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Role of presynaptic metabotropic glutamate receptors in the induction of long-term synaptic plasticity of vesicular release

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Abstract

While postsynaptic ionotropic and metabotropic glutamate receptors have received the lions share of attention in studies of long-term activity-dependent synaptic plasticity, it is becoming clear that presynaptic metabotropic glutamate receptors play critical roles in both short-term and long-term plasticity of vesicular transmitter release, and that they act both at the level of voltage-dependent calcium channels and directly on proteins of the vesicular release machinery. Activation of G protein-coupled receptors can transiently inhibit vesicular release through the release of Gβγ which binds to both voltage-dependent calcium channels to reduce calcium influx, and directly to the C-terminus region of the SNARE protein SNAP-25. Our recent work has revealed that the binding of Gβγ to SNAP-25 is necessary, but not sufficient, to elicit long-term depression (LTD) of vesicular glutamate release, and that the concomitant release of Gαi and the second messenger nitric oxide are also necessary steps in the presynaptic LTD cascade. Here, we review the current state of knowledge of the molecular steps mediating short-term and long-term plasticity of vesicular release at glutamatergic synapses, and the many gaps that remain to be addressed.

Keywords

Group II metabotropic glutamate receptors; G protein-coupled receptors; Giα; Gβγ; long-term synaptic depression; SNAP-25; SNARE protein; synaptic plasticity; vesicular release

1. Introduction

Neurons communicate with one another via chemical messengers called neurotransmitters. Synaptic transmission, i.e. the process of transmitting electrical impulses from one neuron to the other via a chemical intermediate, is crucial for the normal function of a neural network, and perturbations of these junctions have been correlated with many neurological diseases, such as Alzheimer's, schizophrenia and Parkinson's (Cook and Leuchter, 1996; Huang et al., 2011; Nathan et al., 2011). Synapse strength can be bi-directionally modified in an activity dependent manner, and these changes can be brief or persistent. William James (1890), at a time when the neuron doctrine and the role of synapses in intercellular communication had

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yet to emerge, postulated that long-lasting alterations in the strength of connections between excitable elements of the brain could be a way in which memories are stored, an early "connectionist" view that is as current over a century later.

Homosynaptic long-term potentiation (LTP) is a persistent, synapse-specific strengthening of synaptic transmission induced by brief bursts of high frequency synaptic activity (100–200 Hz), whereas long-term synaptic depression (LTD) is weakening of synaptic strength, usually triggered by low frequency activation (1–5 Hz) for prolonged periods (10–15 min). These persistent changes in synaptic strength are widely considered to be leading candidates for cellular mechanisms of memory storage (Bliss et al., 2006; Pastalkova et al., 2006; Whitlock et al., 2006). Glutamate serves as the main excitatory neurotransmitter in the central nervous system and uses a myriad of receptor subtypes to activate ionic channels to change membrane potential, and G protein-coupled receptors to initiate downstream signal transduction. The glutamate receptor family can be divided into two types: **a)** fast, ionotropic glutamate receptors that include 2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid (AMPA), N-Methyl-D-aspartate (NMDA) and kainate receptors (Madden, 2002) and **b)** G-protein coupled metabotropic receptors that act via second messenger cascades (mGluR) (Nicoletti et al., 2011; Niswender and Conn, 2010; Pinheiro and Mulle, 2008). The mGluR superfamily can be divided into eight different receptor subtypes on the bases of their sequence homology, pharmacologic properties and activation of downstream signal transduction pathways (Conn and Pin, 1997). Group I metabotropic receptors consist of $mGluR_1$ and $mGluR_5$, which are primarily expressed postsynaptically with a somatodendritic distribution. These receptors are selectively activated by 3,5-dihydroxyphenyl-glycine (DHPG) and coupled to heterotrimeric G_q proteins that stimulate PLC-β to produce the intracellular messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG) through phosphatidylinositol hydrolysis. IP_3 triggers release of calcium from intracellular endoplasmic reticulum calcium stores (Fagni et al., 2000), while DAG recruits protein kinase C (PKC) to the membrane and activates it (Ferraguti et al., 2008; Houamed et al., 1991; Masu et al., 1991), resulting in enhanced excitability of hippocampal neurons via modulation of Ca^{2+}, K^+ and non-selective cation channels, as well as many longer term effects mediated by serine-threonine phosphorylation of numerous target proteins.

Group II mGluRs ($mGluR_2$ and $mGluR_{3)}$, and group III mGluRs ($mGluR_4$, $mGluR_7$ and $mGluR_8$) are coupled to $G_{i/o}$ proteins that inhibit adenylate cyclase, preventing the formation of cyclic adenosine 3'5'-monophosphate (cAMP). These receptors are expressed both presynaptically and postsynaptically. Presynaptically, their activation can decrease transmitter release by reducing voltage-dependent Ca^{2+} channel conductance through direct binding of Gβγ to the channel, by interfering directly with the presynaptic release apparatus, or both (Anwyl, 1999; Cartmell and Schoepp, 2000). In the hippocampus, group II mGluRs are believed to be localized primarily in presynaptic terminals, while group III mGluR are located in or near presynaptic active zones (Shigemoto et al., 1997). While these mGluRs are perfectly positioned to regulate synaptic transmission, the role of presynaptic mGluR in persistent plasticity of vesicular transmitter release is still relatively under-explored.

