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Discriminative stimulus effects of GHB and $GABA_B$ agonists are differentially attenuated by CGP35348

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Abstract

The aim of this study was to examine the possible heterogeneity of mechanisms that contribute to the discriminative stimulus and rate-decreasing effects of γ-hydroxybutyrate (GHB). Dose effect curves were determined for GHB and two $GABA_B$ receptor agonists (baclofen and SKF97541) alone and together with the selective $GABA_B$ receptor antagonist CGP35348 in rats discriminating GHB. In a second study, GHB and SKF97541 dose effect curves were determined alone and together with baclofen. CGP35348 attenuated the discriminative stimulus and rate-decreasing effects of SKF97541 and baclofen to a greater extent than those of GHB. In the second study, baclofen enhanced the discriminative stimulus and rate-decreasing effects of GHB and SKF97541; however, the GHB dose effect curve was not shifted in a parallel manner. Taken together, these data suggest that multiple mechanisms, possibly including GHB receptors and $GABA_B$ receptor subtypes, are involved in the discriminative stimulus and rate-decreasing effects of GHB.

Keywords: Gamma-hydroxybutyrate; GHB; Baclofen; SKF97541; GABA; Xyrem; Sodium oxybate; Drug discrimination

1. Introduction

Gamma-hydroxybutyrate (GHB) is an endogenous molecule and putative neurotransmitter (Maitre, 1997) that is involved in the regulation of sleep/wake cycles (Mamelak et al., 1977; Van Cauter et al., 1997) and energy metabolism (Boyd et al., 1992; Mamelak, 1997; Ottani et al., 2004). Although its precise mechanism of action is unknown, GHB is used in the US and Europe to treat narcolepsy (Tunnicliff and Raess, 2002; Fuller and Hornfeldt, 2003) and alcoholism (Addolorato et al., 1996; Poldrugo and Addolorato, 1999). GHB [sodium oxybate, marketed as Xyrem® (Schedule III formulation)] increases slow wave (stages III and IV) sleep and the latency to REM sleep, which is thought to be responsible for its therapeutic effect of decreasing daytime episodes of cataplexy in patients with narcolepsy (Mamelak et al., 1986). In addition to its novel therapeutic indications (alcoholism and narcolepsy), GHB is also used recreationally in many countries for its reputed anabolic and euphorigenic effects (Bellis et al., 2003; Caldicott et al., 2004; Couper et al., 2004; Rodgers et al., 2004; Gonzalez and Nutt, 2005). The recreational use of GHB and its alleged involvement in drug-facilitated sexual assaults (ElSohly and Salamone, 1999; Schwartz et al., 2000) led to the placement of GHB into Schedule I of the Controlled Substances Act in 2000 (US Federal Register, 2000).

GHB binds to $GABA_B$ receptors (Mathivet et al., 1997; Lingenhoehl et al., 1999) and specific GHB receptors (Benavides et al., 1982; Snead and Liu, 1984) in brain that are thought to be important for the behavioral effects of GHB. Studies examining the mechanism of action of GHB in vivo (including studies in rats, pigeons and baboons) have shown that $GABA_B$ receptors are important for many of the effects of GHB such as discriminative stimulus effects (e.g., Winter, 1981; Colombo et al., 1998; Carter et al., 2003, 2004a; Koek et

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al., 2004), increased EEG spike wave discharges (Bernasconi et al., 1992; Snead, 1996), loss of righting (Carai et al., 2001), hypothermia (Queva et al., 2003), hypolocomotion (Kaupmann et al., 2003), increased mean arterial pressure, tachycardia and renal sympathetic nerve activity (Hicks et al., 2004), lethality (Carai et al., 2004), catalepsy (Carter et al., 2005b), ataxia (Goodwin et al., 2005), decreased intestinal motility (Carai et al., 2002) and decreased operant responding (Goodwin et al., 2005). Alternatively, a role for GHB receptors in the effects of GHB is less clear, in part, because the study of these receptors has been limited by the lack of selective GHB receptor ligands; however, there is evidence that GHB receptors are involved in some of the behavioral effects of GHB (e.g., Hechler et al., 1993; Carter et al., 2005b).

Despite the similar behavioral profile of baclofen and GHB, baclofen did not increase the latency to REM sleep in rats (Ulloor et al., 2004) or (decerebrate) cats (Takakusaki et al., 2004) and it is not used recreationally. Thus, it is possible that mechanisms of action of GHB, in addition to agonism at $GABA_B$ receptors (e.g., activity at GHB receptors), contribute to the differences between GHB and baclofen. Selective GHB receptor ligands have been shown to produce some GHB-like effects that are not blocked by the selective $GABA_B$ receptor antagonist 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP35348), suggesting that GHB receptors mediate some of the effects of GHB in vivo (Gobaille et al., 2002; Castelli et al., 2003; Carter et al., 2005b). Similarly, there is evidence for $GABA_B$ receptor subtypes (Bonanno and Raiteri, 1992), which might also contribute to some of the differences between GHB and baclofen.

