Molecular Neurobiology

ISSN0893-7648/05/32(3): 237–249/$30.00

Phosphorylation of AMPA Receptors

Mechanisms and Synaptic Plasticity

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Abstract

The ionotropic α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor is densely distributed in the mammalian brain and is primarily involved in mediating fast excitatory synaptic transmission. Recent studies in both heterologous expression systems and cultured neurons have shown that the AMPA receptor can be phosphorylated on their subunits (GluR1, GluR2, and GluR4). All phosphorylation sites reside at serine, threonine, or tyrosine on the intracellular C-terminal domain. Several key protein kinases, such as protein kinase A, protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase II, and tyrosine kinases (Trks; receptor or nonreceptor family Trks) are involved in the site-specific regulation of the AMPA receptor phosphorylation. Other glutamate receptors (*N*-methyl-D-aspartate receptors and metabotropic glutamate receptors) also regulate AMPA receptors through a protein phosphorylation mechanism. Emerging evidence shows that as a rapid and short-term mechanism, the dynamic protein phosphorylation directly modulates the electrophysiological, morphological (externalization and internalization trafficking and clustering), and biochemical (synthesis and subunit composition) properties of the AMPA receptor, as well as protein–protein interactions between the AMPA receptor subunits and various intracellular interacting proteins. These modulations underlie the major molecular mechanisms that ultimately affect many forms of synaptic plasticity.

Index Entries: Glutamate; dopamine; *N*-methyl-D-aspartate (NMDA); kainate; mGluR; GluR; striatum; serine; tyrosine.

Received March 25, 2005; Accepted May 17, 2005.
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Introduction

The excitatory amino acid L-glutamate (glutamate) is a major neurotransmitter in the developing and adult central nervous system (CNS) and participates in the regulation of a variety of synaptic and cellular activities related to signal transduction, survival, and neuroplasticity. The glutamate action is achieved by its specific interactions with two families of surface receptors: ionotropic and metabotropic receptors *(1–4)*. The former is the ligand-gated ion channel and classified into *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propioni c acid (AMPA), and kainate receptors *(2,5,6)*. The NMDA receptor interacts with endogenous ligands (glycine and glutamate) *(7)* and is permeable to Ca^{2+} as well as other ions (Na^+ and K^+) *(8)*. The AMPA/kainate receptor is also an ion channel that promotes Na^+ influx and, to a lesser extent, Ca^{2+} influx *(9)*.

Glutamate receptors are densely expressed in the CNS. High levels of these receptors are distributed over broad areas of the mammalian brain, especially in the cortex, hippocampus, cerebellum, and striatum *(10,11)*. At least six gene families defined by sequence homology encode three subtypes of ionotropic receptors: a single gene family for AMPA receptors, two for kainate receptors, and three for NMDA receptors *(3)*. These genes are scattered over numerous chromosomes. The protein products of these genes generate different subunits for each receptor: four subunits (GluR 1–4 or GluRA-D) for AMPA receptors, five subunits (GluK 5–7, KA-1, and KA-2) for kainate receptors, and six subunits (NR1, NR2A-D, and NR3A) for NMDA receptors *(3)*. Each functional receptor is a heteromeric assembly of multiple subunits. The most generally accepted model of subunit stoichiometry for the NMDA receptor is a pentameric or tetrameric structure *(3)*. The former consists of three NR1 and two NR2 subunits, whereas the latter consists of two NR1 and two NR2 subunits. Based on a spectrum of heteromeric subunit composition, the NMDA receptor can show multiple subtypes with different channel properties. Compared with the NMDA receptor, the AMPA receptor can be even more diversified in terms of its subunit composition and thus functional distinction. The AMPA receptor appears to possess an unfixed subunit stoichiometry, although heteromeric tetramerization is a favorable model of its assembly (refs. *12* and *13*; Fig. 1). Variable AMPA receptor subunit stoichiometry endows an existence of multiple subtypes within the AMPA receptor and excitatory synapses with a much wider range of responses *(3)*.

