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Differential regulation of AMPA receptor GluA1 phosphorylation at serine 831 and 845 associated with activation of NMDA receptor subpopulations

Heng Ai[1], Wei Yang[1], Mao Ye, Wen Lu, Li Yao, Jian-hong Luo*

Department of Neurobiology, Key Laboratory of Medical Neurobiology of Ministry of Health of China, Zhejiang Province Key Laboratory of Neurobiology, Zhejiang University School of Medicine, 388 Yu Hang-tang Road, Hangzhou, Zhejiang, 310058, China

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ABSTRACT

AMPA receptors and NMDA receptors are the main subtypes of ionotropic glutamate receptors in the vertebrate central nervous system. Accumulating evidence demonstrates that two serine sites, S831 and S845, on the AMPA receptor GluA1 subunit, are phosphorylation-regulated and profoundly involved in NMDA receptor-dependent synaptic plasticity. On the other hand, recent studies have revealed distinct functional consequences of activating synaptic or extrasynaptic NMDA receptors, or of activating GluN2A- or GluN2B-containing NMDA receptors. Therefore, it is essential to determine how phosphorylation of the GluA1 at S831 and S845 is regulated by NMDA receptor subpopulations. In this study, we demonstrated transiently increased phosphorylation of GluA1 at S831 and persistently decreased phosphorylation of GluA1 at S845 by bath application of NMDA to hippocampal slices from rats. Interestingly, we also found a differential regulation of phosphorylation of GluA1 at S831 and S845 by activation of NMDA receptor subpopulations: the synaptic and/or the GluN2A-containing NMDA receptors were more likely to mediate up-regulation of GluA1 phosphorylation at S831 and down-regulation of GluA1 phosphorylation at S845, while the extrasynaptic NMDA receptors down-regulated GluA1 phosphorylation at S831. Taken together, our results suggest the NMDA receptor subpopulations differentially regulate GluA1 phosphorylation, which may contribute to NMDA receptor-dependent synaptic plasticity.

AMPA receptors and NMDA receptors are the main subtypes of ionotropic glutamate receptors and play critical roles in synaptic plasticity [3]. AMPA receptors are tetrameric structures consisting of four homomeric or heteromeric subunits (GluA1-4) [21]. In contrast, NMDA receptors are heteromeric complexes assembled from GluN1 subunits and GluN2 and/or GluN3 subunits [8,19]. The GluN2A- and GluN2B-containing NMDA receptors have been intensively studied because of their significant differences in both function and expression in major brain regions [18].

Accumulating evidence demonstrates AMPA receptor function is regulated by subunit composition as well as phosphorylation [4]. For example, using site-directed mutagenesis and phosphopeptide mapping, two important sites of serine phosphorylation (S831 and S845) in the GluA1 C terminus have been identified [20]. S831 is phosphorylated by PKC and CaMKII, the phosphorylation of which potentiates the single-channel conductance of GluA1-containing AMPA receptors [6,5], while S845 is phosphorylated by PKA, which increases the open probability [1] and surface expression of the receptors [7]. The regulation of GluA1 phosphorylation, therefore, is an important mechanism underlying synaptic plasticity.

GluA1 is profoundly involved in NMDA receptor-dependent synaptic plasticity [15,24]. The induction of NMDA receptor-dependent LTP is accompanied by a persistent increase in phosphorylation of GluA1 at S831 [14]. Phosphorylation of S845 is down-regulated during LTD [13]. More recently, it was shown LTD is deficient in mice in which only S845 is mutated to alanine to prevent its phosphorylation [12]. A persistent dephosphorylation of GluA1 at S845, but not at S831, is found in the chemical LTD induced by a brief application of NMDA to hippocampal slices [11,13].

Recent studies have revealed distinct functional consequences of activation of synaptic or extrasynaptic NMDA receptors, or of activation of GluN2A- or GluN2B-containing NMDA receptors, though the results appear complicated and controversial [2,16]. Therefore, we address how GluA1 phosphorylation at S831 and S845 is regulated by activation of the NMDA receptor subpopulations.

