Minireview

N-Methyl-D-aspartate Receptors: Subunit Assembly and Trafficking to the Synapse*

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NMAD1 receptors (NMDARs) are members of the glutamate receptor family of ion channels, which also includes the AMPA, kainate, and delta subtypes of receptors. NMDARs are present at excitatory synapses in the mammalian central nervous system and have specialized characteristics, including voltage-dependent block by magnesium, calcium permeability, and slow deactivation kinetics (1). Biophysical studies of NMDARs outline a complex pharmacology with multiple modulatory sites (1). Calcium entry through the NMDAR regulates numerous downstream signaling pathways leading to both short term and long term neuronal changes (recently reviewed in Ref. 2). This calcium flux is also necessary for inducing long term potentiation, a molecular model of memory (3), suggesting that NMDARs are key players in memory formation and synaptic plasticity. In addition, NMDAR dysfunction is implicated in numerous neurologic and psychiatric disorders, and therapeutic agents that target NMDARs have been studied. The present review will focus on the subunit assembly and trafficking of NMDA receptors, areas of significant progress in recent years.

NMDA Subunits and Splice Variants

The NMDAR family is made up of NR1, NR2, and NR3 subunits. Functional NMDARs are heteromeric complexes containing both NR1 and NR2 subunits (1). NR3 subunits can assemble with NR1-NR2 complexes to depress NMDAR responses and can also assemble with NR1 alone to form a glycine receptor, with the role of this receptor in the central nervous system still unclear (1). The structure of the NMDAR is outlined in Fig. 1.

There is a single NR1 subunit that can exist in eight different variants generated by alternative splicing at three sites within the protein, one in the N terminus and two in the C terminus (4). The N-terminal cassette (the N1 cassette or exon 5) is extracellular and affects regulation of the channel with modulators like protons and spermine (1), whereas the C-terminal cassette is mediators of protein interactions (4, 5). Splicing in the C terminus regulates expression of three different cassettes, the C1, C2, and C2′ cassettes (as shown in Fig. 1). NR1 subunits can be formed either containing or lacking the C1 cassette. If the C2 cassette is absent, the first stop codon is lost and the separate C2′ cassette is expressed in the subunit instead. Therefore, all NR1 contains either the C2 or C2′ cassette. The C1 cassette contains an endoplasmic reticulum (ER) retention signal, which can be overcome by expression of the C2′ cassette, suggesting that splicing of the C-terminal tail of NR1 may regulate receptor trafficking (as reviewed in Refs. 4 and 5). Alternatively spliced forms of NR1 have different regional and developmental expression profiles (6) and are regulated by neuronal activity (7), generating a large degree of channel diversity.

NR2 subunits contain the glutamate-binding site, and four genes encoding NR2 subunits (NR2A–D) have been cloned. The specific NR2 subunits within the receptor complex regulate NMDAR properties in heterologous cells (1, 5, 8). NR2A and NR2B (which form high conductance channels) are highly expressed in the mammalian forebrain (1). NR2B subunit expression predominates early in development, whereas NR2A expression increases with development (8). As the NR2A subunit produces NMDARs with the fastest kinetics, the increasing speed of NMDAR current decay kinetics over development is likely because of its increased expression (1). The NR2C subunit expression is confined mainly to the mature cerebellum, whereas the NR2D subunit is most highly expressed in the young thalamus (1). Regional and developmental regulation of NR2 subunit expression underlies much of the diversity of NMDAR responses in the central nervous system (5, 8).

Subunit Assembly into Functional Complexes

Functional NMDA receptors contain at least 2 glutamate-binding sites and 2 glycine-binding sites, implying a minimum of 4 subunits within the functional channel (1). NMDARs can also assemble with 2 different NR1 splice isoforms and 2 different NR2 subunits (5). Studies on the AMPA receptor (AMPAR), another member of the ionotropic glutamate receptor family, give insights into the structure of the NMDA receptor. Crystallographic analysis coupled with electrophysiologic studies indicate a tetrameric structure of the AMPARs formed by GluR2 composed of a dimer of dimers (9). In the NMDAR, regions of NR2 and NR1 subunits necessary for transmitting allosteric signals between the glutamate and glycine-binding sites are analogous to the areas of dimer interactions in AMPARs (10), suggesting that NMDARs have a similar dimer-dimer interaction. The properties of NMDAR tandems (with a fusion of NR1 and NR2 subunits) also support the idea of the channel being formed of a dimer of dimers (an NR1 dimer and an NR2 dimer) (11). Therefore, the preponderance of evidence now indicates that functional NMDAR complexes are tetramers of 2 NR1 and 2 NR2 subunits, with an evolutionary link between glutamate receptors and K+ channels (12). The actual process of assembly of the individual subunits into the functional channel is not yet well characterized; however, critical residues are known to be located in the N terminus of the receptor (13).

