GABA<sub>B</sub> Receptor-Positive Modulators: Enhancement of GABA<sub>B</sub> Receptor Agonist Effects In Vivo

Wouter Koek, Charles P. France, Kejun Cheng, and Kenner C. Rice

Departments of Psychiatry and Pharmacology, University of Texas Health Science Center, San Antonio, Texas (W.K., C.P.F.); and Chemical Biology Research Branch, National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland (K.C., K.C.R.)

ABSTRACT

In vivo effects of GABA<sub>B</sub> receptor-positive modulators suggest that they have therapeutic potential for treating central nervous system disorders such as anxiety, depression, and drug abuse. Although these effects generally are thought to be mediated by positive modulation of GABA<sub>B</sub> receptors, such modulation has been examined primarily in vitro. The present study was aimed at further examining the in vivo positive modulatory properties of the GABA<sub>B</sub> receptor-positive modulators, 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol (CGP7930) and (R,S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one (rac-BHFF). Both compounds enhanced loss of righting induced by baclofen in mice. However, CGP7930 was less effective and rac-BHFF was less potent for enhancing loss of righting induced by γ-hydroxybutyrate (GHB), which, like baclofen, has GABA<sub>B</sub> receptor agonist properties. In contrast with baclofen- and GHB-induced loss of righting, the hypothermic effects of baclofen and GHB were not enhanced by rac-BHFF but were enhanced by CGP7930 only at doses that produced hypothermia when given alone. CGP7930-induced hypothermia was not attenuated by the GABA<sub>B</sub> receptor antagonist 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP35348), at doses that blocked baclofen-induced hypothermia, and was not increased by the nitric-oxide synthase inhibitor N<sup>ϩ</sup>-nitro-L-arginine methyl ester, at doses that increased the hypothermic effects of baclofen and GHB. The results provide evidence that CGP7930 and rac-BHFF act in vivo as positive modulators at GABA<sub>B</sub> receptors mediating loss of righting, but not at GABA<sub>B</sub> receptors mediating hypothermia. Conceivably, CGP7930, but not rac-BHFF, acts as an allosteric agonist at these latter receptors. Taken together, the results provide further evidence of pharmacologically distinct GABA<sub>B</sub> receptor subtypes, possibly allowing for a more selective therapeutic interference with the GABA<sub>B</sub> system.

Introduction

Allosteric modulators alter the activity of the endogenous ligand by binding to receptor sites that are different from the orthosteric site where the endogenous ligand acts (Christopoulos, 2002; Pin and Prézeau, 2007; Conn et al., 2009; Wang et al., 2009). There is currently much interest in allosteric modulators, because by discriminating between activated and nonactivated receptors, they may have a broader therapeutic window than ligands that indiscriminately alter the activity of all receptors. Allosteric modulators have been identified for various receptors, including GABA<sub>A</sub> and GABA<sub>B</sub> receptors. Because GABA<sub>B</sub> receptors are implicated in various psychiatric disorders (Kerr and Ong, 1995; Pile and Nowak, 2005; Frankowska et al., 2007), including drug dependence (Markou et al., 2004; Addolorato et al., 2009), modulation of these receptors could provide new treatments. Several novel compounds have been characterized as positive modulators of GABA<sub>B</sub> receptors in vitro [e.g., 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol (CGP7930) (Urwyler et al., 2001; Adams and Lawrence, 2007), N,N'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) (Urwyler et al., 2003), (R,S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one (rac-BHFF) (Mal-
baclofen (Carai et al., 2004; Frankowska et al., 2007; Jacobson and Cryan, 2008). In addition, positive modulators of GABA<sub>B</sub> receptors reduce self-administration of alcohol (Orr et al., 2005; Liang et al., 2006; Maccioni et al., 2008, 2009), cocaine (Filip et al., 2007), and nicotine (Momberou et al., 2007; Paterson et al., 2008). Although all of these effects generally are thought to be mediated by positive modulation of GABA<sub>B</sub> receptors, to date such modulation has been examined almost exclusively in vitro. Examination of positive modulating properties in vivo may help to further understand the mechanism by which these compounds exert their potential therapeutic effects.

