2	Flip	4.6 ± 0.3	13.8 ± 1.2
GluR1 + 2	Flop	42.8 ± 3.8	−11.8 ± 1.3
GluR1 L503Y	Flip	6.7 ± 0.2	28.4 ± 1.7
GluR1 L503Y	Flop	11.2 ± 0.7	19.8 ± 1.8
GluR4 L507Y	Flop	10.0 ± 0.6	49.7 ± 3.7

* Expressed in *Xenopus* oocytes and activated by glutamate.

AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

oocytes (table 1). The rates of receptor desensitization and deactivation were not altered in the presence of 80% xenon.

Ideally, we would have investigated the effects of xenon on rapidly evoked kainate currents, but a saturating concentration of kainate typically elicited a peak current only approximately 2% of the peak glutamate current, and, although larger than the steady-state current elicited by glutamate, it was too small to measure in most patches.

Because volatile anesthetics have been reported to inhibit AMPARs in *Xenopus* oocytes[16] as well as inhibiting postsynaptic excitatory currents,[5–8] we anticipated that these agents would inhibit AMPARs activated by glutamate currents, at least at high concentrations. We applied pulses (120 ms) of 3 mM glutamate to patches containing GluR1 receptors in the presence and absence of isoflurane (up to 1,224 μM or 4 MAC). We observed no concentration-dependent inhibition of the peak current or increase in decay rate (fig. 6). We repeated the same experiment with GluR4 (flip) receptors with essentially identical results (data not shown). We tested a second volatile anesthetic, halothane, and found it had no significant effect, either, on glutamate-evoked responses from GluR1 receptors at up to 1,250 μM. These data are shown in figure 7. Additional measurements of the sensitivity of AMPARs to thiopental also showed no inhibitory effects at up to 125 μM, many times higher than the concentration required[26] to cause general anesthesia (n = 3 patches, data not shown).

Discussion

The primary aim of this work was to determine the sensitivity of AMPARs under well-defined conditions using *in vitro* expression systems. These data would help to assess the role that AMPARs might play in the widespread depression of the nervous system that is caused by inhalational anesthetics. Although there is little doubt that these agents can inhibit excitatory synaptic transmission, the role played by postsynaptic AMPARs is unclear. Our main emphasis was on the "inert" anesthetic gas xenon. This was for two reasons. First, xenon is unusual in having little or no effect on γ-amino butyric acid type A receptors, which are sensitive to many other general anesthetics, so other targets must be responsible for its anesthetic action. Second, xenon is able to substantially inhibit the NMDA subtype of glutamate receptors so that effects on other members of the glutamate receptor superfamily might be anticipated. Indeed, in preliminary measurements, we found that xenon could substantially inhibit AMPARs expressed in *Xenopus* oocytes.

This study has confirmed those findings. However, the degree of inhibition varied greatly, depending on both the receptor subunit composition and the agonist used to activate the current. When kainate was used as the agonist, AMPARs were inhibited by approximately 50%, essentially independent of subunit composition (table 1). Although widely used as an agonist of glutamate receptors because it elicits a nondesensitizing response, kainate is a partial agonist,[22] and single-channel recordings indicate that subconductance states may be activated.[40] Also, high-resolution structural data[41] suggest that when kainate binds in the ligand-binding domain of AMPARs, it promotes an intermediate level of domain closure, less than that caused by glutamate.

When we used the neurotransmitter glutamate as the agonist (with cyclothiazide present to preserve the peak response), differences in sensitivity between the various receptor subunit compositions were observed. Overall, there seemed to be an inverse correlation between xenon sensitivity and the extent to which cyclothiazide blocked receptor desensitization. Those subunit combinations that were relatively insensitive to xenon corresponded with those that are known to have a relatively high affinity for cyclothiazide.[33]

We investigated the possibility that the sensitivity of AMPARs to xenon is affected by the extent of receptor desensitization by determining inhibition at different cyclothiazide concentrations (fig. 3). At saturating concentrations of cyclothiazide, the GluR4 (flip) homomer was

