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Phosphorylation of group I metabotropic glutamate receptors (mGluR1/5) *in vitro* and *in vivo*

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Abstract

Group I metabotropic glutamate receptors (mGluR1 and mGluR5 subtypes) are densely expressed in mammalian brain. They are actively involved in the regulation of normal cellular activity and synaptic plasticity, and are frequently linked to the pathogenesis of various mental illnesses. Like ionotropic glutamate receptors, group I mGluRs are subject to the regulation by protein phosphorylation. Accumulative data demonstrate sufficient phosphorylation of the intracellular mGluR1/5 domains at specific serine/threonine sites by protein kinase C in heterologous cells or neurons, which serves as an important mechanism for regulating the receptor signaling and desensitization. Emerging evidence also shows the significant involvements of G protein-coupled receptor kinases, Ca²⁺/calmodulin-dependent protein kinase II, tyrosine kinases, and protein phosphatases in controlling the phosphorylation status of group I mGluRs. This review analyzes the recent data concerning group I mGluR phosphorylation and the phosphorylation-dependent regulation of group I mGluR function. Future research directions in this area with newly available high throughput and proteomic approaches are also discussed in the end.

Keywords

mGluR; phosphorylation; CaMKII; PKC; phosphatase; desensitization

Introduction

L-Glutamate (glutamate) is a major excitatory neurotransmitter in mammalian brain. Through interacting with two classes of surface expressed glutamate receptors, i.e., ionotropic and G protein-coupled metabotropic glutamate receptors (mGluRs), glutamate regulates cellular and synaptic activity and plasticity related to cell death and survival, learning and memory, pain perception, and motor activity (Dingledine et al., 1999; Wang et al., 2007). Among three groups of mGluRs, group I mGluRs (mGluR1 and mGluR5 subtypes) have drawn the most attention and have been most extensively studied for their regulation and function in terms of

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physiological, pharmacological, and biochemical properties (Conn and Pin, 1997). Group I mGluRs are widely distributed throughout brain regions. They are usually localized postsynaptically in an area known as postsynaptic density (PSD) at excitatory synaptic sites as opposed to group II mGluRs (mGluR2/3) which are enriched both presynaptically and postsynaptically and group III mGluRs (mGluR4/6/7/8) which are usually predominant presynaptically. Since group I mGluRs are coupled to $G_{\alpha_{q/11}}$ proteins, activation of them with the selective agonists activates phospholipase C β 1 (PLC β 1) and increases hydrolysis of membrane-bound phosphoinositide (PI). This yields diacylglycerol (DAG), which activates protein kinase C (PKC), and inositol-1,4,5-trisphosphate (IP $_3$), which releases Ca^{2+} from intracellular stores. Active PKC and Ca^{2+} signals could then engage in the modulation of diverse metabotropic activities. As a class of G protein-coupled receptors (GPCRs), group I mGluRs have a typical membrane topology for a GPCR, an extracellular N-terminus, an intracellular C-terminus, and seven transmembrane domains. Noticeably, among six splice variants cloned so far for mGluR1 (1a or 1 α , 1b or 1 β , 1c, and 1d) and mGluR5 (5a and 5b), only the long-form group I mGluRs (1a, 5a, and 5b) have a characteristically large C-terminal tail. A detailed rat mGluR5a topology is illustrated in Fig. 1 based on the amino acid sequence reported by Abe et al. (1992). This long C-terminus provides these splice variants with a spacious basis for direct protein-protein interactions with a number of synaptic and cytoplasmic proteins, including protein kinases and protein phosphatases (PPs) (reviewed in Fagni et al., 2004; Enz, 2007).

