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Xenon blocks AMPA and NMDA receptor channels by different mechanisms

Henry U. Weigt[1*], Karl J. Föhr[2], Michael Georgieff[2], Eva M. Georgieff[2], Uwe Senftleben[2], and Oliver Adolph[2]

[1]Department of Anaesthesiology, SLK-Kliniken Heilbronn, Bad Friedrichshall, Germany, *Email: henry.weigt@gmx.de;
[2]Department of Anaesthesiology, Ulm University, Ulm, Germany

The noble gas xenon (Xe) inhibits not only NMDA receptors (NMDARs), but also the two other subtypes of glutamate receptor i.e. AMPA (AMPARs) and kainate receptors. Preliminary studies on AMPARs suggest that Xe sensitivity might be coupled to receptor desensitization. In order to find out if this hypothesis can be applied to all glutamate receptors, we analyzed additional "non-desensitizing" AMPARs mutants and compared these with homologous mutants of NMDARs. Membrane currents of Neuro2A or SH-SY5Y cells transfected with cDNA encoding AMPA- or NMDA receptors were investigated by whole cell recordings under voltage clamp conditions. Agonists (glutamate, kainate, NMDA) were applied to the cells by means of a rapid perfusion system. Xenon was preincubated for 20 s before testing it in combination with the particular agonist. Xe (3.5 mM) reduced peak and plateau currents of AMPA wild-type receptors [GluR1(i); GluR2(i,Q)], activated for 5 s with 3 mM glutamate, by 45 and 55%, respectively. With mutant AMPARs showing greatly diminished or abolished desensitization i.e. GluR1(i)_L497Y, GluR1(i)_A636T(Lc), GluR2(i,Q)_R649E and GluR2(i,Q)_A643T(Lc), the reduction by Xe was significantly smaller and varied by between 4 and 20%. In contrast, no difference in the blocking capacity of Xe was observed comparing wild-type NR1-1a/NR2A receptors with receptors having point mutations within NR2A that substantially slowed (NR2A_A651T(Lc)) or accelerated (NR2A_M823W) receptor desensitization. Thus, our data indicate that in AMPARs channel blockade by Xe is related to desensitization, whereas in NMDARs no evidence for such a relation was found. Thus, Xe seems to exert its inhibiting effect on various ionotropic glutamate receptors by different molecular mechanisms.

Key words: xenon, AMPA, NMDA, kainate, SH-SY5Y cells, Neuro2A cells

INTRODUCTION

Although the anesthetic properties of xenon (Xe) have been known for more than 50 years (Cullen and Gross 1951) it was the pioneer work of Franks's group at the end of the last century to identify a molecular target for this noble gas (Franks et al. 1998). According to their findings, the anesthetic potency of Xe was ascribed to its blocking capacity of N-methyl-D-aspartate receptor channels (NMDARs). Meanwhile, other targets were identified which might add to the anesthetic potency of Xe. These include two-pore K^+ channels, nicotinic acetylcholine receptors, as well as receptors for serotonin, glycine and GABA (deSousa et al. 2000, Hapfelmeier et al. 2000, Gruss et al. 2004). The two other subtypes of glutamate receptors (GluRs), i.e. AMPA receptors (AMPARs) and kainate receptors (KainatRs), were long believed to be no targets for Xe. However, further studies have shown that Xe also blocks these channels (Plested et al. 2004, Dinse et al. 2005, Nagele et al. 2005, Haseneder et al. 2008). Thus, Xe seems to be among the rare glutamate-receptor antagonists which affect both NMDARs and non-NMDARs.

The knowledge of the structural elements as well as the mechanism whereby Xe affects ion channel proteins is scarce. Studies with AMPARs indicate that Xe sensitivity might be coupled with receptor desensitization, although Xe does not affect the time course of AMPAR desensitization (Plested et al.

Correspondence should be addressed to H. U. Weigt
Email: henry.weigt@gmx.de

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2004, Dinse et al. 2005). Different sites within AMPARs are known to be involved in the process of receptor desensitization. These are the dimer interfaces formed by the ligand-binding domains, the linker regions connecting the ligand-binding domains to transmembrane region M3 and the outermost part of M3 (Sun et al. 2002, Klein and Howe 2004, Yelshansky et al. 2004). Best analyzed in this respect are the dimer interfaces of homomeric GluR2 which were analyzed by means of high-resolution crystallographic studies (Sun et al. 2002). Accordingly, the four agonist-binding subunits of one receptor are organized as a dimer of dimers. One dimer is composed of two subunits which interact extracellularly with their amino-terminal domains and, probably more important, with the backbone of their ligand-binding domains (S1S2), forming the dimer interface. Agonist binding to the S1S2 domains induces conformational changes within each subunit which are transferred *via* linkers to the channel gate thus opening the channel. Following rearrangements within the S1S2 domains, the dimers become instable whereby the receptor desensitizes, i.e. the channel closes (Sun et al. 2002). Initially, cyclothiazide was recognized to abolish desensitization of AMPARs (Partin et al. 1994). Later on a first point mutation within GluR3 (L483Y) was identified which showed a similar behaviour (Stern-Bach et al. 1998). Interestingly both, cylothiazide and the point mutation interact with AMPAR at closely related sites at the dimer interface and abolish receptor desensitization by stabilizing the dimer configuration via hydrophobic interactions (Sun et al. 2002). When cyclothiazide or homologous mutations were tested in GluR1 or GluR4, the channel-blocking capacity of Xe was reduced. This led to the speculation that xenon sensitivity might be correlated with the ability of AMPARs to desensitize (Plested et al. 2004). In order to find out whether this is a general mechanism for AMPARs it remains to be shown whether the other "non-desensitizing" AMPARs mutations, located at different sites outside the ligand-binding domain, behave in a similar manner.

In the group of NMDARs various forms of desensitization are distinguished. The list includes glycine-, zink- and calcium-dependent as well as -independent forms which may be further differentiated by reason of their dependence on the membrane potential (Sather et al. 1990, Krupp et al. 1996, Zheng et al. 2001). Although the underlying structural elements are confined, no clear picture of the molecular mechanism of desensitization has been available for these receptor channels until now. However, high-resolution crystallographic structure analysis revealed that the arrangements of the subunits in the S1S2 heterodimeric NMDAR (composed of the glycine-binding NR1 and the glutamate-binding NR2A subunits) and the S1S2 homodimeric GluR2 receptor are very similar (Furukawa et al. 2005). Accordingly, the dimer interface of NMDARs is also regarded as a crucial site for receptor modulation (Furukawa et al. 2005). Compared to AMPARs, regions in the pre-M1 domain as well as regions at the outermost parts of M3 and M4 seem to play a role in NMDAR desensitization (Krupp et al. 1998, Khoda et al. 2000, Ren et al. 2003).

