Metabotropic Glutamate Receptors in the Trafficking of Ionotropic Glutamate and GABA_A Receptors at Central Synapses

Min-Yi Xiao*, Bengt Gustafsson and Yin-Ping Niu

Institute of Physiology and Pharmacology, Göteborg University, Box 432, 405 30 Göteborg, Sweden

Abstract: The trafficking of ionotropic glutamate (AMPA, NMDA and kainate) and GABA_A receptors in and out of, or laterally along, the postsynaptic membrane has recently emerged as an important mechanism in the regulation of synaptic function, both under physiological and pathological conditions, such as information processing, learning and memory formation, neuronal development, and neurodegenerative diseases. Non-ionotropic glutamate receptors, primarily group I metabotropic glutamate receptors (mGluRs), co-exist with the postsynaptic ionotropic glutamate and GABA_A receptors. The ability of mGluRs to regulate postsynaptic phosphorylation and Ca²⁺ concentration, as well as their interactions with postsynaptic scaffolding/signaling proteins, makes them well suited to influence the trafficking of ionotropic glutamate and GABA_A receptors. Recent studies have provided insights into how mGluRs may impose such an influence at central synapses, and thus how they may affect synaptic signaling and the maintenance of long-term synaptic plasticity. In this review we will discuss some of the recent progress in this area: i) long-term synaptic plasticity and the involvement of mGluRs; ii) ionotropic glutamate receptor trafficking and long-term synaptic plasticity; iii) the involvement of postsynaptic group I mGluRs in regulating ionotropic glutamate receptor trafficking; iv) involvement of postsynaptic group I mGluRs in regulating GABA_A receptor trafficking; v) and the trafficking of postsynaptic group I mGluRs themselves.

Key Words: Metabotropic, ionotropic, glutamate receptor, GABA_A receptor, receptor trafficking, endocytosis, hippocampus, synaptic plasticity.

1. INTRODUCTION

Synaptic transmission between neurons in the mammalian central nervous system (CNS) is constantly modified in activity-dependent or independent ways. This plasticity of central synapses has considerable implications for brain function. It is generally believed that such plasticity, especially the long-lasting activity-dependent ones, underlies learning and memory processes and the developmental maturation of neural circuitry, as well as is involved in pathological conditions and neurodegenerative diseases [2, 12, 74]. Most excitatory central synapses use glutamate as neurotransmitter to activate primarily postsynaptically located ionotropic and metabotropic glutamate receptors. The ionotropic glutamate receptors include AMPA receptors (AMPA), NMDA receptors (NMDARs) and kainate receptors, and mediate (mostly) fast excitatory synaptic transmission. The metabotropic glutamate receptors (mGluRs), a class of G-protein coupled receptors, do not directly mediate fast synaptic transmission but have an essential role in regulating many cellular functions, including synaptic transmission (Fig. 1). At inhibitory synapses, fast synaptic inhibition is primarily mediated through the activation of postsynaptic ionotropic GABA_A receptors by the neurotransmitter GABA [2].

The hippocampus is a CNS structure which is not only crucial for information processing and learning and memory formation, but is also prone to diseases such as Alzheimer and epilepsy. The ionotropic and metabotropic glutamate

receptors and GABA_A receptors mediate much of the synaptic transmission in the hippocampus and are indispensable for its physiological function and for the development of its pathology. Therefore, the synaptic transmission mediated *via* glutamate and GABA_A receptors has been the subject of extensive studies. Recent studies have indicated that trafficking of ionotropic glutamate and GABA_A receptors in and out of, or laterally along, the postsynaptic membrane of hippocampal neurons is an important mechanism underlying synaptic plasticity [16, 19, 23, 72]. Important progress has been made in revealing how this trafficking of ionotropic receptors is regulated, and an involvement of postsynaptic metabotropic glutamate receptors, specifically groups I mGluRs, in this trafficking has been suggested [60, 107, 125]. The mGluRs affect synaptic function and plasticity *via* their diverse actions on neurons/synapses, and in the present review the overall influence of mGluR activation on synaptic plasticity will be briefly described. However, the main focus will be the recent progress concerning the role of group I mGluRs in glutamate and GABA_A receptor trafficking, and thereby in the long-term synaptic plasticity. The review will be based largely on studies on hippocampal synapses, but when found relevant, will include data from other central synapses or cells.

