NMDA receptor subunits: diversity, development and disease

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N-methyl-D-aspartate receptors (NMDARs) are present at many excitatory glutamate synapses in the central nervous system and display unique properties that depend on their subunit composition. Biophysical, pharmacological and molecular methods have been used to determine the key features conferred by the various NMDAR subunits, and have helped to establish which NMDAR subtypes are present at particular synapses. Recent studies are beginning to address the functional significance of NMDAR diversity under normal and pathological conditions.

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Abbreviations
EPSC excitatory postsynaptic current
NMDA *N*-methyl-D-aspartate
NMDAR *N*-methyl-D-aspartate receptor
NMDAR-EPSC NMDA receptor-mediated EPSC

Introduction

N-methyl-D-aspartate receptors (NMDARs) have critical roles in excitatory synaptic transmission, plasticity and excitotoxicity in the CNS. The involvement of NMDARs in these diverse processes reflects their unique features, which include voltage-sensitive block by extracellular Mg^{2+}, a high permeability to Ca^{2+} and unusually slow 'activation/deactivation' kinetics. NMDARs also display sensitivity to an array of endogenous ligands and modulators present in the vicinity of the synapse: the co-agonist glycine must bind before the receptors can be activated, whereas physiological levels of protons suppress NMDAR activation. Extracellular Zn^{2+} and polyamines also act on the receptor to modify its behaviour. Furthermore, NMDAR subunits interact with various intracellular scaffolding, anchoring and signalling molecules associated with the postsynaptic density.

Several distinct NMDAR subtypes have now been identified in central neurons, differing in their sensitivity to endogenous and exogenous ligands, permeation and block by divalent ions, kinetic properties, and interaction with intracellular proteins. Biophysical, pharmacological and molecular methods are all providing a clearer picture of the key features defined by particular subunits and are furnishing tools that can be used to determine the involvement of specific subunits in synaptic transmission. Appreciating the roles played by distinct NMDAR subtypes is essential in understanding normal transmission in the CNS, and should provide information about how NMDAR subunit multiplicity can be exploited for therapeutic advantage. Several recent publications have reviewed the assembly and targeting of NMDARs and their role in developmental plasticity, learning and memory [1–4]. Our aim here is to consider recent advances in the functional and pharmacological identification of the various NMDAR subtypes — including the relationship between subunit composition and receptor properties — and to consider the implications of this receptor diversity in normal and disease states.

NMDAR subunits and splice variants

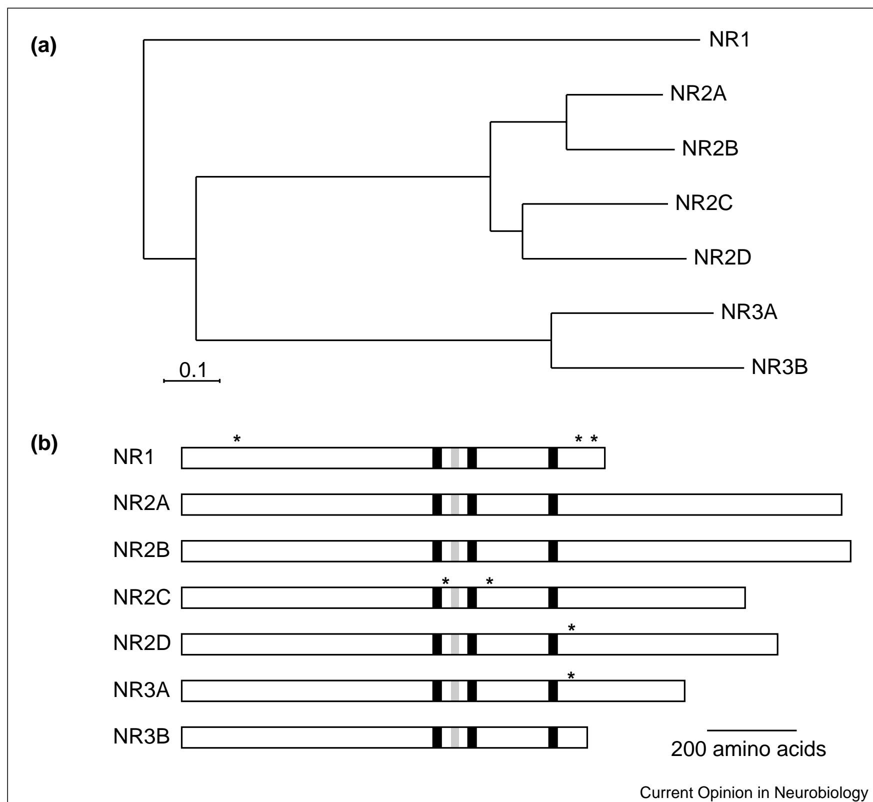
Over the past decade, a variety of NMDAR subunits have been identified: the ubiquitously expressed NR1 subunit; a family of four distinct NR2 subunits (A, B, C and D); and two NR3 subunits [3,5–7]. NR1 occurs as eight distinct isoforms owing to the presence of three independent sites of alternative splicing [1]. Similarly, each of the NR2 and NR3 subunits (apart from NR2A) has several splice variants (see Figure 1), although the functional relevance of the different splice forms remains uncertain. *In situ* hybridization studies have shown that mRNAs for NMDAR subunits are differentially distributed throughout the brain, with patterns of expression that change strikingly during development (see [8,9]). NR2B and NR2D subunits predominate in the neonatal brain, but over the course of development these are supplemented with, or replaced by, NR2A and in some regions NR2C subunits.

