Discriminative Stimulus Effects of γ-Hydroxybutyrate (GHB) in Rats Discriminating GHB from Baclofen and Diazepam

Wouter Koek, Lawrence P. Carter, R. J. Lamb, Weibin Chen, Huifang Wu, Andrew Coop, and Charles P. France

Departments of Psychiatry (W.K., R.J.L., C.P.F.) and Pharmacology (W.K., L.P.C., R.J.L., C.P.F.), University of Texas Health Science Center at San Antonio, San Antonio, Texas; and Department of Pharmaceutical Sciences (W.C., H.W., A.C.), University of Maryland, Baltimore, Maryland

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ABSTRACT

γ-Hydroxybutyrate (GHB) is a drug of abuse with actions at GHB and GABA receptors. This study tried to increase the selectivity of the discriminative stimulus effects of GHB by training animals to discriminate GHB from compounds that share pharmacological mechanisms with GHB. In comparison with a previous GHB versus saline discrimination (group 1), rats were trained to discriminate GHB (200 mg/kg) either from saline and the GABAB agonist baclofen (3.2 mg/kg) (group 2) or from saline, baclofen, and the positive GABA_A modulator diazepam (1 mg/kg) (group 3). In all groups, GHB produced more than 80% GHB-appropriate responding. Baclofen produced 84% GHB-appropriate responding in group 1 but less than 30% in groups 2 and 3. Diazepam produced 68% GHB-appropriate responding in group 1, 30% in group 2, and only 5% in group 3. The GABA_B receptor antagonists CGP35348 [3-[aminopropyl(diethoxymethyl)phosphinic acid] and CGP52432 [3-[3,4-dichlorophenyl][methyl][amino]propyl][diethoxymethyl]phosphinic acid] attenuated the discriminative stimulus effects of GHB; CGP35348 did so with similar potency in all groups, but CGP52432 was significantly less potent in groups 2 and 3 than in group 1. In all groups, the GHB antagonist NCS-382 [(2E)-5-hydroxy-5,7,8,9-tetrahydro-6H-benz[a][7]annulen-6-ylidene ethanoic acid] partially attenuated the discriminative stimulus effects of GHB. The selective GHB receptor ligand UMB86 (4-hydroxy-4-naphthylbutanoic acid sodium) tended to attenuate the discriminative stimulus effects of GHB more in group 3 than in the other groups. The finding that animals can discriminate GHB from baclofen is further evidence that the effects of GHB and baclofen are not identical. Effects that GHB does not share with baclofen may involve GHB receptors or differential interactions with GABA_B receptors.

γ-Hydroxybutyrate (GHB) is used to treat narcolepsy, but it is also a drug of abuse, as are some of its precursors (Nicholson and Balster, 2001). GHB occurs naturally in the brain, where it may function as a neurotransmitter. Its specific binding sites, commonly investigated with the selective radioligand [3H]NCS-382 (Mehta et al., 2001), seem to be G protein-coupled receptors. The precise roles of GHB in central nervous system function, however, are not well understood.

GHB may exert its effects not only through GHB receptors but also through other mechanisms. GHB interacts directly with γ-aminobutyric acid (GABA)_B receptors (Lingenhoehl et al., 1999) and indirectly with all GABA receptor subtypes, because it is metabolized to GABA (Vayer et al., 1985). GABA_B receptors may play a role, because the discriminative stimulus effects of GHB are mimicked by the GABA_B receptor agonist baclofen and are antagonized by the GABA_B receptor antagonist CGP35348 (Colombo et al., 1998; Carter et al., 2003). GABA_A systems are involved, suggested by partial overlap in the actions of GHB and positive GABA_A receptor modulators such as diazepam (Colombo et al., 1998; Carter et al., 2004). Thus, GHB likely has multiple mechanisms of action.

Drug discrimination has proved useful in identifying mechanisms of action, because it can provide sensitive and pharmacologically selective assays of in vivo effects (Colpaert and Balster, 1988). Rats can discriminate GHB from saline (Winter, 1981; Colombo et al., 1995a,b, 1998; Metcalf et al., 2001; Carter et al., 2003; Baker et al., 2004), and the discriminative

ABBREVIATIONS: GHB, γ-hydroxybutyrate; NCS-382, [(2E)-5-hydroxy-5,7,8,9-tetrahydro-6H-benz[a][7]annulen-6-ylidene ethanoic acid; CGP35348, 3-aminopropyl(diethoxymethyl)phosphinic acid; NMDA, N-methyl-D-aspartate; 1,4-BD, 1,4-butanediol; GBL, γ-butyrolactone; SKF87541, 3-aminopropyl(methyl)phosphinic acid hydrochloride; CGP52432, 3-[[3,4-dichlorophenyl][methyl][amino]propyl][diethoxymethyl]phosphinic acid; DTT, dl-dithiothreitol; UMB86, 4-hydroxy-4-naphthylbutanoic acid sodium; PR, potency ratio; CL, confidence limits.
stimulus effects are selective, because pharmacologically unrelated drugs (e.g., phencyclidine and ketamine) do not substitute for GHB (Winter, 1981; Carter et al., 2003; Baker et al., 2004). Also, GHB does not substitute for training drugs that are pharmacologically unrelated to GHB (Beardsey et al., 1996; Woolverton et al., 1999). Together, these studies suggest that the discriminative stimulus effects of GHB involve multiple mechanisms, with a prominent role for GABA<sub>B</sub> receptors, a less important role for GABA<sub>A</sub> receptors, and a role for GHB receptors that has not been clearly delineated.