The aim of our study was to elucidate if the sensitivity to Xe might generally be coupled with the desensitization of ionotropic glutamate receptors. To this end we analyzed additional "non-desensitizing" AMPAR point mutations located outside the ligand binding domain. Furthermore, as all GluR channels are highly homologous we also tested the corresponding NMDAR mutants for receptor desensitization and Xe sensitivity.

METHODS

Molecular biology and heterologous expression

The neuroblastoma cell lines Neuro2A (ATCC#CLL 131) and SH-SY5Y (ATCC#CRL 2266) were used for transient transfection. The cells were cultured at 37°C in a humidified atmosphere of 95% air and 5 % CO_2. Neuro2A cells were cultured in DMEM and SH-SY5Y cells in a 1:1 mixture of HAM-F12 and DMEM, each supplemented with 2 mM L-glutamine (Boehringer, Mannheim, Germany), 10% (v/v) fetal calf serum (Gibco), 50 U penicillin/ml and 50 µg/ml streptomycin (Gibco, Eggenstein, Germany) on polyornithine-coated culture dishes. When the cells had reached 40% confluency, they were transfected with the cDNAs encoding either NMDARs or AMPARs using the Trans-Fektion™ kit (Bio-Rad, München, Germany). For the construction of wild type NMDA receptors the subunits NR1-1a (Moriyoshi et al. 1991) and NR2A (Kutsuwada et al. 1992, Monyer et al. 1992) were used. Heteromeric NMDAR mutants were composed by

combining NR1-1a with either NR2A_M823W (Ren et al. 2003) or NR2A_A651T(Lc) (Khoda et al. 2000). Lc indicates the lurcher mutation within transmembrane region M3 where the SYTANLAAF motif is mutated to SYTANLATF. Homomeric wild type AMPARs were assembled from the flip(i) variant of GluR1(i) or GluR2(i,Q) (Partin et al. 1994). The unedited GluR2(i,Q) was used because only this form conducts appreciable currents when homomeric ion channels are expressed (Koike et al. 2000). Homomeric AMPAR mutants were formed from GluR1(i)_L497Y (Stern-Bach et al. 1998) or GluR2(i, Q)_R649E which is identical with GluR2(i, Q)_R628E (Yelshansky et al. 2004). The following mutants were kindly provided by colleagues: NR2A_M823W (R. Peoples, Millwaukee, USA); GluR1(i)_L497Y (Y. Stern-Bach, Jerusalem, Israel); GluR2(i, Q)_R649E (L. Wollmuth, Stony Brook, USA); NR2A_A651T(Lc) (F. Zheng, Little Rock, USA). All other receptor mutants were engineered using the stratagene quickchange site-directed mutagenesis kit (Stratagene, Amsterdam, The Netherlands) and confirmed by sequence analysis. The positions of the analyzed AMPARs and NMDARs mutants are illustrated in Fig. 1. Non-transfected cells did not respond to agonist applications, indicating that these cells are not equipped with functional AMPARs or NMDARs.

Electrophysiological recordings

Membrane currents were recorded in the whole-cell recording mode using an EPC-9 amplifier and TIDA

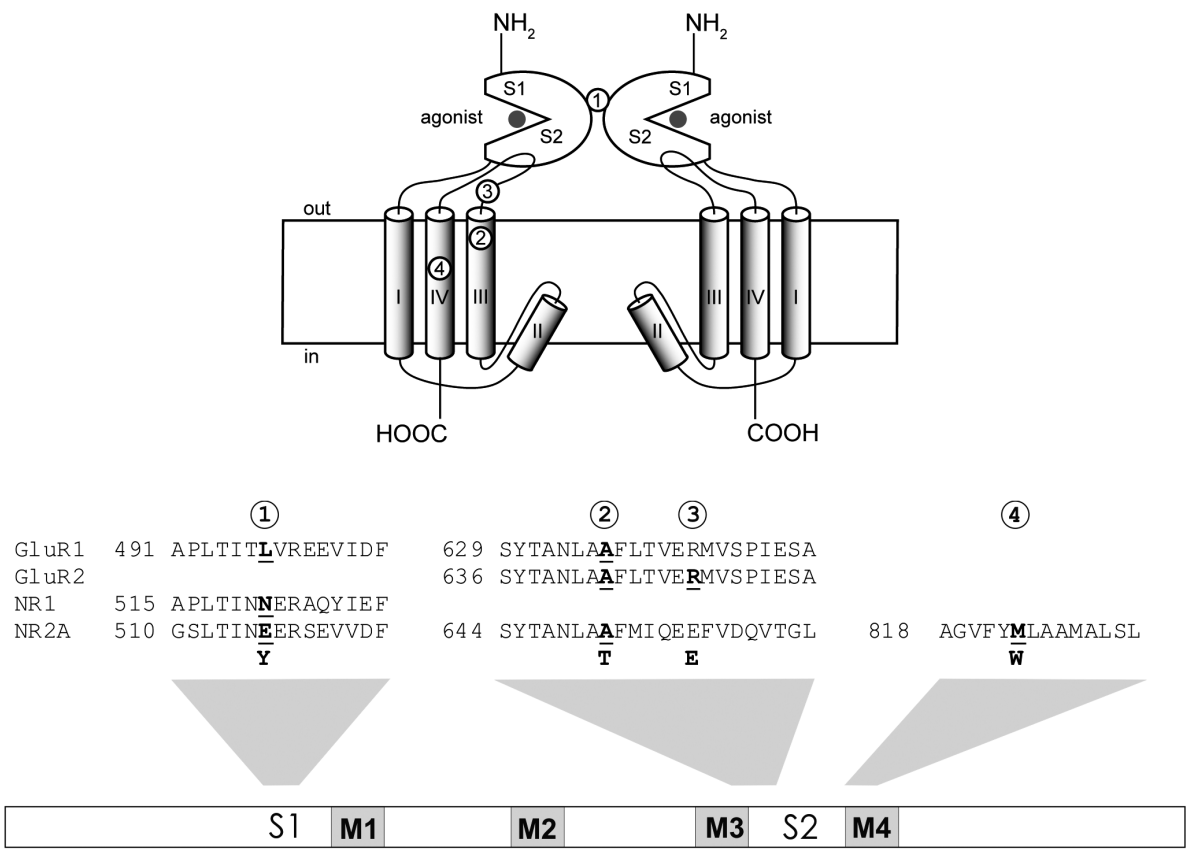

Fig. 1 Alignment of amino acid sequences of AMPA and NMDA receptors. (a) Schematic model of glutamate receptors with the agonist binding sites, located within the S1 and S2 segment, the transmembrane domains (TM) I, III and IV and the reentrant loop of TM II. The positions of the mutations are indicated by numbers (1–4). (b) Selected amino acid sequences of AMPARs and NMDARs and their position in relation to the transmembrane regions M1 to M4 are shown. The positions of the amino acids to be mutated are highlighted in bold and by underlining. The substituted amino acids are indicated in the lower line.