All NMDARs appear to function as heteromeric assemblies composed of multiple NR1 subunits in combination with at least one type of NR2. The NR3 subunit does not form functional receptors alone, but can co-assemble with NR1/NR2 complexes [7,10]. As each of the constituent subunits confers distinct properties to the receptor assembly, to a greater or lesser extent, many of the important functional attributes of the native receptors are determined by the expression of the various subunits and isoforms.

NMDAR functional properties are determined by subunit composition

Studies of recombinant receptors [8,11–13] have provided an understanding of how receptor properties are defined by individual NMDAR subunits. The likely subunit composition of native receptors has been inferred by examining subunit mRNA or protein distribution [8,9,14–17], by using animals with specific subunit genes deleted [7,18–20], and bycomparing the functional properties of native and recombinant NMDARs [21–27]. Together, these approaches have allowed the receptor-channel properties associated with specific subunits to be determined, and have demonstrated the influence of NMDAR subtypes on the characteristics of transmission at specific synapses.

Figure 1



NMDAR subunit diversity. **(a)** Dendrogram of complete amino-acid sequences for rat NMDAR subunits (except NR3B; human). Sequences were aligned using ClustalX and the tree was generated with NJPlot (http://pbil.univ-lyon1.fr/software/njplot.html) ClustalX (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/). **(b)** Representation of NMDAR subunit polypeptides. Black boxes indicate transmembrane domains, and grey boxes show the transmembrane TM2 re-entrant loop. Asterisks denote regions at which alternative splicing takes place. This is best characterized for the NR1 subunit, which has three regions of alternative splicing: the amino-terminal N1 cassette (exon 5); and the carboxy-terminal C1 (exon 21) and C2 (exon 22) cassettes. Splicing at these sites can generate eight distinct isoforms (NRI-1a, -1b, -2a, -2b, -3a, -3b, -4a and -4b; see [1,3]). Splicing of the NR2C subunit leads to truncated polypeptides ending after TM1 or TM3. The NR2D subunit can be spliced in the carboxyl terminus, producing a 33-amino-acid insert. Likewise, NR3A splicing leads to a 20-amino-acid insert in the carboxy-terminal domain. NR2B, NR2C and NR2D also have splice sites in their 5′-untranslated regions but no splice variants have been reported for NR2A (see [3,74]).

