The expanding roles and mechanisms of G protein–mediated presynaptic inhibition

DOI 10.1074/jbc.TM118.004163

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Edited by Henrik G. Dohlman

Throughout the past five decades, tremendous advancements have been made in our understanding of G protein signaling and presynaptic inhibition, many of which were published in the Journal of Biological Chemistry under the tenure of Herb Tabor as Editor-in-Chief. Here, we identify these critical advances, including the formulation of the ternary complex model of G protein–coupled receptor signaling and the discovery of Gβγ as a critical signaling component of the heterotrimeric G protein, along with the nature of presynaptic inhibition and its physiological role. We provide an overview for the discovery and physiological relevance of the two known Gβγ–mediated mechanisms for presynaptic inhibition: first, the action of Gβγ on voltage-gated calcium channels to inhibit calcium influx to the presynaptic active zone and, second, the direct binding of Gβγ to the SNARE complex to displace synaptotagmin downstream of calcium entry, which has been demonstrated to be important in neurons and secretory cells. These two mechanisms act in tandem with each other in a synergistic manner to provide more complete spatiotemporal control over neurotransmitter release.

The development and characterization of selective agonists and antagonists for cell surface receptors was instrumental in formulating the current model of G protein receptor signaling. Radioligand antagonist-binding studies for numerous cell surface receptors, including the α- and β-adrenergic receptors (1–7), muscarinic acetylcholine receptors (8), dopaminergic receptors (9), and glucagon receptors (10), demonstrated the existence of two affinity states within the receptor for agonist binding, a high-affinity state and a low-affinity state, but only a single state with respect to antagonist binding. This critical finding enabled Robert Lefkowitz to formulate and propose a ternary complex model in JBC using early computer-modeling techniques, where a high-affinity state for agonist binding consisting of the agonist, the receptor, and a yet-undetermined guanine nucleotide–binding component was needed for downstream adenylate cyclase activation (11).

The guanine nucleotide–binding component, known today as the heterotrimeric G protein, previously thought of as the “regulatory subunit” of adenyl cyclase, was then purified in sufficiently large quantities to study. It was shown to consist of three polypeptides, one of which bound guanine nucleotides (12, 13). The isolation of purified β-adrenergic receptors (14–16) and heterotrimeric G protein enabled the determination that the presence of these two components was necessary and sufficient for inducing two-state agonist binding in the receptor and nucleotide exchange for the G protein (17, 18). Building upon this model, it was the purification of adenylate cyclase that enabled several articles in JBC and elsewhere from Lefkowitz, Gilman, and Smigel. These articles were instrumental for the initial model of GPCR signaling, with critical reconstitution experiments showing that a minimum of three cellular proteinaceous complexes—the β adrenergic receptor, the heterotrimeric G protein, and the adenylate cyclase—were required for cAMP accumulation in response to agonists (19–21). This effectively reproduced the cellular response in a strictly defined system.

The discovery of Gβγ as a signaling molecule

This initial model of GPCR signaling was predicated upon the GTP-bound Ga subunit conveying the transduced signal to the effector, whereas the Gβγ subunit’s only role was to obstruct the Ga effector-binding domains in the absence of ligand or act as an anchor to the plasma membrane (22). An abrupt challenge to the model came from the critical finding of David Clapham’s group (23) that purified Gβγ subunits from the brain, and not Ga subunits, were responsible for transducing the signal of muscarinic acetylcholine receptors to activate GIRK channels in cardiac pacemaker and atrial cells. This novel finding was vehemently disputed by the groups of Birnbaumer and Brown (24–26), who showed evidence that the activating component of the heterotrimer, termed “Gk” after the potassium channel, was a Ga subunit and that the purified Gβγ preparation of Clapham’s group may have been slightly contaminated with a high-affinity Ga, or interference was generated somehow from CHAPS detergent. The initial finding of Clapham’s group was echoed by other groups (27). The dispute persisted until the work of Jan and Lester (28–30), facilitated by

3 The abbreviations used are: GPCR, G protein–coupled receptor; PAD, primary afferent depolarization; BNST, bed nuclei of the stria terminals; AR, adrenergic receptor; mGluR, metabotropic glutamate receptor; EPSC, excitatory postsynaptic current; s restraint; 5-HT, 5-hydroxytryptamine.