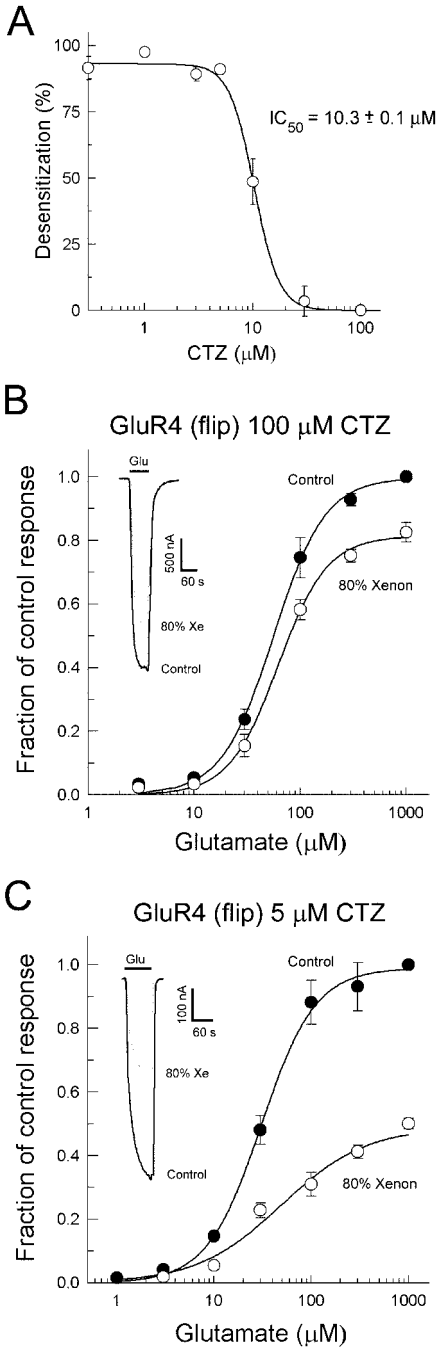

Fig. 3. Inhibition of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors by xenon is related to block of desensitization by cyclothiazide (CTZ). (*A*) Reduction of desensitization by cyclothiazide for GluR4 (flip) receptors expressed in *Xenopus* oocytes. Cyclothiazide (0.3–100 μM) was coapplied with 1 mM glutamate. Cyclothiazide blocked desensitization with an IC_{50} of 10.1 ± 0.1 μM. Currents were normalized to the 100-μM cyclothiazide response. Assuming maximal block of desensitization at this concentration, data from five oocytes were fitted by the equation: $RD = [IC_{50}]^n/([IC_{50}]^n + [\text{cyclothiazide}]^n)$, where *RD* is the fraction of GluR4 (flip) receptors in desensitized states, and *n* is the slope parameter. (*B*) GluR4 (flip) receptors (control data plotted as *filled circles*) are not strongly inhibited by 80% xenon if a saturating concentration of cyclothiazide (100 μM) is included (*open circles*; reduction of maximum current [I_{MAX}]: 18 ± 1%). The *inset* shows xenon depression of GluR4 (flip) currents (*gray trace*) activated by glutamate with 100 μM cyclothiazide, compared with control (in presence of 80% N_2; *black trace*). (*C*) If coapplied cyclothiazide is reduced to 5 μM (approximately IC_{10} for block of desensitization), 80% xenon inhibits control responses (*filled circles*) by an average of 51 ± 4% (*open circles*). The *inset* shows GluR4 (flip) receptor currents activated by 1 mM glutamate with 5 μM cyclothiazide, in the presence (*gray trace*) and absence (*black trace*) of 80% xenon.