software (HEKA, Lambrecht, Germany) (Hamill et al. 1981). Before recording, the cells were rinsed twice with extracellular solution composed of (in mM): 140 NaCl, 2.7 KCl, 1.5 $CaCl_2$, 10 glucose, 0.01 glycine and 12 HEPES; pH 7.3. Patch pipettes were drawn from borosilicate glass with tip resistances between 3–6 MΩ when filled with (in mM) 140 $CsCl_2$, 2 $MgCl_2$, 2 ATPx2Na, 2 EGTA, 10 HEPES; pH 7.2. To improve sealing, tips were briefly dipped into 2% dimethylsilan dissolved in methylene chloride.

Preparation and application of test solutions

Xe-containing solutions were prepared by equilibrating 15 ml Xe gas with 40 ml of the particular test solution. The monitored average concentrations of Xe were 91.4 ± 10.0 µl/ml during the experiments. A Xe concentration of 90 µl/ml corresponds to 3.5 mM at standard conditions in our laboratory (~500 m above sea level, room temperature 22–25°C), a partial pressure of 0.84 atm or an anesthetic potency of about 1.1 MAC value

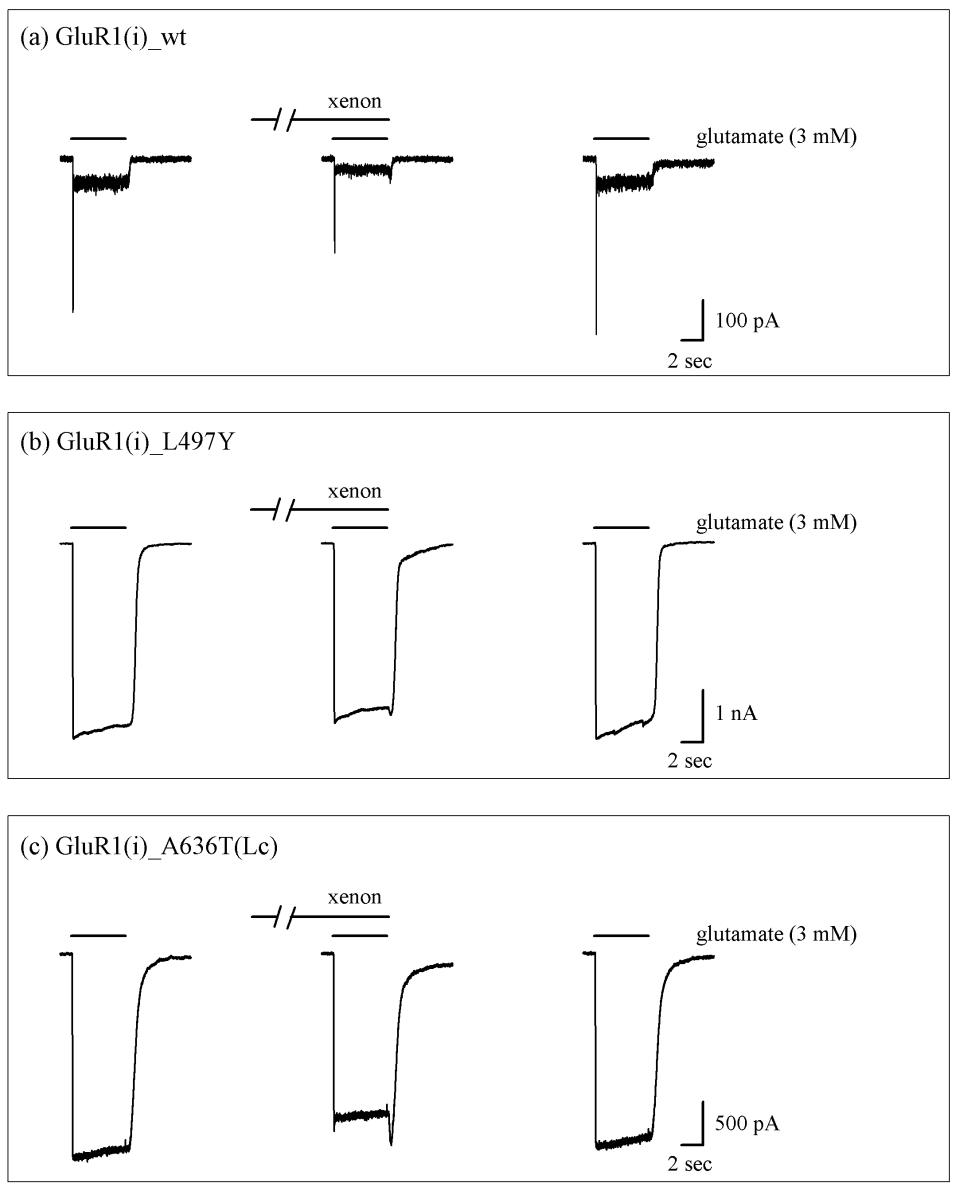

Fig. 2. Effect of xenon on GluR1(i) wt and "non-desensitizing" receptor mutants. Representative current traces obtained from the application of pulses (5 s) of glutamate (3 mM) to GluR1 transfected Neuro2A cells in the absence of (controls left and right) and with xenon (3.5 mM), pre-applied for 20 s (middle) (n=5–8 cells). Applications of glutamate and xenon are denoted by horizontal bars. Cells were voltage-clamped at –80 mV.

for humans. This concentration was chosen from preliminary experiments in order to achieve reliabe effects. Higher concentrations are not compatible with patch clamp recordings as they would require hyperbaric conditions and lower concentrations would result in undistinguishable small effects (Haseneder et al. 2008). The Xe concentration in the fluid actually superfusing the cells was measured using static headspace gas chromatography mass-spectrometry (headspace GCMS).

