Discriminative Stimulus Effects of the GABA_B Receptor-Positive Modulator rac-BHFF: Comparison with GABA_B Receptor Agonists and Drugs of Abuse

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ABSTRACT

GABA_B receptor-positive modulators are thought to have advantages as potential medications for anxiety, depression, and drug addiction. They may have fewer side effects than GABA_B receptor agonists, because selective enhancement of activated receptors could have effects different from nonselective activation of all receptors. To examine this, pigeons were trained to discriminate the GABA_B receptor-positive modulator (R,S)-5,7-di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-3*H*-benzofuran-2-one (rac-BHFF) from its vehicle. The discriminative stimulus effects of rac-BHFF were not mimicked by the GABA_B receptor agonists baclofen and γ -hydroxybutyrate (GHB), not by diazepam, and not by alcohol, cocaine, and nicotine, whose self-administration has been reported to be attenuated by GABA_B receptor-positive modulators. The discriminative stimulus effects of rac-BHFF were not antagonized by the GABA_B receptor antagonist 3-aminopropyl (diethoxymethyl)phosphinic acid

(CGP35348) but were attenuated by the less efficacious GABA_B receptor-positive modulator 2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol (CGP7930), suggesting the possibility that rac-BHFF produces its discriminative stimulus effects by directly activating GABA_{B2} subunits of GABA_B receptors. At a dose 10-fold lower than the training dose, rac-BHFF enhanced the discriminative stimulus effects of baclofen, but not of GHB. This study provides evidence that the effects of GABA_B receptor-positive modulators are not identical to those of GABA_B receptor agonists. In addition, the results suggest that positive modulation of GABA_B receptors does not produce discriminative stimulus effects similar to those of benzodiazepines, alcohol, cocaine, and nicotine. Finally, the finding that rac-BHFF enhanced effects of baclofen but not of GHB is consistent with converging evidence that the populations of GABA_B receptors mediating the effects of baclofen and GHB are not identical.

Introduction

GABA_B receptors, which are present throughout the central nervous system, are implicated in various central nervous system disorders (Cryan and Kaupmann, 2005; Bowery, 2006), including drug dependence (Maccioni et al., 2008; Addolorato et al., 2009; Vlachou and Markou, 2010). They couple through G $\alpha_{i/o}$ to inhibit adenylyl cyclase, close voltage-dependent calcium channels, and open inwardly rectifying K⁺ channels (Bowery et al., 2002; Bettler et al., 2004), and function as autoreceptors and heteroreceptors, modulating neurotransmitter release and neuronal firing, and influencing long-term changes in synaptic strength (Pinard et al., 2010).

GABA_B receptors are heterodimers of GABA_{B1a} or GABA_{B1b} subunits where GABA and other GABA_B receptor ligands bind, combined with GABA_{B2} subunits where allosteric modulators have been proposed to act (Calver et al., 2002; Bettler et al., 2004; Pin et al., 2004). Allosteric modulators alter effects of an endogenous transmitter or orthosteric agonist by binding to regions on the receptor that are different from the orthosteric site where the endogenous transmitter binds (Jensen and Spalding, 2004). By altering activated receptors only, allosteric modulators may have a broader therapeutic window than ligands that alter all receptors. Because GABA_B receptors are thought to be involved in various psychiatric disorders (Kerr and Ong, 1995; Markou et al., 2004; Pilc and Nowak, 2005; Frankowska et al., 2007; Addolorato et al., 2009), allosteric modulation of these receptors could provide new treatments.

Several compounds have been shown to have positive GABA_B modulatory activity in vitro [e.g., 2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol (CGP7930) (Urwiler

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ABBREVIATIONS: GHB, γ -hydroxybutyrate; CGP7930, 2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol; rac-BHFF, (R,S)-5,7-di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-3*H*-benzofuran-2-one; BHF177, *N*-[(1*R*,2*R*,4*S*)-bicyclo[2.2.1]hept-2-yl]-2-methyl-5-[4-(trifluoromethyl)phenyl]-4-pyrimidinamine; CGP35348, 3-aminopropyl(diethoxymethyl)phosphinic acid; CCK, cholecystokinin; CCK-8, sulfated cholecystokinin-octapeptide; GS39783, *N,N'*-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine; GTP(γ)³⁵S, guanosine 5'-O-(3-[³⁵S]thiotriphosphate; MK-801, (5*S*,10*R*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine hydrogen maleate.

et al., 2001; Adams and Lawrence, 2007), *N,N'*-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (Urwyler et al., 2003), (R,S)-5,7-di-*tert*-butyl-3-hydroxy-3-trifluoromethyl-3*H*-benzofuran-2-one (rac-BHFF) (Malherbe et al., 2008), *N*-[(1*R*,2*R*,4*S*)-bicyclo[2.2.1]hept-2-yl]-2-methyl-5-[4-(trifluoromethyl)phenyl]-4-pyrimidinamine (BHF177) (Guery et al., 2007; Maccioni et al., 2009), methyl-2-(1-adamantanecarboxamido)-4-ethyl-5-methylthiophene-3-carboxylate and methyl-2-(cyclohexanecarboxamido)-4-ethyl-5-methylthiophene-3-carboxylate (Castelli et al., 2011), and 2-[1-[2-(4-chlorophenyl)-5-methylpyrazolo[1,5-*a*]pyrimidin-7-yl]-2-piperidinyl]ethanol (Perdona et al., 2011)]. Their positive GABA_B modulatory activity in vitro was evidenced by enhancing GABA, and, for all compounds except BHF177, also by enhancing the GABA_B receptor agonist baclofen. Several of these compounds have been shown to have positive modulatory properties not only in vitro but also in vivo. CGP7930 and rac-BHFF enhanced loss of righting in mice induced by baclofen (Carai et al., 2004; Malherbe et al., 2008; Koek et al., 2010), which probably involves cerebellar GABA_B receptors that are especially sensitive to positive modulation (Hensler et al., 2012). GS39783 enhanced effects of baclofen on hamster circadian activity rhythms (Gannon and Millan, 2011). Recently, evidence was obtained in pigeons that CGP7930 and rac-BHFF also enhance the discriminative stimulus effects of baclofen (Koek et al., 2012). The generality of the positive modulatory effects of CGP7930 and rac-BHFF in vivo increases the likelihood that these effects are involved in their therapeutic-like activity.