2. Presynaptic component of LTD of synaptic transmission

Homosynaptic LTD is an input-specific, long-lasting reduction in synaptic strength induced by prolonged low-frequency stimulation that has been observed at a variety of glutamatergic synapses in the hippocampus (Bear and Abraham, 1996), neocortex and other brain regions (Collingridge et al., 2010). Homosynaptic, associative LTD can be evoked at a synaptic input that is activated out of phase with a second bursting input that converges on the same neuron (Chattarji et al., 1989; Stanton and Sejnowski, 1989), or temporally mismatching

presynaptic and postsynaptic activation to mimic such activity (Debanne et al., 1994; Stanton and Sejnowski, 1989). The N-methyl-D-aspartate subtype of glutamate receptor (NMDAR) has been found to be essential for the induction of some forms of long-term synaptic plasticity because a) it gates the influx of Ca^{2+} and b) its voltage-dependent Mg^{2+} channel block allows the NMDAR to detect the level of coincident pre- and postsynaptic activity at individual synapses (Yuste et al., 1999). NMDAR-gated Ca^{2+} influx leads to the downstream activation of kinases and phosphatases required for inducing LTP and/or LTD in frequency-dependent patterns that elicit differing levels of [Ca^{2+}] increase in dendritic spines. Prolonged stimulation of Schaffer collateral-CA1 synapses at low frequencies (LFS: 1–5 Hz for 10–15 min) elicits a form of LTD (LFS-induced LTD) whose induction is blocked by the NMDAR-selective antagonist D-AP5 (Dudek and Bear, 1992; Mulkey et al., 1993). This stimulation has been suggested to elicit LTD, rather than LTP, because the smaller amplitude and slower rate of postsynaptic [Ca^{2+}] increase selectively activates the high affinity calcium sensitive phophatases calcineurin (PP2B) and PP1, that mediate postsynaptic changes resulting in LTD (Collingridge et al., 2010; Lisman, 1989; Mulkey et al., 1993). This hypothesis is supported by studies showing that inhibition of PP1/PP2A (Mulkey et al., 1993), or of PP2B (Mulkey et al., 1994), blocks the induction of LTD at Schaffer collateral-CA1 synapses. Postsynaptically, calcineurin dephosphorylates and inactivates inhibitor-1 (Mulkey et al., 1994), leading to activation of PP1, which dephosphorylates AMPAR GluR1 subunits at serine-845, thereby both decreasing AMPAR open probability (Lee et al., 1998) and triggering AMPAR internalization (Beattie et al., 2000; Ehlers, 2000). PP1 can also regulate gene expression via the dephosphorylation and the inactivation of the transcriptional factor cAMP response element-binding protein (CREB) (Bito et al., 1996; Deisseroth et al., 1996; Hagiwara et al., 1992), preventing activity-dependent expression of early genes such as c-fos, BDNF and Arc, all found to promote LTP (Barco et al., 2005). Additional mechanisms involved in the postsynaptic induction and expression of a significant component of LTD have been addressed in previous reviews and the reader is referred to them for these considerations (Collingridge et al., 2010; Kessels and Malinow, 2009; Poschel and Stanton, 2007)

Calcineurin can also dephosphorylate presynaptic proteins such as synapsin I (Chi et al., 2003; Jovanovic et al., 2001), whose dephosphorylated state is associated with reduced neurotransmitter release through decreasing the size of the readily-releasable vesicle pool (RRP) (Bykhovskaia, 2011; Hilfiker et al., 1999). Activation of NMDAR (Stanton et al., 2003; Zhang et al., 2006), group I (Zakharenko et al., 2002) and group II (Santschi et al., 2006) mGluR have all been shown to play roles in a presynaptic component of LFS-induced LTD at Schaffer collateral synapses. This presynaptic component of LTD appears to require a retrograde, diffusible intercellular messenger (Bolshakov and Siegelbaum, 1994; Stanton et al., 2003), perhaps arachidonic acid (Bolshakov and Siegelbaum, 1995) and/or nitric oxide (NO) (Stanton et al., 2003; Zhang et al., 2006) that are generated in the postsynaptic neuron. Retrograde messengers such as NO are membrane permeable, rapidly diffusible gaseous messenger that can diffuse from the postsynaptic compartment to presynaptic terminals within a small three-dimensional volume (≤50 μm^3; Wood and Garthwaite, 1994). A principal physiological enzyme target of NO is soluble guanylate cyclase (Southam and Garthwaite, 1993) and studies have shown that NO mediated elevation of presynaptic [cGMP] requires release of Ca^{2+} from ryanodine-sensitive intracellular stores via the second-messenger cyclic ADP ribose, and that this cascade is a key component of the induction of stimulus-evoked LTD at Schaffer collateral–CA1 synapses (Gage et al., 1997; Izumi and Zorumski, 1993; Reyes-Harde et al., 1999).