2. MGLURS AND LONG-TERM SYNAPTIC PLASTICITY AT CENTRAL SYNAPSES

2.1. mGluRs at Central Synapses

The mGluRs differ from ionotropic glutamate receptors in that they are coupled to and exert their effects *via* G-proteins. The mGluRs are divided into three groups based on

*Address correspondence to this author at Institute of Physiology and Pharmacology, Göteborg University, Box 432, Medicinaregatan 11, 405 30 Göteborg, Sweden; Tel: +46 31 7733516; Fax: +46 31 7733505; E-mail: xiao@physiol.gu.se

Fig. (1). Schematic structure and function of a hippocampal CA1 glutamatergic synapse. Postsynaptic AMPARs, NMDARs and kainate receptors are depicted as tetrameric complexes. AMPARs are linked to scaffolding proteins GRIP/ABP. NMDARs are associated with scaffolding and signaling proteins such as PSD-95, CaMKII, nNOS and GKAP. Kainate receptors are depicted alone for simplification. The mGluRs are illustrated as proteins with seven transmembrane segments. They are linked to a scaffolding protein Homer which in turn can mediate an interaction between mGluRs and NMDARs *via* Shank protein as well as mediate an interaction between mGluRs and intracellular Ca²⁺ stores *via* IP₃R. Cell adhesion molecules such as cadherins and neuroligin/neurexin link the presynaptic and postsynaptic membranes. Glutamate released from presynaptic vesicles by exocytosis mainly binds to postsynaptic AMPARs, NMDARs or kainate receptors which open channels, allowing various ions to pass. A rise in Ca²⁺ in the postsynaptic terminal can trigger intracellular signaling cascades which can alter the efficacy of synaptic transmission. The ion passage through the receptor channels results in synaptic currents and potentials which can be detected and recorded using electrophysiology techniques. Meanwhile, the released glutamate binds also to postsynaptic and presynaptic metabotropic glutamate receptors which results in changes in the protein kinase C (PKC) activity, Ca²⁺ release from intracellular stores or cAMP levels. A representative intracellular pool of AMPARs is also shown.

the homology of their amino acid sequences. The group I includes mGluR1 and mGluR5, group II includes mGluRs2-3, and group III includes mGluR4 and mGluRs6-8. Activation of group I mGluRs causes phosphoinositide hydrolysis by phospholipase C (PLC), resulting in protein kinase C (PKC) activation *via* diacylglycerol (DAG) and Ca²⁺ mobilization from intracellular Ca²⁺ stores *via* inositol 1,4,5-trisphosphate (IP₃). On the other hand, activation of group II and III mGluRs results in inhibition of adenylyl cyclase and reduced levels of cAMP. These actions in turn have diverse cellular effects, such as modulation of intrinsic postsynaptic excitability (e.g. *via* voltage-dependent K⁺ channels), of presynaptic neurotransmitter release (e.g. *via* voltage-dependent Ca²⁺ channels) or direct modulation of postsynaptic ionotropic receptors [4, 24, 25, 109]. In the CNS, group I mGluRs are primarily located at the postsynaptic side, whereas group II and III mGluRs are primarily located at the presynaptic membrane. Since mGluRs often co-localize with the ionotropic glutamate and GABA_A receptors at the same synapses, their roles in regulating the synaptic functioning of

ionotropic glutamate and GABA_A receptors have been much studied. Even though the main focus of this review will be the role of group I mGluRs in glutamate and GABA_A receptor trafficking in long-term synaptic plasticity, we will first introduce some basic facts concerning synaptic transmission and plasticity, and effects thereupon by mGluR activation.

2.2. Long-Term Synaptic Plasticity Induction

At excitatory central synapses, certain types of stimulation, e.g. a repetitive stimulation, can lead to long-lasting changes in synaptic strength, preferentially related to AMPAR-mediated synaptic signaling [12]. These changes manifest themselves as alterations in the total amount of passage of ions through the ionotropic glutamate receptors, which can be detected and recorded using electrophysiological techniques. The changes are normally seen as long-lasting (on a time scale of hours to weeks/months) increase or decrease in excitatory postsynaptic currents (EPSCs) or