Positive GABA_B receptor modulators have anxiolytic- and antidepressant-like properties in elevated maze and forced swimming tests, respectively (Cryan et al., 2004; Frankowska et al., 2007; Jacobson and Cryan, 2008), and exhibit antipsychotic-like effects in (5*S*,10*R*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine hydrogen maleate (MK-801)- and amphetamine-induced hyperactivity tests (Wieronska et al., 2011). In addition, they reduce self-administration of alcohol (Orzu et al., 2005, 2012; Liang et al., 2006; Maccioni et al., 2007, 2008, 2009, 2010a, b, 2012; Agabio et al., 2012), cocaine (Filip et al., 2007), and nicotine (Mombereau et al., 2007; Paterson et al., 2008; Vlachou et al., 2011). GABA_B receptor-positive modulators are thought to have advantages as potential medications for anxiety, depression, and drug addiction, because they may have a better side-effect profile than GABA_B receptor agonists, based on the notion that selective enhancement of activated receptors has effects that differ from indiscriminate activation of all receptors. Unlike baclofen, GABA_B receptor-positive modulators do not seem to interfere with motor coordination (Cryan et al., 2004; Jacobson and Cryan, 2008), do not produce loss of righting (Carai et al., 2004; Malherbe et al., 2008; Koek et al., 2010) and, with the possible exception of CGP7930 (Koek et al., 2010), do not induce hypothermia. Also, CGP7930 does not have baclofen-like discriminative stimulus effects, and does not have discriminative stimulus effects similar to those of γ -hydroxybutyrate (GHB), a compound with GABA_B receptor agonist properties (Koek et al., 2012). In contrast, rac-BHFF produced a level of drug-appropriate responding in baclofen-trained animals near the level observed with the training drug, and, like baclofen, substituted partially for GHB (Koek et al., 2012). Thus, the discriminative stimulus effects of rac-BHFF, unlike CGP7930, may be similar to those of baclofen. The present study was aimed at a more comprehensive characterization of

the discriminative stimulus properties of rac-BHFF by attempting to use rac-BHFF as training drug. This study is the first to show that animals can discriminate effects of a GABA_B receptor-positive modulator, and that these effects differ from those of GABA_B receptor agonists.

Establishing rac-BHFF as training drug also made it possible to examine whether rac-BHFF shares discriminative stimulus effects with compounds other than GABA_B receptor agonists. The present study examined the effects of the cholecystokinin (CCK) A receptor agonist CCK-8 (sulfated cholecystokinin-octapeptide), because in a broad radioligand binding screen rac-BHFF was found to be inactive at all non-GABA_B targets tested except CCK_A receptors (Malherbe et al., 2008). Also, because positive modulation of GABA_B receptors has been reported to produce anxiolytic-like effects comparable with diazepam (Frankowska et al., 2007) and to decrease self-administration of alcohol, cocaine, and nicotine, the present study examined whether the discriminative stimulus effects of rac-BHFF resemble those of diazepam, ethanol, cocaine, or nicotine.

Materials and Methods

Animals. Eight adult white Carneau pigeons (*Columba livia*; Palmetto, Sumter, SC) were individually housed under a 12/12-hour light/dark cycle. They had free access to water and were maintained between 80 and 90% of their free-feeding weight by food (Purina Pigeon Checkers, St. Louis, MO) received during experimental sessions and supplemental postsession feedings (Purina Pigeon Checkers or mixed grain). The animals were maintained and the experiments were conducted in accordance with the Institutional Animal Care and Use Committee (The University of Texas Health Science Center at San Antonio) and with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

Apparatus. Experiments were conducted in sound attenuating, ventilated chambers (BRS/LVE, Laurel, MD) equipped with two response keys that could be illuminated by a red light. After completion of each fixed ratio, the key light was extinguished for 4 seconds, during which time a white light illuminated the hopper where food (Purina Pigeon Checkers) was available. Chambers were connected by an interface (MED Associates Inc., St. Albans, VT) to a computer that used MED-PC IV software (MED Associates Inc.) to monitor and control inputs and outputs and to record the data.

Procedure. The discrimination training and testing procedure was similar to that described in detail elsewhere (Koek et al., 2004). Briefly, before each daily session, subjects received either the training dose of rac-BHFF or vehicle using the same route (i.e., oral) and the same interval before being placed into the chambers (i.e., 45 minutes) that were used in the previous study of the effects of rac-BHFF in GHB- and in baclofen-discriminating pigeons (Koek et al., 2012). Drug and vehicle training sessions occurred with equal frequency. Sessions started with a period of 15 minutes, during which the lights were off and key pecks had no programmed consequence. Subsequently, the left and the right keys were illuminated red and 20 consecutive responses on the injection appropriate key resulted in 4-second access to food. These responses had to be consecutive because responses on the injection inappropriate key reset the fixed ratio requirement on the injection appropriate key. The response period ended after 30 food presentations or 15 minutes, whichever occurred first. Initially, pigeons had to satisfy the following criteria for at least seven of nine consecutive sessions: $\geq 90\%$ of the total responses on the injection appropriate key and fewer than 20 responses on the injection inappropriate key before the first food presentation. Thereafter, tests were conducted when these criteria were satisfied during two

consecutive (drug and vehicle) training sessions. Test sessions were the same as training sessions (i.e., a 15-minute period, followed by a response period that ended after 30 food presentations or 15 minutes, whichever occurred first), except that food was available after completion of 20 consecutive responses on either key. These responses had to be consecutive, because switching from responding on one of the keys to responding on the other key reset the fixed ratio requirement.

All compounds were injected i.m. [either immediately (agonists) or 45 minutes (antagonists) before the session], except GABA_B receptor-positive modulators and ethanol, which were administered orally 45 minutes before the session. For each compound, administration routes and times were the same as used previously in GHB- and baclofen-discriminating pigeons (Koek et al., 2012).