Transmembrane topology of a single subunit in NMDA or AMPA receptor assemblies is unique. Unlike G protein-coupled metabotropic glutamate receptors (mGluRs) that usually have seven transmembrane domains, an ionotropic glutamate receptor subunit only has three transmembrane domains (M1, M3, and M4) as well as a cytoplasm-facing re-entrant membrane loop (M2; Fig. 1). The mechanism for threading a receptor subunit through the membrane determines segments facing the extracellular or cytoplasmic fluids. According to the model depicted in Fig. 1, the N-terminus of a glutamate receptor subunit is located extracellularly, and the C-terminus is located intracellularly. The N-terminus and an extracellular loop between M3 and M4 specify the protein domain for ligand recognition, whereas the C-terminus specifies the domain for cytoplasmic modification (phosphorylation, etc.) and interactions with submembranous proteins *(3)*.

Phosphorylation is an important posttranslational modification process for many biologically active proteins. Similarly, glutamate ion channel phosphorylation in neurons occurs as an imperative regulatory mechanism that controls many aspects of channel properties. Therefore, identification of phosphorylation sites on glutamate receptor subunits and evaluation of the functional consequences of altered phosphorylation have drawn considerable attention and have been extensively studied in the recent years. Indeed, glutamate receptors, like many other proteins, are under tight modification by a large number of protein kinases and protein phosphatases *(3,14–16)*. The dynamic phosphorylation by various mechanisms contributes to a

