Review

Diversity of structure and function of $GABA_B$ receptors: a complexity of $GABA_B$-mediated signaling

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Abstract: γ-aminobutyric acid type B ($GABA_B$) receptors are broadly expressed in the nervous system and play an important role in neuronal excitability. $GABA_B$ receptors are G protein-coupled receptors that mediate slow and prolonged inhibitory action, *via* activation of Gαi/o-type proteins. $GABA_B$ receptors mediate their inhibitory action through activating inwardly rectifying K^+ channels, inactivating voltage-gated Ca^{2+} channels, and inhibiting adenylate cyclase. Functional $GABA_B$ receptors are obligate heterodimers formed by the co-assembly of R1 and R2 subunits. It is well established that $GABA_B$ receptors interact not only with G proteins and effectors but also with various proteins. This review summarizes the structure, subunit isoforms, and function of $GABA_B$ receptors, and discusses the complexity of $GABA_B$ receptors, including how receptors are localized in specific subcellular compartments, the mechanism regulating cell surface expression and mobility of the receptors, and the diversity of receptor signaling through receptor crosstalk and interacting proteins.

Keywords: $GABA_B$ receptors, G protein-coupled receptors, di-/oligomerization, trafficking, interacting proteins, posttranslational modification

Introduction

γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS). As many as one-third of CNS neurons in the brain use GABA as their primary neurotransmitter.[1),2)] Most of these neurons are interneurons, which are capable of altering the excitability of neural circuits by regulating glutamatergic neurons and preventing hyperexcitation. GABA provides strong inhibitory effects by acting on two distinct classes of receptors based on their physiological and pharmacological properties. GABA type A ($GABA_A$) receptor is a ligand-gated chloride channel

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Abbreviations: AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPK: 5′AMP-dependent protein kinase; ATF: activating transcription factor; ATP: adenosine triphosphate; BBB: blood-brain barrier; BDNF: brain-derived neurotrophic factor; CaMKII: calcium/calmodulin-dependent protein kinase II; cAMP: cyclic AMP; Ca_V: voltage-gated Ca^{2+}; CGP35348: 3-Aminopropyl-(diethoxymethyl) phosphinic acid; CGP36742: 3-Aminopropyl-butylphosphinic acid; CGP46381: (3-Aminopropyl)(cyclohexylmethyl)phosphinic acid; CGP51176: 3-amino-2(*R*)-hydroxypropyl-cyclohexylmethyl-phosphinic acid; CGP7930: 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol; CHOP: C/EBP-homologous protein; CNS: central nervous system; CREB: cAMP response element-binding protein; C/EBP: CCAAT/enhancer-binding protein; DAG: diacylglycerol; ER: endoplasmic reticulum; GABA: γ-aminobutyric acid; $GABA_A$: GABA type A; $GABA_B$: GABA type B; GDP: guanosine diphosphate; GHB: γ-hydroxy-butyric acid; GIRK: G protein-activated inwardly-rectifying K^+; GISP: GPCR interacting scaffolding protein; GPCR: G protein coupled receptor; GRKs: G protein-coupled receptor kinases; GS39783: N,N′-dicyclopentyl-2-methylsulfanyl-5-nitropyrimidine-4,6-diamine; GTP: guanosine triphosphate; IGF-1: insulin-like growth factor-1; IP_3: inositol trisphosphate; KCTD: Potassium Channel Tetramerization Domain-containing; mGluRs: metabotropic glutamate receptors; Mupp1: Multi-PDZ domain protein 1; NGF: nerve growth factor; NMDA: N-methyl-D-aspartate; NSF: N-ethylmaleimide-sensitive factor; PAM: positive allosteric modulator; PKA: cAMP-dependent protein kinase; PKC: protein kinase C; PLC: phospholipase C; PTM: posttranslational modification; RGS: G protein signaling; RTKs: receptor for tyrosine kinases; TrkB: tropomyosin-related receptor kinase B; VFT: Venus flytrap domain; 7TM: heptahelical transmembrane domain.

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which mediates fast inhibitory signals through rapid postsynaptic membrane hyperpolarization,[2] whereas the metabotropic $GABA_B$ receptor produces slow and prolonged inhibitory signals *via* G proteins and second messengers.[3] Altered $GABA_B$ receptor function has been reported in a variety of neurological and psychiatric disorders, including epilepsy, depression, drug addiction, cognition, and nociception. This review will summarize our current knowledge of $GABA_B$ receptor structure, function, and binding partners, and how $GABA_B$ receptor trafficking is modulated by posttranslational modification. In addition, the relevance of $GABA_B$ receptors in various diseases will be discussed, along with current therapeutic attempts with $GABA_B$ receptor drugs.

1. Structure of $GABA_B$ receptors

$GABA_B$ receptors were first identified by Dr. Norman Bowery in 1979 as a receptor that reduces norepinephrine release through bicuculline- and isoguvacine-insensitive receptors.[4,5] The first $GABA_B$ receptor was only cloned in 1997, nearly 20 years after their discovery.[6] $GABA_B$ receptors are members of class C G protein-coupled receptor (GPCR) family. GPCRs are commonly divided into four classes (A, B, C, and F) based on the sequence homology levels of their transmembrane domains,[7] and class A is by far the largest and most studied GPCRs. Class C GPCRs are composed of metabotropic glutamate receptors (mGluRs), $GABA_B$ receptors, Ca^{2+}-sensing receptors, taste receptors, pheromone receptors, and several orphan receptors.[8] $GABA_B$ receptors and taste receptors are obligatory heterodimers, whereas others are traditionally considered to function as homodimers, although recent studies discovered the assembly of class C GPCRs with other classes of GPCRs.[9,10]

$GABA_B$ receptors are prototypical heterodimers of R1 and R2 subunits.[11–13] $GABA_B$ receptor subunits are composed of three domains: a long extracellular N-terminal domain called Venus flytrap domain (VFT), which contains the orthosteric binding site for GABA; a heptahelical transmembrane domain (7TM); and a C-terminal intracellular tail (Fig. 1). Among these domains, the three-dimensional structures have been solved for the extracellular N-terminal domain and a fragment of the C-terminal intracellular tail.[14–16] The VFT of R1 subunits binds to orthosteric ligands but not R2 subunits, although R2 subunits share 54% similarity with R1 subunits.[17,18] Instead, R2 subunits couple with G protein to produce G protein-mediated signaling.[19–21] Therefore, it is necessary for $GABA_B$ receptors to form R1/R2 heterodimers to produce GABA-mediated GPCR functions. The VFT is a shared structural feature among all class C GPCRs and is also found in bacterial periplasmic binding proteins.[8,22] The existence of numerous alternatively spliced variants of $GABA_B$ receptor subunits have been described.[3] R1 subunits comprise several splice variants designated as R1a, R1b, R1c, R1e, R1j, R1k, R11, R1m, and R1n.[23] In the human CNS, the two major splice variants are the R1a and R1b isoforms, which have been studied intensively and are known to provide the molecular diversity of $GABA_B$ receptors.[3,24] Structurally, the isoforms differ in their N-terminal domain, with a pair of sushi domains present in R1a (961aa) but not in R1b (844aa) (Fig. 1).[25] Sushi domains have been found in several GPCRs[26] and can mediate protein interactions in a wide variety of adhesion proteins.[27] Possibly due to the presence of these sushi domains, R1a subunit-containing $GABA_B$ receptors are preferentially targeted to the axon terminals of excitatory synapses. Postsynaptically, both R1a and R1b isoforms are found in dendrites, but only the R1b subunit seems to localize in spine heads.[28,29] Other splice variants of R1 subunits also exhibit some unique features. The R1c isoform has a single sushi domain and is widely expressed in the brain.[30,31] The R1e/g/h/i/j/l/m/n isoforms do not have the 7 transmembrane domains, G-protein coupling region nor the C-terminal tail; therefore, they are thought to be secreted from cells. The R1e isoform (578aa), which is mainly expressed in peripheral tissues, strongly interacts with R2 subunits and disturbs normal R1/R2 heterodimer formation.[32] Purified sushi domains of the R1j isoform impairs the inhibitory effect of $GABA_B$ receptors on evoked and spontaneous glutamate release.[33] The R1g/h/i isoforms show similar sequences to R1j (190aa) containing sushi domains followed by a unique C-terminal sequence,[34] but their function remains to be elucidated. Other isoforms such as R1d/f are mostly found in transcription expression profiles and so far, no function has been confirmed.[34] Taken together, R1 subunit alternative splicing provides a diverse range of structural and functional $GABA_B$ receptors, and further studies are necessary to understand the physiological role of these isoforms.

