Biochemical Pharmacology, Vol. 46, No. 11, pp. 1877–1885, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00
Pergamon Press Ltd

COMMENTARY

STUDYING BLOCK IN CLONED *N*-METHYL-D-ASPARTATE (NMDA) RECEPTORS

J. PETER RUPPERSBERG,* JOHANNES MOSBACHER, WILLY GÜNTHER, RALF SCHOEPFER† and BERND FAKLER

Max-Planck-Institute for Medical Research, 69028 Heidelberg; and †Center for Molecular Biology, University of Heidelberg, 69120 Heidelberg, Germany

Excitatory synaptic transmission in the vertebrate CNS is mediated predominantly by glutamate, which activates glutamate receptor ion channels. These are divided into two functional subtypes: the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)‡ receptors and kainate receptors, which mediate fast excitatory synaptic currents, and the *N*-methyl-D-aspartate (NMDA) receptors [1], which mediate excitatory synaptic currents that are slower and longer lasting [2–4]. Besides their slow kinetics, NMDA receptors have several other characteristic properties that distinguish them from other ligand-gated ion channels. The NMDA receptor channel can discriminate between different divalent cations in a highly selective way. It is more permeable for Ca^{2+} than for monovalent cations but is rather impermeable for magnesium Mg^{2+} [5, 6]. On the other hand, the NMDA receptor channel is highly sensitive to block by micromolar concentrations of Mg^{2+} [7, 8] but not by Ca^{2+} or Ba^{2+} [5]. Permeability for Ca^{2+}, as well as block by Mg^{2+}, is assumed to be crucial for the physiological function of NMDA receptors [9, 10]. Another unique property of the NMDA receptor is that it needs two different agonists at the same time to become activated. Besides glutamate, glycine also has to be present in micromolar concentrations to open the channel [11–14]. Micromolar concentrations of glycine, however, seem to be always present in the synaptic cleft so that the physiological relevance of the glycine binding site of the NMDA receptor is not yet clear. Some further ways to modulate the NMDA receptor have been described. Polyamines [15], fatty acids [16], nitrogen monoxide donors [17, 18] and oxidizing and reductive agents [19–21] modulate the NMDA receptor by acting from the extracellular side, whereas protein kinases seem to modulate NMDA receptors from the cytoplasmic side of the membrane [22, 23]. Each of these modulation sites can down-regulate channel activity and may, therefore, be the target of substances that block NMDA receptors. Possibly one and the same blocking substance may act on more than one site at the same time, which has to be taken into account when mechanisms of block are discussed. Thus, analysis of the various types of block of the NMDA receptor is a challenging task.

Native or cloned NMDA receptors?

Most of the experiments to study block and inhibition of NMDA receptors have been carried out in native neurons. Since native neurons generally harbour all types of glutamate receptors, the current response mediated by NMDA receptors can only be separated from the responses of other glutamate receptors either if NMDA is used as a selective agonist, or if all other glutamate-activated currents are selectively blocked by their specific antagonists. This restricts the experimental conditions under which NMDA receptors can be studied in native neurons. Moreover, if regulation, block or modulation is to be studied, it is important to work in a homogeneous population of NMDA receptor channels. However, one cannot be sure that the population of NMDA receptors found in a native neuron is homogeneous and not composed of distinct subpopulations with differential properties.

The possibility to work on pure NMDA receptors belonging to only one distinct subtype has been opened up recently by cloning and functional expression of NMDA receptor subunit cDNAs [23–28]. For example, the recombinant heteromeric NR1/NR2A NMDA receptor [24], which is composed of subunits of the NR1 and of the NR2A type, has properties that correspond nicely to the properties of native NMDA receptors as found, for example, in hippocampal neurons [24, 29, 30]. Figure 1 compares current responses to brief glutamate applications measured in outside-out patches from native [3] and cloned NR1/NR2A receptors, which show similar time courses of decay. As shown in panels A and B of Fig. 2, the characteristic voltage-dependent Mg^{2+} block, as measured in outside-out patches from hippocampal CA3 cells, is reproduced in outside-out patches from oocytes expressing cloned NR1/NR2A channels. Thus, it seems attractive in the future to study biophysical mechanisms on these recombinant NMDA receptors instead of using native neurons.

However, since it is difficult to ensure that these cloned receptor channels are indeed completely equivalent to certain native NMDA receptor

* Corresponding author. FAX 6221 486 459.

‡ Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-D-aspartate; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; D-APV, D-(−)-2-amino-5-phosphonovalerate; 7-Cl-KYNA, 7-chlorokynurenate; and ATX argiotoxin$_{636}$.