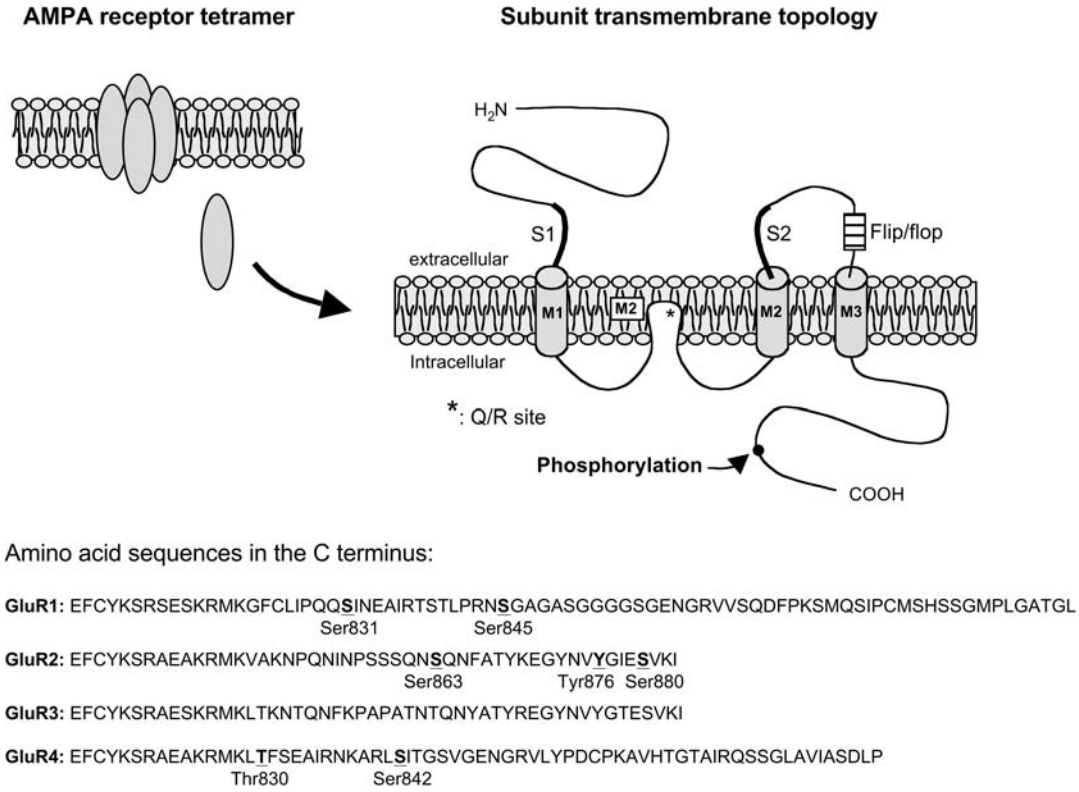

Fig. 1. Schematic diagrams illustrating structure of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor subunits *(16)*. AMPA receptors exist as heteromeric tetramers through cell membrane (top left panel). The transmembrane topology of a single subunit (top right panel) includes the flip/flop alternatively spliced exon, the two ligand-binding domains (S1 and S2) on the extracellular domain, and a Q/R site (Q, glutamine-specific for GluR1, GluR3, and GluR4; R, arginine-specific for GluR2). The cytoplasmic C-terminus provides amino acid sites for phosphorylation and interactions with submembranous proteins. Phosphorylation of GluR1 subunits occurs at serine (Ser) residues 831 and 845, GluR2 subunits at serines 863 and 880 and tyrosine (Tyr) 876, and GluR4 at threonine (Thr) 830 and serine 842. Bold and underlined amino acids represent phosphorylation sites. NH_2, amino terminus; COOH, carboxy terminus.

sophisticated control of many forms of synaptic plasticity in excitatory synaptic transmission. Although NMDA and AMPA receptors both undergo active phosphorylation, this article focuses on AMPA/kainate receptors for the phosphorylation event and functional consequences of the receptor phosphorylation in regulating their synaptic plasticity.

Phosphorylation of AMPA Receptors

The identification of the phosphorylation sites on AMPA receptor subunits was controversial because of a previously suggested receptor topology model that proposed the C-terminus of the receptors to be extracellular and the loop between M3 and M4 to be the major intracellular segment. In 1996, Huganir's group made a first attempt to identify the phosphorylation sites on GluR1 subunits (the most well-characterized subunit of AMPA receptors) based on a current transmembrane topology model of glutamate receptors, in which the C-terminus is intracellular (Fig. 1; ref. *17*). They found that reliable phosphorylation occurred on the GluR1 subunit transfected in HEK cells,

and all phosphorylation sites reside on the intracellular C-terminus. Using site-directed mutagenesis and phosphopeptide mapping analysis, they further identified the two major phosphorylation sites on the serine residues of the GluR1 C terminus: serine 831 and serine 845. The former is specifically phosphorylated by protein kinase C (PKC), whereas the latter is specifically phosphorylated by protein kinase A (PKA). PKA has been found to augment the AMPA receptor current *(17)*. Therefore, phosphorylation of the GluR1 subunit on the PKA-sensitive site (serine 845) is likely to underlie the PKA-induced potentiation of the AMPA receptor current.

A different study conducted by Mammen et al. *(18)* in heterologous expression systems and hippocampal slices demonstrated that the PKC phosphorylation site (serine 831) can also be phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), a kinase abundantly expressed with the GluR1 subunit in the postsynaptic density (PSD) *(19–21)*. Using deletion and site-specific mutants of GluR1, Barria et al. *(22)* observed a similar result in transfected HEK-293 cells. Therefore, a single serine site on the GluR1 subunit is subject to be phosphorylated by two different kinases. This raises the possibility of synergistic (or any other type of) interactions between the two kinases in the regulation of GluR1–Ser831 phosphorylation. Similarly to PKC, CaMKII has long been shown to potentiate the GluR1 current *(23–25)*. Therefore, CaMKII-regulated phosphorylation of GluR1 subunits on serine 831 may contribute to this potentiation. Indeed, the Ser831-to-Ala (S831A) mutant failed to show potentiation of the GluR1 current after active CaMKII was infused into cells expression GluR1 *(22)*.

