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Roles of subunit phosphorylation in regulating glutamate receptor function

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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 as reversible events. An increasing number of synapse-enriched protein kinases have been found to phosphorylate iGluR. 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.

Keywords

Excitatory amino acid; AMPA; NMDA; PKA; PKC; CaMKII; Cdk5; tyrosine kinase

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-

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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) (Figure 1). The first set of phosphorylation sites identified include 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 (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 amino acids identified so far (Mao et al., 2011; Sanz-Clemente et al., 2013b) (Figure 1). In the GluN1 CT, PKC phosphorylates S890 and S896 probably via different PKC isoforms, while PKA phosphorylates a neighboring site S897 (Tingley et al., 1997; Sanchez-Perez and Felipo, 2005). The GluN2A CT contains two PKA phosphorylation sites (S900 and S929) (Krupp et al., 2002), three PKC sites (S1291, S1312, and S1416) (Gardoni et al., 2001; Jones and Leonard, 2005), and a site (S1232) phosphorylated by cyclin dependent kinase-5 (Cdk5) (Li et al., 2001). GluN2B CT S1303 is a substrate site of CaMKII (Omkumar et al., 1996). PKC is another kinase for this site as well as S1323 (Liao et al., 2001). S1480 is a recently identified residue in GluN2B, which is phosphorylated by casein kinase (CK2) (Sanz-Clemente et al., 2010). Tyrosine phosphorylation is restricted to GluN2 (Lau and Huganir, 1995). Multiple tyrosine sites within the GluN2A CT (842, 1292, 1325, and 1387) and GluN2B CT (1252, 1336, and 1472) are phosphorylated by Src and/or Fyn NRTKs (Nakazawa et al., 2001; Vissel et al., 2001; Yang and Leonard, 2001; Taniguchi et al., 2009). Other phosphorylation sites may exist according to a recent study, although their functional relevance has not been determined (Ghafari et al., 2012).

As expected, phosphorylation significantly modulates trafficking and distribution of NMDA receptors. PKC-mediated S890 phosphorylation dispersed the surface clusters of GluN1 (Tingley et al., 1997). S896 and S897, when phosphorylated together, increased surface expression of NMDA receptors (Scott et al., 2001). CK2 phosphorylation of S1480 within the PDZ domain binding site disrupted the interaction between GluN2B and PSD-95, driving GluN2B endocytosis (Chung et al., 2004) and facilitating a well-known developmental switch from GluN2B to GluN2A at synapses (Sanz-Clemente et al., 2010). A recent study showed that activated CaMKII coupled GluN2B and CK2 to form a tri-molecular complex and increased CK2-mediated phosphorylation of GluN2B S1480 (Sanz-Clemente et al., 2013a). Tyrosine phosphorylation site-selectively impacts the receptor. Y1472 and Y1336 phosphorylation seems to enrich NMDA receptors at synaptic and extrasynaptic compartments, respectively (Goebel-Goody et al., 2009). Fyn phosphorylation of Y1336 site-dependently regulated GluN2B cleavage by calpain (Wu et al., 2007). As a major regulator, Fyn is believed to act as a point of convergence for many signaling pathways to modulate GluN2B/NMDA receptors (Trepanier et al., 2012).

PKC potentiated GluN1/GluN2A-mediated currents by phosphorylating GluN2A S1291 and S1312 (Jones and Leonard, 2005). PKCζ seems to be the isoform carrying out this potentiation based on a recent study (Jones et al., 2012). Similarly, PKC augmented GluN1/GluN2B currents via GluN2B S1303 and S1323 analogous to GluN2A S1291 and S1312 (Liao et al., 2001). S1323 may also be a unique site regulating GluN1/GluN2B stretch sensitivity (Singh et al., 2012). GluN2A S900 and S929 phosphorylation modulates desensitization of GluN2A/NMDA receptors according to early and recent studies (Krupp et al., 2002; Maki et al., 2013). GluN2A S1232 phosphorylation may contribute to NMDA receptor currents and NMDA receptor-dependent LTP induction (Li et al., 2001). Recently, NRTKs (Src and Fyn) were found to differentially regulate GluN2A versus GluN2B receptors (Yang et al., 2012). While Src selectively links pituitary adenylate cyclase activating peptide 1 receptors (PAC1R) to tyrosine phosphorylation of GluN2A which in turn potentiates NMDA receptor currents and lowers the LTP threshold, Fyn connects dopamine D1 receptors to GluN2B phosphorylation, leading to augmented NMDA receptor currents and enhanced LTD.

