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Perspectvie

Roles of subunit phosphorylation in regulating glutamate receptor function

John Q. Wang [a,b,*], Ming-Lei Guo [a], Dao-Zhong Jin [a], Bing Xue [a], Eugene E. Fibuch [b], Li-Min Mao [a]

[a] Department of Basic Medical Science, School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, USA
[b] Department of Anesthesiology, School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, USA

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ABSTRACT

Protein phosphorylation is an important mechanism for regulating ionotropic glutamate receptors (iGluRs). Early studies have established that major iGluR subtypes, including α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors and *N*-methyl-D-aspartate (NMDA) receptors, are subject to phosphorylation. Multiple serine, threonine, and tyrosine residues predominantly within the C-terminal regions of AMPA receptor and NMDA receptor subunits have been identified as sensitive phosphorylation sites. These distinct sites undergo either constitutive phosphorylation or activity-dependent phosphorylation induced by changing cellular and synaptic inputs. An increasing number of synapse-enriched protein kinases have been found to phosphorylate iGluRs The common kinases include protein kinase A, protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase II, Src/Fyn non-receptor tyrosine kinases, and cyclin dependent kinase-5. Regulated phosphorylation plays a well-documented role in modulating the biochemical, biophysical, and functional properties of the receptor. In the future, identifying the precise mechanisms how phosphorylation regulates iGluR activities and finding the link between iGluR phosphorylation and the pathogenesis of various brain diseases, including psychiatric and neurodegenerative diseases, chronic pain, stroke, Alzheimer's disease and substance addiction, will be hot topics and could contribute to the development of novel pharmacotherapies, by targeting the defined phosphorylation process, for suppressing iGluR-related disorders.

1. Introduction

Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels and are classified into α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, *N*-methyl-D-aspartate (NMDA) receptors, and kainate receptors (Traynelis et al., 2010). These receptors become functional upon a homomeric and mainly heteromeric assembly of multiple subunits. AMPA receptors, for example, are assembled into a tetrameric structure composed of four subunits (GluA1–4, formerly known as GluR1–4), whereas NMDA receptor tetramers are composed of two obligatory GluN1 (or NR1) and two modulatory GluN2 (or NR2) subunits. All subunits share the similar conformation in the plasma membrane which includes three membrane-spanning domains (M1, M3, and M4), a hydrophobic hairpin domain (M2), an extracellular N-terminus, and an intracellular C-terminus (CT). Intracellular domains, including loop 1, loop 2 and mainly CT, are key zones for phosphorylation. Multiple serine, threonine, and tyrosine residues in the CT of AMPA receptor and NMDA receptor subunits have been identified as sensitive sites that are phosphorylated by a set of synapse-enriched protein kinases, including protein kinase A (PKA), protein kinase C (PKC), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), non-receptor tyrosine kinases (NRTK), and others (Mao et al., 2011; Lu and Roche, 2012; Sanz-Clemente et al., 2013b). Phosphorylation at a specific site is either largely constitutive or activity-dependent as a dynamic and reversible modification in nature. By regulating phosphorylation levels, protein kinases control the biochemistry, biophysics, and physiology of iGluRs, usually in a fashion associated with the concomitant modulation of synaptic plasticity. This perspective provides a brief overview on the role of phosphorylation in regulating iGluRs with a focus on recent progress, which is followed by a perspective on future studies linking phosphorylation biology of iGluRs to neurological disorders.

2. Phosphorylation of AMPA receptors

Reliable serine or threonine phosphorylation occurs in AMPA receptor subunit CT regions (Mao et al., 2011; Lu and Roche, 2012) (Fig. 1). The first set of phosphorylation sites identified include

* Corresponding author at: Department of Basic Medical Science, University of Missouri-Kansas City, School of Medicine, 2411 Holmes Street, Kansas City, Missouri 64108, USA. Tel.: +816 235 1786; fax: +816 235 5574.
E-mail address: wangjq@umkc.edu (J.Q. Wang).

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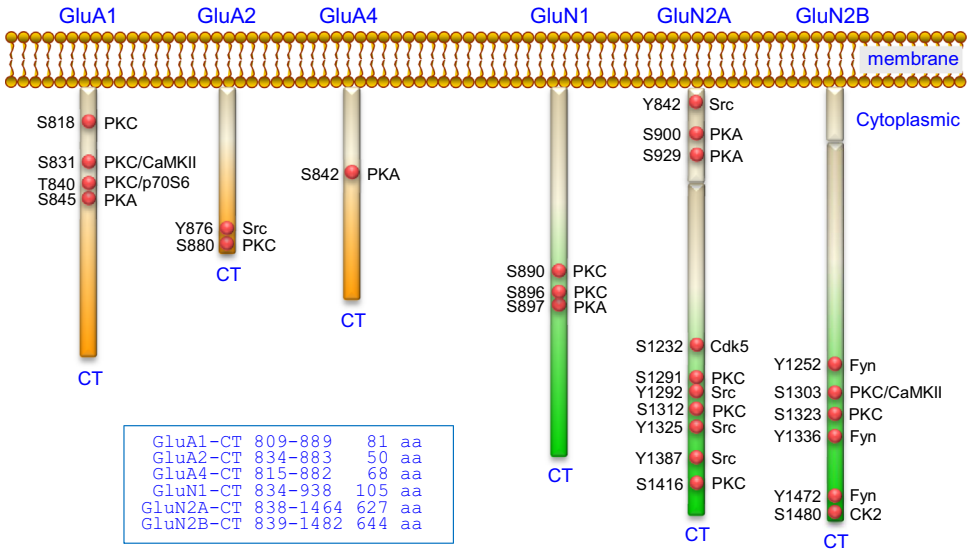

