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MINIREVIEW

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The organizing principle of $GABA_B$ receptor complexes: Physiological and pharmacological implications

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Abstract

$GABA_B$ receptors (GBRs), the G protein-coupled receptors for the neurotransmitter γ-aminobutyric acid (GABA), regulate synaptic transmission at most synapses in the brain. Proteomic approaches revealed that native GBR complexes assemble from an inventory of ~30 proteins that provide a molecular basis for the functional diversity observed with these receptors. Studies with reconstituted GBR complexes in heterologous cells and complementary knockout studies have allowed to identify cellular and physiological functions for obligate and several non-obligate receptor components. It emerges that modular association of receptor components in space and time generates a variety of multiprotein receptor complexes with different localizations, kinetic properties and effector channels. This article summarizes current knowledge on the organizing principle of GBR complexes. We further discuss unanticipated receptor functions, links to disease and opportunities for drug discovery arising from the identification of novel receptor components.

KEYWORDS

GABA, GABA-B, $GABA_B$ receptors, proteomics, γ-aminobutyric acid

1 | GBR FUNCTIONS IN THE BRAIN

$GABA_B$ receptors (GBRs) are G protein-coupled receptors for the main inhibitory neurotransmitter in the central nervous system, γ-aminobutyric acid (GABA).[1-3] The GABA metabolite γ-hydroxybutyrate (GHB), a psychoactive drug of abuse, is a second endogenous ligand of GBRs.[4] Whether the concentrations of GHB in the brain are sufficiently high to activate GBRs is a matter of debate. However, GBRs clearly mediate most of the physiological effects observed with recreational use of GHB. GBRs activate heterotrimeric Gi/o-type G proteins that inhibit adenylyl cyclase through the Gα subunit.[1-3] The consequences of inhibiting cAMP production in neurons through GBRs include the inhibition of spontaneous neurotransmitter release and the disinhibition of two-pore domain K^+ channels.[1] The best-known neuronal GBR functions are the gating of voltage-sensitive Ca^{2+} (Cav) channels and inwardly rectifying Kir3-type K^+ channels by the Gβγ subunits of the G protein.[1,2] GBRs inhibit N- and P/Q-type Ca^{2+} channels, which dampens neurotransmitter release at many terminals, including GABAergic and glutamatergic terminals. Contrasting the dogma that GBRs inhibit Ca^{2+} channels and neurotransmitter release, recent reports suggest that GBRs activate R-type Ca^{2+}-channels to trigger neurotransmitter release in habenular projections to the interpeduncular nucleus.[5,6] Since GBRs conventionally inhibit R-type Ca^{2+} channels in heterologous cells, it remains to be elucidated how GBRs activate these channels in neurons. It is possible that activation of R-type Ca^{2+} channels relates to

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receptor-interacting proteins, intracellular signalling cross-talks and/or GBR effects on network activity. GBRs activate Kir3 channels in the dendrites of most neurons, which hyperpolarizes the membrane and shunts excitatory neurotransmission.[1] Astrocytes in the brain also express GBRs. However, the physiological consequences of GBR signalling in astrocytes are largely unknown. Recent data support that parvalbumin- and somatostatin-expressing interneurons induce GBR-mediated Ca^{2+} elevations in astrocytes.[7] These GBR-induced Ca^{2+} responses decrease and increase upon repetitive stimulation of parvalbumin and somatostatin interneurons, respectively, revealing that GBR responses in astrocytes are plastic. The fact that Gi/o-coupled GBRs induce Ca^{2+} responses in astrocytes is surprising and likely involves a signalling crosstalk with other receptors.[1]