This JBC Review is part of a collection honoring Herbert Tabor on the occasion of his 100th birthday. The authors declare that they have no conflicts of interest with the contents of this article.

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the development of heterologous expression systems such as *Xenopus* oocytes, and the cloning of the individual cDNAs responsible for the G proteins \( \alpha_i \), \( \beta \), and \( \gamma \), which implicated \( \text{G} \beta \gamma \) as the signal transducer of GIRK.

These technological advances paved the way for the group of Catterall (31) to determine that the active G protein subunit responsible for \( \text{G}_{i/o} \)-coupled GPCRs’ inhibitory action upon N- and P/Q-type voltage-gated calcium channels was \( \text{G} \beta \gamma \) and not \( \text{G} \alpha \). This finding was echoed by Ikeda (32) for N-type channels in the same issue of *Nature*. The contemporaneous findings of Harden and Gilman (33) that phospholipase \( \text{C} \beta \) and type II adenylyl cyclase (34) were activated by \( \text{G} \beta \gamma \) provided more widespread acceptance that \( \text{G} \beta \gamma \)–effector interactions were a critical aspect of G protein signaling. Many more direct \( \text{G} \beta \gamma \)-binding effectors would be identified in that period, including the GRK family of kinases (35) and phosphoinositide-3 kinase (36), the molecular basis for many of which were characterized through identification of key residues on the \( \alpha \)-binding surface of \( \text{G} \beta \gamma \) (37). The widespread acceptance of \( \text{G} \beta \gamma \) as a critical signaling protein with a multitude of effectors was instrumental to our studies of its role in the presynaptic regulation of neurotransmitter release. The development and characterization of selective agonists and antagonists for cell surface receptors was instrumental to formulating the current model of G protein receptor signaling, including the field of signaling that modulates neurotransmitter release from the presynaptic terminal.

**The historical basis for presynaptic regulation of neurotransmitter release**

Physiological mechanisms of control over neurotransmitter release are essential for the orderly transduction of signals from presynaptic to postsynaptic neurons. Our understanding of these mechanisms has expanded considerably over the past half-century. Presynaptic regulation of transmitter release is vital to the plasticity of a neuron and the signaling network in which it functions. In various studies, the phenomenon of presynaptic inhibition studies in vertebrates started from analysis of sensory inputs to the spinal cord. As early as the 1930s, presynaptic depolarization from sensory stimulation was shown as a mechanism of physiological modulation of these inputs (38). This was later characterized as primary afferent depolarization (PAD) (39) and first described as a presynaptic mechanism of inhibition by Frank and Fuortes (40). Eccles *et al.* (41, 42) went on to firmly link the phenomenon of PAD to presynaptic inhibition as well as to demonstrate that higher brain centers use this mechanism to control sensory inflow (43). It was also determined by Eccles *et al.* (44) that this inhibition is mediated by GABA receptors, although the identity of GABA as an amino acid neurotransmitter was only later proven in vertebrates (45) following extensive work in invertebrate models (46–49). Ultrastructural and immunohistochemical data showing GABAergic axo-axonic synapses later reinforced the existence of axo-axonic synapses that mediate presynaptic inhibition (50–52).

Supporting evidence for the role of GABA in presynaptic inhibition was provided by studies in the periphery on sympathetic nerve terminals and ganglia in which GABA receptors caused depolarization of sympathetic ganglia neurons. In these neurons, the first example of GPCR-mediated presynaptic inhibition was found in response to noradrenaline (53–56). Strikingly, however, GABA-mediated inhibition at these terminals was not mediated by the ionotropic GABA\( \alpha \) receptor, but rather by a \( \text{G}_{i/o} \)-coupled GPCR (57–60), the \( \text{GABA} \beta \) receptor (61), which responded to the anti-convulsant (–)–baclofen (62). \( \text{GABA} \beta \) receptors are distributed throughout the central nervous system, where they also mediate presynaptic inhibition. Indeed, it is now clear that \( \text{GABA} \beta \) receptors, along with many other GPCRs, including but not limited to opioid receptors (63, 64), cannabinoid receptors (65), \( \alpha_2 \), adrenergic receptors (66), and metabotropic glutamate receptors (67), inhibit glutamate release at spinal sensory synapses, but also throughout the central nervous system.