The aim of this study was to examine the possible heterogeneity of mechanisms that contribute to the discriminative stimulus and rate-decreasing effects of GHB by comparing the effects of GHB with those of well-characterized $GABA_B$ receptor agonists. Previous studies have shown similar substitution profiles for different compounds in rats trained to discriminate 200 mg/kg GHB or 3.2 mg/kg baclofen (i.e., the training stimulus was pharmacologically selective and qualitatively similar in each procedure; Carter et al., 2003, 2004a). In the current studies, dose effect curves were determined for GHB and the $GABA_B$ receptor agonists, baclofen and SKF97541, given alone, and together with the $GABA_B$ receptor antagonist CGP35348, in rats discriminating 200 mg/kg GHB from saline. If the effects of these compounds are mediated by the same CGP35348-sensitive $GABA_B$ receptors, then the magnitude of antagonism of these compounds should be the same. In a second study, GHB and SKF97541 were studied together with baclofen to examine whether the effects of GHB and SKF97541 were differentially enhanced by baclofen.

2. Materials and methods

2.1. Subjects

Adult male Sprague–Dawley rats (Harlan, Indianapolis, IN) were housed individually on a 14:10-h light/dark cycle (experiments conducted during the light period) with free access to water in the home cage. Rats ($N=7$) were maintained at 340 to 360 g by providing rodent chow (Rodent sterilizable diet, Harlan Teklad, Madison, WI) in the home cage after daily sessions. The amount of chow that was provided to each animal (5–16 g) was adjusted daily in order to maintain body weights at or near 350 g. The animals in this study had previously been trained to discriminate 200 mg/kg GHB (i.p.) from saline; among the rats trained and used in prior studies (Carter et al., 2003) and this study, a median of 36 (range: 16–72) sessions was required to satisfy testing criteria. All animals were maintained and 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 1996 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences).

2.2. Procedures

Experiments were conducted in commercially available chambers (Model #ENV-008CT; MED Associates Inc., St. Albans, VT) located within sound-attenuating, ventilated enclosures (Model #ENV-022M; MED Associates Inc.) that have been described in detail elsewhere (Carter et al., 2003). Data were collected using MED-PC IV software and interface (MED Associates Inc.). Rats were trained to discriminate 200 mg/kg GHB i.p. from saline as described previously (Carter et al., 2003). In the current studies, the daily session was changed from one 30-min cycle (15-min time out period, followed by a 15-min response period) to multiple (between one and six) 20-min cycles, each consisting of a 15-min time out period, followed by a 5-min response period. During the time out period, the chamber was dark and lever presses had no programmed consequence, whereas during the response period the stimulus lights above both levers were illuminated and 10 consecutive responses (fixed ratio 10) on the correct lever resulted in the delivery of a food pellet (45 mg; Research Diets, New Brunswick, NJ). A response on the incorrect lever reset the fixed ratio requirement on the correct lever. The response period ended after 5 min or the delivery of 10 food pellets, whichever occurred first. The minimum and maximum duration of a test session was 20 (one cycle) and 120 (six cycles) min, respectively.

Under training conditions, an injection of saline or 200 mg/kg GHB (i.p.), or a sham injection (pressure applied to the abdomen with a capped needle) was given at the start of each cycle (15 min prior to the response period). Training sessions generally consisted of two to six cycles. On saline training days, animals received an injection of saline prior to the first cycle and a sham injection at the beginning of each subsequent cycle; only responding on the saline-associated lever resulted in food delivery during these cycles. On drug training days, animals received 200 mg/kg GHB in one of the cycles (typically the first or third cycle) followed by a sham injection at the start of a subsequent cycle; only responding on the GHB-associated lever resulted in food delivery during both of these cycles. Sessions

were conducted 5–7 days a week, the order of training sessions was generally double alternation (e.g., saline, saline, drug, drug), and the scheduling of saline and drug training sessions was independent for individual animals.

All rats had satisfied the following criteria under single cycle training conditions before this study: at least 90% of the total responses on the correct lever and fewer than 10 responses on the incorrect lever before delivery of the first food pellet for five consecutive sessions, or six out of seven sessions (Carter et al., 2003). After training the animals to respond during multiple cycles, they were required to satisfy the same criteria for all cycles in five consecutive sessions, or six out of seven sessions and for all cycles during at least one training day on which both saline and GHB were administered (i.e., animals responded on the saline-and GHB-paired levers in different cycles within a single session). All subjects acquired the multiple cycle discrimination and were subsequently required to satisfy the training criteria for at least one saline and one drug training session in two of the three sessions before a test (including the day immediately before the test); thus, training sessions occurred more frequently than test sessions regardless of performance in the training session. When individual animals did not satisfy training criteria additional training sessions were scheduled until the aforementioned criteria were satisfied.

