Journal Club

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A Prominent Role for Triheteromeric GluN1/GluN2A/GluN2B NMDARs at Central Synapses

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Review of Tovar et al.

NMDA receptors (NMDARs) are a major subclass of ionotropic glutamate receptors at excitatory synapses in the brain. These receptors are critical for activity-dependent synaptic plasticity and therefore play an important role in network development and information storage in the brain. NMDARs are tetrameric assemblies composed of two obligatory GluN1 subunits together with varying combinations of GluN2(A-D) and GluN3(A-B) subunits. The expression of GluN2 and GluN3 subunits is tightly regulated during development and varies across brain regions, leading to the formation of NMDARs with distinct subunit composition and thus with distinct biophysical and pharmacological properties (Paoletti et al., 2013). The predominant expression of GluN2A and GluN2B subunits in cortical and hippocampal pyramidal neurons, for example, leads to the formation of both diheteromeric (GluN1/GluN2A, "A-type" and GluN1/GluN2B, "B-type") and triheteromeric (GluN1/GluN2A/GluN2B, "AB-type") NMDARs (Tovar and Westbrook, 1999). Whereas the biophysical and pharmacological properties of diheteromeric A-type and B-type NMDARs are well characterized, very little is known about triheteromeric AB-type NMDARs. Nonetheless, recent evidence indicates that AB-type receptors are likely the predominant NMDAR subtype at synapses of the adult hippocampus (Rauner and Kohr, 2011), and may contribute to synaptic responses in other cortical and subcortical regions (Delaney et al., 2013). Because NMDARs are implicated in a variety of neuropsychiatric disorders such as schizophrenia, addiction and stroke, it is important to develop a comprehensive understanding of the function and distribution of different NMDAR subtypes, including triheteromeric AB-type NMDARs, to design novel and effective therapeutic strategies for these debilitating diseases (Paoletti et al., 2013).

Biophysical properties such as deactivation kinetics and probability of channel opening have long been used to determine NMDAR subunit composition in neurons. Studies based on these properties have led to several models of subunit-dependent NMDAR function both during normal synaptic transmission and in pathological states (Paoletti et al., 2013). However, interpretation of these data are complicated by the unknown properties of AB-type NMDARs, since limitations in experimental strategies have rendered these NMDARs notoriously difficult to study. Although AB-type NMDARs retain sensitivity to zinc and ifenprodil, which bind with high affinity to GluN2A and GluN2B subunits respectively, these antagonists fail to produce maximal inhibition of AB-type receptors even when administered simultaneously (Hatton and Paoletti, 2005). Moreover, the isolation of triheteromeric NMDAR currents in heterologous systems has relied on subunit mutagenesis, which alters their biophysical properties (Hatton and Paoletti, 2005). Therefore, a method to isolate native AB-type NMDAR currents in neuronal preparations is necessary to better understand their intrinsic kinetic properties and roles in synaptic transmission.

In a recent issue of *The Journal of Neuroscience*, Tovar et al. (2013) provide the first biophysical characterization of triheteromeric AB-type NMDARs in an intact neuronal culture preparation (Tovar et al., 2013). Autaptic hippocampal synapses were repetitively stimulated in the presence of two antagonists: (1) NVP-AAM007 (NVP), a competitive and reversible antagonist that displays approximately tenfold greater selectivity for A-type over B-type NMDARs (Neyton and Paoletti, 2006); and (2) MK-801, a use-dependent and irreversible NMDAR open-channel blocker. Using this approach, B-type receptors, along with some proportion of AB-type receptors, were irreversibly blocked by MK-801. Those sensitive to NVP (i.e., A-type and some fraction of AB-type), however, were reversibly blocked and recovered from inhibition following washout of both antagonists. A lower dose of NVP was then applied, sufficient to block >80% of A-type receptors (Tovar et al. 2013, their Fig. 3A), leaving a

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residual EPSC dominated by AB-type NMDARs (Tovar et al., 2013; their Fig. 6*A*). The AB-type receptors exhibited deactivation kinetics that more closely resembled that of fast A-type receptors than of the long-decaying B-type receptors (Tovar et al., 2013; their Table 1). These results imply that the presence of a single GluN2A subunit in the triheteromeric configuration is sufficient to accelerate the kinetics of NMDAR deactivation by hundreds of milliseconds. Interestingly, AB-type NMDARs display kinetics leading to the open states that are similar to that of B-type receptors (Tovar et al., 2013; their Table 1). Thus, AB-type NMDARs exhibit qualities of both diheteromeric A- and B-type NMDARs.