The structure of a heterodimeric complex of R1b VFT and R2 VFT has been solved by X-ray crystallography.[16] The two subunits bind in a side-

by-side manner through non-covalent interactions between the N-terminal lobe structure within the VFT but facing the opposite direction.[16] The VFT contains two lobes, LB1 and LB2, reaching further into the extracellular space, and LB1–LB1 interaction serves to facilitate heterodimer formation.[15] In addition, $GABA_B$ receptor subunits lack the cysteine-rich region found at the C-terminal end of mGluR ectodomains, which are involved in the propagation of signals induced by the binding of orthosteric agonists to mGluRs.[16),35]

Dimers, tetramers, or even higher-order oligomers of $GABA_B$ receptors can be detected both in heterologous systems and in native neurons.[36)–39] $GABA_B$ receptors are present in equilibrium between heterodimers and higher-order oligomers with a relative preference for tetramers (dimers of dimers) and octamers (tetramers of dimers).[37] Of note, $GABA_B$ receptor heterodimers are stable due to strong non-covalent interactions, and the higher-order oligomers are the result of weaker and possibly transient interactions among heterodimers.[37] Agonist stimulation does not alter receptor di-/oligomerization, but increases the lateral mobility of $GABA_B$ receptor complexes.[37),40] Therefore, it is possible that the association and dissociation of $GABA_B$ receptors occurs in specific locations on the cell surface, and the dynamic re-arrangement of $GABA_B$ receptor complexes allow the expression of a variety of $GABA_B$-mediated signaling pathways in various cellular locations.

2. $GABA_B$ receptor function

$GABA_B$ receptor-mediated signaling pathways involve one of three effector proteins: G protein-activated inwardly-rectifying K^+ (GIRK) channels, voltage-gated Ca^{2+} (Ca_V) channels, and adenylyl cyclase (Fig. 1).[3] The downstream effects of $GABA_B$ receptors include inhibition of neurotransmitter release and modulation of neuronal excitability.[3] The $GABA_B$ receptors couple to pertussis toxin-sensitive G proteins (Gαi/o family).[41] Following $GABA_B$ receptor activation, G proteins dissociate into their Gα and G$\beta\gamma$ subunits. The Gαi/o subunits inhibit adenylyl cyclase to reduce cyclic AMP (cAMP) levels, whereas G$\beta\gamma$ subunits inhibit Ca^{2+} channels and activate GIRK channels.[42)–44] It is known that Gαi/o proteins inhibit adenylyl cyclase types I, III, V, and VI, whereas G$\beta\gamma$ stimulates adenylyl cyclase types II, IV, and VII. This stimulation depends on the presence of Gαs, which results from the activation of GPCRs.[45),46] $GABA_B$ receptor agonist stimulation inhibits basal and forskolin-stimulated neuronal adenylyl cyclase and reduces intracellular cAMP levels.[47] However, it has been reported that the activation of $GABA_B$ receptors can enhance cAMP formation through Gs-coupled GPCR activation.[48] Both the inhibition and enhancement of cAMP levels by $GABA_B$ receptors have been confirmed *in vivo*.[49] $GABA_B$ receptors have been implicated in synaptic plasticity and memory formation.[50] Because cAMP-dependent protein kinase (PKA) expresses a specific form of synaptic plasticity, which is associated with hippocampal long-term memory,[51] the cAMP-PKA signaling pathways regulated through $GABA_B$ receptors are likely to be a mechanism for fine-tuning synaptic plasticity.

Ca_V channels mediate calcium influx in response to membrane depolarization, thus regulating intracellular processes such as muscle contraction, release of hormones and neurotransmitters, excitation of neurons, and gene expression.[52] One of the first confirmed ion channel effectors of the $GABA_B$ receptor is the Ca_V channel. $GABA_B$ receptors decrease calcium conductance in neuronal membranes, and this action appears to be linked primarily with presynaptic receptors.[53] Presynaptic $GABA_B$ receptors inhibit the opening of Ca_V channels, mainly N-type ($Ca_V2.2$) and P/Q-type ($Ca_V2.1$), through G$\beta\gamma$ subunits to repress calcium influx and trigger neurotransmitter release.[54] Ca_V channels are formed as a complex of several different subunits, $\alpha1$, $\alpha2\delta$, $\beta1$-4, and γ. The structural subunit of Ca_V channels is $\alpha1$, which forms an ion channel pore and regulates ion gating properties.[55] The electrophysiological and pharmacological diversity of Ca_V channels also arises from the existence of $\alpha1$ subunits, which encode at least 10 distinct genes that are further divided into three subfamilies (Ca_V1, Ca_V2, and Ca_V3).[56] The Ca_V1 subfamily includes $Ca_V1.1$, $Ca_V1.2$, $Ca_V1.3$, and $Ca_V1.4$, which are known as L-type channels, are typically high voltage-activated and dihydropyridine-sensitive. $Ca_V2.1$, $Ca_V2.2$, and $Ca_V2.3$ are high voltage-activated and dihydropyridine-insensitive channels mediating P/Q-type, N-type, and R-type Ca^{2+} currents. Ca_V3 channels $Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$ are low voltage-activated and dihydropyridine-sensitive channels, which are called T-type for their transient currents production.[52] L-type and T-type Ca_V families are expressed in many cell types, whereas N-, P/Q-, and R-types are predominantly expressed in neurons. There is also some evidence suggesting that $GABA_B$ receptors inhibit N-type and

P/Q-type Ca_V channel subtypes at postsynaptic sites.[57] These channels are likely to have a role in the generation of dendritic spikes and the amplification of excitatory postsynaptic potentials.[58]

GIRK channels are widely expressed within the CNS and constitute a key determinant of membrane excitability because they mediate the postsynaptic inhibitory effects of many neurotransmitters, including $GABA_B$ receptors.[59] When activated, postsynaptic $GABA_B$ receptors increase potassium conductance in neuronal membranes by opening GIRK channels to promote K^+ efflux (Fig. 1).[60] This reaction occurs through $G\beta\gamma$ subunits, resulting in a hyperpolarization of the neuron that underlies slow and prolonged inhibitory postsynaptic potentials.[59] There is also a convincing evidence that $G\alpha$ subunits can directly interact with intracellular domains of GIRK channels and control their gating.[61),62] In mammals, there are four different GIRK channel subunits (GIRK1, GIRK2, GIRK3, and GIRK4); each consists of two transmembrane spanning domains with both the N- and C-terminus on the intracellular side of the membrane, and a pore domain located between the two transmembrane domains.[63] These GIRK channel subunits form functional homotetrameric or heterotetrameric channels.[64),65] Three GIRK channel subunits (GIRK1, GIRK2, and GIRK3) exhibit broad distributions in the CNS, whereas GIRK4 expression is found primarily in the heart.[66] Consisting of a functional interaction between GIRK channels and $GABA_B$ receptors, these two receptors are highly colocalized in dendritic spines,[67] and the oligomerization of $GABA_B$ receptors with GIRK channels (GIRK1 and GIRK3) has also been reported.[68]