The discriminative stimulus effects of ethanol, like those of GHB, likely involve multiple mechanisms (Grant, 1994; Kostowski and Bienkowski, 1999), with the GABA<sub>B</sub> and N-methyl-D-Aspartate (NMDA) receptor complexes being particularly important. Discriminations between ethanol and other drugs have shown that ethanol can be discriminated from the positive GABA<sub>B</sub> receptor modulator pentobarbital (Bowen et al., 1997) and from the NMDA antagonist dizocilpine (Gatto et al., 1995) and that the resultant ethanol discriminations were devoid of apparent GABA<sub>A</sub>- and NMDA-mediated components, respectively. Here, a similar approach was used in an effort to obtain a GHB discrimination devoid of GABA components, and, possibly, involving only GHB receptor-mediated effects, which would be useful to examine novel GHB receptor ligands.

Discriminations between ethanol and other drugs have been trained in three-lever procedures by reinforcing responding on one lever after ethanol, responding on another lever after the other drug, and responding on the third lever after saline (Gatto et al., 1995; Bowen et al., 1997). In the present study, multiple drug training was conducted in a two-lever procedure, in which animals are trained to discriminate a primary training drug (GHB) from any of several other drugs and from the no-drug condition by reinforcing responding on one lever after the primary training drug, and responding on the other lever after other drugs and after saline (Overton, 1982; Overton et al., 1989). In this procedure, the number of other drugs that can be trained to be discriminated from the primary drug is not limited by the number of levers, in contrast with the three-lever procedure, which typically involves only one other drug.

Different groups of rats were trained to discriminate GHB (200 mg/kg) from saline (group 1); from saline and the GABA<sub>B</sub> agonist baclofen (3.2 mg/kg) (group 2); or from saline, baclofen, and the positive GABA<sub>A</sub> modulator dizepam (1 mg/kg) (group 3). Training doses of GHB, baclofen, and diazepam were the same as in previous drug versus saline discrimination studies (Winter, 1981; Carter et al., 2003, 2004). In the study by Carter et al. (2004), 3.2 mg/kg was selected as the training dose for baclofen and 1 mg/kg for diazepam because these doses occasioned drug lever responding in rats discriminating 200 mg/kg GHB from vehicle (Carter et al., 2003). Here, the following compounds were examined: GHB and its metabolic precursors 1,4-butanediol (1,4-BD) and 4-hydroxy-4-naphthylbutanoic acid sodium (UMB86, Carter et al., 2005); and compounds pharmacologically unrelated to GHB, i.e., cocaine, ketamine, and morphine.

Materials and Methods

Animals. Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN; n = 24) were housed individually in 45 × 24 × 20-cm plastic cages containing rodent bedding (Sani-chips; Harlan Teklad, Madison, WI) in a room maintained on a 14:10-h light/dark cycle (experiments conducted during the light period). Rats were fed 5 to 15 g of chow (Rat Sterilizable Diet; Harlan Teklad) after daily experimental sessions to maintain body weights. Individual weights were initially decreased to 80% of the free-feeding weight and were subsequently allowed to increase to 350 g according to normal growth curves established for Sprague-Dawley rats. Water was available continuously in the home cage. All rats were experimentally naive prior to training. 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).

Apparatus. Daily experimental sessions were conducted in sound-attenuating, ventilated enclosures (model ENV-022M and ENV-008CT; MED Associates, St. Albans, VT), which contained an operant chamber with a stainless steel response panel equipped with two metal levers 11.5 cm apart. The floor of the operant chamber was a grid consisting of 19 stainless steel rods that were 4.8 mm in diameter, spaced 1.6 cm apart, and oriented parallel to the response panel. A 2.5-cm-diameter translucent circle that could be illuminated was located on the response panel above each lever and a 5 × 5-cm opening located equidistant between the two levers was available for food pellet delivery. Food pellets (45 mg; PJA1-0045; Noyes Precision Pellets, Research Diets Inc., New Brunswick, NJ) were delivered by a food hopper external to the operant chamber, but within the enclosure. Chambers were connected by an interface (MED Associates) to a computer that used MED-PC IV software (MED Associates) to monitor and control inputs and outputs and to record the data.