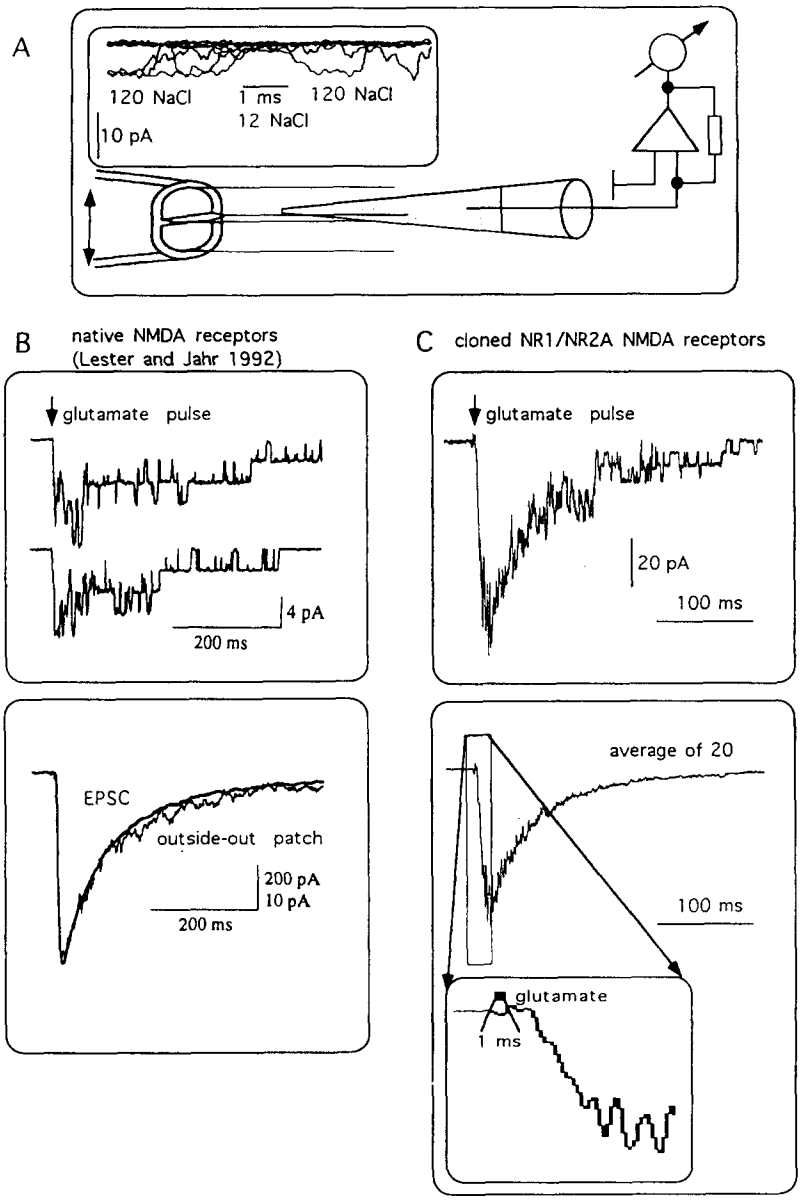


Fig. 1. Glutamate pulses applied to outside-out patches from native and cloned NMDA receptors. (A) Piezo-controlled fast application can mimic fast glutamate release of a synapse. To check application speed, a patch with single-channel openings is briefly (1 msec) exposed to a ten-times-diluted frog Ringer solution (NFR). The reduction of single-channel amplitude is almost as short as the time interval during which the double-barrel pipette is moved in order to expose the patch to the diluted solution. (B) Responses of native NMDA receptors to brief applications of glutamate, redrawn from Lester and Jahr [3]. The decay of the current obtained from averaged single channel data has a time constant between 100 and 200 msec and resembles very much the decay of the excitatory postsynaptic potential (EPSC). (C) Responses of cloned NMDA receptor channels to a brief application of glutamate decay were similarly slow. Current activation is slow compared to the duration of the glutamate pulse (inset). All traces were measured in the presence of 10 μM glycine.

channels, it seems a reasonable strategy first to check whether a certain property characterized in native NMDA receptors is fully reproduced in the cloned channels. If so, the next step would be to carry out biophysical studies in cloned channels to determine the molecular mechanism of this property under well-defined experimental conditions. It is, however, also questionable whether cloned NMDA receptors can really be studied under better defined conditions than native NMDA receptors of neuronal cells.

Two major problems occur in the expression systems used for NMDA receptors, namely *Xenopus*

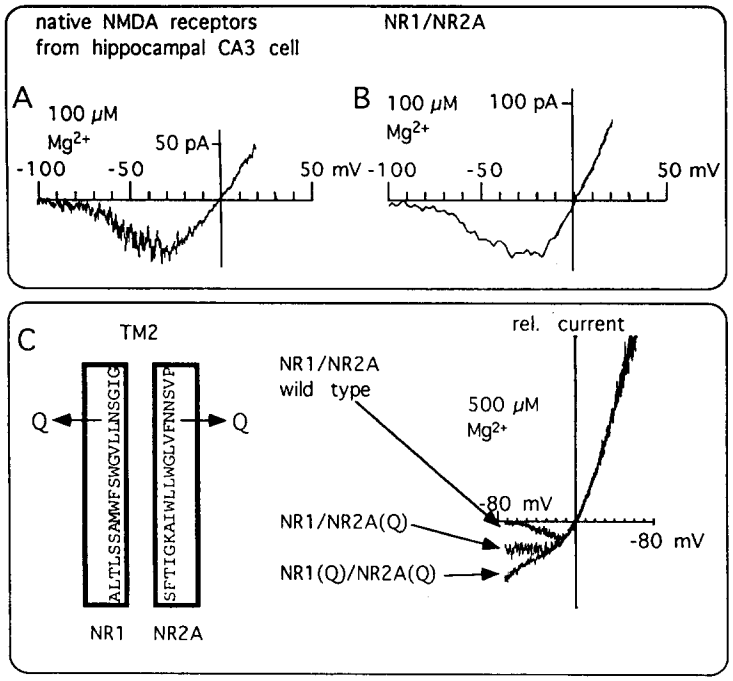


Fig. 2. Mg^{2+} block in native, cloned and mutated NMDA receptor channels. (A) Voltage-dependence of Mg^{2+} block in native NMDA receptors from a hippocampal neuron (provided by P. Jonas) is similar to that in the cloned NR1/NR2A channel. (B) The block in response to 100 μM Mg^{2+} shows the typical N-shape of a highly voltage-dependent pore block. (C) Mutating the N residue at the Q/R/N site of the NR2A subunit to Q decreases sensitivity to 500 μM Mg^{2+}. If, in addition, the N residue in the NR1 subunit is mutated to Q, the Mg^{2+} sensitivity is reduced even more.