Protein-Protein Interactions and Trafficking of NMDARs

Retention in the ER is a common quality control mechanism to block surface delivery of proteins that are not properly assembled or folded. When expressed alone in heterologous systems, NR2 subunits are retained in the ER (14). The ER reten-
the receptor. One of the most highly studied interactions of the NMDAR is with membrane-associated guanylate kinases (MAGUKs) (reviewed in Refs. 22 and 23). MAGUKs are a family of proteins (including SAP102, SAP97, PSD-93, and PSD-95) that are highly expressed in neurons, contain multiple protein-protein interacting domains, and appear to play a role in scaffolding of the postsynaptic density (PSD) (22, 23). The NMDAR/MAGUK interaction is mediated by the PDZ-binding domain of the NR2 subunit (-ESDV, which is found on the extreme distal region of NR2A and NR2B, or -ESEV on NR2C and NR2D) and the first and second PDZ domains of MAGUKS (22, 23). Although the NR2A and NR2B subunits contain identical PDZ-binding domains, there are indications of preferences for MAGUK interactions between NR2A and NR2B (24). NR2 subunits lacking the PDZ-binding domain show decreased expression at the synapse, suggesting that MAGUKs stabilize NMDARs at the synapse (18, 25).

Although they are concentrated at the PSD and are generally thought to anchor NMDARs, there is increasing evidence that MAGUKs are also involved in earlier trafficking of NMDARs. PSD-95 associates directly with the kinesin KIF1B and may be involved in NMDAR trafficking (26). mLin10 (a PDZ-containing protein) works as a scaffold linking the NR2B subunit with KIF17, another kinesin motor, through a multiprotein complex critical for NR2B delivery to the synapse (27). MAGUKs also interact with Sec8, a protein of the exocyst complex, through a PDZ interaction (28, 29). This interaction, which begins in the ER, is necessary for the delivery of the NMDAR to the synapse and shows that an exocyst-MAGUK-NMDAR complex forms early in the delivery of NMDARs (28). Recent work has also implicated MAGUKs (including PSD-95) as molecular adaptors to bring NMDAR modulators into close proximity with the channel; for example, the kinase Pyk2 binds the SH3 domain of MAGUKs (30). PSD-95 also links the protein huntingtin to the NMDAR, an interaction that may underlie the excitotoxicity seen in Huntington’s disease models (31). Interfering with the association of PSD-95 and the NMDAR can reduce ischemic brain damage (32), indicating that studies of MAGUK/NMDAR interactions may yield novel approaches to the design of therapeutics for modulating NMDAR function.

Numerous other proteins interact with NMDARs (as reviewed recently in Ref. 5). Of particular interest is CaMKII, which has characteristics consistent with a molecule critical for memory formation in its ability to modulate AMPA receptors and organize the synapse (33). CaMKII, a serine/threonine kinase that is necessary for long term potentiation induction, is activated by NMDAR-mediated calcium influx (33), and an interaction with the NR2B subunit appears to stabilize CaMKII in an active conformation (34). The protein RACK1 is another critical interactor with the NR2B subunit that controls ethanol and Fyn kinase modulation of the NMDAR (35). The functional significance of these interactions and the interplay of multiple binding partners at a single site on the NMDAR remain active areas of research.

Control of NMDAR Localization

Although NMDARs are concentrated at the postsynaptic membrane, a second population of receptors, extrasynaptic NMDARs, has been identified and may have important functional roles distinct from those of synaptic receptors. For example, extrasynaptic receptors are important mediators of excitotoxicity, whereas synaptic NMDAR activation appears neuroprotective (2, 36). Synaptic and extrasynaptic receptors are also differentially regulated in response to phosphorylation changes (37), but the relationship between synaptic and extrasynaptic receptors remains unclear. Synaptic receptors can be replaced rapidly through lateral diffusion in the plasma mem-
branched, presumably from extrasynaptic receptors (38). On the other hand, synaptic and extrasynaptic NMDARs differ in their subunit compositions, with extrasynaptic receptors being mainly NR2B-containing whereas synaptic receptors are mainly NR2A-containing at ages when both subunits are expressed (see Fig. 2) (39, 40). The rules governing receptor localization at the synapse are still unclear.

Compared with AMPA receptors, NMDARs are stable components of the synapse and are not readily changed in number. NMDARs also appear to be present at immature synapses before AMPARs (41), producing “silent synapses” that are inactive in the absence of sufficient depolarization to remove the magnesium block of the NMDAR. However, a number of manipulations have been shown to alter the number of surface NMDARs (Table I), suggesting that surface expression of NMDARs in oocytes (51) and synaptic receptors is dependent upon GLUT4 binding, although insertion of NR2B/NR1 constructs (15) and association with MAGUKs and the exocyst protein, Sec8 (28, 29). NMDARs assemble in the endoplasmic reticulum and associate with several scaffolding proteins (22, 23). Mature and immature synapses differ in their relative amounts of NR2A and NR2B subunits (1, 8) as do synaptic versus extrasynaptic NMDARs (39, 40). Although both NR2A and NR2B can bind CaMKII, NR2B binding can specifically produce sustained activity of CaMKII (33, 34). Integrin signaling appears critical for determining the developmental synaptic NMDAR subunit composition and presynaptic release probability (43). NMDARs are internalized by clathrin-mediated endocytosis, although the fate of the internalized receptors in neurons is unknown (47, 48). The NR1–1 splice form, containing the C1 and C2 cassettes, is retained within the ER unless assembled with NR2 subunits.

