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Research Report

Glutamate receptor composition of the post-synaptic density is altered in genetic mouse models of NMDA receptor hypo- and hyperfunction

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ABSTRACT

The *N*-methyl-D-aspartate receptor (NMDAR) and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor (AMPAR) are ionotropic glutamate receptors responsible for excitatory neurotransmission in the brain. These excitatory synapses are found on dendritic spines, with the abundance of receptors concentrated at the postsynaptic density (PSD). We utilized two genetic mouse models, the serine racemase knockout (SR−/−) and the glycine transporter subtype 1 heterozygote mutant (GlyT1+/−), to determine how constitutive NMDAR hypo- and hyperfunction, respectively, affect the glutamate receptor composition of the PSD in the hippocampus and prefrontal cortex (PFC). Using cellular fractionation, we found that SR−/− mice had elevated protein levels of NR1 and NR2A NMDAR subunits specifically in the PSD-enriched fraction from the hippocampus, but not from the PFC. There were no changes in the amounts of AMPAR subunits (GluR1, GluR2), or PSD protein of 95 kDa (PSD95) in either brain region. GlyT1+/− mice also had elevated protein expression of NR1 and NR2A subunits in the PSD, as well as an increase in total protein. Moreover, GlyT1+/− mice had elevated amounts of GluR1 and GluR2 in the PSD, and higher total amounts of GluR1. Similar to SR−/− mice, there were no protein changes observed in the PFC. These findings illustrate the complexity of synaptic adaptation to altered NMDAR function.

1. Introduction

Ionotropic glutamate receptors (AMPA, kainate, and NMDA subtypes) serve as the mediators of excitatory glutamatergic signaling in the brain. AMPARs are the principal transducers of fast excitatory neurotransmission that regulate the strength of glutamatergic excitatory synapses. Most AMPARs exist as tetramers comprised of four glutamate receptor subunits, GluR1–4 (Derkach et al., 2007). Receptor subunit composition is brain region dependent and determines the functional properties of the channel, including Ca^{2+} permeability. While AMPARs are involved in rapid excitatory synaptic transmission and determine synaptic strength, NMDARs mediate a slower component of excitatory transmission and are critical postsynaptic mediators of activity-dependent synaptic plasticity (Lau and Zukin, 2007). Throughout most of the brain, the heterotetrameric receptor is composed of two NR1 subunits and two NR2 subunits, all of which contribute transmembrane domains to form the pore of the ion channel that is characterized by high Ca^{2+}

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permeability. The NR1 subunit has eight different splice variants, which may affect channel function. NR2 subunits may be expressed in four different forms (NR2A–D), each of which confers different biophysical and pharmacologic properties to the channel (Lynch and Guttmann, 2001). NMDARs are considered "molecular coincidence detectors" because activation requires postsynaptic depolarization (removal of Mg^{2+} block from the channel pore at resting membrane potential), and the binding of two agonists, glutamate, and either glycine or D-serine at the glycine modulatory site (GMS) (Tsien, 2000). The influx of Ca^{2+} through the NMDAR triggers a cascade of intracellular events that regulate many types of neuroplasticity (Greer and Greenberg, 2008; Wayman et al., 2008), including long-term potentiation (LTP), dendritic patterning, spine elaboration and synaptogenesis, of which the latter three are perturbed in schizophrenia and animal models of the disease (Ross et al., 2006).

Our laboratory has previously generated genetic models of both NMDAR hypofunction (serine racemase knockout (SR–/–) mouse) and hyperfunction (glycine transporter 1 heterozygote (GlyT1+/–) mouse). The SR enzyme converts L-serine to D-serine. As a result, SR–/– mice have >80% reduction in brain D-serine, exhibit reduced global NMDAR-mediated neurotransmission, and impair LTP at the Schaffer collateral-CA1 pyramidal neuron synapse (Basu et al., 2009). Pyramidal neurons in the medial prefrontal cortex (mPFC) of these mutants also have reduced dendritic arborization and spine density (Devito et al., 2011). GlyT1+/– mice have a 50% reduction in the expression and activity of the glycine high-affinity sodium-dependent transporter, GlyT1, which is expressed in brain with a distribution that is complementary to SR (Tsai et al., 2004). In the hippocampus of GlyT1+/– mice, the GMS is virtually fully occupied. In addition, whole-cell patch-clamp recordings in the CA1 region of the hippocampus demonstrate that NMDARs in GlyT1+/– mice display faster decay kinetics and suggest an increase in the contribution of NR2A, as compared to NR2B subunits, to synaptic glutamatergic neurotransmission (Martina et al., 2005).