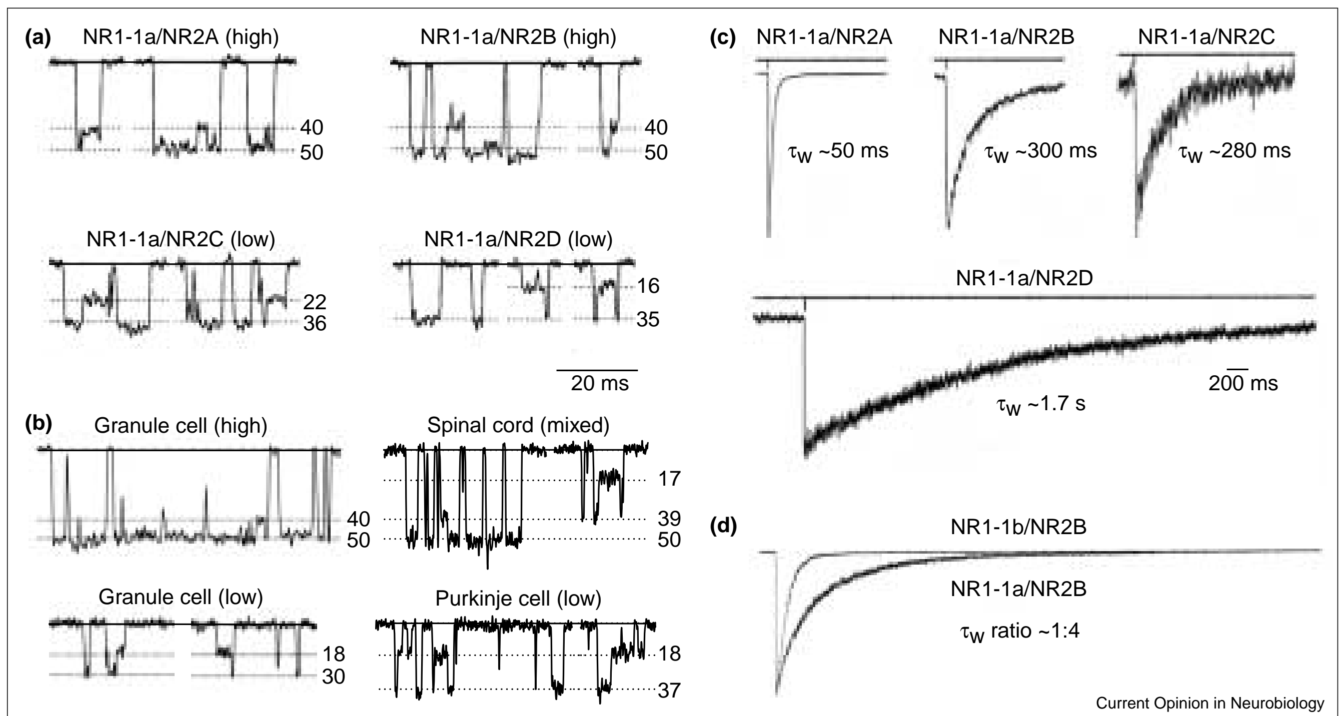
NR2 and NR3 subunits

During synaptic transmission, NMDAR activation generates a current with a slow rise and an exceptionally slow decay time, which exceeds that of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor (AMPAR)-mediated component by at least two orders of magnitude. NMDAR channels first open about 10 ms after glutamate is released into the synaptic cleft, and continue to open and close for hundreds of milliseconds until glutamate unbinds from receptor [13,28]. The time course of decay of NMDAR-mediated excitatory postsynaptic currents (EPSCs), and the apparent affinity of the receptors for glutamate, are both strongly influenced by the identity of the NR2 subunits involved. Consistent with this observation, there is general agreement that glutamate molecules bind with high affinity to the NR2 subunits of the NMDAR [29–31] while glycine molecules bind to the NR1 subunits [32].

For diheteromeric NMDARs (NR1/NR2) the deactivation times span a 50-fold range, following the sequence: NR2A < 2C = 2B << 2D (see Figure 2). Thus, brief application of glutamate onto NR1/NR2A assemblies generates a macroscopic current with a deactivation time constant of tens of milliseconds, compared with several seconds for NR1/NR2D receptors [8,11,12]. The same ranking of subunits has been found in measurements of the steady-state EC_{50} (concentration producing half-maximal response) for glutamate, although here the difference between subunits is only about fourfold. Differences in deactivation time and EPSC decay have also been used to infer the NR2 subunit composition of native NMDARs [24,26,33].

Aside from these kinetic differences, the most obvious subunit dependent properties of NMDARs are their single-channel conductances and their block by extracellular Mg^{2+}. Thus, diheteromeric NMDARs containing NR2A or NR2B subunits generate ‘high-conductance’ channel openings with a high sensitivity to block by Mg^{2+}, whereas NR2C- or NR2D-containing receptors give rise to ‘low-conductance’ openings with a lower sensitivity to extracellular Mg^{2+}. There are also subtle differences in the gating characteristics of NR2C- and NR2D-containing receptors [22,24,34]. Although the characteristic Ca^{2+} permeability of NMDAR channels is not greatly affected by their NR2 subunit composition (fractional Ca^{2+} current varies between 8–14%, [35,36]), it seems likely that the marked difference in Mg^{2+} sensitivity would affect the Ca^{2+} influx generated by synaptic activation of the different NMDAR subtypes. Finally, recent experiments have shown that the NR3 subunit can also give rise to low-conductance channel openings, when co-assembled with NR2A (i.e. NR1/NR2A/NR3) and these channels show a roughly fivefold reduction in relative Ca^{2+} permeability as compared with NR1/NR2A assemblies [7,10]. Thus, the general principle that a low single-channel conductance can provide a clear ‘single-channel signature’ for NR2C- or NR2D- containing receptors applies only in the absence of NR3.