The receptor agonists were applied to the cells using the L/M-SPS-8 superfusion system (List, Darmstadt, Germany). To restrict the presence of the agonists to a small volume within the dish, a combination of two perfusion systems was installed, i.e., (i) a global bath perfusion with the inflow set at 4.5 ml/min and an outflow that removed any excess fluid, and (ii) a local inlet for the generation of a continuous stream of test solution. For a quick change between various test solu-

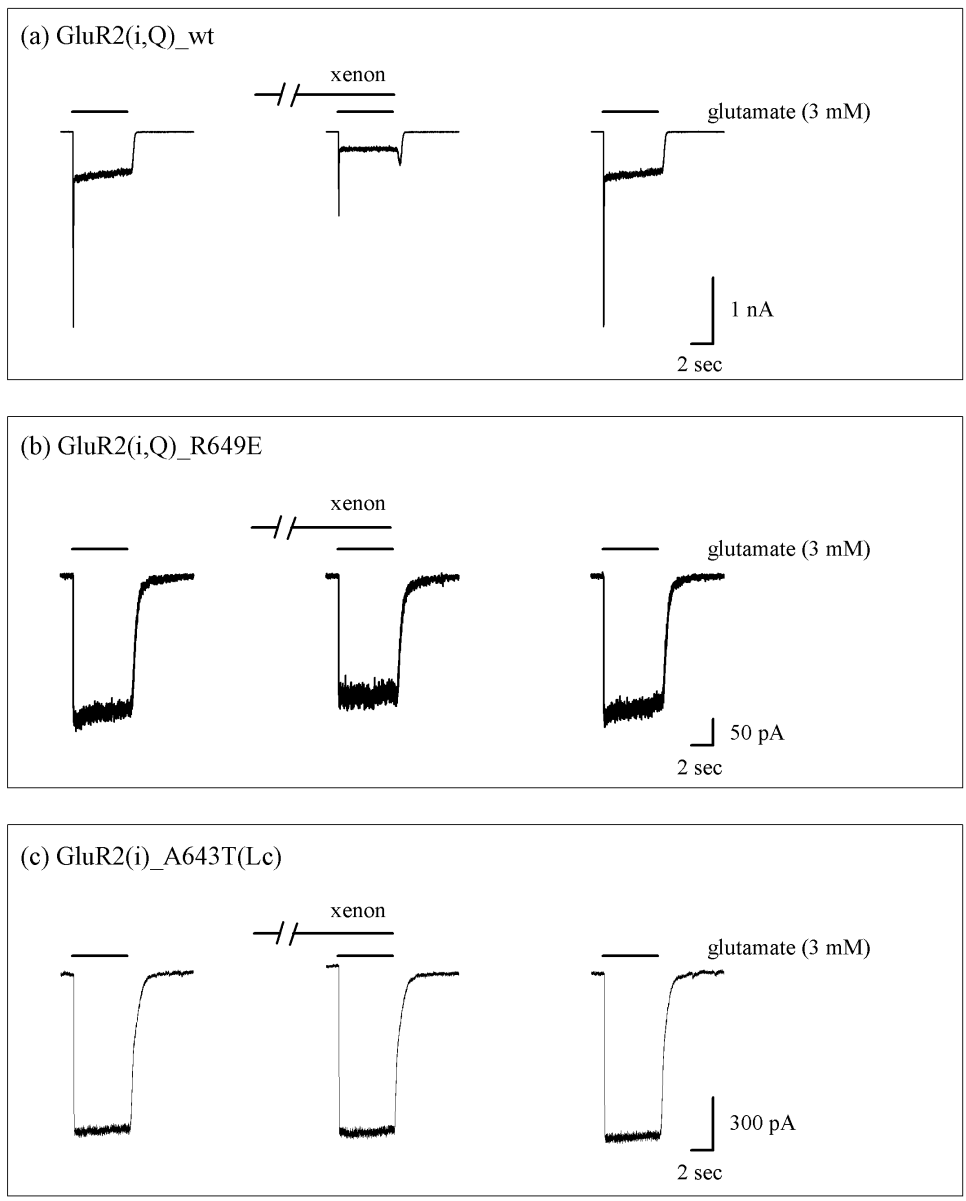

Fig. 3. Effect of xenon on GluR2(i) wt and "non-desensitizing" receptor mutants. Representative current traces obtained from the application of pulses (5 s) of glutamate (3 mM) to GluR2-transfected Neuro2A cells in the absence of (controls left and right) and with xenon (3.5 mM), pre-applied for 20 s (middle) (n=5–8 cells). Applications of glutamate and xenon are denoted by horizontal bars. Cells were voltage-clamped at –80 mV.

tions the local inlet consisted of the tip of an eight-barrelled pipette that was positioned at a distance of 50–100 µm upstream of the measuring field. All test solutions were administered at 1 ml/min using infusion pumps (Braun, Melsungen, Germany). For the pre-application of Xe, an additional inlet, positioned 50 to 100 µm upstream from the measuring field, was used. The time of solution exchange was estimated from the changes in the liquid junction potential to be about 1 ms. All chemicals were obtained from Sigma (Deisenhofen, Germany) if not otherwise stated.

Statistical analysis

The unpaired *t*-test was used when comparing the mean results from different samples. A difference between results was considered significant when $P<0.05$. Results are presented as means ± SD.

RESULTS

Blockade of AMPA receptors by xenon

Application of 3 mM glutamate for 5 s to homomeric GluR1(i) or GluR2(i,Q) receptors resulted in fast inward currents (10–90 % rise time: 7.16 ± 2.01 ms and 9.1 ± 2.2 ms, respectively) which rapidly declined to a plateau value towards the end of agonist application. Plateau to peak ratio was 0.22 for GluR1(i) and 0.24 for GluR2(i,Q), (*n*=6–8 cells). When Xe (3.5 mM) was pre-applied for 20 s the peak current conducted by GluR1(i) was reduced by 42.5 ± 10.5% and the plateau current by 55.5 ± 5.4%. The corresponding values with GluR2(i,Q) were 49.2 ± 4.8% and 54.8 ± 7.9%, respectively (see also Table I). In all experiments the blocking effect of Xe was completely reversible upon a brief washout period of 30 s (Figs 2a, 3a).

Next we analyzed mutants of AMPARs characterized by showing less desensitization than wild-type receptors. The plateau to peak ratio of the conducted currents upon application of 3 mM glutamate was 0.94 with GluR1(i)_L497Y; 0.94 with GluR1(i)_A636T(Lc); 0.97 with GluR2(i, Q)_A643T(Lc) and 0.87 with GluR2(i,Q)_R649E (see also Figs 2bc, 3bc). When Xe (3.5 mM) was pre-applied, the conducted currents were significantly less reduced (by 4 to 20%) than in experiments with the corresponding wild-type receptors (55%). In detail, the amounts of reduction at the end of agonist application were: GluR1(i)_L497Y (5.1 ± 6.1%); GluR1(i)_A636T(Lc) (14.4 ± 7.4%); GluR2(i,Q)_A643T(Lc) (4.0 ± 4.3%) and GluR2(i,Q)_R649E (19.7 ± 8.5%); (*n*=6–8 cells each).