Protein phosphorylation is one of decisive post-translational modification steps for modulating protein expression and function. Synaptic receptors including ion channels and GPCRs are known to be modulated by phosphorylation. Ionotropic glutamate receptors are certainly the targets for phosphorylation by various protein kinases (Lee et al., 2006; Liu et al., 2006; Wang et al. 2006). Emerging evidence also reveals that group I mGluRs are among mGluRs that are readily regulated by phosphorylation in heterologous cells and brain cells (Kim et al., 2008). PKC is a key kinase that has been extensively investigated for phosphorylation of group I mGluRs and the phosphorylation-dependent regulation of receptor function. Other protein kinases, such as G protein-coupled receptor kinases (GRKs), Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), tyrosine kinases, and PPs are also involved in group I mGluR phosphorylation. This mini-review summarizes recent progress in a research topic concerning group I mGluR phosphorylation by various protein kinases and PPs, which is followed by some suggestions for future research directions in this rapidly advancing area.

Phosphorylation of mGluR1/5 by PKC

The phosphorylation of mGluR1/5 by PKC has been most thoroughly investigated in the last decade. An early study shows a significant constitutive phosphorylation of mGluR1a that was stably expressed in heterologous cells and immunoprecipitated by a specific antibody (Alaluf et al., 1995). In addition to constitutive phosphorylation, stimulated phosphorylation of mGluR1a can also be induced according to an observation that activation of mGluR1a with a selective agonist induced a rapid and transient increase in the phosphorylation level. More interestingly, the agonist-induced phosphorylation could be abolished by a specific PKC inhibitor Ro318220 (Alaluf et al., 1995) and the PKC activator mimicked the effect of the mGluR1/5 agonist in phosphorylating mGluR1c (Ciruela et al., 1999). This suggests that PKC is a responsible kinase that mediates phosphorylation of the receptor in response to agonist stimulation. The pharmacologically defined role of PKC was supported by the subsequent identification of a PKC-mediated phosphorylation site at rat mGluR5a in heterologous cells: a threonine residue at position 840 (T840) within the proximal region of mGluR5a C-terminal tail, a region also harboring G proteins (Kawabata et al., 1996, but see below). Other PKC-sensitive serine/threonine site(s) are believed to exist because a truncated mGluR5 peptide lacking T840 still displayed the PKC-mediated phosphorylation *in vitro* (Minakami et al.,

1997). Indeed, multiple PKC consensus phosphorylation sites (K/RxXS/T) (Pearson and Kemp, 1991) can be found on intracellular regions of mGluR5a, including the first and second intracellular loops in addition to the C-terminus (Fig. 1). Several of them appear to be phosphorylated by PKC for producing a rapid desensitization of mGluR5 in *Xenopus* oocytes (see below).

The PKC-mediated phosphorylation can be regulated by calmodulin (CaM), a mobile Ca^{2+} -binding protein and intracellular Ca^{2+} transducer that is involved in a broad array of cellular and synaptic activities. Ubiquitous CaM has been found to directly bind to two distinct sites on the C-terminus of human mGluR5 (H845-L875 and W884-S936) which seem to have different affinities for CaM (Minakami et al., 1997). Such binding to either site was Ca^{2+} -dependent and was able to inhibit the PKC-mediated phosphorylation of mGluR5 (Minakami et al., 1997). Conversely, the PKC phosphorylation antagonized the binding of CaM to mGluR5. Thus, PKC and CaM can reciprocally regulate each other's binding to mGluR5 to accurately control phosphorylation of the receptor. Through this reciprocal loop, the mGluR5 phosphorylation event is tightly linked to changing Ca^{2+} signals by Ca^{2+} -sensitive CaM and PKC.