Figure 2



Functional properties of NMDARs conferred by specific subunits. **(a)** Representative single-channel records of recombinant NMDARs expressed in *Xenopus* oocytes, showing the high-conductance openings of NR2A- and NR2B-containing receptors, and the low-conductance openings of NR2C- and NR2D-containing receptors. Solid lines indicate the closed state, and dotted lines indicate open states. Numbers indicate conductance in picosiemens (data from [13]). **(b)** Example of single-channel records of native NMDARs from cerebellar granule cells, a dorsal horn neuron of the spinal cord, and a cerebellar Purkinje cell, showing openings of high- and low-conductance (data from [21,22]). In these examples, the high-conductance channels are thought to arise from NR1/NR2B receptors and the low-conductance channels from NR1/NR2D receptors in spinal cord and Purkinje cells, and from NR1/NR2C receptors in granule cells. **(c)** Macroscopic currents from recombinant NMDARs expressed in HEK293 cells, illustrating the NR2 subunit-dependent deactivation seen in response to 1-ms applications of 1 mM glutamate (data from [12]). **(d)** Macroscopic currents from recombinant NMDARs illustrating the influence of NR1 splice varaiants on deactivation (4-ms applications of 1 mM glutamate; data from [40]) τ_w is the weighted deactivation time constant.

NR1 isoforms

Although the effects of NR2 subunits have received most attention, NR1 splice variants also strongly influence NMDAR properties. For example, the pH sensitivity of NMDARs is determined by the presence of exon 5 (in the amino terminus). At physiological pH, splice variants that include exon 5 are fully active, whereas those that lack exon 5 are partially blocked [37]. It has been suggested that the exon 5 cassette forms a surface loop, with structural similarities to polyamines, and acts as a tethered modulator to shield the proton-sensor of NR1 [37]. Isoforms containing exon 5 are therefore neither potentiated by polyamines nor inhibited by Zn^{2+} (which produces a similar type of voltage-independent block [38]).

Variations in the proton sensitivity of postsynaptic NMDAR subtypes might be expected to have an important influence on synaptic transmission. However, both proton and Zn^{2+} inhibition are also affected by the identity of the NR2 subunit(s) within the NMDAR complex. Thus, assemblies incorporating NR1 variants that lack exon 5 are much less sensitive to inhibition by H^+ (or Zn^{2+}) when co-assembled with NR2C or NR2D [38,39].

Recently, it has also been shown that splicing of exon 5 can influence the deactivation properties of NMDARs [40]. Unlike NR2A-containing receptors[12], the deactivation time of recombinant NR2B-containing receptors is dependent on whether or not NR1 contains the exon 5 insert. The deactivation rate is roughly four times faster for NR1-1b/NR2B (exon-5-containing) receptors than for the NR1-1a/NR2B (exon-5-lacking) receptors (see Figure 2). This observation may well be relevant to the change in time course of the NMDAR-EPSC decay that occurs at many synapses during development. This seems particularly pertinent to the thalamus and cerebellum, where there is evidence of a developmental increase in mRNA for exon-5-containing NR1 (NR1-1b) isoforms during development [41].

NMDAR subtypes differ in their pharmacology

One way in which the functions of the various NMDAR subunits may be assessed is through the use of subunit selective agonists and antagonists. A number of pharmacological agents

Table 1

Agents selective for NMDAR subtypes.

Agent	Action	Subunit selectivity	References
Ifenprodil	Non-competitive inhibition of NR2B	NR2B >> 2A/2B >> 2D = 2C = 2A	[46,51]
Haloperidol	Non-competitive inhibition of NR2B	NR2B >> 2A/2B >> 2D = 2C = 2A	[12,47]
Ro 8-4304	Non-competitive inhibition of NR2B	NR2B > 2A	[75]
CP 101,606	Non-competitive inhibition of NR2B	NR2B >> 2C = 2A	[76]
Felbamate	Non-competitive inhibition of NR2B	NR2B >> 2A = 2C	[77]
Conantokin-G	Competitive inhibition of NR2B	NR2B > 2D = 2C = 2A	[49]
D-CPPene	Competitive inhibition of NR2B and 2A	NR2A = 2B > 2C = 2D	[50]
PPDA	Competitive inhibition of NR2C and 2D	NR2C = 2D > 2A = 2B	[50]
Protons	Inhibition with NR1 lacking exon 5	NR2A > 2B >> 2D > 2C	[37]
Zn^{2+}	Non-competitive inhibition of NR2A	NR2A>NR2B>NR2C	[39]
Spermine	Glycine-independent potentiation	NR2B only (with NR1-1a)	Reviewed in [1]