Procedure. The procedure was similar to that described in detail previously (Carter et al., 2003). Briefly, before each daily training session, three different groups of subjects (n = 8 per group) received (i.p.) 200 mg/kg GHB or saline (group 1; results reported previously in Carter et al. [2003], except those obtained with SKF97541, CGP52432, UMB86, and DTT); 200 mg/kg GHB or saline or 3.2 mg/kg baclofen (group 2); or 200 mg/kg GHB or saline or 3.2 mg/kg baclofen or 1 mg/kg diazepam (group 3) and were immediately placed into the chamber. Sessions began with a period of 15 min, during which the lights were off and lever presses had no programmed consequence. Subsequently, the lights above both levers were illuminated, and 10 consecutive responses on the injection-appropriate lever resulted in food delivery. Food pellets (45 mg; PJA1-0045; Noyes Precision Pellets, Research Diets Inc., New Brunswick, NJ) were delivered by a food hopper external to the operant chamber, but within the enclosure. Chambers were connected by an interface (MED Associates) to a computer that used MED-PC IV software (MED Associates) to monitor and control inputs and outputs and to record the data.

Discriminating GHB from Other Drugs 171
training sessions were equally divided among the other training conditions (i.e., saline in group 1; saline and baclofen in group 2; and saline, baclofen, and diazepam in group 3). GHB training sessions alternated with the other training sessions and the same training session was generally not conducted for more than two consecutive days. Initially, subjects had to satisfy the following criteria for five consecutive sessions or for six of seven consecutive sessions: at least 90% of the total responses on the injection-appropriate lever and fewer than 10 responses on the incorrect lever before the delivery of the first food pellet. Thereafter, tests were conducted when these criteria were met during two consecutive training sessions, with the right lever correct during one session and the left lever correct during the other session. Test sessions were the same as training sessions (i.e., a 15-min period followed by a response period that ended after the delivery of 100 food pellets or 15 min, whichever occurred first) except that food was delivered after completion of 10 consecutive responses on either lever. Drug (or vehicle) treatments were given immediately before the session, and pretreatments were given 10 min before treatments.

Data Analysis. Differences among groups 1 to 3 in the speed with which they acquired the discrimination, measured as number of sessions to criterion, were analyzed by means of randomization tests for independent samples (Siegel and Castellan, 1988; Edgington, 1995), with p values corrected for multiple comparisons using the step-down Holm-Bonferroni procedure (Ludbrook, 1998).

Test sessions generated data on the following two variables: 1) the percentage 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 session 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, discrimination data from that test were not included in the average. Mean percentage of responses on the GHB lever values were calculated only when they were based on at least half of the animals tested. Differences among the three groups in percentage of GHB-appropriate responding and in response rate observed during tests of the training conditions were analyzed by means of Holm-Bonferroni-corrected randomization tests.

Differences among the dose-response curves in groups 1 to 3 to produce GHB-appropriate responding and to decrease response rate 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 Inc., San Diego, CA; www.graphpad.com), using the following equation: effect = slope × log(dose) + intercept. Straight lines were fitted to the linear portion of dose-response 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. Dose-response curves that failed to cross the 50% level, or that crossed it twice, were not analyzed by linear curve fitting. Response rate data were analyzed by fitting straight lines to the results obtained at all doses tested, except the results obtained in group 1 at the lowest dose of baclofen (i.e., 0.32 mg/kg), BDL (i.e., 10 mg/kg), and GBL (i.e., 10 mg/kg), which were not included in the analyses.

An important feature of the GraphPad program is the possibility to fit models of varying complexity to the data. With GraphPad, a model can be simplified by selecting common parameters (e.g., common slope and common intercept) and simpler models can be compared with more complex models by means of an F-ratio test. If the calculated F for two models is statistically significant, this indicates that the more complex model is required to fit the data. However, if the value of F is not significant, the simple model should be used (for detailed examples of this approach, see Kenakin, 1997). For best-fitting models, doses corresponding to the 50% level of responding on the GHB lever (D50), potency ratios (PRs), and their 95% confidence limits (CL) 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.

Drugs. Drugs were administered i.p. (pH 6–8) and dissolved or diluted in sterile water or saline unless otherwise noted. Compounds studied included GHB, GBL, 1,4-BD, (±)-baclofen, pentobarbital sodium, DTT (Sigma-Aldrich, St. Louis, MO); NCS-382 (sodium salt), morphine sulfate, cocaine hydrochloride (National Institute on Drug Abuse, Research Technology Branch, Rockville, MD); ketamine hydrochloride (Ketaset; Fort Dodge Laboratories, Fort Dodge, IA); diazepam (Sigma/RBI, Natick, MA); and UMB86, CGP35348 (sodium salt), CGP52432 (sodium salt), and SKF97541 (synthesized at the University of Maryland). Diazepam was dissolved at different concentrations in the same vehicle consisting of 70% Emulphor (EL-620, a polyoxymethylated vegetable oil; GAF Corporation, Linden, NJ), 20% sterile water, and 10% ethanol (by volume). All compounds were injected i.p. in a volume of 0.25 to 2.5 ml. Doses are expressed as the form of the drug listed above.