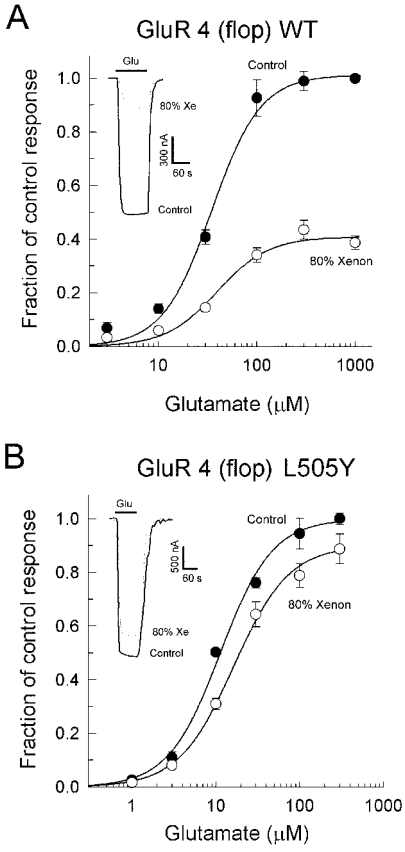

Fig. 4. A point mutation that blocks α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor desensitization eliminates xenon sensitivity. (*A*) Homomeric GluR4 (flop) receptors expressed in *Xenopus* oocytes are very sensitive to xenon when activated by glutamate with 100 μM cyclothiazide. The reduction in maximum current (I_{MAX}) of the fitted Hill curves by 80% xenon was 60 ± 3% (control data, *filled circles*, + 80% xenon, *open circles*; n = 5 cells). The *inset* shows current records for wild type receptors, activated by 300 μM glutamate (with 100 μM cyclothiazide) in the presence of 80% xenon (*gray traces*) and under control conditions (+ 80% N_2, *black traces*). (*B*) Mutant (L505Y) receptors show a much-reduced sensitivity to 80% xenon. Control responses (*filled circles*) were inhibited on average 10 ± 1% (+ 80% xenon, *open circles*; n = 5 cells). Equivalent mutations in the GluR1 subunit (flip and flop isoforms) eliminated xenon sensitivity altogether (table 2). The *inset* shows representative traces for GluR4 L505Y receptors in the presence (*gray traces*) and absence (*black traces*) of 80% xenon.

relatively insensitive to xenon, but a greatly increased sensitivity to xenon was observed as the cyclothiazide concentration was decreased, and the peak response was increasingly compromised as a result of increasing degrees of receptor desensitization. Furthermore, we found that a point mutation that is known[27] to block

