Novel γ-Hydroxybutyric Acid (GHB) Analogs Share Some, but Not All, of the Behavioral Effects of GHB and GABA<sub>B</sub> Receptor Agonists


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ABSTRACT

γ-Hydroxybutyrate (GHB), a therapeutic for narcolepsy and a drug of abuse, has several mechanisms of action that involve GHB and GABA<sub>B</sub> receptors, metabolism to GABA, and modulation of dopaminergic signaling. The aim of these studies was to examine the role of GHB and GABA<sub>B</sub> receptors in the behavioral effects of GHB. Three approaches were used to synthesize GHB analogs that bind selectively to GHB receptors and are not metabolized to GABA-active compounds. Radioligand binding assays identified UMB86 (4-hydroxy-4-napthylbutanoic acid, sodium salt), UMB72 [4-(3-phenylpropyloxy)butyric acid, sodium salt], UMB73 (4-benzyloxybutyric acid, sodium salt), 2-hydroxyphenylacetic acid, 3-hydroxyphenylacetic acid (3-HPA), and 4-hydroxy-4-phenylbutyric acid as compounds that displace [3H]NCS-382 [5-[^3H]-(2E)-(5-hydroxy-5,7,8,9-tetrahydro-6H-benzo[a][7]annulen-6-ylidene) ethanoic acid] from GHB receptors at concentrations that do not markedly affect [3H]GABA binding to GABA<sub>B</sub> receptors. In rats and pigeons, GHB discriminative stimulus effects were not mimicked or attenuated by UMB86, UMB72, or 3-HPA up to doses that decreased responding. In mice, GHB, GHB precursors (γ-butyrolactone and 1,4-butanediol) and GABA<sub>B</sub> receptor agonists [SKF97541 [3-aminopropyl(methyl)phosphinic acid hydrochloride] and baclofen] dose-dependently produced hypolocomotion, catalepsy, ataxia, and loss of righting. The GABA<sub>B</sub> receptor antagonist CGP35348 (3-aminopropyl(diethoxymethyl)phosphinic acid) attenuated catalepsy and ataxia that was observed after GHB and GABA<sub>B</sub> receptor agonists SKF97541 and baclofen. UMB86, UMB72, UMB73, and 3-HPA, like GHB, produced hypolocomotion, ataxia, and loss of righting; however, catalepsy was never observed with these compounds, which is consistent with the cataleptic effects of GHB being mediated by GABA<sub>B</sub> receptors. Ataxia that was observed with UMB86, UMB72, UMB73, and 3-HPA was not antagonized by CGP35348, suggesting that ataxia induced by these analogs is not mediated by GABA<sub>B</sub> receptors and might involve GHB receptors.

γ-Hydroxybutyrate (GHB) is a putative neurotransmitter and neuromodulator in brain (Maitre, 1997), a therapeutic for alcoholism (Poldrugo and Addolorato, 1999) and narcolepsy (Fuller and Hornfeldt, 2003), and a drug of abuse (Deegenhardt et al., 2003; McDonough et al., 2004). GHB binds to GHB receptors (Benavides et al., 1982; Mehta et al., 2001) and GABA<sub>B</sub> receptors (Xie and Smart, 1992; Lingenhoehl et al., 1999), and it is metabolized to GABA, which can act at GABA receptors. GHB has also been hypothesized to modulate GABA<sub>B</sub> receptor function through GABA<sub>B</sub> receptor-mediated increases in neurosteroid concentrations in rat brain (Barbaccia et al., 2002). Furthermore, several studies have reported that GHB modulates dopaminergic transmission, although the mechanism of this modulation is not well understood (Peigenbaum and Howard, 1996).

Effects of exogenously administered GHB include sedation,
seizures, catalepsy, ataxia, amnesia, coma, and death (Snead and Liu, 1993; Navarro et al., 1998; Mason and Kerns, 2002). The wide range of behavioral effects that can be observed following administration of GHB is likely due to its multiple mechanisms of action. Specific GHB receptors have been hypothesized (Benavides et al., 1982; Snead and Liu, 1984; Hechler et al., 1987; Castelli et al., 2000; Mehta et al., 2001) and recently cloned (Andriamampandry et al., 2003). However, behavioral studies suggest an important role for GABAB receptors (Colombo et al., 1998; Lobina et al., 1999; Hechler et al., 1987; Castelli et al., 2000; Mehta et al., 2001) and recently cloned (Andriamampandry et al., 2003). However, behavioral studies suggest an important role for GABAA receptors (Bernasconi et al., 1992; Xie and Smart, 1992; Mathivet et al., 1997; Lingenhoehl et al., 1999).