Since the diminished receptor desensitization is also coupled to an increased sensitivity to glutamate, we performed additional experiments with the lurcher mutations of GluR1(i) and GluR2(i,Q) using a much lower, but still saturating agonist concentration (glutamate, 10 µM). The percentage of reduction of the conducted current by pre-applied Xe was about the same as obtained with 3 mM glutamate (not shown).

Because desensitization and Xe sensitivity of AMPARs might depend on the agonist, we also applied kainate when investigating the blocking effect of Xe on these lurcher-mutants. The currents were reduced with GluR1(i)_A636T(Lc) by 14.0 ± 4.1% and with GluR2 (i, Q)_A643T(Lc) by 13.4 ± 8.6% (*n*=5–6 cells each). Again, the currents were significantly less reduced than with the corresponding wild-type receptors (55%).

Blockade of NMDA receptors by xenon

The current response to a 5-s pulse of 100 µM NMDA in the continuous presence of 10 µM glycine to NR1-1a/NR2A receptors expressed in SH-SY5Y cells was characterized by a fast peak followed by a slow decline so that the current did not reach steady state at the end of agonist application (Fig. 4a). The ratio of late to peak current was 0.46. In the presence of Xe both peak and late currents were reduced by 42.4 ± 9.4 and 44.4 ± 8.3%, respectively (*n*=8 cells).

In analogy to the experiments performed with AMPARs we tested homologous NMDAR mutations which were characterized by an altered behavior of desensitization. Two of the mutations corresponded directly to the above non-desensitizing AMPAR mutations. At first we introduced the mutation N521Y in NR1-1a and E516Y in NR2A which are homologous to L497Y of GluR1. The mutants desensitized as much as the wild-type receptor with a late to peak current ratio of 0.28. In the presence of Xe the peak and late currents were reduced by 34.5 ± 5.1% and 48.2 ± 2.1%; *n*=6 cells, respectively (Fig. 4b). Next we analyzed the lurcher mutation of NR2A in combination with NR1-1a wild type. The evoked currents were almost constant with a ratio of peak to late current of 0.94. Peak currents were inhibited by 40.6 ± 6.7% and late currents by 49.0 ± 9.7%; *n*=5 cells (Fig. 4c). With these

two mutants the inhibitory potency of Xe was not significantly different from that with the wild-type receptor.

Finally we tested the NR2A mutation M823W which is characterized by a very fast and nearly total desensitization (Fig. 4c). The late to peak current ratio was 0.02. Since the late current was only slightly above baseline we evaluated the effect of Xe only for the peak value. The current reduction produced by Xe with this mutant was significantly smaller (32.9 ± 4.3%; n=8 cells) than with the NR1-1a/NR2A wild type (42.4%).

DISCUSSION

AMPA receptors

All AMPARs undergo fast desensitization upon agonist binding. Differences in the degree of desensitization and their sensitivity to cyclothiazide, the most prominent blocker of AMPA receptor desensitization, arise from alternative splicing at the C-terminal end of the ligand binding domain S2 whereby flip and flop variants are discriminated (Sommer et al. 1990, Partin et al. 1994). Further point mutations at quite different locations within the amino acid chain of AMPARs were identified which also affect desensitization (Stern-Bach et al. 1998, Schwarz et al. 2001, Klein and Howe 2004, Yelshansky et al. 2004). So far, one of these non-desensitizing mutants was analyzed for Xe sensitivity. It turned out that homomeric ion channels composed of either GluR1_L503Y or GluR4_L507Y were almost insensitive to Xe. From this result a correlation between receptor desensitization and Xe sensitivity was postulated (Plested et al. 2004).

In this study we have investigated the Xe sensitivity of additional "non-desensitizing" homomeric mutants of the flip variant of GluR1 [L497Y; A636T(Lc)] and GluR2 [R649E; A643T(Lc)]. In all of them the blockade of the conducted currents by Xe was strongly reduced or absent. These data support the hypothesis that in various AMPARs the Xe sensitivity might be related to the amount of desensitization. So far, the mechanism by which these mutations abolish desensitization is clarified only for GluR1_L497Y. According to the three-dimensional model, based on crystallographic structure analysis, the position of the L to Y substitution is located at the backbone of the S1S2 ligand binding domain (Sun et al. 2002). All other analyzed mutations are located outside the ligand binding

Table I

Blockade of wild-type and mutant AMPA and NMDA receptors by xenon. Overview of results.

Receptor	plateau/peak ratio	inhibition peak current (%)	inhibition plateau current (%)
AMPA receptors			
GluR1(i) wild-type	0.22	42.5 ± 10.5	55.5 ± 5.4
GluR1(i)_L497Y	0.94		5.1 ± 6.1*
GluR1(i)_A636T(Lc)	0.97		14.4 ± 7.4*
GluR2(i) wild-type	0.24	49.2 ± 4.8	54.8 ± 7.9
GluR2(i)_R649E	0.87		19.7 ± 8.5*
GluR2(i)_A643T(Lc)	0.97		4.0 ± 4.3*
NMDA receptors			
NR1-1a/NR2A wild-type	0.46	42.4 ± 9.4	44.4 ± 8.3
NR1-1a_N521Y/NR2A_E516Y	0.28	34.5 ± 5.1	48.2 ± 2.1
NR1-1a/NR2A_A651T(Lc)	0.94	40.6 ± 6.7	49.0 ± 9.7
NR1-1a/NR2A_M823W	0.02	32.9 ± 4.3*	

*P<0.05 in comparison to control

domain. While the lurcher mutation (A-T substitution) is positioned at the outer part of the putative third transmembrane region, the R-E substitution is located in the adjacent linker region connecting M3 with S2 of the ligand-binding domain (Klein and Howe 2004, Yelshansky et al. 2004).