laevis oocytes and human embryonic kidney cells (HEK 293). While the membrane of native neurons has a high density of NMDA receptors, such that outside-out patches with multiple channel openings can be obtained easily, in transfected HEK 293 cells expression density of recombinant NMDA receptors is lower so that recordings normally have to be made in the whole-cell recording mode of the patch-clamp technique [24]. *Xenopus* oocytes, which in some laboratories provide much higher channel densities than HEK 293 cells, allow one to isolate outside-out patches with multiple channels to perform, for example, ultra-fast agonist application experiments. However, work in *Xenopus* oocytes is complicated by the presence of Ca^{2+}-dependent chloride channels in the oocyte membrane. These endogenous channels are activated by low intracellular concentrations of Ca^{2+} and even by barium ions, which appear at the cytoplasmic face of the membrane because of the Ca^{2+} or Ba^{2+} inward currents mediated by the heterologous NMDA receptor channels. These chloride currents, which contaminate the currents mediated by the artificially expressed channels, complicate the interpretation of results obtained from any Ca^{2+}-permeable channel type studied after heterologous expression in *Xenopus* oocytes. This problem, however, can be overcome by using cell-free patches and 10 mM 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) instead of ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) as a Ca^{2+} chelator on the cytoplasmic side of the patch, rather than the two-microelectrode voltage-clamp technique used by the majority of groups working on cloned glutamate receptor channels in *Xenopus* oocytes. We used cell-free patches and BAPTA to study NMDA receptors in oocytes in most of the cases. Only for agonist competition experiments (last section; see also Fig. 5) did we use two-microelectrode measurements, which therefore typically display a Ca^{2+}-dependent peak of chloride current at the beginning of each record.

Types of block found in cloned NMDA receptor channels

The first NMDA receptor subunit (NR1) was isolated with the help of an expression cloning protocol. RNA samples obtained by serial subdivision of a cRNA pool from rat brain were tested for their potency to express NMDA-activated currents in *Xenopus* oocytes [31] until the particular clone encoding NR1 was isolated. While it was possible to detect NMDA-activated currents in the nanoampere range even upon injection of a pool containing about 3000 different cRNAs, surprisingly, expression of the pure cRNA encoding the cloned NR1 subunit did not yield much stronger currents. Larger NMDA-activated currents in the microampere range had not been detected in *Xenopus* oocytes before efforts were made to coexpress the NR2 subunits with the

NR1 subunit [24, 26]. Since none of the NR2A to C subunits expressed detectable currents as homomultimers and neither the NR1 nor the NR2 subunits expressed homomeric channels in HEK 293 cells [24], it is now generally assumed that the natural NMDA receptor channels are composed of, at least, both NR1 and NR2 subunits.

However, it was a remarkable discovery that block by D(−)-2-amino-5-phosphonovalerate (D-APV), MK801, Zn^{2+}, Mg^{2+} and 7-chlorokynurenate (7-Cl-KYNA), as well as modulation by glycine, could be found for the nanoampere currents of the homomeric NR1 channels expressed in *Xenopus* oocytes. Although expression of homomeric NR1 channels was rather low in *Xenopus* oocytes, all other measurable properties matched to a certain extent what was determined later in NR1/NR2A heteromultimeric channels, while NR1/NR2C channels showed a reduced Mg^{2+} sensitivity.

Molecular determinants of the pore block of NMDA receptors

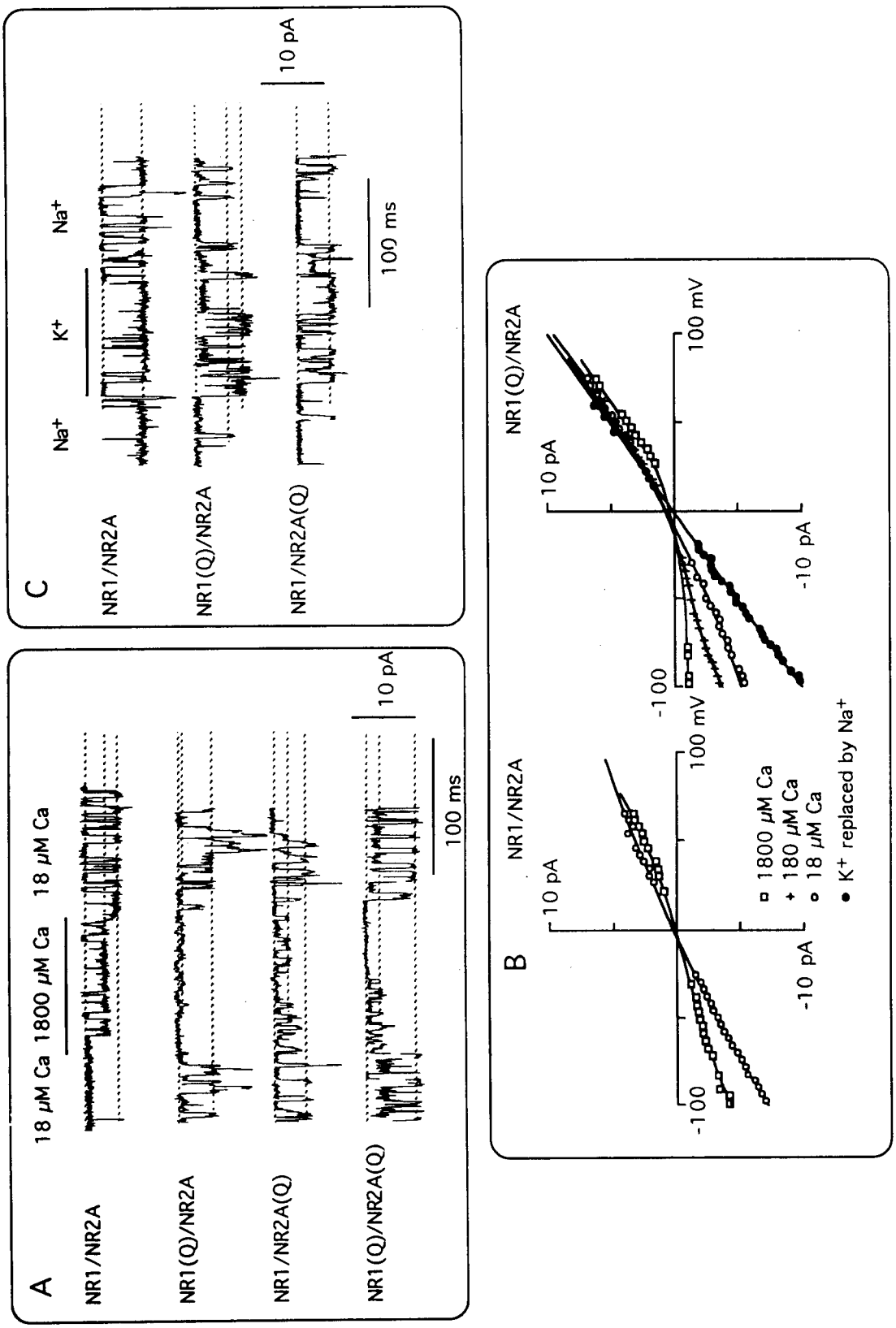
While D-APV and 7-Cl-KYNA are competitive blockers that interact with the binding sites for glutamate and glycine, respectively, MK801, Zn^{2+} and Mg^{2+} are assumed to block the channel pore. By homology to acetylcholine and AMPA receptor subunits, the second hydrophobic segment (TM2) of the NMDA receptor sequence was suspected to line the pore of the NMDA receptor channel [24, 26, 31]. Molecular determinants of pore block thus were expected to be located in the TM2 sequence. The focus of interest was placed on an amino acid residue in TM2 of the NMDA receptor subunits which is homologous to the so-called Q/R site of the AMPA receptor subunits [6, 32]. In AMPA receptor subunits, this site, which is prone to an RNA-editing process, controls Ca^{2+} permeability. If the mRNA encoding AMPA receptor subunits exhibits a glutamine residue (Q) at this site, the corresponding AMPA receptor channels are permeable to Ca^{2+}. If, however, the GluR-B mRNA sequence is edited at this site and the Q in TM2 of the corresponding channel subunits is changed to the positively charged amino acid arginine (R), Ca^{2+} permeability of the resulting AMPA receptor channels is found to be reduced drastically [32].