Although the C-terminus of GluR1 shows limited homology to the other AMPA receptor subunits (Fig. 1), the other subunits are also substrates for protein kinases. The majority of AMPA receptors in the brain contain the GluR2 subunit, a subunit that also contains potential sites for protein phosphorylation. Site-directed mutagenesis has confirmed that phosphorylation of the ubiquitous GluR2 subunit occurs on serines 863 and 880 of the C-terminus *(26,27)*. Because serine 880 phosphorylation occurs in response to PKC activation *(26,27)*, this phosphorylation may mediate the PKC regulation of AMPA receptor function. The GluR4 is the most rapidly desensitizing AMPA receptor subunit. Phosphorylation of this subunit was found on serine 842 within the C-terminal domain in vitro and in vivo *(28)*. Serine 842 is phosphorylated in vitro by PKA, PKC, and CaMKII and in transfected HEK cells by PKA. Additionally, threonine 830 is located in a consensus site (KXT) for PKC phosphorylation *(29)* and, therefore, is a potential phosphorylation site by PKC *(28)*. The GluR3 subunit has a limited number of serine residues on its intracellular C-terminal domain (Fig. 1). Phosphorylation of this subunit on any residue has not been reported.

In addition to the phosphorylation on the serine and threonine residues, tyrosine phosphorylation of the AMPA receptor occurs in vivo and in vitro. Nonreceptor Src family tyrosine kinases (Trks) induced tyrosine phosphorylation of GluR1 *(30,31)*. Brain-derived neurotrophic factor, a ligand for a receptor Trk (TrkB), also increased GluR1 tyrosine phosphorylation—probably via a mechanism involving NMDA receptor activation *(32)*. However, among the last 75 amino acids of the GluR1 C-terminus (where phosphorylation on a specific residue—serine or threonine—typically occurs), there is no single tyrosine residue (Fig. 1). Therefore, the site of tyrosine phosphorylation on GluR1 remains to be identified from its intracellular domains. Unlike GluR1, the end region of the GluR2 C-terminus has multiple tyrosine residues. Src family Trks phosphorylated tyrosine 876 *(33)*, a last tyrosine residue near the end of C-terminus and within the C-terminal PDZ ligand domain (Fig. 1). Tyrosine phosphorylation on this site appears to inhibit the association of the GluR2 subunit with the PDZ domain-containing proteins and glutamate receptor interacting proteins 1 and 2 (GRIP1/2) but not protein interacting with C-kinase-1 (PICK1) *(33)*. The inhibition of the GluR2 binding to GRIP1/2

resulted in internalization of GluR2 *(33)*. This internalization can be prevented by site mutation of tyrosine 876 *(33)*. Similarly to Src, insulin and low-frequency stimulation increased tyrosine phosphorylation of the GluR2 subunits in hippocampal slices *(34)*. The enhanced phosphorylation appears to be necessary for insulin-induced AMPA receptor endocytosis and depression of AMPA receptor-mediated excitatory currents *(34)*. No apparent tyrosine phosphorylation on GluR3 and GluR4 has been noted in synaptic membranes *(35)*. Trks (receptor and nonreceptor families) and insulin have been well-documented to modulate the two major forms of activity-dependent synaptic plasticity: long-term potentiation (LTP) and long-term depression (LTD) *(36,37)*. Therefore, tyrosine phosphorylation of AMPA receptor subunits by these Trks may at least partly mediate their modulation of the AMPA receptor.