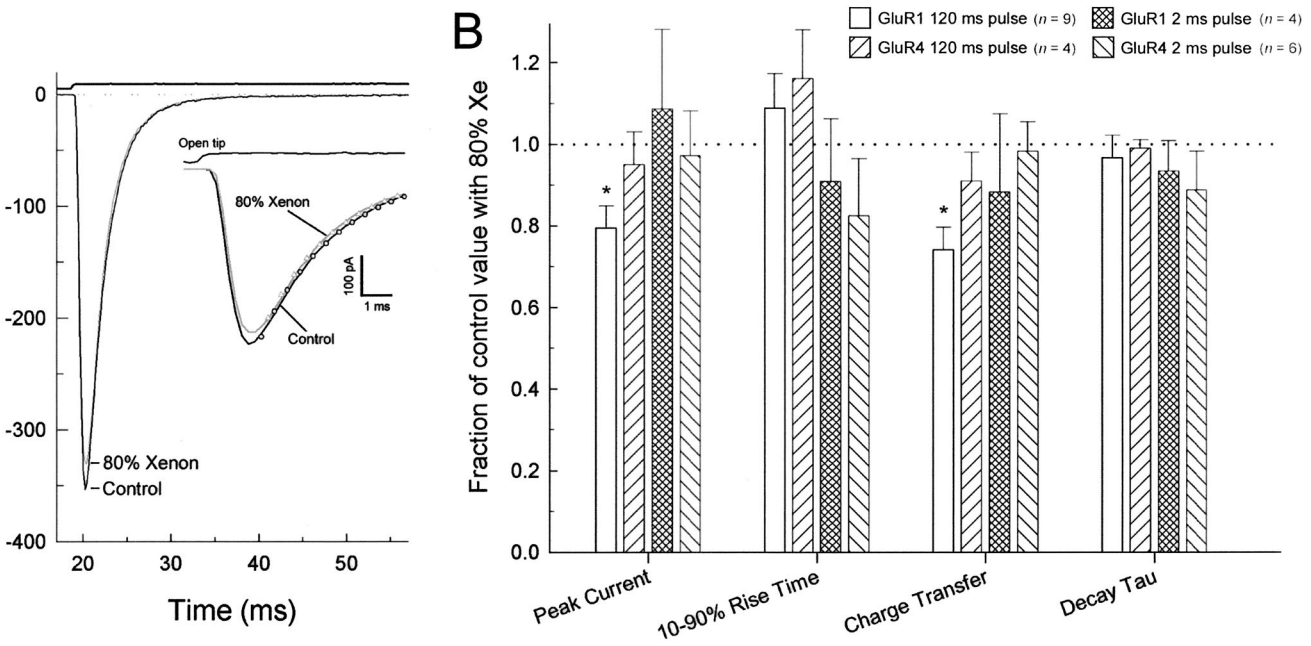

Fig. 5. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors activated by rapid pulses of glutamate are not inhibited by 80% xenon. (*A*) Representative traces of GluR1-containing patch responses to rapidly applied 120-ms pulses of glutamate in control (*black trace*) and test (*gray trace*) conditions. Outside-out patches were excised from HEK-293 cells. Traces are the average of 10 responses to glutamate applied at 0.2 Hz. Solutions containing 80% xenon and those for washout were preapplied for 90 s. Patch current ran down on average less than 1% per application. The *top trace* shows the time course of agonist application, as measured by the change in liquid junction potential at the open tip of the patch pipette after the experiment (pulse duration, 120 ms; 10–90% increase time, approximately 150 μs). The *inset* shows the same traces on an expanded scale, with single-exponential fits to control (*circles*; τ = 2.48 ms) and test (*triangles*; τ = 2.34 ms) responses, filtered at 2 kHz. (*B*) Summary of effects of 80% xenon on AMPA receptors in rapid agonist application experiments. Patches containing either GluR1 or GluR4 (both flip) were exposed to 80% xenon and activated by short (2-ms) or long (120-ms) pulses of glutamate. Only in the case of GluR1 receptors were any of the fitted parameters altered significantly. The number of patches for each experiment is given in parentheses.

AMPAR desensitization essentially abolished sensitivity to xenon (table 2).

Taken together, these results strongly suggest a link between xenon sensitivity and receptor desensitization. It is possible that xenon influences the equilibrium distribution of receptors in open, closed, and desensitized states in ways that have physiologic relevance. For example, xenon could slow recovery from a desensitized state, which would alter postsynaptic responses to rapid stimulation by a train of action potentials. On the other hand, the changes in sensitivity that occur as a result of the mutation of the receptor or binding of cyclothiazide may not be relevant to the activation and desensitization of native receptors. Further experiments using rapid applications of glutamate could address this point.

Because weakly interacting anesthetics such as xenon might exert their effects by binding to and stabilizing certain conformational states of ion channels, the anesthetic sensitivity of a putative channel target should be assessed using an endogenous agonist that is applied with a time course and at concentrations that are physiologically relevant. This can be accomplished, or at least approximated, by using an ultrarapid perfusion system to apply brief high concentrations of neurotransmitter with a time of application that is as fast as that found at synapses.

Perhaps unexpectedly, given the xenon sensitivity of the AMPARs when expressed in oocytes, rapidly activated AMPARs were remarkably insensitive to 80% xenon. The peak current activated by 3 mM glutamate was reduced (20 ± 5%, n = 9 patches) in the case of GluR1 receptors, but patch currents due to GluR4 receptors were not affected at all. There were no significant changes in the rate of desensitization or the rate of activation. Responses of either homomeric receptor (GluR1 and GluR4) activated by short (2-ms) or long (120-ms) pulses of glutamate were equally insensitive. The deactivation rate of the receptors after a 2-ms pulse was also not affected by 80% xenon. Unfortunately, it was not possible to test the GluR1/GluR2 combination, which showed some sensitivity in *Xenopus* oocytes, because of the very small currents obtained in patches. Although we suspect this sensitivity has more to do with the relative affinity of this subunit combination to cyclothiazide,[33] the possibility of this receptor being sensitive to xenon remains, strictly speaking, open. Because the steady-state current after a 120-ms application of glutamate or kainate might be a correlate of the slowly activated AMPAR currents seen with bath application in oocytes, we tried to ascertain whether this residual current was inhibited. However, the magnitude of the steady-state current generated by a 120-ms pulse of ei-