2 | MARKETED GBR DRUGS

It is a widely accepted concept that disturbances in the excitation/inhibition balance underlie numerous neurological and neuropsychiatric disorders. Too little inhibition is linked to epilepsy, spasticity, anxiety, sleep disorders, depression, addiction and chronic pain while too much inhibition is associated with schizophrenia and cognitive deficits.[8] GBRs modulate synaptic transmission and neuronal activity in most neurons of the brain. Reciprocally, GBR expression is down-regulated in response to sustained neuronal excitation.[1] It is therefore no wonder that dysregulated GBR signalling has long been associated with neurological and mental health disorders.[1,2,9] Despite the well-documented involvement of GBRs in disease, only two GBR drugs are currently on the market. The prototypical GBR agonist baclofen (Lioresal®) is prescribed to reduce muscle rigidity and spasms associated with multiple sclerosis.[1] The small doses of intrathecal baclofen used to treat spasticity do not cause systemic side effects. GHB (also known as sodium oxybate, Xyrem®), the second marketed GBR drug, is prescribed to decrease daytime sleepiness and reduce sudden attacks of weak/paralysed muscles (cataplexy) in narcoleptic patients.[4] The exact mechanism of action of Xyrem is unknown but it is generally assumed that Xyrem exerts its therapeutic effects by activating GBRs. Xyrem is administered orally during the night when potential side effects from systemic activation of GBRs, such as weakness and muscle relaxation, are less problematic. Differences in the dose, time and route of drug administration therefore allow using GHB and baclofen in different indications even though both activate GBRs. Baclofen is increasingly used off-label to treat alcohol dependence.[10] In France, baclofen is currently the most prescribed pharmacotherapy for alcohol use disorders. While clinical trials support the efficacy of high doses of baclofen for alcohol use disorders, they also note sedation as a major side effect.[10] Recent trials support that GHB, like baclofen, may also be useful in the therapy of alcohol dependence.[11] In fact, GHB (Alcover®) is already marketed in Italy to alleviate withdrawal symptoms and reduce the craving for alcohol.

3 | FUNCTIONS OF OBLIGATE RECEPTOR COMPONENTS

The molecular structure of GBRs remained for a long-time elusive. In 1997, expression cloning using a high-affinity radioligand antagonist allowed to identify the GB1 subunit, which by itself did not form a functional receptor.[1] However, GB1 featured the typical seven-transmembrane topology of G protein-coupled receptors and exhibited homology to metabotropic glutamate receptors. Database searches and yeast-two-hybrid screens subsequently identified the sequence-related GB2 subunit, which by itself again was non-functional.[1] Most neurons in the brain co-expressed GB1 and GB2, which suggested that they act together in a complex. Electrophysiological and biochemical studies in transfected heterologous cells indeed revealed that co-expression of GB1 with GB2 subunits was necessary to generate a functional receptor. This represented the first example of an obligate heterodimeric G protein-coupled receptor. Confirmation that native GB1 and GB2 subunits form heterodimers was obtained in subsequent studies with knockout mice showing that lack of GB1 or GB2 subunits abrogated all electrophysiological and biochemical GBR responses.[1] Analysis of knockout mice further revealed that the GB1/GB2 complex stabilizes its constituent proteins. The behavioural phenotypes of GB1 and GB2 knockout mice include epilepsy, cognitive impairments, hyperactivity, hyperalgesia, increased anxiety and a reduced threshold for fear responses.[1] Recent studies addressed the functions of GBRs in identified neuronal populations. Genetic deletion of GBRs in principal neurons of the input layer of the auditory cortex produces deficits in auditory map remodelling, indicating that GBRs gate auditory critical period plasticity.[12] Genetic deletion of GBRs in dopamine neurons of the ventral tegmental area (VTA) markedly increased cocaine-induced locomotion without affecting general or morphine-induced locomotor activity.[13] It appears that long-range GABAergic inputs from the nucleus accumbens to the VTA activate GBRs on dopamine neurons to regulate cocaine-induced locomotion.[13] Altogether, knockout studies confirmed that heterodimeric GB1/GB2 complexes represent the minimal functional unit required for receptor signalling and showed that neuronal GBRs regulate a wide array of physiological functions and behaviours.

A large body of evidence supports that GB1 and GB2 assume distinct and non-redundant functions in the

heterodimeric receptor. Most notably, GB1 binds GABA while GB2 couples to the G protein and increases GABA affinity at GB1.[1-3] Allosteric interactions between GB1 and GB2 are therefore necessary for activating the G protein at GB2 after binding of GABA to GB1.[2,3] X-ray structures of the heterodimeric GB1/GB2 ectodomains in the resting and active states showed that the so-called "venus fly-trap domain" of GB1 closes upon GABA binding.[2,3] Conversely, antagonists stabilize the open inactive conformation of the GB1 venus fly-trap domain. The GB2 venus fly-trap domain remains constitutively open during the activation process.[2,3] The GB1/GB2 heterodimer exhibits high intrinsic conformational flexibility. In the absence of ligands, GB1 spontaneously oscillates between inactive and active states.[2] Because of these frequent conformational changes, the receptor exhibits high basal activity in the absence of agonist.[14]