CGP7930 and rac-BHFF have been reported to increase loss of righting in mice induced by a subthreshold dose of the GABA<sub>B</sub> receptor agonist baclofen (Carai et al., 2004; Malherbe et al., 2008). These findings, together with the observation that CGP7930 and rac-BHFF did not produce loss of righting when given alone, were taken as evidence that CGP7930 and rac-BHFF have positive modulating properties at GABA<sub>B</sub> receptors in vivo. To characterize these in vivo effects in more detail, the present study established dose-response curves for GABA<sub>B</sub> receptor agonists and used shifts of these curves to quantify the relative potency and effectiveness of the positive modulators.

GABA<sub>B</sub> receptors can be activated by baclofen, but also by other drugs, such as γ-hydroxybutyrate (GHB) (Mathivet et al., 1997). However, the GABA<sub>B</sub> receptor mechanisms underlying the effects of baclofen and GHB do not seem to be identical. First, the GABA<sub>B</sub> receptor antagonist 3-aminopro-pyl(diethoxymethyl)phosphinic acid (CGP35348) is often less potent in antagonizing the effects of GHB than the effects of baclofen (Koek et al., 2004, 2007b, 2009; Carter et al., 2006). Second, N-methyl-D-aspartate antagonists enhance the behavioral effects of GHB but not baclofen (Koek et al., 2007a; Koek and France, 2008). Preferential activity of GHB at GABA<sub>B</sub> autoreceptors on glutamatergic neurons and preferential activity of baclofen at GABA<sub>B</sub> autoreceptors on GABAergic neurons could conceivably account for some of these differences (Carter et al., 2009). Recent in vitro evidence suggests that CGP7930 and its analog 2,6-di-2-ethylpentyl-4-(3-hydroxy-2-spiropentylpropyl)-phenol (BSPP) selectively potentiate activity at GABA<sub>B</sub> autoreceptors, but not at heteroreceptors (Chen et al., 2006; Parker et al., 2008). This suggests the possibility, examined here, that CGP7930, and perhaps rac-BHFF, preferentially enhance in vivo effects of baclofen compared with those of GHB.

GABA<sub>B</sub> receptor activation not only produces loss of righting, but also other in vivo effects, such as hypothermia (Kaupmann et al., 2003). Hypothermia, which occurs at lower doses than loss of righting, probably is mediated by a population of GABA<sub>B</sub> receptors in a particular brain region (i.e., hypothalamus) that differs from the population of GABA<sub>B</sub> receptors involved in loss of righting. To examine whether these GABA<sub>B</sub> receptor populations differ in their susceptibility to enhancement by positive modulators, the present study characterized the effects of CGP7930 and rac-BHFF on baclofen- and GHB-induced hypothermia, which have not been studied before, and compared these effects with those on loss of righting, which to date have been studied at a single agonist dose.

Surprisingly, the present study found that CGP7930 produced hypothermia when given alone. To study the involvement of GABA<sub>B</sub> receptors in these effects, their antagonism by CGP35348 was examined, in comparison with antagonism of baclofen- and GHB-induced hypothermia. In rats, the NOS inhibitor N<sub>R</sub>-nitro-arginine methyl ester (L-NAME) enhances baclofen-induced hypothermia (Rawls et al., 2004, 2006). Our preliminary observations (unpublished) showed this enhancement to also occur in mice. Thus, the present study further examined the mechanisms underlying CGP7930-, baclofen-, and GHB-induced hypothermia by testing whether L-NAME enhanced the hypothermic effects of CGP7930 in a manner similar to that observed with baclofen and GHB.

Materials and Methods

Animals. A total of 160 adult male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME), weighing 26 to 37 g at the beginning of the experiments, were housed in groups of four in an environmentally controlled room (temperature, 24°C; relative humidity, 45%) under a 14/10-h light/dark cycle (light on at 7:00 AM) with food (rodent sterilizable diet; Harlan Teklad, Madison, WI) and water continuously available. The animals were maintained and the experiments were conducted in accordance with the Institutional Animal Care and Use Committee at the University of Texas Health Science Center, San Antonio, TX and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

Apparatus. Body temperature was measured with a digital thermometer (model BAT7001H) and a thermistor probe (model RET-3), both manufactured by Physitemp Instruments, Inc. (Clifton, NJ).

