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COMMENTARY

Activation of excitatory glycine NMDA receptors: At the mercy of a whimsical GluN1 subunit

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Preview

Cell-to-cell communication in the nervous system depends on chemical or neurotransmitter-activated ion channels. *N*-methyl-D-aspartate receptors (NMDAR) are a prominent class of ligand-gated ion channels (Hansen et al., 2017) and are typically viewed as "glutamate-gated," since it is synaptic release of glutamate that often drives their activity. There is however another subclass of NMDARs that is composed of only glycine-binding GluN1 and GluN3 subunits (Chatterton et al., 2002; Perez-Otano et al., 2001). Like their glutamate-gated siblings, these glycine (or D-serine)-gated NMDARs are excitatory and are implicated in numerous brain functions and disorders (Bossi et al., 2022; Crawley et al., 2022; Larsen et al., 2011; Marco et al., 2013; Otsu et al., 2019). But glycine acts in an odd way. Binding of the "agonist" glycine to the GluN3 subunit activates the receptor, whereas its binding to GluN1 induces strong desensitization, blocking receptor function (Awobuluyi et al., 2007; Grand et al., 2018; Kvist et al., 2013). This odd action of glycine makes studying GluN1/N3 NMDARs in vitro and in vivo challenging. Pharmacological tools have helped to further our understanding, but again oddness pervades: some GluN1 competitive antagonists—but not all—prevent glycine binding and permit activation of GluN1/N3 receptors (Grand et al., 2018; Kvist et al., 2013). In this issue, using patch clamp electrophysiology and molecular dynamic simulations, Rouzbeh et al. (2023) help clarify the odd pharmacology of the GluN1 subunit in GluN1/N3 receptors, further highlighting that the properties of the GluN1 subunit depend on what other subunits it is associated with and laying the groundwork to develop tools to target this key subclass of NMDARs in the clinic.

There are seven NMDAR subunits encoded by distinct genes. These subunits come together to form tetramers that share a common topology (Fig. 1). Every NMDAR contains two glycine-binding GluN1 subunits. The other subunits can be two glutamate-binding GluN2(A–D; Fig. 1, left) or two glycine-binding GluN3(A–B) subunits (Fig. 1, right; Hansen et al., 2021). NMDARs that contain both a GluN2 and a GluN3 subunit have been suggested, but their structure, function, and expression remain elusive (Crawley et al., 2022). Numerous structural and functional studies have focused on GluN1/N2 NMDARs (Glasgow et al., 2015; Hansen et al., 2021). In GluN1/N2 NMDAR, glycine acts as a "co-agonist." Because of its high affinity, ambient glycine saturates the GluN1 glycine site in GluN1/N2 NMDARs, and the synaptic release of glutamate drives their activation. The activation of GluN2-containing NMDARs is also subject to a voltage-dependent blockade by extracellular Mg^{2+}, and they display a high Ca^{2+} permeability with channel opening (Fig. 1, left; Paoletti et al., 2013; Wollmuth 2018). On the other hand, GluN1/N3 receptors are essentially insensitive to Mg^{2+}, and while they are cation-selective and hence excitatory, they are poorly permeable to Ca^{2+} (Chatterton et al., 2002). GluN1/N3 receptors are predominantly extrasynaptic (Bossi et al., 2022; Crawley et al., 2022). Compared to GluN2-containing NMDARs, much less is known about the physiology and pharmacology of these excitatory glycine NMDARs. Given their critical role in brain function and disorders including being a key element of Huntington's disease (Marco et al., 2013), expanding the pharmacological toolkit for these receptors is essential.