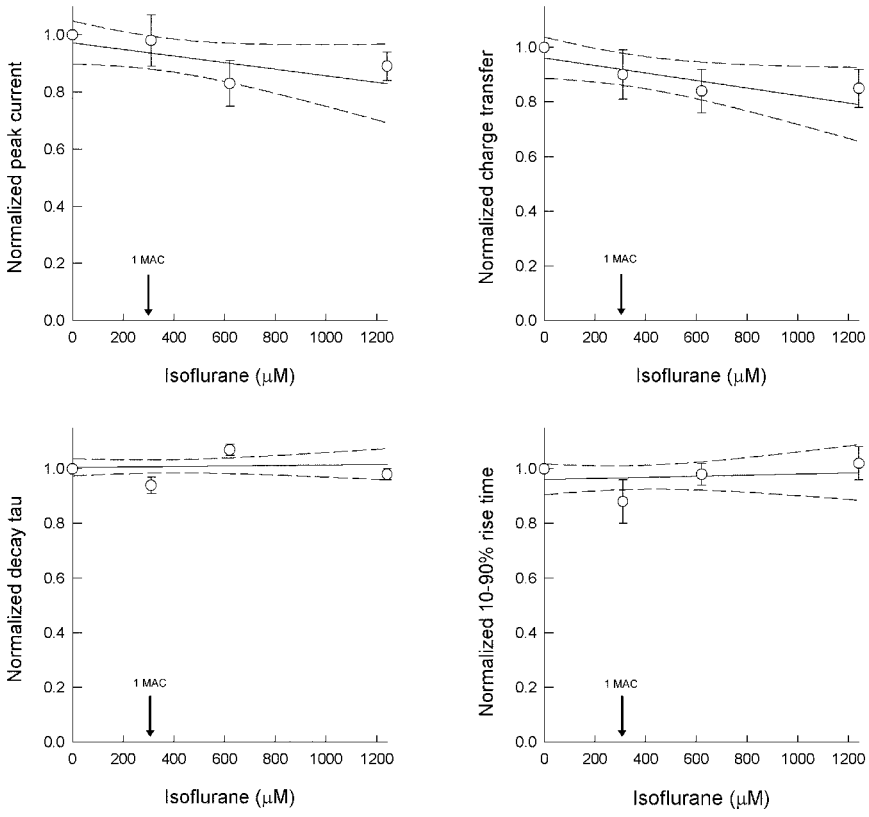

Fig. 6. Isoflurane does not inhibit rapidly activated GluR1 (flip) receptor currents. Responses of outside-out patches (excised from HEK-293 cells) to 120-ms pulses of 3 mM glutamate are not significantly affected by isoflurane up to 1,224 μM (4 minimum alveolar concentration [MAC]). At a clinically relevant concentration, the effects on the peak current, charge transfer, decay rate, and increase time were negligible. Each *point* represents the mean of n ≥ 5 patches. The *dashed lines* represent the 95% confidence interval of the fitted linear regression to all points.

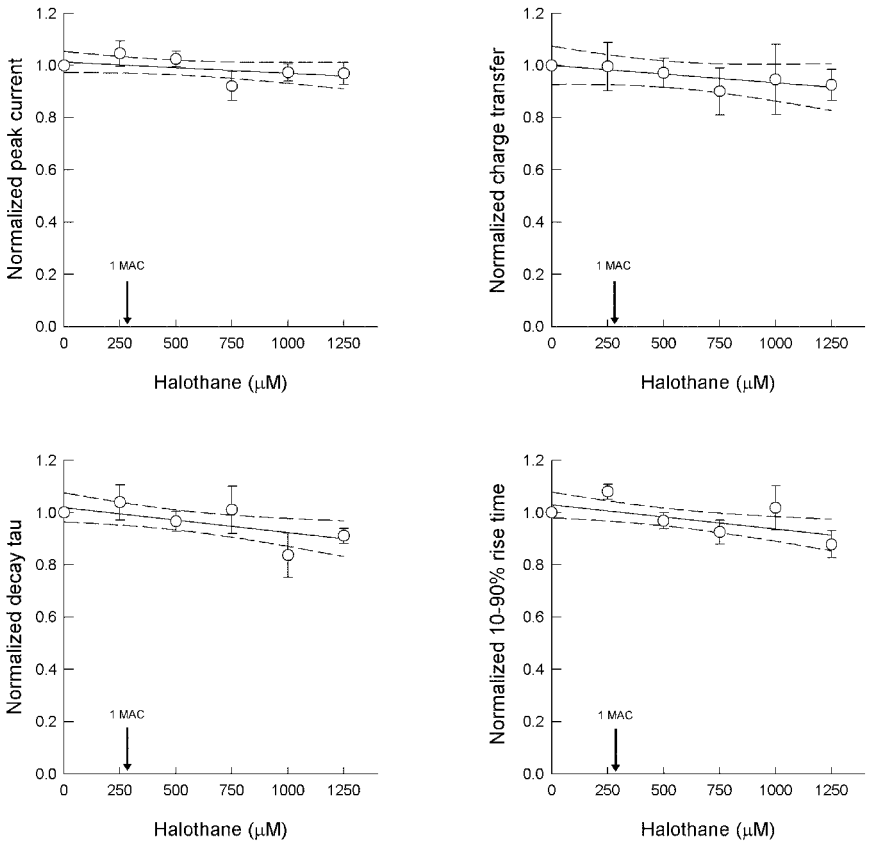

Fig. 7. Halothane does not inhibit rapidly activated GluR1 (flip) receptors. Responses of outside-out patches (excised from HEK-293 cells) to 120-ms pulses of 3 mM glutamate were not inhibited by halothane up to 1,250 μM (5 MAC). Neither the peak current nor rate of desensitization was affected by the presence of halothane. Each *point* represents the mean of n ≥ 5 patches. The *dashed lines* represent the 95% confidence interval of the fitted linear regression to all points.

ther agonist was often comparable with the noise in the patch (typically approximately 100 times smaller than the peak glutamate response), so this was not possible to determine. Further investigations may be required to clarify the extent and impact of these putative inhibitory effects.