The relative importance of GHB receptors in mediating behavioral effects of GHB is not clear, in part because GHB has direct and indirect effects at GABA receptors. Thus, GHB receptor-selective ligands are critical tools to examine the role of GHB receptors in the various effects of GHB and related compounds. We recently reported the synthesis of the GHB receptor-selective analog UMB68. UMB68 and GHB have similar affinities for [3H]NCS-382-labeled GHB receptors; however, unlike GHB, UMB68 does not have affinity for GABA_B receptors and does not have GHB-like discriminative stimulus effects in rats (Wu et al., 2003).

In the present study, three approaches introduced aromatic substituents on the GHB molecule to prevent metabolism to compounds with affinity for GABA receptors. First, it has recently been shown (Macias et al., 2004) that the 4-hydroxyl group of GHB acts as a hydrogen bond acceptor when bound to GHB receptors, and not as a donor, as suggested previously (Bourguignon et al., 2000). Protecting the 4-hydroxyl of GHB as an aryl alkyl ether (UMB72, UMB73, and UMB87; Fig. 1) simultaneously converts the readily oxidizable alcohol function to the more inert ether function (Block and Beale, 2004), introduces an aromatic substituent, and allows for the retention of a hydrogen bond acceptor. Second, a phenolic hydroxyl cannot undergo metabolism to an amino group and also contains the required aromatic group. The presence of such a group in 2-hydroxyphenylacetic acid (2-HPA) and 3-hydroxyphenylacetic acid (3-HPA) (Fig. 1) results in a conformationally restricted analog of GHB with a similar distance between the acid and the hydroxyl group. Third, 4-hydroxy-4-phenylbutyric acid has a sterically demanding phenyl ring in the 4-position and was previously shown to have affinity for [3H]GHB-labeled receptors (Bourguignon et al., 2000). The same report showed that introducing 4-benzyl groups in the GHB molecule further increases the affinity for [3H]GHB-labeled receptors. UMB86 (Fig. 1) has a napthyl group that occupies the same space as the phenyl group in the 4-benzyl analogs, whereas its steric bulk close to the alcohol would be expected to hinder enzymatic oxidation of the alcohol (Block and Beale, 2004).

Of the compounds synthesized, first, two or three compounds from each of the three synthetic approaches were evaluated in the binding assays to determine whether compounds from different structural classes bound selectively to GHB receptors. [3H]NCS-382 was used to examine the specific binding of GHB analogs to GHB receptors in rat cerebrocortical membranes (Mehta et al., 2001). The same analogs were also evaluated in different membrane preparations developed to study the specific binding of [3H]GABA to GABA_A or GABA_B receptors. Second, a representative compound from each chemical approach was examined in drug discrimination experiments. Drug discrimination data in rats have shown that GHB precursors and compounds that act at GABA_B receptors occasion substantial GHB-appropriate responding, whereas pharmaco-}

![Fig. 1. Structures of GHB, UMB68, 4-hydroxy-4-phenylbutyric acid, UMB86, UMB73, UMB87, UMB72, 2-HPA, and 3-HPA.](image-url)
exert their GHB-like effects through conversion to GHB, and they do not displace \(^{3}H\)GABA or \(^{3}H\)NCS-382 from rat cerebral cortex (Carter et al., 2002, 2003). Similarly, the prototypical GABA\(_A\) receptor agonists SKF97541 and baclofen serve as positive controls. Given that GHB can produce effects through multiple mechanisms of action, it is important to compare the effects of GHB to those produced by conventional GABA\(_A\) receptor agonists, as well as to related compounds that are used recreationally.

