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Research Report

Tyrosine phosphorylation of the *N*-methyl-D-aspartate receptor is enhanced in synaptic membrane fractions of the adult rat hippocampus

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Abstract

Hippocampal *N*-methyl-D-aspartate receptors (NMDARs) contribute to the expression of certain types of synaptic plasticity, such as long-term potentiation (LTP). It is well documented that tyrosine kinases increase NMDAR phosphorylation and potentiate NMDAR function. However, it is unclear how these phosphorylation changes result in enhanced NMDAR activity. We previously reported that NMDAR surface expression can be increased by LTP-inducing stimulation via tyrosine kinase-dependent mechanisms in the adult hippocampus [D.R. Grosshans, D.A. Clayton, S.J. Coultrap, M.D. Browning, Nat. Neurosci., 5 (2002) 27–33]. We therefore hypothesized that tyrosine phosphorylation of the NMDAR may enhance the trafficking of the receptor to the synaptic membrane. Here, we show that the stoichiometry of NR2A and NR2B tyrosine phosphorylation is significantly higher in synaptosomal membranes than intracellular microsomal/light membranes. Interestingly, NR2B tyrosine-1472, but not NR1 serine-896 or -897, phosphorylation is significantly higher in synaptosomal membranes than intracellular microsomal/light membranes. Furthermore, treatment of hippocampal slices with either a tyrosine phosphatase inhibitor or a tyrosine kinase inhibitor alters NMDAR tyrosine phosphorylation and produces a corresponding change in the concentration of NMDARs in the synaptosomal membrane fraction. Taken together, these data support the hypothesis that tyrosine phosphorylation may enhance NMDAR activity by increasing the number of NMDARs at the synaptic membrane.

Theme: Neurotransmitters, modulators, transportors, and receptors
Topic: Regional localization of receptors and transmitters

Keywords: NMDA receptor; Localization; Tyrosine phosphorylation; Serine phosphorylation; Tyrosine phosphatase inhibitor; Tyrosine kinase inhibitor; Sacrifice; Tyrosine-1472; Serine-896; Serine-897

1. Introduction

Long-term potentiation (LTP), a form of synaptic plasticity proposed as a mechanism underlying memory, is characterized by a persistent increase in the size of synaptic responses following repetitive stimulation [5]. At the hippocampal Schaffer collateral-CA1 synapse, two types of ionotropic glutamate receptors are primarily involved in LTP induction and maintenance: the *N*-methyl-D-aspartate receptor (NMDAR) and the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor (AMPAR) [5]. NMDAR activation is required for LTP induction at this synapse [12], whereas functional increases in AMPAR current constitute, for the most part, the increase in postsynaptic responses seen

Abbreviations: NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptor; LTP, long-term potentiation; bpV(phen), potassium bisperoxo(1,10-phenanthroline)oxovanadate(V); LavA, Lavendustin A; AMPAR, α-amino-3-hydroxy-5-methyl-4-isolxazole propionate receptor; CaMKII, calcium/calmodulin protein kinase II; aCSF, artificial cerebrospinal fluid; SDS, sodium dodecyl sulfate; LP1, synaptosomal membrane fraction; P3, intracellular microsomal/light membrane fraction; EPSP, excitatory postsynaptic potential; ER, endoplasmic reticulum

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with LTP [39]. Interestingly, the NMDAR requires two events for the channel to open and trigger LTP induction: ligand binding and membrane depolarization [2,5]. The ability of the NMDAR to act as a coincidence detector of these two events has contributed to the growing interest in the regulation of NMDARs in synaptic plasticity and even learning and memory.

Glutamate receptor trafficking between the plasma membrane and intracellular compartments has been proposed as a mechanism involved in LTP expression [5,7]. Previous work has shown that AMPARs move rapidly to the membrane in neonatal animals following LTP [22,55]. During this early stage in development, most synapses are 'silent synapses', or immature synapses containing NMDARs but lacking AMPARs [48]. Thus, the membrane insertion of AMPARs seen after LTP induction in neonatal animals may likely be responsible, at least in part, for the potentiated synaptic response that is observed [24]. Despite the fact that 'silent synapses' rarely exist in adult animals [42,48,50], relatively few studies have examined AMPAR and NMDAR movement following LTP induction in adults.