The PKC phosphorylation has functional implications. While mGluR1 and 5 both trigger the release of Ca^{2+} from intracellular stores, they induce different response patterns of Ca^{2+} transients, which seem to be controlled by the PKC-mediated phosphorylation. In transfected cells, mGluR1a activation induced a single-peaked Ca^{2+} rise whereas mGluR5a activation elicited characteristic Ca^{2+} oscillations (Kawabata et al., 1996). The latter depends on the phosphorylation at a single amino acid (Kawabata et al., 1996; Uchino et al., 2004; Kim et al., 2005; but Dale et al., 2001). Early studies suggest that the phosphorylation of the mGluR5a C terminus at T840 by PKC (likely the Ca^{2+} -independent PKC δ isoform rather than Ca^{2+} -dependent PKC γ) is responsible for the generation of Ca^{2+} oscillations in mGluR5a-expressing cells (Kawabata et al., 1996; Uchino et al., 2004). A more recent biochemical study performed in a different laboratory identifies that the serine 839 (S839) is probably the real residue at which phosphorylation occurs while the adjacent T840 only plays a permissive role in the PKC-dependent phosphorylation of S839 (Kim et al., 2005). The permissive role of T840 is unique to mGluR5a since this site is not conserved in mGluR1a (D854). As a result, PKC did not phosphorylate the same site at mGluR1a despite that S839 is conserved in mGluR1a (S853) (Kim et al., 2005). In cultured astrocytes that solely express mGluR5, oscillatory responses of intracellular Ca^{2+} to an agonist were converted to non-oscillatory responses by a PKC inhibitor (Nakahara et al., 1997), indicating the gating role of PKC-promoted phosphorylation in determining the response pattern of Ca^{2+} signals in native mGluR5 expressed in brain cells as compared to cloned mGluR5 expressed in heterologous cells. How the PKC phosphorylation of a single amino acid switches the Ca^{2+} response pattern is unclear. The fact that the phosphorylation site (S839) lies in the G protein-coupling region of the mGluR5 C terminus is noteworthy.

Another physiological consequence of the PKC-mediated phosphorylation of mGluR1/5 involves the homologous (agonist-dependent) and heterologous (agonist-independent) desensitization of the receptor (Dhami and Ferguson, 2006). Like other GPCRs, group I mGluR desensitization occurs as a consequence of covalent receptor modification due to phosphorylation usually by two families of intracellular kinases: second messenger-dependent protein kinases and GPCR-specific GRKs (see below). In the case of homologous desensitization, stimulation of mGluR1/5 with the selective agonist leads to the DAG-dependent activation of PKC. Activated PKC can then phosphorylate mGluR1/5 to promote the homologous type of desensitization, an attenuation of receptor responsiveness to repeated or prolonged agonist stimulation (Lefkowitz, 1993). The existence of such negative feedback is clearly supported by a number of pharmacological studies. The PKC inhibitors reduced the

agonist-dependent mGluR1/5 desensitization in cultured neurons (Catania et al., 1991; Aronica et al., 1993) or *Xenopus* oocytes expressing mGluR5a (Gereau and Heinemann, 1998). In contrast, the PKC activators induced a desensitization-like reduction of mGluR1/5 signaling in hippocampal slices (Schoepp and Johnson, 1988) or *Xenopus* oocytes (Gereau and Heinemann, 1998). The role of PKC in the mGluR5 desensitization is selective since the protein kinase A (PKA) selective agents (an activator or inhibitor) had no effect on the desensitization (Gereau and Heinemann, 1998). In the effort of identifying the phosphorylation sites necessary for the agonist-stimulated and PKC-mediated desensitization, Gereau and Heinemann (1998) found that mutation of multiple sites in the first and second intracellular loops and the C-terminus of mGluR5a (T606, S613, T665, S881, and S890) seems to be sufficient to block the PKC-dependent desensitization. Since most of these sites align well with the PKC phosphorylation consensus sequence, PKC is thought to phosphorylate these residues to inhibit the receptor function, although actual phosphorylation status of these sites was not tested in this model. Interestingly, the T840 that is essential for generating the oscillatory Ca^{2+} responses to mGluR5 activation had no functional impact to the desensitization of mGluR5 (Gereau and Heinemann, 1998). Thus, the distinct PKC phosphorylation sites on mGluR1/5 may control the specificity of function. The PKC phosphorylation of mGluR1/5 is believed to disrupt normal G protein-coupling and thereby reduce the efficacy of mGluR1/5 signaling. Consistent with this, an IP_3 pathway-selective desensitization was achieved by the PKC-mediated phosphorylation of an mGluR1a threonine residue (T695; T681 in mGluR5a) that is involved in the receptor coupling to $\text{G}\alpha_{q/11}$ (Francesco and Duvoisin, 2000). Mutation of this residue almost completely abolished mGluR1 α -mediated rapid desensitization. T695 (mGluR1a) or T681 (mGluR5a) is located within a hinge region of the second intracellular loop (Fig. 1), that connects two putative α -helices (Francesco and Duvoisin, 1998) and is present only in group I mGluRs. This region is rich in basic amino acids (R/K) and T695 or T681 falls within a consensus site for PKC-mediated phosphorylation. In addition to the homologous desensitization, PKC mediates the heterologous desensitization since PKC inhibitors blocked the group I mGluR desensitization induced by stimulation of heterologous $\text{G}\alpha_{q/11}$ -coupled muscarinic M1 receptors (Mundell et al., 2002; 2004).