potentials (EPSPs). Long-term potentiation (LTP) and long-term depression (LTD) are two prominent forms of such synaptic plasticity, and are seen as the cellular basis for learning and memory formation and for neural development [12, 73, 74]. At inhibitory synapses such long lasting changes in synaptic strength, expressed as an increase or decrease in the inhibitory postsynaptic currents (IPSCs) or potentials (IPSPs), can modulate the excitability of excitatory neurons, cause neuronal oscillation and synchronization, and regulate the synaptic plasticity at excitatory synapses [36]. There are, in the hippocampus and other brain areas, different forms of LTP and LTD, generally subdivided into NMDAR-dependent, or -independent, after whether their induction depends critically on the activation of NMDARs or not. In the case of NMDAR-dependent LTP/LTD, their induction mechanisms are relatively well defined. It is commonly agreed that their induction requires postsynaptic NMDAR channel opening and Ca^{2+} influx through these channels, this influx giving rise to LTP or LTD depending on the temporal and spatial characteristics of the Ca^{2+} rise in the postsynaptic compartment [131]. It has also been suggested that NMDARs with different subunit composition (subtype NR2A versus NR2B) or synaptic locations (synaptic versus extrasynaptic) by coupling to distinct intracellular signaling machineries should be critical for determining whether LTP or LTD is induced [66, 79]. However, this notion has not been consistent with earlier experiments carried out in genetically modified mice [57, 58, 111, 121], and has been questioned by a recent study showing that both NR2A and NR2B subtypes of NMDARs are capable of inducing LTP, and possibly also LTD [10]. In the case of NMDAR-independent LTP/LTD, their induction mechanisms are less clear, but may also involve synaptic Ca^{2+} rise which can be achieved by Ca^{2+} influx through other channels, e.g. via voltage-dependent Ca^{2+} channels, or/and by Ca^{2+} release from intracellular stores [12, 74, 110, 112]. This can also occur presynaptically, such as for LTP at the hippocampal mossy fiber-CA3 pyramidal neuron synapse [73, 89] (but see Yeckel *et al.* [127] and Henze *et al.* [41]). There have been a considerable number of studies investigating how different factors may influence the induction of both NMDAR-dependent and -independent long-term synaptic plasticity, and mGluRs have been shown to play an important role under certain conditions.

2.3. mGluRs Involvement in Long-Term Synaptic Plasticity Induction

Various studies have shown that mGluRs are either necessary, or dispensable, or irrelevant, for the induction of different forms of long-term synaptic plasticity. There are many reasons that can account for such discrepancies, including different forms of synaptic plasticity studied, different experimental conditions/parameters, different developmental stages of glutamate/GABA_A receptors, specific agonists/antagonists used, and the timing of mGluR activation etc. [4, 13, 81]. Nonetheless, mGluR activation has been found to modulate the induction of NMDAR-dependent long-term synaptic plasticity. For example, in the hippocampal CA1 area the broad spectrum mGluR agonist 3R-dicarboxylic acid (ACPD) was found to facilitate, or prime,

the subsequent induction of LTP induced by high frequency stimulation, an effect involving group I mGluRs since it was antagonized by the group I mGluR antagonist L-2-amino-3-phosphonopropionic acid [21], as well as was induced by the specific group I mGluR agonist 3,5-dihydroxyphenylglycine (DHPG) [22]. By using the selective mGluR1 antagonist +/- 2-methyl-4-carboxyphenylglycine (LY367385) and the selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP), mGluR5 has been found to be involved in the induction of the late (protein synthesis-dependent) phase of NMDAR-dependent LTP induced by strong stimulation, while both mGluR1 and mGluR5 were involved in the induction of LTP obtained using weak stimulation [34]. A blockade of group I mGluRs, specifically mGluR5, has also been shown to impair LTP induced by high frequency stimulation as well as the spatial learning in animals *in vivo* [86, 87]. All these effects were observed at concentrations (of mGluR agonists or antagonists) which did not affect basal synaptic transmission [21, 30, 34]. Neither did the priming effect depend on altered NMDA signaling since e.g., a blockade of the DHPG-induced potentiation of NMDA responses by mGluR5 antagonist LY344545 failed to affect the priming effect [30]. These observations raise the possibility that group I mGluRs may affect plasticity induction *via* e.g. influencing certain phases of receptor trafficking so that receptors are more easily available when the plasticity induction is applied. The genetic approach has also been used for studying the involvement of mGluR subtypes in synaptic plasticity induction [53]. For example, mice lacking mGluR5 showed impaired learning and reduced CA1 NMDAR-dependent LTP [69]. The mGluR1- and mGluR5-knockout mice have also been demonstrated to have reduced corticostriatal LTP [39].