Phosphorylation of Kainate Receptors

Protein phosphorylation can also be a regulatory mechanism for the kainate receptor. Two early studies revealed that recombinant GluK6 homomeric receptors are phosphorylated by PKA, and this phosphorylation may underlie an enhancement of whole-cell current responses of GluK6 *(38,39)*, similarly to that observed with GluR1. A specific change in channel properties that contributes to the PKA potentiation appears to be an increase in the open probability without any apparent changes in the time-course of responses. Two extracellularly localized serine residues (serines 684 and 666) were previously proposed to be important sites for the PKA modification *(38,39)*, but they are unlikely sites for phosphorylation by intracellular PKA *(40)*. Therefore, the phosphorylation sites on GluK6 by PKA remain to be explored experimentally. Two recent studies show that the GluK5 subunit was phosphorylated by PKA and/or PKC in cultured cortical neurons *(41)* or in vitro *(42)*. A series of overlapping truncation mutants followed by site-directed mutation suggests that serine 880 and/or serine 886 on the GluK5 C-terminus are the specific phosphorylation sites by PKC *(42)*. Active PKC enhances the kainate current and mediates the mGluR5 potentiation of kainate receptor responses *(41)* as well as stabilization of kainate receptors at the synapse by interacting with anchoring proteins GRIP1/2 and PICK1 *(42)*. These PKC actions may involve serine phosphorylation of the GluK5 subunit.

Direct Impacts of Protein Phosphorylation on AMPA Receptors

Following the identification of the phosphorylation sites, direct impacts of altered phosphorylation status on these sites to AMPA receptor activity have been extensively studied for the past 5 yr. Possible direct impacts include, but are not limited to, the regulation of electrophysiological, morphological (externalization and internalization trafficking and clustering), and biochemical (synthesis and subunit composition) properties of the AMPA receptor as well as protein–protein interactions between the AMPA receptor subunits and intracellular PDZ-domain- and non-PDZ-domain-containing synaptic proteins. Electrophysiologically, phosphorylation of the GluR1 subunit on the PKA-sensitive site (serine 845) increased the channel open probability *(43)* and the peak amplitude of the current *(17)*. On the other hand, phosphorylation of the GluR1 subunit on the PKC/CaMKII site (serine 831) increased the single-channel conductance *(44)*. These positive impacts may contribute to the potentiation of synaptic efficacy and strength following the protein phosphorylation. Morphologically, PKA phosphorylation of GluR4 drove AMPA receptors into synapses *(45)*. Both PKA and CaMKII phosphorylation of GluR1 produced the same effect *(45)*. Therefore, serine phosphorylation of GluR1/4 facilitates the incorporation (insertion trafficking) of AMPA receptors into synapses. It is unclear whether the C-terminal phosphorylation of a given subunit has

any influence over biochemical synthesis and subunit composition of the AMPA receptor.

In addition to its ion channel signaling, the AMPA receptor interacts with numerous submembranous synaptic proteins through its cytoplasmic C-terminus to (a) organize into macromolecular (multiprotein) complexes coordinating the postreceptor signaling and (b) regulate the membrane distribution pattern of the AMPA receptor by controlling the trafficking and clustering of the receptor *(46–48)*. The relatively long-form C-terminus of the GluR1 subunit interacts with the PDZ-domain-containing proteins SAP97 in a membrane-associated guanylate kinase family *(49,50)* and A-kinase-anchoring proteins 79/150 (AKAP79/150) *(51, 52)* in transfected COS7 cells and hippocampal neurons. The interactions with these proteins allow SAP97 and AKAP79/150 to target PKA to GluR1 for the basal phosphorylation of serine 845 *(49,51)*. In the course of LTD development, SAP97–AKAP–PKA complexes recruit and activate protein phosphatase 2B to dephosphorylate serine 845, leading to the internalization of the AMPA receptor and the expression of LTD *(50,51)*. A recent report showed that neuronal pentraxin 1, a member of a newly recognized subfamily of long pentraxins in the hypoxia-ischemia injury cascade, interacts with GluR1, which is critical for the role of pentraxin 1 in mediating ischemic injury *(53)*.