A critical result in determining a mechanism by which GPCRs mediate presynaptic inhibition came from recordings from dorsal root ganglion cells in culture. These are the cells that give rise to spinal sensory synapses, which demonstrate PAD, thus understanding their cellular biology provides insight into the biochemistry of their presynaptic terminals. Various GPCR agonists (68, 69) that target G proteins in dorsal root ganglia cells (59, 60, 70) directly inhibited Ca\( ^{2+} \) currents, including both noradrenaline \( \alpha_2 \)-adrenergic and \( \text{GABA} \beta \) receptors. This led to the hypothesis that GPCRs cause presynaptic inhibition by inhibiting presynaptic Ca\( ^{2+} \) entry, although this has not been demonstrated directly at spinal sensory synapses. Nevertheless, it is now clear that presynaptic inhibition is ubiquitous and is found throughout the central nervous system (61, 71–73). Whereas some presynaptic GPCRs clearly inhibit neurotransmission by inhibiting presynaptic Ca\( ^{2+} \) entry (74–77), it is also clear that other mechanisms targeting the release machinery directly are also important (78, 79).

**Identification of the \( \text{G} \beta \gamma \)–SNARE interaction as a critical inhibitory mechanism of exocytosis downstream of Ca\( ^{2+} \) entry**

Many \( \text{G}_{i/o} \)-coupled GPCRs were shown to inhibit exocytosis via the action of \( \text{G} \beta \gamma \) on voltage-gated calcium channels to inhibit Ca\( ^{2+} \) fluxes (80–84). Other researchers found that inhibition occurred at a distinct site downstream of Ca\( ^{2+} \) entry (78, 85, 87). The large size of the sea lamprey (*Petromyzon marinus*) giant axon preparation, utilized by our group as a model of synaptic transmission, facilitated the direct axonal injection of proteins, such as \( \text{G} \beta \gamma \), or critical \( \text{G} \beta \gamma \)-chelating peptides, such as \( \text{BARK} \). Injection of \( \text{G} \beta \gamma \) inhibited EPSCs without perturbing voltage-gated Ca\( ^{2+} \) currents, whereas the chelating peptide blocked the inhibitory effect of the lamprey serotonin receptor, a receptor homologous to human 5-HT\( _{1B} \) (79). Later studies expanded upon this initial finding, showing that the step inhibited by \( \text{G} \beta \gamma \) was late in the vesicular docking and priming cycle and could occur within tens of milliseconds after the uncaging of agonist (88). In addition, 5-HT action on the lamprey serotonin receptor reduced quantal size and prevented full fusion (89), reinforcing the notion that \( \text{G} \beta \gamma \) was acting at a late step and not influencing vesicular pool sizes via this mechanism.
No data were shown pertaining to the effector target of Gβγ in the original study from 2001 (79), but in 2005, the presynaptic release machinery was shown to be the target (88), because botulinum neurotoxin type A (BoNT/A) cleaves the nine C-terminal residues of the peripheral membrane t-SNARE SNAP-25 (90, 91), leaving a truncated SNAP-25Δ9 that is still fusogenic (92, 93), albeit to a greatly reduced extent. BoNT/A completely eliminated any inhibition by 5-HT. This was confirmed in mammalian tissue, by recording effects of 5-HT1B receptors on glutamate release from CA1 pyramidal neurons (76). We also showed direct in vitro binding of Gβγ to the t-SNARE proteins SNAP-25 and syntaxin 1A and synaptobrevin, as well as t-SNARE and ternary SNARE complexes (94, 95). These studies showed further that competition occurred between Gβγ and the synaptic calcium sensor synaptotagmin 1 for binding sites upon SNAP-25. From this, it was hypothesized that Gβγ could displace synaptotagmin 1 at docked and primed SNARE complexes, preventing it from performing its fusogenic lipid-mixing activity. At higher levels of Ca2+, the fully Ca2+-occupied synaptotagmin would bind more tightly to the membrane-bound SNARE complex (94–96) and be more able to overcome the inhibition of Gβγ (94, 95, 97).