To address the activation dynamics and pharmacology of GluN1/N3 receptors, Rouzbeh et al. (2023) studied two compounds: CGP-78608 and L-689,560. In terms of GluN1 pharmacology in GluN1/N2 receptors, these compounds are classified as "competitive antagonists" and hence block binding of glycine to the GluN1 agonist binding site. For GluN1/N3 receptors, CGP-78608 potentiates currents (Grand et al., 2018), an outcome that presumably reflects that it antagonizes glycine binding to GluN1, preventing its strong desensitization or inhibitory action (Fig. 2). Rouzbeh and colleagues confirm these general observations both in native and recombinant GluN1/N3. However, comparable experiments with L-689,560 highlight that it has a very different action: in contrast to CGP-78608, the

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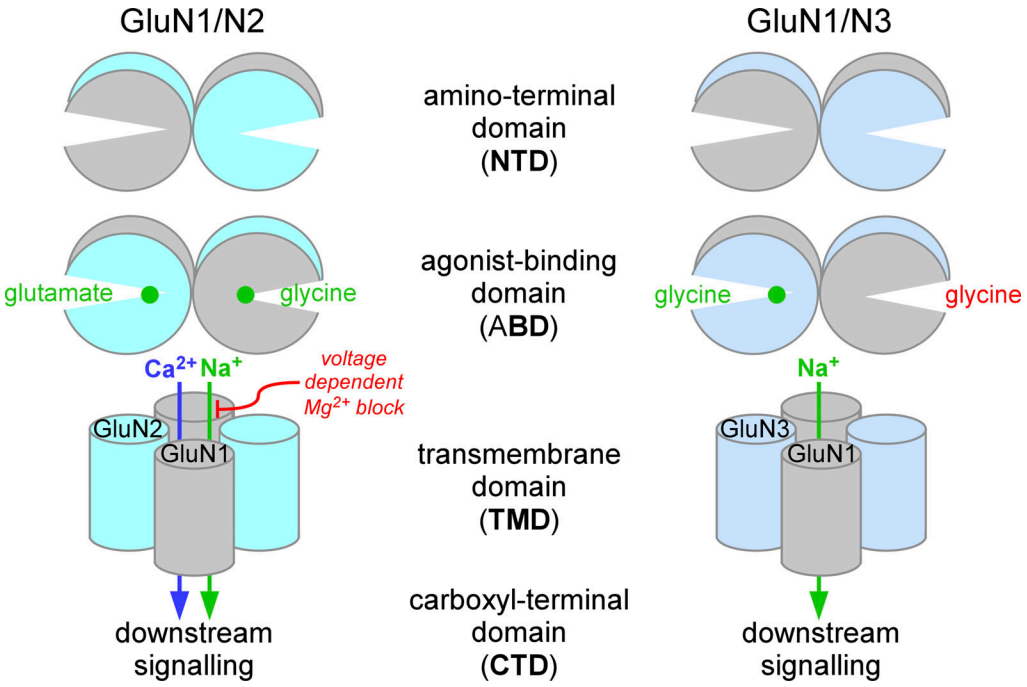

Figure 1. **Features of GluN1/N2 and GluN1/N3 NMDA receptors (NMDAR).** NMDARs function as tetramers with four domains in each subunit: extracellular amino-terminal (NTD) and agonist-binding (ABD) domains; transmembrane domain (TMD) forming the ion channel; and an intracellular carboxyl-terminal domain (CTD). The clamshell-like NTD and ABD regulate ion channel activity. Left: GluN1/N2 receptors. Glutamate (GluN2) and glycine (GluN1) binding are required for channel opening. Current flow is also regulated by a voltage-dependent block by extracellular Mg^{2+}. When open and at potentials near the resting membrane potential, GluN1/N2 receptors predominantly produce inward current carried by Na^+ and Ca^{2+} and hence are excitatory. Right: GluN1/N3 receptors are composed of only glycine-binding subunits and display an odd activation mechanism. Glycine binding to GluN3 by itself can activate the receptor whereas binding to GluN1 leads to strong desensitization and inhibition of receptor function. GluN1/N3 receptor mainly conduct inward Na^+.

competitive antagonist L-689,560 does not "awaken" or potentiate native GluN1/N3-mediated currents in hippocampal neurons and, in fact, has no discernable action. It is not inert, however, in that the potentiating actions of CGP-78608 are reversed by the co-application of L-689,560. Hence, there are two presumed competitive GluN1 antagonists with opposite actions on GluN1/N3 receptors. Again, the pharmacology of GluN1/N3 receptors is odd. Rouzbeh et al. (2023) ultimately provide a framework to understand these differences.