Results

All eight animals in each of the three groups acquired the GHB discrimination (Fig. 1). Animals in groups 2 and 3, trained to discriminate GHB not only from saline but also from baclofen (groups 2 and 3), and from diazepam (group 3), required significantly more sessions to reach the discrimination criterion than those trained to discriminate GHB from saline (group 1). The median sessions to criterion (range) in groups 1, 2, and 3 were 35 (23–41), 55 (33–108), and 53 (43–107), respectively.

Under test conditions, GHB dose-dependently increased responding on the GHB-appropriate lever (Fig. 2, top left) and decreased response rate (bottom left), in all three groups. Results obtained in tests of saline (not shown) and of the training dose of GHB (Fig. 2, left) did not differ significantly among the groups (randomization test); the mean (S.E.M.) percentage GHB-appropriate responding across groups was

![Fig. 1. Acquisition of a GHB discrimination in three different groups of rats (n = 8 per group) trained to discriminate 200 mg/kg GHB from saline (group 1, GHB versus SAL), saline or 3.2 mg/kg baclofen [group 2, GHB versus (SAL or BAC)], or saline, 3.2 mg/kg baclofen, or 1 mg/kg diazepam [group 3, GHB versus (SAL or BAC or DZP)] using a two-lever food-reinforced procedure. The median sessions to criterion, interquartile range, and range are plotted for each of the three GHB discriminations. Data marked with an asterisk are significantly different from those obtained in the GHB- versus SAL-discriminating animals. Data obtained in rats discriminating 200 mg/kg GHB from saline are replotted from Carter et al. (2003).](image-url)
0.03 (0.02) after saline and 91.7 (4.9) after 200 mg/kg GHB, and the overall response rate was 1.07 (0.04) responses/s after saline and 0.74 (0.05) after 200 mg/kg GHB. Linear curves fitted to the dose-response data obtained with GHB in each of the groups had slope values significantly different from zero, indicating that GHB significantly increased GHB-appropriate responding and decreased response rate in each group. The simplest model that could be fitted to the GHB discrimination data was one with a common slope and intercept, indicating that the effects of GHB on GHB-appropriate responding did not differ significantly among the three groups (common D50 (95% CL) = 130 (113–151) mg/kg). The effects of GHB on response rate seemed to differ among the groups, with the slope and intercept values for group 1 being significantly different (p < 0.005) from those of groups 2 and 3, which did not differ significantly among themselves. At doses higher than 100 mg/kg, however, GHB affected response rate similarly in all groups.

In group 1, baclofen and diazepam dose-dependently increased responding on the GHB-appropriate lever to a maximum of 84.1 and 68.4%, respectively (Fig. 2, top middle and top right). Compared with group 1, baclofen produced less GHB-appropriate responding in groups 2 and 3, in which 3.2 mg/kg baclofen was an additional training condition, and diazepam produced less GHB-appropriate responding in group 3, in which 1 mg/kg diazepam was an additional training condition. The percentage of GHB-appropriate responding after 3.2 mg/kg baclofen differed significantly among the groups (randomization test, p < 0.005; Fig. 2, top middle) and was significantly (p < 0.05) lower in groups 2 and 3 than in group 1. Similarly, the percentage of GHB-appropriate responding after 1 mg/kg diazepam differed among the groups (randomization test, p < 0.05; Fig. 2, top right), but only in group 3 it was significantly lower than in group 1. Linear curves fitted to the response rate data of baclofen and diazepam in each of the groups (Fig. 2, bottom middle and bottom right) had slope values significantly different from zero, indicating that both drugs significantly decreased response rate in each group. The simplest model that could be fitted to the response rate data of baclofen and diazepam was one with a common slope and intercept, indicating that these effects did not differ significantly among the groups. Similarly, the effects of diazepam on response rate in each of the groups were not significantly different.

The GHB precursor 1,4-BD dose-dependently increased responding on the GHB-appropriate lever to a maximum of 84.1 and 68.4%, respectively (Fig. 2, top middle and top right). Compared with group 1, 1,4-BD increased responding on the GHB-appropriate lever in groups 2 and 3, in which 3.2 mg/kg baclofen was an additional training condition, and diazepam produced less GHB-appropriate responding in group 3, in which 1 mg/kg diazepam was an additional training condition. The percentage of GHB-appropriate responding after 3.2 mg/kg baclofen differed significantly among the groups (randomization test, p < 0.005; Fig. 2, top middle) and was significantly (p < 0.05) lower in groups 2 and 3 than in group 1. Similarly, the percentage of GHB-appropriate responding after 1 mg/kg diazepam differed among the groups (randomization test, p < 0.05; Fig. 2, top right), but only in group 3 it was significantly lower than in group 1. Linear curves fitted to the response rate data of baclofen and diazepam in each of the groups (Fig. 2, bottom middle and bottom right) had slope values significantly different from zero, indicating that both drugs significantly decreased response rate in each group. The simplest model that could be fitted to the response rate data of baclofen and diazepam was one with a common slope and intercept, indicating that these effects did not differ significantly among the groups. Similarly, the effects of diazepam on response rate in each of the groups were not significantly different.