Lipid-mixing assays in reconstituted systems with pure components lent critical support to this hypothesis. Gβγ inhibited Ca2+-synaptotagmin and SNARE-dependent lipid mixing in a defined system of v- and t-SNARE–harboring liposomes, in a concentration-dependent manner (97). This inhibitory action of Gβγ on lipid mixing was more potent at lower concentrations of synaptotagmin 1 C2AB and was greatly diminished in the complete absence of synaptotagmin 1 C2AB. In addition, Gβγ could displace fluorescent synaptotagmin 1 C2AB from t-SNARE–harboring supported lipid bilayers (97). These studies, much like the original GPCR reconstitution experiments of Lefkowitz and Gilman’s groups (19–21) published in JBC, show the requirements for a critical GPCR signaling pathway in a defined system.

The molecular requirements for the Gβγ–SNARE interaction

As stated above, the initial insight that the formed SNARE complex was the effector of Gβγ downstream of Ca2+ entry came from studies of the action of BoNT/A. After BoNT/A treatment, 5-HT1B receptor–mediated presynaptic inhibition was lost in both lamprey giant synapses (88) and in outputs from CA1 pyramidal neurons (76). Subsequent binding studies with recombinant SNAP-25Δ9 showed only a partial 1.5-fold loss of affinity for Gβγ relative to full-length SNAP-25, however (95), implying that other residues were involved.

In light of this finding, we utilized a peptide-mapping approach to find residues on SNAP-25 that were instrumental to bind Gβγ and confirmed these studies with scanning Ala mutagenesis (98). We found nine residues located in two clusters upon SNAP-25. The first cluster was adjacent to the N terminus, whereas the second cluster was adjacent to the C terminus. Mutation of eight of the nine residues to Ala abolished both the ability of Gβγ to bind SNAP-25 and the ability of the lamprey serotonin receptor to inhibit neurotransmission, creating a key mechanistic linkage between Gβγ binding and Gi/o-coupled GPCR-mediated inhibition of exocytosis (98). Despite this, mutation of the two residues highlighted by the peptide mapping screen within the C-terminal nine residues to Ala did not phenocopy the effect of BoNT/A with respect to Gi/o-coupled GPCRs (99), implying that more of the C terminus was involved. Screening of truncated SNAP-25 constructs for Gβγ binding showed that the extreme C-terminal three residues were involved in the mechanism (99). Given the two glycine residues within the extreme C terminus, we speculated that the high flexibility of this region may be required to stabilize Gβγ binding.

The binding of Gβγ to SNARE may be splice variant-dependent; the two splice variants of SNAP-25, SNAP-25A and SNAP-25B, differ by 9 residues via differential splicing of exon 5 within the palmitoylation domain of the linker region between the two SNARE-forming helices (100–102). The two splice variants exhibit time-dependent expression, with SNAP-25A predominating in embryonic development and early postnatal life and SNAP-25B being the predominant isoform in adults, although SNAP-25a expression is retained in the adult hypothalamus and cortex (102–104). These two splice variants appear to differ in their ability to interact with certain Gβγ subunits. Co-immunoprecipitation studies show that genetic ablation of SNAP-25B expression partially reduces the ability to co-immunoprecipitate Gβ2, but not Gβ1, with SNAP-25 in the hippocampus (105). The region of SNAP-25 where the two splice variants differ is adjacent to only one of the nine residues critical for Gβγ binding, residue Glu-62 (98). Scanning Ala mutagenesis showed no reduction in Gβγ binding after mutagenizing any of the residues that differ between each splice variant. Two possible explanations could account for this phenomenon: first, that this differential binding is due to perturbations in the local environment adjacent to residue Glu-62 and, second, that isoform-dependent palmitoylation results in more efficient localization of SNAP-25 to the G-protein/GPCR complex within the active zone.