Although the macroscopic effect on receptor desensitization appears to be the same, there are several differ-

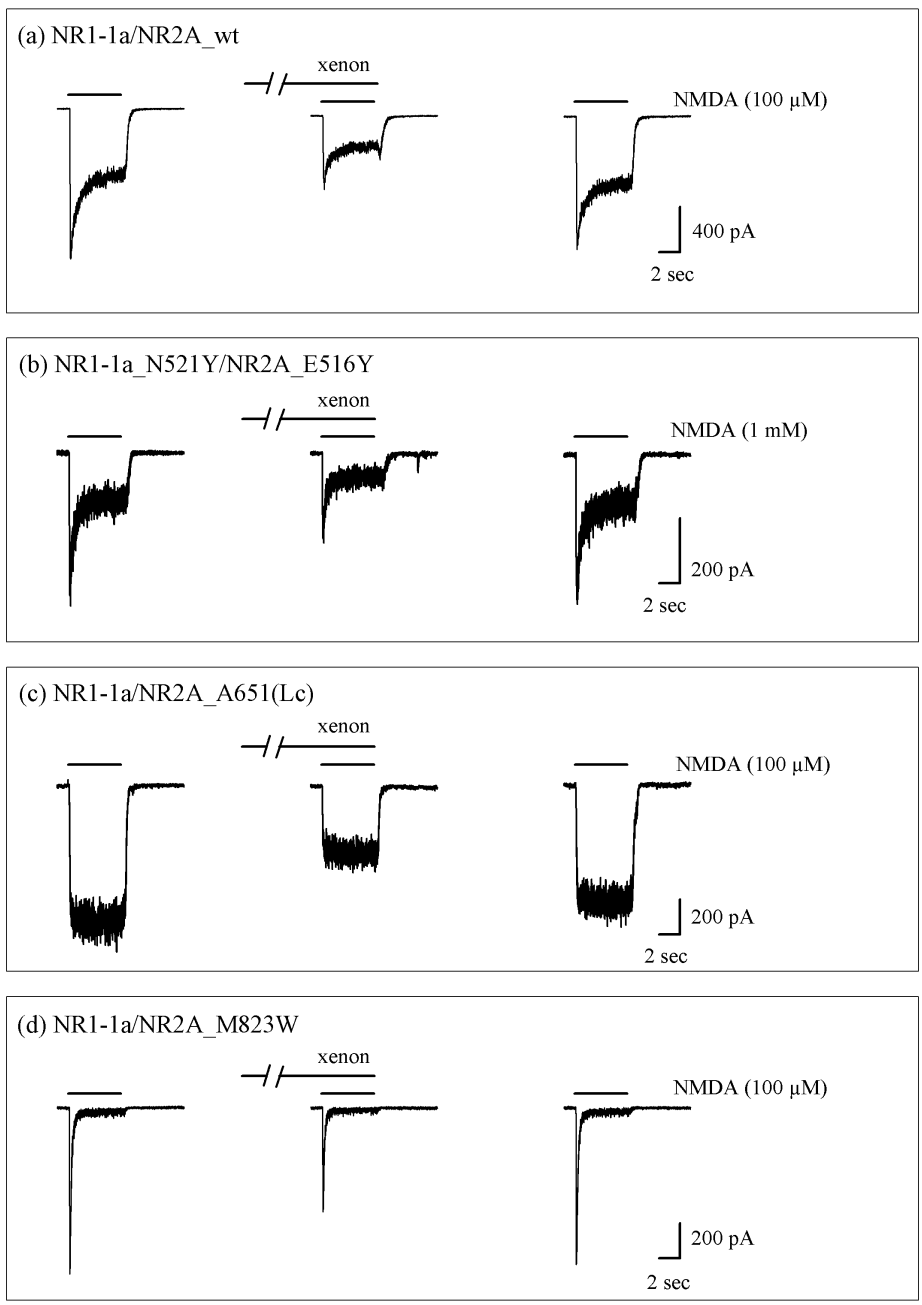

Fig. 4. Inhibition of NMDA-induced membrane currents in NR1-1a/NR2A transfected SH-SY5Y cells by xenon. Representative current traces obtained from the application of pulses (5 s) of of NMDA (100 µM) in the continuous presence of glycine (10 µM) were applied to NR1-1a/NR2A transfected SH-SY5Y cells in the absence of (controls left and right) and with xenon (3.5 mM), pre-applied for 20 s (middle) (n=6–8 cells). For measurements as shown in (b) agonist concentrations were increased to 1 mM NMDA and 100 µM glycine due to a decreased agonist affinity of this mutant. Applications of NMDA and xenon are denoted by horizontal bars. Cells were voltage-clamped at –80 mV

ences concerning receptor behavior between the L-Y and the R-E substitutions. The former completely abolishes desensitization in all GluRs whereas the latter discriminates between GluR1 and GluR2, having a much stronger effect on GluR2 (Stern-Bach et al. 1998, Robert et al. 2001, Yelshansky et al. 2004). Furthermore, in both substitutions the increase in apparent agonist affinity and the decrease in the rate of receptor desensitization are affected to a different degree (Robert et al. 2001, Yelshansky et al. 2004). Altogether, these differences led to the conclusion that the L-Y and R-E substitutions block desensitization in GluRs by fundamentally different mechanism (Yelshansky et al. 2004). In the L-Y substitution, desensitization is abolished by stabilizing the dimer conformation due to concatenating two S1S2 domains *via* hydrophobic interactions at the dimer interface (Sun et al. 2002). In the R-E substitution, electrostatic interactions are made responsible for influencing the gating of GluRs (Yelshansky et al. 2004).

The lurcher mutations (A-T substitutions) in AMPARs are also characterized by an increase in apparent agonist affinity. Although the inhibiting potency of Xe was reduced in all A-T substitutions, a difference in Xe sensitivity between the GluR1 and GluR2 subtypes was observed. This might be explained by the different degree by which these mutations abolish receptor desensitization (Schwarz et al. 2001, Klein and Howe 2004). On the molecular level suppression of desensitization seems to be achieved by a strengthening of helix interactions between M3 and M1 whereby channel gating is influenced (Klein and Howe 2004). Thus, although located at quite different sites of the receptor, all substitutions have in common an increase in agonist affinity and stabilization of conformations which prevent desensitization. In all these non-desensitizing mutants, Xe sensitivity is profoundly reduced.

An apparent contradiction to the hypothesis of a correlation between AMPAR desensitization and Xe sensitivity was raised by the finding that Xe inhibited non-desensitizing currents induced by kainate. It was suggested that Xe sensitivity depends on the agonist used (Plested et al. 2004). Therefore we tested the non-desensitizing lurcher mutations for Xe sensitivity using kainate as agonist. In this case, Xe failed to suppress kainate-mediated AMPAR responses. This may be best explained by the fact that kainate-activated AMPAR responses do show desensitization at a time scale that is too rapid to be resolved in the whole-cell configuration. An indirect clue to this hypothesis is that application of cyclothiazide increases the currents induced by kainate in non-desensitizing AMPARs several fold (Patneau et al. 1993).