In this study, hippocampal slices were exposed to NMDA in combination with other pharmacological reagents to activate NMDA receptors [10]. We found a differential regulation of GluA1 phosphorylation at S831 and S845 by activation of NMDA receptor subpopulations.

* Corresponding author. Tel.: +86 571 88208002; fax: +86 571 88208045.
E-mail address: luojianhong@zju.edu.cn (J.-h. Luo).
[1] These authors contributed equally to this work.

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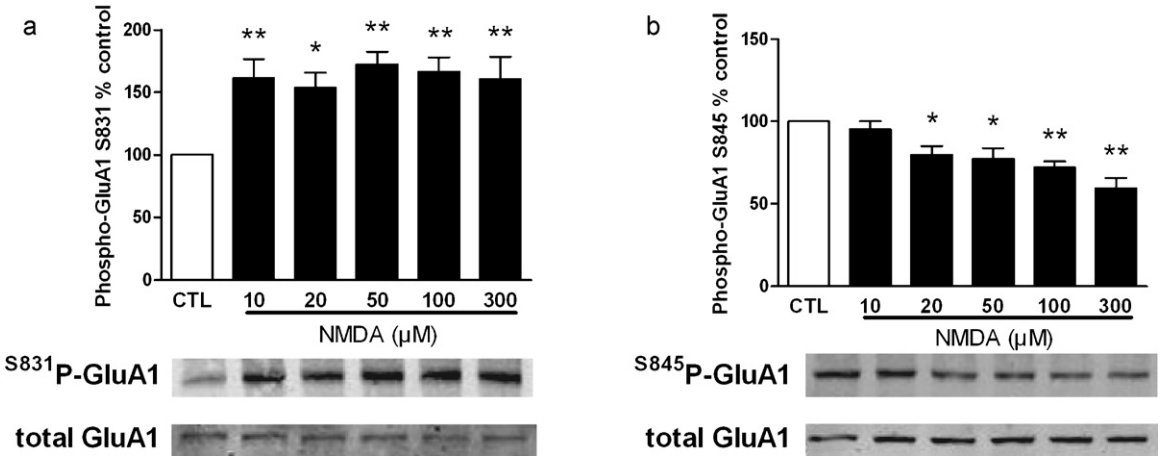

Fig. 1. Dose-related effects of NMDA on phosphorylation of GluA1 S831 and GluA1 S845 in hippocampal slices from rats. In the presence of TTX, CNQX, and nimodipine, different concentrations of NMDA were added, and then the phosphorylation levels of GluA1 S831 and S845 and the total GluA1 were analyzed by Western blotting. (a) Various concentrations of NMDA (10–300 μM, 3 min) increased the phosphorylation levels of S831 relative to total GluA1 ($n = 5$–6). (b) NMDA application (10–300 μM, 3 min) decreased the phosphorylation levels of S845 relative to total GluA1 ($n = 5$–6). Data presented as mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$ compared with control.

Monoclonal anti-GluA1 was from Santa Cruz (San Francisco, CA, USA). Both rabbit anti-phosphoGluA1 S831 and anti-phosphoGluA1 S845 were from Millipore Corp. (Billerica, MA, USA). The secondary antibodies, goat anti-mouse IgG Dylight™680 and goat anti-rabbit IgG Dylight™800, were from Thermo Electron Corp. (San Jose, CA, USA). All reagents were from Sigma (St. Louis, MO, USA) unless otherwise indicated.

All animal experiments were carried out in accordance with the ethical guidelines of the Zhejiang University Animal Experimentation Committee and were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Preparation of hippocampal slices was performed as described previously [24]. Four- to six-week-old male Sprague-Dawley rats were anesthetized with ether. The brain was immediately removed and placed in ice-cold oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 4 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 10 glucose, 1 KH_2PO_4, 25.7 $NaHCO_3$. Both hippocampi were carefully dissected out on a plate soaked with ice-cold oxygenated ACSF and unrolled on an agar block. Subsequently, 400 μm transverse slices were made in ice-cold oxygenated ACSF with a Vibratome 1000 Plus tissue chopper (St. Louis, MO, USA). The slices were transferred to a chamber perfused with 26 °C ACSF bubbled with a 95% O_2:5% CO_2 mix and equilibrated for at least 1 h before use.

