CGP7930: A Positive Allosteric Modulator of the GABA_B Receptor

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ABSTRACT

CGP7930 (3-(3',5'-Di-*tert*-butyl-4'-hydroxy)phenyl-2,2-dimethylpropanol) is a positive allosteric modulator of the metabotropic GABA_B receptor. CGP7930 has been found to modulate the GABA_B receptor in the open, or high affinity, state increasing agonist affinity for the receptor and signal transduction efficacy following agonist stimulation. The GABA_B heteromeric subunit B2, involved in signal transduction but not ligand binding, seems to be the site of action of CGP7930 and similar allosteric modulators. When administered alone in naïve animals, CGP7930 acts as an anxiolytic in rodents without other overt behavioral effects and has also been demonstrated to reduce self-administration of nicotine, cocaine, or alcohol in rodents, suggesting that “fine tuning” of the GABA_B receptor by positive allosteric modulators may be able to regulate abuse of these drugs. Baclofen, the GABA_B agonist, is currently finding use in treating addiction and various other disorders, but this can result in off-target effects and tolerance. CGP7930 when co-administered with baclofen enhances its potency, which could in theory minimize deleterious effects. Further study of CGP7930 is required, but this compound, and others like it, holds potential in a clinical setting.

INTRODUCTION

γ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter within the central nervous system. To date, there are three main receptor types for GABA to act upon: the

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ionotropic GABA_A and GABA_C receptors, and the metabotropic GABA_B receptor. For the purposes of this review, we will consider only the metabotropic GABA_B receptor.

The GABA_B receptor is a member of "Family 3" G protein-coupled receptors, which also includes calcium-sensing, metabotropic glutamate, and some pheromone and taste receptors. These receptors exhibit large, extracellular, ligand-binding domains containing a so-called "Venus flytrap module," the site at which agonists bind to stabilize the active conformation of the receptor (Binet et al. 2004a). GABA_B receptors can couple to G_i and G_o proteins that, when stimulated, activate K⁺ and inhibit Ca²⁺ channels via the $\beta\gamma$ complex, with the α subunit regulating adenylate cyclase and therefore influencing cyclic adenosine monophosphate (cAMP) levels (Kornau 2006). The GABA_B receptor can also directly interact via a C-terminal tail with cAMP responsive element binding protein (CREB-2) and activating transcription factor (ATFx) (White et al. 2000). The GABA_B receptor structure is an obligatory dimeric complex consisting of B1 and B2 subunits, both of which appear to be prerequisite for receptor function. Both subunits seem to be required not only for receptor and, therefore, G protein activation, but for cell-surface expression of the receptor (Binet et al. 2004a). Further evidence of heterodimerization has been demonstrated using fluorescent resonance energy transfer studies (Pin et al. 2004).

There are similarities between the GABA_B receptor subunits, including a seven-transmembrane domain, and a long, extracellular, N-terminus chain (Onali et al. 2003). However, the subunits play quite a distinct functional role in the working GABA_B receptor. The GABA_{B1} subunit appears responsible for ligand binding, while the GABA_{B2} subunit couples to pertussis toxin-sensitive G proteins, G_i and G_o (Chen et al. 2006). The GABA_{B1} subunit has been suggested to contain an endoplasmic reticulum retention signal causing it to be unable to reach the cell surface alone. Mutation of this region allows the subunit to approach the cell surface, but it is functionally inert (Pin et al. 2004). The GABA_{B2} subunit, when in heterodimeric conformation with the GABA_{B1} subunit, seems to mask this retention signal, therefore, selecting the heteromeric receptor complex expression. Mice lacking the GABA_{B1} or GABA_{B2} subunit gene show reduced expression in the corresponding GABA_B receptor subunit, that is, GABA_{B2} or GABA_{B1}, respectively (Bettler and Tiao 2006), further suggesting that cell surface expression of one GABA_B subunit is dependent on the expression of the other. Additionally, two isoforms of the GABA_{B1} subunit (GABA_{B1a} and GABA_{B1b}) have been described but to date do not seem to reproducibly exhibit unique functional or pharmacological properties (Bettler and Tiao 2006). However, recent work has found differences in cellular localization between these two isoforms, which suggests differing functional consequences (Vigot et al. 2006). The GABA_{B2} subunit contains a heptahelical domain critical for G protein activation. This domain has also been suggested to interact with the extracellular domain of the GABA_{B1} subunit, altering agonist affinity and coupling efficacy (Binet et al. 2004a).