As an initial step to distinguish the actions of CGP-78608 and L-689,560, Rouzbeh and colleagues took advantage of mutations (F484A and T518L or FA and TL) in the GluN1 glycine binding site (i.e., the orthosteric site) that abolish glycine binding. Since these mutations remove glycine binding to GluN1, they too can permit robust activation of GluN1F^{FA+TL}/N3 receptors (Kvist et al., 2013). Surprisingly and in contrast to what is observed with CGP-78608, L-689,560 robustly inhibits GluN1F^{FA+TL}/N3 receptors-mediated currents. This is not what one would expect if L-689,560 acted as a pure competitive antagonist. Indeed, these experiments as well as others (e.g., Schild analysis and kinetic analysis of binding and unbinding of CGP-78608 and L-689,560) led the authors to conclude that while CGP-78608, L-689,560, and glycine overlap in terms of sites of action in GluN1, L-689,560 is not a competitive antagonist in GluN1/N3 receptors but rather a negative allosteric modulator.

The authors also identified an additional key point: both the identity of the ligand that occupies the GluN1 site, as well as mutations in this site, impact the efficacy and potency of glycine at the GluN3 site. Indeed, the authors demonstrated experimentally that the inhibition mediated by L-689,560 binding arises via an alteration in the glycine potency and efficacy which limits the activation of GluN1/N3 receptors at low (0.1 mM) but not at high (10 mM) glycine concentrations, in contrast to CGP-78608. These experimental results suggest a mechanism in which positive allosteric interactions exist between binding of CGP-78608 to GluN1 and glycine to GluN3A, whereas negative allosteric interactions exist between binding of L-689,560 to GluN1 and glycine to GluN3A (Fig. 2).

To provide additional mechanistic insights into their observations, the authors carried out a series of molecular dynamics simulations to study the movements of the GluN1 ABD clamshell in different binding states. The protein conformational landscapes of the simulations revealed that CGP-78608 binding promotes more open GluN1 ABD conformations with a broader energy basin, whereas L-689,560 binding promotes more closed ABD conformations. Together with functional data, it suggests that distinct conformations of the GluN1 ABD affect glycine binding at the GluN3 site: more open conformations in GluN1 promote glycine binding to GluN3 allowing the channel to fully awaken, while more closed conformations negatively affect agonist efficacy at the GluN3 site which leads to channel inhibition. Hence, the authors propose that GluN1 and GluN3 ABD use complex crosstalk between agonist binding sites, to allosterically modulate the receptor's function.