Since all NMDA receptor subunits exhibit an asparagine (N) at the homologous position [24], and since they are even more permeable for Ca^{2+} than unedited AMPA receptors, the N at this position in the NR1 and NR2 subunits was artificially mutated to Q and R. The striking result of these experiments was that the introduction of the positively charged R at this site drastically reduced the sensitivity of the mutated NMDA receptors to Mg^{2+} and MK801 [6, 33]. Moreover, the permeability to Ca^{2+} was practically abolished [6]. Interestingly, the mutations by which the N in the wild-type subunits was changed to Q showed differential effects in the NR1 and the NR2 subunit. If the mutation (N to Q) was introduced in the NR1 subunit, Mg^{2+} block was only weakly reduced, Mg^{2+} permeability was virtually unchanged, but Ca^{2+} permeability was decreased markedly. On the other hand, when the same mutation was introduced into the NR2A or NR2C subunit, the mutated channels had unchanged Ca^{2+} permeability, but an increased Mg^{2+} permeability and a reduced sensitivity to block by Mg^{2+} (Fig. 2C) [6]. Sensitivity to Mg^{2+} block almost completely disappeared when both mutated subunits were coexpressed to form channels where the N residues of all subunits at this site were mutated to Q (Fig. 2C).

An independent possibility for constructing channels that have the N mutated to Q in all subunits would be to express homomeric NR1 channels that have been mutated to Q at this position (NR1(Q)). Surprisingly, channels obtained in this way measured in whole *Xenopus* oocytes by two-microelectrode voltage-clamp showed a Mg^{2+} block that was similar to NR1(Q)/NR2A channels where only the NR1 subunit has been mutated to Q (not shown). There are two possibilities to explain this. First, the mutations at the Q/R/N site may somehow be critical for the structure of the channel subunits and influence properties such as pore size and permeabilities in a subunit-specific and complicated way. The second possibility would be that *Xenopus* oocytes may express an endogenous NR2 subunit at a low density which coassembles with the heterologous NR1 subunits expressed from injected RNA to form functional channels. Although this possibility is rather speculative, it would explain not only why channels forming upon expression of the NR1(Q) subunit alone resemble NR1(Q)/NR2A channels, but also why the NR1 subunits cannot form homomeric channels in HEK 293 cells and why, when expressed in *Xenopus* oocytes, NR1 yielded enough channels to allow an expression cloning approach even with fractionated mRNA. If, according to the first possibility the Q/R/N site of the NMDA receptor were a critical determinant of protein structure, one might expect that mutations at this site also should influence other properties of the channels. Figures 3 and 4 summarize properties of the mutants at the Q/R/N site with respect to Ca^{2+} block, monovalent-ion permeability and channel kinetics.

NR1(Q)/NR2A channels are most efficiently blocked by extracellular Ca^{2+}. They are much more sensitive to Ca^{2+} than wild-type channels (Fig. 3, A

Fig. 3. Changes in Ca^{2+} block and monovalent permeability of NR1/NR2A channels where the N at the Q/R/N site was mutated to Q. (A) A sudden change from 18 to 1800 μM Ca^{2+} applied to an outside-out patch with wild-type NR1/NR2A receptors gave a reduction of single channel amplitude to 60% (at −80 mV). The same increase in Ca^{2+} concentration reduced single channel amplitude to 15% if the N residue in the NR1 subunit was mutated to Q. The same mutation in the NR2A subunit gave a single-channel redution to 40%, while the double mutant resulted in a block to 25% of the control value. (B) The blocking effect of Ca^{2+} on the single-channel amplitude is voltage-dependent. In the current-voltage relations of single-channel amplitudes at different Ca^{2+} concentrations, Ca^{2+} block is much more pronounced at negative potentials. NR1(Q)/NR2A channels show different permeabilities to Na^+ and K^+. The single-channel amplitude is markedly smaller in external Na^+ solution than in K^+ solution and the reversal potential is shifted to −10 mV in Na^+ solution. (C) Exchange of extracellular Na^+ against K^+ increases single-channel amplitude of NR1(Q)/NR2A but not of NR1/NR2A(Q) or wild-type channels.


