Abstract

N-methyl-D-aspartate (NMDA) receptors are a subclass of glutamate receptors that play an essential role in mediating excitatory neurotransmission and synaptic plasticity in the mammalian central nervous system (CNS). The activation of NMDA receptors plays a key role in brain development and memory formation. Abnormal regulation of NMDA receptors plays a critical role in the etiology of many neuropsychiatric disorders. NMDA receptors form a heterotetrameric complex composed of GluN1, GluN2(A-D) and GluN3(A, B) subunits. The NMDA receptors surface expression is regulated at multiple levels including early processing (synthesis, subunit assembly, endoplasmic reticulum (ER) processing, intracellular trafficking to the cell surface), internalization, recycling and degradation. NMDA receptors are regulated by the availability of GluN subunits within the ER, the presence of ER retention and export signals, and posttranslational modifications including phosphorylation and palmitoylation. However, the role of *N*-glycosylation in regulating of NMDA receptor processing has not been studied in detail. The aim of this study was to clarify the mechanisms of regulation of surface expression and functional properties of NMDA receptors. We used a combination of molecular biology, microscopy, biochemistry and electrophysiology in heterologous (COS-7 and HEK293 cells) and native (cerebellar granule cells (CGC) and hippocampal cultures) expression systems, which allow us the complex view of the issue. Our results show that surface delivery of GluN1/GluN2C receptors is regulated by three distinct regions within the i) A2 segment of amino-terminal domain (ATD), ii) M3 transmembrane domain and iii) proximal part of C-terminal domain (CTD), including SLPSP motif. iv) We also found that NMDA receptors are released from the ER only when two asparagine residues in the GluN1 subunit (Asn-203 and Asn-368) are *N*-glycosylated. v) Furthemore, we found that removing *N*-glycans from native NMDA receptors altered the receptor affinity for glutamate. vi) Our experiments also identified 23 different types of lectins which associate with both GluN1 and GluN2B NMDA receptor subunits and altered their biophysical properties. vii) Finally we found, that 11 out of 12 predicted *N*-glycosylation sites in GluN1 subunit and 7 out of 7 *N*-glycosylation sites in GluN2B subunit are occupied by *N*-glycans.

Key words: glutamate receptor; cerebellar granule cells; HEK293 cells; ion channel; intracellular trafficking; endoplasmic reticulum; *N*-glycosylation; biochemistry; electrophysiology