Differential Gi/o-signaling mechanisms in secretory cells: synergy between Gβγ’s action on VGCC and SNARE

Subsequent to the initial discovery of the Gβγ–SNARE mechanism in the lamprey giant synapse, numerous studies have demonstrated its applicability to several families of Gi/o-coupled GPCRs in a variety of mammalian secretory cells. Over the past 18 years of Gβγ–SNARE research, the development of pharmacological and genetic tools for dissection of presynaptic GPCR mechanisms has allowed researchers to demonstrate its importance in an increasingly sophisticated manner. The release of GABA from Purkinje cells in the cerebellum was shown to occur via two mechanisms, including a spontaneous and cAMP-dependent form of release that was inhibited by group II metabotropic glutamate receptors (mGluRs) under conditions where Ca2+ entry from the extracellular space and internal release are both blocked (106).

The discovery that the Gβγ–SNARE mechanism could be bypassed via treatment with BoNT/A or the delivery of the SNAP-25 193–206 C-terminal peptide (88) was critical for distinguishing this mechanism from other pathways that regulate presynaptic release. Biochemical studies showed that the SNAP-25Δ9 produced by BoNT/A had lower affinity for Gβγ than full-length SNAP-25 (95). Single fiber inputs from the
nociceptive pontine parabrachial nucleus form synapses upon the neurons of the central amygdala, and neurotransmission at this synapse is regulated presynaptically by norepinephrine acting on the α2-adrenergic receptor (107). The action of this receptor was abolished after treatment with BoNT/A, phenocopying that of the lamprey serotonin receptor. In the paraventricular nuclei of the hypothalamus, α-opioid receptors regulate release from magnocellular neurosecretory cells with a mechanism that the authors attributed to the action of Gβγ on the release machinery; however, intriguingly, quantal size was not affected by α-opioid receptor agonism, differing from results observed with the lamprey serotonin receptor (108). Moreover, this α-opioid receptor agonism was abolished via treatment with ionomycin to permeabilize the plasma membrane to 2.5 mM Ca2+.

These latter results using ionomycin are consistent with effects in synapses, where high presynaptic Ca2+ prevents Gβγ-mediated SNARE interactions (76, 88), but create an intriguing contrast to results observed with amperometry in chromaffin cells, in which μ-opioid agonism remains inhibitory to release subsequent to treatment with ionomycin in 5 mM Ca2+ (109). Nevertheless, the charge of the chromaffin cell amperometric spike, a measure homologous to quantal size in neurons, was reduced by overexpression of Gβγ, as was quantal size itself by the action of serotonin receptors in the lamprey giant synapse (89). In peripheral tissue, the Gβγ–SNARE mechanism also inhibits release. This was first demonstrated to occur in the β cells of the islets of Langerhans, where the α5 adrenergic receptor inhibited insulin release in a manner that could be overcome by BoNT/A or via intracellular chelation of Gβγ with the SNAP-25 193–206 peptide (110).

In the hippocampus at CA1-subicular synapses, the 5-HT1B receptor was shown to inhibit exocytotic release without altering Ca2+ fluxes. Destruction of the exocytotic apparatus with BoNT/C was shown to liberate Gβγ from its site on SNARE; Gβγ is then capable of modulating Ca2+ entry through VGCC (76). GABA_B receptors on the same presynaptic terminals acted to reduce Ca2+ fluxes (Fig. 1). Also, in the hippocampus, long-term depression of synaptic strength in presynaptic Schaffer collateral terminals, as well as presynaptic inhibition mediated by group II mGluRs, was similarly shown to be dependent upon Gβγ and the C terminus of SNAP-25 (111).