Heterodimerization of GB1 with GB2 occurs in the endoplasmic reticulum and regulates targeting to the plasma membrane.[1] The endoplasmic reticulum-resident prenylated Rab acceptor family 2 (PRAF2) protein binds to an endoplasmic reticulum retention signal in GB1.[15] Assembly of GB1 with GB2 releases PRAF2, which allows the heterodimeric receptor to exit the endoplasmic reticulum and to traffic to the cell surface. GB1/GB2 heterodimers at the plasma membrane can form transient higher-order complexes via interaction of their GB1 subunits.[16] Higher-order complexes assemble by random collision of heterodimers in an activity-independent manner. Consequently, higher-order complexes are more abundant at higher densities of heterodimers.[2] Assembly into higher oligomers limits receptor signalling via G proteins because neighbouring G protein binding sites cannot be simultaneously occupied.[16]

Two main variants of the GB1 subunit exist, GB1a and GB1b, which differ by the presence of two sushi domains at the N-terminus of GB1a.[1-3] When expressed in heterologous cells, GB1a/GB2 and GB1b/GB2 receptors are functionally and pharmacologically alike.[1] However, studies with GB1a and GB1b knockout mice showed that the lack of GB1a and GB1b subunits differentially influences synaptic plasticity processes,[1] network oscillations[1,17] and behaviour.[1] Biochemical and electrophysiological experiments further revealed that GB1a/GB2 receptors accumulate at axon terminals while GB1b/GB2 receptors accumulate in the somatodendritic compartment. Mechanistically, the sushi domains act as axonal trafficking signals[1] and stabilize GB1a/GB2 receptors at the cell surface.[18] Proteomic work identified the β-amyloid precursor protein (APP), the adherence junction-associated protein 1 (AJAP-1) and the PILRα-associated neural protein (PIANP) as interactors of the sushi domains of GB1a.[19] APP, AJAP-1 and PIANP may therefore play a role in axonal trafficking and localization of GB1a/GB2 receptors (see below).

4 | FUNCTIONS OF NON-OBLIGATE RECEPTOR COMPONENTS

Electrophysiology revealed that GBR-mediated K^+ currents in non-neuronal cells exhibit a slower rise time than in neurons.[20] Moreover, GBR-induced K^+ currents exhibit little desensitization in heterologous cells while they exhibit pronounced desensitization in some neurons.[20] Differences between cloned and native GBR responses suggested early on the existence of receptor-associated proteins that influence receptor kinetics. The search for GBR-associated proteins sparked biochemical experiments showing that native GBRs form high-molecular-weight complexes of >500 kDa.[1] Individual GB1/2 heterodimers of 220 kDa were not observed, corroborating that GB1/2 heterodimers execute their functions in combination with associated proteins. Quantitative proteomic approaches eventually identified approximately 30 proteins that stably or transiently associate with native GB1 or GB2 subunits[19] (Figure 1). The protein inventory comprises sushi domain-interacting proteins and signalling components, such as G protein subunits, ion channels and elements of the presynaptic release machinery. In addition, the protein inventory includes proteins of unknown functions. Most of the GBR-associated proteins only show a partial spatial and temporal overlap with the expression patterns of GB1 and GB2 in the brain, indicating that they are non-obligate receptor components. The anatomically and temporally restricted expression of most GBRs components suggests a highly diverse and modular receptor composition. Interestingly, many of the GBR-associated proteins previously identified in yeast-two-hybrid screens were absent in the GBR proteome,[2,19] possibly because yeast-two-hybrid screens are better at detecting low-affinity or transient pairings. However, it is also possible that yeast-two-hybrid screens erroneously detect protein interactions that do not occur in vivo, for example because the supposed partner proteins are expressed in different cellular or subcellular compartments. Below, we discuss progress made in dissecting the neuronal, cellular and behavioural functions of the GBR-associated proteins identified using proteomic approaches and native tissue. The available data are compatible with a modular organization of GBR complexes, in which the GB1a/2 and GB1b/2 heterodimers can associate with a variable repertoire of proteins regulating trafficking, signalling and localization of the receptor complex (Figure 1).

4.1 | KCTD proteins

The cytosolic K^+ channel tetramerization domain (KCTD) proteins KCTD8, KCTD12, KCTD12b and KCTD16 bind to the C-terminal domain of GB2[1,2] (Figure 1). Recent cryo-electron microscopy and crystallization studies indicate that