One study has suggested that NMDARs can be rapidly recruited to the membrane in mature rats following experience-dependent synaptic activity in the visual cortex [49]. Consistent with this hypothesis, we previously reported that LTP induction rapidly increases the surface expression of NMDARs, but not AMPARs, via tyrosine phosphorylation-dependent mechanisms in area CA1 of the adult hippocampus [20]. Interestingly, these findings highlight differential involvement of AMPARs and NMDARs in LTP induction between developing and adult animals. The data also suggest that tyrosine kinase activity is required for NMDAR membrane surface expression. While the precise mechanism(s) regulating NMDAR trafficking are still unclear, we have been very interested in the possibility that NMDAR surface expression may be mediated by tyrosine phosphorylation of the receptor itself.

Functional NMDARs are heteromeric assemblies that require an obligatory NR1 subunit in addition to one or more NR2 subunits [25]. The NR1 subunit is derived from a single gene that can exist as eight different variants based on alternative splicing [16]. Four separate genes have been cloned for the NR2 subunit (NR2A–D) [25,37], and in the hippocampus, the NR2A and NR2B subunits are the principal NR2 subunits that are expressed [38]. The intracellular C-terminal tails of the NR2A and NR2B subunits contain tyrosine residues that appear to be phosphorylated by tyrosine kinases, including the Src-family kinase Fyn [40,62,73]. Conversely, the NR1 intracellular C-terminus does not appear to be tyrosine phosphorylated [27] but can be phosphorylated on serine and threonine residues [63].

NMDAR function can be modulated by tyrosine kinases and phosphatases [8,35,67,68,74]; however, it is not known how this modulation is achieved. Several groups have provided evidence that phosphorylation of the NMDAR may regulate movement of the receptor in and out of the membrane [4,15,20,26,66]. For example, in striatal synaptosomes, dopamine D1 receptor activation leads to NMDAR movement to the membrane and this effect seems to be dependent on tyrosine phosphorylation [15]. Likewise, we reported that Src tyrosine kinases are required for the movement of NMDARs to the membrane after LTP induction in area CA1 of the adult hippocampus [20]. Consistent with these reports, Besshoh et al. (2005) recently showed that NR2A and NR2B tyrosine phosphorylation is positively correlated with an increase in the number of these receptors in the postsynaptic density of adult rats exposed to an ischemic challenge [4].

Additional studies provide support for the hypothesis that specific tyrosine residues are involved in NMDAR trafficking and function [28,29,51,66]. In particular, one study showed that endocytosis of NR2B-containing NMDARs in heterologous cells and dissociated neurons appears to be dependent on a consensus tyrosine-specific internalization motif (YXXØ) at the extreme C-terminus of the protein [51]. Binding of clathrin-associated endocytic protein complexes, including adaptor protein-2 (AP-2), to the NR2B subunit seems to require the tyrosine residue (Tyr1472) located within the YXXØ motif [28]. In agreement, recent work by Snyder and colleagues suggests that dephosphorylation of NR2B Tyr1472 correlates with amyloid-β-induced internalization of the NMDA receptor in dissociated cortical neurons [58]. Furthermore, mutation of tyrosine residue 842 on the NR2A subunit, proposed to be contained within a YXXØ motif, to phenylalanine stabilizes NR1/NR2A peak current decline, an effect that appears to be due to clathrin-mediated endocytosis of functional NR1/NR2A receptors, in HEK293 cells [66].

In the present study, our aim was to test the hypothesis that phosphorylation of the NMDAR influences its membrane surface expression in the hippocampus. Based on this hypothesis, we predicted that if tyrosine phosphorylation enhances NMDAR trafficking to the synapse, then synaptosomal membrane fractions would exhibit significantly greater relative stoichiometry of NR2A and NR2B tyrosine phosphorylation than would the intracellular microsomal/light membrane fractions. We also predicted that treatments such as tyrosine phosphatase and kinase inhibitors, which alter NMDAR tyrosine phosphorylation, would produce concomitant changes in NMDAR surface expression. The data presented in this report confirm these predictions and support the hypothesis that tyrosine phosphorylation of the NMDAR can influence the membrane surface expression of the NMDAR in the adult hippocampus.

2. Materials and methods

2.1. Materials

Rabbit polyclonal antibodies against the NR2A and NR2B subunits were generated in our laboratory and