A
18 μM Ca 1800 μM Ca 18 μM Ca
NR1/NR2A
NR1(Q)/NR2A
NR1/NR2A(Q)
NR1(Q)/NR2A(Q)
10 pA
100 ms
C
Na+
K+
Na+
NR1/NR2A
NR1(Q)/NR2A
NR1/NR2A(Q)
10 pA
100 ms
B
NR1/NR2A
NR1(Q)/NR2A
10 pA
-10 pA
-100
100 mV
□ 1800 μM Ca
+ 180 μM Ca
○ 18 μM Ca
● K+ replaced by Na+

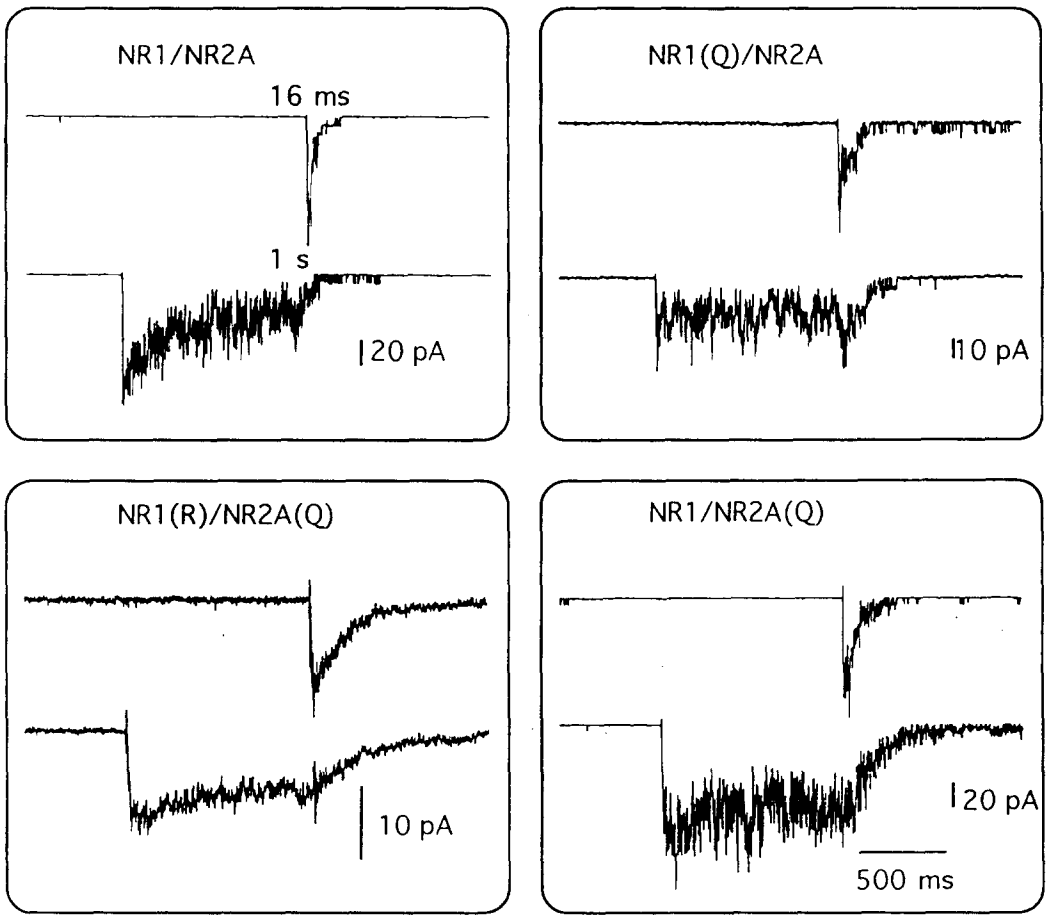


Fig. 4. Kinetic differences between cloned NMDA receptors mutated at the Q/R/N site. Pulses of 16 and 1000 msec of 100 μM glutamate plus 10 μM glycine are applied to outside-out patches with NMDA receptor channels of the NR1(Q)/NR2A, of the NR1/NR2A(Q) and of the NR1(R)/NR2A(Q) type. Mutant channels show a slower deactivation upon wash-out of glutamate and glycine and look more flickery. Desensitization during the long glutamate pulse seems to be less pronounced in the mutant channels. The R mutation of the NR1 subunit decreases single-channel amplitude drastically so that individual channels can no longer be resolved.

and B) and also more sensitive than NR1/NR2A(Q) channels. NR1(Q)/NR2A(Q) channels have a Ca^{2+} sensitivity that ranges between the sensitivities of NR1(Q)/NR2A and NR1/NR2A(Q) channels (Fig. 3A).

The current traces shown in Fig. 3A have been measured in ionic conditions where the extracellular medium contained predominately Na^{2+}, (NaCl 115 mM, KCl 2.5 mM, $CaCl_2$ 18 μM, sodium-HEPES 10 mM, pH 7.2), whereas the internal medium contained exclusively K^+ (KCl 100 mM, K_3BAPTA 10 mM, potassium-HEPES 10 mM, pH 7.2) as positive ions. Under these conditions the single-channel amplitude of both NR1(Q)/NR2A and NR1/NR2A(Q) channels is smaller than for wild-type channels, while NR1(Q)/NR2A(Q) channels exhibit a larger single-channel amplitude and an unusually frequent occurrence of subconductance levels. An increase in single-channel amplitude was observed in NR1(Q)/NR2A channels when external Na^+ was replaced by K^+ (Fig. 3, B and C) which did not influence the single channel amplitude of NR1/NR2A(Q) or wild-type channels. Correspondingly, the reversal potential of NR1(Q)/NR2A channels is shifted to the left by about 10 mV in the presence of external Na^+ (Fig. 3B).

Single-channel kinetics of mutated NR1/NR2A channels were not studied systematically, although it was obvious that mutated channels generally showed shorter openings and exhibited more flickery behaviour than wild-type channels.