Some of the agents shown to exhibit a degree of subunit-selective action on recombinant NMDARs. Symbols in subunit-selectivity rank orders indicate roughly one (>) or two (>>) orders of magnitude difference in reported IC_{50} values. For more details, see also [1,44].

have been shown to distinguish between certain NMDAR subtypes (see Table 1). Competitive antagonists such as AP5 (2-amino-5-phosphonpentanoic acid) and D-CPPene (3-[2-carboxypiperazine-4-yl]-propenyl-1-phosphonic acid), channel blockers such as MK-801 (dizocilpine), ketamine, phencyclidine, amantadine and memantine, and novel non-competitive antagonists such as felbamate show moderate selectivity for certain subunit combinations. For example, sensitivity to MK-801 is greater for recombinant NR1/NR2A and NR1/NR2B receptors than for NR1/NR2C (see [42]). More effective discrimination between receptors containing different NR2 subunits can be achieved with non-competitive antagonists that act through the proton sensor of the NR1 subunit [43–45]. The best characterised of these compounds is ifenprodil, which has an IC_{50} (concentration producing half-maximal inhibitor) that is about 400-fold lower for NR2B- than for NR2A-, NR2C- or NR2D-containing receptors[46].

Several related phenylethanolamines and their derivatives are thought to act in a similar manner (Table 1); thus both haloperidol and the ifenprodil derivative CP101,606, are highly effective at suppressing responses from NR1/NR2B receptors [47,48]. These drugs suppress the activation of NMDARs containing the NR2B subunit by enhancing their sensitivity to inhibition by protons [43]. As a consequence, inhibition by phenylethanolamines can be overcome by increasing pH. Recently, conantokin-G, a 17-amino-acid peptide extracted from cone snail venom, has been identified as a highly selective competitive antagonist of NR2B-containing NMDARs [49].

Although pharmacological agents that selectively block NR2A-, NR2C- or NR2D-containing receptors have not been described, PPDA ([±]-cis-1-[phenanthren-2yl-carbonyl]piperazine-2,3-dicarboxylic acid) has been suggested to preferentially block NR2C- and NR2D-containing NMDARs [50]. Also, NR2A-containing receptors can be identified with the Zn^{2+} chelator TPEN (*N,N,N′,N′*-tetrakis-[2-pyridylmethyl]-ethylenediamine), which enhances the NMDAR response by removing the tonic inhibition caused by low levels of Zn^{2+} present in experimental solutions [39]. By selectively potentiating responses from NR2A-containing receptors, TPEN provides a convenient distinction between NR2A- and NR2B-containing synaptic NMDARs [24,33]. Although the use of these subunit selective antagonists may appear straightforward, consideration needs to be given to the existence of triheteromeric NMDAR subunit assemblies, for which drug selectivity may not have been determined (see below).

Developmental changes in synaptic NMDAR subtypes

One indicator of the functional importance of NMDAR subunit diversity comes from examining the subunit mRNA changes seen during development. At embryonic stages, the NR2B subunit is found in most brain regions, whereas the NR2D subunit is present in the diencephalon and brainstem. Soon after birth, NR2A mRNA is found in most regions, whereas NR2C appears later and is prominent in the cerebellum (see [8,9]). Functional studies have now examined the possible subunit composition of NMDARs in a number of identified neurons in various regions of the CNS. There appears to be a general trend towards a decreasing (but still significant) contribution from the NR2B subunit during development, which is associated with an increasing contribution of NR2A-containing NMDARs to the synaptic current. As discussed below, however, the exact changes in subunit expression vary with brain region, and there is also evidence for variation in the NR2 subunit composition of NMDARs at different sites even within a single cell [20,23,24,51,52].

Expression of the NR2A subunit and its role in synaptic plasticity

A gradual replacement or supplementation of NR2B by NR2A during postnatal development has been implicated in the speeding of NMDAR-EPSC decay — a phenomenon often linked with the ability of neuronal circuits to exhibit experience-dependent synaptic plasticity [4]. For example, in visual cortex the NMDAR-EPSCs are sensitive to

NR2B-selective antagonists when the NMDAR-EPSC decay is slow (postnatal day [P] 3–5), and this sensitivity is lost by P7 when the NMDAR-EPSC decays rapidly. For some time it has been known that acceleration of these NMDAR-EPSCs is delayed if animals are deprived of light. A recent study has shown that light exposure of dark-reared animals results in the rapid insertion at the synapse of new NMDARs with a higher NR2A:NR2B ratio [53] (see Update).