The short-form C-terminus of the GluR2 subunit interacts with the PDZ-domain-containing scaffold proteins: AMPA receptor binding protein/GRIP *(54,55)*, ATPase *N*-ethylmaleimide-sensitive fusion protein or *N*-ethylmaleimide-sensitive factor (NSF) *(56–58)*, α- and β-soluble NSF attachment protein *(57)*, and PICK1 *(48)*. A large number of studies have shown the importance of these interactions in regulating trafficking of AMPA receptors *(48,54–59)*. Because the binding domain at the C-terminus of GluR2 for these interacting proteins overlaps with the phosphorylation sites on tyrosine 876 and serine 880, phosphorylation status on these sites affects the association of the AMPA receptor subunit with the interacting proteins, thereby modifying trafficking and redistribution of the receptor. Indeed, phosphorylation of GluR2 on tyrosine 876 by Trks or serine 880 by PKC reduced the affinity of GluR2 for GRIP in vitro and in transfected cells *(28,35,60,61)*. Disrupted interactions of GluR2 with GRIP trigger internalization, leading to reduced surface expression of GluR2. This process has been suggested to mediate the expression of hippocampal LTD *(62)*. In this process, PICK1 appeared to stabilize the internalized GluR2 and, therefore, intracellular perfusion of a synthetic peptide that disrupts the GluR2–PICK1 interaction inhibited the expression of hippocampal LTD *(63)*. Finally, the C-terminus of GluR4c subunit interacts with NSF *(58)* and PICK1 *(59)* and the GluR3 subunit interacts with AMPA receptor binding protein and PICK1 *(59)*. More studies are needed to illustrate functional implications of these interactions.

Regulation of AMPA Receptor Phosphorylation

Generation of phospho-specific antibodies against the phosphorylation sites on the GluR1 or other subunits provides a useful tool to study the regulation of AMPA receptor phosphorylation in vivo or in cultured neurons under different experimental manipulations. In hippocampal neurons—particularly in the CA1 subregion—phosphorylation of postsynaptic AMPA receptors has been demonstrated to play important roles in regulating synaptic plasticity (LTP and LTD). Through the modulation of synaptic plasticity, protein phosphorylation of AMPA receptors is actively involved in the learning and memory processes in the brain. Strong, brief (≤2 s), and high-frequency (≥50 Hz) stimulation that specifically induces LTP increased a recruitment of active CaMKII to the PSD to phosphorylate various proteins, including the CaMKII-sensitive site on the GluR1 subunit (serine 831) *(22,64)*. Therefore, CaMKII-mediated serine 831 phosphorylation

is believed to contribute to the induction of LTP. The other phosphorylation site on GluR1, serine 845, is phosphorylated by PKA under basal conditions and may not participate in LTP *(65)*. Conversely to LTP, LTD is usually induced by weak, prolonged (≥30 s), and low-frequency (<10 Hz) stimulation and is related to a persistent dephosphorylation of GluR1–Ser845, but not GluR1–Ser831 (refs. *64*–*67*). Therefore, phosphorylation of serine 831 and dephosphorylation of serine 845 at the GluR1 subunit are differentially involved in the modulation of LTP and LTD, respectively. Additionally, serine 880 phosphorylation at GluR2 by PKC disrupted the interaction of GluR2 with GRIP and triggered endocytosis, leading to the induction of LTD *(62)*.

As a major structure in the basal ganglia, the dorsal (caudate putamen) and ventral (nucleus accumbens) striatum regulates extrapyramidal motor behaviors and behaviors unrelated to movement—that is, cognitive behaviors (learning and memory, affection, addiction, rewarding, reinforcement, etc.). During the recent decades, the striatum has been the focus of studies on brain mechanisms underlying various neurological disorders (Parkinson's disease, Huntington, drug addiction, etc.). The striatum is anatomically enriched with excitatory afferents. The dorsal striatum receives abundant glutamatergic projections from widespread areas of the cerebral cortex and thalamus, whereas the ventral striatum receives excitatory inputs from the amygdala, hippocampus, and prefrontal cortex *(68,69)*.