Whether there is a specific mechanism for delivering NMDARs to the synapse is still not clear. The recent discovery that the NMDAR is associated with the exocyst complex (as discussed above) and that interfering with this interaction decreases synaptic NMDAR responses (28) may outline a process of regulated delivery to the synapse. In addition, binding of the motor protein KIF17 to a multiprotein complex containing the NMDAR is associated with the exocyst complex (as discussed above) and that interfering with this interaction decreases synaptic NMDAR responses (28) may outline a process of regulated delivery to the synapse. In addition, binding of the motor protein KIF17 to a multiprotein complex containing the NR2B subunit is critical for delivery of NR2B-containing receptors to the dendrite, although KIF17 may only regulate delivery of NMDARs to the periphery of the synapse (27). Interestingly, the interaction of NMDARs with both the exocyst and KIF17 has been shown for the NR2B subunit, so the questions of whether NR2A trafficking is similar to that of NR2B and how individual NMDARs with multiple NR2 subunits traffic remain unanswered. Introduction of light stimuli to dark-reared animals quickly changes synaptic subunit composition in rats, indicating a process allowing for very rapid movement of NR2A-containing receptors into the synapse (46). The mechanism of how neurons differentially traffic NR2A- and NR2B-containing NMDARs remains a basic question of critical importance in shaping the synaptic response.

Endocytosis is a common mechanism used to regulate the number of ion channels that are present on the plasma mem-

| Activity | Modulators of NMDAR localization |
|----------|----------------------------------|
| Blocking synaptic activity increases NMDAR number at the synapse (49) | |
| LTP in mature rats increases surface NMDAR number (20) | |
| Agonist binding | |
| Glutamate or glycine antagonists inhibit synaptic NR1/NR2A delivery (25) | |
| Glycine primes NMDARs for internalization (50) | |
| Insulin | |
| Insulin increases surface expression of NMDARs in oocytes (51) | |
| PSD-95 coexpression eliminates insulin potentiation of NR1/NR2A (52) | |
| Metabotropic glutamate receptors (mGluRs) | |
| Group I mGluR stimulation internalizes NMDARs in neurons (53) | |
| Group I mGluR stimulation increases surface NMDARs in oocytes (54) | |
| PKC | |
| PKC potentiates NMDA currents but with complex effects (55) (reviewed in Ref. 56) | |
| PKC phosphorylation in the C1 cassette increases surface NR1 constructs (15) | |
| PKC moves NMDAR from synaptic to extrasynaptic sites in neurons (57) | |
| PSD-95 attenuates PKC effects in oocytes (58) | |
| Tyrosine phosphorylation | |
| Multiple effects of Src kinase on NMDARs have been recently reviewed (59) | |
| In striatum, tyrosine phosphorylation moves NMDARs into synaptosomes (60) | |
| Dephosphorylation of Tyr-842 in NR2A promotes NMDAR rundown (47) | |
| Tyrosine dephosphorylation causes extrasynaptic NMDAR rundown (37) | |

**Table I.** Modulators of NMDAR localization.

**Fig. 2.** Summary of trafficking and subunit composition of synaptic NMDARs. NMDARs assemble in the endoplasmic reticulum and associate with MAGUKs and the exocyst protein, Sec8 (28, 29). NMDAR transport may involve a kinesin motor that moves cargo along microtubules (26, 27). Synaptic receptors are directly or indirectly associated with several scaffolding proteins (inset) (22, 23). Mature and immature synapses differ in their relative amounts of NR2A and NR2B subunits (1, 8) as do synaptic versus extrasynaptic NMDARs (39, 40).

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brane. Two sites have been implicated in the clathrin-mediated internalization of NR2 subunits. The first is in the C terminus close to the last transmembrane domain (Tyr-842 in NR2A) with a similar motif present in all NR2 subunits as well as NR1. Dephosphorylation of this tyrosine leads to AP-2 binding and clathrin-mediated endocytosis (47). The second site is near the distal C terminus (Tyr-1472 of NR2B, with a similar motif present in NR2A). Constructs containing the NR2B C terminus are stabilized on the cell surface by loss of Tyr-1472, implying that an interaction with AP-2 is involved in receptor internalization (48). Interaction of the NR2 C terminus with PSD-95 also decreases internalization suggesting that stabilization of the receptor on the cell surface is another important function of the NMDAR/MAGUK interaction (48).

Conclusions

The importance of the NMDAR to synaptic function has been recognized for more than two decades but only recently have the tools been available to investigate the mechanisms that regulate the expression of functional receptors at the synapse. Events that appear distant from the ultimate function at the synapse, such as the assembly of subunits in the ER, are critical because the properties of the receptor are determined by the subunit composition. The trafficking of the receptors to the synapse and their organization at the synapse appear to depend on two major factors, phosphorylation of the receptor and its interaction with other proteins. As expected for a molecule playing a key role at the synapse, multiple interacting proteins are emerging, and the determination of how the various interactions with these proteins are coordinated lies ahead.

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