A functional GABA_B receptor seems to bind only a single GABA molecule despite the sequence similarity between the GABA_{B1} and GABA_{B2} subunits. Amino acid sites responsible for binding GABA do not appear to be conserved in the GABA_{B2} subunit, whereas the GABA_{B1} binding pocket sequence is highly conserved between species. Site-directed mutagenesis against the GABA_{B2} putative binding pocket has been unable to change the receptor function (Pin et al. 2004). In contrast, mutations of key residues within the GABA_{B1} subunit significantly alter ligand affinity and G protein activation (Galvez et al. 2000).

The mRNA and protein of both subunits are widely expressed in almost all neuronal cell populations (Kornau 2006). Western blotting conducted on postmortem human frontal cortex tissue has revealed the presence the GABA_{B2} subunit and two isoforms of the GABA_{B1} subunit, suggesting the presence of a functional GABA_B receptor (Olianas et al. 2005). The highest known concentrations of the GABA_B receptor are found in the thalamus, cortex, and cerebellum (Pilc and Nowak 2005).

GABA_B receptors are an interesting pharmacological target, the stimulation of which in humans has been linked to analgesia, hypotension, and anxiolysis (as reviewed in Bowery 1989). There have been a multitude of studies conducted with the prototypical GABA_B receptor agonist baclofen. This compound has found use as a centrally acting muscle relaxant, but can also induce sedation and hypothermia (Pilc and Nowak 2005). Activation of the GABA_B receptor by the selective agonist baclofen leads to a translocation and accumulation of CREB2 from the cytoplasm into the nucleus. Such a signaling mechanism is unique among “Family 3” G protein-coupled receptors and likely has a role in the long-term changes within the central nervous system (White et al. 2000).

POSITIVE ALLOSTERIC MODULATORS OF GABA_B RECEPTORS

Allosteric modulators are drugs that interact with hormones or neurotransmitter receptors at a site separate from the orthosteric binding pocket for an agonist or competitive antagonist (Urwyler et al. 2004). They are a promising area of drug discovery, given their capacity to “fine-tune” signaling mechanisms. Such compounds typically exhibit no intrinsic activity on their own, but rather enhance the effects of relevant ligands. At present, the most promising GABA_B ligand available is baclofen, a compound that can result in significant off-target effects due to the often quite high doses required to achieve efficacious functional effects.

One strategy to attempt to overcome the various problems associated with baclofen is the development of positive allosteric modulators of GABA_B receptors, which may represent a more subtle and controllable means of altering receptor function, or allow the use of lower doses of agonists to reduce unwanted drug effects (Christopoulos 2002). The most studied compound that acts in this manner to facilitate GABA_B receptor-mediated signaling is CGP7930 (3-(3',5'-Di-*tert*-butyl-4'-hydroxy)phenyl-2,2-dimethylpropanol), Fig. 1, Urwyler et al. 2001). This agent has been shown to interact with a discrete domain of the GABA_{B2} subunit apparently by stabilizing the active state involved in G-protein activation. The GABA_B receptor antagonist CGP54626 ([*S*-(*R*^{*},*R*^{*})]-[3-[[1-(3,4-Dichlorophenyl)ethyl]amino]-2-hydroxypropyl](cyclohexylmethyl) phosphinic acid) binds competitively to the GABA binding site of the GABA_B receptor, yet does not competitively inhibit the effects of CGP7930, indicating that CGP7930 in all likelihood does not bind within the GABA_{B1} extracellular domain site. In work on chimeric GABA_B receptor subunits, it was discovered that CGP7930 does not require GABA_{B1} or GABA_{B2} extracellular domains at the present. Rather, the binding site seems to lie within the heptahelical domain, and although more likely to be within the GABA_{B2} subunit, the exact binding location remains unclear (Binet et al. 2004b).

FIG. 1. The chemical structure of CGP7930.