Test sessions were identical to training sessions, except that 10 consecutive responses on either lever resulted in the delivery of food. For test sessions, saline, CGP35348 (antagonism studies) or baclofen (agonist combination studies) was given at the start of the first cycle, followed by cumulative doses of GHB, baclofen or SKF97541 at the start of subsequent cycles. For individual animals, doses of GHB, baclofen and SKF97541 were studied up to a dose that occasioned greater than 90% GHB-appropriate responding or resulted in fewer than 10 responses in a single cycle. The order of treatment with different doses of CGP35348 or baclofen in the first cycle was unsystematic.

2.3. Data analyses

Test sessions generated data on the following two variables: (1) the percentage of responses on the GHB-appropriate lever, calculated by dividing the number of responses on the GHB-appropriate lever by the total number of responses on both levers and multiplying the result by 100; and (2) the response rate, calculated by dividing the total number of responses made on both levers by the duration of the response period in seconds. The mean percentage of responses on the GHB lever ± 1 S.E.M. and the mean rate of responding ± 1 S.E.M. during test sessions were plotted as a function of dose. When an animal responded at a rate less than 20% of its vehicle control rate (i.e., average rate during the five most recent saline training sessions), discrimination data from that test were not included in the average. The mean percentage of responses on the GHB lever was calculated only when that value was based on at least half of the animals tested.

Differences among the dose effect curves for drug-appropriate responding and rate of responding were analyzed by simultaneously fitting straight lines to the individual dose–response data by means of GraphPad Prism version 4.02 for Windows (GraphPad Software, San Diego, CA), using the following equation: effect = slope * log(dose) + intercept. For drug-appropriate responding data, straight lines were fitted to the linear portion of the dose effect curves that crossed the 50% level of responding on the GHB lever; the linear portion comprised data points at doses with effects immediately below and above 50%, and included not more than one dose with ≥ 75% effect and not more than one dose with ≤ 25% effect (Koek et al., 2005). Dose effect curves that failed to cross the 50% level (i.e., 320 mg/kg CGP35348 + SKF97541 and 320 mg/kg CGP35348 + baclofen) were not analyzed by linear curve fitting.

Response rate data were transformed to a percent of control and straight lines were fitted to the linear portion of the dose effect curves that crossed the 50% level of responding, using the same criteria to define the linear portion of the dose effect curve as described above. Dose effect curves that failed to cross the 50% level (i.e., 178 mg/kg CGP35348 + baclofen and 560 mg/kg CGP35348 + SKF97541) were not analyzed by linear curve fitting. To analyze the dose effect curves for the rate of responding for GHB alone and together with 178 and 320 mg/kg CGP35348, and for the control GHB and SKF97541 dose effect curves when studied together with baclofen, it was assumed that the next (quarter log-unit) larger dose would eliminate responding in all animals. This was done to estimate the minimum magnitude of shift in the dose effect curves for GHB and SKF97541.

Differences among the dose effect curves were analyzed by selecting common parameters (e.g., slope, intercept) and comparing simpler models (i.e., those with common parameters, such as a shared slope) with more complex models (i.e., a model allowing for different slopes) by means of an F-ratio test. If the calculated F-value for two different models was statistically significant, the more complex model was used to fit the data (i.e., using different or best-fit slopes or intercepts). However, if the calculated F-value was not significant, the simpler model was used (i.e., using a common slope or intercept; for detailed examples of this approach, see Kenakin, 1997). When dose effect curves could be fitted with a common slope (i.e., dose effect curves were parallel), doses corresponding to the 50% level of responding (D_{50}), potency ratios and their 95% confidence limits were calculated by parallel line analysis (Tallarida, 2000) of data from individual subjects. Slope values and their 95% confidence intervals were obtained by means of GraphPad.

Repeated measures analysis of variance (ANOVA) and Tukey–Kramer multiple-comparison tests (for post hoc comparisons when the F-statistic of the ANOVA was significant; NCSS, Kaysville, UT) were used to test for differences between the maximum percent drug-appropriate responding (drug as factor), the rate of responding in different cycles under control conditions (cycle as factor), the effects of different doses of CGP35348 alone (dose of CGP35348 and replication as factors), and the effects of CGP35348 on individual doses of the GHB dose effect curve (treatment as factor). The

isobolographic analyses were conducted as described by Tallarida (2000) using commercially available software (Pharm Tools Pro v1.1.27, The McCary Group Inc., Elkins Park, PA).

2.4. Drugs

3-Aminopropyl(methyl)phosphinic acid hydrochloride (SKF97541) and 3-aminopropyl(diethoxymethyl)phosphinic acid sodium (CGP35348) were synthesized as described previously (Froestl et al., 1995). All chemicals used in the synthesis of SKF97541 and CGP35348 were purchased from Sigma-Aldrich (USA). Gamma-hydroxybutyrate sodium (GHB) and (±)baclofen were purchased from Sigma-Aldrich (USA). Drug solutions were prepared by dissolving GHB, SKF97541 and CGP35348 in sterile water and baclofen in physiological saline (pH 5–9). Doses are expressed as the forms indicated above and injection volumes varied from 0.1 to 1.0 ml.