Procedure. Righting was assessed repeatedly in the same animals before and at different intervals (15–120 min) after drug administration. Loss of the righting reflex was scored as 1, otherwise the score was 0. Righting was considered absent when a mouse, after having been placed on its back, did not right itself within 15 s (i.e., the plantar surface of none of the feet made full contact with the floor).

Immediately before drug administration, baseline temperature was measured by inserting the lubricated probe 2 cm into the rectum. Thereafter, body temperature was recorded repeatedly in the same animals at different intervals (ranging from 15 to 120 min) after drug administration.

Drug tests were conducted in groups of mice (body temperature: n = 4–6; righting: n = 8) selected nonsystematically from the population of mice (body temperature: 80; righting: 80) available for the present studies. Individual mice were tested with drugs on average 10 times (range = 5 to 15), and no mouse received the same drug test twice. In an effort to control for the effects of repeated testing, drug doses were tested in a nonsystematic order with at least 1 week between tests.

Data Analysis. The effects of drugs given alone on loss of righting were examined by calculating the percentage of animals showing loss of righting for each dose and each postadministration interval. To further examine drugs given alone and drugs given together, for each dose (or dose combination) the loss of righting scores obtained from 0 to 120 min postadministration was summed for each animal (maximum total score = 6). Total scores were averaged across animals, and mean values ± S.E.M. were plotted as a function of dose. Dose-response data were analyzed by log-linear regression (Tallarida, 2000) of individual values by using Prism (GraphPad Software, Inc., San Diego, CA), with the following equation: effect = slope × log(dose) + intercept. Deviations from linearity were examined by the replicates test. P ratio tests in Prism were used to compare dose-response curves with respect to their slopes and inter-
Results

Baclofen and GHB produced loss of righting in a dose- and time-dependent manner (Fig. 1, left). Using the total of the loss of righting scores at each of the time points as a measure of drug effect, dose-response data were collected for baclofen and GHB, given alone and together with 320 mg/kg CGP35348 (Fig. 1, top right, filled and unfilled symbols, respectively). None of the dose-response data obtained in the present study deviated significantly from linearity, unless stated otherwise. The dose-response curves of GHB and baclofen had a common slope \([F_{(1,52)} = 2.89, P < 0.05]\) and significantly different ED\(_{50}\) values [baclofen: 34 (95% confidence limits: 28–41) mg/kg; GHB: 870 (750–2890) mg/kg; \(F_{(1,53)} = 67.53, P < 0.0001\)]. Thus, baclofen was almost 30-fold more potent than GHB in producing loss of righting. CGP35348 significantly increased the ED\(_{50}\) values for baclofen \([F_{(1,37)} = 37.63, P < 0.0001]\) and GHB \([F_{(1,45)} = 37.37, P < 0.0001]\) in a similar manner (3- and 3.3-fold, respectively). Unlike baclofen and GHB, the positive GABA\(_B\) receptor modulators CGP7930 and rac-BHFF did not produce any loss of righting (Fig. 1, bottom right).

CGP7930 dose-dependently enhanced both baclofen- and GHB-induced loss of righting, but did so in a different manner (Fig. 2, top). CGP7930 shifted the dose-response curve of baclofen to the left in a parallel manner. The dose-response curves of baclofen in the presence of CGP7930 had a common slope \([F_{(3,80)} = 1.16, P > 0.20]\) and significantly different ED\(_{50}\) values \([F_{(3,83)} = 14.82, P < 0.0001]\). At the highest dose of 320 mg/kg, CGP7930 decreased the ED\(_{50}\) of baclofen 2.8-fold, from 34 (28–41) to 12 (10–15) mg/kg. In contrast, CGP7930 shifted the dose-response curve of GHB in a non-parallel manner, as evidenced by significant different slope values for the dose-response curves of GHB in the presence of CGP7930 \([F_{(2,81)} = 3.54, P < 0.05]\). As a result, the extent to which CGP7930 increased the potency of GHB depended on the effect level, with an almost 2-fold shift at maximal effect levels, but no apparent shift at intermediate and minimal effect levels. CGP7930 enhanced the effects of baclofen in a different manner than the effects of GHB; however, in both cases CGP35348 attenuated the enhanced effects. A dose of 320 mg/kg CGP35348 significantly attenuated the effects of 320 mg/kg CGP7930 combined with 17.8 mg/kg baclofen \((t = 2.90, df = 14, P < 0.05)\) and with 1000 mg/kg GHB \((t = 2.51, df = 13, P < 0.05)\) (Fig. 2, top, downward triangles).