4. General conclusions and future perspectives

The phosphorylation-dependent posttranslational modification of iGluRs has been intensively investigated since 1996. Multiple serine, threonine, and tyrosine amino acids have been identified primarily in the CT regions of AMPA receptor and NMDA receptor subunits. Early studies show that either constitutive or induced phosphorylation at a specific site exerts the distinct regulation of the biochemical, biophysical and functional properties of modified receptors. These previous observations have paved the solid way for future studies aimed to advance our understanding of glutamate receptor phosphorylation in many perspectives. First, mechanistic insights into the phosphorylation-mediated regulation are poorly understood at present. More studies are needed to elucidate how phosphorylation alters the biochemical features of phosphorylated molecules and how this biochemical alteration leads to stepwise changes in receptor expression and function. Second, structural biology could be included in the future multidisciplinary studies to map new phosphorylation sites in combination of discovering the structural state acquired for interactions between substrates and kinases or between iGluRs and submembranous proteins. The knowledge of structural biology is useful to predict and characterize potential phosphorylation sites and protein-protein interactions. Also, crystallographic analysis provides a powerful tool to reveal a structural basis for kinase-iGluR binding and to discover phosphorylation-triggered structural changes. Third, given the fact that the same kinase phosphorylates multiple iGluR subunits while different kinases can phosphorylate the same

subunit and sometimes even at the same site, it is essential to investigate the distinct role of each individual kinase versus the role of multiple kinases when converged at the same site. It is expected that various kinases work in concert to phosphorylate and regulate iGluRs. To add an additional layer of the mechanism, protein phosphatases that have been less studied as compared to protein kinases need more attention as the phosphorylation level of iGluRs is most likely determined by the balance between kinases and phosphatases. Additionally, other types of posttranslational modifications, such as palmitoylation, ubiquitination and sumoylation, co-occur with phosphorylation to iGluRs. Like phosphorylation, these modifications are an enzymatic process catalyzed by discrete enzyme systems. They are inducible, regulatable, and reversible and are common mechanisms for regulating iGluRs and excitatory synapses (Mao et al., 2011). Thus, how these different types of modifications interact with each other will be an interesting topic for future studies. Finally and more importantly, future disease-based studies are in need of intensification to directly link iGluR phosphorylation to a state of disease. Altered phosphorylation levels of iGluRs in relevant brain regions have been associated with a variety of neurological disorders (Mao et al., 2011). Recently increasing lines of loss-of-function mutation mice (by replacing serine, threonine, or tyrosine alone or together by alanine or phenylalanine) provide a direct tool to evaluate the importance of a specific phosphorylation site or a set of defined phosphorylation sites in the pathogenesis or progression of various brain illnesses, including psychiatric and neurodegenerative diseases, chronic pain, stroke, Alzheimer's disease and substance addiction. The increasing use of these mice will steadily advance the iGluR phosphorylation research to the desired functional level.

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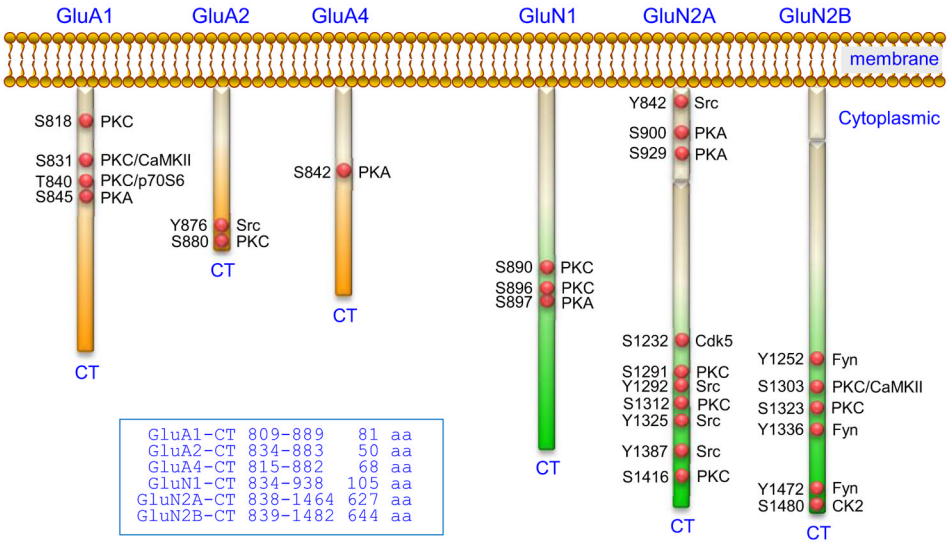

Figure 1. Phosphorylation sites in the CT regions of AMPA receptor and NMDA receptor subunits

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.