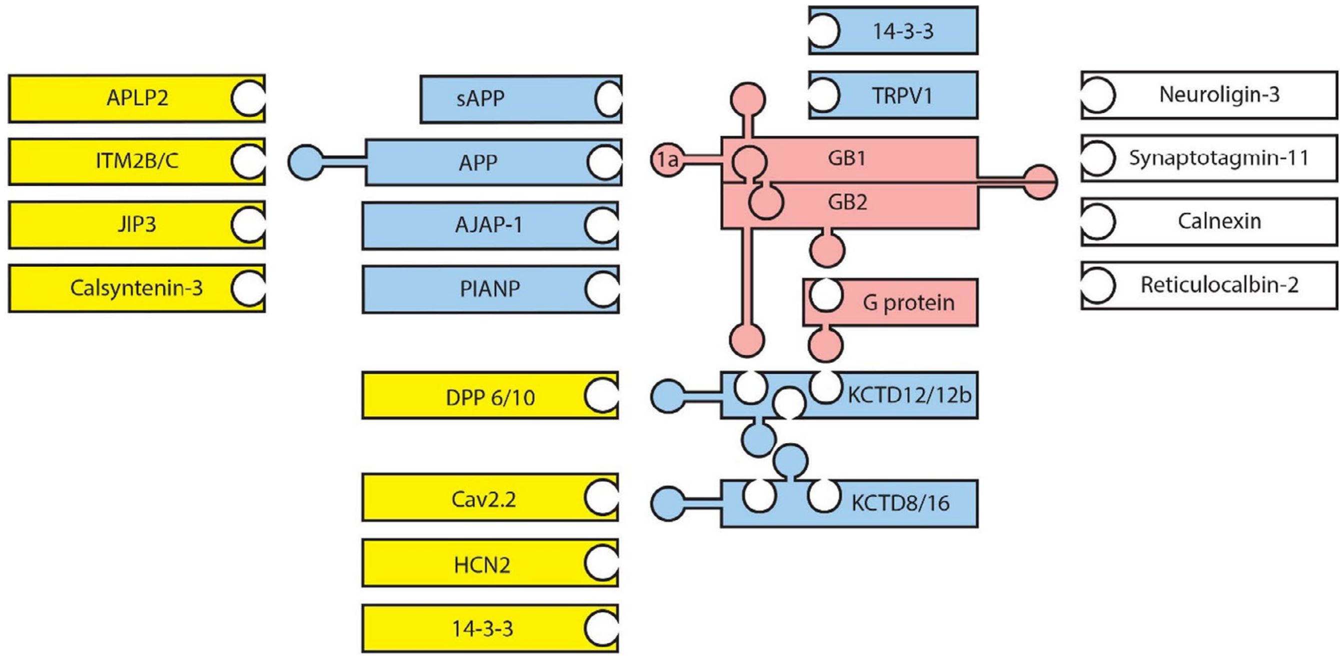

FIGURE 1 Organizing principle of modular GBR complexes. GB1 and GB2 are obligate receptor components that, together with the heterotrimeric G protein, constitute a fully functional GB1/GB2 receptor core (red). Modular association of non-obligate receptor components with the receptor core generates complexes of varying composition and properties. Primary interactors (blue) of the receptor are the auxiliary KCTD proteins that bind as homo- and hetero-pentamers to GB2 and the G protein. Multiple interactions between GB2, G protein and KCTD proteins stabilize a ternary complex whose components act in concert to regulate receptor kinetics. All KCTD proteins accelerate receptor signalling to the G protein. KCTD12 and KCTD12b additionally induce fast desensitization of receptor-activated K^+ currents, most likely by interfering with Gβγ binding to the channel. APP, the soluble form of APP (sAPP), AJAP-1 and PIANP bind to the N-terminal sushi domains of GB1a. APP acts as an axonal trafficking factor for GBRs. JIP and calsyntenin proteins bind to APP and link the APP/GBR complex to the axonal trafficking motor. Binding of sAPP to the N-terminal sushi domain of GB1a is reported to activate GBRs and to inhibit neurotransmitter release. TRPV1 channels bind to GB1a, which reverts TRPV1 sensitization. 14-3-3 proteins associate with the ER retention signal in the C-terminal domain of GB1 subunits. DPP6/10 proteins are secondary interactors (yellow) that assemble with KCTD12, while N-type Ca^{2+} (Cav2.2) channels, HCN2 channels and 14-3-3 proteins bind to KCTD8/16. APLP2 and ITM2B/C are secondary interactors that assemble with APP into a complex that together with the heterodimeric GBR complex forms a supercomplex (complex of complexes). The binding partners of neuroligin-3, synaptotagmin-11, calnexin and reticulocalbin-2 in the receptor complex are unknown (white), as are possible effects on receptor signalling or localization. Only proteins identified in the proteome of native GBRs are shown