3. Results

The median number of sessions under multiple cycle training conditions to satisfy testing criteria was 8 (range: 6–16). During the course of these studies, animals satisfied testing criteria in an average (SEM) of 80.0% (4.0) of training cycles in which 200 mg/kg GHB was administered. Under test conditions, saline occasioned less than 5% GHB-appropriate responding (Fig. 1, filled symbols above "V"). GHB dose-dependently occasioned responding on the drug-appropriate lever up to 94.0% at a dose of 320 mg/kg (Fig. 1, upper left panel); the training dose of GHB (200 mg/kg) was omitted from the cumulative dosing sequence in order to maintain equal dose increments across drugs. Baclofen and SKF97541 occasioned a maximum of 62.8% and 58.9% drug-appropriate responding, respectively, which was less than, but not significantly different from, the maximum drug-appropriate responding occasioned by GHB ($p>0.05$).

The mean (S.E.M.) response rate following saline administration in the first cycle under training conditions was 0.98± 0.01 responses/s and did not differ significantly from response rates in subsequent cycles (1–4; $F_{(3,1688)}=0.45$, $p>0.05$; data not shown). For GHB, baclofen and SKF97541, the dose that occasioned the most GHB-appropriate responding (320, 3.2 and 0.32 mg/kg, respectively) decreased response rate to 90%, 80% and 60% of control, respectively, and the quarter log-unit larger dose (when studied) eliminated responding in all animals (Fig. 1, lower panels).

When given alone, CGP35348 occasioned some (maximum of 28%) GHB-appropriate responding that was not dose-dependent and not significantly different from saline at any dose studied ($F_{(3,77)}=1.47$, $p>0.05$; Fig. 1, open symbols above "V"). Each dose of CGP35348 significantly decreased rate of responding compared with control ($F_{(3,79)}=12.55$, $p<0.05$, Tukey–Kramer multiple-comparison test), an effect that was dose-dependent (i.e., 560 mg/kg CGP35348 was significantly different from 178 and 320 mg/kg CGP35348; $p<0.05$, Tukey–Kramer multiple-comparison test) and not significantly

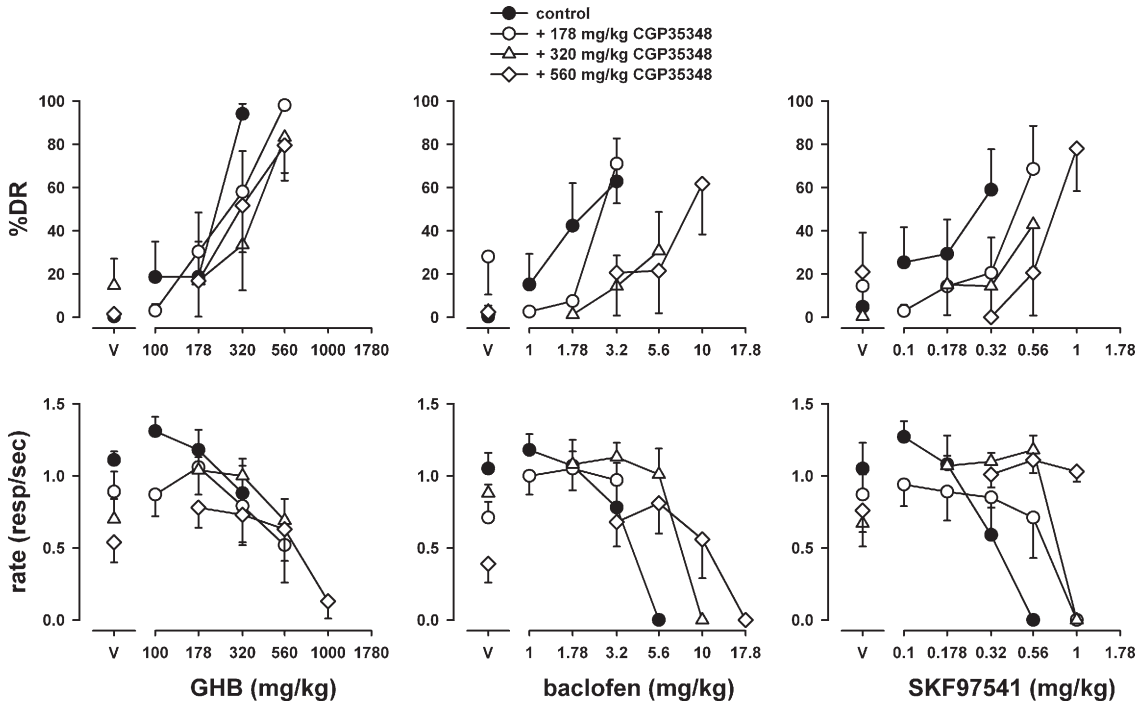

Fig. 1. Discriminative stimulus and rate-decreasing effects of GHB (left panels) and the $GABA_B$ receptor agonists baclofen (center panels) and SKF97541 (right panels) alone (closed symbols) and together with three doses of the $GABA_B$ receptor antagonist CGP35348 (open symbols). Ordinates: top panel, the percentage of responses on the drug-appropriate lever [%DR]; bottom panel, rate of responding in responses per second. Abscissa: dose in mg/kg body weight; data above "V" show the effects of saline or a dose of CGP35348 alone. Shown are averaged data for at least six out of seven rats±1 S.E.M., except for discrimination data under the following conditions: $N=5$ for 560 mg/kg CGP35348+SKF97541 and $N=5$ for 560 mg/kg CGP35348+baclofen.

different between determinations (i.e., prior to each of the three agonist dose effect curves; $F_{(2,79)}=0.80$, $p>0.05$).