The dose of rac-BHFF that was initially chosen for training (i.e., 178 mg/kg) occasioned the greatest amount of drug key-responding in pigeons discriminating baclofen from saline (Koek et al., 2012).

Data Analysis. The mean percentage of responses on the training drug-appropriate key $\pm$ 1 S.E.M. was plotted as a function of dose. When an animal responded at a rate less than 20% of the vehicle control rate, discrimination data from that test were not included in the average. Mean percentages of responses on the training drug-appropriate key were calculated only when they were based on at least one-half of the animals tested.

Dose-response curves that attained at least 80% training drug-appropriate responding were analyzed by nonlinear regression of individual values by means of GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, CA) using the sigmoid equation: $\text{response} = \text{bottom} + (\text{top} - \text{bottom}) / (1 + 10^{-(\log \text{ED}_{50} - \log (\text{dose})) \times \text{slope}})$, with bottom = 0 and top = 100. F ratio tests in Prism were used to compare dose-response curves with respect to their slopes. Parallel shifts of dose-response curves were examined by simultaneously fitting sigmoid models to the control and the shifted curves and expressing the $\log \text{ED}_{50}$ of the shifted curve as the sum of the $\log \text{ED}_{50}$ of the control curve and the log of the potency ratio, which yielded an estimate of the potency ratio and its 95% confidence limits (see "EC₅₀ shift" equation in GraphPad Prism). Shifts of dose-response curves were considered statistically significant if the 95% confidence interval of the potency ratio did not include 1. Dose-response curves that attained a maximum between 50 and 80% training drug-appropriate responding were analyzed in the same manner, except that instead of fitting a sigmoid curve to all dose-response data, a straight line was fitted only to the data at doses with effects immediately below and above 50%, to estimate the ED_{50} and slope. Possible deviations from the regression models were examined by the replicates test implemented in GraphPad Prism. None of the dose-response data obtained in the present study deviated significantly from the regression models used.

Drug effects on response rate were examined by calculating for each dose the 95% confidence interval around the mean rate of responding (expressed as percentage of vehicle control). If this interval did not contain 100, the response rate was considered significantly different from control.

Drugs. Baclofen, diazepam, and (-)-nicotine hydrogen tartrate were purchased from Sigma-Aldrich (St. Louis, MO), and sulfated CCK-8 from Tocris Bioscience (Bristol, UK). GHB and cocaine hydrochloride were provided by the National Institute on Drug Abuse (Bethesda, MD). CGP7930 and rac-BHFF were synthesized by K. Cheng at the National Institute on Drug Abuse, and CGP35348 (3-aminopropyl(diethoxymethyl) phosphinic acid) was synthesized by J. Agyin at the University of Texas Health Science Center (San Antonio, TX). All compounds were dissolved in sterile water or saline, except diazepam, which was dissolved in sterile water with 70% castor oil ethoxylate(30) [Rhodia (Novecare), Charleston, SC] and 10% ethanol (by volume); CGP7930, which was suspended in sterile water with 0.6% methylcellulose; and rac-BHFF, which was suspended in a 4:1:15 mixture containing Cremophor EL (Kolliphor EL; Sigma-Aldrich), 1,2-propanediol and sterile water (Malherbe et al., 2008). All

compounds were injected intramuscularly in a volume of 0.1–1 ml, except CGP7930, rac-BHFF, and ethanol, which were administered orally by a feeding needle, in a volume of 0.5–5 ml. The dose of ethanol was manipulated by varying the volume of a 20% (v/v) solution of ethanol in sterile water. Doses are expressed as the form of the compound listed above.

Results

Because 178 mg/kg rac-BHFF had marked rate-decreasing effects in drug-naïve pigeons, for which little tolerance occurred within 20 sessions (unpublished data), the training dose was decreased to 100 mg/kg. At this dose, seven of the eight animals acquired the discrimination (median sessions to criterion 31, range 3–61, excluding sessions that were used to calculate criterion performance).

Rac-BHFF produced discriminative stimulus effects in a dose- and time-dependent manner, without affecting response rate (Fig. 1). Under test conditions, rac-BHFF dose-dependently increased responding on the rac-BHFF-appropriate key from 1% after vehicle to a maximum of 100% at the training dose of 100 mg/kg (upper left panel, solid circles), without significantly affecting the rate of responding (lower left panel, solid circles). A sigmoid curve fitted to the dose-response data yielded an ED_{50} value of 26 [95% confidence limits: 15–45] mg/kg rac-BHFF. The GABA_B antagonist CGP35348, at a dose (320 mg/kg) that attenuated the effects of baclofen and GHB (see below), did not significantly shift the dose-response curve of rac-BHFF to produce training drug-appropriate responding (dose ratio: 1.3; 95% confidence limits: 0.7–2.2). However, CGP35348 together with rac-BHFF significantly decreased the rate of responding. When the training dose of rac-BHFF (i.e., 100 mg/kg) was given at various times before the session, its discriminative stimulus effects were not apparent after 15 minutes, were maximal after 1 hour, and decreased to 30% after 8 hours (upper right panel), without significantly affecting response rate at any of the intervals (lower right panel).

Baclofen and GHB did not produce full rac-BHFF-like discriminative stimulus effects (Fig. 2). They increased rac-BHFF-appropriate responding to at most 64% (baclofen) and 57% (GHB) (upper panels, solid circles) when tested at doses up to and including those that significantly decreased response rate (lower panels, solid circles). CGP35348 antagonized the discriminative stimulus- and response rate-decreasing effects of baclofen and GHB (Fig. 2, compare open squares with solid circles). Rac-BHFF, at a dose that when given alone produced little training drug-appropriate responding (i.e., 10 mg/kg; Fig. 1, upper left panel), enhanced the discriminative stimulus effects of baclofen (Fig. 2, upper left panel, compare solid squares with solid circles) and did not enhance, but appeared to attenuate, the discriminative stimulus effects of GHB (Fig. 2, upper right panel, compare solid squares with solid circles). A dose ratio for the enhancement of baclofen by rac-BHFF was not calculated, because the difference between the slopes of the ascending part of the dose-response curves approached statistical significance ($P = 0.06$); the enhancement was evidenced by the ED_{50} values of the dose-response curves being significantly different ($P = 0.03$).