the T1 tetramerization domain of KCTD16 assembles into an open pentameric ring with an inner diameter of ~25 Å.[21-23] Adjacent KCTD16 T1 subunits are arranged side-by-side with similar C- and N-terminal orientation. The high-resolution crystal structure of the T1 pentamer in complex with a C-terminal peptide of GB2 shows that a single GB2 peptide binds to the inner surface of the open pentameric ring, with the interface having exceptionally high shape complementarity.[22,23] The GB2 peptide is located asymmetrically, off centre, away from the opening of the ring.[23] The GB2 peptide loops around inside the ring structure of the pentamer with the N- and C-termini of GB2 pointing to the KCTD16 N-terminus.[22] In addition to binding to GB2, KCTDs also interact with the Gβγ subunits of the G protein via their H1 homology domain.[20,22] Structural analysis revealed that a KCTD12 H1-pentamer interacts with five copies of the Gβγ heterodimer in a near perfect C5 symmetry.[22] Interactions between KCTD12 and Gβγ are confined to the Gβ subunit. The five Gβγ subunits each interact with two KCTD12 H1 domains. Lack of formation of partial KCTD12/Gβγ oligomers suggested that KCTD12 binding to Gβγ is highly cooperative.[22]

Importantly, combining the KCTDs with GB1 and GB2 subunits in heterologous cells confers the missing fast kinetics to recombinant GBRs.[20,24] Conversely, genetic ablation of the KCTDs in neurons leads to a slowing of GBR signalling.[20,24] In addition, the KCTDs shorten the delay between agonist application and onset of the receptor response.[20] This acceleration of receptor signalling likely relates to the KCTD's ability to scaffold the G protein at the receptor, which renders diffusion of the G protein to the receptor during the activation process obsolete.[20] On the other hand, scaffolding of the G protein may also prevent the receptor from activating multiple G proteins by random collision, which will reduce the signal amplification typically observed with G protein-coupled receptors. KCTD12 and KCTD12b additionally induce a pronounced desensitization of the receptor response by

activity-dependent uncoupling of the Gβγ subunits from effector K^+ channels.[20,22] Biochemical experiments and bimolecular bioluminescence resonance energy transfer (BRET) experiments revealed that not only KCTD homomers but also KCTD heteromers associate with the receptor (Figure 1).[24] Of note, charged interactions at the pentameric interface of the KCTD16 T1 structure are conserved among all $GABA_B$-related KCTD proteins, which explains how pentameric heteromers can form. Moreover, conservation of amino acids in the GB2/KCTD interface is compatible with the observation that KCTDs can form heteromers that regulate GBR responses.[23,24] In fact, the formation of KCTD heteromers enables a fine-tuning of receptor kinetics. KCTD12/16 heteromers, for example, increase the duration of slow inhibitory post-synaptic currents (IPSCs) in hippocampal neurons.[24] In addition to their kinetic effects on the receptor response, the KCTDs promote surface expression of the receptor complex and shift the EC_{50} value of GBR-mediated K^+ currents towards lower concentrations.[1] The KCTDs exert per se little allosteric influence on the orthosteric GABA binding site,[25] suggesting that the observed increase in GABA potency relates to KCTD effects on the G protein cycle. In addition to their kinetic effects, KCTD proteins scaffold effector channels and other proteins at the receptor. KCTD16, for example, recruits N-type Ca^{2+} channels, hyperpolarization-activated cyclic nucleotide-gated 2 (HCN2) channels and 14-3-3 proteins to the receptor[19] (Figure 1).

The KCTD proteins are non-obligatory GBR components. However, they stably associate with the receptor and co-immunopurify with GB1 and GB2 under stringent solubilization conditions.[19,20] KCTDs should therefore be viewed as auxiliary receptor subunits that regulate surface expression and receptor kinetics. Of note, the GB2 C-terminal domain of invertebrates lacks a KCTD binding site,

TABLE 1 Potential links of GBR components to human traits and disease

Receptor component	Disease	Molecular link	Reference
GB1	Encephalitis	Autoantibodies	59,61,62
	Alzheimer's disease	Protein expression post-mortem	63
GB2	Rett syndrome	Mutations in TM3 and TM6	33,34
	Epileptic encephalopathy	Exome sequencing	26
KCTD8	Type 2 diabetes	GWAS	39
	Brain size	GWAS	35
KCTD12	Type 2 diabetes	GWAS	40
	Bipolar I disorder	GWAS	38
	Pain	Proteomic	41
	Major depressive disorder	Gene expression post-mortem	37
	Gastrointestinal tumours	Proteomic and gene mutation	42,43
AJAP-1	Migraine	GWAS	46
	Glioblastoma multiform	Gene deletion, down-regulated	47
	Adolescent idiopathic scoliosis	GWAS	45
PIANP	Intellectual disability	Exome sequencing	44
APP	Alzheimer's disease	Amyloid plaques	63
Nlgn-3	Pain	Proteomic	41
Syt-11	Schizophrenia	Patient sequencing	64
	Parkinson's disease	GWAS	65,66
Cav subunit β2	Bipolar I disorder	GWAS	38
	Major depressive disorder	Gene expression post-mortem	37
HCN2	Generalized epilepsy	Exome sequencing	67
TRPV1	Inflammatory pain	Proteomic	30

