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

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**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-<i>tert</i>-butyl-4-(3-hydroxy-2,2-dimethylpropyl) phenol (CGP7930) and (R,S)-5,7-di-<i>tert</i>-butyl-3-hydroxy-3-trifluoromethyl-3<i>H</i>-benzofuran-2-one (rac-BHFF). Both compounds enhanced loss of rigidity induced by baclofen in mice. However, CGP7930 was less effective and rac-BHFF was less potent for enhancing loss of rigidity 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 rigidity, 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>ω</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.

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**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 Prêteau, 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-<i>tert</i>-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-<i>tert</i>-butyl-3-hydroxy-3-trifluoromethyl-3<i>H</i>-benzofuran-2-one (rac-BHFF) (Mal-
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>ω</sub>-nitro-L-arginine methyl ester (l-NNAME) 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-NNAME 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 (Tilalida, 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. F ratio tests in Prism were used to compare dose-response curves with respect to their slopes and inter-
cept. For example, a nonsignificant $F$ ratio for slopes and a significant $F$ ratio for intercepts show that dose-response curves are parallel but occupy different positions on the dose axis. Effects of 320 mg/kg CGP35348 on loss of righting produced by CGP7930 or rac-BHFF together with baclofen or GHB were analyzed by Student’s $t$ test.

Effects of drugs on body temperature when given alone were analyzed by two-way analysis of variance with dose as between-subjects factor and time as within-subjects factor, followed by Dunnett’s test (NCSS 2007 program; NCSS Statistical Software, Kaysville, UT). To examine the effects of drugs when given together, for each dose combination the area under the body temperature time curve (AUC) from 0 to 120 min postadministration was calculated for each animal by using Prism version 5.02 for Windows (GraphPad Software, Inc.), which used the trapezoid rule to calculate the area below the baseline value obtained immediately before drug administration. AUC values were averaged across animals, and the mean values ± S.E.M. were plotted as a function of dose. Dose-response data were analyzed by log-linear regression as described for loss of righting. In addition, pretreatment interval effects were analyzed by two-way analysis of variance with interval and dose for loss of righting. In addition, pretreatment interval effects were analyzed by two-way analysis of variance with dose as between-subjects factor and time as within-subjects factor, followed by Dunnett’s test.

**Fig. 1.** Effects of the GABAB receptor agonist baclofen, GHB, and the GABAB receptor-positive modulators CGP7930 and rac-BHFF on righting in C57BL/6J mice ($n$ = 8 per dose). Left, symbols represent the percentage of animals showing loss of righting at different times after intraperitoneal administration of baclofen or GHB. Right, symbols represent mean ± S.E.M righting scores, totaled for each animal across the six different times after injection. Open symbols in the upper right represent data obtained when baclofen and GHB were given together with 320 mg/kg of the GABAB receptor antagonist CGP35348.

**Drugs.** Baclofen and L-NAME HCl were purchased from Sigma-Aldrich (St. Louis, MO). GHB was 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 (Bethesda, MD), and CGP35348 was synthesized by J. Agin at the University of Texas Health Science Center (San Antonio, TX). All compounds were dissolved in sterile water, except CGP7930, which was suspended in sterile water with 0.6% methylcellulose, and rac-BHFF, which was suspended in a 4:1:13 mixture containing Cremophor EL, 1,2-propanediol, and distilled water (Malherbe et al., 2008). All compounds were injected intraperitoneally, except CGP7930, which was also administered orally, in a volume of 5 to 20 ml/kg. When more than one drug was administered, they were administered at the same time (except where noted). Doses are expressed as the form of the compound listed above.

**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 GABAB 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 combined 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 [$F_{(3,107)} =$...
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: $F_{(6,128)} = 19.42$, $P < 0.001$; dose $\times$ time: $F_{(24,192)} = 8.28$, $P < 0.001$; 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: $F_{(6,180)} = 1.15$, $P > 0.20$; time: $F_{(6,108)} = 21.46$, $P < 0.001$; 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 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, and 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 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 \( F(2,54) = 1.17, P = 0.20; \) GHB: \( F(2,50) = 0.90, P = 0.05 \) and a common \( ED_{50} \) \( F(2,56) = 2.26, P = 0.10 \; \) 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 \( F(1,12) = 1.32, P > 0.20 \). 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 (Fig. 4, top). 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 \( F(2,40) = 0.07, P = 0.20 \); GHB: \( F(2,36) = 0.92, P = 0.20 \), respectively) and common \( ED_{50} \) values \( F(2,42) = 0.13, P = 0.20; \) GHB: \( F(2,38) = 0.87, P = 0.20 \).