The results of Tovar et al. (2013) clarify our understanding of synaptic NMDAR function and help to resolve a long-standing inconsistency in the literature of NMDAR pharmacology. The GluN2B-selective antagonist ifenprodil and its derivatives have been widely used to characterize the "GluN2B-fraction" of synaptic NMDAR EPSCs. However, while some reports demonstrate that ifenprodil alters both the amplitude and deactivation kinetics of NMDAR currents (Bellone and Nicoll, 2007; Wang et al., 2008; de Marchena et al., 2008), others have shown that the change in amplitude by ifenprodil is not accompanied by a change in kinetics (Gray et al., 2011). Importantly, Tovar et al. convincingly demonstrate that ifenprodil reduces the amplitude but does not alter the kinetics of AB-type NMDAR EPSCs (Tovar et al., 2013; their Fig. 7*A*). Collectively, these results suggest that the acceleration of NMDAR deactivation kinetics by ifenprodil likely reflects the presence of a high proportion of diheteromeric B-type NMDARs. Consistent with this notion, ifenprodil significantly reduces the amplitude and accelerates the kinetics of NMDAR EPSCs from synapses in early developing hippocampal circuits, which are known to express a high proportion of B-type receptors (Bellone and Nicoll, 2007; de Marchena et al., 2008; Gray et al., 2011). Furthermore, NMDAR EPSCs from pyramidal neurons in the adult prefrontal cortex, which maintain a high proportion of B-type receptors into adulthood, also display ifenprodil-accelerated kinetics (Wang et al., 2008). Finally, ifenprodil reduces the amplitude of NMDAR EPSCs from mature neurons, without altering their kinetics (Bellone and Nicoll, 2007; Gray et al., 2011), in line with the developmental reduction of B-type NMDARs at synapses. Together, these results support the notion that AB-type NMDARs underlie a large fraction of NMDAR EPSCs at mature hippocampal synapses (Rauner and Kohr, 2011; Tovar et al., 2013) and further suggest that the classic NMDAR subunit switch during development may not reflect a B-type to A-type replacement per se, but rather a change from B-type to predominately AB-type. Furthermore, these data highlight that changes in both amplitude and kinetics of NMDAR currents should be examined when using ifenprodil to more accurately determine the contribution of B-type and AB-type receptors. It is necessary to note here that the efficacy of ifenprodil is highly dependent on extracellular Mg^{2+}, with maximal inhibition achieved under Mg^{2+}-free conditions (Rauner and Kohr, 2011). This important detail should be considered in the design of future experiments using ifenprodil and its derivatives, as well as in the interpretation of previous results.

The hybrid nature of AB-type NMDARs, which exhibit features of both A-type and B-type NMDAR function, raises interesting possibilities regarding their role in synaptic transmission and plasticity. First, NMDAR kinetics can powerfully influence the temporal characteristics of synaptic integration (Paoletti et al., 2013). Long-decaying B-type NMDARs extend, whereas GluN2A-containing NMDARs shorten, the temporal window for synaptic integration and the initiation of regenerative dendritic and NMDA spikes (Larkum et al., 2009). The relatively rapid decay kinetics of AB-type receptors can thereby enhance the temporal precision of coincidence detection and stimulus representation during fast excitatory neurotransmission. Furthermore, GluN2A and GluN2B subunits, although very similar in structure, exhibit key differences in amino-acid sequence of their intracellular C-terminal tails. These regions are sites of interaction with intracellular scaffolds and distinct downstream effector molecules, thereby conferring specific signaling properties to GluN2A- and GluN2B-containing NMDARs (Paoletti et al., 2013). For instance, the preferential interaction between GluN2B subunits and calcium/calmodulin-dependent kinase II (CaMKII) is critical for the expression of long-term potentiation (Barria and Malinow, 2005). Interestingly, during the so-called "critical period" in early cortical development, neurons more readily express synaptic plasticity compared with mature neurons (Crair and Malenka, 1995). Since B-type NMDARs predominate during early development and preferentially associate with plasticity-related machinery, the reduction of synaptic GluN2B-containing NMDARs is generally thought to underlie this developmental change in synaptic plasticity (Paoletti et al., 2013). By virtue of their GluN2B subunit, the expression of AB-type NMDARs may help to explain why mature synapses generally retain some degree of ifenprodil sensitivity and synaptic plasticity. Future studies will likely shed light on these intriguing possibilities.

Because of its relatively poor subunit-selectivity compared with GluN2B-selective antagonists, the use of NVP to distinguish GluN2A- from GluN2B-containing NMDARs has been challenged (Neyton and Paoletti, 2006). Tovar et al. (2013) have demonstrated the utility of this GluN2A-selective antagonist in a series of well designed pharmacological experiments to isolate AB-type NMDARs and have provided important insight into their intrinsic biophysical properties (Tovar et al., 2013). Based on these data, we have outlined a potential procedure for determining the relative contributions of NMDAR subtypes to central synapse function (i.e., by examining the effects of ifenprodil on both amplitude and kinetics of NMDAR EPSCs). Although NMDARs are implicated in several neuropsychiatric disorders, with subunit-composition hypothesized to play a critical role in their pathogenesis, several subunit-selective NMDAR-based clinical therapies have largely failed (Paoletti et al., 2013). It is tempting to speculate that these clinical shortcomings resulted in part from complexities associated with the presence of AB-type NMDARs, in light of the prominent role these receptors play in synaptic transmission (Rauner and Kohr, 2011; Tovar et al., 2013). The coming years will undoubtedly witness refinements in our understanding of both the function and subcellular distribution of distinct NMDAR subtypes, and perhaps the development of novel therapeutic strategies that accommodate for the enigmatic AB-type NMDARs.

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