Besides the fact that, as discussed above, group I mGluR activation can affect the induction properties, such as the threshold, of the NMDAR-dependent long-term synaptic plasticity, group I mGluR activation can under certain conditions also induce synaptic plasticity. This plasticity is also primarily expressed as a long-lasting change of AMPAR-mediated synaptic signaling and does not require NMDAR activation for its induction. For example, synaptic activation by low frequency stimulation (1-2 Hz) for several minutes under the blockade of NMDARs resulted in a form of LTD which is solely dependent on activation of mGluRs [102] or more specifically group I mGluRs [107], indicating that synaptically released glutamate is sufficient to activate the group I mGluRs to induce synaptic plasticity. Application of exogenous mGluR agonists, especially group I agonists alone, or in combination of synaptic activation, has also been used to study the role of mGluR activation in synaptic plasticity. For example, in hippocampal slices, ACPD application together with a synaptic activation resulted in LTP while ACPD application alone resulted instead in LTD. Thus mGluRs may co-activate with NMDARs to induce LTP but induce LTD alone *via* its action on IP3 receptors [35]. In hippocampal slices, DHPG (group I mGluR agonist) application for a few minutes resulted in LTD [102, 107, 125, 129] which was dependent on dendritic protein synthesis [107]. Application of DHPG was also found to depotentiate a previously established LTP and this

depotentialization was mediated primarily by mGluR5 and depended also on protein synthesis [129]. In the hippocampus *in vivo*, DHPG was found to induce a slow-onset LTP which correlated with increased expression levels of certain proteins and immediate early genes, and which may contribute to late phases of synaptic plasticity [14]. This slow-onset LTP was associated with an up-regulation of mGluR5 (and down-regulation of mGluR2/3), as seen by using Western blot or *in situ* hybridization [77]. Notably, in hippocampal slices, activation of group I mGluRs by a prior preconditioning stimulation can produce a PKC-dependent inactivation of these mGluRs, which in turn inhibits the induction of group I mGluR-induced LTD/depotentialization, resulting in a form of metaplasticity [122].

3. IONOTROPIC GLUTAMATE RECEPTOR TRAFFICKING AS AN IMPORTANT MECHANISM FOR LONG-TERM SYNAPTIC PLASTICITY EXPRESSION

The induction of long-term synaptic plasticity has overall been relatively well defined, and, as discussed above, group I mGluRs have been found to play a role in this induction. However, how the synaptic modifications (normally a persistent up- or down-regulation of AMPAR-mediated synaptic signaling) are sustained during the course of hours to weeks/months is much less understood. Recently, it has been increasingly realized that membrane receptor trafficking is an important mechanism by which neurons can rapidly modify their responses to external/internal stimuli. The membrane trafficking of ionotropic glutamate and GABA_A receptors has thus emerged as an important mechanism for long-lasting synaptic plasticity [16, 23]. Before discussing the possible role of group I mGluRs in it, we will briefly introduce the synaptic trafficking of ionotropic glutamate receptors in the context of long-term synaptic plasticity.

3.1. AMPAR Trafficking

New evidence suggests that AMPARs are constitutively cycling in and out of the synapse, or laterally along the synaptic membrane, on a rapid time scale (seconds to minutes), and that a change in such trafficking is an important mechanism for expressing and sustaining long-lasting synaptic plasticity [16, 76]. In hippocampal slices, LTP was demonstrated to be associated with exocytosis of postsynaptic AMPARs [67], and in cultured neurons, NMDAR activation was shown to induce membrane insertion of new AMPARs and LTP [68]. For the endocytosis of AMPARs, the clathrin-mediated endocytosis pathways are involved, and redistribution of synaptic AMPARs can be regulated by neuronal activity [75]. NMDAR activation can trigger AMPAR endocytosis *via* activation of the calcium-dependent protein phosphatase calcineurin [7]. Another form of receptor trafficking involves a lateral movement of AMPARs at the postsynaptic membrane on a very fast time scale (seconds) [19], and theoretical work predicts that such lateral movement can significantly regulate synaptic strength [101]. Endocytosis of synaptic AMPARs may in fact follow a two-step model in which receptors first diffuse laterally to extrasynaptic sites before they are subsequently internalized [96, 130]. One protein that has convincingly been shown to

regulate AMPAR trafficking is stargazin, a membrane AMPAR interacting protein [18] which, in addition, can regulate AMPAR gating [95, 113]. Recently, AMPAR-mediated signaling was shown to be quickly silenced on a fast time scale (seconds) at hippocampal CA3-CA1 synapses in the developing hippocampus, presumably *via* removal of postsynaptic AMPARs [124]. This result indicates that a very low level of synaptic activity can result in a rapid change in AMPAR trafficking, at least at developing central synapses.