Experiments using single-cell PCR with reverse transcription to correlate the expression of individual subunits with the functional properties of NMDA receptors in neocortical cells [25] have indicated that relatively low levels of NR2A mRNA may be sufficient to generate rapidly decaying NMDAR-EPSCs. This implies that NR2A subunits are preferentially targeted to the synapse, or that NR2A co-assembles with NR2B to form receptors with fast kinetics. But it is less clear in visual cortical cells whether this NR2A-dependent kinetic change signals the end of ocular dominance plasticity within the thalamo-cortical projection or, as has also been proposed, the onset of the peak of cortical plasticity [54].

Similar changes in the functional and pharmacological properties of NMDAR-EPSCs have been described in other neurons, including hippocampal CA1 pyramidal cells [55], anterior neostriatum and archistriatum (during song learning in the zebra finch [56,57]), and in cerebellar granule cells during the first 3 weeks of their development [23,26]. All of these cells display a change in the NMDAR-EPSC kinetics and ifenprodil sensitivity consistent with a decreasing contribution of NR2B subunits and an increasing synaptic involvement of NR2A. In each of these disparate systems the functional consequence of the switch to a faster NMDAR-EPSC needs further examination, as does the significance of the altered Ca^{2+} signal that this change in the synaptic conductance would be expected to produce.

Subcellular variation in NMDAR subunit composition

Growing evidence indicates that in some cells extrasynaptic and synaptic NMDARs differ in their subunit composition. Whereas NMDAR-EPSCs in visual cortex lose their sensitivity to NR2B-selective antagonists by P7, the extrasynaptic receptors are still blocked at this stage, suggesting that NR1/NR2B-containing receptors are present but are no longer targeted to the synapse [58]. Differences have also been noted between synaptic and extrasynaptic NMDARs in young cerebellar granule cells [23]. In dorsal horn spinal neurons both NR1/NR2D and NR1/NR2B receptors are present extrasynaptically, whereas the kinetic and pharmacological properties of the NMDAR-EPSCs indicate that NR2A receptors predominate at the primary afferent inputs to mature cells [52]. Other cell types, including cerebellar Golgi, Purkinje and stellate cells also express extrasynaptic NMDAR subtypes that are absent from the synapse (see [59]).

Finally, it is clear that NMDAR subtypes can be distributed in a ‘synapse-selective manner’ within a single cell. Targeted disruption of the NR2A subunit gene selectively reduces the NMDAR-EPSCs at distal apical dendrites of CA3 pyramidal neurons, while disruption of the NR2B subunit gene reduces the NMDAR-EPSC at synapses on basal dendrites [20]. At present, the functional significance of these differences in subunit targeting is far from clear.

The presence of NR2C subunits in synaptic NMDARs

As described above, NMDARs containing NR2C (or NR2D) exhibit a low sensitivity to Mg^{2+}. The functional significance of this reduced Mg^{2+} sensitivity has not been examined in detail, but it would be expected to allow these NMDARs to operate at more negative membrane potentials than conventional NR2A/B-containing receptors. This difference may explain, in part, the ability of antagonists with moderate selectivity for NR2A/B- or NR2C/D-containing receptors to differentially block long-term potentiation (LTP) and long-term depression (LTD) in the hippocampus [50]. It is important to note, however, that the NR2C subunit is present at high levels only in cerebellar granule cells.

The gradual increase in mRNA for NR2C and NR2A seen during granule cell development is accompanied by the expression of a mixed population of low- and high-conductance NMDAR channels [21]. Only low-conductance openings are observed in granule cells from mice lacking NR2A [18], whereas only high-conductance openings are present in mice lacking NR2C [19]. Recent studies have indicated that, although NMDAR-EPSC sensitivity to Mg^{2+} is low in the third postnatal week, consistent with the presence of NR2C-containing receptors, their decay time is fast and matches that of NR1/NR2A NMDARs [23,26]. It is not until around maturity that the NMDAR-EPSC decay time slows to a value characteristic of NR1/NR2C receptors, suggesting that a high level of NR2C expression is required for the switch from triheteromeric (NR1/NR2A/NR2C) to diheteromeric (NR1/NR2C) synaptic receptors [26].