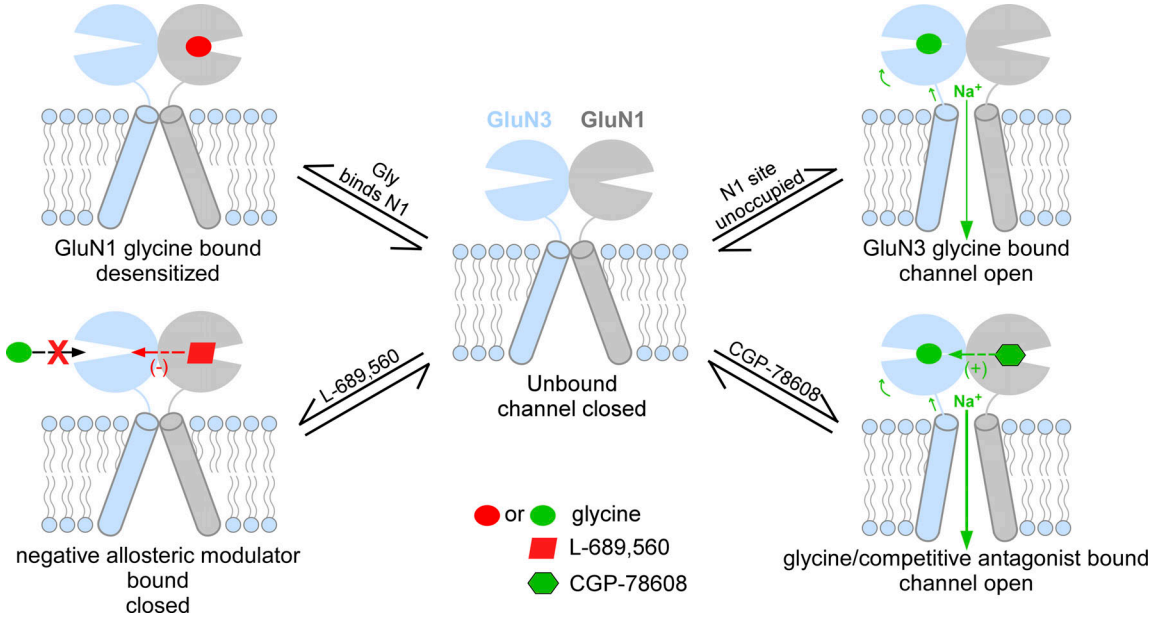

Figure 2. **GluN1 is a modulatory subunit in the GluN1/N3 complex.** Cartoons illustrating the modulatory action of GluN1 on GluN1/N3 receptor activation. Top left: Glycine binding to GluN1 induces desensitization and prevents channel opening. Bottom left: In the GluN1/N3 complex, L-689,560 acts as a negative allosteric modulator reducing the efficacy and potency of glycine binding to the GluN3 site. Top right: Glycine binding to GluN3 alone can induce channel opening. It may be glycine unbinding from the GluN1 site, with the GluN3 site occupied, that allows receptor activation. Bottom right: The competitive antagonist CGP-78608 prevents glycine binding to GluN1 but also enhances efficacy and potency of glycine binding to the GluN3 site, greatly enhancing current flow.

Conclusions

The results of Rouzbeh et al. (2023) provide insights into the structural basis of GluN1/N3 receptor function and may inform the design of new drugs targeting these receptors. Still, life seemed more straightforward with GluN1/N2 receptors. Sure, there is interaction between the GluN1 and GluN2 ligand-binding domains, but glycine and glutamate act as co-agonists. On the other hand, the interaction between the ligand-binding domains in GluN1/N3 receptors appears much stronger. Indeed, one of the major conclusions the authors arrive at is that the GluN1 ligand-binding site in the GluN1/N3 complex acts as a modulatory element (Fig. 2): (1) agonist binding here is not required for receptor activation; (2) mutations in this site affect agonist potency and efficacy in GluN3 subunit; and (3) ligand binding at this site—either the negative allosteric modulators (L-689,560) or the competitive antagonist (CGP-78608) can modulate agonist potency and efficacy in GluN3.

Future directions

The results presented in Rouzbeh et al. (2023) provide new insights into the pharmacology and mechanism of activation of GluN3-contaning receptors. However, many questions about GluN1/N3 receptors remain unanswered. Notably, how are the unique spatial interactions achieved between the orthosteric sites in the GluN1 and GluN3? Also, how are these conformational interactions propagated to the ion channel to drive receptor desensitization or channel opening? And what are the molecular determinants, in terms of the ABD, for the differential effects of glycine or other pharmaceutical compounds?

One major challenge to delineating subunit interactions and gating mechanism of GluN1/N3 NMDARs is the absence of any full-length structure of GluN3-containing NMDARs. At present we can only assume that the overall topology matches that of GluN1/N2 receptors (Fig. 1), yet the strong interaction between agonist-binding domains and the rapid and strong desensitization upon glycine binding to the GluN1 subunit—this process is very slow in GluN2-containing receptors—suggests there may be some key differences in the relationship between subunits and domains. Further, in terms of activation mechanism, GluN1/N3 receptors display features more akin to non-NMDARs than GluN2-containing NMDARs in that they can be activated by binding of agonist to only one or two subunits. In addition, zinc and protons potentiate GluN1/N3 function (Madry et al, 2008; Cummings and Popescu, 2016), but appear to use different mechanisms than those that mediate zinc and proton inhibition of GluN2-containing receptors. These observations raise questions on how exactly the structure supports a gating pattern unlike that in GluN1/N2 receptors and correspondingly how pharmacological tools might modulate this gating pattern. The experiments by Rouzbeh and colleagues provide new information about GluN1/N3 receptors and suggest mechanistic details that may eventually help resolve the pharmacology, function, and biological roles of GluN1/N3 in brain physiology.

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