3.2. NMDAR Trafficking

Recent studies indicate that NMDARs are also very mobile, both capable of cycling in and out of postsynaptic membranes [5, 38, 94, 98] and of moving laterally between synaptic and extrasynaptic pools [115]. A study of endoplasmic reticulum (ER) retention of NMDAR subunits and of their transportation from ER to the plasma membrane showed that activity-dependent mRNA splicing can control the export from ER and the synaptic delivery of NMDARs [82]. However, how the synaptic trafficking of NMDARs is regulated under physiological and pathological situations remains less clear [120]. PKC can induce a rapid delivery of functional NMDARs and an increase in NMDAR clusters on the surface of dendrites and dendritic spines, and activation of mGluRs can enhance such an NMDAR delivery to the postsynaptic membrane [60]. However, in another study, using cultured rat hippocampal neurons, the opposite effect was found in that a selective activation of PKC with phorbol esters induced a rapid dispersal of NMDARs from synaptic to extrasynaptic plasma membrane [32]. By using single-molecule tracking in hippocampal neurons, it was demonstrated that, like AMPARs, NMDARs displayed a high degree of lateral mobility at synapses. This NMDAR trafficking was shown to be regulated by PKC activation but not by neuronal activity [37].

3.3. Kainate Receptor Trafficking

Kainate receptors contribute to excitatory postsynaptic currents in many CNS regions including the hippocampus. They can play both post- and presynaptic roles, and be involved in short- and long-term synaptic plasticity as well as in pathological conditions [48]. A change in the trafficking of kainate receptors would alter their regulatory functions [28, 50, 51]. Indeed, it has been found that kainate receptor subunits GluR5-6 interact with postsynaptic PDZ domain-containing proteins at mossy fiber-CA3 synapses [46], making kainate receptors possible targets for trafficking. Nevertheless, kainate receptor trafficking seems to employ mechanisms distinct from those of the AMPARs [18] and multiple trafficking signals are involved in the trafficking of kainate receptors subtypes, e.g. GluR5-6 and KA2 [97, 126]. Different splice variant isoforms of GluR5 and GluR6 (GluR5a-c, GluR6a-b) have different surface expression at the neuronal membrane. For example, GluR6a is rich at the membrane while GluR6b, GluR5a-c are rich in ER, suggesting that the splice variants and subunit composition of kainate receptors will affect their trafficking from the ER to the plasma membrane [52].

4. GROUP I MGLURS REGULATE IONOTROPIC GLUTAMATE RECEPTOR TRAFFICKING

4.1. Interaction Between Group I mGluRs and Scaffolding/Receptor Proteins

Group I mGluRs (mGluR1 and mGluR5) are believed to exist primarily postsynaptically at hippocampal synapses and to be linked to postsynaptic PSD-95 *via* two scaffolding proteins Homer and Shank [42, 105]. Shank, as well as GKAP/SAPAP (a family of proteins highly concentrated in the PSD and bound to the guanylate kinase domain of PSD-95), cross-link the mGluR1/5/Homer complex with the NMDAR/PSD-95 complex, and therefore plays a role in the signaling mechanisms of both mGluRs and NMDARs [88, 116]. Both mGluR1 and mGluR5 undergo alternative splicing to generate isoforms, such as mGluR1a-d, and mGluR5a-b, differing in their C-terminal sequence, which may be differentially regulated. The Homer/Ves1 proteins have been increasingly indicated as major regulating proteins for group I mGluRs [31, 123]. A PSD-95/PDZ domain-containing protein termed tamalin was shown to interact with group I mGluRs, especially mGluR1a and mGluR5, *via* its PDZ domain-containing terminal [55]. At the same time, PSD-95 family members also interact with NMDAR and AMPAR subunits (NR2 and GluR1). Postsynaptic mGluR5 and mGluR1 are thereby well positioned such that their activation can influence NMDAR and AMPAR functions. It has been suggested that there is a reciprocal positive feedback interaction between mGluR5 and NMDARs [1].