3. Pharmacology of $GABA_B$ receptors

The ligands for $GABA_B$ receptors can be divided into three types, agonists, antagonists, and allosteric modulators. Baclofen, an analogue of GABA, was the first selective agonist, which was synthesized in 1962, to enhance blood-brain barrier (BBB) penetration.[3] Up to now, baclofen is the only drug that targets $GABA_B$ receptors on the market, and it is used as a muscle relaxant to treat spasticity due to spinal cord injury, cerebral palsy, and multiple sclerosis.[69] Phenibut, a deschloro analogue of baclofen, was developed in the Soviet Union in the 1960s and introduced as a neuropsychotropic drug.[70] It is also a potent blocker of $\alpha 2\delta$ subunit-containing voltage-dependent calcium channels.[71] Today, phenibut is marketed for medical use in Russia, Ukraine, and Latvia, but is not approved for clinical use in the United States and in most of European countries. The anticonvulsant and analgesic drug gabapentin interacts with the sushi domain of R1a/R2 heterodimer.[72)–74] However, the inhibitory effect of gabapentin was found to be independent of the activation of $GABA_B$ receptors.[75)–78] The phosphinic acid analogue of GABA, 3-aminopropyl-phosphonic acid (CGP27492) and its methyl homolog CGP35024 are more potent than baclofen.[79)–81] Of note, CGP27492 and CGP35024 also act as antagonists for $GABA_C$ receptors, the newly identified members of the Cl^--permeable ionotropic GABA receptors that mediate slow and sustained neural inhibition.[82)–84] Other methyl phosphinic acid-based agonists include CGP44532 and its (R)-(+)-enantiomer CGP44533. CGP44532 has a longer lasting inhibitory effect than CGP44533 with more potent analgesic response than baclofen.[85]

There is a rapid transition from γ-aminopropyl-methyl-phosphinic acid CGP35024 acting as a $GABA_B$ receptor agonist to its homolog γ-aminopropyl-ethyl-phosphinic acid CGP36216 acting as a $GABA_B$ receptor antagonist.[79),86),87] It has been shown that CGP47656 increases the release of GABA on presynaptic $GABA_B$ autoreceptors in the rat neocortex but it also acts as a full agonist at presynaptic $GABA_B$ heteroreceptors by inhibiting the release of somatostatin.[88] γ-Hydroxy-butyric acid (GHB) is a minor metabolite of GABA synthesized by GABA transaminase and succinic semialdehyde reductase. GHB is known to act as a weak $GABA_B$ receptor partial agonist and is used to treat excessive daytime sleepiness and cataplexy in patients with narcolepsy.[89]

$GABA_B$ receptor antagonists that block slow inhibitory signaling have been developed.[90] Notably, the first available selective $GABA_B$ receptor antagonists are all baclofen analogues, namely phaclofen, sacrofen, and 2-hydroxy-sacrofen. Although these antagonists display low potency, they cannot penetrate the BBB. The first $GABA_B$ receptor antagonists capable of penetrating the BBB were 3-aminopropyl-diethoxymethyl-phosphinic acid (CGP35348), 3-aminopropyl-butylphosphinic acid (CGP36742), 3-aminopropyl-cyclohexylmethyl-phosphinic acid (CGP46381), and 3-amino-2(R)-hydroxypropyl-cyclohexylmethyl-phosphinic acid (CGP51176). These antagonists are called first-generation $GABA_B$ receptor antagonists.[69] Currently there are numerous $GABA_B$ receptor antagonists, which display IC_{50} values from the nanomolar

to micromolar range and their therapeutic potentials are currently being studied.

Allosteric modulators are molecules that bind to a site on a neurotransmitter receptor that is topographically distinct from the orthosteric binding pocket for agonists.[91] Allosteric agents have little or no intrinsic agonistic activity on their own but induce conformational changes in the receptor, and affect the interaction of receptors with agonists and associated proteins. Allosteric modulators of $GABA_B$ receptors constitute a good pharmacological alternative to gain selectivity for the treatment of various disorders, because of their unique structure. The $GABA_B$ receptor positive allosteric modulator (PAM) acts by stabilizing the active conformation of the 7TM domain in R2 subunits and thus induces the closure of the VFT domain in the R1 subunit, which is associated with $GABA_B$ activity (Fig. 1). The first available PAMs for $GABA_B$ receptors were 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and N,N′-dicyclopentyl-2-methylsulfanyl-5-nitropyrimidine-4,6-diamine (GS39783), both of which enhance agonist-stimulated responses by binding at the 7TM domain of the R2 subunit.[91] CGP7930 shows antidepressant-like effects and reduces alcohol intake in rodents.[92] CGP7930 directly acts as a PAM and a partial agonist through R2 subunits, which can facilitate agonist responses at low concentrations, and activate the receptor at higher concentrations.[93–95] A more potent compound has also been identified, GS39783, which enhances $GABA_B$ receptor-mediated inhibition of cAMP formation and shows anxiolytic-like effects and attenuates rewarding properties of the substances of abuse.[96–98]

4. $GABA_B$ receptor interacting proteins

GPCR function can be attributed to receptor interacting molecules that are expressed and function in distinct cell types.[99] A number of interacting proteins for $GABA_B$ receptors have been identified (Fig. 2).[99] These proteins are important not only for regulating receptor activity but also for modulating receptor trafficking. This section summarizes the types of proteins that interact with $GABA_B$ receptors and discusses their roles in $GABA_B$ receptor function (Table 1).

G proteins and RGS proteins. $GABA_B$ receptor-mediated signal transduction requires G proteins and G protein signaling (RGS) proteins.[100] From biochemical studies, it is evident that $GABA_B$ receptors predominantly couple to $G\alpha i$- and $G\alpha o$-type G proteins.[101] Activated receptors catalyze the exchange of guanosine diphosphate to guanosine triphosphate (GTP) on $G\alpha$ subunits, promoting conformational changes of heterotrimeric G proteins ($G\alpha\beta\gamma$) and dissociation of $G\alpha$ subunits from $G\beta\gamma$ subunits. The GTP-bound $G\alpha i/o$ subunit then inhibits adenylyl cyclase, decreases intracellular cAMP levels and reduces PKA-mediated signaling.[100] $G\beta\gamma$ subunits, on the other hand, couple with two types of ion channels, GIRK and Ca^{2+} channels. These channels modulate second messengers such as cAMP, diacylglycerol (DAG), or inositol trisphosphate (IP_3) and regulate multiple signaling cascades.

It is well known that the family of RGS proteins are essential for GPCR-GIRK channel signaling pathway.[102] RGS proteins negatively regulate GPCR signaling by serving as $G\alpha$ GTPase-activating proteins.[103] At least 37 RGS proteins have been identified in humans, with a conserved RGS homology domain that is crucial for GTPase activity.[104] They are classified into eight subfamilies (RZ, R4, R7, R12, RA, GEF, GRK, and SNX) based on their structure and amino acid sequence similarities. RGS2 protein reduces $GABA_B$-GIRK signaling sensitivity in dopaminergic neurons of the ventral tegmental area.[105] RGS6 protein plays a role in motor coordination by modulating $GABA_B$ receptor signaling in the cerebellum.[106] Furthermore, RGS4 protein directly associates with the R2 subunit of the $GABA_B$ receptor in the prefrontal cortex and hypothalamus.[107] RGS4 protein also induces a faster form of desensitization within a second of agonist application *in vitro*.[108]

Transcription factors. Two transcription factors, activating transcription factor (ATF)/cAMP response element binding-protein (CREB) family and CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) have been reported to associate with $GABA_B$ receptor subunits.[109–112] The C-terminus of R1 subunit interacts with the leucine zipper motif of ATF4/CREB2 and ATFx. Translocation of ATF4/CREB2 into or out of the nucleus is seen following $GABA_B$ receptor activation; however, the physiological significance of ATF4/CREB2 interaction with $GABA_B$ receptors is yet to be determined.[110,111] The interaction of $GABA_B$ receptor and CHOP is reported to regulate $GABA_B$ receptor surface expression. The C-terminal leucine zipper of CHOP associates with the leucine zipper present in the C-terminal domain of R2 subunits, and the N-terminal domain of CHOP associates with an unidentified intracellular site of the R1a