GHB, baclofen and SKF97541 were studied alone and together with 178, 320 and 560 mg/kg CGP35348. CGP35348 appeared to shift the GHB dose effect curve to the right (Fig. 1, upper left panel); however, the simplest model that could be fitted to the dose effect curves for the discriminative stimulus effects of GHB alone and together with CGP35348 had a common slope ($F_{(3,67)}=1.20$, $p>0.05$) and a common intercept [$F_{(3,71)}=1.89$, $p>0.05$; common D_{50} (95% confidence limits)= 279.8 (231.2–338.6)], indicating that the GHB dose effect curve was not significantly shifted rightward; however, drug-appropriate responding occasioned by 320 mg/kg GHB was significantly attenuated by 320 mg/kg CGP35348 ($F_{(7,48)}=7.67$, $p<0.05$, Tukey–Kramer multiple-comparison test). When CGP35348 was studied together with baclofen or SKF97541, the simplest model that could be fitted to each group of dose effect curves for discriminative stimulus effects was one with a common slope ($F_{(2,38)}=1.18$, $p>0.05$ and $F_{(2,31)}=0.32$, $p>0.05$ for baclofen and SKF97541, respectively) and different intercepts (Table 1), indicating a significant parallel rightward shift in (i.e., antagonism of) the baclofen ($F_{(2,41)}=12.20$, $p<0.001$) and SKF97541 ($F_{(7,48)}=7.67$, $p<0.05$) dose effect curves by CGP35348. The dose of 178 mg/kg CGP35348 shifted the baclofen and SKF97541 dose effect curves to a similar extent [potency ratio (95% confidence limits)=1.4 (0.7–2.6) and 1.8 (1.1–2.9)], respectively. The larger dose of 560 mg/kg CGP35348 further shifted the baclofen [potency ratio (95% confidence limits)=4.1 (2.1–8.0)] and SKF97541 dose effect curves [potency ratio (95% confidence limits)=2.9 (1.7–5.1)].

For response rate data, the group of dose effect curves for GHB alone and together with CGP35348 could be fitted with a common slope ($F_{(3,42)}=2.05$, $p>0.05$) and a common intercept ($F_{(3,46)}=1.72$, $p>0.05$), indicating that CGP35348 did not significantly attenuate the effects of GHB on rate of responding (Fig. 1, lower left panel). Response rate data for the groups of baclofen and SKF97541 dose effect curves (each drug alone and together with CGP35348) could both be fitted with a common slope ($F_{(2,25)}=2.35$, $p>0.05$ and $F_{(2,26)}=2.44$, $p>0.05$ for baclofen and SKF97541, respectively) and different intercepts (Table 1), indicating that CGP35348 significantly attenuated the rate-decreasing effects of baclofen ($F_{(2,28)}=9.81$, $p<0.001$) and SKF97541 ($F_{(2,29)}=24.69$, $p<0.001$) over the same dose range (Fig. 1, lower center and right panels) and to a similar extent at 320 mg/kg CGP35348 [baclofen potency ratio (95% confidence limits)=2.0 (0.7–5.2), SKF97541 potency ratio (95% confidence limits)=2.6 (2.0–3.3)].

The smallest dose of baclofen that did not occasion GHB-like discriminative stimulus effects or markedly decrease responding by itself (i.e., 1.0 mg/kg) was studied together with doses of GHB and SKF97541 to examine whether these dose effect curves could be shifted leftward and whether the magnitude of the shift was the same for GHB and SKF9741. The simplest model that could be fitted to the GHB dose effect curves for drug-appropriate responding and rate-decreasing effects was one with different slopes ($F_{(1,51)}=7.68$, $p<0.01$ and $F_{(1,40)}=4.31$, $p<0.05$ for drug-appropriate responding and rate-decreasing effects, respectively), indicating that baclofen did not shift the GHB dose effect curve leftward in a parallel manner, but enhanced the discriminative stimulus effects of smaller doses of GHB (Fig. 2, upper left panel). In contrast, the dose effect curves for drug-appropriate responding and rate-decreasing effects of SKF97541 alone and together with baclofen could be fitted to a model with a common slope ($F_{(2,46)}=1.50$, $p>0.05$ and $F_{(1,30)}=3.83$, $p>0.05$ for drug-appropriate responding and rate-decreasing effects, respectively) and different intercepts ($F_{(2,49)}=29.63$, $p<0.001$ and $F_{(1,32)}=55.54$, $p<0.001$ for drug-appropriate responding and rate-decreasing effects, respectively), indicating that baclofen significantly shifted the SKF97541 dose effect curve to the left (Fig. 2, right panels).