Responses of the mutated channels to brief pulses of glutamate also showed differences from responses of wild-type channels. The decay phase of the ensemble currents measured in outside-out patches in response to brief (16 msec) wash-in and wash-out of 100 μM glutamate and 10 μM glycine was about twice as slow in all mutated channels than in wild-type channels (Fig. 4). This suggests a prolonged dissociation of agonists from the mutated channels.

In the traces in Fig. 4 measured with NR1(R)/NR2A(Q) channels, no single channels can be resolved even though the current scale is larger than in the other traces. This reflects the reduction of single-channel amplitude by about a factor of 5 (not shown) found in mutants carrying an R at the Q/R/N site.

From the data presented in Figs. 3 and 4 one may conclude that the mutations at the Q/R/N site indeed have a complex influence on many properties of the channels. However, they influence kinetics of the channel less severely than permeability and block.

Differential effects of mutations at the Q/R/N site on the effect of channel blockers of NR1/NR2A channels

As shown above, there are two distinct ways by which sensitivity to Mg^{2+} block of NR1/NR2A channels can be reduced by mutating the asparagine residue at the Q/R/N site. The introduction of the positively charged arginine residue, which removes Mg^{2+} block completely, is associated with loss of divalent permeability and reduction of single-channel amplitude. A mechanistic interpretation of this mutant effect would be that the positive charge located near the conducting pathway does not allow any type of divalent ion to pass the channel and also does not allow magnesium ions to enter to reach their blocking site. On the other hand, introduction of a Q instead of a N in NR2 decreased Mg^{2+} block but in contrast to the R, increased Mg^{2+} permeability. This could be interpreted as a permeant block of the channel by magnesium ions which may get off from their binding site through the channel with an off-rate that increases with hyperpolarizing membrane voltage.

Is there any further evidence for these two concepts? Mutations at the Q/R/N site introducing the positively charged R residue reduced sensitivity not only to block by Mg^{2+} but also to other positively charged pore blockers such as MK801 [33] and argiotoxin$_{636}$ (ATX) in AMPA receptors [34] and in cloned NMDA receptors [35]. In contrast to this mutation where the N was mutated to R, the mutation to Q, which also reduced sensitivity to Mg^{2+}, increased sensitivity to ATX. Wild-type NR1/NR2A channels had an IC_{50} value for ATX block of 10 nM which was changed by the R mutation in the N2A subunit to values larger than 10,000 nM; the mutation of N to Q in the NR2A subunit decreased the IC_{50} to 1 nM. If NR1 and NR2A subunits were both mutated from N to Q, ATX sensitivity increased to an IC_{50} value of 0.2 nM (measured at a membrane potential of −70 mV) [35]. Taking into account that ATX is a large molecule with 29 carbon atoms that certainly may not permeate the NMDA receptor pore, the increase in sensitivity might be caused by facilitation of ATX entry into the channel due to the Q mutation. The change of the channel pore structure that facilitates entry of ATX might also be the reason for the higher Mg^{2+} permeability which, however, causes a reduction of Mg^{2+} block because Mg^{2+} can leave its binding site in the channel by permeation. Following this interpretation, the apparent contradiction that one and the same mutation can increase blocking efficacy of one pore blocker and reduce sensitivity to another pore blocker would be easily resolved.

Studying competitive antagonists in cloned NMDA receptors

Many studies have been performed to analyze the

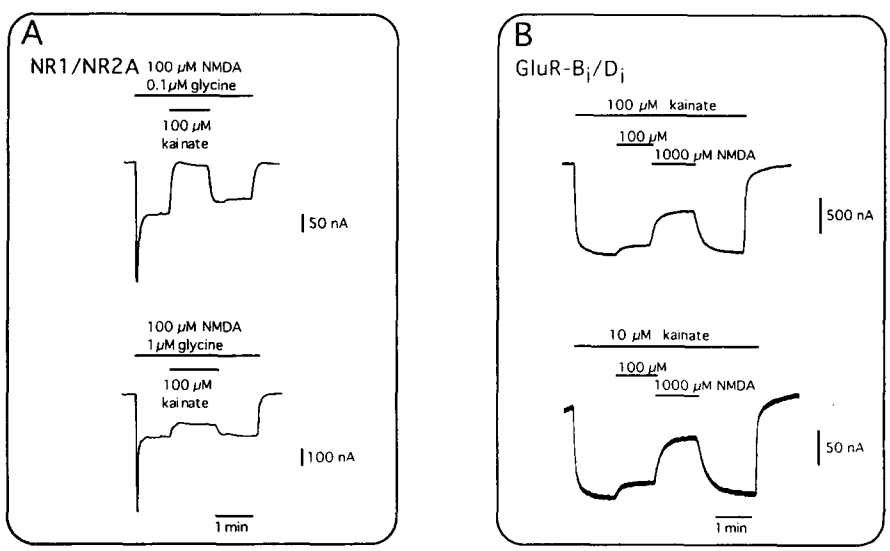


Fig. 5. Effects of kainate on NMDA receptors and of NMDA on AMPA receptors. (A) Whole-cell current mediated by NR1/NR2A channels expressed in a *Xenopus* oocyte is blocked by 100 μM kainate. The amount of block depends on the glycine concentration. It is almost complete at 0.1 μM glycine and about 30% at 1 μM glycine. (B) AMPA receptors of the GluR-B_i/D_i heteromeric type are blocked by 1000 μM NMDA. The effect of NMDA is slightly dependent on the concentration of kainate by which the receptors are activated.

actions of various agonists and antagonists of the glutamate and glycine binding sites of the NMDA receptor. There are two reasons why it might be interesting to also study this issue in cloned NMDA receptor channels.

First, it would be quite exciting to determine glutamate and glycine binding sites in the primary sequence of the NMDA receptor by site-directed mutagenesis. Such studies, however, have yet to be carried out.