In view of these results with xenon, we studied the effects of the inhalational anesthetics halothane and isoflurane under conditions in which AMPARs were activated by ultrarapid application of glutamate. We found that isoflurane failed to show any inhibitory actions on GluR1 or GluR4 receptors at up to 4 MAC (MAC for isoflurane for rats[42] corresponds to 0.31 mM). A similar lack of sensitivity was observed with halothane at up to approximately 5 MAC (MAC for halothane[42] corresponds to 0.27 mM).

Reports of AMPAR inhibition by inhalational anesthetics have generally used kainate or other artificial agonists to evoke currents. For example, AMPAR currents evoked by kainate in *Xenopus* oocytes are sensitive to inhalational anesthetics.[16,43] Similarly, quisqualate-evoked currents have also been reported[19] to be sensitive to enflurane and isoflurane. This sensitivity is consistent with the results we report here for xenon when kainate is used as an agonist. However, it would seem that AMPARs are much less sensitive to inhalational general anesthetics when activated by the natural neurotransmitter under conditions approximating to those found at synapses. This is certainly consistent with the absence of any effect of xenon on the fast component of the glutamatergic excitatory postsynaptic currents observed in cultured hippocampal synapses.[24] Therefore, it seems probable that the inhibition[5–8] of excitatory synaptic responses by volatile anesthetics will be accounted for largely, if not entirely, by presynaptic mechanisms.[8,44] This is in contrast to the actions of xenon[24] and nitrous oxide[45] on glutamatergic synapses, where inhibition can be entirely accounted for in terms of antagonism of postsynaptic NMDA receptors.

Based on the general insensitivity that we have observed for inhalational anesthetics acting on AMPARs when brief pulses of glutamate are used to evoke currents, we conclude that AMPARs are unlikely to play a crucial role in the production or maintenance of the anesthetic state. However, in coming to this conclusion, certain limitations of our study must be recognized. First, some excitatory synapses[46] are thought to involve the release of aspartate rather than, or together with, glutamate, and it is possible that AMPARs activated by aspartate are more sensitive. If such synapses show an enhanced sensitivity to inhalational anesthetics, this may warrant further investigation. Second, our experiments were performed at room temperature rather than at physiologic temperatures, and it is conceivable that this may have affected sensitivity. However, the available evidence[42,47,48] shows that sensitivity generally decreases with increasing temperature, so it is likely that our experiments would have, if anything, exaggerated the sensitivity. Finally, the obvious caveat applies when extrapolating results from *in vitro* expression systems to the enormously more complex environment of intact synapses.

An important corollary to the main conclusion of this study is that the pharmacologic sensitivity of AMPARs can depend greatly on the agonists used. This study is the first to determine the anesthetic sensitivity of glutamate receptors using an ultrarapid application system, and our results emphasize the importance of studying receptors in *in vitro* expression systems under conditions that mimic, as closely as possible, the exposure of synaptic receptors to neurotransmitters.

The authors thank Raquel Yustos, M.Sc. (Research Technician, Department of Biological Sciences, Imperial College London, London, United Kingdom), for technical assistance and Robert Dickinson, Ph.D. (Lecturer, Department of Anaesthetics and Intensive Care, Imperial College London), and Stephen Brickley, Ph.D. (Lecturer, Department of Biological Sciences, Imperial College London), for helpful comments on the manuscript.