Note: Disease-related alterations in receptor components, where known, are indicated.

indicating that auxiliary KCTD subunits represent a functional specialization of GBRs during vertebrate evolution. Likely, the KCTDs evolved to quickly initiate and terminate receptor signalling to effector channels. In addition, preassembly of the G protein at the receptor may increase constitutive activity and contribute to G protein/receptor specificity.

4.2 | Sushi domain-associated proteins

APP, AJAP-1 and PIANP are transmembrane proteins that co-purify with native GB1a/2 receptors and bind in a mutually exclusive manner to the sushi domains of the presynaptic GB1a subunit[19] (Figure 1). APP is the source of β-amyloid (Aβ) peptides, a hallmark of Alzheimer's disease (Table 1). A recent report by Rice et al[26] shows that binding of the soluble form of APP (sAPP) to the N-terminal sushi domain of GB1a inhibits GBR-mediated neurotransmitter release. A GBR antagonist disinhibits sAPP-inhibited release, supporting that sAPP acts as GBR agonist or positive allosteric modulator. A related report shows that binding of full-length APP to the N-terminal sushi domain of GB1a is necessary for vesicular trafficking of GBRs to axon terminals.[27] Consistent with vesicular GBR transport, kinesin-1 adaptors of the c-Jun N-terminal kinase-interacting protein (JIP) and calsyntenin (CSTN) protein families are shown to bind to APP and to link the APP/GBR complex to kinesin-1 motors. In contrast to the report by Rice et al, no functional effects of sAPP at GBRs were observed. Functional effects of sAPP at GBRs therefore need to be independently confirmed. AJAP-1 and PIANP share sequence similarity in their intracellular domains. The two proteins are expected to localize to adherens junctions that mediate adhesion between pre- and post-synaptic membranes.[28,29] AJAP-1 and PIANP do not play a role in vesicular axonal trafficking of GBRs.[27] Possibly, these proteins anchor GB1a/2 receptors at synaptic sites by binding to the sushi domains in *cis* or in *trans*. Amyloid-like protein 2 (APLP2), integral membrane protein 2B (ITM2B) and ITM2C are additional transmembrane proteins that selectively co-purify with the GB1a subunit[19] (Figure 1). Since these proteins associate with APP, they probably represent secondary interactors of GBRs (Figure 1). It therefore appears that GBRs can assemble with multiprotein APP complexes into supercomplexes (complexes of complexes).

4.3 | Effector channels

GBRs gate Kir3-type K^+ channels and voltage-sensitive Ca^{2+} channels in most neurons of the central nervous system.[1,5,6] Kir3 channels do not appear to physically associate with GBRs while N-type Ca^{2+} channels co-purify with native GBRs by interacting with KCTD16 (Figure 1).[19] Surprisingly, proteomic work indicates that transient receptor potential vanilloid 1 (TRPV1) and HCN2 channels also associate with GBRs (Figure 1).[19,30] Interestingly, activation of GB1 reverts the sensitized state of TRPV1 channels in a G protein-dependent manner.[30] Similarly, GBRs also inhibit transient receptor potential melastatin-3 (TRPM3) channels.[31,32] However, no direct interaction of TRPM3 channels with GB1 has been reported. HCN2 channels, like N-type Ca^{2+} channels, associate via KCTD16 with the receptor (Figure 1).[19] Dopaminergic neurons of the VTA co-express HCN2 channels, KCTD16 and GBRs and thus provided a cellular system to study the physiological consequences of the HCN2/GBR interaction. It was shown that GBRs activate HCN2 currents and shorten the duration of inhibitory post-synaptic potentials[19] (Figure 2). HCN2 channels are dissociated from GBRs in KCTD16 knockout mice, which prevents HCN2 activation and prolongs the duration of inhibitory post-synaptic potentials. The mechanism(s) underlying GBR-induced activation of HCN2 channels is still unknown. Possible mechanisms include (a) membrane hyperpolarization via Kir3 channels, (b) allosteric interactions between receptor and channel, and/or