rac-BHFF dose-dependently enhanced baclofen- and GHB-induced loss of righting (Fig. 2, middle), as evidenced by parallel \([F_{(3,104)} = 2.25, P > 0.05]\), leftward shifts \([F_{(3,107)} = 2.25, P > 0.05]\).
7.49, \( P < 0.0001 \) of the dose-response curves. At the highest dose of 100 mg/kg, rac-BHFF decreased the ED_{50} of baclofen 1.9-fold, from 31 (27–35) to 16 (10–15) mg/kg and decreased the ED_{50} of GHB 1.6-fold, from 740 (640–850) to 460 (400–540) mg/kg. A dose of 320 mg/kg CGP35348 attenuated the enhanced effects of 100 mg/kg rac-BHFF combined with 17.8 mg/kg baclofen (\( t = 2.90, df = 14, P < 0.05 \)) and 320 mg/kg GHB (\( t = 2.51, df = 13, P < 0.05 \)) (Fig. 2, middle, upward triangles).

To characterize the enhancing properties of CGP7930 and rac-BHFF, ED_{50} values for baclofen and GHB in the presence of different doses of the modulators were used to calculate dose ratios for each modulator/agonist combination, except for the combination of CGP7930 and GHB, which did not yield parallel shifts. These ratios, shown in a Schild-like plot (Fig. 2, bottom), could be fitted with straight lines with a common slope \([F_{(2,3)} = 0.60, P > 0.20]\) not significantly different from 1 (i.e., 1.2 (0.92–1.5)) and significantly different intercepts \([F_{(2,5)} = 6.53, P < 0.05]\). These lines were used to estimate the dose of the modulator needed to shift the agonist dose-response curve 2-fold to the left, which was 220 mg/kg for CGP7930 combined with baclofen, 89 mg/kg for rac-BHFF combined with baclofen, and 120 mg/kg for rac-BHFF combined with GHB. Thus, rac-BHFF was 2.5-fold more potent than CGP7930 in enhancing the effects of baclofen and 1.3-fold more potent in enhancing baclofen than in enhancing GHB.