In the brain, axonal processes may form synapses on neuronal cell bodies or other axons, but in most cases they form on dendrites. More than 90% of all dendritic excitatory synapses in the central nervous system are on dendritic spines (Fiala et al., 2002). The distal tip of these spine heads contains a PSD, an electron dense structure specialized for postsynaptic signaling and plasticity that consists of glutamate receptors, signaling molecules, and scaffolding proteins (Sheng and Hoogenraad, 2007). The PSD dynamically changes its structure and composition in response to synaptic activity (Sheng and Hoogenraad, 2007). Regulation of glutamate receptor composition in the PSD is complex and modulated at many levels. The number of NMDARs at the surface is determined by trafficking, as well as clathirin-mediated internalization and insertion, all of which can be influenced by alternate splicing and the phosphorylation state of receptor subunits (Lau and Zukin, 2007). Under basal conditions, AMPARs undergo constitutive recycling between synapses and the cytosol, but during LTP induction they are more actively recycled through an endosome pathway to enhance exocytosis. AMPAR subunit composition, phosphorylation state, PSD proteins (protein interacting kinase 1 (PICK1),

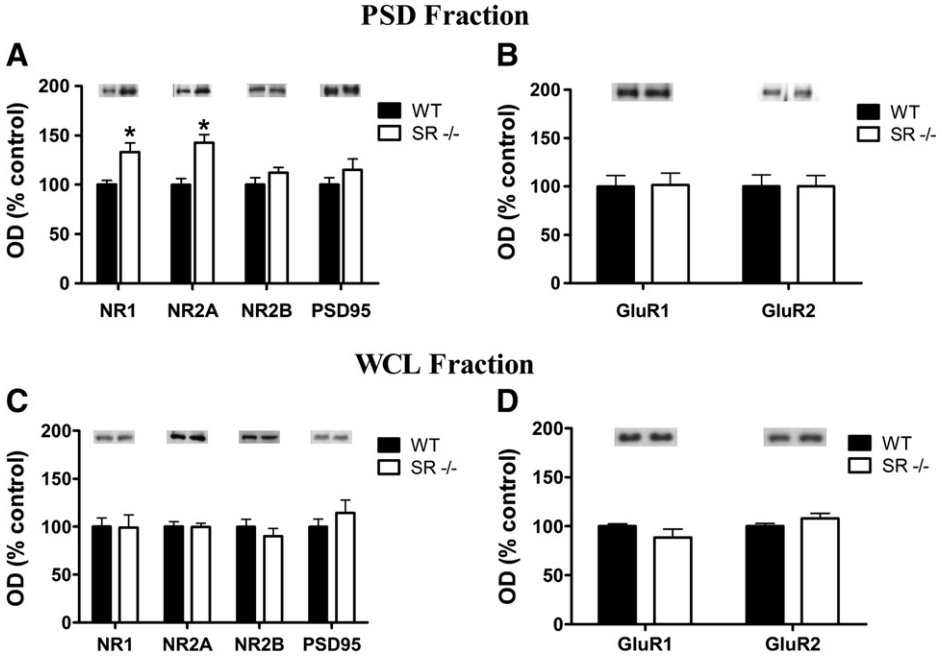

Fig. 1 – Altered hippocampal NMDAR expression selectively in the PSD of SR–/– mice. (A, B) Postsynaptic density enriched (PSD) and (C, D) whole cell lysate (WCL) hippocampal fractions were collected from adult wild type (WT; black bars; $n=6–12$) and mutant (SR–/–; white bars; $n=6–12$) mice. The amount of NMDAR subunits (NR1, NR2A, NR2B), postsynaptic density protein of 95 kDa (PSD95), and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor (AMPAR) subunits (GluR1, GluR2) were quantified by Western blot analysis. Values are expressed as the optical density (OD) normalized to WT values (%control). Bars represent mean values ± s.e.m. Asterisk (*) indicates that SR–/– differed significantly from WT ($p<0.05$).