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themselves. The response rate data obtained with 1,4-BD, however, could be fitted with a common slope and intercept, indicating that these effects did not differ significantly among the groups. In contrast with 1,4-BD, the GHB precursor GBL had effects that seemed to differ more markedly among the groups (Fig. 3, middle). Specifically, GBL seemed to be generally less potent in groups 2 and 3 than in group 1. The response rate data of GBL could be fitted with a common slope, and GBL was significantly less potent in groups 2 and 3 than in group 1 \[PR\ (95\% \text{ CL}) = 2.5 (1.8–3.4)\] and \[2.3 (1.6–3.1)\], respectively, but had similar potencies in groups 2 and 3 \[PR\ (95\% \text{ CL}) = 1.1 (0.8–1.6)\].

The GABAB receptor agonist SKF97541 dose-dependently increased responding on the GHB-appropriate lever in group 1 to a maximum of 51.7%. In groups 2 and 3, the maximum effects of SKF97541 seemed to be lower (i.e., 16.7 and 24.9% GHB-appropriate responding, respectively), but the differences failed to reach statistical significance (randomization test). In contrast with possible group differences in the effects of SKF97541 on GHB-appropriate responding, SKF97541 had similar dose-dependent effects in decreasing response rate in all groups, because all response rate data could be fitted with a common slope and intercept.

The GABAB receptor antagonist CGP35348 significantly decreased the percentage GHB-appropriate responding observed with the training dose of 200 mg/kg GHB in all three groups (Fig. 4, top left), as shown by the slope of each curve being significantly different from zero. These effects of CGP35348 did not differ significantly among the three groups, because all data could be fitted with a common slope and intercept (common \(D_{50} = 45 \text{ mg/kg}, 95\% \text{ CL 39–51}\)). CGP35348 had no significant effect on response rate in animals treated with the training dose of 200 mg/kg GHB, because none of the slope values differed significantly from zero. In tests with 200 mg/kg GHB, the mean response rate was 0.66 (0.04) (data not shown), significantly lower than with tests of saline (i.e., 1.07 (0.04) (data not shown)), and similar to the values obtained when CGP35348 was given before 200 mg/kg GHB (Fig. 4, bottom left). Thus, although CGP35348 blocked the discriminative stimulus effects of GHB, it failed to attenuate its effects on response rate.

Like CGP35348, another GABAB receptor antagonist, CGP52432, dose-dependently decreased the percentage GHB-appropriate responding observed with the training dose of 200 mg/kg GHB (Fig. 4, top right), i.e., the slopes were significantly different from zero. The data obtained in each group could be fitted with a common slope, and CGP52432 was significantly less potent in group 2 than in group 1 \[PR\ (95\% \text{ CL}) = 2.5 (1.2–5.5)\], significantly less potent in group 3 than in group 1 \[PR\ (95\% \text{ CL}) = 5.5 (3.2–9.5)\], and significantly less potent in group 3 than in group 2 \[PR\ (95\% \text{ CL}) = 2.2 (1.1–4.6)\] \(D_{50} (95\% \text{ CL}) = 19 (13–28), 49 (20–121),\) and 107 (71–161), in groups 1, 2, and 3, respectively). In group 1, CGP52432 did not significantly attenuate the response rate effects of 200 mg/kg GHB, i.e., the slope was not significantly different from zero. In groups 2 and 3, however, CGP52432
further decreased response rate (slopes significantly different from zero) and did so in a similar manner in both groups (data could be fitted with a common slope and intercept).

In animals trained to discriminate GHB from saline (group 1), the GHB receptor antagonist NCS-382 decreased the percentage GHB-appropriate responding observed with the training dose of GHB to less than 50% at 32 mg/kg, but not at higher doses (Fig. 5, top left), and it increased the percentage GHB-appropriate responding to 50% when given alone (Fig. 5, top right). Although NCS-382 did not produce any GHB-appropriate responding in groups 2 and 3 when given alone, NCS-382 did not decrease GHB-induced drug-appropriate responding more extensively in these groups than in group 1. NCS-382 dose-dependently decreased response rate in animals treated with the training dose of GHB (Fig. 5, bottom left), as shown by the slope of each curve being significantly different from zero. The data of each group could be fitted with a common slope, and NCS-382 was significantly more potent in group 2 than in group 1 \([PR (95\% \text{ CL}) = 2.0 (1.1–4.8)]\), significantly more potent in group 3 than in group 1 \([PR (95\% \text{ CL}) = 2.5 (1.3–4.6)]\), and equipotent in groups 2 and 3 \([PR (95\% \text{ CL}) = 1.3 (0.7–2.2)]\). Thus, although NCS-382 partially attenuated the discriminative stimulus effects of GHB, it did not attenuate the effects of GHB on response rate. NCS-382 decreased response rate when given alone (Fig. 5, bottom right), as shown by the slope of each curve being significantly different from zero. The data of group 2 and 3 could be fitted with a common slope, but different from the slope in group 1. NCS-382 was significantly more potent in group 2 than in group 3 \([PR (95\% \text{ CL}) = 1.7 (1.2–2.4)]\).