References

1. Pocock G, Richards CD: Excitatory and inhibitory synaptic mechanisms in anaesthesia. Br J Anaesth 1993; 71:134–47
2. McFarlane C, Warner DS, Todd MM, Nordholm L: AMPA receptor competitive antagonism reduces halothane MAC in rats. ANESTHESIOLOGY 1992; 77:1165–70
3. Dall V, Orntoft U, Schmidt A, Nordholm L: Interaction of the competitive AMPA receptor antagonist NBQX with hexobarbital. Pharmacol Biochem Behav 1993; 46:73–6
4. Sawada S, Yamamoto C: Blocking action of pentobarbital on receptors for excitatory amino acids in the guinea pig hippocampus. Exp Brain Res 1985; 59:226–31
5. El-Beheiry H, Puil E: Anaesthetic depression of excitatory synaptic transmission in neocortex. Exp Brain Res 1989; 77:87–93
6. Miu P, Puil E: Isoflurane-induced impairment of synaptic transmission in hippocampal neurons. Exp Brain Res 1989; 75:354–60
7. Perouansky M, Baranov D, Salman M, Yaari Y: Effects of halothane on glutamate receptor–mediated excitatory postsynaptic currents: A patch-clamp study in adult mouse hippocampal slices. ANESTHESIOLOGY 1995; 83:109–19
8. MacIver MB, Mikulec AA, Amagasu S, Monroe F: Volatile anesthetics depress glutamate transmission *via* presynaptic actions. ANESTHESIOLOGY 1996; 85:823–34
9. MacIver MB, Roth SH: Barbiturate effects on hippocampal excitatory synaptic responses are selective and pathway specific. Can J Physiol Pharmacol 1987; 65:385–94
10. Dickinson R, de Sousa SLM, Lieb WR, Franks NP: Selective synaptic actions of thiopental and its enantiomers. ANESTHESIOLOGY 2002; 96:884–92
11. Fujiwara N, Higashi H, Nishi S, Shimoji K, Sugita S, Yoshimura M: Changes in spontaneous firing patterns of rat hippocampal neurones induced by volatile anaesthetics. J Physiol (Lond) 1988; 402:155–75
12. Pearce RA, Stringer JL, Lothman EW: Effect of volatile anesthetics on synaptic transmission in the rat hippocampus. ANESTHESIOLOGY 1989; 71:591–8
13. Taverna FA, Cameron BR, Hampson DL, Wang LY, MacDonald JF: Sensitivity of AMPA receptors to pentobarbital. Eur J Pharmacol 1994; 267:R3–5
14. Yamakura T, Sakimura K, Mishina M, Shimoji K: The sensitivity of AMPA-selective glutamate receptor channels to pentobarbital is determined by a single amino acid residue of the alpha 2 subunit. FEBS Lett 1995; 374:412–4
15. Yamakura T, Sakimura K, Shimoji K, Mishina M: Effects of propofol on various AMPA-, kainate- and NMDA-selective glutamate receptor channels expressed in Xenopus oocytes. Neurosci Lett 1995; 188:187–90
16. Dildy-Mayfield JE, Eger EI II, Harris RA: Anesthetics produce subunit-selective actions on glutamate receptors. J Pharmacol Exp Ther 1996; 276: 1058–65
17. Minami K, Wick MJ, Stern-Bach Y, Dildy-Mayfield JE, Brozowski SJ, Gonzales EL, Trudell JR, Harris RA: Sites of volatile anesthetic action on kainate (glutamate receptor 6) receptors. J Biol Chem 1998; 273:8248–55
18. Yamakura T, Harris RA: Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels: Comparison with isoflurane and ethanol. ANESTHESIOLOGY 2000; 93:1095–101
19. Wakamori M, Ikemoto Y, Akaike N: Effects of two volatile anesthetics and

a volatile convulsant on the excitatory and inhibitory amino acid responses in dissociated CNS neurons of the rat. J Neurophysiol 1991; 66:2014–21

20. Rosenmund C, Stern-Bach Y, Stevens CF: The tetrameric structure of a glutamate receptor channel. Science 1998; 280:1596–9

21. Dingledine R, Borges K, Bowie D, Traynelis SF: The glutamate receptor ion channels. Pharmacol Rev 1999; 51:7–61

22. Patneau DK, Vyklicky L Jr, Mayer ML: Hippocampal neurons exhibit cyclothiazide-sensitive rapidly desensitizing responses to kainate. J Neurosci 1993; 13:3496–509

23. Mayer ML, Westbrook GL: The physiology of excitatory amino acids in the vertebrate central nervous system. Prog Neurobiol 1987; 28:197–276

24. de Sousa SLM, Dickinson R, Lieb WR, Franks NP: Contrasting synaptic actions of the inhalational general anesthetics isoflurane and xenon. ANESTHESIOLOGY 2000; 92:1055–66