Second, as already mentioned, data about agonist and antagonist effects at the ligand binding sites measured in native neurons might suffer from the problem of mixed channel populations. It has been discussed recently that hybrid channels composed of AMPA and NMDA receptor subunits with mixed pharmacological properties may exist in native neurons [36]. One argument in favour of the existence of such hybrid channels was that the responses evoked by the co-application of kainate and NMDA on native neurons are smaller than the sum of responses obtained with either kainate or NMDA separately. The authors argue against an interpretation of this result by inhibitory interaction of NMDA with kainate receptors and of kainate with NMDA receptors by showing that there is no competition between both agonists.

With cloned channels it is now possible to investigate the effect of NMDA on AMPA receptors and the effect of kainate on NMDA receptors either separately or by mixing two populations of NMDA and AMPA receptor subunits to test the hypothesis of the occurrence of AMPA–NMDA hybrid receptors. Figure 5 shows the effects of kainate on the current responses of cloned NMDA receptors to NMDA and glycine application and those of NMDA on current responses mediated by AMPA receptor channels evoked by kainate application. As shown in Fig. 5A, 100 μM kainate had an inhibitory effect of about 95% on the amplitude of NMDA-evoked currents mediated by NR1/NR2A channels in the presence of 0.1 μM glycine. The blocking effect of kainate was much smaller if the experiment was done in the presence of 1 μM glycine (30%). This indicates that the mechanism of the kainate-block of NMDA receptor is a competitive binding of kainate at the glycine binding site. Variations of the NMDA concentration did not change the kainate block significantly (not shown).

The opposite experiment, to block AMPA receptors by NMDA co-application, was also successful. A 1 mM concentration of NMDA blocked 40% of the response of heteromeric GluR-B_i/GluR-D_i AMPA receptors (Fig. 5B) or homomeric GluR-D_i AMPA receptors (not shown) to 100 μM kainate. If kainate was reduced to 10 μM, which resulted in 10-fold smaller responses, the block by 1 mM NMDA was increased slightly to 60%. This suggests that in this case NMDA competes to some extent with kainate at the ligand binding site. Competition, however, seems to be too weak to explain the blocking effect completely. Since at the AMPA receptor a second site for the binding of amino acids such as the glycine site of the NMDA receptor is not yet defined, there is no alternative hypothesis for the NMDA effect on AMPA receptors.

According to the results shown in Fig. 5, the observation that the effect of co-applied kainate and NMDA on mixed populations of receptors is smaller than the sum of the separated effects would be expected also in the absence of NMDA–AMPA hybrid channels. It is, therefore, no argument for the existence of AMPA–NMDA hybrid receptors. The lack of competition observed might be explained with an effect on modulatory amino acid sites such as the glycine site of the NMDA receptor where the blocking amino acid interacts with the receptor molecule.

Discussion

Although a large number of excellent studies analyzing the complex biophysics of block of NMDA receptors have been carried out in neurons, it appears useful to study these properties also in cloned NMDA receptor channels. First of all, this can be very helpful in understanding the mechanism of a blocking agent on the molecular level. The example pointed out here is divalent block and block by ATX, which was found to be controlled completely by the so-called Q/R/N site of the NMDA receptor. This site is either able to shield the receptor against entry of blocking ions if it is mutated to carry a positive charge, or, on the other hand, it may determine divalent permeability and control blocking efficacy by changing the off-rate through the pore. According to this view, a blocking ion is one that may enter the channel but cannot permeate.

Second, it may be an advantage to check physiological functions of the receptors by expressing pure cloned NMDA receptors in pure culture. Effects like the sum-up effect of different agonists discussed above can be understood much more easily if the system under investigation does not contain a complex mixture of different glutamate receptor subtypes.

Nevertheless, it would be dangerous to rely too much on the properties of cloned NMDA receptor channels, as long as it is unclear in a particular case whether a phenomenon or property to be studied in the cloned receptor is completely reproduced also in the native receptor. As long as one cannot be absolutely sure about the molecular composition of the native NMDA receptors, the comparison of native and cloned receptors should not be neglected but rather put ahead of any planned experimental effort to study biophysics of cloned channels.

Summary

The biophysics of block of NMDA receptor channels has been investigated extensively during the past 8 years. In the last few years, cloned NMDA receptor channels have become available. Here we have discussed advantages and disadvantages of studying block phenomena in cloned NMDA receptors. Some recent work on the pore block of the cloned NMDA receptor channels was critically reviewed and extended by data about the calcium block. Novel effects of kainate on cloned NMDA receptors and of NMDA on cloned AMPA receptors were reported and discussed with respect to recent work concerning possible occurrence of NMDA–AMPA hybrid channels.

Acknowledgements—We thank Prof. P. H. Seeburg for providing AMPA and NMDA receptor clones and Dr M. Häusser for reading the manuscript.

