Allosteric Modulators of GABA<sub>B</sub> Receptors: Mechanism of Action and Therapeutic Perspective

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Abstract: γ-aminobutyric acid (GABA) plays important roles in the central nervous system, acting as a neurotransmitter on both ionotropic ligand-gated Cl<sup>-</sup>-channels, and metabotropic G-protein coupled receptors (GPCRs). These two types of receptors called GABA<sub>A</sub> (and C) and GABA<sub>B</sub> are the targets of major therapeutic drugs such as the anxiolytic benzodiazepines, and antispastic drug baclofen (lioresal®), respectively. Although the multiplicity of GABA<sub>B</sub> receptors offer a number of possibilities to discover new selective drugs, the molecular characterization of the GABA<sub>B</sub> receptor revealed a unique, though complex, heterodimeric GPCR. High throughput screening strategies carried out in pharmaceutical industries, helped identifying new compounds positively modulating the activity of the GABA<sub>B</sub> receptor. These molecules, almost devoid of apparent activity when applied alone, greatly enhance both the potency and efficacy of GABA<sub>B</sub> agonists. As such, in contrast to baclofen that constantly activates the receptor everywhere in the brain, these positive allosteric modulators induce a large increase in GABA<sub>B</sub>-mediated responses only WHERE and WHEN physiologically needed. Such compounds are then well adapted to help GABA to activate its GABA<sub>B</sub> receptors, like benzodiazepines favor GABA<sub>A</sub> receptor activation. In this review, the way of action of these molecules will be presented in light of our actual knowledge of the activation mechanism of the GABA<sub>B</sub> receptor. We will then show that, as expected, these molecules have more pronounced in vivo responses and less side effects than pure agonists, offering new potential therapeutic applications for this new class of GABA<sub>B</sub> ligands.

Key Words: Baclofen, anxiety, drug addiction, allosteric modulators, class C GPCRs.

INTRODUCTION

As one of the major neurotransmitters in the brain, γ-aminobutyric acid (GABA) plays critical roles in brain development and physiology. By activating GABA<sub>A</sub> receptors, which are Cl<sup>-</sup>-gated channels, this neurotransmitter prevents neuronal depolarization, and as such controls the transmission of excitatory signals. In young animals, these GABA<sub>A</sub> receptors generate instead excitatory responses, and replace the glutamatergetic system not yet fully established. Controlling GABA<sub>A</sub> receptor activity soon appeared as an interesting way for the treatment of brain dysfunction. This led to the discovery of benzodiazepines that allosterically enhance GABA<sub>A</sub> receptor activation by acting at a site distinct from the GABA binding site. These positive modulators act by increasing GABA affinity and potency, and by facilitating Cl<sup>-</sup>-channel opening, and are widely used for the treatment of insomnia, anxiety and epilepsies.

GABA also acts on G protein-coupled GABA<sub>B</sub> receptors [6]. These receptors limit neurotransmitter release at many synapses, including most GABAergic and glutamatergic ones, by inhibiting at least Ca<sup>2+</sup>-channel opening. They are also located in post-synaptic elements where they activate G protein-regulated inward-rectifying K<sup>-</sup>-channels (GIRK channels) [42]. These receptors were pharmacologically identified in the early 80’s, being selectively activated by baclofen (β-p-chlorophenyl-GABA) [31], a molecule that is used for the treatment of spasticity in multiple sclerosis patients due to its muscle-relaxant properties [9]. The GABA<sub>B</sub> receptors are also responsible for most effects of the drug of abuse gamma-hydroxybutyrate (GHB) that acts as a GABA<sub>B</sub> partial agonist at high doses [34, 48]. GABA<sub>B</sub> agonists also demonstrated a number of beneficial effects both in animals and in humans [5]. Indeed, activation of GABA<sub>B</sub> receptors exerts analgesic/antinociceptive effects in animal models of chronic inflammation and neuropathy (see [5]), suppresses drug seeking behavior [15], and has some anxiolytic activity both in animal models and in human [17]. However, undesired side effects such as hypothermic and sedative effects, greatly limits the use of GABA<sub>B</sub> agonists in therapeutics [5]. Moreover, tolerance to baclofen chronic treatment is well established [44]. In addition to agonists, GABA<sub>B</sub> antagonists were also shown to have potential therapeutic effects, such as antidepressant activity [17], cognition improvement [22], and beneficial effects in rat models of absence epilepsy [53].