The discriminative stimulus effects of rac-BHFF were not mimicked by the GABA_B receptor-positive modulator CGP7930 (Fig. 3, upper left panel). CGP7930 produced at most 20% rac-BHFF-appropriate responding at a dose of 320 mg/kg; because of limited solubility, higher doses could not be tested.

Fig. 1. Effects of the GABA_B receptor positive modulator rac-BHFF in pigeons trained to discriminate between 100 mg/kg rac-BHFF and vehicle using a two-key food-reinforced procedure. The mean ($\pm$ S.E.M.; if not shown, S.E.M. values are contained by the symbol) percentage of responses on the rac-BHFF-appropriate key (top panels) and the mean ($\pm$ S.E.M.) rate of responding (expressed as percentage control; bottom panels) are plotted as a function of dose ($n = 4-6$ per dose) in the left panels and as a function of time after the administration of 100 mg/kg rac-BHFF in the right panels. rac-BHFF was tested alone (solid circles) and together with the GABA_B receptor antagonist CGP35348 (320 mg/kg; open squares). For dose-response data that attained the 80% level, nonlinear regression was used to obtain the best-fitting sigmoid curve; for other dose-response data, and for time-response data, the individual points were connected. Stars indicate mean rates of responding that were significantly ($P < 0.05$) different from control.

When given together with the training dose, CGP7930 attenuated the discriminative stimulus effects of rac-BHFF.

The CCK_A receptor agonist CCK-8 did not produce full rac-BHFF-like responding (Fig. 3, upper middle panel). CCK-8 increased rac-BHFF-appropriate responding to at most 49% at doses that decreased response rate. To examine possible CCK_A antagonist properties of rac-BHFF, it was administered together with a response rate-reducing dose of CCK-8. The training dose of rac-BHFF did not attenuate the effects of CCK-8 on response rate (Fig. 3, lower middle panel). Effects on rac-BHFF-appropriate responding were not calculated, because when rac-BHFF and CCK-8 were administered together, more than half of the animals responded at a rate less than 20% of the vehicle control rate.

The discriminative stimulus effects of rac-BHFF were not mimicked by the GABA_A receptor-positive modulator diazepam (Fig. 3, upper right panel). At the lowest dose tested (i.e., 1 mg/kg), diazepam significantly increased the rate of responding (Fig. 3, lower right panel). When tested at doses up to and including those that significantly decreased response rate, diazepam increased rac-BHFF-appropriate responding to at most 25%.

Ethanol, cocaine, and nicotine substituted at most partially for rac-BHFF (Fig. 4, upper panels). Rac-BHFF-appropriate responding was maximally increased to 52% by ethanol, to 20% by cocaine, and to 38% by nicotine when tested at doses

up to and including those that significantly decreased response rate (Fig. 4, lower panels).

Discussion

This study is the first to show that animals can be trained to discriminate a positive GABA_B receptor modulator from vehicle. Seven of the eight pigeons met the training criterion after a median number of sessions (i.e., 31; range 3–61), similar to that observed previously with 100 mg/kg GHB (median 32, range 19–75; Koek et al., 2004), suggesting that rac-BHFF is as discriminable as GHB. Conceivably, interactions with CCK_A receptors could mediate in the discriminative stimulus effects of rac-BHFF, because in a broad ligand binding screen, rac-BHFF was found to be inactive at all non-GABA_B targets tested except CCK_A receptors (Malherbe et al., 2008). However, the CCK_A receptor agonist CCK-8 substituted only partially for rac-BHFF, suggesting that rac-BHFF does not have marked CCK_A receptor agonist properties. Also, the response rate-decreasing effects of CCK-8 were not antagonized by rac-BHFF, suggesting that rac-BHFF does not have CCK_A receptor antagonist properties. Thus, it appears unlikely that interactions of rac-BHFF with CCK_A receptors play a major role in its discriminative stimulus effects.

To examine the role of GABA_B receptors in the discriminative stimulus effects of rac-BHFF, antagonism tests were

Fig. 2. Effects of the GABA_B receptor agonist baclofen (left) and GHB (right) in pigeons trained to discriminate between 100 mg/kg rac-BHFF and vehicle using a two-key food-reinforced procedure. Baclofen and GHB were tested alone (solid circles), together with the GABA_B receptor antagonist CGP35348 (320 mg/kg; open squares), and together with the GABA_B receptor positive modulator rac-BHFF (10 mg/kg; solid squares). Stars indicate mean rates of responding that were significantly ($P < 0.05$) different from control. See Fig. 1 for other details.

conducted with CGP35348, which acts at the GABA site of the GABA_B receptor. CGP35348 did not antagonize the effects of rac-BHFF examined in the present study. Previously, CGP35348 did not antagonize the effects of rac-BHFF in baclofen-discriminating pigeons (Koek et al., 2012). In the present study, lack of antagonism of rac-BHFF by CGP35348 was observed at dose of CGP35348 (i.e., 320 mg/kg) that blocked the response rate-decreasing effects of the GABA_B receptor agonists baclofen and GHB. Although CGP35348 generally acts as a silent antagonist, in vitro conditions have been reported in which it acts as a partial agonist (Urwylers et al., 2005; Hensler et al., 2012). Consistent with this, combining CGP35348 with rac-BHFF enhanced their effects on response rate. However, combining CGP35348 with rac-BHFF did not enhance the rac-BHFF-like discriminative stimulus effects (present study) or the baclofen-like discriminative stimulus effects (Koek et al., 2012) of CGP35348. Thus, the partial agonist properties of CGP35348 evidenced in vitro are not always apparent in vivo. Nevertheless, under conditions in which CGP35348 appears to have partial agonist properties, such as in the study by Urwyler et al. (2005), which showed that the maximum stimulation produced by CGP35348 in the presence of GABA_B receptor positive modulators was at most 40% of the stimulation observed with GABA, CGP35348 should still behave as an antagonist of GABA. Taken together, the lack of antagonism of the discriminative stimulus effects of rac-BHFF by CGP35348 suggests that these effects do not result from enhanced activity of endogenous GABA at GABA_B receptors. Instead, the discriminative stimulus effects of rac-BHFF

could involve receptor activation through other sites of the GABA_B receptor, consistent with in vitro evidence that rac-BHFF can activate GABA_B receptors in the absence of GABA (Malherbe et al., 2008; Hensler et al., 2012).