4.2. Group I mGluRs Regulate Glutamate Receptor Trafficking at Central Synapses

As described above, hippocampal CA3-CA1 excitatory synapses display a form of LTD which is dependent on group I mGluR activation [102, 107, 125]. This activation has been found to lead to an internalization of AMPARs/NMDARs and a loss of receptors from the surface membrane of synapses [107, 125]. This result indicates that group I mGluRs have the ability to regulate the trafficking of AMPARs and NMDARs at hippocampal synapses. In a further study, activation of the p38 mitogen-activated protein kinase (p38 MAPK) was shown to be involved in the induction of this LTD. A potential downstream process of the p38 MAPK activation is the formation of the guanyl nucleotide dissociation inhibitor-Rab5 complex leading to an accelerating AMPAR endocytosis [47]. The DHPG (group I mGluR agonist) induced-depotentiation of previously established LTP was found to be associated with a reduced increase of the surface expression of AMPARs seen with LTP, suggesting that activation of mGluR5 triggers a protein synthesis-dependent internalization of synaptic AMPARs [129]. An mGluR-dependent LTD based on an internalization of AMPARs has also been indicated in other structures than hippocampus. For example, in nucleus accumbens (NAc) neurons, glutamate-induced AMPAR internalization was affected by the group I mGluR agonist DHPG and antagonist PHCCC [78]. In neostriatal neurons, activation of group I mGluRs by DHPG can regulate the activity of casein kinase 1 (CK1) which has been implicated in a variety of cellular functions, including intracellular trafficking [63]. DHPG

activates CK1 *via* Ca^{2+} -dependent stimulation of calcineurin followed by a subsequent dephosphorylation process [65] which resembles the process of DHPG-induced AMPAR endocytosis. At the same time, activation of mGluR1 accelerates NMDAR insertion into the membrane [60]. The mGluR5 is also subjected to endogenous phosphorylation and dephosphorylation which can be influenced by NMDAR activation. This suggests that endogenous phosphorylation regulatory mechanisms can be used to mediate crosstalk between synaptic ionotropic and metabotropic glutamate receptors [92]. It has been previously shown that ionotropic (GABA_A) and metabotropic (dopamine D5) receptors can have direct physical interaction in hippocampal neurons [64]. It will be interesting to see whether there can be direct protein-protein interaction between group I mGluRs and ionotropic glutamate receptors, and how this would influence the trafficking of the latter in the hippocampal neurons. However, the recently described AMPAR silencing (and trafficking) in the developing hippocampus appears not to be regulated by mGluR activation [124]. This would imply that there might be multiple mechanisms or pathways for the trafficking of ionotropic glutamate receptors. From a study using a fluorescent marker to monitor presynaptic activity during mGluR-induced LTD at hippocampal CA3-CA1 excitatory synapses, it was suggested that the LTD can be explained by an altered presynaptic transmitter release, even though the group I mGluRs are activated postsynaptically [128]. Morphological changes in terms of synapse elimination was also reported for this LTD [106]. However, a presynaptic expression for mGluR-induced synaptic plasticity may be limited to the neonatal period, there being a developmental switch from presynaptic to postsynaptic expression with age [90].

4.3. Molecular Mechanisms for Group I mGluRs in Regulation of Receptor Trafficking

The molecular mechanisms for receptor (especially AMPAR) trafficking have been extensively investigated. Numerous studies have identified several receptor interacting proteins and shown that they play important roles in the receptor trafficking and clustering at the synaptic membrane [23, 76]. These are proteins that either contain, or do not contain, PDZ domains, a protein-protein interaction motif. The glutamate receptor interacting proteins/AMPA binding protein (GRIP/ABP), protein interacting with C-kinase (PICK1), and synapse associated protein 97 (SAP97) are examples of PDZ containing proteins. The N-ethylmaleimide sensitive fusion protein (NSF), soluble NSF attachment proteins (α , β -SNAP), Lyn (a Src-family non-receptor protein tyrosine kinase), the guanine nucleotide binding protein (G α i1) and stargazin are examples of proteins which do not contain PDZ domains [15, 16, 28, 40, 54, 105].

There are several possible ways for mGluRs to intervene in the trafficking of ionotropic receptors to the synaptic membrane. First, the activation of group I mGluRs can, *via* IP_3 , result in Ca^{2+} concentration changes in the postsynaptic compartment, thus possibly regulating the receptor interacting proteins mentioned above. Endocytosis of AMPARs may involve the Ca^{2+} -dependent protein phosphatase calcineurin [7, 62]. The NR2B subunit of the NMDAR can also be