Parallel with enriched glutamatergic afferents to the striatum, high levels of glutamate receptors are expressed in this region, including AMPA receptors *(70–73)*. In addition to glutamatergic inputs, dopamine is another major input that interacts with glutamatergic transmission in coregulating the γ-aminobutyric acid (GABA)ergic medium-sized projection neurons (the most common cell type in the striatum) and several types of interneurons *(74,75)*. In fact, both glutamatergic and dopominergic inputs can converge onto a same neuron to cooperatively regulate its activity. This is supported by the colocalization of postsynaptic dopamine and glutamate receptors, including AMPA receptors, on the same projection neurons or interneurons *(76,77)*. Among many forms of the crosstalking between the two major transmitters, dopamine inputs show the ability to modulate the phosphorylation status of AMPA receptors in cultured striatal neurons and striatal slices. Pharmacological stimulation of D1 dopamine receptors that are positively coupled to adenylate cyclase *(78,79)* promoted the phosphorylation of AMPA receptor GluR1 subunits at the PKA phosphorylation site (serine 845) *(80–82)*. Systemic administration of the indirect dopamine receptor agonists (i.e., psychostimulants, cocaine, and methamphetamine) also increased striatal phosphorylation of GluR1–Ser845 in vivo *(80,81)*. Similar enhancement of the GluR1–Ser845 phosphorylation was observed in the nucleus accumbens upon stimulation of dopamine release in vivo using electrical stimulation of dopamine cell bodies in the ventral tegmental area *(83)*. The mechanism for the enhanced serine 845 phosphorylation appears to involve both PKA and protein phosphatase 1 (PP1), an anchoring protein binding to the AMPA receptors through spinophilin *(84,85)*. Under the resting state, the AMPA channel is kept in the dephosphorylated state ("low activity") by constitutively active PP1. Activation of D1 dopamine receptors converts the channel to the phosphorylated state ("high activity") by a dual mechanism, involving a direct phosphorylation of GluR1–Ser845 by PKA and the inhibition of PP1 by dopamine and cyclic adenine monophosphate (cAMP)-regulated phosphoprotein DARPP-32 *(80,81)*. Together, dopamine inputs can rapidly modulate AMPA channel currents in striatal neurons through a postsynaptic mechanism involving protein phosphorylation. Increased GluR1 phosphorylation by the intracellular D1–PKA pathway may trigger externalization of AMPA receptors, resulting in the potentiation of synaptic strength in addiction-related neuronal circuits *(86)*.

Purkinje neurons in the cerebellum receive the excitatory glutamatergic projection from parallel fibers, the axons from cerebellar granule neurons. Individual Purkinje neurons establish synapses with climbing parallel fibers. A well-characterized form of synaptic plasticity, LTD, has been documented at the Purkinje neuron-parallel fiber synapses with the active involvement of AMPA receptors *(87,88)*. PKC has been documented to participate in the development of LTD in cultured Purkingje neurons *(87–90)*. Therefore, phosphorylation of AMPA receptor subunits on PKC-sensitive sites may underlie the PKC-mediated synaptic plasticity. GluR2 is an AMPA receptor subunit densely expressed at the Purkinje neuron-parallel fiber synapses *(91)*. Phosphorylation of GluR2 on the PKC site (serine 880) resulted in a decrease in the association of the receptor with the anchoring protein GRIP, followed by disruption of receptor clusters and internalization of the receptor in cultured Purkinje neurons *(28,92)*. Such phosphorylation-triggered receptor endocytosis is believed to underlie the induction of LTD at the Purkinje neuron-parallel fiber synapses.

