Neuropharmacology 112 (2017) 29–33

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Modulating synaptic NMDA receptors

Kenneth R. Tovar**, Gary L. Westbrook*

Vollum Institute, Oregon Health & Science University, Portland, OR 97239, United States

ARTICLE INFO

Article history:
Received 17 August 2016
Accepted 17 August 2016
Available online 23 August 2016

Keywords:
glutamate receptors
Neurons
Synapses

ABSTRACT

Recent structural information on ligand-gated glutamate receptors and newly-discovered clinical uses for NMDA receptor antagonists has renewed interest in understanding the mechanisms of drug action at these receptors. Although the voltage-dependence and calcium permeability of NMDA receptors are well-studied, the mechanisms affecting the time course of synaptic NMDA receptor activation may be of more therapeutic value by serving as a rheostat for the total synaptic response. The NMDA receptor-mediated EPSC time course has been thought of as a fixed parameter based simply on receptor subunit composition as variably constrained by anatomical and developmental expression patterns, albeit subject to modification by kinetic behaviors such as modal gating. However, the EPSC time course also can be manipulated by endogenous and exogenous ligands. In this commentary we discuss insights into the *in situ* composition and kinetic behavior of synaptic NMDA receptors and propose new opportunities to target modulatory sites on NMDA receptors and to develop useful therapeutics. The emerging data on the atomic structure of NMDA receptors and knowledge of the kinetics of native receptors in neurons provide a roadmap in this regard.

This article is part of the Special Issue entitled 'Ionotropic glutamate receptors'.

1. The evidence for modulation of NMDA receptors

Drugs that target ligand- or voltage-gated ion channels of excitable cells have long been used to understand how channels work. Our understanding of neuronal excitability and synaptic transmission has relied on drugs that targeted voltage-gated ion channels such as tetrodotoxin and tetramethylammonium. Likewise, drugs such as barbiturates and benzodiazepines acting on $GABA_A$ receptors prolong inhibitory postsynaptic currents (IPSCs), resulting in their efficacy as anti-convulsants and anxiolytics. As the primary mediators of excitatory synaptic transmission, ionotropic glutamate receptors in the central nervous system are the targets for a long list of agonists and antagonists. This work began with the studies of Jeff Watkins and colleagues in the early 1980s (Evans et al., 1982) and has continued in the years since the cloning of glutamate receptor subunits in the early 1990s (reviewed in Traynelis et al., 2010). NMDA receptors were considered an especially promising therapeutic target because of their unique role in synaptic transmission and plasticity, and as mediators of the after-effects of hyperexcitability in neurological diseases (Lau and Tymianski, 2010; Zhou and Sheng, 2013). As a result, considerable drug discovery efforts resulted in many potent NMDA receptor antagonists. However, the use of these competitive antagonists and channel blockers has been thwarted by undesirable side effects or therapeutic ineffectiveness. Perhaps surprisingly, relatively low efficacy NMDA receptor antagonists have found use for symptomatic treatment of Alzheimer's disease (memantine), and the dissociative anesthetic ketamine is coming into use as an acute-acting antidepressant (reviewed in Johnson et al., 2015; Lodge and Mercier, 2015; Monteggia and Zarate, 2015). Interestingly, recent results show that the time course of the NMDA receptor activation can be manipulated by endogenous and exogenous ligands. Taking advantage of this opportunity for modulation of NMDA receptor function requires consideration of subunit composition as well as the kinetic (i.e. non-equilbrium) behavior of native receptors - the topics we highlight in this commentary.

NMDA receptors are non-selective cation channels that flux calcium and are blocked by extracellular magnesium, resulting in extrinsic voltage sensitivity. A less well-appreciated aspect of NMDA receptor function is the long-lasting duration of the synaptic response that can vary by 2–3 orders of magnitude following neurotransmitter binding. The duration has important implications for signaling imposed by these kinetic parameters. Functional NMDA receptors are multimeric proteins and their constituent

* Corresponding author.
** Corresponding author. Neuroscience Research Institute, University of California, Santa Barbara, CA 93106-5060, United States.
E-mail addresses: Ken.tovar@lifesci.ucsb.edu (K.R. Tovar), westbroo@ohsu.edu (G.L. Westbrook).

http://dx.doi.org/10.1016/j.neuropharm.2016.08.023
0028-3908/

subunits confer unique pharmacological, kinetic and gating properties (Moriyoshi et al., 1991; Kutsuwada et al., 1992; Meguro et al., 1992; Monyer et al., 1992, 1994; Vicini et al., 1998). Cloning of NMDA receptor subunits also revealed that their expression patterns were regulated anatomically and developmentally, hinting that multiple NMDA receptor subtypes exist *in vivo*. Subsequent work confirmed the existence of multiple synaptic NMDA receptor subtypes with distinct functional properties and anatomical distribution (Cull-Candy and Leszkiewicz, 2004). These discoveries led to the idea that the molecular heterogeneity of NMDA receptors could be used to develop modulatory ligands targeting specific combinations of subunits or in specific neuronal populations. This attractive idea is yet to be fully realized, in part because of a lack of knowledge of the native NMDA receptor composition.