At dentate granule cell mossy fiber-CA3 synapses, it is generally agreed that, like LTP, LTD induced by electrical stimulation leads to a largely presynaptically expressed form of LTD. A low frequency stimulus train of 1–2 Hz for 10–15 min can induce LTD at mossy fiber

synapses (Kobayashi et al., 1996; Nicholls et al., 2006). This form of LTD depends on the presynaptic G-protein coupled receptors (GPCR) mGluR2 and A1 adenosine receptors, which, upon activation, release Gα subunits that inhibit adenylate cyclase (Kobayashi et al., 1996; Nicholls et al., 2006) and reduce activity of the cAMP-PKA pathway (Tzounopoulos et al., 1998).

2.1 Role of G-protein coupled receptors and $G\alpha_{i2}$ in presynaptic LTD

Studies have shown that inhibition of PKA can augment the magnitude of LTD induced by a subthreshold low-frequency stimulus train (1Hz/400seconds; (Santschi et al., 1999), and that pairing inhibition of PKA with production of cGMP induces a robust form of chemical LTD (CLTD) with a presynaptic locus of expression requiring activation of cyclic GMP-dependent protein kinase (PKG; Santschi et al., 1999). These studies also found that presynaptic GPCRs such as A1 adenosine receptors and group II mGluRs can supply adenylate cyclase inhibition necessary for induction of LTD (Santschi et al., 1999). Indeed, simultaneous activation of A1 adenosine receptors and elevation of [cGMP] is sufficient to elicit CLTD, and LTD is impaired by selective antagonists for either group II mGluR (EGLU) or A1 receptors (DPCPX) (Santschi et al., 2006), supporting a role for increased PKG and reduced PKA activity in the induction of presynaptic LTD (Broome et al., 1994; Gage et al., 1997; Izumi and Zorumski, 1993; Reyes-Harde et al., 1999; Santschi et al., 1999).

A presynaptic locus of expression of cyclic GMP-dependent LTD is further supported by experiments using the styryl dye FM1-43 to visualize vesicular release rates from Schaffer collateral presynaptic terminals (Pyle et al., 1999; Stanton et al., 2003; Stanton et al., 2005; Tyler et al., 2006; Zakharenko et al., 2002), and by measurements of release of [^{3}H]glutamate from isolated presynaptic hippocampal synaptosomes (Bailey et al., 2003). Transient inhibition of glutamate release is thought to be due to modulation of voltage-dependent calcium channels, particularly N-type presynaptic calcium channels that are downstream targets of GPCRs (Tedford and Zamponi, 2006), but whether the same signal transduction and expression mechanisms are involved in any forms of LTD was unknown. Transgenic mice that express a mutant, constitutively active $G\alpha_{i2}$ which inhibits adenylate cyclase, exhibit enhanced stimulus-induced LTD at both mossy fiber-CA3 (MF-CA3; Nicholls et al., 2006) and Schaffer collateral-CA1 (SCH-CA1; Bailey et al., 2008) synapses without significant changes in basal synaptic transmission. Interestingly, pharmacological elevations in [cGMP] which elicit a transient presynaptic depression in wildtype rodent slices (Boulton et al., 1994) evokes a presynaptic LTD in slices from these $G\alpha_{i2}$ transgenic animals (Bailey et al., 2008; Nicholls et al., 2006). Furthermore, LFS-induced LTD at both MF-CA3 and SCH-CA1 synapses is also enhanced in slices from these animals (Bailey et al., 2008), implying a decreased threshold for chemically and stimulus-evoked LTD. Overall, these studies suggest that activation of inhibitory $G\alpha_i$ subunits by presynaptic G-protein coupled receptors contribute to inhibition of presynaptic adenylate cyclase/ suppression in cyclic AMP production in the presynaptic terminal that is essential for induction of a presynaptic form of LTD. There are multiple presynaptic GPCRs that liberate $G\alpha_i$, (Santschi et al., 2006) found that antagonists of either A1 adenosine receptors or group II mGluRs (both coupled to $G_{i/o}$) impair the induction of stimulus-evoked SCH-CA1 LTD. The adenylate cyclase isoforms AC1 and AC8 are candidates as immediate targets of inhibitory $G\alpha_i$ subunits, because they are sensitive to inhibition by Gi-coupled receptors (Nielsen et al., 1996). Gα-mediated inhibition of presynaptic adenylate cyclase would lower both basal and stimulus-evoked PKA activity, in turn altering phosphorylation states of a wide range of presynaptic PKA substrate proteins. These substrates could include, but are not restricted to, proteins that are essential in synaptic vesicle recycling, such as Rab3A and its effector protein Rim1, both believed to regulate presynaptic activity downstream of PKA

(Chevaleyre et al., 2007; Huang et al., 2005; Lonart et al., 1998), or members of the SNARE complex (Nagy et al., 2005).

In the above-referenced study at mossy fiber-CA3 synapses in transgenic mice expressing constitutively active $G\alpha_{i2}$ (Nicholls et al., 2006), these investigators observed both a doubling of the magnitude of stimulus-evoked LTD, and an occlusion of the transient suppression of mossy fiber synaptic transmission normally elicited by pharmacological activation of group II mGluRs, suggesting that, while group II mGluR activation elicits only transient depression of presynaptic glutamate release, there must be additional factors, such as NO activation of guanylyl cyclase and release from interterminal Ca^{2+} stores (Reyes and Stanton, 1996; Gage et al., 1997; Reyes-Harde et al., 1999), required to convert this depression to LTD. Similarly, constitutively active $G\alpha_{i2}$ leads to suppression of MF-CA3 and SCH-CA1 LTP, which was rescued by exogenous application of S_P-adenosine 3',5'-monophosphorothioate (SP-cAMPS), implying that the cAMP-PKA pathway is important for presynaptic LTP, at least at these synapses (Bailey et al., 2008; Nicholls et al., 2006). Previous studies have shown LTP at MF-CA3 synapses to be expressed presynaptically and depend on cAMP and PKA (Huang et al., 1994; Villacres et al., 1998), consistent with presynaptic group II mGluRs acting via inhibitory $G\alpha_{i2}$ to suppress activity in the cAMP-PKA pathway.