NR1/NR2D receptors have been identified extrasynaptically, but not at the synapse

Although there is good evidence that diheteromeric NR2A-, NR2B- and NR2C-containing NMDARs participate in synaptic transmission, there is no evidence for NR1/NR2D-containing receptors at any central synapse, despite the fact that the distinctive single-channel properties of NR1/NR2D receptors have enabled their identification in the extrasynaptic membrane of several cell types [22,59]. Recombinant NR1/NR2D receptors exhibit a remarkably slow macroscopic deactivation [8,11,12]. Therefore, if native NR1/NR2D receptors also exhibit prolonged deactivation kinetics, their involvement in synaptic transmission should be apparent from a conspicuously slow EPSC decay that would occur on the timescale of seconds rather than hundreds of milliseconds.

Recently, rapid application of glutamate onto Purkinje cell patches, at a stage when these cells express a pure population of NR1/NR2D receptors extrasynaptically, has shown that these receptors do indeed deactivate very slowly [27], consistent with data from recombinant receptors. In other cell types that express an extrasynaptic population of NR1/NR2D receptors, the decay of NMDAR-EPSCs is fast [22,24,52], leading to the conclusion that NR1/NR2D receptors are absent from the postsynaptic membrane.

The possibility still exists that the NR2D subunit is present at the synapse but is preferentially co-assembled with other NR2 subunits. Such triheteromeric assemblies (NR1/NR2B/NR2D) may not exhibit the slow deactivation of pure NR1/NR2D receptors but may display other NR2D-like properties. In this context it is of interest to note that immunohistochemical data suggest that all NR2D-containing receptors in the midbrain are triheteromeric [14]. Finally, although apparently excluded from the synapse, the unusually high affinity of NR1/NR2D receptors for glutamate may allow them to serve some novel function associated with an extrasynaptic location.

Evidence for functionally distinct triheteromeric NMDARs in neurons

Molecular biological and immunoprecipitation studies have provided compelling evidence that some native NMDARs contain more than one type of NR2 subunit in the same assembly [14,60,61]. As described above, there is also evidence from studies of recombinant receptors that the NR3 subunit co-assembles with NR1/NR2 receptors to produce a functionally distinct triheteromeric NMDAR [7,10]. However, the issue of whether triheteromeric assemblies represent a sizeable fraction of the synaptic NMDARs, or whether these are predominantly diheteromeric (NR1 plus one type of NR2) remains unresolved.

Until recently it has also been unclear to what extent the inclusion of a second NR2 subunit type influences the functional properties of the receptor. NMDARs are thought to contain two copies of NR1 ([62]; but see also [63]), and two copies of NR2 [31,63]. If the receptors are indeed tetrameric, then we would expect cells expressing two types of NR2 to display only one form of triheteromeric receptor. Co-expression of NR2A and NR2D has been shown to generate three recombinant channels types: high- and low-conductance openings, and a novel receptor channel thought to arise from a triheteromeric assembly [64]. In contrast, studies of native NMDARs in cells expressing a mixture of high- (NR2A- or NR2B-containing) and a low-conductance (NR2C- or NR2D-containing) channels have generally identified only these two types of channel openings ([21,22,24,52,59]; but see also [19]). This might suggest that any additional channel types represent only a small fraction in the extrasynaptic population.

Recombinant NMDARs in cells transfected with NR1, NR2A and NR2B subunits, display reduced ifenprodil sensitivity and exhibit kinetics of recovery from ifenprodil block that differ from those of NR1/NR2B receptors, suggesting the formation of triheteromeric assemblies [12,51]. From their pharmacological and kinetic properties, the extrasynaptic NMDARs in cultured hippocampal cells are thought to be NR1/NR2B assemblies [51]. In contrast, the NMDAR-EPSCs in these cells arise from two populations of receptors, most of which show a response to ifenprodil that is consistent with the presence of NR1/NR2A/NR2B triheteromeric assemblies. Similarly, the presence of triheteromeric assemblies (NR1/NR2A/NR2C) could also explain the apparently discrepant kinetic behaviour and Mg^{2+} sensitivity of NMDAR-EPSCs at immature mossy fibre-granule cell synapses [26]. Therefore, there is growing support for the presence at certain synapses of triheteromeric assemblies that exhibit distinct functional and pharmacological properties.

NMDAR diversity and disease

Inappropriate activation of NMDARs has been implicated in the aetiology of several disease states. In particular, excessive calcium influx through NMDARs can cause excitotoxic neuronal death, and thus blockade of NMDARs is neuroprotective in animal models of both stroke and seizure [65]. Stroke was, therefore, the first clinical indication considered for NMDAR antagonists, but the usefulness of most drugs was limited by their actions on normal synaptic transmission or by additional side effects. Most dramatically, reduction of NMDAR activity by non-competitive antagonists such as ketamine or phencyclidine resulted in dopaminergic hyperactivity and behavioural changes characteristic of schizophrenia. Parenthetically, although the mechanism linking NMDAR hypofunction and psychosis remains to be established (see [66]), mice with reduced NR1 subunit expression [67] or NR2A subunit deletion [68] have been proposed as useful animal models of schizophrenia.

