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Comparison of the effects of the $GABA_B$ receptor positive modulator BHF177 and the $GABA_B$ receptor agonist baclofen on anxiety-like behavior, learning, and memory in mice

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ABSTRACT

γ-Aminobutyric acid B ($GABA_B$) receptor activation is a potential therapeutic approach for the treatment of drug addiction, pain, anxiety, and depression. However, full agonists of this receptor induce side-effects, such as sedation, muscle relaxation, tolerance, and cognitive disruption. Positive allosteric modulators (PAMs) of the $GABA_B$ receptor may have similar therapeutic effects as agonists with superior side-effect profiles. The present study behaviorally characterized *N*-([1*R*,2*R*,4*S*]-bicyclo[2.2.1]hept-2-yl)-2-methyl-5-(4-[trifluoromethyl]phenyl)-4-pyrimidinamine (BHF177), a $GABA_B$ receptor PAM, in mouse models of anxiety-like behavior, learning and memory. In addition, the effects of BHF177 were compared with the agonist baclofen. Unlike the anxiolytic chlordiazepoxide, baclofen (0.5, 1.5, and 2.5 mg/kg, intraperitoneally) and BHF177 (10, 20, and 40 mg/kg, orally) had no effect on anxiety-like behavior in the elevated plus maze, light/dark box, or Vogel conflict test. Baclofen increased punished drinking in the Vogel conflict test, but this effect may be attributable to the analgesic actions of baclofen. At the highest dose tested (2.5 mg/kg), baclofen-treated mice exhibited sedation-like effects (i.e., reduced locomotor activity) across many of the tests, whereas BHF177-treated mice exhibited no sedation-like effects. BHF177 exhibited pro-convulsion properties only in mice, but not in rats, indicating that this effect may be species-specific. At doses that were not sedative or pro-convulsant, baclofen and BHF177 had no selective effects on fear memory retrieval in contextual and cued fear conditioning or spatial learning and memory in the Barnes maze. These data suggest that BHF177 has little sedative activity, no anxiolytic-like profile, and minimal impairment of learning and memory in mice.

1. Introduction

γ-Aminobutyric acid (GABA) is the most widely distributed amino acid neurotransmitter in the central nervous system and acts as the primary inhibitory neurotransmitter (Johnston, 1978; Sivilotti and Nistri, 1991). GABA is critically involved in the function of multiple brain sites, and thus dysfunction of the GABAergic system is likely to be involved in the development of dependence on drugs of abuse, such as nicotine and cocaine, and other psychiatric disorders, including anxiety and depression (Cryan and Slattery, 2010; Koob, 2000; Millan, 2003; Reynolds, 2008; Vlachou and Markou, 2010; Xi and Gardner, 2008). GABA signaling is mediated through ionotropic $GABA_A$ and $GABA_C$ receptors and metabotropic $GABA_B$ receptors (Bormann, 1988; Bowery, 1989). $GABA_A$ receptors are ligand-gated ion channels responsible for the rapid component of inhibitory postsynaptic potentials. $GABA_B$ receptors are G-protein-coupled receptors that inhibit adenylate cyclase activity and mediate the slow and prolonged component of synaptic inhibition (Bormann, 1988; Bowery et al., 2004). The prototypical $GABA_B$ receptor agonist baclofen, which is used for the treatment of spasticity and skeletal muscle rigidity, has shown therapeutic promise in a wide range of other indications, including drug dependence, anxiety disorders, and depression (Bettler et al., 2004; Cousins et al., 2002; Cryan and Kaupmann, 2005; Cryan and Slattery, 2010). However, baclofen induces sedation and muscle

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relaxation, limiting its use as a tool for behavioral research and therapeutic agent, although tolerance develops to some of these effects (Ong and Kerr, 2005).