The discriminative stimulus effects of rac-BHFF were not mimicked by the GABA_B receptor agonist baclofen and GHB, consistent with the finding that rac-BHFF did not fully substitute for the training drug in pigeons trained to discriminate baclofen or GHB from vehicle (Koek et al., 2012). Together, these findings are further evidence that the effects of GABA_B receptor positive modulators are not identical to those of GABA_B receptor agonists. Interestingly, the GABA_B receptor positive modulator CGP7930 produced little rac-BHFF-appropriate responding when tested at doses that take into account that CGP7930 is about 3-fold less potent than rac-BHFF (Koek et al., 2012). Instead, CGP7930 attenuated the discriminative stimulus effects of rac-BHFF. This suggests the possibility that both compounds have agonist activity at the receptor sites mediating the discriminative stimulus effects of rac-BHFF, but that CGP7930 has less intrinsic efficacy at these sites than rac-BHFF. In addition to enhancing endogenous GABA at GABA_{B1} subunits, CGP7930 also directly activates GABA_{B2} subunits (Binet et al., 2004). Like CGP7930, rac-BHFF is able to activate GABA_B receptors in the absence of GABA, and is more efficacious than CGP7930 in stimulating GTP(γ)³⁵S binding in membranes of transfected Chinese hamster ovary cells expressing GABA_B receptors (Malherbe et al., 2008) and in brain using quantitative autoradiography (Hensler et al., 2012). Thus, although a role for non-GABA_B receptors in the discriminative stimulus effects of rac-BHFF

Fig. 3. Effects of the GABA_B receptor positive modulator CGP7930 (left panels), the CCK_A receptor agonist CCK-8 (middle panels), and the GABA_A receptor positive modulator diazepam (right panels) in pigeons trained to discriminate between 100 mg/kg rac-BHFF and vehicle using a two-key food-reinforced procedure. All compounds were tested alone (solid circles), and CGP7930 and CCK-8 were also tested together with the training dose of rac-BHFF (open circles). Stars indicate mean rates of responding that were significantly ($P < 0.05$) different from control. See Fig. 1 for other details.

cannot be ruled out, the present findings suggest the possibility that at the training dose examined here (i.e., 100 mg/kg) rac-BHFF produces its discriminative stimulus effects not by enhancing endogenous GABA at GABA_{B1} subunits, but by directly activating GABA_{B2} subunits.

Rac-BHFF, at a dose 10-fold lower than the training dose, enhanced the discriminative stimulus effects of baclofen. This

observation confirms and extends findings that rac-BHFF enhanced the discriminative stimulus effects of baclofen in pigeons trained to discriminate baclofen and in pigeons trained to discriminate GHB (Koek et al., 2012), and agrees with other evidence of the in vivo effectiveness of rac-BHFF as GABA_B receptor-positive modulator (Malherbe et al., 2008; Koek et al., 2010). Here, rac-BHFF enhanced the potency of

Fig. 4. Effects of ethanol (left), cocaine (center), and nicotine (right) in pigeons trained to discriminate between 100 mg/kg rac-BHFF and vehicle using a two-key food-reinforced procedure. Stars indicate mean rates of responding that were significantly ($P < 0.05$) different from control. See Fig. 1 for other details.

baclofen to substitute for rac-BHFF about 3-fold. However, rac-BHFF did not similarly enhance the potency of baclofen to decrease responding, because in the presence of rac-BHFF 10 mg/kg baclofen did not decrease responding, whereas 32 mg/kg baclofen given alone did. The observation that rac-BHFF did not similarly enhance different GABA_B receptor-mediated effects is consistent with previous findings in mice that rac-BHFF enhanced baclofen-induced loss of righting but not baclofen-induced hypothermia, whereas both effects of baclofen were antagonized by CGP35348 (Koek et al., 2010). Using quantitative autoradiography, GABA_B receptor-positive modulators have been found to act in a brain region-dependent manner (Hensler et al., 2012). Together, these observations suggest that rac-BHFF may preferentially modulate particular GABA_B receptor populations.

Rac-BHFF enhanced the discriminative-stimulus effects of baclofen, but not of GHB. This differential enhancement, which suggests that the baclofen-enhancing effects of rac-BHFF do not result from the drug-appropriate responding it produces when given alone, is consistent with previous findings in baclofen- and GHB-discriminating pigeons (Koek et al., 2012). Effects of allosteric modulators can be agonist-dependent (e.g., Kenakin, 2009). Thus, the present and the previous results could be explained by assuming that the same GABA_B receptors mediate effects of baclofen and GHB, and that rac-BHFF enhances effects at these receptors in an agonist-dependent manner. However, there is accumulating evidence that the GABA_B receptor mechanisms underlying the effects of baclofen and GHB are not identical. Behavioral effects of baclofen and GHB are differentially enhanced by *N*-methyl-D-aspartate antagonists (Koek et al., 2007a; Koek and France, 2008) and differentially antagonized by CGP35348 (Koek et al., 2004, 2007b, 2009, 2012; Carter et al., 2006). In these studies, CGP35348 completely antagonized baclofen and GHB, but antagonized baclofen more potently than GHB. Because the antagonism of GHB, like that of baclofen, was complete, it seems unlikely that receptors other than GABA_B receptors are involved in the effects of GHB examined in the aforementioned studies. Thus, the different potencies with which CGP35348 antagonized baclofen and GHB suggests that different GABA_B receptor populations mediate these effects of baclofen and GHB. Therefore, the differential enhancement of effects of baclofen and GHB by rac-BHFF may not involve agonist-dependent enhancement of a single population of GABA_B receptors, but may result from preferential modulation of different GABA_B receptor populations. Consistent with this latter possibility, *in vitro* studies show that CGP7930 enhances activity at GABA_B autoreceptors, but not at GABA_B heteroreceptors (Chen et al., 2006; Parker et al., 2008), and show that CGP7930 and rac-BHFF enhance GTP(γ)³⁵S binding stimulated by GABA_B receptor agonists in a brain region-dependent manner (Hensler et al., 2012). Such selective enhancement is further evidence of pharmacologically distinct GABA_B receptor populations.