2.2 mGluR-mediated LTD in different stages of brain development

Immature synapses (postnatal days 1–2) exhibit a form of mGluR-dependent LTD that is induced postsynaptically via co-activation of group I mGluRs and voltage-dependent calcium channels, and expressed presynaptically at SCH-CA1 synapses in the hippocampus (Bolshakov and Siegelbaum, 1994). Other studies have reported that, in neonatal rats, low-frequency stimulus-evoked LTD is mGluR-dependent, and not affected by NMDAR blockade (Feinmark et al., 2003; Li et al., 2002). mGluR-LTD in neonates is also independent of protein synthesis and does not require changes in the surface expression of AMPARs. This form of LTD is expressed as a long-term decrease in transmitter release, as demonstrated by increases in paired-pulse facilitation (Nosyreva and Huber, 2005), coefficient of variation of EPSC's (Bolshakov and Siegelbaum, 1994) and FM1-43 destaining (Zakharenko et al., 2002). The retrograde messenger in this neonatal mGluR-LTD has been suggested to be arachidonic acid (Bolshakov and Siegelbaum, 1994; Feinmark et al., 2003), and to involve the postsynaptic p38 MAPK-PLA2 pathway in the generation of this intercellular messenger.

In juvenile rats, mGluR-LTD can be induced by low frequency trains of paired-pulse stimulation (paired-pulse interval 50ms, delivered at a frequency of 1–2 Hz) in the presence of an NMDAR antagonist; (Huber et al., 2002; Kemp et al., 2000) or by a 5Hz/3min LFS train (Oliet et al., 1997). This group 1 mGluR-mediated LTD most likely involves postsynaptic activation of the mGluR5 subtype of mGluRs (Huang and Hsu, 2006; Huber et al., 2001; Oliet et al., 1997). Group 1mGluR-LTD that far outlasts washout of drug can be induced chemically by the group I agonist (S)-3,5-dihydroxyphenylglycine (Huber et al., 2001), is independent of both NMDAR and group II mGluR activation (Huang and Hsu, 2006) and occludes paired-pulse stimulus-evoked LTD, suggesting overlapping induction/expression mechanisms. The induction of group 1 mGluR-LTD required a postsynaptic increase in $[Ca^{2+}]$ and activation of PKC (Nicholls et al., 2006; Oliet et al., 1997). Interestingly, expression of DHPG-LTD also requires *persistent* activation of group I mGluRs, since established DHPG-LTD can be reversed by mGluR antagonists, but is reestablished once these blockers are removed from the extracellular perfusate (Huang and Hsu, 2006). Group 1 mGluR-LTD in juvenile animals requires protein synthesis (Connelly et al., 2011; Huber et al., 2001), and is thought to be mediated by endocytosis of postsynaptic AMPA and NMDA receptors (Nosyreva and Huber, 2005; Snyder et al., 2001).

Additional evidence for a postsynaptic site of expression of DHPG-LTD comes from variance-mean analyses of release probability (Pr) at Schaffer collateral presynaptic terminals (Zhang et al., 2006) and studies of FM1-43 destaining kinetics from the readily releasable vesicle pool (Zhang et al., 2006). A presynaptic site of expression has also been reported for group 1 mGluR-LTD (Connelly et al., 2011; Fitzjohn et al., 2001; Manzoni and Bockaert, 1995) accompanied by enhanced paired-pulse facilitation (Fitzjohn et al., 2001; Manzoni and Bockaert, 1995), increased EPSC failures, changes in EPSC coefficient of variation, decreased mEPSC frequency (Fitzjohn et al., 2001) and a lack of postsynaptic change in responses to focal application of glutamate by uncaging (Rammes et al., 2003). Taken together, the above data lead to the conclusion that DHPG-induced LTD is expressed postsynaptically in the young brain, can co-exist with presynaptic alterations in release probability, and can also lead to postsynaptic endocytosis of AMPARs that creates silent synapses appearing to be presynaptic increases in release failures (Liao et al., 1995).

In adult rats, mGluR-LTD is not affected by inhibiting PKC or PKA, (Schnabel et al., 1999, 2001) or depleting intracellular calcium stores (Schnabel et al., 1999). This form of LTD is induced postsynaptically and may require CaMKII-mediated protein synthesis (Mockett et al., 2011) and postsynaptic protein tyrosine phosphatases (PTPs) dependent internalization of surface GluR2-containing AMPARs (Gladding et al., 2009), but also entail persistent presynaptic inhibition of voltage-sensitive Ca^{2+} and K^{+} channels (Watabe et al., 2002) that leads to reduced presynaptic Ca^{2+} influx (Tan et al., 2003). LTD cascades can include crosstalk with other receptors, as has been shown in a study of CA3 pyramidal neuron-specific M1 muscarinic receptor knockout animals, which exhibited deficiencies in mGluR-LTD (induced by bath application of DHPG), but normal stimulus-evoked LTD (Kamsler et al., 2010) This loss of mGluR-LTD was attributed to increased presynaptic release probability as a result of M1 receptor knockout, suggesting a presynaptic modulatory role for M1 receptors in mGluR-LTD (Kamsler et al., 2010).