2. What are the native receptors?

Understanding the patterns of expression and their impact on NMDA receptor function is complex. The seven NMDA receptor subunits are grouped into three families; GluN1, GluN2 (A- D) and GluN3 (A, B). The rules of association of NMDA receptor subunits were worked out in heterologous expression systems. From these studies we know that functional receptors are composed of GluN1 and one or more of the GluN2 subunits. Receptors are tetramers (Clements and Westbrook, 1991; Laube et al., 1998; Furukawa et al., 2005) with two GluN1 subunits per receptor (Behe et al., 1995). NMDA receptors are unique among ligand-gated channels in requiring two different agonists, glutamate and glycine (or serine), for activation (Johnson and Ascher, 1987; Forsythe et al., 1988). The GluN1 subunit binds glycine (Kuryatov et al., 1994) and the GluN2 subunits bind glutamate. Each unique GluN1-GluN2 pair introduced into heterologous expression systems produces receptors with distinctive pharmacological and kinetic properties (Kutsuwada et al., 1992; Vicini et al., 1998). Expressing GluN1 and either of the GluN3 subunits produces glycine-gated channels with strikingly different properties than neuronal NMDA receptors, including a lack of sensitivity to many common NMDA receptor ligands (Chatterton et al., 2002).

GluN1 is expressed throughout the brain, in almost all neurons and in some glia. GluN2A and GluN2B have overlapping expression in the cortex and hippocampus, with expression primarily, though not exclusively, in principal neurons (Monyer et al., 1994). GluN2C is expressed in much of the midbrain and cerebellum (Takahashi et al., 1996) and GluN2D is expressed embryonically, throughout brainstem and midbrain structures (Monyer et al., 1994) with expression into the adult in some cell types (Suárez et al., 2010). The GluN2C and GluN2D subunits have been the targets for development of drugs acting as allosteric modulators, but we will base our discussion here around what is known for GluN2A and GluN2B, which are expressed at high levels and with a broad distribution throughout the cortex and hippocampus. GluN2A is barely detectable embryonically whereas GluN2B expression is high and decreases slightly during the first few postnatal weeks as GluN2A expression increases, but many neurons co-express both subunits into adulthood (Monyer et al., 1994). Expression of more than one GluN2 subunit type in single neurons is likely the rule rather than the exception, complicating the characterization of native receptors. Indeed, not long after NMDA receptor subunits were cloned, receptor complexes containing two different GluN2 subunits were biochemically detected in cortical neurons (Sheng et al., 1994). In retrospect, this suggested that the properties of *in vivo* NMDA receptors are unlikely to be fully explained by the properties of heterologously expressed diheteromeric receptors.

Nonetheless, many fundamental insights into the basic properties of NMDA receptors have resulted from studies of recombinant receptors, of known subunit composition, expressed in heterologous systems. This strategy was also used to find and characterize receptor/ligand interactions. Because NMDA receptors are obligate heteromers, introduction of pairs of subunit types into heterologous expression systems produces a single diheteromeric receptor type whereas introduction of three subunit types could result in three receptor types (two di- and one triheteromeric). Results from the latter type of experiment are difficult to interpret because of the inability to control the receptors produced, a limitation that only recently has been overcome (Hansen et al., 2014; Stroebel et al., 2014). In neurons, NMDA receptor subunit expression is controlled anatomically and developmentally and functional proteins are assembled and processed to synaptic sites in a non-random manner (Kumar and Huguenard, 2003; Tovar et al., 2013). Importantly, recent experiments on native receptors has revealed that triheteromeric NMDA receptors are the major contributor to excitatory postsynaptic currents (EPSCs) in hippocampal neurons (Rauner and Köhr, 2011; Gray et al., 2011; Tovar et al., 2013). Moreover, isolated triheteromeric synaptic NMDA receptors, which until then were not experimentally accessible, have novel kinetic and pharmacological properties (Tovar et al., 2013).

3. Modifying the NMDA receptor synaptic time course

The trigger of course for NMDA receptor activation at synapses is the time course of glutamate in the synaptic cleft. However, the glutamate transient is quite brief, rising to a peak of around 1 mM and decaying with a predominant time constant of a millisecond (Clements, 1996). Although the response associated with synaptic activation of colocalized AMPA receptors is also brief (Hestrin, 1993; Jonas and Spruston, 1994), the time course of synaptic NMDA receptor activation is orders of magnitude slower with rise times ranging from 5 to 15 ms and deactivation time constants that can be hundreds of milliseconds or longer (Lester et al., 1990; Hestrin et al., 1990; Gray et al., 2011). Pulsatile neurotransmitter release emphasizes the importance of the non-equilibrium behavior of NMDA receptor activation. Because subunit composition controls this kinetic behavior, subunit composition also governs the time course of the NMDA receptor EPSC. This characteristic is more than a biophysical curiosity as the long time course is critical to synaptic function by acting as a memory device that associates a fast presynaptic signal with a much longer postsynaptic signal. This long duration can govern signaling events ranging from computation at single synapses in the olfactory bulb (Schoppa and Westbrook, 1999) to network dynamics underlying working memory in prefrontal cortex (Wang, 2001). Natural stimulus patterns from cells that are presynaptic to hippocampal pyramidal neurons (Dobrunz and Stevens, 1999) often occur at frequencies that produce overlap of synaptic NMDA receptor responses, creating an ongoing NMDA receptor 'tone' that given their voltage-dependence can serve as a booster device.

The time course of the NMDA response for diheteromeric receptors has been thoroughly examined (Vicini et al., 1998). These experiments revealed that the deactivation in response to brief agonist applications is highly characteristic of the diherteromeric receptor type. The primary time constant (τ_D) of recombinant diheteromeric receptors varied from ~20 ms for A-type (GluN1/GluN2A) receptors to more than a second for D-type (GluN1/GluN2D) receptors (Vicini et al., 1998). These approaches have also been used to explore the microscopic kinetics of channel gating including modal behavior, which results from differences in transitions between fully occupied closed states (Popescu et al., 2004; Zhang et al., 2008). These approaches in heterologous expression systems have been invaluable in understanding the gating of different NMDA subunit combinations. However, because of the