For drug-appropriate responding (i.e., discriminative stimulus effects), the D_{50} of SKF97541 [0.19 (0.14, 0.26)] was markedly enhanced by 1.0 mg/kg baclofen [D_{50}=0.06 (0.04, 0.08), potency ratio (95% confidence limits)=3.5 (2.2–5.5)], although this combination of baclofen and SKF97541 appeared to be subadditive. Isobolographic analysis predicted a D_{50} of 0.01 mg/kg SKF97541 when given together with 1.0 mg/kg

Table 1
Mean D_{50} values (mg/kg) and 95% confidence limits (CL) for compounds alone and together with doses of the selective $GABA_B$ receptor antagonist CGP35348

Drug	Control			178 mg/kg CGP35348			320 mg/kg CGP35348			560 mg/kg CGP35348		
%DR	D_{50}	95% CL		D_{50}	95% CL		D_{50}	95% CL		D_{50}	95% CL	
GHB	214.2	7.6 [a]	6078.3 [a]	248.5	186.1	331.9	348.3	237.5	510.9	320.8	210.7	488.3
Baclofen	2.10	1.39	3.18	2.88	1.44	5.77	ND [b]			8.66	4.11	18.24
SKF97541	0.26	0.17	0.38	0.46	0.31	0.67	ND [b]			0.76	0.49	1.16
Rate	D_{50}	95% CL		D_{50}	95% CL		D_{50}	95% CL		D_{50}	95% CL	
GHB	396.2 [c]	18.9 [a]	8307.1 [a]	517.9 [c]	332.4	806.8	611.0 [c]	377.7	988.5	543.0 [c]	163.6	1802.4
Baclofen	3.90	2.43	6.24	ND [b]			7.73	3.48	17.15	9.50	5.56	16.22
SKF97541	0.33	0.27	0.41	0.67	0.42	1.06	0.85	0.73	0.99	ND [b]		

[a] In general, the range of the 95% CLs was proportional to the D_{50} (mean range/D_{50}±S.E.M.=1.07±0.14, N=18), except for %DR and rate for GHB under control conditions where the range of the CLs was 28.3 and 20.9 times greater than the respective D_{50} values. This result is likely due to constraining steep dose effect curves comprised of only two points to a common slope, because D_{50} values calculated using individual slopes yielded D_{50} and 95% CLs of 227.2 (195.6, 263.8) and 409.8 (356.8, 470.7) for %DR and rate, respectively.

[b] ND—these values were not determined because the dose response curve failed to cross the 50% level.

[c] These values were calculated assuming that the next (quarter log-unit) largest dose would completely suppress responding in all animals.

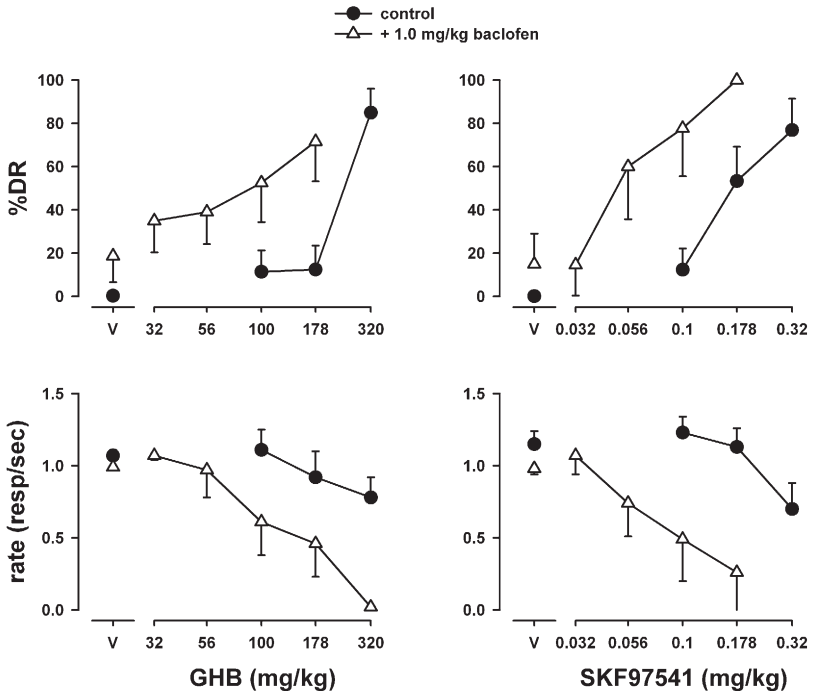

Fig. 2. Discriminative stimulus and rate-decreasing effects of GHB (left panels) and SKF97541 (right panels) alone (closed symbols) and together with 1.0 mg/kg baclofen (open symbols). Ordinates: top panel, the percentage of responses on the drug-appropriate lever [%DR]; bottom panel, rate of responding in responses per sec. Abscissa: dose in mg/kg body weight; data above "V" show the effects of saline or baclofen alone. Shown are doubly determined data from 6 rats and represent $N=8$–12, with the exception of 0.1 and 0.178 mg/kg SKF97541 + 1.0 mg/kg baclofen, $N=4$.