When given alone, baclofen, GHB, and CGP7930, but not rac-BHFF, decreased body temperature in a dose- and time-dependent manner (Fig. 3, left and center) [baclofen and GHB, dose: \( F_{(2,3)} = 9.48, P < 0.001 \); time: \( F_{(6,138)} = 19.42, P < 0.001 \); dose \( \times \) time: \( F_{(12,276)} = 8.28, P < 0.001 \); CGP7930, dose: \( F_{(5,21)} = 25.15, P < 0.001 \); time: \( F_{(6,126)} = 30.43, P < 0.001 \); dose \( \times \) time: \( F_{(30,126)} = 7.27, P < 0.001 \); rac-BHFF, dose: \( F_{(6,138)} = 1.15, P > 0.20 \); time: \( F_{(6,138)} = 21.46, P < 0.001 \); dose \( \times \) time: \( F_{(30,108)} = 1.91, P < 0.01 \)]. The lowest dose that produced statistically significant hypothermia was 3.2 mg/kg for baclofen, 178 mg/kg for GHB, and 100 mg/kg for CGP7930. Baclofen and GHB produced maximal hypothermia at 30 to 60 min after injection, and maximal effects of CGP7930 were apparent approximately 90 min after injection. The lowest body temperature observed with CGP7930 was 34 (0.3) °C, which was not significantly different \([t (8) = 1.96, P > 0.05]\) from that obtained with baclofen [32.6 (0.5)] or GHB [32.3 (1.2)]. None of the values obtained with rac-BHFF differed significantly from vehicle control. Using AUC as a measure of drug effect, dose-response data were collected for baclofen and GHB, given alone and together with 320 mg/kg CGP35348 (Fig. 3, top right, filled and unfilled symbols, respectively). The dose-response curves of GHB and baclofen had a common slope \([F_{(1,44)} = 0.41, P > 0.20]\) and significantly different ED_{50} values [baclofen: 4.7 (3.8–5.8) mg/kg; GHB: 250 (210–280) mg/kg; \( F_{(1,45)} = 72.41, P < 0.0001 \)]. Thus, baclofen was 53-fold more potent in producing hypothermia than GHB. CGP35348 significantly increased the ED_{50} value for baclofen 3.4-fold \([F_{(1,29)} = 45.02, P < 0.0001]\), but did not significantly alter the ED_{50} for GHB \([F_{(1,29)} = 0.01, P > 0.20]\). CGP7930 produced hypothermia \([ED_{50} = 100 (73–130)]\), such as baclofen and GHB, but its dose-response curve (Fig. 3, bottom right) was significantly shallower \([F_{(1,39)} = 19.78, P < 0.0001]\). CGP7930 also produced hypothermia when administered orally (data not shown), with an ED_{50} [i.e., 140 (104–201) mg/kg] that did not differ significantly \([F_{(1,35)} = 2.64, P > 0.10]\) from its ED_{50} after intraperitoneal administration [i.e., 100 (73–130) mg/kg], and with a common slope \([F_{(1,34)} = 2.52, P > 0.10]\).
contrast with CGP7930, rac-BHFF did not produce hypothermia, as evidenced by the slope of the regression line not being significantly different from zero \[ F(1,18) = 2.29, P = 0.10 \].

CGP7930 enhanced the hypothermic effects of baclofen and GHB (Fig. 4, top). At 320 mg/kg, CGP7930 significantly shifted the dose-response curves of baclofen \[ F(1,36) = 18.84, P < 0.001 \] and GHB \[ F(1,37) = 14.39, P < 0.001 \] to the left, in a parallel manner for GHB \[ F(1,36) = 2.08, P = 0.10 \] but not for baclofen \[ F(1,36) = 6.50, P = 0.05 \]. At lower doses of CGP7930 (i.e., 32 and 100 mg/kg), the dose-response curves of baclofen and GHB were similar to control and had a common slope \[ F(2,54) = 1.17, P > 0.20 \] and a common ED50 \[ F(2,56) = 2.26, P > 0.10 \]; GHB: \[ F(2,52) = 0.07, P > 0.20 \]. None of the dose-response curves deviated from linearity, except the dose-response curve of baclofen in the presence of 32 mg/kg CGP7930 (\( P < 0.001 \)).

When 100 mg/kg CGP7930 was given 60 min before baclofen (3.2, 5.6 mg/kg) or GHB (178, 320 mg/kg) (data not shown), the results were not significantly different \[ F(1,12) = 1.32, P > 0.20 \] from those obtained when CGP7930 was coadministered with baclofen or GHB. In contrast with CGP7930, rac-BHFF did not alter the hypothermic effects of baclofen and GHB (Fig. 4, bottom). The dose-response curves of baclofen and GHB obtained in the presence of different doses of rac-BHFF could be fitted with common slopes \[ F(2,40) = 0.07, P > 0.20 \] and common ED50 values \[ F(2,36) = 0.92, P > 0.20 \] for baclofen and GHB:

CGP7930-induced hypothermia was not significantly attenuated by the GABAB receptor antagonist CGP35348; the dose-response curves shown in Fig. 5, top left had a common slope \[ F(2,37) = 1.62, P > 0.20 \]. In contrast, CGP35348 shifted the dose-response curve for baclofen-induced hypothermia to the right \[ F(3,59) = 29.33, P < 0.001 \], in a parallel manner \[ F(3,56) = 1.62, P > 0.20 \] (Fig. 5, top center). These antagonist effects of CGP35348 were quantified by means of a Schild regression plot (not shown). The plot, with a slope [i.e., –0.54 (–0.77, –0.31)] significantly different from –1 (\( P < 0.05 \)), yielded an apparent pA2 value, as an empirical potency estimate, of 3.55
CGP35348 significantly decreased body temperature when injected in a nonparallel manner ($P < 0.20$) from the plot based on data obtained only at 60 min after the injection of the antagonist (data not shown). Doses of CGP35348 that antagonized the effects of baclofen failed to antagonize the effects of GHB (Fig. 5, top right). At the highest dose tested (i.e., 1000 mg/kg), CGP35348 significantly ($P < 0.05$) shifted the dose-response curve of GHB to the left in a nonparallel manner ($P = 0.05$). This dose of CGP35348 significantly decreased body temperature when given alone [dose: $F(4,25) = 2.51, P = 0.067$; time: $F(6,150) = 7.93, P < 0.001$; dose $\times$ time: $F(24,150) = 2.72, P < 0.001$] to a minimum value of 36.7 (S.E.M. = 0.35) °C, 45 min after injection (data not shown).

CGP7930-induced hypothermia was not significantly affected by l-NAME. The dose-response curves shown in Fig. 5, bottom left had a common slope [$F(2,105) = 1.87, P > 0.10$] and a common ED$_{50}$ [$F(2,107) = 1.75, P > 0.10$]. In contrast, l-NAME dose-dependently enhanced baclofen- and GHB-induced hypothermia (Fig. 5, bottom center and right) at doses that did not lower body temperature when given alone [data not shown; dose: $F(4,25) = 0.09, P > 0.20$; time: $F(6,150) = 11.42, P < 0.001$; dose $\times$ time: $F(24,150) = 1.67, P = 0.084$]. At 10 to 100 mg/kg, l-NAME significantly shifted the dose-response curves of baclofen [$F(3,79) = 11.10, P < 0.001$] and GHB [$F(3,71) = 7.58, P < 0.001$] to the left, in a parallel manner [baclofen: $F(3,76) = 0.44, P > 0.20$; GHB: $F(3,68) = 0.85, P > 0.20$]. l-NAME maximally shifted the baclofen dose-response curve 2.4-fold to the left at 32 and 100 mg/kg and maximally shifted the GHB dose-response curve 2.5-fold to the left at 100 mg/kg. Thus, l-NAME seemed to be more potent in enhancing the hypothermic effects of baclofen than those of GHB.

**Discussion**

The main finding of the present study is that the positive GABA$_B$ receptor modulators CGP7930 and rac-BHFF enhanced baclofen- and GHB-induced loss of righting, but not hypothermia. These results suggest that CGP7930 and rac-BHFF act in vivo as positive modulators at GABA$_B$ receptors involved in loss of righting, but not at GABA$_B$ receptors mediating hypothermic effects of GABA$_B$ receptor agonists. Thus, different GABA$_B$ receptor populations may differ in their susceptibility to positive modulatory effects, possibly allowing for a more selective therapeutic interference with the GABA$_B$ system.

Baclofen and GHB induced loss of righting in mice, consistent with previous observations (Carter et al., 2005), and antagonism by CGP35348 confirmed a role for GABA$_B$ receptors in these effects. CGP7930 and rac-BHFF enhanced baclofen- and GHB-induced loss of righting without producing loss of righting when given alone, in agreement with previous findings (Carai et al., 2004; Malherbe et al., 2008). Antagonism of the enhancement by CGP35348 indicated the involvement of GABA$_B$ receptors. In contrast with previous studies using a subthreshold dose of baclofen (Carai et al., 2004; Malherbe et al., 2008) and GHB (Carai et al., 2004), dose-response curves for baclofen and GHB were established. CGP7930 shifted the baclofen dose-response curve to the left in a parallel manner, but increased the slope of GHB dose-response curve. Conceivably, this may be related to different GABA$_B$ receptors mediating the effect of baclofen and GHB (i.e., GABA$_B$ autoreceptors and heteroreceptors, respectively; e.g., (Carter et al., 2009) and CGP7930 selectively potentiating autoreceptor activity (Chen et al., 2006). BHFF was 2.5-fold more potent than CGP7930 in enhancing baclofen and was less potent in enhancing GHB than baclofen. Taken together, these results suggest the possibility that GABA$_B$ receptor-positive modulators preferentially enhance in vivo effects of baclofen compared with those of GHB. If confirmed, this would be further evidence that the GABA$_B$ receptor mechanisms involved in the effects of baclofen and GHB are not identical.