The merit of CGP7930 has been examined in both *in vitro* and *in vivo* paradigms, administered alone, and also in combination with baclofen. Studies include preclinical examination in relation to sedation and hypnosis, depression, and drug-seeking behaviors.

CGP7930: *IN VITRO* STUDIES

The bullfrog [^3H]CGP54626 GABA_B receptor antagonist binding site is positively modulated by CGP7930. The increased affinity of GABA for GABA_B receptors was demonstrated by the biphasic displacement curve in the presence of CGP7930. Of note was the finding that there was no increase in affinity for antagonists, as CGP7930 acts to stabilize the receptor in a high affinity, open conformation, while antagonists prevent the closure of this conformation (Asay and Boyd 2006).

CGP7930 seems to effect GABA_B receptor-induced inhibition of adenylate cyclase activity by increasing the potency of baclofen and GABA in the presence of forskolin (in rat frontal cortex) or Ca^{2+} /calmodulin (within rat dorsal striatum), although in this paradigm CGP7930 does not significantly alter maximal responses (Onali et al. 2003). Plausibly, the authors suggested that differences within the signal transduction pathway may be responsible for the seeming lack of change in efficacy in the inhibitory model. Compounds that stimulate adenylate cyclase possibly have a larger pool of G proteins (mediated by both G_O and G_S) than those that inhibit it (mediated by G_i). Importantly, Onali et al. (2003) note that there was no effect of CGP7930 alone; that is, it exhibits no agonistic properties in this model, indicating that the doses used in the study were low enough for CGP7930 not to act as an agonist at the GABA_B receptor as previously reported (Binet et al. 2004b).

In rat olfactory bulb granule cell layer and in frontal cortex, baclofen, GABA, and corticotrophin-releasing hormone (CRH) all stimulate basal adenylate cyclase activity. CRH-stimulated adenylate cyclase activity is increased by both baclofen and GABA, an effect that is potentiated when CGP7930 is present (Onali et al. 2003). In transfected HEK 293 cells, the EC_{50} for GABA was increased three-fold by 0.1 nM of CGP7930 and ten-fold by 1.0 nM as measured via a [^{35}S]GTP γ S assay (Binet et al. 2004b). In membranes of rat granule cell layer, CGP7930 increased the pEC_{50} and maximal effect of baclofen. Similar

results were obtained using GABA as the agonist with CGP7930 increasing the pEC_{50} and maximal activity of GABA (Onali et al. 2003). Locally applied baclofen in the striatum of freely moving rats produced inhibition of extracellular cAMP accumulation. This effect was blocked by the administration of CGP56999 (((3-(1-(3-carboxyphenyl)ethyl)amino)-2-hydroxypropyl)cyclohexylmethyl phosphinic acid), a GABA_B receptor antagonist. This inhibition of cAMP accumulation was potentiated by orally applied GS39783 (N,N'-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine), a compound similar to CGP7930 (a positive allosteric modulator of the GABA_B receptor), and again blocked by CGP56999, supporting the mechanism of action as allosteric modulation, *in vivo* (Gjoni et al. 2006).

Olianas et al. (2005) found that CGP7930 (100 μ M) added to human frontal cortex in the presence of free Ca^{2+} ion enhanced the potency of GABA in stimulating [35 S]GTP γ S binding by four-fold and increased the maximal stimulatory response by $82.5 \pm 8.9\%$. Similar results were observed when using the potent GABA_B agonist 3-aminopropylphosphinic acid with a 4.8-fold increase in potency and $96.6 \pm 7.1\%$ increase in maximal response and baclofen with a 6.2-fold increase in potency and $77 \pm 6.5\%$ increase in maximal response. Again, it is important to note that CGP7930 failed to produce any significant effects on basal binding of [35 S]GTP γ S. Other positive allosteric GABA_B receptor modulators such as GS39783 have similar effects. In Chinese hamster ovary cells expressing the functional GABA_B receptor and also in rat brain membranes, an increase in both potency and maximal efficacy was noted at the GABA_B receptor in the presence of GS39783, and a net increase in affinity for GABA_B agonists. This is perhaps due to enhancement of the G protein coupling of the receptor (Urwyler et al. 2003). The authors also note that GS39783 was found to suppress paired pulse inhibition in HEK193 cells, a phenomenon reversed by the addition of the GABA_B receptor antagonist CGP55845A ((2S)-3-[[[(1S)-1-(3,4-Dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl)phosphinic acid), and speculate that this effect is probably due to enhancing the effect of synaptically released GABA at presynaptic GABA_B receptors (Urwyler et al. 2003).