25. Franks NP, Dickinson R, de Sousa SL, Hall AC, Lieb WR: How does xenon produce anaesthesia? (letter) Nature 1998; 396:324

26. Franks NP, Lieb WR: Molecular and cellular mechanisms of general anaesthesia. Nature 1994; 367:607–14

27. Stern-Bach Y, Russo S, Neuman M, Rosenmund C: A point mutation in the glutamate binding site blocks desensitization of AMPA receptors. Neuron 1998; 21:907–18

28. Kozak M: An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res 1987; 15:8125–48

29. Franks NP, Lieb WR: Stereospecific effects of inhalational general anesthetic optical isomers on nerve ion channels. Science 1991; 254:427–30

30. Raventós J: The action of Fluothane: A new volatile anaesthetic. Br J Pharmacol Chemother 1956; 11:394–410

31. Washburn MS, Numberger M, Zhang S, Dingledine R: Differential dependence on GluR2 expression of three characteristic features of AMPA receptors. J Neurosci 1997; 17:9393–406

32. Verdoorn TA, Burnashev N, Monyer H, Seeburg PH, Sakmann B: Structural determinants of ion flow through recombinant glutamate receptor channels. Science 1991; 252:1715–8

33. Partin KM, Patneau DK, Mayer ML: Cyclothiazide differentially modulates desensitization of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor splice variants. Mol Pharmacol 1994; 46:129–38

34. Diamond JS, Jahr CE: Transporters buffer synaptically released glutamate on a submillisecond time scale. J Neurosci 1997; 17:4672–87

35. Koblin DD, Fang Z, Eger EI II, Laster MJ, Gong D, Ionescu P, Halsey MJ, Trudell JR: Minimum alveolar concentrations of noble gases, nitrogen, and sulfur hexafluoride in rats: Helium and neon as nonimmobilizers (nonanesthetics). Anesth Analg 1998; 87:419–24

36. Cullen SC, Eger EI II, Cullen BF, Gregory P: Observations on the anesthetic effect of the combination of xenon and halothane. ANESTHESIOLOGY 1969; 31:305–9

37. Weathersby PK, Homer LD: Solubility of inert gases in biological fluids and tissues: A review. Undersea Biomed Res 1980; 7:277–96

38. Fleck MW, Bahring R, Patneau DK, Mayer ML: AMPA receptor heterogeneity in rat hippocampal neurons revealed by differential sensitivity to cyclothiazide. J Neurophysiol 1996; 75:2322–33

39. Sun Y, Olson R, Horning M, Armstrong N, Mayer M, Gouaux E: Mechanism of glutamate receptor desensitization. Nature 2002; 417:245–53

40. Swanson GT, Kamboj SK, Cull-Candy SG: Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition. J Neurosci 1997; 17:58–69

41. Armstrong N, Gouaux E: Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: Crystal structures of the GluR2 ligand binding core. Neuron 2000; 28:165–81

42. Franks NP, Lieb WR: Temperature dependence of the potency of volatile general anesthetics: Implications for *in vitro* experiments. ANESTHESIOLOGY 1996; 84:716–20

43. Li L-H, Chen L, Harris A: Enflurane inhibits NMDA, AMPA, and kainate-induced currents in Xenopus oocytes expressing mouse and human brain mRNA. FASEB J 1993; 7:479–85

44. Schlame M, Hemmings HC Jr: Inhibition by volatile anesthetics of endogenous glutamate release from synaptosomes by a presynaptic mechanism. ANESTHESIOLOGY 1995; 82:1406–16

45. Mennerick S, Jevtovic-Todorovic V, Todorovic SM, Shen W, Olney JW, Zorumski CF: Effect of nitrous oxide on excitatory and inhibitory synaptic transmission in hippocampal cultures. J Neurosci 1998; 18:9716–26

46. Gundersen V, Chaudhry FA, Bjaalie JG, Fonnum F, Ottersen OP, Storm-Mathisen J: Synaptic vesicular localization and exocytosis of L-aspartate in excitatory nerve terminals: A quantitative immunogold analysis in rat hippocampus. J Neurosci 1998; 18:6059–70

47. Dickinson R, Lieb WR, Franks. NP: The effects of temperature on the interactions between volatile general anaesthetics and a neuronal nicotinic acetylcholine receptor. Br J Pharmacol 1995; 116:2949–56

48. Jenkins A, Franks NP, Lieb WR: Effects of temperature and volatile anesthetics on $GABA_A$ receptors. ANESTHESIOLOGY 1999; 90:484–91