Baclofen decreased body temperature, in agreement with previous observations (Gray et al., 1987; Jacobson and...
This has been taken to indicate that the GABAB receptor
baclofen (Koek et al., 2004, 2007b, 2009; Carter et al., 2006).
However, the regression plot was not different when, instead
of the 2-h AUC data, only data were used that were obtained
60 min after administration of CGP35348, when agonist ef-
effects of baclofen and antagonist effects of CGP35348 are
probably maximal (Koek et al., 2007b). This suggests that
other factors, such as a heterogeneous receptor population,
might be involved (Kenakin, 1997). An apparent pA2 value of
3.55 was calculated as an empirical estimate of the potency
with which CGP35348 antagonized the hypothermic effects
of baclofen. This potency estimate, which is equivalent to 70
mg/kg CGP35348, agrees with the previous finding that 100
mg/kg CGP35348 shifted the dose-response curve for the
cataleptic effects of baclofen 2-fold to the right (Koek et al.,
2007b). These results obtained with CGP35348, together
with the finding that baclofen does not produce hypothermia
in GABA_B receptor knockout mice (Quéva et al., 2003), indi-
cate that baclofen-induced hypothermia is mediated by
GABA_B receptors and, therefore, useful as an in vivo assay of
GABA_B receptor activation.

Like baclofen, GHB produces hypothermia by activating
GABA_B receptors, evidenced by its lack of hypothermic ef-
effects in GABA_B receptor knockout mice (Kaupmann et al.,
2003). Consistent with the involvement of GABA_B receptors,
CGP35348 antagonizes many of the effects of GHB, but is
often less potent in antagonizing the effects of GHB than
baclofen (Koek et al., 2004, 2007b; Carter et al., 2006).
This has been taken to indicate that the GABA_B receptor
mechanisms underlying the effects of GHB are not identical
to those of prototypical GABA_B agonists such as baclofen
(Koek et al., 2009). Consistent with this, in the present study
CGP35348 failed to antagonize GHB-induced hypothermia at
doses that antagonized baclofen-induced hypothermia. At
the highest dose, CGP35348 shifted the GHB dose-response
curve to the left and produced hypothermia when given
alone. Thus, its hypothermic effects seemed to limit its an-
tagomism of GHB-induced hypothermia. The finding that
high doses of CGP35348 produced hypothermia, like GABA_B
agonists, may be related to its partial agonist properties at
GABA_B receptors (Urwyler et al., 2005). The present results
differ from a report that CGP35348 antagonized hypothermia
produced by γ-butyrolactone, a prodrug of GHB, (Carai
et al., 2008) and a report that GHB-induced hypothermia was
anagonized by the GABA_B antagonist (2S)-(+)-5,5-dimethyl-
2-morpholineacetic acid (SCH50911) (van Nieuwenhuijzen
and McGregor, 2009). These differences could be related
in part to the use of γ-butyrolactone, which has pharmacoki-
etic properties different from GHB, and SCH50911, which
may have pharmacological properties different from
CGP35348. Be that as it may, these studies clearly indicate
an important role for GABA_B receptors in GHB-induced
hypothermia. The present results with CGP35348 suggest that
baclofen and GHB produce hypothermia through different
GABA_B receptor mechanisms.