GABA<sub>B</sub> receptors have therefore been used as a target in high throughput screening strategies with the aim at identifying new ligands acting at this receptor. As already well documented for the related metabotropic glutamate (mGlu) receptors [26, 28], this strategy leads to the discovery of allosteric modulators acting at the GABA<sub>B</sub> receptor [76, 77]. In contrast to mGlus for which both positive and negative allosteric modulators were identified, only positive allosteric modulators (PAMs) have been described so far for the GABA<sub>B</sub> receptor. These compounds display no or very partial agonist activity, but enhance both the potency and efficacy of GABA<sub>B</sub> agonists. As such, these molecules appear as a better alternative to GABA<sub>B</sub> agonists, allowing the specific enhancement of GABA<sub>B</sub> receptor activity when and where needed, and as such, are less prone to tolerance in contrast to the pure agonists that constantly activate the receptor in any region where it is expressed.

In the present chapter, we aimed at describing the mechanism of action of the identified allosteric modulators of the GABA<sub>B</sub> receptor. We will first describe our current knowledge of the functioning of this complex receptor (for reviews see [2, 65]). We will then highlight the potential new therapeutic possibilities offered by these molecules, as based on the recent preclinical studies reported in the literature.

STRUCTURE AND ACTIVATION MECHANISM OF THE GABA<sub>B</sub> RECEPTOR

The GABA<sub>B</sub> receptor is part of the class C of GPCRs that also includes the mGlus, the Ca<sup>2+</sup>-sensing, and the sweet and umami taste receptors among others [64]. These receptors are dimers, either homodimers linked by a disulphide bond (mGlus and Ca<sup>2+</sup>-sensing receptors), or heterodimers made of two similar, but distinct subunits (the GABA<sub>B1</sub> and taste receptors). Indeed, the GABA<sub>B</sub> receptor was the first GPCR to be identified that requires two distinct subunits to function: the GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits [33, 36, 79] (Fig. 1). Although the GABA<sub>B1</sub> subunit was soon shown to bind all known GABA<sub>B</sub> ligands (both agonists and antagonists), this protein did not form a functional GABA<sub>B</sub> receptor when expressed alone [35]. Only when GABA<sub>B1</sub> was co-expressed with the homologous GABA<sub>B2</sub> subunit was a functional GABA<sub>B</sub> receptor observed, either in cell lines or in cultured neurons. The GABA<sub>B</sub> dimeric entity was confirmed in native tissue [36]. Indeed, both
Each GABA B receptor subunit is made of two main domains: a large extracellular domain structurally similar to bacterial periplasmic amino-acid binding proteins often called a Venus Flytrap domain (VFT) [24], linked to the GABA B2 HD through a coiled-coil interaction [8, 10, 54, 61]. Such a system is assumed to control the trafficking to the cell surface of correctly assembled GABA B heterodimers.

Each GABA B receptor subunit is made of two main domains: a large extracellular domain structurally similar to bacterial periplasmic amino-acid binding proteins often called a Venus Flytrap domain (VFT) [24], linked to a 7 transmembrane domain (the heptahelical domain (HD)) typical of all GPCRs (Fig. 1). Moreover, co-immunoprecipitation of GABA B proteins and their targeting to the cell surface. COPI binding to the GOPII domain (VFT) and the heptahelical domain (HD). GABA B and other orthosteric GABA B ligands are known to bind in the GABA B VFT. No known ligand bind at the equivalent site in GABA B2. Only the GABA B1 HD appears to be responsible for G-protein coupling. These images were made using the coordinates of the dimer of mGlu1 VFTs in the inactive empty state (left) and those of the active Glu occupied state (right) [43], in association with a dimer of HD generated based on the proposed model of the dimer of rhodopsin [47].

GABA B and GABA B2 mRNAs are co-localized in most brain regions. Second, both proteins are found in the same neurons, even in the same subcellular compartments as observed at the electron microscopic level. Moreover, co-immunoprecipitation of GABA B1 with a GABA B2 antibody could be demonstrated from brain membranes. Eventually, mice lacking either GABA B1 or GABA B2 share very similar phenotypes, and none of the known GABA B-mediated responses could be measured in either mice [66, 69]. Although unusual baclofen-mediated inhibition of GIRK channels could be observed in mice lacking GABA B2, it is still not known whether this represents a natural response, or is the consequence of the absence of the GABA B2 subunit. Taken together, these data demonstrate that the assembly between these two proteins is required to get a functional GABA B receptor in native tissues.