GABA_B receptor positive modulators are thought to have advantages as potential medications for anxiety, depression, and drug addiction (Cryan et al., 2004; Frankowska et al., 2007; Jacobson and Cryan, 2008; Vlachou and Markou, 2010). They may have a better side-effect profile than GABA_B receptor agonists, based on the notion that selective enhancement of activated receptors has effects that differ from

indiscriminate activation of all receptors. Unlike baclofen, GABA_B receptor positive modulators do not appear to interfere with motor coordination (Cryan et al., 2004; Jacobson and Cryan, 2008), do not produce loss of righting (Carai et al., 2004; Malherbe et al., 2008; Koek et al., 2010) and, with the possible exception of CGP7930 (Koek et al., 2010), do not induce hypothermia (Jacobson and Cryan, 2008; Malherbe et al., 2008; Koek et al., 2010). rac-BHFF and CGP7930 did not mimic the discriminative-stimulus effects of baclofen and GHB (Koek et al., 2012) and in the present study, baclofen and GHB did not mimic the discriminative-stimulus effects of rac-BHFF. Also, the discriminative-stimulus effects of rac-BHFF were not mimicked by diazepam, suggesting that rac-BHFF can induce anxiolytic-like effects (Malherbe et al., 2008) without producing benzodiazepine-like discriminative-stimulus effects. The discriminative-stimulus effects of rac-BHFF were also not mimicked by alcohol, cocaine, and nicotine, whose self-administration has been reported to be attenuated by GABA_B receptor-positive modulators. This suggests that rac-BHFF suppresses alcohol self-administration (Maccioni et al., 2010b) without producing alcohol-like discriminative stimulus effects. It suggests also, together with the finding that CGP7930 did not produce cocaine-like discriminative stimulus effects (Filip et al., 2007), that positive modulation of GABA_B receptors does not produce discriminative stimulus effects similar to those of alcohol, cocaine, or nicotine.

In summary, the discriminative stimulus effects of the positive GABA_B receptor modulator rac-BHFF differ from those of GABA_B receptor agonists, of a benzodiazepine, and of drugs of abuse whose self-administration is reportedly attenuated by GABA_B receptor-positive modulators. The discriminative stimulus effects of rac-BHFF were not antagonized by CGP35348 but were attenuated by CGP7930, suggesting the possibility that at the training dose examined here (i.e., 100 mg/kg) rac-BHFF produces its discriminative stimulus effects not by enhancing endogenous GABA at GABA_{B1} subunits, but by directly activating GABA_{B2} subunits. At a dose 10-fold lower than the training dose, rac-BHFF enhanced the discriminative stimulus effects of baclofen. Thus, rac-BHFF acts *in vivo* as GABA_B receptor-positive modulator and, at higher doses, conceivably also as allosteric GABA_B receptor agonist. Together with previous evidence that the GABA_B receptor populations involved in the *in vivo* effects of baclofen and GHB are not identical, the findings that rac-BHFF enhanced effects of baclofen, but not of GHB, suggest these populations differ in their susceptibility to positive modulatory effects. Such differential susceptibility could allow for more selective therapeutic targeting of GABA_B receptors.

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Authorship Contributions

Participated in research design: Koek.

Contributed new reagents or analytic tools: Cheng, Rice.

Performed data analysis: Koek.

Wrote or contributed to the writing of the manuscript: Koek.

References

- Adams CL and Lawrence AJ (2007) CGP7930: a positive allosteric modulator of the GABA_B receptor. *CNS Drug Rev* 13:308–316.
- Addolorato G, Leggio L, Cardone S, Ferrulli A, and Gasbarrini G (2009) Role of the GABA(B) receptor system in alcoholism and stress: focus on clinical studies and treatment perspectives. *Alcohol* 43:559–563.