rac-BHFF did not alter baclofen- and GHB-induced hypo-
thermia at doses that enhanced baclofen- and GHB-induced
loss of righting. CGP7930 enhanced baclofen- and GHB-in-
duced hypothermia, but only at a dose that produced hypo-
thermia when given alone. In a recent study in mice,
CGP7930 did not significantly affect body temperature ex-
ccept for a moderate increase of less than 1°C at the highest
dose tested, i.e., 300 mg/kg p.o. (Jacobson and Cryan, 2008).
In the present study, CGP7930 decreased body temperature
with similar potency when administered intraperitoneally
(ED_{50} = 100 mg/kg) and orally (ED_{50} = 140 mg/kg). Thus,
differences between the effects of CGP7930 in the study by
Jacobson and Cryan (2008) and in the present study do not
seem to be related to the route of administration, but may be
related to mouse strain (C57BL/6J in the present study; OF1
in the prior study), because strains can differ in their sensi-
tivity to the hypothermic effects of baclofen (Jacobson and
Cryan, 2005). In the present study, the hypothermic effects
of CGP7930 were not altered by doses of CGP35348 that antag-
onized the hypothermic effects of baclofen. This suggests that
the hypothermic effects of CGP7930 do not result from en-
hancement of the effects of endogenous GABA at GABA_B
receptors. Instead, they could result from activation of
the GABA_B receptor by CGP7930 through a site different from
the site where GABA acts, suggested by the observation that
CGP7930 can directly activate the receptor (Binet et al.,
2004). Thus, although the present results do not provide
evidence that CGP7930 acts in vivo as a selective GABA_B receptor mediator of hypothermia, they are consis-
tent with the possibility that CGP7930 behaves as an allo-
steric agonist at these receptors.

The NOS inhibitor L-NAME dose-dependently enhanced
the hypothermic effects of baclofen and GHB in mice without
affecting body temperature when given alone. These results
are in agreement with previous observations on the enhance-
ment of baclofen-induced hypothermia by L-NAME in rats
(Rawls et al., 2004, 2006) and extend these results to mice
and hypothermia induced by GHB. The mechanism underly-
ing these synergistic effects has been suggested to involve
GABA_B receptor-mediated suppression of NO synthesis in
brain regions that regulate body temperature, with NO pro-
duction diminished further by L-NAME (Rawls et al., 2006).
This mechanism may be involved not only in the hypothermic
synergy of baclofen and L-NAME, but also in that of GHB
and L-NAME, because L-NAME enhanced the hypothermic effects
of baclofen and GHB in a similar manner. In contrast, the
hypothermic effects of baclofen and GHB were differentially
agonized by CGP35348. These results suggest the possi-
bility that baclofen and GHB produce hypothermia through
different GABA_B receptor populations that are similarly cou-
pled to NO production.

CGP7930-induced hypothermia was not affected by L-
NAME, suggesting CGP7930 acts through GABA_B receptors
not coupled to NO production. Although CGP7930 seems to
be a selective GABA_B receptor modulator (Urwyler et al.,
2001), the possible involvement of other non-GABA_B recep-
tors in its hypothermic effects can at present not be ruled out.
Be that as it may, the finding that rac-BHFF did not produce
hypothermic effects suggests rac-BHFF to be a more selective
in vivo GABA_B receptor modulator than CGP7930.

Taken together, the present results show that CGP7930
and rac-BHFF enhance baclofen- and GHB-induced loss of
righting, but not hypothermia. Effects of GABA_B agonists on
motor coordination and body temperature probably are me-
diated by different GABA_B receptor populations, in brain
regions such as motor cortex and cerebellum and in the
hypothalamus, respectively. There is evidence that the spe-
cific GABA<sub>B</sub> receptor populations that mediate ataxia and hypothermia are under differential genetic control (Jacobson and Cryan, 2005). Based on the results of the present experiments, it is tempting to speculate that the pharmacological properties of these receptor populations differ as well. Differential enhancement of GABA<sub>B</sub> receptor populations by positive modulators has been shown in vitro: presynaptic GABA<sub>B</sub> autoreceptors seem to be sensitive to CGP7930 and the specific GABA<sub>B</sub> receptor populations that mediate ataxia and specific GABA<sub>B</sub> receptor populations that mediate hypothermia. If different GABA<sub>B</sub> receptor populations differ in their susceptibility to positive modulatory effects, this could allow a more selective therapeutic interference with the GABA<sub>B</sub> system.

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References


**Address correspondence to:** Dr. Wouter Koek, Departments of Psychiatry and Pharmacology, University of Texas Health Science Center, 7703 Floyd Curl Drive, Mail Code 7792, San Antonio, TX 78229-3900. E-mail: koek@uthscsa.edu