When expressed alone, the GABA B1 subunit is mostly retained in the endoplasmic reticulum (ER), both in transfected cell lines and in neurons [16]. This is due to the presence of an intracellular retention signal (RRR) located in its intracellular tail that constitutes a binding site for the coat protein I complex (COP) [8, 10, 54, 61]. COPI is known to target back to the ER proteins that were assembled in the ER. Of interest, this Arg is found in the GABA B2, but not in the GABA B1, similar to that of the conserved D/ERY motif of class A GPCRs. Of interest, this Arg is found in the GABA B2, but not in the GABA B1, further highlighting the pivotal role played by GABA B2 in G-protein activation.

How can agonist binding in GABA B1 VFT activate the GABA B2 HD? Much information to answer that question came from the solved crystal structure of the mGlu1 VFT dimer with and without bound agonist or antagonist [43, 73]. These structures revealed that agonist binding in the VFT stabilizes a closed conformation that is also associated with a major change in the relative orientation of the two VFTs in the dimer (Fig. 1). This relative movement is expected to induce a relative movement of the HDs, a proposal that is consistent with FRET studies [72]. Of interest, although both HDs in a mGlu homodimer are identical, this process leads to the active state of only one of them [32], likely because a single G-protein can interact at a time with such dimeric entities [19]. This model perfectly fits with all mutational analysis of GABA B receptor function. Indeed, the closure of the GABA B1 VFT has been shown to be responsible for GABA B receptor activation [40], and such a closure activates the GABA B2 HD whether it is part of the associated subunit (like in the wild-type heterodimer) or linked to the GABA B1 VFT [23]. Moreover, point mutations introduced into either the GABA B1 VFT or the GABA B2 HD were found to increase constitutive activity of this receptor, consistent with these two domains playing a critical role in receptor activation [56].

In summary, the GABA B receptor is a complex allosteric protein made or four main domains working "de concert" to allow GABA binding in the VFT of one subunit (GABA B1) to activate the HD of the associated subunit (GABA B2), likely through relative movement between these domains (Fig. 1). As we will see now, such a complex structure offers a number of possibilities to modulate GABA B receptor function.

ALLOSTERIC MODULATORS OF THE GABA B RECEPTOR: PROPERTIES AND MECHANISM OF ACTION

Early studies following the molecular characterization of the GABA B receptor heterodimer indicated that Ca²⁺ ions act as enhancers of this receptor [80]. Indeed, few hundred micromolar of Ca²⁺ increased the potency of GABA in stimulating GTPaS binding or G-protein activation measured in second messenger assays [25]. This effect is observed both with the recombinant and the native receptor [25], even in post-mortem human tissues [58], and results from a direct increase in GABA affinity. Of interest, this effect of
Ca\(^{2+}\) was not observed with baclofen, suggesting that the chlorophenyl group of baclofen prevents the action of Ca\(^{2+}\) ions, pointing to the possibility that Ca\(^{2+}\) directly binds within the GABA binding site in the GABA\(_B\) VFT. This was further validated using site directed and 3D modeling studies [25]. According to the expected physiological Ca\(^{2+}\) concentration range, the GABA\(_B\) receptor is expected to be always potentiated under physiological condition. Only under pathological conditions, when the extracellular Ca\(^{2+}\) concentration reaches values as low as few micromolar, can this effect disappear. Whatever, these data revealed that it is possible to positively modulate GABA\(_B\) receptor function with small molecules.

Few years before this observation, a number of allosteric modulators of the other class C GPCRs, and especially mGlu receptors, were identified, including both negative and positive allosteric modulators [26, 28] (see this issue). The negative modulators first identified for mGlul and mGlul5, were found to inhibit in a non-competitive manner the activity of the receptors, and to display in most cases inverse agonist activity [12, 62]. In contrast, PAMs were found to have no, or weak agonist activity when applied alone, but to greatly enhance both the potency and the efficacy of agonists [41, 57]. Both types of modulators were found to bind in a cavity within the HD, contacting residues of TM3, TM5 TM6 and TM7, therefore at a site clearly distinct from the glutamate binding site located in the VFT. Residues that constitute this binding site differ between receptor subtypes, such that most modulators identified so far, either positive or negative, were found to be highly subtype selective, in contrast to the orthosteric ligands that usually do not discriminate between mGlul receptors from the same group [26, 28].