modulated by Ca^{2+} via Ca^{2+} -dependent CaMKII [6]. Thus, group I mGluRs are likely to take part in the regulation of receptor trafficking through Ca^{2+} release from intracellular Ca^{2+} stores. Second, activation of PKC, via DAG, can also regulate certain receptor interacting proteins, e.g., disrupting the interaction between PICK1 and intracellular AMPAR clusters resulting in a rapid translocation of AMPARs to the membrane surface. PKC can also induce a rapid delivery of functional NMDARs to the synaptic surface membrane [59, 60]. Thus, the activation of group I mGluRs can modulate AMPAR and NMDAR trafficking also through PKC activation. Finally, tyrosine kinases have been indicated to interact with GRIP and PICK1 [15]. GRIP/ABP have also been shown to interact with Ephrin receptors and their ligands which activate also Src tyrosine kinase [17, 114]. At the mossy fiber-CA3 synapse, synaptic activation of mGluR1 can lead to G-protein-independent activation of a Src-family tyrosine kinase [44, 45].

For kainate receptors, scaffolding proteins that contain PDZ domains, such as PSD-95, SAP97, PICK1 and GRIP were shown to interact largely with GluR6a, and to less extent with other subtypes. The PKC-dependent phosphorylation of the C-terminal domain of GluR6 can stabilize its binding with GRIP, thus indicating that group I mGluR can regulate kainate receptor trafficking and clustering through regulating PKC activity in the synapse [51]. Kainate receptors have also been shown to interact with cadherin/catenin [26], implying that group I mGluR may also intervene in kainate receptor function/trafficking by regulating the Ca^{2+} -dependent intercellular adhesion molecules cadherin/catenin.

Interestingly, group I mGluR activation can lead to both LTP and LTD which both have been shown to involve receptor trafficking in their expression. As discussed above, group I mGluR activation may regulate receptor trafficking via different signaling pathways, therefore it is possible that different stimulation protocols such as those used for inducing LTP and LTD, respectively, may engage those pathways differentially, contributing to either LTP or LTD. For example, the high frequency stimulation used to induce LTP was suggested to activate PKC pathways while DHPG induces LTD via activation of the p38 MAPK pathway [100]. Besides, group I mGluR activation may only be required for certain aspects of LTP induction, e.g. to lower the induction threshold while LTD may require group I mGluR activation to trigger induction as well as to maintain the receptor trafficking during its expression. Finally, group I mGluR activation by LTD-inducing stimulation triggers protein synthesis process that is required for the receptor trafficking [107] while there is so far no evidence that receptor trafficking during LTP requires protein synthesis.

5. GABA_A RECEPTOR TRAFFICKING AND GROUP I MGLURS

Each GABA_A receptor consists of several subunits in a heteromeric complex. There are multiple GABA_A receptor subtypes (predominantly composed of α , β and γ subunits) differentially expressed in distinct neuronal populations [103]. The inhibitory activity mediated by ionotropic GABA_A receptors normally imposes an effective constraint on

hippocampal activity, a structure known to be prone to CNS pathology such as epilepsy. An altered GABA transmission by changed GABA_A receptor trafficking can thus have a profound effect on normal synaptic transmission, as well as situations associated with epileptogenesis. In fact, GABA transmission can be both up- and down-regulated in a long-term manner [70, 91]. Similar to the ionotropic glutamate receptors, a change in GABA_A receptor number on the postsynaptic membrane is an efficient means of regulating GABA_A signaling [56, 72, 91, 117, 119]. The surface number and subunit stability of GABA_A receptors is regulated by the ubiquitin-like protein Plic-1 which blocks the degradation of ubiquitinated receptors and enhance the insertion of GABA_A receptors into the membrane [9]. PKB(also called Akt) can phosphorylate GABA_A receptors both *in vitro* and *in vivo*, and increase the surface number of receptors [119]. A 17 kDa GABA_A receptor-associated protein (GABARAP) was identified to interact with the GABA_A $\gamma 2$ subunit, microtubules, and NSF which is involved in receptor endocytosis. GABARAP can affect GABA_A receptor trafficking in cultured hippocampal neurons [61]. By using antibody tagging in rat hippocampal neurons in culture, internalized GABA_A receptors were found mainly at postsynaptic sites where the GABA_A receptor anchoring protein gephyrin interacts with cytoplasmic proteins [118]. It has also been shown that GABA_A receptor trafficking can be regulated by the same glial-generated protein as for AMPA receptor trafficking, but with the opposite effect. Thus, in hippocampal pyramidal cells tumor necrosis factor- α (TNF α) causes a rapid exocytosis of AMPARs but endocytosis of GABA_A receptors [8, 108]. Similar to ionotropic glutamate receptors, the trafficking of GABA_A receptors may also be regulated through PKC, or calcineurin, pathways through the activation of group I mGluRs [56, 72]. Indeed, activation of PKC modulates GABA_A receptor internalization via a $\beta 2$ subunit motif, in both cerebral cortical neuronal slices and cultures [43]. Functionally, group I mGluRs were suggested to contribute to the sustained potentiation of GABA_A synaptic transmission in pyramidal cells induced by theta burst stimulation [93].

