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Regulation of extrasynaptic signaling by polysialylated NCAM: Impact for synaptic plasticity and cognitive functions

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ABSTRACT

The activation of synaptic *N*-methyl-D-aspartate-receptors (NMDARs) is crucial for induction of synaptic plasticity and supports cell survival, whereas activation of extrasynaptic NMDARs inhibits long-term potentiation and triggers neurodegeneration. A soluble polysialylated form of the neural cell adhesion molecule (polySia-NCAM) suppresses signaling through peri-/extrasynaptic GluN2B-containing NMDARs. Genetic or enzymatic manipulations blocking this mechanism result in impaired synaptic plasticity and learning, which could be repaired by reintroduction of polySia, or inhibition of either GluN1/GluN2B receptors or downstream signaling through RasGRF1 and p38 MAP kinase. Ectodomain shedding of NCAM, and hence generation of soluble NCAM, is controlled by metalloproteases of a disintegrin and metalloprotease (ADAM) family. As polySia-NCAM is predominantly associated with GABAergic interneurons in the prefrontal cortex, it is noteworthy that EphrinA5/EphA3-induced ADAM10 activity promotes polySia-NCAM shedding in these neurons. Thus, in addition to the well-known regulation of synaptic NMDARs by the secreted molecule Reelin, shed polySia-NCAM may restrain activation of extrasynaptic NMDARs. These data support a concept that GABAergic interneuron-derived extracellular proteins control the balance in synaptic/extrasynaptic NMDAR-mediated signaling in principal cells. Strikingly, dysregulation of Reelin or polySia expression is linked to schizophrenia. Thus, targeting of the GABAergic interneuron-principle cell communication and restoring the balance in synaptic/extrasynaptic NMDARs represent promising strategies for treatment of psychiatric diseases.

Contents

1. Introduction

In the developing central nervous system, cell adhesion molecules of the immunoglobulin superfamily (IgCAMs) are essential for the

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guidance of axonal growth cones to their cellular targets and for the formation of new synapses with a high temporal and spatial precision. Among IgCAMs are neural cell adhesion molecule (NCAM), neuroplastin, L1, close homolog of L1 (CHL1), Thy-1, telencephalin (TLCN), contactins, NrCAM, and others (reviewed in Dityatev et al., 2008). NCAM is a transmembrane glycoprotein that has been discovered as the first member of the IgCAMs superfamily more than 40 years ago (Jorgensen and Bock, 1974). In the central nervous system, it is expressed on the cell surface of both neurons and glia. NCAM is known to promote Ca^{2+}-independent cell-cell and cell-extracellular matrix (ECM) adhesion via homophilic and heterophilic interactions. During brain development, NCAM has been implicated in several essential events, such as neurite outgrowth and fasciculation, cell migration, proliferation, and synaptogenesis (Dityatev et al., 2000; Maness and Schachner, 2007). In the adult rodent brain, NCAM plays an important role in synaptic plasticity, learning, and memory (reviewed in Senkov et al., 2012). In humans, abnormalities in NCAM and polySia expression are associated with major psychiatric and neurodegenerative diseases, such as schizophrenia, bipolar disorder, depression, and Alzheimer's disease (reviewed in Brennaman and Maness, 2010; Hildebrandt and Dityatev, 2015).

Importantly, two complementary polysialyltransferases ST8SIA2 and ST8SIA4 add the unique glycan polysialic acid (polySia) to the extracellular domain of NCAM in a cell type-, age-, and activity-regulated fashion (reviewed in Hildebrandt and Dityatev, 2015; Schnaar et al., 2014). PolySia is a linear homopolymer composed of α2,8-glycosidically linked residues of sialic acid, which represents a negatively charged nine-carbon acidic monosaccharide. Notably, the number of sialic acid residues in the polySia chains can range from 8 to more than 90 residues, and attachment of polySia dramatically changes the properties of NCAM (Hildebrandt and Dityatev, 2015; Schnaar et al., 2014; Weinhold et al., 2005). NCAM is the major carrier of polySia in the mature nervous system, as indicated by the nearly complete loss of polySia in the brains of adult NCAM knockout mice (Cremer et al., 1994).

Here, we briefly review polySia-dependent and independent mechanisms of synaptic plasticity and put forward a view that shed polysialylated NCAM acts as an inhibitory signaling molecule in communication between GABAergic interneurons and extra-/perisynaptic domain of principal cells, which may represent a new transcellular signaling pathway important for cognitive functions.

2. The role of NCAM and polySia in synaptic plasticity in the adult brain

In 1994, a seminal study by Lüthi and colleagues showed that NMDAR-dependent long-term potentiation (LTP) in the CA1 subregion of the hippocampus is markedly reduced following injection of antibodies against NCAM (Luthi et al., 1994). Strikingly, mice with constitutive ablation of the *NCAM* gene (*NCAM*$^{-/-}$) show impaired LTP and long-term depression (LTD) in the CA1 subregion (Kochlamazashvili et al., 2010; Muller et al., 2000; Muller et al., 1996) and reduced LTP at mossy fibers-CA3 synapses in the hippocampus, which is consistent with abnormal lamination of mossy fibers in these mutants (Cremer et al., 1998). To distinguish the developmental effects of NCAM from those in the mature brain, transgenic mice conditionally deficient for NCAM (*NCAMff+*) have been generated (Bukalo et al., 2004). In *NCAMff+* mice, NCAM is ablated by a Cre-recombinase under the control of calcium-calmodulin-dependent kinase II (αCaMKII) promotor, which leads to reduced NCAM expression during late postnatal development. *NCAMff+* mice exhibit reduced LTP and LTD in the CA1 region but normal LTP in the hippocampal CA3 region, and this finding is in agreement with the normal lamination of mossy fibers in these knockout mice (Bukalo et al., 2004).