Accumulating evidence indicates that allosteric positive modulators (PAMs), which act at sites distinct from the orthosteric binding site, may have a better side-effect profile compared with full agonists because PAMs are devoid of substantial intrinsic agonist activity in the absence of the orthosteric agonist. Instead, PAMs potentiate the potency and maximal efficacy of full agonists, such as endogenously released GABA (Christopoulos, 2002; Jensen and Spalding, 2004), thus offering more physiological means to activate the receptors than agonists. This notion is supported by the demonstrated effects of benzodiazepines that are $GABA_A$ receptor PAMs. Compared with orthosteric agonists at the $GABA_A$ receptor, benzodiazepines have similar pharmacological effectiveness in treating anxiety and sleep disorders, while having improved side-effect profiles (Mohler et al., 2002). Moreover, the $GABA_B$ receptor PAM GS39783 showed similar efficacy as the agonist baclofen in rodent models of drug dependence (Lhuillier et al., 2007; Maccioni et al., 2008, 2007; Mombereau et al., 2007; Paterson et al., 2008; Slattery et al., 2005) and anxiety-like behavior (Cryan et al., 2004; Mombereau et al., 2004). In contrast to baclofen, GS39783 showed few adverse effects, having no effect on locomotor activity, rotarod performance (Cryan et al., 2004), or food- and sucrose-maintained responding (Maccioni et al., 2008, 2007; Paterson et al., 2008). Thus, $GABA_B$ receptor PAMs may exhibit similar pharmacological effectiveness as the full agonist baclofen and therefore serve as a useful tool for the evaluation of the role of $GABA_B$ receptors in psychiatric disorders.

Although the important role of $GABA_A$ receptors in anxiety has been well documented, findings on the involvement of $GABA_B$ receptors in anxiety-like behavior have been inconsistent (Cryan and Kaupmann, 2005; Frankowska et al., 2007; Partyka et al., 2007). Thus, we evaluated the effects of $GABA_B$ receptor activation by the novel PAM *N*-([1*R*,2*R*,4*S*]-bicyclo[2.2.1]hept-2-yl)-2-methyl-5-(4-[trifluoromethyl]phenyl)-4-pyrimidinamine (BHF177) on anxiety-like behavior in various mouse models and compared the effects of BHF177 and baclofen. BHF177 is a potent ($pEC_{50} = 5.78 \pm 0.03$; E_{max} (%) $= 183 \pm 4$) and selective $GABA_B$ receptor PAM with good metabolic stability and ability to cross the blood–brain barrier (Guery et al., 2007). Similar to GS39783, BHF177 showed efficacy in animal models of drug dependence (Maccioni et al., 2009; Paterson et al., 2008; Vlachou et al., 2011a). However, little is known about whether BHF177 has anxiolytic efficacy. The present study evaluated the potential anxiolytic properties of BHF177 in the elevated plus maze, light/dark box, and Vogel conflict test in mice. These tests are standard "first pass" preclinical tests for anxiolytic efficacy and commonly used to evaluate the putative anxiolytic properties of compounds (Cryan and Sweeney, 2011; Treit et al., 2010). Both the light/dark box and elevated plus maze are approach-avoidance-based behavioral paradigms (i.e., they are based on the conflict between innate exploratory drive and avoidance of brightly lit or open, elevated environments in rodents) (Rodgers et al., 1997). The Vogel conflict test is a conflict-based anxiety test, in which the drinking behavior of thirsty rodents is punished by aversive shock delivery (Millan and Brocco, 2003). Although the neurobiology that underlies these models is not identical, all of these tests are sensitive to anxiolytic compounds with a wide spectrum of putative mechanisms, including benzodiazepines, $5\text{-}HT_{1A}$ receptor agonists, and selective serotonin reuptake inhibitors (Cryan and Sweeney, 2011; Treit et al., 2010).