Phosphorylation of the AMPA receptor subunit may be implicated in other neurological disorders. Transient global ischemia in rats enhanced phosphorylation of GluR1–Ser831, but not GluR1–Ser845, in the hippocampal CA1 and CA3/dentate gyrus *(93,94)*. A parallel increase in activity of CaMKII, but not PKA, was observed in the same area. Therefore, the serine 831 phosphorylation probably is catalyzed by CaMKII, and this event likely contributes to the development of pathogenic activities occurring in the vulnerable subfield of the hippocampus after transient global ischemia. Capsaicin injection significantly induced a PKC-dependent increase in GluR1–Ser831 and a PKA-dependent increase in GluR1–Ser845 in the ipsilateral spinal cord to the injection *(95,96)*. Similar results were found in the rostral ventromedial medulla after Freund's adjuvant-induced hindpaw inflammation *(97)*. These results support a role of the phosphorylated GluR1 subunit in the regulation of brain neuronal responses to peripheral noxious inflammation.

Regulation of AMPA Receptors Through Protein Phosphorylation by Glutamate Receptors

NMDA receptors, another subtype of ionotropic glutamate receptors, shows the ability to regulate AMPA receptors. This regulation may be mediated through a protein phosphorylation mechanism. Two recent studies explored this possibility. Activation of NMDA receptors dephosphorylated serine 845 of the GluR1 subunit in hippocampal slices *(52,98)*. This dephosphorylation resulted from the removal of PKA driving force from the serine 845, because NMDA caused displacement of PKA from synapses and inhibition of PKA mimicked the NMDA effect *(52)*. Additionally, activation of phosphatase may contribute to this process because NMDA-induced dephosphorylation of serine 845 was blocked by the phosphatase inhibitor *(98)*. Together, a coupling exists from NMDA receptors to AMPA receptors for the regulation of GluR1 phosphorylation on a PKA site (serine 845) via a signaling mechanism involving both PKA and phosphatase.

Compared with NMDA receptors, the regulation of AMPA receptors by mGluRs could be a more intriguing issue. This is because G protein-coupled mGluRs have connections to multiple second messenger systems (including PKA, PKC, and CaMKII) that are highly active in regulating AMPA receptor phosphorylation *(4)*. Through these connections, mGluRs are postulated to play an active role in the regulation of AMPA receptor phosphorylation and, therefore, their functions. Among eight subtypes of mGluRs cloned to date, group I mGluRs (mGluR1/5 subtypes) are positively coupled to phosphoinositide (PI) hydrolysis. Activation of mGluR1/5 leads to enhanced PI hydrolysis, resulting in production of inositol-1,4,5-triphosphate, which releases intracellular Ca^{2+} and diacylglycerol, thus activating PKC. Group II (mGluR2/3) and III (mGluR4/6/7/8) mGluRs are negatively linked to adenylyl cyclase. Activation of these two groups of mGluRs leads to inhibited adenylate cyclase and reduced

cAMP/PKA activity. Linkages to the multiple intracellular effectors allow mGluRs to preferentially participate in the metabotropic modulation of their targets (including AMPA receptors), especially when both mGluRs and AMPA receptors are densely expressed in the PSD. Although study on this issue remains at its infant stage, available data show that selective activation of group I receptors with the mGluR1/5 agonist DHPG increased GluR1 phosphorylation on serine 831 in cultured cortical and striatal neurons (Arora, Yang, and Wang, unpublished observations). An increase in serine 845 was also seen 5 min (Arora, Yang, and Wang, unpublished observations, 2005), but not 30 min *(99)*, after incubation of the group I receptor agonist (DHPG or CHPG). Therefore, group I receptors possess the ability to regulate GluR1 phosphorylation in a facilitatory manner. DHPG has been found to trigger GluR1 internalization in cultured striatal neurons *(100)*. Thus, DHPG-stimulated GluR1 phosphorylation may be a triggering event for this process. Future studies are needed to characterize the mGluR1/5 regulation of AMPA receptor phosphorylation and to elucidate the signaling pathways transmitting mGluR1/5 signals to AMPA receptors and physiological consequences of the mGluR1/5-regulated AMPA receptor phosphorylation in controlling AMPA receptor signaling.

Acknowledgments

The studies of the author mentioned in the review were supported by grants from the National Institutes of Health (DA10355 and MH61469 to J. Q. Wang).

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