Tyrosine phosphorylation of mGluR1/5

In addition to serine and threonine, tyrosine phosphorylation could occur to mGluR1/5. Electrophysiological evidence in midbrain dopamine neurons has shown that the protein tyrosine kinase inhibitors inhibited the mGluR1/5 response to the agonist DHPG (Tozzi et al., 2001). Hence group I mGluR signaling relies on an intact protein tyrosine kinase activity. Using an anti-phosphotyrosine antibody along with an mGluR1a or 5 antibody, tyrosine phosphorylation of mGluR1a and 5 was investigated in striatal neurons *in vivo* and *in vitro* (Orlando et al., 2002). It was found that mGluR5 was tyrosine phosphorylated while no tyrosine phosphorylation of mGluR1a was detected. Tyrosine phosphorylation of mGluR5 was enhanced by the tyrosine phosphatase inhibitor pervanadate, indicating the receptor is normally subject to an active endogenous cycle of phosphorylation and dephosphorylation. Further biochemical results show that the tyrosine phosphorylation level of mGluR5 is positively correlated to the efficacy of receptor signaling as the tyrosine phosphatase inhibitor produced parallel increases in tyrosine phosphorylation and mGluR5-mediated PI hydrolysis (Orlando et al., 2002). Apparently, tyrosine phosphorylation supports mGluR5 function as opposed to the inhibitory regulation of it by PKC-mediated phosphorylation. In the case of crosstalk well known between *N*-methyl-D-aspartate (NMDA) receptors and mGluR5, NMDA could potentiate mGluR5 function through either enhancing tyrosine phosphorylation or inhibiting PKC phosphorylation or both. In fact, NMDA treatment increased the level of tyrosine phosphorylation of mGluR5 (Orlando et al., 2002) contrast to reduced PKC phosphorylation of the receptor in response to NMDA (Alagarsamy et al. 1999; 2002). Another difference between PKC-mediated and tyrosine phosphorylation of mGluR5 is that the former is sensitive

to the group I mGluR agonists whereas the latter is not (Orlando et al., 2002). At present, accurate tyrosine phosphorylation site(s) have not been mapped in detail while there are only a few tyrosine sites in the C-terminus of mGluR5a (Fig. 1). It is unknown whether any specific subtype of tyrosine kinases and tyrosine phosphatases could directly interact with mGluR5 in heterologous cells or neurons.