REFERENCES

1. Dingledine R, Excitatory amino acids: Modes of action on hippocampal pyramidal cells. *Fedn Proc* **42**: 2881–2885, 1983.
2. Forsythe ID and Westbrook GL, Slow excitatory postsynaptic currents mediated by *N*-methyl-D-aspartate receptors on cultured mouse central neurones. *J Physiol (Lond)* **396**: 515–533, 1988.
3. Lester RAJ and Jahr CE, NMDA channel behavior depends on agonist affinity. *J Neurosci* **12**: 635–643, 1992.
4. Lester RA, Clements JD, Westbrook GL and Jahr CE, Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents. *Nature* **346**: 565–567, 1990.
5. Mayer ML and Westbrook GL, Permeation and block of *N*-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. *J Physiol (Lond)* **394**: 501–527, 1987.
6. Burnashev N, Schoepfer R, Monyer H, Ruppersberg JP, Günther W, Seebrug PH and Sakmann B, Control by asparagine residues of calcium permeability and magnesium blockade in the NMDA receptor. *Science* **257**: 1415–1419, 1992.
7. Nowak L, Bregestovski P, Ascher P, Herbet A and Prochiantz A, Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307: 462–465, 1984.
8. Mayer ML, Westbrook GL and Guthrie PB, Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* **309**: 261–263, 1984.
9. Collingridge GL and Singer W, Excitatory amino acid receptors and synaptic plasticity. *Trends Pharmacol Sci* **11**: 290–296, 1990.
10. Kauer JA, Malenka RC and Nicoll RA, NMDA application potentiates synaptic transmission in the hippocampus. *Nature* **334**: 250–252, 1988.
11. Bonhaus DW, Burge BC and McNamara JO, Biochemical evidence that glycine allosterically regulates an NMDA receptor-coupled ion channel. *Eur J Pharmacol* **142**: 489–490, 1987.
12. Forsythe ID, Westbrook GL and Mayer ML, Modulation of excitatory synaptic transmission by glycine and zinc in cultures of mouse hippocampal neurons. *J Neurosci* **8**: 3733–3741, 1988.
13. Thomson AM, Walker VE and Flynn DM, Glycine enhances NMDA-receptor mediated synaptic potentials in neocortical slices. *Nature* **338**: 422–424, 1989.
14. Johnson JW and Ascher P, Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* **325**: 529–531, 1987.
15. Williams K, Dawson VL, Romano C, Dichter MA and Molinoff PB, Characterization of polyamines having agonist, antagonist, and inverse agonist effects at the polyamine recognition site of the NMDA receptor. *Neuron* **5**: 199–208, 1990.
16. Miller B, Sarantis M, Traynelis SF and Attwell D, Potentiation of NMDA receptor currents by arachidonic acid. *Nature* **355**: 722–725, 1992.
17. Manzoni O, Prezeau L, Desagher S, Sahuquet A, Sladeczek F, Bockaert J and Fagni L, Sodium nitroprusside blocks NMDA receptors via formation of ferrocyanide ions. *Neuroreport* **3**: 77–80, 1992.
18. Manzoni O, Preseau L, Marin P, Deshager S, Bockaert J and Fagni L, Nitric oxide-induced blockade of NMDA receptors. *Neuron* **8**: 653–662, 1992.
19. Sucher NJ and Lipton SA, Redox modulatory site of the NMDA receptor-channel complex: Regulation by oxidized glutathione. *J Neurosci Res* **30**: 582–591, 1991.
20. Aizenman E, Hartnett KA, Zhong C, Gallop PM and Rosenberg PA, Interaction of the putative essential nutrient pyrroloquinoline quinone with the *N*-methyl-D-aspartate receptor redox modulatory site. *J Neurosci* **12**: 2362–2369, 1992.
21. Aizenman E, Lipton SA and Loring RH, Selective modulation of NMDA responses by reduction and oxidation. *Neuron* **2**: 1257–1263, 1989.
22. Chen L and Huang L-YM, Protein kinase C reduces Mg^{2+} block of NMDA-receptor channels as a mechanism of modulation. *Nature* **356**: 521–523, 1992.
23. Yamazaki M, Hori H, Araki K, Mori KJ and Mishina M, Cloning, expression and modulation of a mouse NMDA receptor unit. *FEBS Lett* **300**: 39–45, 1992.
24. Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B and Seeburg PH, Heteromeric NMDA receptors: Molecular and functional distinction of subtypes. *Science* **256**: 1217–1221, 1992.
25. Nakanishi N, Axel R and Shneider NA, Alternative splicing generates functionally distinct *N*-methyl-D-aspartate receptors. *Proc Natl Acad Sci USA* **89**: 8552–8556, 1992.
26. Meguro H, Mori H, Araki K, Kushiya E, Kutsuwada T, Yamazaki M, Kumanishi T, Arakawa M, Sakimura K and Mishina M, Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature* **357**: 70–74, 1992.
27. Ikeda K, Nagasawa M, Mori H, Araki K, Sakimura K, Watanabe M, Inoue Y and Mishina M, Cloning and expression of the $\varepsilon 4$ subunit of the NMDA receptor channel. *FEBS Lett* **313**: 34–38, 1992.
28. Durand GM, Gregor P, Zheng X, Bennett MV, Uhl GR and Zukin RS, Cloning of an apparent splice variant of the rat *N*-methyl-D-aspartate receptor NMDAR1 with altered sensitivity to polyamines and activators of protein kinase C. *Proc Natl Acad Sci USA* **89**: 9359–9363, 1992.
29. Burnashev N, Monyer H, Seeburg PH and Sakmann B, Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. *Neuron* **8**: 189–198, 1992.
30. Stern P, Behe P, Schoepfer R and Colquhoun D, Single-channel conductances of NMDA receptors expressed from cloned cDNAs – Comparison with native receptors. *Proc R Soc Lond [Biol]* **250**: 271–277, 1992.
31. Moriyoshi K, Masu M, Ishii T, Shigemoto R, Mizuno N and Nakanishi S, Molecular cloning and characterization of the rat NMDA receptor [see comments]. *Nature* **354**: 31–37, 1991.
32. Sommer B, Köhler M, Sprengel R and Seeburg PH, RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. *Cell* **67**: 11–20, 1991.
33. Mori H, Masaki H, Yamakura T and Mishini M, Identification by mutagenesis of a $Mg^{(2+)}$-block site of the NMDA receptor channel. *Nature* **358**: 673–675, 1992.
34. Herlitze S, Raditsch M, Ruppersberg JP, Jahn W, Monyer H, Schoepfer R and Witzemann V, Argiotoxin detects molecular differences in AMPA receptor channels. *Neuron* **10**: 1131–1140, 1993.
35. Raditsch M, Ruppersberg JP, Kuner T, Günther W, Schoepfer R, Seeburg PH, Jahn W and Witzemann V, Subunit-specific block of cloned NMDA receptors by $argiotoxin_{636}$. *FEBS Lett* **324**: 63–66, 1993.
36. Henley JM, Ambrosini A, Rodriguez-Ithurralde D, Sudan H, Brackley P, Kerry C, Mellor I, Abutidze K, Usherwood PNR and Barnard EA, Purified unitary kainate/α - amino - 3 - hydroxy - 5 - methylisooxazole - propionate (AMPA) and kainate/AMPA/*N*-methyl-D-aspartate receptors with interchangeable subunits. *Proc Natl Acad Sci USA* **89**: 4806–4810, 1992.