The equilibrated hippocampal slices were transferred to 26 °C oxygenated ACSF with 1 μM TTX, 40 μM CNQX, 100 μM APV, and 5 μM nimodipine for 3 h [10]. Healthy hippocampal slices were simply screened by examining pyramidal neuron morphology under a microscope, and were then treated with NMDA in different conditions as described below.

The slices were incubated in various concentrations of NMDA (10–300 μM) in 26 °C oxygenated and Mg^{2+}-free ACSF with 1 μM TTX, 40 μM CNQX, and 5 μM nimodipine for 3 min to assess dose-related effects, or incubated in 3 μM NMDA in the above solution to assess time-course effects.

Extrasynaptic NMDA receptors were selectively activated by procedures described previously [10]. Briefly, after washing for 20 min after blockade in ACSF with 1 μM TTX, 40 μM CNQX, and 5 μM nimodipine for 3 h, the slices were transferred to 50 μM MK801 in 26 °C oxygenated ACSF containing 10 μM bicuculline and 5 μM nimodipine for 15 min. After rinsing in ACSF for at least 15 min to remove MK801, the slices were incubated in 20 μM NMDA in Mg^{2+}-free ACSF containing 1 μM TTX, 40 μM CNQX, and 5 μM nimodipine for 3 min.

To selectively inhibit the GluN2B-contaning NMDA receptors, the slices after blockade were treated with 20 μM NMDA in Mg^{2+}-free ACSF containing 1 μM TTX, 40 μM CNQX, 5 μM nimodipine, and 10 μM ifenprodil for 3 min.

Sample preparation was performed as described previously [17]. Briefly, slices were homogenized in ice-cold homogenization buffer (10 mM Tris–HCl buffer, pH 7.4, containing (in mM) 320 sucrose, 1 Na_3VO_4, 5 NaF, 1 EDTA and 1 EGTA). The tissue homogenate was centrifuged at 700 × *g* for 10 min at 4 °C. The supernatant was transferred to another tube and centrifuged at 37,000 × *g* at 4 °C for 40 min. The pellet, thought to be a crude membrane fraction, was resuspended in 10 mM Tris–HCl, pH 7.4. Then the same volume of 2× loading buffer (125 mM Tris–HCl, pH 6.8, 4% SDS, 10% dithiothreitol, 15% glycerol) was added to generate the samples. The samples were boiled for 5 min before SDS-PAGE and proteins were transferred to nitrocellulose membranes. The proteins were probed with specific primary antibody at 4 °C overnight, and detected by the secondary antibodies. The signal intensity in each blot was quantified using LI-COR/Odyssey (Li-Cor Biosciences, Lincoln, NE). Phospho-GluA1 protein signals were normalized to GluA1 protein signals.

Results from at least three independent experiments were averaged and differences were tested by one-way ANOVA, followed by the Bonferroni Test or Dunnett's Multiple Comparison Test. All data are represented as mean ± SEM. Statistical significance was determined as $^{*}p < 0.05$ or $^{**}p < 0.01$.

To define the direct effect of NMDA treatment on GluA1 phosphorylation, we pretreated hippocampal slices with TTX, CNQX, APV, and nimodipine for 3 h to minimize neuronal activity and maintain the slices in a basal condition before NMDA application. Some slices without any further treatment were collected as controls. Different concentrations of NMDA were added to the ACSF containing TTX, CNQX and nimodipine to stimulate the slices. The results showed the relative phosphorylation levels of GluA1 at S831 in all groups treated with NMDA were significantly increased (Fig. 1a). Compared to the control group, the relative levels of S831 phosphorylation for those treated with different concentrations of NMDA were 161.3 ± 15.3% for 10 μM NMDA ($p < 0.01$), 153.5 ± 12.2% for 20 μM ($p < 0.05$), 172.1 ± 10.5% for 50 μM ($p < 0.01$), 166.4 ± 11.7% for 100 μM ($p < 0.01$), and 160.5 ± 18.0% for 300 μM ($p < 0.01$). However, there was no significant difference among those treated with different concentrations of NMDA.