The GHB receptor ligand UMB86 seemed to decrease the percentage GHB-appropriate responding observed with the training dose of GHB more potently and more extensively in group 3 than in the other groups (Fig. 6, top left), although the differences among the groups failed to reach statistical significance (randomization test). When given alone, UMB86 did not produce more than 20% GHB-appropriate responding.
in any of the three groups (Fig. 6, top right). UMB86 decreased response rate, when given together with GHB and when given alone, and was equipotient in groups 2 and 3, but was significantly more potent in groups 2 and 3 than in group 1 [PR (95% CL) ranged from 2.1 (1.7–2.8) to 2.4 (1.8–3.2)] when given alone.

When given before 200 mg/kg GHB, DTT decreased the percentage of GHB-appropriate responding partially and seemed to do so similarly in the three groups (Table 1). DTT failed to attenuate the effects of GHB on response rate; instead, DTT further decreased response rate (slope significantly different from zero in each group), in a similar manner in each group (all data could be fitted with a common slope and a common intercept value).

The maximum percentage of GHB-appropriate responding observed with other drugs ranged from 20% (cocaine) to 42% (morphine) (Table 1). Pentobarbital, cocaine, ketamine, and morphine were all tested up to and including doses that decreased response rate, and their effects on rate (slopes of all curves significantly different from zero) were similar across the different groups (curves of each drug could be fitted with a common slope and a common intercept value).

The main finding of the present study is that animals can be trained to discriminate GHB from compounds that share pharmacological actions with GHB, i.e., the GABA<sub>B</sub> agonist baclofen and the positive GABA<sub>A</sub> modulator diazepam. This finding is further evidence that the behavioral effects of GHB and baclofen are not identical. Studying differences between GHB and baclofen may help to understand why GHB is used to treat narcolepsy, and is abused, whereas baclofen is not.

The present study explored the possibility of establishing a GHB discrimination devoid of GABA components and involving only GHB receptors. Such a discrimination would be useful as an in vivo assay of agonist and antagonist properties of GHB receptor ligands. The present study showed that animals can be trained to discriminate GHB from the GABAergic compounds baclofen and diazepam. The GHB versus other drug(s) discriminations were acquired relatively rapidly, in about 1.5 times the number of sessions needed for the acquisition of a GHB versus saline discrimination. In all three discriminations, GHB and its precursor 1,4-BD produced more than 80% GHB-appropriate responding. The GHB-like discriminative stimulus effects of GBL were less consistent than those of 1,4-BD, perhaps because of different pharmacokinetics (Carter et al., 2003). The GHB-like effects of the GABA<sub>B</sub> agonists baclofen and SKF977541 were decreased to less than 30% in rats trained to discriminate GHB from baclofen. Similarly, the GHB-like effects of diazepam were attenuated in rats trained to discriminate GHB not only from baclofen but also from diazepam. The effects of compounds pharmacologically unrelated to GHB (i.e., cocaine, ketamine, and morphine) in the GHB versus other drug(s) discriminations were similar to their effects in the GHB versus saline discrimination. Together, these findings are further evidence that animals can be trained to discriminate a particular compound from a group of other compounds and that such discriminations may increase pharmacological selectivity (Overton, 1982; Overton et al., 1989). Other methods to enhance selectivity in drug discrimination include three-lever procedures (White and Holtzman, 1981), drug versus drug training (Boja and Schechter, 1990), and the AND-OR procedure (Stolerman and Mariathasan, 1990), in which animals are trained to discriminate between a mixture and either of its component drugs alone. The AND-OR procedure is perhaps closest to the present approach, except that in the current study a drug with multiple effects was used instead of a drug mixture and that a response for saline was defined. The lack of a specific response alternative during tests of conditions that are unlike any of the training conditions can induce “nonresponding” as an alternative (Jarbe and Swedenberg, 1982). The present procedure avoids this complication by reinforcing responses on the non-GHB lever not only after drugs other than GHB but also after saline.