Fig. 1. Phosphorylation sites in the CT regions of AMPA receptor and NMDA receptor subunits. Multiple serine, threonine, and tyrosine phosphorylation sites have been identified in the CT regions of AMPA receptor subunits (GluA1, GluA2, and GluA4) and NMDA receptor subunits (GluN1, GluN2A, and GluN2B). The GluN2A CT and GluN2B CT are particularly large, containing 627 and 644 amino acids (aa), respectively. Most phosphorylation sites in the GluN2A/B CT are located in the distal segments.

serine 831 (S831) and S845 in GluA1 (Roche et al., 1996; Barria et al., 1997; Mammen et al., 1997). The former is phosphorylated by PKC and CaMKII, whereas the latter is phosphorylated by PKA. Additionally, GluA1 is phosphorylated at S818 by PKC (Boehm et al., 2006) and threonine 840 (T840) by PKC (Lee et al., 2007) and p70S6 kinase (Delgado et al., 2007). Other subunits are also subject to phosphorylation. GluA2 contains a PKC site (S880) (Matsuda et al., 1999; Chung et al., 2000) and GluA4 has a primary site (S842) sensitive to PKA and possibly other kinases (Carvalho et al., 1999). In addition to serine and threonine, phosphorylation occurs at tyrosine 876 (Y876) in GluA2 in response to Src NRTKs (Hayashi and Huganir, 2004).

Phosphorylation at these sites has a significant impact on AMPA receptors. Biochemically, phosphorylation regulates trafficking of modified subunits, resetting the number of the receptor among different subcellular/subsynaptic compartments. S845 is obviously a key site controlling GluA1 trafficking. Phosphorylation at this site consistently traffics receptors to extrasynaptic membranes and primes extrasynaptic receptors for synaptic insertion based on early and recent studies (Estaban et al., 2003; He et al., 2009; Incontro et al., 2013). Other phosphorylation sites, including GluA1 S818 and GluA4 S842, exert the same effect (Estaban et al., 2003; Boehm et al., 2006; Gomes et al., 2007). S818 phosphorylation was recently shown to achieve this effect by increasing the GluA1 interaction with a neuronal specific actin-binding protein 4.1N (Lin et al., 2009). In contrast to accelerated exocytosis with increased synaptic insertion of receptors, phosphorylation also enables endocytosis and reduces the abundance of synaptic receptors. In GluA2, the two major phosphorylation sites (Y876 and S880) are noticeably adjacent to the end of CT and overlap with the binding domain (880-SVKI) for PDZ domain-containing scaffold proteins, such as glutamate receptor interacting proteins 1 and 2 (GRIP1/2). Thus, enhanced phosphorylation at Y876 or S880 disrupted the association of GluA2 with GRIP1/2, thereby accelerating endocytosis of GluA2 and reducing the abundance of surface-expressed AMPA receptors (Matsuda et al., 1999, 2000; Chung et al., 2000; Seidenman et al., 2003; Hayashi and Huganir, 2004). However, complex of the role of GRIPs in regulating AMPA receptor trafficking is underscored by the finding that GRIP interactions with GluA2 were not required for surface expression of GluA2 in cultured hippocampal neurons (Braithwaite et al., 2002).

Phosphorylation also alters biophysical properties of AMPA receptor channels. An early study found that GluA1 S831 phosphorylation by CaMKII increased single channel conductance (Derkach et al., 1999). This effect was recently replicated in PKC-phosphorylated S831 (Jenkins and Travnelis, 2012). Moreover, the S831 regulation relies on coexpression of GluA1/A2 with transmembrane AMPA receptor regulatory proteins (TARPs) (Kristensen et al., 2011). S845 phosphorylation enhanced the channel open probability and the current peak (Roche et al., 1996; Banke et al., 2000). Recently, it was shown that adenosine A(2A) receptors seem to engage this PKA-S845 pathway to increase the availability of GluA1-containing AMPA receptors at extrasynaptic pools for synaptic insertion and augment AMPA currents in hippocampal neurons (Dias et al., 2012).

Synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD), is evidently regulated by phosphorylation according to a large number of previous and recent studies (Lee, 2006; Lu and Roche, 2012). The regulation is largely based on a metaplastic basis that AMPA receptors undergo activity-dependent and phosphorylation-mediated recruitment to or removal from synapses during synaptic plasticity. S818, S831 or S845 phosphorylation alone or in combination seems to underlie or potentiate LTP expression (Estaban et al., 2003; Boehm et al., 2006; Oh et al., 2006; Lee et al., 2010; Makino et al., 2011). S845 is also critical for LTD expression since S845A but not S831A mutant mice lacked LTD (Lee et al., 2010). In contrast, GluA2 S880 phosphorylation that reduces GluA2 surface expression promoted LTD (Seidenman et al., 2003). Recent studies support the similar role of phosphorylation at these sites in synaptic plasticity (Dias et al., 2012; Fernandez-Monreal et al., 2012; Halt et al., 2012; Sanderson et al., 2012; Kohda et al., 2013; Ren et al., 2013). Interestingly, S845 phosphorylation serves as a prerequisite step for homeostatic synaptic plasticity (Goel et al., 2011) and a mechanism for sorting endocytically-removed AMPA receptors to endosomes for reinsertion or lysosomes for degradation (Fernandez-Monreal et al., 2012).

3. Phosphorylation of NMDA receptors

The CT domains of NMDA receptor subunits, especially GluN2A/B, are relatively large and accommodate most if not all phosphorylated