Because the Vogel conflict test is a conflict-based anxiety test that involves aversive learning to a painful stimulus (Millan and Brocco, 2003), the flinch–jump test was conducted to determine whether analgesic effects of the compound are involved in the effects obtained in this conflict test. Furthermore, activation of the $GABA_B$ receptor by baclofen has been reported to disrupt learning and memory (Castellano et al., 1989; McNamara and Skelton, 1996; Nakagawa and Takashima, 1997; Pitsikas et al., 2003; Stackman and Walsh, 1994; Zarrindast et al., 2004). Therefore, the effects of BHF177 and baclofen on fear-related learning and memory, evaluated in the contextual and cued fear conditioning tests, and spatial learning and memory, evaluated in the Barnes maze, were investigated to characterize any potential cognitive deficits induced by these compounds. Finally, we noticed that several mice experienced mild seizures after administration of the highest dose of BHF177 in these behavioral tests. Therefore, the pentylenetetrazole (PTZ)-induced seizure test was conducted to evaluate the potential pro-convulsant properties of this compound in both mice and rats. We conducted the PTZ test in rats to test the hypothesis that the pro-convulsant effects of BHF177 may be species-specific because BHF177-induced seizures in rats have never been observed in our behavioral studies and have not been reported by others who used similar dose ranges (Paterson et al., 2008; Vlachou et al., 2011a, 2011b).

2. Materials and methods

2.1. Subjects

Experimentally naive male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME, USA), 12–14 weeks old, and naive male Wistar rats (Charles River, Raleigh, NC, USA) that weighed 300–320 g upon arrival in the laboratory were housed in humidity- and temperature-controlled animal facilities on a reverse 12 h/12 h light/dark cycle (lights off at 7:00 AM) with *ad libitum* access to food and water except during testing. Behavioral testing was conducted during the dark phase of the light/dark cycle. All of the procedures were conducted in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care and National Research Council's Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees of the University of California San Diego and The Scripps Research Institute. An independent group of naive mice was used for each experiment using a between-subjects design for the factor drug *Dose*, with the exception of a few vehicle-treated mice that were used in both the probe and retention tests in the Barnes maze (see below).

2.2. Drugs

Chlordiazepoxide and R(+)-baclofen hydrochloride (Sigma–Aldrich, St. Louis, MO, USA) were dissolved in sterile 0.9% saline and injected intraperitoneally (i.p.) 30 min before testing. *N*-([1*R*,2*R*,4*S*]-bicyclo[2.2.1]hept-2-yl)-2-methyl-5-(4-[trifluoromethyl]phenyl)-4-pyrimidinamine (BHF177) was synthesized as described below. This compound was suspended in 0.5% methylcellulose and administered orally (p.o.) 60 min before testing. The convulsant drug PTZ (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in sterile 0.9% saline and injected i.p. immediately before testing. The doses of baclofen (0.5, 1.5, and 2.5 mg/kg) and route of administration (i.p.) were chosen based on previously published studies (Cryan et al., 2004; Dalvi and Rodgers, 1996; Frankowska et al., 2007; Umezu, 1999; Zarrindast et al., 2001) that all reported a narrow effective dose range of this compound before sedative effects were manifested. The doses of BHF177 (10, 20, and 40 mg/kg) and route of administration (p.o.) were also chosen based on previous studies (Maccioni et al., 2009; Paterson et al., 2008; Vlachou et al., 2011a, 2011b). All of the drugs were administered in volumes of 0.1 ml/10 g body weight in mice and 0.1 ml/100 g body weight in rats. The effects of baclofen and BHF177 were investigated using a between-subjects design for the factor *Dose*, with $n = 12$ per group for most of the behavioral tests, with the exception of the flinch–jump test and rat PTZ-induced seizure test, in which eight subjects per group were used.

2.2.1. Synthesis of BHF177

The previous synthesis of BHF177 (Guery et al., 2007) required seven steps from commercially available 2,4-dichloro-5-bromobenzene, during which the C2-methyl, C5-aryl, and C4-amine groups were introduced in succession. We chose instead to assemble the pyrimidine core with the methyl group pre-installed, resulting in the five-step procedure shown in Fig. 1 (see Supplemental material for detailed synthesis and compound characterization information). The final product showed nuclear magnetic resonance, mass spectrometry, and chromatographic data identical to BHF177 prepared previously by the published route, and consistent with its proposed structure. Under similar administration parameters and doses, the same batch of BHF177 synthesized following this procedure had behavioral effects on startle reactivity in rats (unpublished observations).