Unique Biology and Single-Channel Properties of GluN2A- and GluN2C-Containing Triheteromeric N-Methyl-D-Aspartate Receptors

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Subhrajit Bhattacharya and Stephen F Traynelis

Department of Pharmacology, School of Medicine, Emory University, Atlanta, GA, USA.

ABSTRACT: Triheteromeric N-methyl-D-aspartate receptors (NMDARs) are assemblies of two different types of GluN2 subunits that endow receptors with properties distinct from their diheteromeric counterparts. Previous studies show an abundance of triheteromeric NMDARs across the central nervous system (CNS), making them an important receptor population to investigate and potential drug target. A recent study by Bhattacharya et al. (1) demonstrated the prevalence of GluN1/GluN2A/GluN2C triheteromeric NMDARs in cerebellar granule cells (CGCs), (2) suggested that GluN2C subunits seldom express as diheteromers, (3) suggested that GluN2A subunits are the preferred partners for GluN2C to functionally express at the cell surface, and (4) revealed unique single-channel properties of these triheteromeric assemblies, which may enable these cells to perform unique tasks. Taken together, this work demonstrates the physiological existence of GluN1/GluN2A/GluN2C receptors in the CGCs.

KEYWORDS: Triheteromeric NMDA receptors, GluN2C trafficking, cerebellum

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CORRESPONDING AUTHOR: Subhrajit Bhattacharya, Department of Pharmacology, School of Medicine, Emory University, 1510 Clifton Road NE, Atlanta, GA 30322, USA.
Email: sbhat40@emory.edu

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Commentary

A study by Bhattacharya et al[1] published in the journal Neuron has shown that the GluN2C subunit of the N-methyl-D-aspartate receptors (NMDARs) exists primarily in triheteromeric GluN1/GluN2A/GluN2C receptors. The study further reveals unique single-channel properties of triheteromeric GluN1/GluN2A/GluN2C receptors that might affect neuronal function.

NMDARs are part of the ionotropic glutamate receptor family that mediates a slow Ca^{2+}-permeable component of excitatory neurotransmission. Ca^{2+} permeability, channel conductance and voltage-dependent blockade by Mg^{2+} vary with the subunit composition, which is under complex regulation in the central nervous system (CNS).[2] Two GluN1 and GluN2 subunits make up the tetrameric NMDAR assembly. The GluN2 subunit, encoded by four different genes, endows the receptor with unique properties. NMDARs are involved in critical processes ranging from circuit development to memory formation.[2] Bhattacharya et al used a combination of electrophysiological and molecular approaches to study the subunit composition of NMDARs in cerebellar granule cells (CGCs). The authors also controlled subunit stoichiometry to explore the effects of co-expression of two different GluN2 subunits within the triheteromeric receptor complex, referred to as such because receptors are composed of three different subunits (eg, GluN1, GluN2A, and GluN2C). Several previous studies have suggested that native NMDARs form triheteromeric assemblies with two different GluN2 subunits.[3,4] Triheteromeric receptors show different functional and pharmacologic properties compared with their diheteromeric counterparts.[5-8] Bhattacharya et al provide direct physiological evidence of presence of GluN1/GluN2A/GluN2C receptors as the major population of NMDARs in CGCs. CGCs express both GluN2A and GluN2C subunits and show some properties consistent with GluN2C-containing NMDARs.[9-12] Although CGCs express GluN2A and GluN2C, NMDAR responses in cerebellar slices were not enhanced by a positive allosteric modulator that is selective for diheteromeric NMDARs containing two GluN2C subunits. This finding suggests that the most of the NMDARs on the cell surface contain only one GluN2C subunit. Utilizing an animal model that genetically lacked the *Grin2A* gene, the study suggested that receptors that contain two GluN2C subunits can be functionally expressed in the absence of GluN2A subunit, confirming the activity of the modulator on native receptors. However, these results do not provide information that would allow us to understand the mechanism underlying the exclusion of diheteromeric GluN2C-containing NMDARs from the functional population.