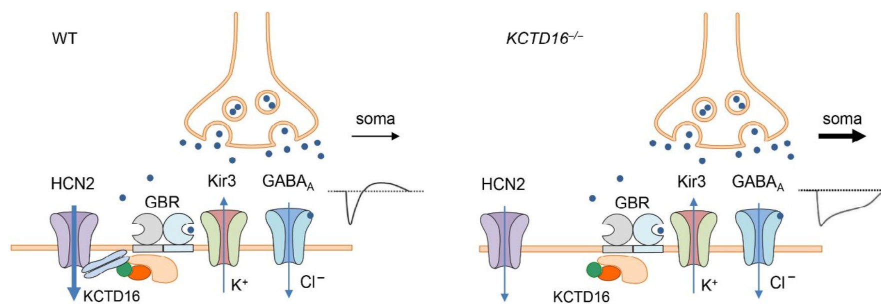

FIGURE 2 Physiological relevance of the newly discovered association of GBRs with HCN2 channels. HCN2 channels interact via KCTD16 with GBRs in wild-type (WT) dopaminergic neurons of the VTA. GBR activation facilitates activation of associated HCN2 channels through the hyperpolarizing influence of receptor-activated Kir3 currents, allosteric interactions or second messenger systems. Activation of HCN2 channels shortens the duration of IPSPs propagating to the soma of dopaminergic neurons (indicated with a weak black arrow), likely through shunting of the IPSP. In KCTD16 knockout neurons, HCN2 channels are dissociated from GBRs, which prevents HCN2 channel activation, promotes IPSP propagation (reduced shunting) and consequently produces larger IPSPs at the soma (indicated with a strong black arrow)

(c) dynamic interactions between the channel and G protein subunits or second messengers.

4.4 | Other receptor components

Additional proteins of the GBR interactome are neuroligin-3 (Nlgn-3), synaptotagmin-11 (Syt-11), calnexin, reticulocalbin-2 and inactive dipetidylpeptidases 6/10 (DPP 6/10; Figure 1).[19] It is unknown whether these proteins represent primary or secondary interactors of GB1 or GB2. Purification of native GBR complexes from knockout mice and reverse-affinity purifications with antibodies against these proteins will reveal whether their presence in receptor complexes depends on other receptor components and hint at physiological functions.

5 | NOVEL LINKS OF RECEPTOR COMPONENTS TO DISEASE

As mentioned above, GBRs have long been associated with neurological and psychiatric conditions.[1,2] Genome-wide association studies (GWAS), proteomic, exome sequencing and microarray studies have provided novel links of receptor components to disease (Table 1). Recently, mutations in the GB2 transmembrane domains 3 and 6 have been associated with Rett syndrome, epileptic encephalopathy and infantile epileptic spasms.[33,34] Some of these mutations increase constitutive receptor activity and therefore reduce the efficacy of GABA in stimulating the receptor. Auxiliary KCTD subunits have been associated with small brain size,[35] schizophrenia,[36] depression,[37] bipolar I disorder,[38] diabetes,[39,40] pain[41] and cancer.[42,43] The sushi domain-interacting proteins APP, AJAP-1 and PIANP are linked to Alzheimer's disease, intellectual disability,[44] adolescent idiopathic scoliosis,[45] migraine[46] and cancer.[47] HCN2 mutations are associated with generalized epilepsy. Additional receptor components link to pain, schizophrenia, Parkinson's disease, bipolar I disorder and depression. For most genetic links, insights into pathophysiological mechanisms are lacking, which hinders the design of straightforward therapeutic concepts. It is also important to note that genetic links to disease in non-obligate GBR components do not necessarily relate to dysfunctional GBR signalling. Nevertheless, it may be interesting to approach disease in terms of protein-protein interactions in receptor complexes. Mutations in the same receptor component may lead to different disease phenotypes by disrupting different protein interactions and functions. Conversely, mutations in different proteins that disrupt the same interaction and receptor function may lead to the same disease. Knowledge about the organizing principle of GBRs may therefore pave the way for more specific therapeutic interference with disease (see below).