Several functional studies have shown that polySia carried by NCAM is involved in activity-induced synaptic plasticity in the hippocampus (Table 1). Acute removal of polySia by endosialidase N (endoN), an enzyme that specifically degrades polySia, leads to reduced levels of LTP and LTD in the CA1 region of the hippocampus *ex vivo* (Becker et al., 1996; Muller et al., 1996) and impaired spatial learning in the Morris water maze (Becker et al., 1996; Venero et al., 2006). Similarly, mice deficient in polysialyltransferase ST8SIA4 (*St8sia4*$^{-/-}$), the enzyme responsible for addition of polySia to NCAM in the mature brain, show impaired LTP and LTD at Schaffer collateral-CA1 synapses in hippocampal slices but normal LTP at mossy fiber-CA3 synapses (Eckhardt et al., 2000). Notably, reduced CA1 LTP in hippocampal slices from *NCAM*$^{-/-}$ mice can be rescued by injection of the extracellular domain of polySia-NCAM and soluble polySia but not by NCAM, supporting the view that polySia is the portion of polySia-NCAM that is necessary and sufficient for the induction of LTP in the CA1 subregion of the hippocampus (Senkov et al., 2006). Moreover, *in vivo* electrophysiological field recordings have shown decreased levels of LTP in the hippocampal dentate gyrus of urethane-anaesthetized *NCAM*$^{-/-}$ mice; however, both *St8sia2*$^{-/-}$ and *St8sia4*$^{-/-}$ mice exhibit normal LTP in this region (Stoenica et al., 2006). In the mPFC, enzymatic removal of polySia or deficiency in NCAM or ST8SIA4, but not of ST8SIA2, resulted in impaired LTP at synapses from layer 2/3 to layer 5 neurons (Varbanov et al., 2016).

Table 1
Effects of NCAM, polySia, and polysialyltransferases on synaptic plasticity in the hippocampus and prefrontal cortex. *NCAM*$^{-/-}$, *St8Sia2*$^{-/-}$, and *St8Sia4*$^{-/-}$ denote mice that are constitutively deficient in *NCAM*, *St8Sia2*, or *St8Sia4*, respectively. *NCAMff+* denotes mice that are conditionally deficient in NCAM in αCaMKII-expressing neurons. Abbreviations: CA, *Cornu Ammonis*; DG, dentate gyrus; Endo, endosialidase N or NF; mPFC, medial prefrontal cortex; LTP, long-term potentiation; LTD, long-term depression; ↓, impaired; =, normal; n.d., not determined. References are given in the text.

Form of synaptic plasticity	*NCAM*$^{-/-}$	*NCAMff+*	Endo	*St8sia4*$^{-/-}$	*St8sia2*$^{-/-}$
CA1 LTP	↓	↓	↓	↓	=
CA1 LTD	↓	↓	↓	↓	n.d.
CA3 LTP	↓	=	=	=	=
DG LTP	↓	n.d.	n.d.	=	=
mPFC LTP	↓	n.d.	↓	↓	=

Altogether, these findings indicate that polySia is required for LTP and LTD in the CA1 region as well as for LTP in the mPFC, whereas synaptic plasticity in the CA3 and dentate gyrus depends on the NCAM glycoprotein rather than on its associated glycan polySia (Table 1). A growing body of evidence suggests that polySia-NCAM might regulate LTP via modulation of NMDARs. Hence, in the next section, we introduce these receptors in details necessary to understand the mechanisms of their modulation by polySia and to discuss possible strategies for pharmacological compensation of polySia deficit.

3. Synaptic and extrasynaptic NMDA receptors

NMDARs show a pronounced diversity in their subunit composition, pharmacological properties, interaction partners, and subcellular localization (Paoletti et al., 2013). Seven different NMDAR subunits have been reported: the GluN1 subunit, four GluN2 subunits (GluN2A, GluN2B, GluN2C, and GluN2D), and two distinct GluN3 subunits (GluN3A and GluN3B). Importantly, functional NMDARs form heterotetramers that are composed of two obligatory GluN1 subunits in combination with two GluN2 subunits, or alternatively, one GluN2 and one GluN3 subunit (Monyer et al., 1992; Ulbrich and Isacoff, 2008).

Notably, the functional activation of NMDARs requires the simultaneous binding of its agonist glutamate and one of its two co-agonists, namely glycine (Forsythe et al., 1988; Johnson and Ascher, 1987) and D-serine (Henneberger et al., 2010; Mothet et al., 2000). The binding site for glutamate is located in the GluN2 subunit (Furukawa et al., 2005), whereas glycine and D-serine bind to the strychnine-insensitive NMDAR glycine binding site in the GluN1 subunit of NMDARs. The identity of the GluN2 subunit represents a determining factor for the