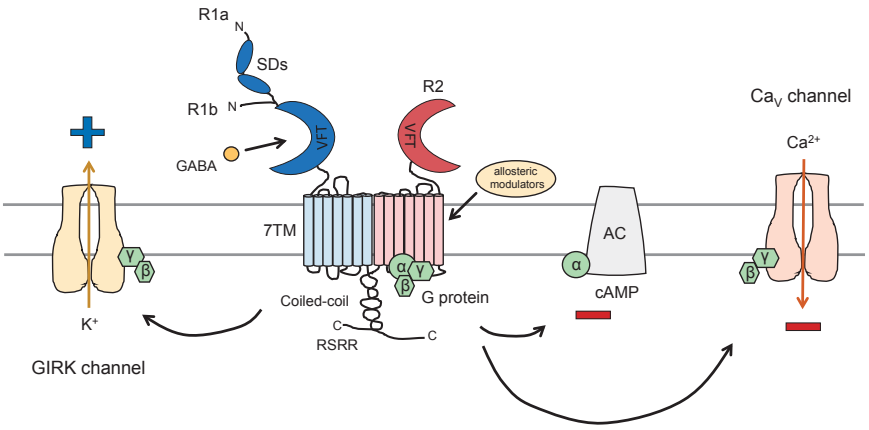

Fig. 1. Structural organization of $GABA_B$ receptors and the primary $GABA_B$ receptor effectors. Functional $GABA_B$ receptors form heterodimers composed of R1 and R2 subunits. Both subunits are heptahelical membrane proteins that have seven-transmembrane (7TM) domains with a large extracellular N-terminal domain containing a Venus flytrap (VFT) domain and a large intracellular C-terminal tail containing a coiled-coil protein–protein interaction module. The R1 subunit is responsible for ligand binding in the VFT domain, whereas the VFT of R2 subunit fails to bind any known ligands. Instead, the heptahelical domain of the R2 subunit contains a binding site for allosteric modulators, which affect the affinity of ligand binding to the R1 subunit. The interaction between the R1 and R2 subunits takes place at their C-terminus through the coiled-coil domains. The R1 subunit exists in two main isoforms. R1a is distinguished from R1b by the presence of two sushi domains (SDs). An endoplasmic reticulum (ER) retention signal (RSRR) is present distal to the coiled-coil domain in the R1 subunit and prevents the ER exit of R1 unless it is masked by an R2 subunit. The binding of GABA results in the recruitment and activation of $G\alpha i/o$ proteins *via* the R2 subunit. The activated $G\alpha i/o$ subunits inhibit adenylyl cyclase, resulting in lowered cAMP levels, while $G\beta\gamma$ subunits activate GIRK channels at postsynaptic sites and inhibit Ca_V channels at presynaptic sites, leading to neuronal inhibition.

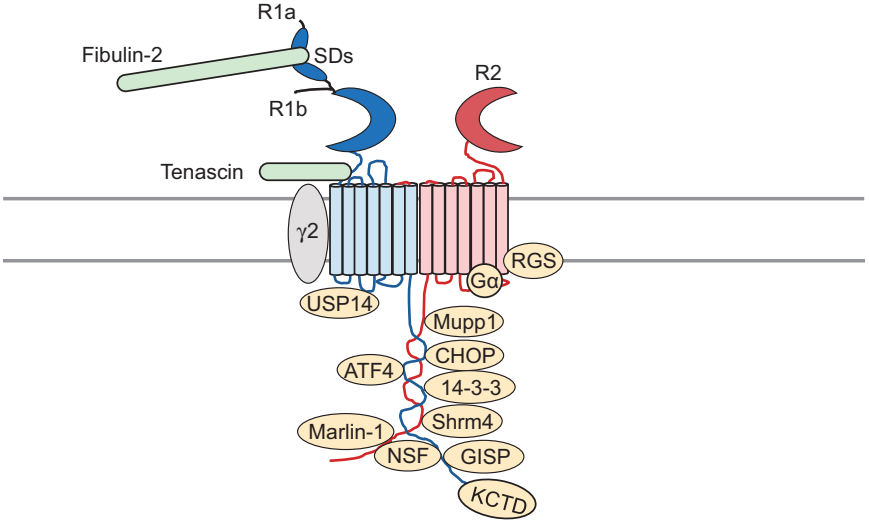

Fig. 2. $GABA_B$ receptor interacting proteins. A number of proteins have been found to interact with the C-terminus of $GABA_B$ receptor subunits. Among the interacting proteins are leucine-zipper transcription factors ATF4/CREB2 and CHOP, scaffolding and adaptor proteins 14-3-3, GISP, NSF, and PDZ domain-containing scaffold proteins Shrm4 and Mupp1. It is proposed that these proteins regulate receptor dimerization, intracellular trafficking, and synaptic localization. The C-terminus of the R2 subunit associates with KCTD proteins, which regulate Ca_V channel activity and $GABA_B$ receptor trafficking. The C-terminus of the R1 subunit associates with the brain-specific RNA binding protein Marlin-1 to target the cytoskeleton and regulate receptor transportation. Neurotransmitter receptors such as $GABA_A$ receptor $\gamma 2$ subunit, mGluRs, and GIRK channels are also $GABA_B$ receptor binding partners although only the $\gamma 2$ subunit has been identified to directly associate with R1 subunits so far. The N-terminus of R1 subunits also interact with proteins such as extracellular matrix protein fibulin-2 and tenascin. The extracellular sushi domains of the R1 subunit interact with fibulin-2, whereas tenascin binds to the extracellular domains of R1 subunits, possibly *via* the second transmembrane domain. Other proteins such as Gi/o proteins and RGS proteins bind to the R2 subunit to induce GPCR signaling.

Table 1. Summary of $GABA_B$ receptor interacting proteins

Proteins	Site of interaction	Function	References
Gi/o	R2 subunit second intracellular loop	Effector binding	3
		Essential for $GABA_B$-mediated signaling	101
RGS	R2 subunit	GIRK channel signaling	102–108
ATF4/CREB2	R1 subunit C-terminus	Receptor-mediated transcriptional regulation	110
			111
CHOP	R2 subunit C-terminus	Receptor-mediated transcriptional regulation	112
	R1a subunit	Accumulation of $GABA_B$ receptors in the ER	
14-3-3	R1 subunit	Interfere R1/R2 heterodimerization	118
GISP	R1 subunit	Enhance $GABA_B$ receptor surface expression	119
NSF	C-terminus of R1 and R2 subunits	Enhance $GABA_B$ receptor signaling and trafficking	120
Shrm4	R1 subunit C-terminus	Enhance $GABA_B$ receptor signaling and trafficking	121
Mupp1	R2 subunit C-terminus	Modulate $GABA_B$ receptor stability and signaling	122
$GABA_A$ receptor $\gamma 2$ subunit	R1 subunit	Enhance R1 subunit surface expression	126
		Enhance R1/R2 heterodimer internalization	
mGluR1	ND	$GABA_B$ receptor-mediated Ca^{2+} signaling	130
		Increase glutamate sensitivity of mGluR1	134
GIRK channel	ND	Receptor signaling	67
			68
KCTD	R2 subunit C-terminus	Reduces $GABA_B$ receptor internalization	145–148
		Increase S892 phosphorylation in R2 subunit	
Marlin-1	R1 subunit C-terminus	$GABA_B$ receptor transport?	149
Fibulin-2	Sushi domain of the R1 subunit	Receptor anchoring	25
Tenascin	Extracellular domains of R1 subunit	Suppress postsynaptic $GABA_B$ receptor activity	151
USP14	R1 subunit second intracellular loop	Regulates post-endocytic sorting of $GABA_B$ receptors	170

ND: not determined.

subunit.[112] In HEK293 cells, overexpression of CHOP induces the accumulation of $GABA_B$ receptors in the endoplasmic reticulum (ER).[112] Ischemia-mediated up-regulation of CHOP down-regulates cell surface $GABA_B$ receptors by preventing their trafficking from the ER to the plasma membrane, and disrupts $GABA_B$ receptor heterodimerization.[113]

Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) genes are thought to be the targets of $GABA_B$-mediated transcriptional regulation. The production of both NGF and BDNF are enhanced following $GABA_B$ receptor antagonist stimulation.[114] However, the linkage of $GABA_B$ receptor to the transcriptional factors such as a transduction signaling mechanism to the nucleus, still needs to be addressed.