6. TRAFFICKING OF GROUP I MGLURS AT POSTSYNAPTIC MEMBRANE

In recent years, the trafficking of mGluRs themselves has also received substantial attention. In non-neuronal systems mGluRs can be transported to and from the surface of cells in response to external stimuli. For example, by using combined imaging of Ca^{2+} and GFP fluorescence in single human embryonic kidney (HEK) 293 cells to simultaneously monitor the movement and function of mGluR1a, it was shown that the surface expression of mGluR1, like that of ionotropic glutamate receptors, can be rapidly regulated in response to agonist activation [29]. The trafficking of group I mGluRs was found to be regulated by Homer-1b and -1c. Whereas Homer-1b inhibits the surface expression of mGluRs [99] Homer-1c induces clustering of mGluR1a in the plasma membrane [20]. Agonist-induced internalization of mGluR1a is arrestin- and dynamin-dependent [83] and is subjected to PKC, CaMKII and PKA regulation [84, 85]. It was also shown in the same cell system that the agonist-dependent mGluR1a internalization is β -arrestin dependent

while the agonist-independent internalization involves the β -arrestin-independent targeting of mGluR1a to clathrin-coated vesicles [27]. When the C6 glioma cells were treated with L-glutamate, quisqualate or ACPD, cell surface mGluRs were down-regulated through clathrin-mediated endocytosis [71].

In neuronal systems, the cell surface expression of mGluR5 in cultured cerebellar granule cells depends on Homer-1 protein [3]. Depolarized Purkinje cells can increase their cell surface mGluR1 by blocking their internalization through MAPK activity and Homer-1a expression [80]. The Homer/Vesl proteins have been implicated in the regulation of group I mGluR trafficking in neurons [31, 123]. For example, Homer-1c was found to reduce the rate of loss of mGluR1a from the cell surface and to increase the extent of dendritic trafficking of the receptor in rat primary cultured neurons [20]. In primary hippocampal neurons in culture mGluR5 is endocytosed by a clathrin-independent pathway [33]. Using single particle tracking to follow mGluR5 movement in real time, mGluR5 was found to be transported actively on the cell surface through actin-mediated retrograde transport of microtubules [104]. The intracellular signaling system in the mGluR endocytosis seems to involve the small GTP-binding protein Ral, its guanine nucleotide exchange factor, and phospholipase D2 which are constitutively associated with mGluR1 and regulate constitutive mGluR1 endocytosis in both HEK cells and neurons [11]. The agonist-stimulated differential sorting of the mGluR1 receptor and β -arrestin, as well as the activation of MAP kinases by mGluR1 agonist, were demonstrated in cultured cerebellar Purkinje cells [49]. Tamalin, a PSD-95/PDZ domain-containing protein, plays a key role in the association of group I mGluRs with members of small GTP-binding proteins and their related proteins. This association contributes to the trafficking and localization of group I mGluRs at hippocampal cell culture synapses [55]. Therefore, mGluR1 and mGluR5 are constitutively or activity-dependently internalized in heterologous cell cultures, neuronal cultures, and intact neuronal tissues. Similar to ionotropic glutamate receptors [23], the proper targeting of mGluRs at synapses is essential for their synaptic functions. Regulation of mGluR trafficking would provide a powerful means to modulate the long-term synaptic plasticity of ionotropic glutamate and GABA_A receptors, and other synaptic functions in which mGluRs are involved.

CONCLUSIONS

The knowledge about the roles of mGluRs in glutamate and GABA_A receptor trafficking can provide a basis for treating many central nervous system diseases, and facilitate potential clinical uses of mGluR agonists/antagonists for such treatments. The challenges in the future will be to establish the specific molecular mechanisms involved in the biochemical signaling cascades for group I mGluRs in the regulation of the trafficking of the ionotropic receptors, to determine the structural determinants underlying interaction between mGluRs and ionotropic receptors at postsynaptic compartments, and to identify the physiological and pathological factors which can contribute to the alterations of mGluRs' interaction with other ionotropic receptors.

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