The selectivity of drug discrimination can be affected also by training animals to discriminate a particular dose of the training drug from a lower dose (i.e., dose-dose discrimination; Colpaert and Janssen, 1986; Kleven and Koek, 1997). For example, in two groups of rats trained to discriminate 0.04 mg/kg fentanyl from saline (drug-saline discrimination) or from 0.02 mg/kg fentanyl (dose-dose discrimination), clazocine, nalorphine, and N-allyl-normetazocine acted as partial agonists in drug versus saline discrimination, but

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**Fig. 6.** Effects of 200 mg/kg GHB 10 min after pretreatment with UMB86 (left) and effects of UMB86 alone (right, in three different groups of rats trained to discriminate 200 mg/kg GHB from saline (group 1, closed circles; n = 8), saline or 3.2 mg/kg baclofen (group 2, open circles; n = 4–5), or saline, 3.2 mg/kg baclofen, or 1 mg/kg diazepam (group 3, open squares; n = 6–8). See Fig. 2 for other details.
they were devoid of 0.04 mg/kg fentanyl-like discriminative stimulus effects and acted as complete antagonists of 0.04 mg/kg fentanyl in dose-dose discrimination (Colpaert and Janssen, 1986). Thus, it is conceivable that in the present study, the pharmacological selectivity of the GHB discrimination was enhanced, not because of qualitative differences between the effects of GHB and the other drugs, but because of quantitative differences. For example, it is possible that the animals discriminate 3.2 mg/kg baclofen from 200 mg/kg GHB because the discriminative stimulus effects of this dose of baclofen are the same as those of a dose of GHB lower than 200 mg/kg. Other findings, however, are inconsistent with the interpretation of the present findings as arising from implicit dose-dose discrimination training. First, in the present study, a significant steepening of the dose-response curve typically produced by dose-dose discrimination (Colpaert and Janssen, 1986; Kleven and Koek, 1997) was not observed. A consequence of this steepening is that the discriminative stimulus effects of the training dose are expected to be completely blocked by a lower dose of an antagonist in a dose-dose discrimination than in a drug-saline discrimination, because the dose-response curve needs to be shifted less far to the right. Indeed, naloxone more potently antagonizes the discriminative stimulus effects of fentanyl in a dose-dose discrimination (Colpaert and Janssen, 1982; Colpaert and Janssen, 1986). In the present study, however, the potency of the GABAB antagonist CGP53548 was not affected by the training conditions, and instead of being more potent, the GABAB antagonist CGP52432 was less potent in the GHB versus other drug(s) discriminations compared with the GHB versus saline discrimination. Together, these findings suggest that the GHB versus other drug(s) discriminations are not based on quantitative differences between the effects of GHB and the other drugs, but involve qualitative differences.

In the GHB versus saline discrimination, the GABAA agonists baclofen and SKF97541 both produced GHB-appropriate responding, but the maximum effect of SKF97541 seemed to be lower than that of baclofen, perhaps related to the affinity of SKF97541 for GABAA receptors, which baclofen lacks (Pan and Lipton, 1995). Although baclofen and SKF97541 no longer produced substantial GHB-appropriate responding in the GHB versus other drug(s) discriminations, GABAB receptors still seem to be involved, because the discriminative stimulus effects of GHB in these discriminations were blocked completely by the GABAB antagonists CGP53548 and CGP52432, which lack affinity for GHB sites labeled with [3H]NCS-382 (M. K. Ticku, unpublished observations). The potency of the antagonists, however, was differentially affected by the training conditions. The potency with which CGP53548 antagonized the discriminative stimulus effects of GHB in each of the three discriminations. In contrast, the potency of CGP52432 differed significantly among the discriminations. As a result, CGP52432, which was approximately 2-fold more potent than CGP53548 in the GHB versus saline discrimination, was equipotent to CGP53548 in the GHB versus baclofen discrimination, and was approximately 2-fold less potent than CGP53548 in the

### Table 1

Test results obtained with saline, GHB, DTT given before 200 mg/kg GHB, and with other drugs given alone in different groups of rats trained to discriminate 200 mg/kg GHB either from saline (group 1), saline and 3.2 mg/kg baclofen (group 2), or saline, 3.2 mg/kg baclofen, and 1 mg/kg diazepam (group 3).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Discrimination Test Compound</th>
<th>Dose</th>
<th>% GHB</th>
<th>Rate</th>
<th>% GHB</th>
<th>Rate</th>
<th>% GHB</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.13</td>
<td>0.03</td>
<td>1.12</td>
<td>0.06</td>
</tr>
<tr>
<td>GHB</td>
<td></td>
<td>200</td>
<td>0.0</td>
<td>0.0</td>
<td>1.13</td>
<td>0.04</td>
<td>0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>DTT before 200 mg/kg GHB</td>
<td></td>
<td>5.6</td>
<td>0.01</td>
<td>0.10</td>
<td>1.19</td>
<td>0.05</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.19</td>
<td>0.14</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.91</td>
<td>0.24</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Cocaine</td>
<td></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.93</td>
<td>0.12</td>
<td>0.0</td>
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<tr>
<td>Ketamine</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.95</td>
<td>0.24</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.95</td>
<td>0.24</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

% GHB, mean (±1 S.E.M.) percentage of responses on the GHB-appropriate lever; N.D., not determined due to rate-decreasing effects at this dose (i.e., response rate was <20% of control in more than half the animals tested); rate, mean (±1 S.E.M.) responses per second; and N.T., not tested at this dose.