Scaffolding proteins. Scaffold proteins are crucial regulators of many key signaling pathways. It offers a simple and flexible mechanisms for regulating selectivity in signaling pathways, shaping output cellular behaviors, and achieving new responses.[115] It is known that GPCRs also function as scaffolds for the recruitment of a variety of proteins that serve to modulate both G protein-dependent and -independent cellular signaling pathways, and regulate GPCR trafficking.[116]

The C-terminus of the R1 subunit contains consensus motifs involved in binding to 14-3-3 proteins, small dimeric proteins (27–32 kDa) with seven highly conserved isoforms (β, γ, ζ, σ, ε, η, and τ).[117] These proteins have been implicated in a variety of cellular processes, including regulation of

synaptic transmission *via* K^+ channels, GPCR-mediated signal transduction, and interactions with phosphoproteins.[117] Only two isoforms of 14-3-3 proteins ζ and η interact with the R1 subunit of the $GABA_B$ receptors, and interfere with R1/R2 heterodimerization.[118] A 130-kDa protein, GPCR interacting scaffolding protein (GISP), associates directly with the R1 subunit *via* a coiled-coil domain. GISP promotes $GABA_B$ receptor surface expression and enhances GIRK currents.[119] The C-terminus of both R1 and R2 subunits interact with the scaffolding protein N-ethylmaleimide-sensitive factor (NSF), an ATPase that is critical for intracellular trafficking.[120] Coordinated action of NSF and protein kinase C (PKC) regulates the activity of $GABA_B$ receptors.

The C-terminus of the R1 subunit contains a putative PDZ domain-binding consensus sequence. A recent study identified that Shrm4, a protein expressed only in polarized tissues and whose mutations have been linked to epilepsy and intellectual disability, interacts with the C-terminus of the R1 subunit and controls their cell surface expression and intracellular trafficking *via* a dynein-dependent mechanism.[121] Shrm4 associates with both R1a and R1b subunits, and Shrm4 knockdown reduced the levels of both isoforms in dendrites. Because the R1a subunit preferentially localizes to axons *via* its sushi domains, only an R1a subunit that could escape from axonal targeting may associate with Shrm4 in the Golgi apparatus and be re-directed to dendrites.[121] $GABA_B$ receptor R2 subunits possess a C-terminal motif VSGL that has the potential to interact with PDZ-domain-containing scaffold proteins. Biochemical analysis confirmed that Multi-PDZ domain protein 1 (Mupp1) interacts with R2 subunits and regulates $GABA_B$ receptor signaling as well as receptor stability.[122]

Neurotransmitter receptors. $GABA_B$ receptors interact with several neurotransmitter receptors and regulate receptor activity. Examples of such receptors are ionotropic $GABA_A$ receptors. Twenty-one $GABA_A$ receptor subunits have been cloned from the mammalian CNS. These have been divided into eight classes based on sequence identity: $\alpha(1–6)$, $\beta(1–3)$, $\gamma(1–3)$, δ, $\varepsilon(1–3)$, π, θ, and $\rho(1–3)$.[123] The majority of $GABA_A$ receptor subtypes in the brain are composed with a likely stoichiometry of $2\alpha{:}2\beta{:}1\gamma$.[124] To a lesser extent, $\delta/\varepsilon/\pi$ subunits replace the γ subunit to form benzodiazepine-insensitive receptor subtypes.[125] The $\gamma2$ subunit of $GABA_A$ receptors was found to interact with the R1 subunits of $GABA_B$ receptors and promote R1 subunit surface expression in the absence of R2 subunits.[126] On the other hand, the $\gamma2$ subunit associates with functional R1/R2 heterodimers and enhances $GABA_B$ receptor internalization in response to agonist stimulation.[126] In contrast, the activation of $GABA_B$ receptors promotes BDNF secretion through increased phospholipase C (PLC)/DAG/PKC activation, and enhances $GABA_A$ receptor cell surface expression.[127] Signaling crosstalk between $GABA_B$ and $GABA_A$ receptors has also been identified. In developing hypothalamic neurons, $GABA_B$ receptor activation depresses $GABA_A$ receptor-mediated Ca^{2+} elevation, both by reducing the presynaptic release of GABA and decreasing postsynaptic Ca^{2+} responses.[128] In dentate gyrus granule cells, $GABA_B$ receptors colocalize with $GABA_A$ receptors on postsynaptic dendritic and somatic membranes, and $GABA_B$ receptor activity enhances tonic inhibition induced by extrasynaptic $GABA_A$ receptors.[129]

mGluR1 is another receptor found to interact with $GABA_B$ receptors.[130] mGluR1 belongs to class C type GPCRs as a $GABA_B$ receptor. It couples with Gq protein to increase IP_3 production and Ca^{2+} signaling when activated by glutamate.[131] Both receptors exhibit a high co-localization in the dendritic spine of Purkinje cells but no oligomerization of $GABA_B$ receptor and mGluR1a is observed, suggesting the existence of a $GABA_B$-mGluR1 receptor complex but with no physical contact.[132),133] Extracellular Ca^{2+} interacts with $GABA_B$ receptors in cerebellar Purkinje cells, leading to an increase in the glutamate sensitivity of mGluR1, and that extracellular Ca^{2+}-mediated crosstalk is not mediated *via* Gαi/o proteins.[134] Precise control of these two receptors is thought to be important for the balance of neuronal inhibition and excitation.

Although no physical contact or complex formation has been reported, there is functional crosstalk between $GABA_B$ receptors and ionotropic glutamate receptors. The major synaptic Ca^{2+} signals in the brain are mediated *via* N-methyl-D-aspartate (NMDA) receptors, which are crucial for activity-dependent changes in synaptic plasticity.[135),136] Ca^{2+} influx *via* NMDA receptors is inhibited by $GABA_B$ receptor activation.[137] This effect on NMDA receptors is independent of GIRK channel or Ca_V channel activation. There are several reports, including from the author, that NMDA receptors can regulate $GABA_B$ receptor endocytosis, trafficking and, degradation.[138)–140] NMDA receptor activation promotes $GABA_B$ receptor phosphoryla-

tion and dephosphorylation depending on the length of NMDA receptor activation, and regulates $GABA_B$ receptor cell surface expression.[139] NMDA receptor-mediated regulation of $GABA_B$ receptors may be important in conditions of neurological diseases, such as epilepsy and ischemia. Another type of ionotropic glutamate receptor, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are the main fast synaptic transduction elements and crucial for synaptic plasticity. It was found that enhanced $GABA_B$ receptor activity increases the number of excitatory synapses and cell surface AMPA receptors.[50]

Functional crosstalk between $GABA_B$ receptors and receptor for tyrosine kinases (RTKs) has also been reported. $GABA_B$ receptors trigger the secretion of BDNF and subsequent activation of the tropomyosin-related receptor kinase B (TrkB) receptor signaling pathway to promote the development of GABAergic synapses.[141] The $GABA_B$ receptor can also transactivate insulin-like growth factor-1 (IGF-1) receptor (IGF-1R) to induce Akt (protein kinase B) phosphorylation and protect cerebellar granule cells from apoptosis.[142] Upon activation of $GABA_B$ receptors, $G\alpha i/o$ and $G\beta\gamma$ subunits are released from $GABA_B$ receptors, followed by recruitment of focal adhesion kinase 1, IGF-1R, and Akt to $GABA_B$ receptors. This dynamic regulation of $GABA_B$ receptor-associated complex formation is critical for signal transduction and transactivation-dependent neuronal survival.[143]