When applied to rat striatal slices, baclofen decreased the maximal NMDA-mediated stimulation of tyrosine hydroxylase activity without significantly changing its EC_{50} , suggesting that the GABAergic system plays a role in the control of striatal dopaminergic function (Arias Montano et al. 1992). In slice preparations from the ventral tegmental area of Sprague–Dawley rats, CGP7930 was effective in facilitating the inhibitory effect of baclofen on the spontaneous firing rates of tegmental dopaminergic cells, an effect that could be prevented by pretreatment with the selective GABA_B receptor antagonist, CGP55845 (Chen et al. 2005). Notably, CGP7930 had no effect on cell firing rates when given alone, although this could be an artifact of the *in vitro* preparation with low levels of endogenous GABA release.

Positive allosteric modulators are also an interesting tool for examining orthosteric ligands. For example, Urwyler et al. (2005) examined a number of these ligands in a Chinese hamster ovary expression system. The partial GABA_B agonist CGP47656 (3-amino-2(S)hydroxypropyl methylphosphinic acid) normally displays both high- and low-affinity binding (probably reflecting populations of the GABA_B receptor in low- and high-affinity conformations). The presence of positive allosteric modulators CGP7930 or GS39783, as would be expected, only increases the high-affinity binding component, in this case by a factor of four. The competitive GABA_B antagonists CGP35348 ((3-aminopropyl)(diethoxymethyl)phosphinic acid) and 2-OH-saclofen do not stimulate

[³⁵S]GTP γ S binding on their own, but in the presence of the allosteric modulators acted as partial agonists (similar to CGP47656) with increased potency and efficacy. This suggests that these two compounds, being structurally similar to GABA_B agonists, bind in the agonist binding pocket within the Venus flytrap module, but ordinarily do not cause the module to close, thus activating the receptor. Comparison between these two ligands and GABA_B agonists may assist in elucidating the functional groups of GABA_B agonists and antagonists (Urwyler et al. 2005).

CGP7930: *IN VIVO* STUDIES

Hypnosis and Sedation

Onset of the loss of righting reflex in DBA mice via baclofen or γ -hydroxybutyric acid administration was dose-dependently reduced by the administration of CGP7930, while the duration of hypnosis was similarly increased (Carai et al. 2004). The loss of righting reflex caused by co-administration of baclofen (40 mg/kg i.p.) or γ -hydroxybutyric acid (500 mg/kg i.p.) combined with CGP7930 (100 mg/kg i.p.) was completely blocked by prior administration of the GABA_B receptor antagonist SCH 50911 (100 mg/kg i.p.) at a dose that did not produce sedation (Carai et al. 2004).

Depression and Anxiety

The GABAergic system almost certainly plays a role in depression and anxiety. Although CGP7930 has not received much attention in these models, other positive allosteric modulators of the GABA_B receptor have. GS39783 produced no effects on behavioral tests of locomotion, cognition, temperature, or narcosis, but did, however, display anxiolytic properties (Cryan et al. 2004). GABA_{B1} knockout mice, or mice chronically treated with the selective GABA_B receptor antagonist CGP56433A (3-[1(S)-[3-(cyclohexylmethyl)hydroxyphosphinyl]2(S)hydroxypropylamino]nethyl} benzoic acid) display decreased immobility in a forced swim test suggesting an antidepressant-like effect, whereas they display high levels of anxiety in the light–dark box, elevated zero maze, and staircase tests compared to their wild-type littermates (Mombereau et al. 2004). Acute and chronic treatment with GS39783 decreased anxiety in both a light–dark box paradigm and elevated zero maze behavioral tests without altering the locomotor activity of the CGP56433A-treated, or GABA_{B1} knockout mice (Mombereau et al. 2004).