baclofen, which was outside of the 95% confidence limits of the D_{50} obtained when combining SKF97541 with 1.0 mg/kg baclofen [0.06 (0.04, 0.08)]. The D_{50} of SKF97541 [0.37 (0.29, 0.46)] to decrease responding was also enhanced by 1.0 mg/kg baclofen [$D_{50}=0.09$ (0.05, 0.18), potency ratio (95% confidence limits)=3.9 (2.3–6.5)], although the combination of baclofen and SKF97541 to decrease responding appeared to be supra-additive. Isobolographic analysis predicted a D_{50} of 0.28 mg/kg SKF97541 when given together with 1.0 mg/kg baclofen, which was outside of the 95% confidence limits of the D_{50} obtained when combining SKF97541 with 1.0 mg/kg baclofen [0.09 (0.05, 0.18)].

4. Discussion

The main finding of this study is that the discriminative stimulus and rate-decreasing effects of GHB were not modified by a selective $GABA_B$ receptor antagonist or agonist to the same magnitude as these drugs modified the effects of other well-characterized $GABA_B$ receptor agonists. The selective $GABA_B$ receptor antagonist CGP35348 significantly shifted the baclofen and SKF97541 dose effect curves to the right and attenuated the discriminative stimulus effects of 320 mg/kg GHB; however, the magnitude of antagonism (i.e., rightward shift) of the GHB dose effect curve was not as large as what was observed for baclofen and SKF97541. Further, baclofen enhanced the discriminative stimulus and rate-decreasing effects of SKF97541, whereas the GHB dose effect curves for discriminative stimulus and rate-decreasing effects were not shifted leftward in a parallel manner. These data are consistent with GHB exerting some of it effects by CGP35348-sensitive $GABA_B$ receptors; however, the present results suggest that additional mechanisms (e.g., GHB receptors or CGP35348-insensitive $GABA_B$ receptors) contribute to the discriminative stimulus and rate-decreasing effects of GHB.

Previous studies that examined the mechanism(s) responsible for the discriminative stimulus effects of GHB used selective antagonists and typically implicated $GABA_B$ receptors as the primary site of action (Colombo et al., 1998; Lobina et al., 1999; Carter et al., 2003, 2004a; Koek et al., 2004). Consistent with these findings, the present studies show that the $GABA_B$ receptor agonists baclofen and SKF97541 reliably occasion GHB-appropriate responding (e.g., the control data in Figs. 1 and 2). Furthermore, the maximum GHB-appropriate responding observed after cumulative doses of GHB, baclofen, and SKF97541 in these studies was not significantly different from the results of a previous study using single dosing conditions (Carter et al., 2003), suggesting that the use of cumulative dosing does not quantitatively affect the discriminative stimulus effects of GHB in rats.

Cumulative dosing was used in this study, in part, because it allows for the determination of a dose effect curve in a single session. Previous reports that examined discriminative stimulus effects of GHB studied single doses of agonists alone and together with different doses of antagonists, whereas the current study determined agonist dose effect curves alone and together with different doses of an antagonist. In other studies that used this approach to investigate the mechanism of action of GHB to decrease operant responding, the analysis of full dose effect curves in the absence and presence of different doses of

CGP35348 revealed that the apparent antagonism of the rate-decreasing effects of GHB by CGP35348, unlike the clear antagonism of baclofen, was not statistically significant (Carter et al., 2004b). This finding suggested that the mechanism(s) of action of GHB and baclofen are not identical, and that a mechanism(s) in addition to agonism at $GABA_B$ receptors contributes to the rate-decreasing effects of GHB (Carter et al., 2004b). One possible explanation for this finding is that GHB receptors contribute to the rate-decreasing effects of GHB. Compounds that bind selectively to GHB receptors share some behavioral effects with GHB, including the ability to decrease operant responding and locomotion, and to produce catalepsy and ataxia (Wu et al., 2003; Carter et al., 2005b).

In vitro evidence suggests that GHB can dose-dependently modulate glutamatergic signaling, which is consistent with GHB binding to specific GHB receptors at lower concentrations and $GABA_B$ receptors at higher concentrations (Maitre, 1997; Mehta et al., 2001; Wu et al., 2004). Stimulation of glutamate release by nanomolar concentrations of GHB is blocked by the purported GHB-receptor antagonist NCS-382 and not by CGP35348 (Ferraro et al., 2001; Castelli et al., 2003), whereas inhibition of glutamate release by millimolar concentrations of GHB is blocked by CGP35348 and not by NCS-382 (Banerjee and Snead, 1995; Ferraro et al., 2001; Castelli et al., 2003). Thus, the effects of smaller doses of GHB might be mediated by GHB receptors, whereas the effects of larger doses of GHB might be mediated by $GABA_B$ receptors. Taken together, these data suggest that attenuation of the effects of GHB with a selective GHB or $GABA_B$ receptor antagonist is likely to depend on the dose at which the effect is observed and on the relative contribution of glutamatergic and GABAergic signaling to that effect.