NMDA receptors

For NMDA receptors much more structural elements are known to be involved in receptor desensitization than for AMPARs. In contrast to AMPARs, these elements are not only located extracellularly but also intracellularly. Additional variability arises from the heteromeric organization of NMDARs since the related structural elements are partly expressed in a subunit-specific manner. However, there are also similarities between AMPARs and NMDARs. Thus, with NMDARs, we focused mainly on those structural elements which have a known counterpart in AMPARs.

The closest similarity concerning amino acid position and behavior of desensitization was given for the lurcher mutation within the C-terminal end of M3, forming the core of the extracellular vestibule. We tested this mutation in the NR2A subunit in combination with wild-type NR1-1a and found no receptor desensitization. This is in accordance with the dominance of the lurcher mutation found for other heteromeric GluRs (Schwarz et al. 2001). However, although desensitization was abolished, Xe sensitivity was the same as with the wild-type receptor. Next we analyzed the newly described mutations N521Y in NR1-1a and E516Y in NR2A. These mutations were originally designed according to L483Y of GluR2 in order to stabilize the dimer interface of the S1S2 domains of NMDA receptors (Furukawa et al. 2005). We found that desensitization of these mutants was not affected. This might be in accordance with previous findings that ion channel activity is not significantly perturbed by these mutations (Furukawa et al. 2005). Anyhow, the mutants showed no reduction in sensitivity for Xe. Finally, we analyzed one mutation in the fourth transmembrane region of NR2A (M823W) which produced an accelerated and almost complete desensitization (Ren et al. 2003). If desensitization of NMDA receptors directly relates to Xe sensitivity we would have expected an increased Xe sensitivity. However, the blocking capacity of Xe was even less. Altogether, none of the above-mentioned manipulations at NMDARs, resulting in reduced or enhanced receptor desensitization, affected Xe sensitivity notably. Thus,

it seems likely that in NMDARs Xe sensitivity is not related to receptor desensitization.

Although homomeric AMPARs and heteromeric NMDARs are similar in the arrangement and interaction of their subunits in the S1S2 ligand-binding domain, minor but probably important differences must be considered. Whereas agonist binding in AMPAR is only coupled to lobe closure, in NMDAR this event may also be transferred to the dimer interface which itself plays a key role in receptor desensitization (Furukawa et al. 2005). Thus, it might be also of relevance that the mutation L483Y in GluR2 increases agonist affinity whereas the corresponding mutations in NMDAR reduce agonist affinity (Furukawa et al. 2005). In case of the lurcher mutation, the agonist affinity is increased in both receptor types (Khoda et al. 2000, Yuan et al. 2005). Furthermore, although the stability of the dimer conformation is promoted in AMPARs and NMDARs by these mutations, it is manifold tighter in AMPARs (Sun et al. 2002, Furukawa et al. 2005). Finally, distinct sites outside the S1S2 domain like the amino terminal domain which are involved in dimer formation and modulation of channel gating might contribute to the different behavior observed between AMPARs and NMDARs (Erreger and Traynelis 2005, Rachline et al. 2005)

Mechanism of xenon block

When we summarize the different effects of Xe on these receptors, we must keep in mind that NMDARs inevitably assemble from heteromeric subunits whereas AMPARs are composed of homo- or heteromeric subunits. In this respect it is interesting to note that Xe blocks heteromeric NMDARs whereas in AMPARs its effect is restricted to homomeric channels – except GluR4(o) heteromers (Plested et al. 2004). Such differences may arise from the so-called staggering of subunits. In NR2CRs, for example, the homologous positions even of the highly conserved SYTANLAAF motif are relocated in the vertical axis of the ion channel and are positioned 4 amino acids more externally than those in NR1 (Sobolevsky et al. 2002). Since this motif within the M3 segment is regarded as part of the molecular link between the ligand-binding domain and the channel gate, the staggering might affect arrangement of the subunits and channel behavior.

If we compare Xe sensitivity in relation to receptor desensitization in the ionotropic glutamate receptor subtypes, NMDARs resemble kainate receptors since GluR6, a subtype of kainate receptors, is inhibited by Xe even though desensitization is abolished by concanavalin A (Dinse et al. 2005). As no manipulation at NMDARs affected Xe sensitivity, no direct proposal about the mechanism can be offered for this receptor.

At AMPARs, Xe shares some important pharmacological similarities with pentobarbital. Xe gives rise to "hump" currents when it is simultaneously withdrawn with the agonist and pentobarbital remains bound or "trapped' to a closed state of the receptor channel after unbinding from the agonist. These effects are in accordance with the behavior of open-channel blockers (Jackson et al. 2003, Dinse et al. 2005). However, xenon does not operate as an open-channel blocker at the NMDA receptor (Weigt et al. 2008). Furthermore, both Xe and pentobarbital are less potent when AMPAR desensitization is suppressed. Because "trapping" of pentobarbital is abolished when desensitization is overcome by cyclothiazide, it is concluded, that the action of pentobarbital requires the transition of AMPARs into a desensitized state. Therefore, a state-dependent interaction between pentobarbital and AMPARs is favored (Jackson et al. 2003). Due to the reduced potency of Xe to block non-desensitizing AMPARs it also satisfies the criteria to antagonize this receptor *via* the preferential binding to an agonist-bound e.g. the desensitized state.

A state-dependent interaction between Xe and non-desensitizing AMPARs or NMDARs might also be the key to understanding the different behavior of these receptors. So far a computer simulation performed for the L497Y mutant of GluR1 indicates that the phenotype of the "non-desensitizing" currents is rather the result of an increased rate of exit from desensitization than that from entry into desensitization (Robert et al. 2001). Thus, the dwell time of these receptors in the desensitized state is largely reduced, whereby a possible interaction of Xe with this state will be impaired. Since the corresponding time constants of NMDARs are orders of magnitude lower, we speculate that sufficient time will be available for the interaction of Xe with a particular state of this receptor type.

CONCLUSION

Although Xe inhibits AMPARs and NMDARs to a similar degree, our results show that the mechanism of actions seems to be different because in AMPARs the

inhibitory potency of Xe is coupled to receptor desensitization whereas in NMDARs such a relation was not found.

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REFERENCES

Cullen C, Gross C (1951) The anesthetic properties of xenon in animals and human beings, with additional observations on krypton. Science 113: 580–582.

deSousa S, Dickinson R, Lieb W, Franks N (2000) Contrasting Synaptic Actions of the Inhalational General Anesthetics Isoflurane and Xenon. Anesthesiology 92: 1055–1066.