Taken together, these data indicated that compounds interacting in the HD of class C GPCRs could allosterically modulate their activity, and these compounds had three main advantages: 1) original chemical structures, different from that of the orthosteric ligands, usually poly-cyclic with a good bioavailability, more prone to chemical modifications, and in agreement with the Lipinski’s rules for drug-likeliness; 2) much higher selectivity among related sequences; and 3) a good respect of the biological activity of the receptors, especially for the PAMs that facilitate agonist action, and therefore enhance receptor activation when and where needed physiologically.

These observations lead a number of pharmaceutical companies to search for new GABA\(_B\) modulators using high throughput functional assays. So far, only 2 PAMs have been reported in the literature (2,6-Di-tert.-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and N,N’-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and some of their derivatives) [76, 77] (Fig. 2), and some others have been reported in patents [49-51]. Aryl-alkylamine (such as fendiline), amino acids like phenylalanine, leucine and isoleucine as well as dipeptides have also been shown to enhance GABA\(_B\) receptor activity in brain slices [14, 37, 38]. However, others reported that fendiline inhibits, rather than mimic, the effect of CGP7930 [58], and Urwyller and colleagues show that the effect of Aryl-alkylamine and amino acids are rather indirect, and do not result from a direct PAM action on the receptor itself [74].

CGP7930 and GS39783 were found to enhance agonist potency as well as efficacy on recombinant GABA\(_B\) receptors in various assays (Fig. 3), on both human and rat receptors [20, 76, 77]. GS39783 was also shown to be active on fish and chicken receptors, but not on the Drosophila one [20], and CGP7930 enhances GABA affinity on the bullfrog receptor [1], demonstrating a good conservation of the allosteric site in vertebrates. In the GTP\(^\gamma\)S binding assay CGP7930 and GS39783 increase GABA potency by 5-10 fold, and increase the maximal effect from 1.5 to up to 2 fold, with potencies ranging from 3 to 5 \(\mu\)M, depending on the agonist concentration. The same positive allosteric effect was also observed when coupling of the GABA\(_B\) receptor to GIRK channels was measured in Xenopus oocytes [76, 77], or when the coupling of the receptor to phospholipase C was made possible with recombinant chimeric Gq/i/o proteins [3, 76]. Very similar enhancing effects were observed with all three well known GABA\(_B\) receptor agonists, GABA, baclofen and APPA. Of interest, the PAMs largely increased the efficacy of partial agonists like CGP47656 to make it a full agonist, with a similar maximal effect as that of GABA. Moreover, among 7 competitive antagonists, two (CGP35348 and 2-OH-saclofen) became partial agonists [75].

![Fig. (2)](image2.png)

Fig. (2). Structure of the two PAMs identified for the GABA\(_B\) receptor.

![Fig. (3)](image3.png)

Fig. (3). The PAMs increase both the potency and efficacy of GABA on the GABA\(_B\) receptor. Data were obtained from membranes prepared from HEK 293 cells expressing GABA\(_B1\), GABA\(_B2\) and the G protein. GTP\(^\gamma\)S binding was measured in the presence of the indicated concentration of GABA with (open triangles) or without (closed squares) 100 \(\mu\)M CGP7930. This figure is adapted from [3].

CGP7930 and GS39783 increased agonist affinity as measured with \(^{[3]}\)H]-APPA or through the displacement of radio-labeled antagonists [75-77]. However, the increase in affinity (2 fold) is lower than the measured increase in potency. In agreement with the allosteric potentiation further stabilizing the closed state of the GB1 VFT, a decrease in both the ON and OFF binding rates of agonists was observed, as well as a slight decrease in the affinity of most antagonists [63]. Only the affinities of the antagonists CGP35348 and 2-OH-saclofen that became partial agonists in the presence of the PAMs, were increased [75].