Whereas GHB receptors might contribute to the rate-decreasing effects of GHB, the discriminative stimulus effects of GHB are not mimicked by compounds that bind selectively to GHB receptors (Wu et al., 2003; Carter et al., 2005a,b). This finding might result from the GHB discriminative stimulus comprising different components that are not mimicked by any one component (e.g., McMillan and Li, 2002), by perceptual masking (e.g., Gauvin and Young, 1989) of a GHB receptor-mediated component by a more salient $GABA_B$ receptor-mediated component (Koek et al., 2006), or by the involvement of multiple $GABA_B$ receptor subtypes. Emerging evidence suggests that $GABA_B$ receptor heterogeneity (subtypes) might play a role; for example, in pigeons discriminating 100 mg/kg GHB (i.m.), a 10-fold larger dose of CGP35348 was needed to block the effects of GHB compared to baclofen (Koek et al., 2004).

Recent drug discrimination studies have attempted to remove the baclofen-like component from the GHB discriminative stimulus by training rats to discriminate GHB from saline or baclofen (Koek et al., 2005). In these studies, baclofen no longer occasions GHB-appropriate responding; however, the potency of CGP35348 to antagonize the effects of GHB is not changed. 3-[[(3,4-Dichlorophenyl)methyl]amino]propyl] diethoxymethyl)phosphinic acid (CGP52432) is a $GABA_B$ receptor antagonist that, unlike CGP35348, exhibits a 100-fold greater affinity at $GABA_B$ heteroreceptors as compared to autoreceptors (Lanza et al., 1993). In contrast to the similar antagonist potency of CGP35348 in rats discriminating GHB from either saline or baclofen, removing the baclofen-like component from the GHB discriminative stimulus decreases the potency of CGP52423 to antagonize GHB (Koek et al., 2005), supporting the notion that the GHB-specific component is more closely related to the modulation of glutamatergic signaling by $GABA_B$ heteroreceptors, whereas the baclofen component is likely related to the activity at $GABA_B$ autoreceptors and heteroreceptors.

The differential enhancement of the discriminative stimulus and rate-decreasing effects of GHB and SKF97541 by baclofen is also consistent with the discriminative stimulus effects of GHB being mediated by $GABA_B$ heteroreceptors and the rate-decreasing effects of GHB being mediated by GHB and $GABA_B$ receptors. If the discriminative stimulus effects of GHB are related to the modulation of glutamatergic signaling and GHB and baclofen have opposing effects on glutamatergic signaling (Banerjee and Snead, 1995; Ferraro et al., 2001; Castelli et al., 2003), then administration of GHB and baclofen together would not result in the enhanced discriminative stimulus effects that would be expected from two compounds acting through the same mechanism (e.g., baclofen and SKF97541). In contrast, compounds that bind selectively to either GHB receptors or $GABA_B$ receptors can decrease response rate (Carter et al., 2004b, 2005b); thus, the enhanced rate-decreasing effects of GHB in the presence of baclofen could be due to the sum of effects of GHB and baclofen at GHB and $GABA_B$ receptors. Moreover, if GHB can suppress behavior through multiple mechanisms, this might account for the relatively steep GHB dose effect curve that is obtained under most conditions. That baclofen appeared to enhance the discriminative stimulus effects of SKF97541 less than predicted and to enhance its rate-decreasing effects more than predicted might be due to antagonist effects of SKF97541 at $GABA_C$ receptors (Ragozzino et al., 1996); however, further study is required to draw conclusions regarding the possible involvement of $GABA_C$ receptors in these effects.

There is growing evidence that some of the effects of GHB (e.g., therapeutic-and abuse-related effects) are not mediated exclusively by direct, agonist actions at $GABA_B$ receptors. Studies over a broad range of GHB doses and using selective GHB and $GABA_B$ receptor-selective compounds have suggested a possible role for GHB receptors in the neurochemical (Banerjee and Snead, 1995; Ferraro et al., 2001; Castelli et al., 2003) and behavioral effects of GHB (Carter et al., 2005b). Moreover, there is evidence for functional $GABA_B$ receptor subtypes (Seabrook et al., 1990; Bonanno and Raiteri, 1992; Lanza et al., 1993; Fassio et al., 1994; Yamada et al., 1999). Differential activity of GHB at $GABA_B$ autoreceptors and heteroreceptors, particularly at larger doses, could account for some of the differences that are observed between GHB and other better-characterized $GABA_B$ receptor agonists. Further investigation of the functional significance of GHB receptors, $GABA_B$ autoreceptors and $GABA_B$ heteroreceptors might lend insight to novel therapeutic effects of GHB and therapeutic potential of different $GABA_B$ receptor ligands.

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