Phosphorylation of mGluR1/5 by CaMKII

CaMKII is an attractive serine/threonine kinase that might be able to phosphorylate mGluR1/5 and regulate their function. CaMKII is highly abundant in brain cells, especially at synaptic sites (Kelly et al., 1984). The enzyme is supersensitive to Ca^{2+} as it is activated by the binding of Ca^{2+} and CaM. The activated kinase accesses and phosphorylates not only exogenous substrates but also its autophosphorylation site (T286 in the α isoform). This renders and sustains a partial Ca^{2+} /CaM-independent (autonomous) kinase activity even after the initial Ca^{2+} stimulus subsides (Hudmon and Schulman, 2002; Colbran and Brown, 2004). As such, CaMKII is capable of integrating information conveyed by diverse forms of local Ca^{2+} transients in a relatively long period of time. CaMKII has been shown to phosphorylate and regulate a large number of receptors and proteins at excitatory synapses (Yoshimura et al., 2002). With regard to mGluR1/5, multiple sites predicted for a phosphorylation substrate consensus, RXX(S/T) (White et al., 1998), can be found in the C-terminus of mGluR1a and mGluR5a (Fig. 1) based on a simple sequence analysis. Given the co-clustering of CaMKII and mGluR1/5 at the defined PSD microdomain, mGluR1 and/or 5 situate well as a substrate of CaMKII. Although no attempt has been reported to investigate the direct binding and phosphorylation of either mGluR1 or 5 by CaMKII, available data so far obtained from pharmacological studies indicate that CaMKII like PKC actively regulates mGluR1a. CaMKII activation is required for the internalization and homologous desensitization of mGluR1a in response to glutamate stimulation (Mundell, 2004). Similarly, active CaMKII contributes to the internalization and heterologous desensitization of mGluR1a in response to stimulation of muscarinic M1 receptors (Mundell et al., 2002; 2004). CaMKII, conventional PKC (α , β , γ), and CaM are all Ca^{2+} -sensitive molecules. If they bind to the intracellular region of group I mGluRs at either overlapping or adjacent sites, significant crosstalk among them is believed to occur for the sake of sophisticatedly coordinating mGluR1/5 responses to changing signals from glutamatergic synapses.

Phosphorylation of mGluR1/5 by GRKs

Several members of the GRK family (GRK1-7), including GRK2, GRK4, and GRK5, may phosphorylate mGluR1a. Constitutive and/or agonist-stimulated mGluR1a phosphorylation was enhanced by co-expression of GRK2 (Dale et al., 2000) or GRK4 (Sallese et al., 2000) in HEK293 cells. The agonist-stimulated mGluR1a phosphorylation was reduced by GRK2 dominant-negative mutants (Dale et al., 2000). These data indicate that the GRK isoforms assayed possess the ability to phosphorylate mGluR1a. A further study with a series of truncation mutants demonstrates that serine/threonine sites within the last 150–333 amino acids of mGluR1a C-terminus are subject to the phosphorylation by GRK2 (Dhami et al., 2002). However, without experiments, accurate residues phosphorylated by GRKs are difficult to predict because, unlike PKC and other second messenger-dependent protein kinases, a rigidly defined consensus sequence has not been established for GRKs.

One of most noticeable roles of GRKs is to mediate the agonist-promoted homologous desensitization of GPCRs, including group I mGluRs (Gainetdinov et al., 2004). GRKs can phosphorylate the receptor to facilitate the binding of β -arrestins that sterically interdict receptor-G protein coupling. GRK2, GRK4, and GRK5 have all been shown to contribute to mGluR1 desensitization in heterologous or Purkinje cells (Dale et al., 2000; 2001; Sallese et

al., 2000; Iacovelli et al., 2003). However, the GRK2-mediated phosphorylation is not required for the GRK2-dependent mGluR1a desensitization, but rather GRK2 desensitizes the receptor by the direct binding to the receptor through its regulator of G protein signaling (RGS) homology (RH) domain (Dhami et al., 2002; Dhami and Ferguson, 2006). The RH domain of GRK2 binds to the second intracellular loop of mGluR1a at K691/K692 (K677/678 in mGluR5a), which overlaps a region essential for G protein-coupling (Dhami et al., 2005). Thus, the binding could disrupt the G protein-coupling and reduce the efficacy of mGluR1 signaling. GRK2 may also play a role in PKC- and CaMKII-mediated desensitization of group I mGluRs as these enzymes seem to trigger GRK2 to associate with mGluR1a to promote an internalization and desensitization of mGluR1a homologously induced by glutamate or heterologously induced by an M1 agonist (Mundell, 2004). Finally, mGluR5, like mGluR1a, can also be regulated by GRK2 in a kinase activity-dependent manner (Sorensen and Conn, 2003). The T840 site in the mGluR5a C-terminus appears to be at least partially important for the GRK2 regulation of mGluR5 function.