3. Gβγ modulation of neurotransmission

Activation of GPCRs leads to the obligatory release of Gα and Gβγ subunits in a 1:1 stoichiometric ratio. While plasticity research has focused on downstream pathways activated by Gα subunits (such as Gs, Gq, Gi/o), the roles of Gβγ subunits in short and long-term plasticity of presynaptic function have been far less studied. Receptors such as CB1 endocannabinoid (Matsuda, 1997; Matsuda et al., 1990), 5HT-1 serotonin (Gerachshenko et al., 2005; Photowala et al., 2006), D2 dopamine (Congar et al., 2002), M4 muscarinic (Shirey et al., 2008), and α2-adrenergic receptors, are known to inhibit evoked transmitter release (Stephens and Mochida, 2005). Gβγ modulation of presynaptic vesicular release can occur in two different ways; ion channel regulation and direct regulation of vesicle exocytosis machinery. Ion channels that can alter their properties due to Gβγ binding include voltage-dependent calcium channels and G-protein coupled inward rectifying potassium channels, both of which can alter presynaptic action potential stimulated influx of calcium, thereby affecting probability of vesicular release. There is strong evidence to suggest that high voltage-activated N and P/Q type calcium channels are direct downstream targets for presynaptic Gβγ proteins (Herlitze et al., 1996; Ikeda, 1996; Tedford and Zamponi, 2006; Zhang et al., 2008). Binding of these G-proteins leads to a voltage-dependent inhibition of calcium currents that decreases vesicular release probability (Ikeda, 1996; Jarvis et al., 2000; Zhang et al., 2008). Another target of Gβγ modulation is G- protein activated inward rectifying potassium (GIRK) channels. GIRK channels are regulated by $G_{i/o}$ coupled GPCRs and present at presynaptic terminals (Ponce et al., 1996) localized to presynaptic active zones (Ladera et al., 2008). Activation of GIRK channels by direct interactions with Gβγ proteins (Sadja et al., 2001) leads to membrane hyperpolarization, thus decreasing local membrane excitability and reducing the amount of

local Ca^{2+} influx into presynaptic terminals, although this has not been demonstrated in a presynaptic terminal.

3.1 Gβγ modulation of vesicular release by direct interaction with SNARE proteins

Evidence suggests that there may be two different actions of Gβγ on the presynaptic release machinery. First, Gβγ can modify spontaneous mEPSP frequency, first demonstrated at the neuromuscular junction (Silinsky, 1984). Secondly, a Ca^{2+}-independent pathway for presynaptic modulation of neurotransmitter release by Gβγ, first identified by studies in the lamprey, where (Blackmer et al., 2001) demonstrated that presynaptic injection of Gβγ into these large terminals mimicked serotonin inhibition of presynaptic transmitter release. To determine the role of Gβγ in serotonin-mediated inhibition, these authors used a potent, selective Gβγ scavenger (ct-GRK2), which completely occluded the ability of serotonin to inhibit chemical neurotransmission, suggesting a crucial role for Gβγ subunits in *transient,* serotonin-mediated presynaptic inhibition of release via GPCRs. Fluorescent calcium imaging revealed that presynaptic Gβγ injection did not alter evoked Ca^{2+} entry through voltage-gated calcium channels (Blackmer et al., 2001) and that serotonin application can still inhibit synaptic transmission when paired with Ca^{2+} release by intraterminal uncaging (Gerachshenko et al., 2005) suggesting that Gβγ is likely to act on binding sites downstream of calcium entry, such as proteins of the presynaptic vesicular release apparatus.

SNAP (soluble NSF attachment protein) receptor (SNARE) proteins seem likely candidates as Gβγ binding targets that modulate transmitter release, since Gβγ can directly bind to syntaxin-1A, SNAP-25 and the ternary SNARE complex (Blackmer et al., 2005; Blackmer et al., 2001; Gerachshenko et al., 2005; Yoon et al., 2007). Indeed, SNAP-25 became a prime candidate Gβγ binding target when it was found that pretreatment of lamprey synapses with type A botulinum toxin (BoNT/A, a protease that selectively cleaves 9 amino acids from the C-terminus of SNAP-25) (Binz et al., 1994; Gerachshenko et al., 2005; Xu et al., 1998), both reduced release probability and prevented the ability of Gβγ to inhibit exocytosis (Gerachshenko et al., 2005). Furthermore, presynaptic infusion with the C-terminal 14-amino-acid fragment of SNAP-25 (ct-SNAP-25) also blocked 5-HT mediated presynaptic inhibition, reinforcing the idea that G-protein binding to the C-terminus of SNAP-25 could serve as a mechanism for inhibition of neurotransmitter release, at least at lamprey synapses (Gerachshenko et al., 2005). This inhibition of vesicle fusion downstream of calcium entry could have been as a result of **a)** decreased efficiency of the SNARE complex, **b)** uncoupling of interactions between the putative calcium sensor synatotagmin and the SNARE complex (Blackmer et al., 2005; Gerachshenko et al., 2005; Yoon et al., 2007; Figure 1B) or **c)** a shift in the preferred mode of vesicle fusion to a kiss-and-run mode of release (Photowala et al., 2006; Schwartz et al., 2007; Gerachshenko et al., 2009), decreasing quantal size and cleft glutamate release concentration. Photowala et al. (2006) used a method of sensing trapped FM1-43 within vesicles by quenching with the hydrophilic fluorophore sulforhodamine101, to conclude that 5-HT led to an enhanced recruitment of a kiss and run mode of release. In addition, it was demonstrated that 5-HT, via Gβγ acting at the SNARE complex, led to transient vesicle fusion of synaptic vesicles that resulted in decreased quantal size (Blackmer et al., 2001; Chen et al., 2005; Photowala et al., 2006).