- Agabio R, Maccioni P, Carai MA, Gessa GL, Froestl W, and Colombo G (2012) The development of medications for alcohol-use disorders targeting the GABA_B receptor system. *Recent Patents CNS Drug Discov* 7:113–128.
- Bettler B, Kaupmann K, Mosbacher J, and Gassmann M (2004) Molecular structure and physiological functions of GABA_B receptors. *Physiol Rev* 84:835–867.
- Binet V, Brajon C, Le Corre L, Acher F, Pin JP, and Prézeau L (2004) The heptahelical domain of GABA_{B2} is activated directly by CGP7930, a positive allosteric modulator of the GABA_B receptor. *J Biol Chem* 279:29085–29091.
- Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M, Bonner TI, and Enna SJ (2002) International Union of Pharmacology. XXXIII. Mammalian γ -aminobutyric acid(B) receptors: structure and function. *Pharmacol Rev* 54:247–264.
- Bowery NG (2006) GABA_B receptor: a site of therapeutic benefit. *Curr Opin Pharmacol* 6:37–43.
- Calver AR, Davies CH, and Pangalos M (2002) GABA_B receptors: from monogamy to promiscuity. *Neurosignals* 11:299–314.
- Carai MA, Colombo G, Froestl W, and Gessa GL (2004) In vivo effectiveness of CGP7930, a positive allosteric modulator of the GABA_B receptor. *Eur J Pharmacol* 504:213–216.
- Carter LP, Chen W, Coop A, Koek W, and France CP (2006) Discriminative stimulus effects of GHB and GABA(B) agonists are differentially attenuated by CGP35348. *Eur J Pharmacol* 538:85–93.
- Castelli MP, Casu A, Casti P, Lobina C, Carai MAM, Colombo G, Solinas M, Giunta D, Mugnaini C, and Pasquini S, et al. (2011) Characterization of COR627 and COR628, two novel positive allosteric modulators of the GABA_B receptor. *J Pharmacol Exp Ther* 340:529–538.
- Chen Y, Menendez-Roche N, and Sher E (2006) Differential modulation by the GABA_B receptor allosteric potentiator 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)-phenol (CGP7930) of synaptic transmission in the rat hippocampal CA1 area. *J Pharmacol Exp Ther* 317:1170–1177.
- Cryan JF, Kelly PH, Chaperon F, Gentsch C, Mombereau C, Lingenhoehl K, Froestl W, Bettler B, Kaupmann K, and Spooner WP (2004) Behavioral characterization of the novel GABA_B receptor-positive modulator GS39783 (N,N'-dicyclopentyl-2-methylsulfonyl-5-nitro-pyrimidine-4,6-diamine): anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. *J Pharmacol Exp Ther* 310:952–963.
- Cryan JF and Kaupmann K (2005) Don't worry 'B' happy! a role for GABA_B receptors in anxiety and depression. *Trends Pharmacol Sci* 26:36–43.
- Filip M, Frankowska M, and Przeglasiński E (2007) Effects of GABA(B) receptor antagonist, agonists and allosteric positive modulator on the cocaine-induced self-administration and drug discrimination. *Eur J Pharmacol* 574:148–157.
- Frankowska M, Filip M, and Przeglasiński E (2007) Effects of GABA_B receptor ligands in animal tests of depression and anxiety. *Pharmacol Rep* 59:645–655.
- Gannon RL and Millan MJ (2011) Positive allosteric modulators at GABA_A receptors exert intrinsic actions and enhance the influence of baclofen on light-induced phase shifts of hamster circadian activity rhythms. *Pharmacol Biochem Behav* 99:712–717.
- Guery S, Floersheim P, Kaupmann K, and Froestl W (2007) Syntheses and optimization of new GS39783 analogues as positive allosteric modulators of GABA_B receptors. *Bioorg Med Chem Lett* 17:6206–6211.
- Hensler JG, Advani T, Burke TF, Cheng K, Rice KC, and Koek W (2012) GABA_B receptor-positive modulators: brain region-dependent effects. *J Pharmacol Exp Ther* 340:19–26.
- Jacobson LH and Cryan JF (2008) Evaluation of the anxiolytic-like profile of the GABA_B receptor positive modulator CGP7930 in rodents. *Neuropharmacology* 54: 854–862.
- Jensen AA and Spalding TA (2004) Allosteric modulation of G-protein coupled receptors. *Eur J Pharm Sci* 21:407–420.
- Kenakin T (2009) *A Pharmacology Primer: Theory, Application and Methods*, 3rd ed, Elsevier Academic Press, Burlington, MA.
- Kerr DI and Ong J (1995) GABA_B receptors. *Pharmacol Ther* 67:187–246.
- Koek W and France CP (2008) Cataleptic effects of gamma-hydroxybutyrate (GHB) and baclofen in mice: mediation by GABA(B) receptors, but differential enhancement by N-methyl-D-aspartate (NMDA) receptor antagonists. *Psychopharmacology (Berl)* 199:191–198.
- Koek W, Flores LR, Carter LP, Lamb RJ, Chen W, Wu H, Coop A, and France CP (2004) Discriminative stimulus effects of gamma-hydroxybutyrate in pigeons: role of diazepam-sensitive and -insensitive GABA(A) and GABA(B) receptors. *J Pharmacol Exp Ther* 308:904–911.
- Koek W, Khanal M, and France CP (2007a) Synergistic interactions between 'club drugs': gamma-hydroxybutyrate and phencyclidine enhance each other's discriminative stimulus effects. *Behav Pharmacol* 18:807–810.
- Koek W, Mercer SL, and Coop A (2007b) Cataleptic effects of gamma-hydroxybutyrate (GHB), its precursor gamma-butyrolactone (GBL), and GABA_B receptor agonists in mice: differential antagonism by the GABA_B receptor antagonist CGP35348. *Psychopharmacology (Berl)* 192:407–414.
- Koek W, Mercer SL, Coop A, and France CP (2009) Behavioral effects of gamma-hydroxybutyrate, its precursor gamma-butyrolactone, and GABA(B) receptor agonists: time course and differential antagonism by the GABA(B) receptor antagonist 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP35348). *J Pharmacol Exp Ther* 330:876–883.
- Koek W, France CP, Cheng K, and Rice KC (2010) GABA_B receptor-positive modulators: enhancement of GABA_B receptor agonist effects in vivo. *J Pharmacol Exp Ther* 335:163–171.
- Koek W, France CP, Cheng K, and Rice KC (2012) Effects of the GABA_B receptor-positive modulators CGP7930 and rac-BHFF in baclofen- and γ -hydroxybutyrate-discriminating pigeons. *J Pharmacol Exp Ther* 341:369–376.
- Liang JH, Chen F, Krstew E, Cowen MS, Carroll FY, Crawford D, Beart PM, and Lawrence AJ (2006) The GABA(B) receptor allosteric modulator CGP7930, like baclofen, reduces operant self-administration of ethanol in alcohol-preferring rats. *Neuropharmacology* 50:632–639.