The two identified GABA\(_B\) PAMs show no or only slight agonist activity when applied alone in most assays, both in recombinant systems, and in native preparations [20, 59, 76, 77]. However, partial agonist activity of CGP7930 could be observed when IP production was measured in HEK293 cells co-expressing the GABA\(_B\) receptor and the chimeric G-protein Gq9 [3]. This was not the
In summary, GABA B receptor activation is due to the closure of the subunit expressed in native brain membranes and in vivo also confirmed the PAM activity of these two compounds at the native GABA B receptor [27, 59]. When examined in brain slices, these compounds potentiated GABA B receptor action on synaptic transmission. GS39783 suppresses the paired pulse inhibition of population spikes recorded on hippocampal CA1 pyramidal cells, an effect that likely results from the potentiation of the action of ambient GABA at pre-synaptic GABA B receptors located on GABAergic terminals [77]. The other GABA B enhancer CGP7930 enhances baclofen-induced depression of dopaminergic neurons in the ventral tegmental area [14] and the GABAergic synaptic transmission in the CA1 area of the hippocampus [13]. Surprisingly, no significant effect on excitatory synaptic transmission in hippocampal CA1 network was observed [13] with CGP7930. It is proposed that this may result from a differential effect of this enhancer on the autoreceptors located in GABAergic terminals, and the heteroreceptors located in glutamatergic terminals. Although GABA B1 and GABA B2 splice variants have been shown to be differentially distributed in these two types of terminals [78], CGP7930 was found to be equally active on both recombinant receptors. Further studies are therefore required to clarify this issue.

Most importantly, both CGP7930 and GS39783 were found to pass the blood brain barrier when injected i.p. (or even when given orally in the case of GS39783) allowing the examination of their behavioral effects in vivo. Indeed, GS39783 decreased cAMP formation in vivo in the striatum only when co-administered orally with a threshold concentration of baclofen [27]. In vivo efficacy of CGP7930 was also illustrated by its marked enhancement of the sedative and hypnotic effect of both baclofen and GHB in DBA mice [11]. Due to the original mechanism of action of these PAMs, it was therefore of interest to examine whether such compounds have different effects than the GABA B agonist baclofen.

### Differential Effects of PAMs and Agonists

Although baclofen is being used in the treatment of spasticity for multiple sclerosis patients, its myorelaxant, sedative, cognitive and hypothermic effects limit its use in a number of other pathologies. In contrast to baclofen and other GABA B agonists that activate constantly and everywhere the receptor, PAMs are expected to enhance receptor activity only when and where needed physiologically (when and where GABA is produced to act on the GABA B receptor) (Fig. 5). As such, differential effects of PAMs and agonists were expected. Indeed, GS39783 given alone did not display sedative, cognitive, myorelaxant activities [18]. However, sedative effects were reported for CGP7930 at high doses [46]. No effect of GS39783 on body temperature was also observed [18]. This document the general idea that PAMs could be a better alternative to baclofen for the treatment of pathologies in which such side effects are not desired. Of interest, as described in more details below, the PAMs display more pronounced anxiolytic effects than GABA B agonists and keep most of the known positive actions of baclofen (Table 1).

### GABA B PAMs as Potential New Anxiolytics

The GABA system is well known to be involved in anxiety, as illustrated by the effect of benzodiazepines. However, the involve-
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...lack the side effects of the commonly used benzodiazepines. 

Moreover, no synergy with alcohol was observed after three weeks of treatment, demonstrating an absence of tolerance [55].

The anxiolytic effect of GS39783 could still be efficient in reducing stress-induced hyperthermia [18], a test that could not be performed with baclofen due to its hypothermic action.

Table 1. Comparison of the Effect and Properties of GABA-B Agonists and PAMs

|                         | Agonists | PAMs                  | Ref.          |
|-------------------------|----------|-----------------------|---------------|
| tolerance               | yes      | Not after 3 weeks     | [44, 55]      |
| Body temperature        | decrease | No effect             | [18]          |
| sedation                | increase | No effect             | [18] but see [46] |
| myorelaxation           | yes      | No effect             | [9, 18]       |
| cognition               | decrease | No effect             | [18]          |
| Anxiety                 | variable | decrease              | [5, 17, 18, 55] |
| Cocaine self-admin      | decrease | decrease              | [15, 45, 70, 71] |
| Alcohol intake          | decrease | decrease              | [15, 46, 60]  |
benzodiazepines acting as PAMs at the GABA\(_B\) receptors, these data represent certainly the second best example of such a class of compounds. A search for similar molecules acting at other receptors is now open.

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