From this group of studies as a whole, we hypothesize that Gβγ–SNARE and Gβγ–VGCC actions are synergistic, receptor-specific mechanisms to provide more complete temporal and scalar control over vesicular release. In hippocampal CA1-subicular paired recordings, greater extent of inhibition was observed by treating with both a 5-HT1B agonist and a GABA_B agonist (Fig. 2). The addition of a GABA_B agonist prevents recovery of EPSC amplitude subsequent to treatment with the 5-HT1B agonist during repetitive stimulation. We interpret these data as the GABA_B agonist blocking the influx of Ca2+ into the terminal, preventing Ca2+ from binding to synaptotagmin and thus reducing its competition with Gβγ on the SNARE complex and allowing augmented inhibition. This paradigm of G_11_α-coupled GPCRs working in tandem to synergistically inhibit release may be present for a multitude of receptor systems at many different synapses.

The development of mouse genetic models deficient in the Gβγ–SNARE interaction

The idea of Gβγ–SNARE–dependent GPCRs and Gβγ–VGCC–dependent GPCRs acting in a synergistic manner was expanded upon with the development of the SNAP-25Δ3 mouse model, which carries a point mutation in SNAP-25 (Gly-
toxic reagents, such as BoNT/A or Cd2+, permits researchers to investigate the mechanism in the absence of expressing secretory cell populations. Critically, the model permits studies at the CA1-subicular synapse in the SNAP-25−/−H9004 SNAP-25 heterozygote phenocopy the results (112) obtained with WT animals (76), indicating a role for GABAB receptor. Moving forward, we anticipate that the −SNARE mechanism in all SNAP-25−/−−SNARE mechanism (99, 112) uniformly inhibited EPSCs throughout the stimulus train. This indicates the least homology to the other G protein–mediated presynaptic inhibition leading to physiological and behavioral phenotypes. Science Signaling 2019, ©American Association for the Advancement of Science.

204), truncating it by three residues. The mutation partially disables the inhibitory Gβγ−SNARE mechanism (99, 112) while maintaining full excitatory activity. This mouse is viable in the homozygous state, enabling researchers to assess the importance of the Gβγ−SNARE mechanism in all SNAP-25−/−−SNARE mechanism (112) obtained with BoNT/A in WT animals (76), indicating a role for Gβγ−SNARE in the 5-HT1B receptor, but not the GABAergic receptor. The animal model was also used to identify a new role for the Gβγ−SNARE mechanism on attenuating stress in the bed nuclei of the stria terminalis (BNST), where presynaptic inhibition upon excitatory parabrachial inputs onto BNST neurons was shown to occur via the α2A adrenergic receptor, but not the GABAergic receptor. Moving forward, we anticipate that the SNAP-25−/−−SNARE mechanism is required for Gβγ−SNARE disruption. In the future, we also plan to develop genetic models that permit tissue-specific ablation of the mechanism to more directly assess its importance in individual circuits. This would allow researchers the ability to identify specific neuronal subtypes where Gβγ−SNARE could be a key regulatory mechanism.

**Specificity of Gβγ signaling**

A growing body of work supports the notion of subunit specificity for a subset of effectors of the five known Gβ and 12 Gγ subunits, most of which can heterodimerize with each other in vitro, although in vivo evidence is more scarce (113, 114). Genetic studies show that despite considerable homology, Gβ and Gγ subunits perform specific roles in development. For example, genetic ablation of Gβ3 leads to microencephaly or neural tube closure deficits: homozygous null mutants died within 2 days of birth (115). Correspondingly, human de novo loss-of-function mutations in Gβi, were associated with hypotonia and seizures along with prominent neurodevelopmental disability (116). Gβ2 is a mediator of neuronal excitability through inhibition of T-type Ca2+ channels (117, 118).