Interestingly, Gβγ has been shown to interact with SNAP-25 and syntaxin1A (both members of the core SNARE complex), but, more importantly at the fully formed ternary SNARE complex, the interaction sites are near those used by synaptotagmin (Gerona et al., 2000; (Yoon et al., 2007). During the resting state, docked presynaptic vesicles are primed by a switch of plasma membrane t-SNARE associated syntaxin from its closed to its open conformation triggered by dissociation of the munc18-syntaxin complex (Carr and Rizo, 2010; Sudhof and Rothman, 2009). In the open conformation, the syntaxin-1 SNARE motif

is unbound from the Habc domain and made available to complex with SNARE motifs in v-SNAREs (synaptobrevin). Once the action potential invades the presynaptic terminal, neurotransmitter release is initiated within 200μs and shows a steep dependence on $[Ca^{2+}]$ influx through high voltage gated Ca^{2+} channels (Schneggenburger and Neher, 2005; Tsien et al., 1991; Uchitel et al., 1992) with a half maximal activation at as high as 190μM $[Ca^{2+}]$ (Heidelberger et al., 1994; Sheng et al., 1996). Synaptotagmin-1 is believed to be the putative Ca^{2+} sensor that intiates synchronized vesicle fusion (Brose et al., 1992; Martens and McMahon, 2008; Yoshihara and Littleton, 2002). GPCR activation close to the active zone has the potential to release Gβγ that could compete with synaptotagmin binding, thus, it is possible that at low to moderate presynaptic $[Ca^{2+}]_i$,, calcium-independent Gβγ/SNARE binding wins out, inhibiting exocytosis (Blackmer et al., 2005; Blackmer et al., 2001; Gerachshenko et al., 2005), while high $[Ca^{2+}]_i$ produces a steep increase in affinity of synaptotagmin for SNARE proteins, promoting exocytosis. Indeed, even after BoNT/A-mediated cleavage of the C-terminus of SNAP-25, exocytosis is restored by high $[Ca^{2+}]_i$ (Gerachshenko et al., 2005), overcoming the loss of C-terminus binding sites by promoting more efficient SNARE fusion. However, the roles of Ca^{2+} and SNARE protein binding of Gβγ could be united if Gβγ binding to one or more SNARE proteins results in a lower affinity of synaptotagmin for Ca^{2+} that leads to reduced probability of release.

In mammalian synapses of the central amygdala, norepinephrine inhibits neurotransmitter release via activation of presynaptic α2 adrenoceptors on excitatory parabrachial inputs (Delaney et al., 2007). Presynaptic application of the Gβγ-binding peptide mSIRK (Chen et al., 2005), as well as incubation with BoNT/A, both significantly reduce noradrenergic inhibition at these terminals (Delaney et al., 2007), supporting an essential role for Gβγ in this presynaptic inhibition. In the hypothalamus, dynorphin mediated inhibition of glutamate release that occurs via activation of presynaptic κ-opioid receptors also appears to recruit direct Gβγ-mediated inhibition of exocytosis, since this action is independent of both inhibition of adenylyl cyclase and voltage-dependent calcium channels (Iremonger and Bains, 2009). In a non-neuronal system (INS 832/13 β-cells), norepinephrine also inhibits exocytosis via Gβγ interaction with SNAP-25, indicated by studies showing that intracellular application of antibodies against Gβγ or BoNT/A block this inhibition, while myristoylated βγ-binding/activating peptide mSIRK inhibited exocytosis to a similar degree as norepinephrine (Zhao et al., 2010).

4. Gβγ and the C-terminus of SNAP-25 are required for LTD of transmitter release

The above studies strongly argue for a role for Gβγ binding to the C-terminus region of SNAP-25 in mediating *transient* inhibition of neurotransmission elicited by a variety of GPCRs (Figure 1A, B), but could a similar mechanism be involved in the induction/expression of *long-term* presynaptic plasticity, and could it involve group II mGluRs and/or other GPCRs that liberate Gβγ?