Regulation of mGluR1/5 phosphorylation by PPs

The regulation of protein phosphorylation requires coordinated interactions between protein kinases and PPs. Phosphorylation of mGluR1/5 is therefore reasoned to be regulated by PPs. Evidence from pharmacological studies supports this notion. Application of okadaic acid, an inhibitor relatively selective for the serine/threonine phosphatase PP1/2A, prevented the recovery of mGluR5a from desensitization. PP2A also forms complexes with mGluR5 in neurons *in vivo* based on the data from coimmunoprecipitation (Mao et al., 2005a). These data together support a role of the okadaic acid-sensitive PPs in dephosphorylation of mGluR5 and recovery of the receptor from the PKC-mediated desensitization (Gereau and Heinemann, 1998).

Since mGluRs and ionotropic glutamate receptors are co-clustered in the localized PSD of excitatory synapses, a reciprocal regulation between them vigorously occurs in response to changing synaptic inputs. Activation of mGluR1/5 enhances NMDA receptor function, one of the most prominent effects of active mGluR1/5 in a number of brain regions (Fitzjohn et al., 1996; Benquet et al., 2002; Heidinger et al., 2002; Kotecha et al., 2003; Huang and van den Pol, 2007). Similarly, activation of NMDA receptors potentiates mGluR5-mediated responses (Lüthi et al., 1994; Challiss et al., 1994; Yang et al., 2004). Such positive crosstalk is important for organizing diverse forms of synaptic plasticity and contributes to the pathogenesis of various mental and neurodegenerative illnesses. To unraveling the mechanism underlying the NMDA receptor regulation of mGluR5, Alagarsamy et al. (1999; 2002) discovered a role of PPs. NMDA at low (5–15 μ M), but not high (50–100 μ M), concentrations enhanced mGluR5 responses through activation of a PP, followed by dephosphorylation of PKC phosphorylation sites on mGluR5 (such as S890) and thereby reduction of PKC-mediated desensitization. In support of this model, a PP2B selective inhibitor cyclosporine-A blocked the effect of NMDA on mGluR5-mediated responses. An mGluR5 mutant (S890G), a form of the receptor resistant to PKC-mediated desensitization, showed the lack of desensitization and the potentiating effect of NMDA. Noticeably, the effect of NMDA was insensitive to okadaic acid (Alagarsamy et al., 1999). This suggests an interesting scenario that the phosphatase responsible for the effect of NMDA may be a phosphatase (likely PP2B, also known as calcineurin) different from the okadaic acid-sensitive phosphatase (likely PP1/2A) responsible for natural recovery of mGluR5 from desensitization (Gereau and Heinemann, 1998).

The significant influence of PPs on mGluR1/5 phosphorylation suggests close vicinity or likely direct physical interactions between the two proteins. Indeed, PP1 γ 1 (PP1C1) in both recombinant and native forms binds to the C-terminus of long-form group I mGluRs, i.e., mGluR1a, mGluR5a, and mGluR5b (Crocì et al., 2003). Further mapping of PP1 γ 1-interacting

domains within mGluR1/5 C termini reveals a conserved PP1 γ 1 recognition motif 891-KSVSW-895 for mGluR1a and 880-KSVTW-884 for mGluR5a (Crocì et al., 2003). The fact that the PP1 γ 1-binding motif on mGluR1/5 falls within or immediately distal to possible PKC phosphorylation sites (such as S881 and S890 on mGluR5a) is noteworthy. It is currently unknown whether other PP isoforms (PP1/2A, PP2B, etc.) can directly bind to mGluR1/5. Nevertheless, by either directly binding to a substrate or by virtue of close physical proximity through binding to a molecule adjacent to a target substrate, protein kinases or PPs can sufficiently catalyze their substrates.