Trafficking

One potential explanation for the absence of diheteromeric GluN2C-containing NMDARs on the surface of CGCs is that GluN1/GluN2C receptors assemble, but do not traffic to the cell surface properly. Although speculative and unsupported by experimental evidence, this suggestion is consistent with decreased trafficking of GluN2C compared with GluN2A in other models and cultured CGCs.[13] So far, only a handful of studies have addressed the issue of differential regulation of GluN2C trafficking. Important cellular proteins like 14-3-3

and sorting nexin 27 have been shown to interact with GluN2C subunits and may regulate GluN2C trafficking.[14-16] It also seems possible that GluN2C contains additional regulatory elements in the form of phosphorylation sites or certain domains that restrict their surface expression. It is further possible that inclusion of GluN2A in the tetramer masks such elements.[13-15] Clearly, there is a need for experimental data to address this gap in existing knowledge regarding why diheteromeric GluN2C-containing NMDARs are not functionally expressed at the surface. Hence, further investigation of trafficking-control mechanisms is warranted to understand the role of trafficking of GluN1/GluN2A/GluN2C NMDARs.

Channel Properties

One important finding of this study is the elucidation of single-channel properties for triheteromeric GluN1/GluN2A/GluN2C NMDARs, which showed unique open times and complex multiple conductance levels that were distinct from diheteromeric receptors. Interestingly, open probability and mean open time of triheteromeric receptors were lower than GluN1/GluN2A/GluN2A, but similar to GluN1/GluN2C/GluN2C receptors, suggesting that the GluN2C subunit may have a dominant effect on the open probability. Furthermore, diheteromeric GluN1/GluN2A/GluN2A and triheteromeric GluN1/GluN2A/GluN2C channels showed prolonged bursts of open-period interspersed by long periods of close-times. However, there was no distinct prolonged closed time component in the GluN1/GluN2C/GluN2C channel recordings that separated open periods into bursts of openings. In addition, diheteromeric GluN1/GluN2C NMDARs showed two conductance levels, while triheteromeric GluN1/GluN2A/GluN2C receptors had three distinct conductance levels, raising the possibility that the distinct sub-conductance levels of the triheteromeric receptor are influenced by GluN2A and GluN2C subunits. Analysis of sub-level transitions in triheteromeric channel supports the idea that these three levels arise from the same receptor. These findings provide insight about the relative GluN2A and GluN2C subunit contribution toward pore properties of triheteromeric receptors. Overall, the work suggests that the triheteromeric receptors have burst properties similar to diheteromeric GluN2A-containing NMDARs. Triheteromeric receptors also have a low open probability and mean open time similar to GluN2C-NMDARs. These are in line with single-channel recordings from CGCs, which show conductance patterns similar to triheteromeric receptors.[17] Furthermore, single-channel conductance levels from CGCs obtained from *Grin2A*$^{-/-}$ mice may arise from GluN1/GluN2C receptors.[10] This is consistent with findings from Bhattacharya et al where a positive allosteric modulator sensitive to presence of two GluN2C subunits enhanced responses in *Grin2A*$^{-/-}$ neurons. Takahashi et al concluded that different GluN2 subunits form distinct NMDARs in CGCs and suggest that NMDAR responses are not exclusively restrained to diheteromeric assemblies in situ. This is consistent with demonstration of different channel properties in receptors that contain GluN1/GluN2A/GluN2A, GluN1/GluN2A/GluN2C, and GluN1/GluN2C/GluN2C.

Conclusions

Triheteromeric NMDARs have a distinct pharmacology.[1,4,6,8,18] GluN1/GluN2A/GluN2B triheteromers make up a significant portion of total NMDA receptors in hippocampus and cortex in adult animals.[4,19] GluN2A and GluN2C subunits are co-expressed across multiple areas such as spinal cord, and locus coeruleus, brainstem nuclei, hypothalamus, thalamus, and retinal ganglion cells.[1] Due to their wide distribution, it is important to understand triheteromeric receptor pharmacology for other GluN2-containing NMDAR combinations as well. GluN2B and GluN2D subunits are expressed in several areas like the hippocampal interneurons, striatal medium spiny neurons, substantia nigra pars compacta dopaminergic neurons, and the spinal cord.[3,20-22] Altered expression ratio of GluN2B and GluN2D subunits in the subthalamic nucleus and striatum has been implicated in neurodegenerative diseases including Parkinsonism.[23,24] Hence, future studies should be aimed at understanding additional triheteromeric receptors, such as GluN1/GluN2B/GluN2D triheteromeric complex and their contribution toward excitatory neurotransmission in the CNS. Ultimately, a comprehensive evaluation of pharmacology for all possible combinations of subunit will be needed if we are to develop new therapeutics that specifically target receptor dysfunction in a broad range of neurological and psychiatric disorders.

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Author Contributions

SB and SFT both wrote the commentary.

ORCID iD

Subhrajit Bhattacharya https://orcid.org/0000-0003-1326-0699

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