Dinse A, Föhr KJ, Georgieff M, Beyer C, Bulling A, Weigt HU (2005) Xenon reduces glutamate-, AMPA- and kainate-induced membrane currents in cortical neurons. Br J Anaesth 94: 479–485.

Erreger K, Traynelis S (2005) Allosteric interaction between zinc and glutamate binding domains on NR2A causes desensitization of NMDA receptors. J Physiol 569: 381–393.

Franks NP, Dickinson R, deSousa SL, Hall AC, Lieb WR (1998) How does xenon produce anesthesia? Nature 396: 324.

Furukawa H, Singh SK, Mancusso R (2005) Subunit arrangement and function in NMDA receptors. Nature 438: 185–192.

Gruss MG, Bushell TJ, Bright DP, Lieb AR, Mathie A, Franks NP (2004) Two-pore-domain K+ channels are a novel target for the anesthetic gases xenon, nitrous oxide, and cyclopropane. Mol Pharmacol 65: 443–452.

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391: 85–100.

Hapfelmeier G, Zieglgänsberger W, Haseneder R, Schneck H, Kochs E (2000) Nitrous oxide and xenon increase the efficacy of GABA at recombinant mammalian $GABA_A$ receptors. Anesth Analg 91: 1542–1549.

Haseneder R, Kratzer S, Kochs E, Eckle VS, Ziegelgänsberger W, Rammes G (2008) Xenon reduces N-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated synaptic transmission in the amygdala. Anesthesiology 109: 998–1006.

Jackson MF, Joo DT, Al-Mahrouki AA (2003) Desensitization of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors facilitates use-dependent inhibition by pentobarbital. Mol Pharmacol 64: 395–406.

Khoda K, Wang Y, Yuzaki M (2000) Mutation of a glutamate receptor motif reveals its role in gating and delta2 receptor channel properties. Nat Neurosci 3: 315–322.

Klein RM, Howe JR (2004) Effects of the lurcher mutation on GluR1 desensitization and activation kinetics. J Neurosci 24: 4941–4951.

Koike M, Tsukada S, Tsuzuki K (2000) Regulation of kinetic properties of GluR2 AMPA receptor channels by alternative splicing. J Neurosci 20: 2166–2174.

Krupp JJ, Vissel B, Heinemann SF (1996) Calcium-dependent inactivation of recombinant N-methyl-D-aspartate receptors is NR2 subunit specific. Mol Pharmacol 50: 1680–1688.

Krupp JJ, Vissel B, Heinemann SF (1998) N-terminal domains in the NR2 subunit control desensitization of NMDA receptors. Neuron 20: 317–327.

Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, Araki K, Meguro H, Masaki H, Kumanishi T, Arakawa M, Mishina M (1992) Molecular diversity of the NMDA receptor channel. Nature 358: 36–41.

Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg P (1992) Heterooligomeric NMDA receptors: molecular und functional distinction of subtypes. Science 256: 1217–1221.

Moriyoshi K, Masu M, Ishii T, Shigemoto R, Mizuno N, Nakanishi S (1991) Molecular cloning und characterization of the rat NMDA receptor. Nature 354: 31–37.

Nagele P, Metz LB, Crowder M (2005) Xenon acts by inhibition of non–N-methyl-D-aspartate receptor–
mediated glutamatergic neurotransmission in Caenorhabditis elegans. Anesthesiology 103: 508–513.

Partin KM, Patneau DK, Mayer ML (1994) Cyclothiazide differentially modulates desensitization of alpha-amino-3 -hydroxy-5-methyl-4-isoxazolepropionic acid receptor splice variants. Mol Pharmacol 46: 129–138.

Patneau DK, Vyklicky Jr. L, Mayer ML (1993) Hippocampal neurons exhibit cyclothiazide-sensitive radpidly desensitizing responses to kainate. J Neurosci 13: 3496–3509.

Plested AJ, Wildman SS, Lieb WR, Franks NP (2004) Determinants of the sensitivity of AMPA receptors to xenon. Anesthesiology 100: 347–358.

Rachline J, Perin-Dureau F, Le Goff A (2005) The micromolar zinc-binding domain on the NMDA receptor subunit NR2B. J Neurosci 25: 308–317.

Ren H, Honse Y, Karp BJ (2003) A site in the fourth membrane-associated domain of the N-methyl-D-aspartate receptor regulates desensitization and ion channel gating. J Biol Chem 278: 276–283.

Robert A, Irizarry SN, Hughes TE, Howe JR (2001) Subunit Interactions and AMPA receptor desensitization. J Neurosci 21: 5574–5586.

Rubin CM, Kimura RH, Schmid CW (2002) Selective stimulation of translational expression by Alu RNA. Nucleic Acids Res 30: 3253–3261.

Sather W, Johnson JW, Henderson G, Ascher P (1990) Glycine-insensitive desensitization of NMDA responses in cultured mouse embryonic neurons. Neuron 4: 725–731.

Schwarz MK, Pawlak V, Osten P, Mack V, Seeburg PH, Köhr G (2001) Dominance of the lurcher mutation in heteromeric kainate and AMPA receptor channels. Eur J Neurosci 14: 861–868.

Sobolevsky AI, Rooney L, Wollmuth LP (2002) Staggering of subunits in NMDAR channels. Biophys J 83: 3304–3314.

Sommer B, Kainänen K, Verdoorn TA, Wisden W, Burnashev N, Huber A, Köhler M, Takagi T, Sakmann B, Seeburg PH (1990) Flip and flop: A cell-specific functional swith in glutamate-operated channels of the CNS. Science 249: 1580–1585.

Stern-Bach Y, Russo S, Neuman M, Rosenmund C (1998) A point mutation in the glutamate binding site blocks desensitizition of AMPA receptors. Neuron 21: 907–918.

Sun Y, Olson R, Horning M (2002) Mechanism of glutamate receptor desensitization. Nature 417: 245–253.

Weigt HU, Adolph O, Georgieff M, Georgieff EM, Föhr KJ (2008) Evidence that xenon does not produce open channel blockade of the NMDA receptor. J Neurophysiol 99: 1983–1987.

Yelshansky MV, Sobolevsky AI, Jatzke C (2004) Block of AMPA receptor desensitization by a point mutation outside the ligand-binding domain. J Neurosci 24: 4728–3476.

Yuan H, Erreger K, Dravid SM, Trynelis ST (2005) Conserved structural and functional control of 'N-methyl-D-asparatate receptor gating by transmembrane domain M3. J Biol Chem 280: 29708–29716.

Zheng F, Erreger K, Low CM (2001) Allosteric interaction between the amino terminal domain and the ligand binding domain of NR2A. Nat Neurosci 4: 894–901.