Despite these initial concerns, many NMDAR antagonists lacking psychotic side-effects have been considered for the treatment of stroke. For example, in recent years the channel blocker aptiganel (CNS 1102), the competitive glutamate antagonist selfotel (CGS 19755) and the competitive glycine site antagonist gavistinel (GV150526) have all completed phase III clinical trials. Unfortunately, all have failed to live up to preclinical expectations showing little or no therapeutic benefit [69,70]. Whether these negative results reflect an initially over optimistic view of the NMDAR's involvement in ischaemic damage, or the difficulties associated with the interpretation of this clinical data, remains an open question [69].

There is evidence to suggest that not all NMDAR subtypes are equally important for producing the neuronal death associated with ischaemia. NMDARs incorporating NR1 subunits that lack exon 5 are much less sensitive to inhibition by H^+ when co-assembled with NR2C or NR2D subunits [38]. It would therefore be anticipated that, during

ischaemia, activity of NR2C- or NR2D-containing NMDARs would not be suppressed to the same extent as that of other NMDARs by the accompanying increase in extracellular H^+ concentration. This conclusion is consistent with experimental results showing that neuronal death after vascular occlusion is reduced in transgenic mice lacking the NR2C subunit [71].

NMDAR antagonists have also been considered to be of potential use in treating several neurodegenerative conditions, as well as chronic pain, and there is some evidence to suggest that NMDAR subunit-selective drugs may be beneficial (see [44]). For example, the high density of NR2B-containing NMDARs in the basal ganglia raises the possibility that NR2B-selective antagonists may be more useful than broad-spectrum antagonists in the development of future treatments for Parkinson's disease (see [72]).

More speculatively, the cognitive enhancement reported to occur in mice following overexpression of the NR2B subunit has led to the suggestion that targeting specific NMDAR subtypes might prove a useful strategy for developing novel drugs to combat cognitive disabilities [73]. As our current knowledge regarding the functional significance of specific NMDAR subtypes is far from complete, however, it is difficult to predict how the future development of subunit-selective drugs will impact on the treatment of CNS disorders.

Conclusions

It is now possible able to make use of the characteristic biophysical and pharmacological properties of NMDARs to establish the subunit composition of many native subtypes. Recent studies using such approaches have described the targeting of particular NMDAR subtypes to specific locations in single cells, and have identified developmental changes occurring in the subunit composition of synaptic and extrasynaptic NMDARs.

Until fairly recently, only four types of functionally distinct NMDARs — associated with the different NR2 subunits — had been clearly distinguished in the CNS. With the recognition of functionally distinct triheteromeric receptors, the identification of the NR3 subunit family, and the added functional complexity conferred by NR1 splice variants, it is now apparent that the functional diversity of native NMDARs is much greater than thought previously. Establishing the significance of this heterogeneity, both in normal and disease states, continues to be a major challenge.

Update

Experience-dependent changes in the subunit composition of synaptic NMDARs have been shown to modify the temporal summation of EPSCs in the visual cortex, although the effect of these changes on neuronal integration and/or Ca^{2+} influx remains unknown [78]. Importantly, however, a temporal dissociation between changes in the pharmacology (subunit composition) and the kinetic behaviour of NMDARs seen during a critical period of development at thalamocortical synapses casts doubt on the idea that acceleration of NMDAR-EPSCs is a direct cause of the loss of LTP [79].

Overexpression of NR2B in the forebrain has been shown to increase sensitivity to inflammatory pain [80], an effect suggested to be distinct from the previously described cognitive enhancement. This has led to the suggestion that NR2B-selective antagonists may be useful in the treatment of chronic pain.

Paradoxically, tissue plasminogen activator (tPA), a clot-busting drug used in the treatment of acute stroke, has been found to potentiate NMDAR-induced Ca^{2+} influx and neuronal death. This action has been suggested to result from cleavage of the N-terminus of the NR1 subunit [81]. In contrast, potentiation of NMDARs by another protease, thrombin, does not involve receptor cleavage, but has been linked to activation of the PAR1 receptor [82]. Unravelling the NMDAR subunit-selective actions of these proteases may identify new therapeutic targets (see [83]).

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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