*From Carter et al. (2005), except the data obtained with DTT.*
GHB versus baclofen or diazepam discrimination. CGP35348 and CGP52432 have different potencies at GABA<sub>B</sub> receptors modulating the release of different neurotransmitters (e.g., Bonanno et al., 1999): CGP52432 is more potent than CGP35348 at GABA<sub>B</sub> receptors modulating cholecystokinin release, equipotent at GABA<sub>A</sub> receptors modulating somatostatin release, and less potent at receptors modulating glutamate release. These findings, together with the present results, suggest the possibility that GABA<sub>B</sub> receptors modulating glutamate release are more prominently involved in the discriminative stimulus effects of GHB when GHB is discriminated from baclofen and diazepam than when GHB is discriminated from saline. Studies with other GABA<sub>B</sub> antagonists will help to further examine this differential involvement.

GHB may exert its effects not only through GABA receptors, but also through specific GHB receptors that have been investigated with [3H]NCS-382 (Mehta et al., 2001). NCS-382 was initially reported to antagonize several behavioral effects of GHB, such as self-administration in mice (Martellotta et al., 1998) and discriminative stimulus effects in rats (Colombo et al., 1995a). However, more recent studies suggest that the GHB antagonist effects of NCS-382 are limited (Carai et al., 2001; Cook et al., 2002; Lamb et al., 2003; Carter et al., 2003; Koek et al., 2004), perhaps by its own agonist effects. NCS-382 has GHB-like effects on intestinal motility (Carai et al., 2002) and GHB-like discriminative stimulus effects (Koek et al., 2004). These GHB-like effects of NCS-382 likely reflect its agonist actions at GABA<sub>B</sub> receptors. Consistent with this, NCS-382 lacked GHB-like discriminative stimulus effects under conditions in which GABA<sub>B</sub> agonists no longer produced GHB-like discriminative stimulus effects, i.e., in the GHB versus other drug(s) discriminations. However, under these conditions NCS-382 was still unable to completely antagonize the discriminative stimulus effects of GHB. Thus, at present, evidence seems to be lacking that GHB receptors are more prominently involved in the discriminative stimulus effects of GHB when animals are trained to discriminate GHB from baclofen (and diazepam). The availability of potent and selective GHB receptor antagonists would greatly facilitate delineating the role of GHB receptors in the discriminative stimulus and other effects of GHB.

Recently, we reported the synthesis of several GHB receptor-selective analogs of GHB that, unlike GHB, displace [3H]NCS-382 from GHB receptors at concentrations that do not markedly affect [3H]GABA binding to GABA<sub>B</sub> receptors and that are not metabolized to GABA-active compounds (Wu et al., 2003; Carter et al., 2005). When tested up to doses that decreased responding, these selective GHB receptor ligands did not mimic or attenuate the discriminative stimulus effects of GHB (Wu et al., 2003; Carter et al., 2005). Here, one of these ligands (i.e., UMB86) was examined in rats trained to discriminate GHB from other drugs. UMB86 did not mimic the discriminative stimulus effects of GHB in any of the three discriminations, but it tended to attenuate the discriminative stimulus effects of GHB more in rats trained to discriminate GHB from baclofen and diazepam than in the other discriminations. This suggests the possibility that UMB86 has antagonist properties at GHB receptors and that GHB receptors may be more prominently involved in the GHB versus baclofen or diazepam discrimination than in the other discriminations examined here.

DTT is thought to alter the structural stability of GABA<sub>B</sub> receptors and reportedly antagonized GABA<sub>B</sub> agonist- and GHB-induced sedation/hypnosis in mice and completely blocked the discriminative stimulus effects of GHB in rats (Carai et al., 2004). The present results, however, showed DTT to attenuate the discriminative stimulus effects of GHB only partially in each of the three discriminations. Procedural factors such as the route by which GHB was administered (p.o. in the study by Carai et al., 2004; i.p. in the present study) may account for this discrepancy. Nevertheless, the generality of the GHB-blocking effects of DTT seems to be limited.

In summary, rats can be trained to discriminate GHB from the GABA<sub>B</sub> agonist baclofen and from the positive GABA<sub>A</sub> modulator diazepam. In addition to demonstrating that the effects of GHB and baclofen are not identical, this discrimination may help to identify compounds with GHB- but not baclofen-like effects. Such compounds would be useful tools to examine the mechanisms underlying the therapeutic and abuse-related effects of GHB that baclofen seems to lack.

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References


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