Gβγ, which shows the least homology to the other Gβ subunits, has a unique capacity to form heterodimers with the Gγ-like domain of the regulator of G protein signaling (RGS) proteins, such as RGS9, in addition to several studies showing heterodimer formation with Gγ1 (119–121). Genetic ablation of Gβ5 shows a very different pattern of phenotypes to Gβ1; Gβ5 knockout mice showed growth deficits, abnormal motor behavior (“tiptoe-walking” in the unstressed state, and hyperactivity (122, 123), despite being viable. Gγγ−null mice exhibit handling-induced seizures with associated mortality, while being less susceptible to diet-induced obesity than WT littermates, whereas Gγγ−null mice have deficits in adenyl cyclase activity and increased startle response, but normal locomotion (124, 125). These variable phenotypes resulting from genetic ablation of individual Gβ and Gγ subunits suggest isofrom dependence for Gβγ dimerization and specificity in signaling roles (126–128). Multiple independent groups have observed heterodimer or isofrom-dependent coupling of receptors to effectors (129, 130). This may be attributable to variations in expression level, localization, or affinity for effectors within each Gβ and Gγ subunit (131); for example, somatostatin receptors in RINm5F cells were specifically shown to inhibit VGCC via Gβγ, but the muscarinic receptor was shown to couple to VGCC via Gγ1 (135). In GH3 cells, a similar result was observed for somatostatin receptors coupling to VGCC with Gγ1, but the muscarinic receptor was shown to couple to VGCC via Gγ1 (135). In addition, Gβγ subunits have distinct expression patterns in the brain (131, 136, 137).

Furthermore, unique Gβγ isoforms play specific roles in mediating interactions with both receptors and effectors (126–128, 130), and isoforms exhibit tissue-dependent specificity for...
individual receptors or effectors (125, 139–141). This has been observed for the t-SNARE complex, where Gβγ has a 14-fold higher affinity than Gβ1γ1 (97) and a 20-fold higher potency at inhibiting exocytosis (94). However, the lack of subunit-specific antibodies for each Gβ and Gγ subunit limits the understanding of Gβγ specificity. Using a powerful quantitative proteomic approach (131), we are beginning to get a glimpse of the specificity by which different Gβγ subunits are recruited to both GPCRs (131) and the SNARE complex (142). For example, we determined the in vivo Gβγ specificity of presynaptic α2AR-adrenergic receptors (α2ARs) in both adrenergic (auto-α2ARs) and nonadrenergic neurons (hetero-α2ARs) (142). This study suggested that auto- and hetero-α2ARs utilize different Gβγ subunits to regulate their downstream signaling mechanisms. We are currently using similar approaches to delineate mechanisms of Gβγ subunit specificity to SNARE complexes to continue to pursue the remarkable specificity of Gβ and Gγ subunits.

Future directions

Negative regulators of signaling of many types are very important for shaping signals. For example, RGS proteins and phosphatases both have very important roles in controlling the turnover rate of signal transduction. The inhibition of secretion is no different. This type of regulation is like a rheostat; it does not turn off rate of signal transduction. The inhibition of secretion is important for shaping signals. For example, RGS proteins and phosphatases both have very important roles in controlling the turnover rate of signal transduction. The inhibition of secretion is no different. This type of regulation is like a rheostat; it does not turn off rate of signal transduction. The inhibition of secretion is important for shaping signals. For example, RGS proteins and phosphatases both have very important roles in controlling the turnover rate of signal transduction. The inhibition of secretion is no different. 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We noticed that the phenotypes resulting from disabling Gβγ–SNARE interaction are of variable strength, with, for example, subtle effects on locomotion but dramatic effects on stress responses (112). This leads us to suggest that targeting the Gβγ–SNARE interaction might have some level of specificity. In addition, specificity might be improved by combining an inhibitor with an antagonist of a presynaptic Gi/o-coupled receptor (Fig. 3). The two agents would be on the same pathway, and we hypothesize that untoward side effects of each could be mitigated. A great deal is known about regulation of exocytosis by Gβγ–VDCC interaction, and this has led to a large class of pharmaceuticals that work as calcium channel modulators (138). The Gβγ–SNARE interaction downstream of calcium entry is much less understood and has been studied in relatively few cases. Enhancement of the release of hormones and neurotransmitters by inhibiting the Gβγ–SNARE interaction could be a novel pharmaceutical strategy. This is an underappreciated locus that has great potential to differentiate various modulatory pathways and to provide fine tuning of hormone or neurotransmitter release within a range of physiological activities. Similarly, regulation of exocytosis through the two Gβγ-mediated modulatory mechanisms enhanced each other’s activity (Fig. 2), leading to a more profound level of inhibition (Fig. 3).

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