Drug-Seeking

GABA_B receptor agonists have been implicated in suppressing alcohol-drinking behavior in rat models (Liang et al. 2006). For example, baclofen has been shown in rats to suppress responding for alcohol in dependent animals (Walker and Koob 2007) and reduce the alcohol deprivation effect (Colombo et al. 2006). In humans, self-reported cravings were attenuated in subjects (Addolorato et al. 2002) even when other treatments had failed (Bucknam 2007), alcohol intake was reduced (Flannery et al. 2004) and the severity of

alcohol withdrawal syndrome was minimized to levels comparable with the current “gold standard” treatment, diazepam (Addolorato et al. 2006).

Considering the acquisition of alcohol-drinking behavior, CGP7930 was shown to dose-dependently reduce daily alcohol intake of sP (Sardinian alcohol preferring) rats without altering their total daily fluid intake during 5 days of treatment. At the highest dose of 100 mg/kg, rats were consuming less than one-fifth of the alcohol untreated rats were drinking. Upon completion of treatment, rats rapidly returned to “baseline” alcohol intake. When rats already drinking alcohol were treated with CGP7930, a reduction of 30–40% was observed during the first day, and this effect diminished resulting in rats treated at the highest dose (100 mg/kg i.p.) drinking only marginally less than untreated rats on the 4th and 5th days (Orri et al. 2005). In a different study, inbred alcohol-preferring (iP) rats were trained to respond to 10% v/v ethanol in a fixed ratio paradigm. Both baclofen and CGP7930 independently reduced voluntary response for 10% ethanol in a dose-related manner. Neither drug had an impact on response to water. A combination of individually subthresholded doses of baclofen and CGP7930 was also able to reduce operant responding for ethanol, suggesting that CGP7930 indeed acts to facilitate GABA_B receptor-mediated signaling in this paradigm. These data demonstrate the potential of positive allosteric modulators of metabotropic GABA_B receptors to regulate alcohol response, and highlight a possible clinical potential of co-administration of baclofen and CGP7930 (Liang et al. 2006).

CGP7930 appears to reduce the motivation to self-administer cocaine, as judged by a reduction in the break-point maintained by 1.5 mg/kg cocaine infusions on a progressive ratio schedule in rats (Smith et al. 2004). Furthermore, CGP7930 was more potent at reducing cocaine self-administration than another allosteric modulator, GS39783 (Smith et al. 2004). GS39783 has been found to have no intrinsic effects on intracranial self-stimulation (ICSS) in rats in contrast to many drugs of abuse, such as cocaine, which lower ICSS thresholds. Baclofen dose-dependently increased ICSS thresholds, and co-administration of GS39783 and baclofen attenuated the threshold-lowering effect of cocaine administration dose-dependently. This work suggests that GABA_B receptor activation opposes the rewarding effects of cocaine, and possibly of other drugs of abuse (Slattery et al. 2005).

When Wistar rats under either fixed or progressive ratio schedules were administered baclofen or the GABA_B receptor agonist CGP44532 (3-amino-2-(S)-hydroxy-propyl)-methyl-phosphinic acid), a dose-dependent reduction in nicotine response occurred, with higher doses producing a similar effect on food responding (Paterson et al. 2004). Interestingly, the positive allosteric modulator GS39783 has been shown to block the rewarding effects of nicotine in rats in a conditioned place preference (CPP) paradigm during the conditioning phase, but not if administered immediately prior to the CPP test (Mombereau et al. 2007). A significant accumulation of Δ FosB was found in the nucleus accumbens of rats treated chronically with nicotine, and this effect was reversed when GS39783 was administered during the acquisition phase of CPP, but not when it was administered immediately prior to the CPP test. This suggests that the neuroadaptive changes initiated by nicotine are relatively long-lived and robust, but can be functionally reversed by GABA_B receptor positive allosteric modulators (Mombereau et al. 2007).

Positive allosteric modulators appear to be an interesting tool to closely examine the GABA_B receptor and associated ligand properties. Moreover, such compounds hold potential in a clinical setting via co-administration by reducing the dose required of drugs

that may otherwise produce off-target effects and/or tolerance. Further study is clearly warranted to gather more information to reveal if these compounds will ultimately have a benefit in medical practice.

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