Other important binding proteins. A recent study showed that $GABA_B$ receptors form macromolecular complexes with members of a subfamily of the potassium channel tetramerization domain-containing (KCTD) proteins. KCTD proteins consist of 26 members that share sequence similarity with the cytoplasmic domain of voltage-gated K^+ (K_v) channels and have roles in various biological processes including transcriptional repression and cytoskeleton regulation.[144] The KCTD protein family members KCTD8, KCTD12, KCTD12b, and KCTD16 are tightly associated with the C-terminus of $GABA_B$ receptor R2 subunit.[145),146] This co-assembly changes the properties of $GABA_B$ receptors in a KCTD subtype-specific manner. For instance, KCTD16 and KCTD8 lead to the persistent inhibition of Ca_V channel activity, whereas KCTD12 and KCTD12b receptors transiently decrease Ca_V channel activity.[146),147] Furthermore, KCTD12 reduces the constitutive receptor internalization to increase the magnitude of receptor signaling.[148]

The C-terminus of the $GABA_B$ receptor R1 subunit associates with brain-specific RNA-binding protein Marlin-1, also designated as Jamip-1 or Jakmip1.[149] The association of $GABA_B$ receptor and Marlin-1 was found in cytoskeleton, thus it is thought to regulate receptor transport.[150] The N-terminus of the $GABA_B$ receptors also interacts with proteins such as fibulin-2. Fibulin-2 is an extracellular matrix protein that binds to the sushi domain of R1a subunit, but not R1b subunit.[25] Because R1a and R1b isoforms of the R1 subunit have been shown to preferentially localize to different subcellular compartments, fibulin-2 may provide evidence for the existence of subtype-specific interacting proteins. Finally, there is some evidence that the HNK-1 carbohydrate carried by many neural extracellular matrix proteins, such as tenascin-R and tenascin-C, binds to an extracellular domain of R1 subunits.[151] HNK-1 carbohydrate may be involve in homeostatic regulation of $GABA_A$ receptor-mediated perisomatic inhibition by suppressing postsynaptic $GABA_B$ receptor activity.[151]

5. Posttranslational modification of $GABA_B$ receptors

Posttranslational modifications (PTMs) of proteins play an important role in cellular functions. PTM is the covalent addition of certain functional groups to proteins. More than 40 PTMs have been identified, and their relation to the diseases such as cancer and neurological disorders have been proposed. This section summarizes two major PTMs found in $GABA_B$ receptors, phosphorylation and ubiquitination, and their role in regulating $GABA_B$ receptor function.

Phosphorylation. Protein phosphorylation is the most common and best studied PTM, in which protein function is regulated in response to extracellular stimuli both inside and outside the nervous system.[152] Regulation of protein phosphorylation requires protein kinases, protein phosphatases, and substrate proteins. Phosphorylation is involved in almost every cellular process, and it modulates the activity of target proteins at various cellular locations, and controls the activity of signaling networks. Protein phosphorylation is achieved by protein kinases that transfer a phosphate group from adenosine triphosphate (ATP) to serine, threonine, and/or tyrosine residues of a target protein, and this can be reversed by a reaction called dephosphorylation by protein phosphatases. Disruption or enhancement of protein phosphorylation is implicated in the

progression of various serious diseases including cancer, neurodegeneration, and immune diseases.[153]

Prolonged agonist stimulation of GPCRs often leads to phosphorylation of multiple intracellular residues, which is largely dependent upon the activity of G protein-coupled receptor kinases (GRKs). In general, phosphorylation of GPCRs by GRKs induces the desensitization of the receptor followed by an interaction with cytosolic cofactor protein β-arrestin, which uncouples G proteins from the receptor and removes them from the plasma membrane *via* clathrin-dependent endocytosis to terminate receptor signaling.[154] This is considered as an important mechanism for GPCRs to regulate receptor signaling efficacy. Emerging evidence for $GABA_B$ receptors suggests that this GPCR does not conform to this type of regulation. Multiple studies using both native and recombinant receptors have demonstrated that $GABA_B$ receptors do not undergo agonist-induced internalization and are not GRK substrates.[155] Although GRKs did not appear to be $GABA_B$ receptor kinases, GRK4 and GRK5 have been reported to play a role in agonist-induced desensitization.[156),157] The suppression of GRK4 levels in cerebellar granule cells strongly inhibits $GABA_B$ receptor desensitization.[156] Similarly, in *Xenopus* oocytes and baby hamster kidney cells, expression of $GABA_B$ receptors and GIRKs does not result in desensitization unless co-expressed with GRK4 or GRK5.[157] Thus, unlike most GPCRs, GRKs may function as anchoring proteins that regulate $GABA_B$ receptor activity but not phosphorylation. Biochemical studies have revealed that $GABA_B$ receptors are phosphorylated by various kinases on multiple serine and threonine residues within the cytoplasmic domains of both R1 and R2 subunits.[158] Moreover, $GABA_B$ receptor exhibits significant levels of basal phosphorylation that are not due to agonist stimulation and undergo clathrin-dependent constitutive endocytosis followed by receptor recycling.[155]

So far, five phosphorylation sites have been identified: serine 867 (S867) and S917/923 on the R1 subunit, and S783 and S892 on the R2 subunit (Fig. 3). S867 on the R1 subunit is subject to phosphorylation by calcium/calmodulin-dependent protein kinase II (CaMKII). S867 phosphorylation promotes dynamin-dependent $GABA_B$ receptor endocytosis particularly to the receptors that cluster with GIRK channels.[140] Phosphorylation of S917/923 on the R1 subunit and S783 on the R2 subunit are all mediated by 5′AMP-dependent protein kinase (AMPK).[159] AMPK acts as an energy sensor to regulate cellular metabolism and directly associates with the R1 subunit *via* residues 910–925 within the coiled-coil domain. The role of S783 phosphorylation in $GABA_B$ receptors has been studied intensively by the author in both native and recombinant receptors. The physiological relevance of all three AMPK substrates have been examined by measuring AMPK-mediated GIRK channel activity, and so far, only S783 phosphorylation is evident in enhancing the cell surface stability of $GABA_B$ receptors.[159] Termination of S783 phosphorylation has also been studied. Activation of NMDA-type glutamate receptors rapidly increase S783 phosphorylation followed by a slower protein phosphatase 2A activity, which transiently switches the state of S783 phosphorylation. Dephosphorylated $GABA_B$ receptors undergo clathrin-mediated endocytosis and divert from a recycling to a proteasomal degradation pathway to attenuate $GABA_B$ receptor signaling.[139] It is evident from the studies using S783A mutant knock-in mice that S783 phosphorylation does not significantly impact presynaptic $GABA_B$ receptor function at glutamatergic neurons but modulate postsynaptic $GABA_B$ receptor activity.[50] S892 on the R2 subunit is a PKA substrate.[160] S892 phosphorylation enhances the membrane stability of $GABA_B$ receptors, and prolonged activation of $GABA_B$ receptors *via* activation of Gαi/o protein, leads to the inhibition of adenylyl cyclase to reduce PKA levels, and consequently a reduction in the phosphorylation of S892.[155] The phosphorylation of S892 can be promoted by the assembly of KCTD12 with R2 subunits.[161] The assembly of receptors with KCTD12 increases basal S892 phosphorylation and stabilizes receptors on the cell surface.[148] Increased tonic S892 phosphorylation attenuates KCTD12-induced fast desensitization. Phosphorylation of S783 and S892 has also been detected in astrocytes, which are the most abundant cells in the CNS and play essential roles in synaptic transmission. ATP-mediated P2Y receptor (P2YR) signaling elevates intracellular calcium levels and enhances both S783 and S892 phosphorylation.[162] S783 phosphorylation is mediated *via* P2YR-Ca^{2+}/CaM-dependent protein kinase kinase (CaMKK)-AMPK signaling, and S892 phosphorylation is induced by pertussis toxin-sensitive P2YRs. These phosphorylation on astrocytic $GABA_B$ receptors are likely to act as a detector to fine-tune astrocyte activity.