Summary

This mini-review highlights the recent progress in analyzing the phosphorylation and regulation of group I mGluRs. The long-form group I mGluRs (mGluR1a, mGluR5a, and mGluR5b) are characterized with a unique long C-terminal tail, which provides a solid basis for protein-protein interactions and phosphorylation. Available data mainly from heterologous expression systems suggest that a panel of protein kinases, including PKC, CaMKII, and GRKs, and PPs play a significant role in controlling the phosphorylation status of group I mGluRs. In addition, mGluR5 is subject to tyrosine phosphorylation. Phosphorylation certainly provides a dynamic mechanism for the regulation of mGluR1/5 function. There is evidence showing that the phosphorylation status of a specific amino acid residue could determine the Ca²⁺ response pattern after agonist stimulation of group I mGluRs. More importantly, the PKC-mediated phosphorylation of multiple serine/threonine sites in the intracellular regions of mGluR1/5 largely controls both homologous and heterologous desensitization of the receptor. Phosphorylation of the receptor is also believed to be an important posttranslational modification step for regulating the clustering, trafficking (internalization and externalization), anchoring, signaling, and turnover of the receptor (Liu et al., 2006). However, the phosphorylation research is still at its early stage as most studies of this kind have just examined phosphorylation-dependent implications through a pharmacological approach, and a direct biochemical detection of receptor phosphorylation itself is largely lacking (Kim et al., 2008). Of note, among numerous potential phosphorylation sites (serine, threonine, and tyrosine) in the long C-terminus, most of them have not been charted to any kinase or phosphatase even though not all of them will serve as phosphorylation sites. Besides PKC, other protein kinases and phosphatases, including those unmentioned in this review, have not been thoroughly explored for their binding and phosphorylation of group I mGluRs and for their roles in regulating receptor function via a phosphorylation mechanism. Future advanced studies will have to focus on biochemical characterizations of phosphorylation reaction and in-depth elucidation of molecular mechanisms for phosphorylation-regulated function in both recombinant receptors expressed in heterologous cells and native receptors in neurons. An example for a kinase-GPCR interaction was shown in our recent investigation concerning phosphorylation of limbic dopamine D3 receptors (D3R) by CaMKII *in vitro* and *in vivo* (Liu et al, unpublished observations). In a series of *in vitro* assays, purified recombinant CaMKII and D3R were first found to directly bind to each other. The two native proteins were next shown to form complexes *in vivo* in neurons. The binding of CaMKII to D3R was supersensitive to Ca²⁺, and the NMDA receptor provides a Ca²⁺ entry to regulate the binding. The CaMKII binding enables the kinase to phosphorylate D3R as detected by tandem mass spectrometry and by a phospho- and site-specific antibody. Finally, the CaMKII-D3R interaction modulates D3R signaling and D3R-sensitive limbic motor activity. Similar comprehensive analyses of endogenous mGluR phosphorylation and functional regulation will be important in the future.

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Fig. 1.

Schematic illustration of membrane topology of rat mGluR5a in terms of amino acid sequence (accession #: NP_058708/NCBI). The mGluR5b subtype contains an insert of 32 additional amino acids (aa) after L875. The illustrated regions include identified (filled circles, red) and potential (filled circles, blue) PKC phosphorylation sites, potential tyrosine phosphorylation sites (filled circles, green), potential CaMKII phosphorylation sites (open squares, red), and binding domains for G $\alpha_{q/11}$ protein-coupling (filled circles, yellow; Dhimi and Ferguson, 2006), CaM (open circles, red; Minakami et al., 1997), Homer (filled circles, black; Tu et al., 1998), PP1 γ 1 (filled circles, black; Croci et al., 2003), tamalin (filled circles, black; Kitano et al., 2002), GRK2 (filled circles, black; Dhimi et al., 2005) or Siah-1A (filled circles, black; Ishikawa et al., 1999). Note the overlap of some binding domains, indicating an existence of active crosstalk. CaM, calmodulin; CaMKII, Ca²⁺/calmodulin-dependent protein kinase II;

GRK, G protein-coupled receptor kinase; IL, intracellular loop; PKC, protein kinase C; PP, protein phosphatase; Siah-A, seven in absentia homolog-1A.