- Maccioni P, Pes D, Orrù A, Froestl W, Gessa GL, Carai MA, and Colombo G (2007) Reducing effect of the positive allosteric modulator of the GABA(B) receptor, GS39783, on alcohol self-administration in alcohol-preferring rats. *Psychopharmacology (Berl)* 193:171–178.
- Maccioni P, Fantini N, Froestl W, Carai MA, Gessa GL, and Colombo G (2008) Specific reduction of alcohol's motivational properties by the positive allosteric modulator of the GABA_B receptor, GS39783—comparison with the effect of the GABA_B receptor direct agonist, baclofen. *Alcohol Clin Exp Res* 32:1558–1564.
- Maccioni P, Carai MA, Kaupmann K, Guery S, Froestl W, Leite-Morris KA, Gessa GL, and Colombo G (2009) Reduction of alcohol's reinforcing and motivational properties by the positive allosteric modulator of the GABA(B) receptor, BHF177, in alcohol-preferring rats. *Alcohol Clin Exp Res* 33:1749–1756.
- Maccioni P, Flore P, Carai MA, Mugnaini C, Pasquini S, Corelli F, Gessa GL, and Colombo G (2010a) Reduction by the positive allosteric modulator of the GABA (B) receptor, GS39783, of alcohol self-administration in Sardinian alcohol-preferring rats exposed to the "sipper" procedure. *Front Psychiatry* 1:20.
- Maccioni P, Thomas AW, Carai MA, Gessa GL, Malherbe P, and Colombo G (2010b) The positive allosteric modulator of the GABA(B) receptor, rac-BHFF, suppresses alcohol self-administration. *Drug Alcohol Depend* 109:96–103.
- Maccioni P, Zaru A, Loi B, Lobina C, Carai MA, Gessa GL, Capra A, Mugnaini C, Pasquini S, and Corelli F, et al. (2012) Comparison of the effect of the GABA(B) receptor agonist, baclofen, and the positive allosteric modulator of the GABA(B) receptor, GS39783, on alcohol self-administration in 3 different lines of alcohol-preferring rats. *Alcohol Clin Exp Res* 36:1748–1766.
- Malherbe P, Masciadri R, Norcross RD, Knoflach F, Kratzenstein C, Zenner MT, Kolb Y, Marcuz A, Huwyler J, and Nakagawa T, et al. (2008) Characterization of (R,S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one as a positive allosteric modulator of GABA_B receptors. *Br J Pharmacol* 154:797–811.
- Markou A, Paterson NE, and Semenova S (2004) Role of gamma-aminobutyric acid (GABA) and metabotropic glutamate receptors in nicotine reinforcement: potential pharmacotherapies for smoking cessation. *Ann NY Acad Sci* 1025:491–503.
- Mombereau C, Lhuillier L, Kaupmann K, and Cryan JF (2007) GABA_B receptor-positive modulation-induced blockade of the rewarding properties of nicotine is associated with a reduction in nucleus accumbens DeltaFosB accumulation. *J Pharmacol Exp Ther* 321:172–177.
- Orrù A, Fujani D, Cassina C, Conti M, Di Clemente A, and Cervo L (2012) Operant, oral alcoholic beer self-administration by C57BL/6J mice: effect of BHF177, a positive allosteric modulator of GABA(B) receptors. *Psychopharmacology (Berl)* 222:685–700.
- Orrù A, Lai P, Lobina C, Maccioni P, Piras P, Scanu L, Froestl W, Gessa GL, Carai MA, and Colombo G (2005) Reducing effect of the positive allosteric modulators of the GABA(B) receptor, CGP7930 and GS39783, on alcohol intake in alcohol-preferring rats. *Eur J Pharmacol* 525:105–111.
- Parker DA, Marino V, Ong J, Puspawati NM, and Prager RH (2008) The CGP7930 analogue 2,6-di-tert-butyl-4-(3-hydroxy-2-spiropropylpropyl)-phenol (BSPP) potentiates baclofen action at GABA(B) autoreceptors. *Clin Exp Pharmacol Physiol* 35:1113–1115.
- Paterson NE, Vlachou S, Guery S, Kaupmann K, Froestl W, and Markou A (2008) Positive modulation of GABA(B) receptors decreased nicotine self-administration and counteracted nicotine-induced enhancement of brain reward function in rats. *J Pharmacol Exp Ther* 326:306–314.
- Perdona E, Costantini VJ, Tessari M, Martinelli P, Carignani C, Valerio E, Mok MH, Zonzini L, Visentini F, and Gionotti M, et al. (2011) In vitro and in vivo characterization of the novel GABA_B receptor positive allosteric modulator, 2-1-[2-(4-chlorophenyl)-5-methylpyrazolo[1,5-a]pyrimidin-7-yl]-2-piperidinylethanol (CMPPE). *Neuropharmacology* 61:957–966.
- Pilc A and Nowak G (2005) GABAergic hypotheses of anxiety and depression: focus on GABA-B receptors. *Drugs Today (Bare)* 41:755–766.
- Pin J-P, Kniazeff J, Binet V, Liu J, Maurel D, Galvez T, Duthey B, Havlickova M, Blahos J, and Prézeau L, et al. (2004) Activation mechanism of the heterodimeric GABA(B) receptor. *Biochem Pharmacol* 68:1565–1572.
- Pinard A, Seddik R, and Bettler B (2010) GABA_B receptors: physiological functions and mechanisms of diversity. *Adv Pharmacol* 58:231–255.
- Urwiler S, Mosbacher J, Lingenhoehl K, Heid J, Hofstetter K, Froestl W, Bettler B, and Kaupmann K (2001) Positive allosteric modulation of native and recombinant gamma-aminobutyric acid(B) receptors by 2,6-Di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13501. *Mol Pharmacol* 60:963–971.
- Urwiler S, Pozza MF, Lingenhoehl K, Mosbacher J, Lampert C, Froestl W, Koller M, and Kaupmann K (2003) N,N'-Dicyclopentyl-2-methylsulfonyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: novel allosteric enhancers of gamma-aminobutyric acidB receptor function. *J Pharmacol Exp Ther* 307:322–330.
- Urwiler S, Gjonj T, Koljatic J, and Dupuis DS (2005) Mechanisms of allosteric modulation at GABA_B receptors by CGP7930 and GS39783: effects on affinities and efficacies of orthosteric ligands with distinct intrinsic properties. *Neuropharmacology* 48:343–353.
- Vlachou S, Guery S, Froestl W, Banerjee D, Benedict J, Finn MG, and Markou A (2011) Repeated administration of the GABA_B receptor positive modulator BHF177 decreased nicotine self-administration, and acute administration decreased cue-induced reinstatement of nicotine seeking in rats. *Psychopharmacology (Berl)* 215: 117–128.
- Vlachou S and Markou A (2010) GABA_B receptors in reward processes. *Adv Pharmacol* 58:315–371.
- Wierońska JM, Kusek M, Tokarski K, Wabno J, Froestl W, and Pilc A (2011) The GABA_B receptor agonist CGP44532 and the positive modulator GS39783 reverse some behavioural changes related to positive syndromes of psychosis in mice. *Br J Pharmacol* 163:1034–1047.

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