PKC is also known to phosphorylate $GABA_B$ receptor R1 subunits, although the phosphorylation

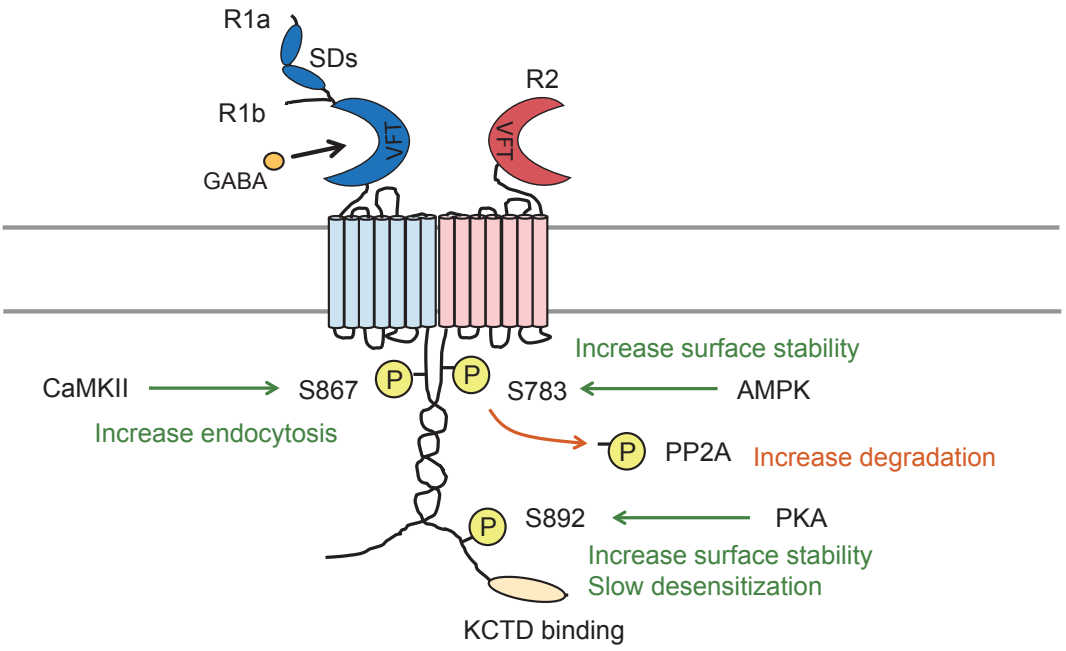

Fig. 3. Phosphorylation of $GABA_B$ receptors and their functional modulation. Five phosphorylation sites have been identified so far: serine 867 (S867) and S917/923 on the R1 subunit, and S783 and S892 on the R2 subunit. Calcium/calmodulin-dependent protein kinase II (CaMKII) phosphorylates S867 on R1 subunit and promotes dynamin-dependent receptor endocytosis. 5′AMP-dependent protein kinase (AMPK) has been found to phosphorylate S917/923 on the R1 subunit and S783 on the R2 subunit. However, only S783 phosphorylation is evident in native tissue. S783 phosphorylation stabilizes $GABA_B$ receptors on the plasma membrane, thereby enhancing GIRK channel activity. The termination of S783 phosphorylation is due to dephosphorylation by protein phosphatase 2A (PP2A), which promotes clathrin-mediated endocytosis of $GABA_B$ receptors followed by proteasomal degradation. S892 phosphorylation by PKA enhances $GABA_B$ receptor cell surface stability, promotes potassium channel tetramerization domain-containing (KCTD) 12 (KCTD12) protein assembly with the R2 subunit and attenuates KCTD12-induced desensitization.

site has not been identified.[120] In Chinese hamster ovary cells, $GABA_B$ receptor activity promotes PKC recruitment to the plasma membrane and induces R1 subunit phosphorylation. Phosphorylation of the R1 subunit fosters the dissociation of NSF protein from $GABA_B$ receptors and enhances desensitization. PKC phosphorylation does not trigger $GABA_B$ receptor internalization similar to PKA phosphorylation.[120]

Ubiquitination. Ubiquitination is a posttranslational modification that generally directs proteins for degradation by proteasomes or by lysosomes, and this modification functions to regulate the number of cellular processes including inflammation, stress responses, and DNA repair. An 8.5 kDa protein ubiquitin associates with the lysine (Lys) residues of target proteins by a sequential reaction of three enzymes: ubiquitin activating enzymes (E1), ubiquitin-conjugation enzymes (E2), and ubiquitin ligases (E3). Ubiquitination of GPCRs and the mechanisms for regulating receptor to undergo lysosomal degradation are well established.[163] Furthermore, recent findings have provided strong evidence for the additional role of ubiquitin in other cellular mechanisms such as receptor trafficking, β-arrestin- and G protein-mediated signaling.[164–166]

Ubiquitination has been reported to regulate the amount of newly synthesized $GABA_B$ receptors that traffic to the plasma membrane *via* endoplasmic reticulum-associated degradation machinery.[167] The Lys-48-linked polyubiquitination of lysines 767/771 in the C-terminal domain of the R2 subunit targets receptors to proteasomes for degradation, and inactivation of these ubiquitination sites increases receptor levels in the plasma membrane as well as $GABA_B$ receptor-mediated signaling. Another type of $GABA_B$ receptor ubiquitination, Lys-63-linked ubiquitination of R1 subunit is known to promote surface receptor degradation.[168] Cell surface $GABA_B$ receptor degradation has been reported upon activation of glutamate receptors, possibly through CaMKII-mediated phosphorylation of S867 on the R1 subunit.[138,169] Lys-63-linked ubiquitination of the R1 subunit is mediated by the E3 ligase Mind

Bomb-2.[169] PKC-induced ubiquitination of $GABA_B$ receptors has also been proposed recently, and the de-ubiquitination enzyme USP14 (ubiquitin-specific protease 14), which associates with the R1 subunit *via* the second intercellular loop, regulates post-endocytic ubiquitination of the $GABA_B$ receptors.[170]

6. $GABA_B$ receptor trafficking and cell surface mobility

Cell-surface trafficking of $GABA_B$ receptors is controlled by an ER retention sequence (RSRR) in the C-terminus of R1 subunits, thus the R1 subunit cannot reach the plasma membrane by itself and is retained in the ER. The C-terminal tail of R2 subunit masks ER retention sequences in the R1 subunit *via* their coiled-coil domain interaction and escort the R1 subunit to the cell surface.[171]

Control of cell surface $GABA_B$ receptor expression plays an important role in the regulation of receptor efficacy. $GABA_B$ receptor cell surface expression is remarkably stable, and baclofen treatment does not induce conventional β-arrestin recruitment.[155),160] However, the $GABA_B$ receptor undergoes constitutive endocytosis *via* clathrin-mediated pathways.[172] In basal conditions, $GABA_B$ receptors internalize as heterodimers *via* clathrin- and dynamin-dependent mechanisms and localize to Rab11-positive recycling endosomes. After constitutive endocytosis, large numbers of $GABA_B$ receptors recycle back to the plasma membrane to maintain steady-state cell surface numbers.[138),155] Of note, endocytosis is detected only in dendrites and not in axons.[138] The balance between insertion and degradation after receptor internalization as well as a rapid recycling processes maintain $GABA_B$ receptor cell surface expression levels.[173] As mentioned earlier, phosphorylation of $GABA_B$ receptors dramatically regulate cell surface stability of the receptors. Exposure to glutamate promotes phosphorylation/dephosphorylation of $GABA_B$ receptors and regulates cell surface number of the receptors.