To test the hypothesis that Gβγ binding to the C-terminus region of SNAP-25 might play a necessary role in the induction of LTD at Schaffer collateral-CA1 synapses in the mammalian hippocampus, (Zhang et al., 2011) treated hippocampal slices with BoNT/A, which, by itself, produced a ~50% reduction in fEPSPs associated with a presynaptic reduction in release probability (demonstrated by variance-mean analysis). In this study, slices pretreated with BoNT/A showed impaired induction of LTD by low frequency (2 Hz/10min) Schaffer collateral stimulation, while homosynaptic LTP induced by theta burst stimulation and, interestingly, dedepression induced by low frequency stimulation at the same synapse were not affected (Zhang et al., 2011). These findings support the notion that,

in addition to GPCR-mediated transient presynaptic inhibition of release, the C-terminus of SNAP-25 is also essential in some way for the induction of LTD.

Zhang et al., (2011) also showed that stimulus-evoked LTD and BoNT/A-mediated presynaptic depression mutually occlude one another, since saturation of SCH-CA1 LTD eliminated any further effect of BoNT/A on synaptic transmission, further suggesting shared mechanisms of induction. Interestingly, the ability of BoNT/A to inhibit vesicular release was restored when, after saturating LTD, theta burst stimulation was applied to repotentiate synaptic strength, a sharp contrast from the lack of effect of BoNT/A pretreatment on *de novo* LTP (Zhang et al., 2011). However, BoNT/A pretreatment did not occlude cGMP-mediated presynaptic depression (Gage et al., 1997; Izumi and Zorumski, 1993; Reyes-Harde et al., 1999) induced by application of the selective type V phosphodiesterase inhibitor zaprinast, suggesting divergent mechanisms for decrease in synaptic strength (Zhang et al., 2011). Moreover, stimulus-evoked LTD was not impaired by reducing extracellular [Ca^{2+}] as it was by BoNT/A, and elevating extracellular [Ca^{2+}] after BoNT/A did not rescue LTD, indicating that the effects of BoNT/A were not simply due to the reduction in probability of release (Zhang et al., 2011).

To directly test whether the presynaptic role of the C-terminus of SNAP-25 in LTD might involve binding of $G\beta\gamma$ in this region, these authors utilized two molecularly distinct methods used previously to scavenge free $G\beta\gamma$, to determine whether they could impair the induction of LTD at SCH-CA1 synapses. Schaffer collateral presynaptic terminals were selectively filled by electroporating pyramidal cell soma in field CA3 to fill them with either a $G\beta\gamma$ scavenging peptide, consisting of 14 amino acids of the C-terminus sequence (Ct-SNAP-25 peptide) of SNAP-25 (Gerachshenko et al., 2005), or the $G\beta\gamma$ binding peptide mSIRK (Chen et al., 2005). In both cases, there was a significant reduction in the magnitude of LTD when compared to LTD induced in the presence of scrambled control peptides, confirming the conclusion that $G\beta\gamma$ binding to its targets is crucial for the full expression of LFS-LTD (Zhang et al., 2011). The Ct-SNAP-25 peptide also markedly reduced the presynaptic inhibition produced by group II mGluR agonists, supporting a role for $G\beta\gamma$ in mGluR-dependent transient synaptic depression at mammalian synapses (Zhang et al., 2011) similar to that observed at lamprey synapses (Blackmer et al., 2005; Blackmer et al., 2001; Gerachshenko et al., 2005). Since $G\beta\gamma$ is known to directly bind to and inhibit voltage-gated calcium channels, this study also tested the possibility that such binding could play a role in presynaptic LTD. Calcium imaging revealed that induction of LTD was associated with only a transient decrease in stimulus-evoked Ca^{2+} influx that, while possibly contributing to short-term plasticity of release, could not underlie LTD, suggesting rather mechanisms downstream of calcium entry (Blackmer et al., 2005; Blackmer et al., 2001; Chen et al., 2005; Delaney et al., 2007; Gerachshenko et al., 2005; Takahashi et al., 2001)

5. Conclusion

Recent work has revealed a critical role for metabotropic glutamate GPCRs in the induction of a presynaptic form of LTD of synaptic transmission at hippocampal synapses, one that appears to recruit the same GPCR-mediated biochemical cascade involved in transient presynaptic depression at both lamprey and mammalian synapses. This cascade requires the simultaneous GPCR-mediated release of $G\alpha_i$, which inhibits adenylate cyclase, and $G\beta\gamma$, which binds to the C-terminus of the SNARE protein SNAP-25 to reduce vesicular glutamate release, and this cascade can be activated by a number of GPCRs, including group II mGluRs and A1 adenosine receptors. It is clear however that, while these G-proteins are essential components in the induction of a presynaptic form of LTD, they are not sufficient to elicit long-term changes in transmitter release. Both at lamprey and mammalian synapses, GPCR activation alone, whether by serotonin, glutamate or adenosine, elicits only transient

presynaptic depression of transmitter release that recovers upon removal of receptor agonist. Previous studies at the SCH-CA1 synapse (Figure 1C), where both presynaptic LTD and LTP require *postsynaptic* activation, have shown that it is the production of the second messenger nitric oxide (NO) that appears to be an essential additional intercellular messenger in presynaptic plasticity (Gage et al., 1997; Izumi and Zorumski, 1993). Postsynaptically-generated NO acts to report to the presynaptic terminal that a "Hebbian" coincident activation of presynaptic and postsynaptic compartments has occurred. This NO, through activation of guanylyl cyclase that leads to increased cyclic GMP-dependent protein kinase activation, causes the production of the ryanodine receptor agonist cyclic ADP ribose that releases Ca^{2+} from intraterminal stores (Reyes-Harde et al., 1999). This Ca^{2+} appears to activate CamKII that is also an essential component of the LTD cascade (Stanton and Gage, 1996). Evidence now supports direct actions on proteins of the vesicular release apparatus underlying long-term plasticity of release (Figure 1C), rather than persistent alterations at the level of voltage-dependent calcium channels (Zhang et al., 2011).