6 | PHARMACOLOGICAL IMPLICATIONS

Disturbances in the excitation/inhibition balance underlie numerous neurological and neuropsychiatric disorders.[8] Many available therapies for these disorders work by restoring a normal excitation/inhibition balance in perturbed neuronal pathways. Since activation or inhibition of GBRs modulates the excitation/inhibition balance, GBRs have been the focus of many drug discovery programs targeting mental health disorders. Unfortunately, baclofen either lacked efficacy (Fragile X syndrome), had a short duration of action, produced tolerance (pain) or exhibited prohibitive side effects (mainly muscle relaxation, sedation and mental confusion) when tested in indications other than spasticity, its prime therapeutic use.[2,9,48-50] It has been argued that positive allosteric modulators of GBRs should produce fewer side effects and less tolerance because they selectively enhance the activity of receptors activated by endogenous GABA. Positive allosteric modulators of GBRs showed promising effects in animal models of drug abuse, schizophrenia, visceral pain, epilepsy, anxiety and overactive bladder, while they tested negative for depression and neuropathic pain.[48,49] Preclinical studies further support that positive allosteric modulators indeed produce less adverse effects, such as sedation and muscle relaxation, than agonists.[1,2,49] Thus far, however, no allosteric modulators for G protein-coupled receptors have been approved for the treatment of psychiatric or neurological disorders, even though several allosteric modulators entered Phase II trials.[51] There is some concern that allosteric modulators for G protein-coupled receptors lack efficacy in human trials, even if preclinical data are positive.[9] Despite this general reluctance in starting new trials, Addex Pharmaceuticals (Geneva, Switzerland) recently announced the first clinical study with a positive allosteric modulator of GBRs, ADX71441, for the treatment of cocaine addiction (https://www.addextherapeutics.com/en/partners-collaboration/). GBR antagonists showed promising nootropic, anti-absence seizures and antidepressant effects in animal models.[48,52] They also showed statistically significant improvements of working memory and attention in a Phase II clinical trial with mild Alzheimer disease patients.[53] However, seizure liability of antagonists remains a main concern. A general shortcoming of agonists, antagonists and positive allosteric modulators is that they do not discriminate GB1a/2 and GB1b/2 receptor subtypes and their effector systems. This is problematic because GBRs mediate pre- and post-synaptic functions at excitatory and inhibitory synapses and thus may have opposite effects on the excitation/inhibition balance depending on the cellular context. Global activation, inhibition or

allosteric modulation of GBRs therefore mitigates desired therapeutic effects and generates unwanted side effects.[9]

Influencing region/circuit specific GBR functions by targeting identified receptor complexes would improve drug selectivity and allow a more specific therapeutic interference with disease.[54] It is possible that inclusion of receptor-associated proteins into high-throughput compound screens uncovers new pharmacological sites for regulating receptor activity, as has been shown for AMPA receptor antagonists blocking certain receptor/TARP combinations.[8] Targeting disease-relevant protein-protein interactions with peptides constitutes another means to influence specific receptor functions without affecting others.[54-56] A good example for this approach is NA-1, a cell-penetrating peptide reducing ischaemic brain damage by interfering with the NMDA receptor/PSD-95 interaction.[57] Interfering with KCTD12 binding to GBRs, for example, would allow increasing and prolonging post-synaptic inhibition, which is expected to have anxiolytic effects. Similarly, preventing binding of APP to the N-terminal sushi domain of GB1a may interfere with GBR-mediated inhibition of glutamate release and enhance cognitive functions. Antibody-based therapeutics that interfere with specific receptor components represent an additional means to regulate the activity of molecularly defined receptor complexes.[58] Proof-of-principle that antibodies can regulate GBR activity is provided by activity-blocking GB1 autoantibodies in the serum of patients with autoimmune encephalitis.[59]

7 | FUTURE DIRECTIONS

Given the fundamental roles that GBRs play in synaptic transmission, behaviour and disease, it is important to study the structural organization of these receptors. The past two decades have seen a constant remodelling of our concept of GBR structure—from the discovery of obligate heterodimers to the recognition that heterodimers can form structurally and functionally diverse multiprotein receptor complexes assembled with distinct repertoires of auxiliary KCTD subunits, ion channels, adhesion and signalling proteins. It emerges that mutual interactions between receptor components stabilize proteins that work together to convey and regulate a specific function (eg GB2, KCTDs and G protein subunits). The core receptor can assemble with itself (GB1/GB2 oligomerization) and with other multiprotein complexes (eg APP with ITM2B/C proteins) into supercomplexes. It will be important to address whether multiprotein GBR complexes are stable over time or whether they dynamically reorganize in response to neuronal activity or developmental cues. Moreover, a spatiotemporal map of GBR complexes at axonal and dendritic sites will be necessary for a detailed understanding of cellular GBR functions. A structural understanding of receptor complexes in different functional states and in association with interacting proteins will largely depend on the success of cryo-electron microscopy and X-ray crystallography efforts.[60] Such studies will also provide information on the stoichiometry of receptor components and spark drug discovery efforts aiming at interfering with specific protein interactions and receptor functions.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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