Lipid rafts are dynamic assemblies of proteins and lipids that float freely within the liquid-disordered bilayer of cellular membranes. These highly dynamic raft domains are essential in signaling processes and also form sorting platforms for targeted protein trafficking. $GABA_B$ receptors and their downstream effectors, $G\alpha i/o$ proteins, are all localized in lipid rafts.[174),175] Notably, $GABA_B$ receptors in raft-enriched fractions exhibited lower $GTP\gamma S$ response to agonist binding than in whole membranes, suggesting that changes in the membrane environment may regulate receptor function.[175] Furthermore, studies of the dynamic lateral diffusion of $GABA_B$ receptors at the cell surface revealed that the restricted mobility of $GABA_B$ receptors is regulated by the C-terminal region in R2 subunits. After activation by baclofen, the levels of mobile receptors are increased significantly.[40] By using single-molecule analysis of fluorescence-labeled $GABA_B$ receptor, it is evident that agonist stimulation increases the mobility of large oligomers of $GABA_B$ receptors on the cell surface.[176] These data suggests the possibility of $GABA_B$ receptor mobility between lipid raft and non-lipid raft domains. Given that the level of cell surface $GABA_B$ receptors is stable after agonist stimulation, lateral diffusion of $GABA_B$ receptors may provide a mechanism for controlling inhibitory strength.

7. $GABA_B$ receptors and diseases

Impaired $GABA_B$ receptor-mediated synaptic transmission underlies a variety of neurological and psychiatric disorders. This section will discuss several diseases in which $GABA_B$ receptors are known to be involved together with some promising indications for treatment using $GABA_B$ receptor drugs.

Anxiety and depression. $GABA_B$ receptors have been implicated in the pathophysiology of emotional disorders such as anxiety and depression.[177] Interest in the role of $GABA_B$ receptors in anxiety has emerged because R1 subunit-deficient mice are more anxious than their wild-type counterparts in several anxiety-related tests, such as the light-dark box and staircase tests.[178] The role of $GABA_B$ receptors in emotional behavior was also suggested by the elevated levels of $GABA_B$ receptor expression in the limbic system.[178] Supporting these observations, baclofen has been shown to have an anxiolytic effect and $GABA_B$ receptor PAMs were found to be promising compounds in the treatment of anxiety disorders.[179] The antagonism of $GABA_B$ receptors may also be a potential therapeutic strategy for depression. R1 subunit-deficient mice display an antidepressant-like phenotype in forced swim tests, and these phenotypes were recapitulated in studies using the $GABA_B$ receptor antagonist CGP56433A.[178] In support of these data, baclofen attenuates the decrease in immobility caused by antidepressants.[180),181]

Addiction. Over the years, a number of clinical observations suggested that baclofen may offer benefit in the treatment of alcohol use and substance use disorders.[182)–185] Multiple preclinical studies have

demonstrated the ability of baclofen to suppress alcohol drinking, oral alcohol self-administration, and intravenous self-administration of cocaine, nicotine, amphetamine, methamphetamine, morphine, and heroin in rodents.[186] Some randomized controlled trials and case reports support the efficacy of baclofen in suppressing alcohol consumption, craving for alcohol, and alcohol withdrawal symptomatology in alcohol-dependent patients.[186] Baclofen attenuates the reinforcing effects of abused drugs by influencing the mesolimbic dopamine system.[187] Recently, interest in testing high doses of baclofen in alcohol use disorder treatment has emerged; however, side-effects such as somnolence, insomnia, dizziness, and paresthesia pose a principle limitation to its administration in alcohol addiction.[188),189] Preclinical research has then extended the anti-addictive properties of baclofen to PAM. In light of their more favorable side-effect profile compared to baclofen, PAMs may represent a major step forward in $GABA_B$ receptor-based pharmacotherapy of alcohol use and substance use disorders.[186]

Epilepsy. $GABA_B$ receptors have been implicated into the etiology of epilepsies.[3] The G1465A polymorphism in the gene for the R1 subunit has been linked to the risk of temporal lobe epilepsy as well as the severity of the disease.[189] mRNA expression and immunoreactivity of $GABA_B$ receptors, as well as $GABA_B$-mediated pre- and post-synaptic responses, are decreased in discrete cortical and hippocampal areas of epileptic patients.[189)–192] In addition, R1 subunit-deficient mice exhibited generalized seizure activities.[193),194] The role of $GABA_B$ receptor-mediated mechanisms in the pathogenesis of seizures depends on neural networks that involve $GABA_B$ receptors, which determine the seizure type. $GABA_B$ receptor agonists have been shown to diminish seizure activity in mouse models of both generalized convulsive and focal seizures; however, generalized non-convulsive seizures such as typical and atypical absence seizures are exacerbated by $GABA_B$ receptor agonists and blocked by $GABA_B$ receptor antagonists.[195)–197] This dichotomy is likely due to the involvement of thalamic circuitry in both typical and atypical absence seizures. Therefore, $GABA_B$ receptor-mediated mechanisms can be pro- or anti-convulsant depending on the nature of the pathological neuronal networks. Recent studies reported that $GABA_B$ receptor PAMs offer anti-convulsive actions in animal models.[198)–200] Considering that PAMs offer beneficial behavioral effects without overt side-effects, PAMs may serve as a clinically relevant strategy for the management of epileptic seizures.[69),201),202]

Cognition. $GABA_B$ receptors are highly expressed in brain regions implicated in learning and memory.[203] Post-mortem $GABA_B$ receptor expression studies in Alzheimer's disease brains has suggested an increase in R1 subunit expression in the hippocampus that correlates with the extent of neurofibrillary tangle pathology.[204] Alternative splicing of $GABA_B$ receptors and GIRK expression have also been suggested a possible changes in $GABA_B$ receptor signaling in Alzheimer's disease.[205] In this respect, a clinical trial using $GABA_B$ receptor antagonist SGS742 (formerly known as CGP36742) was progressed to Phase II in an attempt to treat mild cognitive impairment.[206] SGS742 was administered orally at 600 mg three times a day for eight weeks in a double-blind trial in 75 patients. SGS742 significantly improved working memory and attention, suggesting that $GABA_B$ receptor antagonism can promote cognitive performance. However, SGS742 failed to progress to a Phase III clinical trial, and currently there are no other $GABA_B$ receptor antagonists in development for cognitive diseases.[207] Identifying novel targets to develop specific drugs for $GABA_B$ receptors will be necessary.

8. Conclusion

This review summarizes how one single receptor, $GABA_B$ receptor, generates multiple functions such as: (1) the presence of heterodimers and large oligomers increase the complexity in cellular localization and function; (2) splice variants of the R1 subunit contribute to the functional diversity of this receptor; (3) interacting proteins for $GABA_B$ receptors provide a vast amount of receptor function by regulating receptor localization, signaling specificity, and pharmacological profiles; (4) crosstalk between various receptors helps to balance neuronal inhibition and excitation as well as signal transduction and transactivation-dependent neuronal survival; (5) PTMs such as phosphorylation and ubiquitination regulate receptor trafficking and the amount of receptors on the plasma membrane; and (6) receptor localization in lipid rafts is involved in regulating the efficacy of receptor signaling. These complexities generated from a single GPCR still need to be clarified, but future studies will help to confirm the mechanisms regulating $GABA_B$ receptor function and reasons for the interactions with multiple proteins. $GABA_B$ receptors are excellent therapeutic targets, because drugs acting on these receptors have

the potential to treat a wide variety of neurological diseases. The full therapeutic benefits of $GABA_B$ receptors need to be elucidated using various methods.

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Profile

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