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 \( F(2,37) = 29.33, P < 0.001 \), in a parallel manner \( F(2,37) = 0.24, P = 0.20 \) and a common \( ED_{50} \) \( F(2,39) = 1.62, P > 0.20 \). In contrast, CGP35348 shifted the dose-response curve for baclofen-induced hypothermia to the right \( F(3,50) = 29.33, P < 0.001 \), in a parallel manner \( F(3,50) = 0.62, P > 0.20 \) (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 \( pA_2 \) value, as an empirical potency estimate, of 3.55.
given alone significantly decreased body temperature when the left in a nonparallel manner (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 × 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 t-NAME. The dose-response curves shown in Fig. 5, bottom left had a common ED50 [F(2,105) = 1.87, P > 0.10] and a common ED50 [F(2,107) = 1.75, P > 0.10]. In contrast, t-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 × time: F(24,150) = 1.67, P = 0.034]. At 10 to 100 mg/kg, t-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(1,76) = 0.44, P > 0.20; GHB: F(3,68) = 0.85, P > 0.20]. t-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, t-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 is often less potent in antagonizing the effects of GHB than CGP35348 antagonizes many of the effects of GHB, but is consistent with the involvement of GABAB receptors, evidenced by its lack of hypothermic effects in GABA<sub>B</sub> receptor knockout mice (Urwyler et al., 2005). The present results with high doses of CGP35348 produced hypothermia, like GABAB receptor-mediated suppression of NO synthesis in brain regions such as motor cortex and cerebellum and in the hypothalamus, respectively. There is evidence that the selective GABA<sub>B</sub> receptor modulator CGP7930 did not significantly affect body temperature except 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<sub>50</sub> = 100 mg/kg) and orally (ED<sub>50</sub> = 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 sensitivity 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 antagonized the hypothermic effects of baclofen. This suggests that the hypothermic effects of CGP7930 do not result from enhancement of the effects of endogenous GABA at GABA<sub>B</sub> receptors. Instead, they could result from activation of the GABA<sub>B</sub> 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 positive modulator at GABA<sub>B</sub> receptors mediating hypothermia, they are consistent with the possibility that CGP7930 behaves as an allosteric 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 enhancement 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 underlying these synergistic effects has been suggested to involve GABA<sub>B</sub> receptor-mediated suppression of NO synthesis in brain regions that regulate body temperature, with NO production 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 antagonized by CGP35348. These results suggest the possibility that baclofen and GHB produce hypothermia through different GABA<sub>B</sub> receptor populations that are similarly coupled to NO production.

CGP7930-induced hypothermia was not affected by L-NAME, suggesting CGP7930 acts through GABA<sub>B</sub> receptors not coupled to NO production. Although CGP7930 seems to be a selective GABA<sub>B</sub> receptor modulator (Urwyler et al., 2001), the possible involvement of other non-GABA<sub>B</sub> receptors 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<sub>B</sub> 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<sub>B</sub> agonists on motor coordination and body temperature probably are mediated by different GABA<sub>B</sub> receptor populations, in brain regions such as motor cortex and cerebellum and in the hypothalamus, respectively. There is evidence that the spec-
cific GABA<sub>B</sub> receptor populations that mediate ataxia and hyothermia 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 GCG7930 analog BSPP, whereas presynaptic GABA<sub>B</sub> heteroceptors are not (Chen et al., 2006; Parker et al., 2008). Conceivably, such differential enhancement could also be involved in the in vivo effects of CGP7930 and rac-BHFF reported here.

In summary, the positive GABA<sub>B</sub> receptor modulators CGP7930 and rac-BHFF enhanced baclofen- and GHB-induced loss of righting, but not hyothermia, suggesting that they act in vivo as positive modulators at some, but not all, GABA<sub>B</sub> receptors. 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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