While a role for GPCRs, including group II mGluRs, in presynaptic activity-dependent plasticity is now becoming evident, the downstream molecular mechanisms are still far from elucidated. One key question is how SNAP-25 is modified molecularly to persistently reduce glutamate release, since persistent binding of $G\beta\gamma$ for many hours or longer is unlikely. Another is whether other SNARE proteins are also targets for persistent plastic modifications. Ca^{2+}-dependent kinases and phosphatases, and PKG, may all play critical roles, suggesting phosphorylation sites on SNAP-25 and other SNARE proteins as prime targets. While occlusion experiments suggest that LTD has something in common with BoNT/A-mediated cleavage of the C-terminus of SNAP-25, the notion of an endogenous BoNT/A-like activity may be too irreversible to be computationally useful. Nevertheless, it is now becoming clear that, in addition to postsynaptic dendritic spines in all their complexity, the presynaptic terminal is also capable of long-term activity-dependent plasticity of transmitter release that offers the potential for far different, complex and frequency-dependent plasticity of synaptic transmission that is likely to play important roles in neural network development, learning and memory and information storage, warranting further investigation of the potential for nootropic therapeutics that target group II mGluRs and other presynaptic GPCRs.

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Highlights

- Presynaptic mGluRs are key to short and long-term plasticity of transmitter release.
- mGluRs transiently inhibit vesicular release via release of Gβγ.
- Gβγ binding the SNAP-25 C-terminus is necessary, not sufficient, for LTD of release.
- Gαi2, Nitric oxide, cyclic GMP, cyclicADP ribose, CaMKinase are all necessary for LTD.

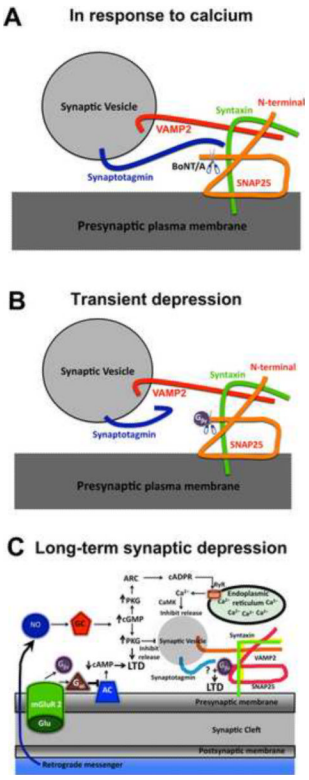

Figure 1.

A: In response to presynaptic calcium entry, synaptotagmin, a synaptic vesicle protein that is the putative calcium sensor controlling evoked neurotransmitter release, binds to the C-terminus of SNAP-25 (Gerona et al., 2000; Zhang et al., 2002), one of three proteins in the SNARE complex of vesicle fusion machinery. Cleavage of the last 9 amino acids of the C-terminus of SNAP-25 with type A Botulinum Toxin (BoNT/A) reduces synaptotagmin binding (Gerona et al., 2000; Zhang et al., 2002), and shifts the calcium dependence of release (Gerona et al., 2000; Capogna et al.,. 1997). **B:** Gβγ binding to the C-terminus of SNAP-25 directly but transiently inhibits release. When G protein-coupled receptors such as group II metabotropic glutamate receptors are activated, Gβγ subunits are liberated and bind to the C-terminus of SNAP-25, where they interfere competitively with calcium-dependent binding of synaptotagmin to inhibit release (Blackmer et al., 2005; Gerachshenko et al., 2005). Cleavage of the last 9 C-terminus amino acids of SNAP-25 with BoNT/A eliminates Gβγ binding (Blackmer et al., 2005; Gerachshenko et al., 2005), mGluR2-mediated presynaptic depression (Zhang et al., 2011), and LTD (Zhang et al., 2011), implicating this site in both transient and long-term presynaptic depression. **C:** Signal transduction cascades necessary for induction of presynaptic long-term depression in the Schaffer collateral-CA1 presynaptic terminal. Abbreviations: mGluR2 - group II metabotropic glutamate receptor; NO – nitric oxide; GC – soluble guanylyl cyclase; AC – adenylate cyclase; cAMP – cyclic adenosine 3',5' monophosphate; cGMP – cyclic guanosine 3',5' monophosphate; LTD – long-term depression; PKG – cyclic GMP-dependent protein kinase; ARC – ADP-ribosyl cyclase/hydrolase; cADPR – cyclic ADP ribose; RyR – ryanodine receptor; CaMK – Ca2+/ calmodulin-dependent protein kinase; VAMP2 – vesicle-associated membrane protein 2; SNAP25 – synaptosomal-associated protein 25.