### Materials and Methods

#### Drugs

All chemicals used in the synthesis of the test compounds were purchased from Sigma-Aldrich (St. Louis, MO). All compounds showed \(^1\)H NMR (300 MHz, D\(_2\)O) and mass spectra (m/z, Finnegan LCQ, negative ion mode) consistent with their assigned structures. Elemental analyses were performed by Atlantic Microlabs Inc. (Norcross, GA) and were within ± 0.4% of theory. UMB86 was prepared by adding sodium hydroxide to a methanol solution of \(\gamma\)-napthylbutyrolactone and stirring for 2 h. After removal of the solvent, UMB86 was recrystallized from methanol (m.p. = 181–182°C). UMB72 and UMB73 were prepared by the method of Sheehan et al. (1971) (monoaalkylation of 1,4-BDL under basic conditions), followed by oxidation of the remaining hydroxyl group with pyridinium dichromate using standard conditions (Corey and Schmidt, 1978). The sodium salts of UMB72 and UMB73 were crystallized from methanol (m.p. = 228–230°C). Application of the same alkylation procedure to the phenethyl analog (UMB87) was unsuccessful, presumably due to basic elimination of phenethyl bromide to give styrene. Thus, UMB87 was prepared using the mercury-assisted alkylation method of McKillop and Ford (1974), followed by pyridinium dichromate oxidation (m.p. = 212–214°C). SKF97541 and CGP35348 were synthesized as described previously (Frostel et al., 1995). The radioligand \(^{3}H\)NCS-382 was synthesized as described previously (Mehta et al., 2001). \(^{3}H\)GABA was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). GHB, GBL, 1,4-BDL, (±)baclofen, 3-HPA, and 2-HPA were purchased from Sigma-Aldrich.

For in vivo studies, drug solutions were prepared as follows: GHB, SKF97541, CGP35348, and 3-HPA were dissolved in sterile water; baclofen was dissolved in physiological saline; GBL and 1,4-BDL were diluted in sterile water; and UMB72, UMB73, and UMB86 were suspended in a solution of physiological saline and 1% Tween 80 (Sigma-Aldrich). The pH of each drug solution was adjusted to between 5 and 9 with lactic acid or sodium hydroxide (Sigma-Aldrich), as necessary.

#### Subjects

All animals were housed individually on a 12-12 light/dark cycle (experiments conducted during the light period) with free access to water in the home cage. Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used for binding studies and drug discrimination experiments. Rats used in the binding studies had free access to food and weighed between 250 and 300 g. Rats discriminating 200 mg/kg GHB i.p. \((n = 9;\) Carter et al., 2003; Wu et al., 2003) were maintained at 340 to 360 g by providing rodent chow (rodent sterilizable diet, Harlan Teklad, Madison, WI) in the home cage after daily experimental sessions. Adult white Carneau pigeons (Columbia livia; Palmetto, Sumter, SC) discriminating 100 mg/kg GHB i.m. \((n = 6;\) Koek et al., 2004) were maintained at 80 to 90% of their free-feeding weight, ranging from 590 to 620 g, by providing mixed grain in the home cage after daily sessions. Eighty male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were used to examine directly observable effects; all mice had free access to food (rodent sterilizable diet; Harlan Teklad), weighed between 17 to 30 g, and were experimentally naive before testing. On arrival, mice were allowed at least 5 days to habituate to the experimental room, then were handled for at least 1 day prior to the start of testing. 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 (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).

#### Binding

Membranes were prepared as described previously (Mehta et al., 2001). Briefly, rats were decapitated, and the cerebral cortex and cerebellum were dissected. Tissue was stored at −80°C until it was thawed and homogenized in ice-cold 0.32 M sucrose, pH 7.4 (20 ml/g tissue), and centrifuged at 1000g for 10 min at 4°C. The supernatant was then centrifuged at 140,000g for 30 min at 4°C to obtain the mitochondrial plus microsomal (P2 + P3) fraction. This fraction was dispersed in ice-cold double-distilled deionized water and homogenized with a Brinkman Polytron at a setting of 6 for two 10-s bursts, 10 s apart. The suspension was centrifuged at 140,000g for 30 min at 4°C. The pellet was then resuspended in ice-cold Tris buffer (50 mM, pH 7.4) and centrifuged at 140,000g for 30 min at 4°C. This step was repeated twice. After the final centrifugation step, the pellet was resuspended in a small volume of ice-cold Tris buffer (50 mM, pH 7.4) and stored at −80°C. On the day of the assay, the tissue was thawed and washed twice with buffer as before (140,000 g, 30 min, 4°C) and then resuspended in the buffer for use in the assay.

\[^{3}H\]NCS-382 binding was measured using a centrifugation assay as described previously (Mehta et al., 2001). Briefly, aliquots (0.3–0.4 mg of protein) of membrane preparation in Tris buffer (50 mM, pH 7.4) were incubated with \[^{3}H\]NCS-382 (16 nM) in triplicate at 4°C for 10 min in a 1 ml total volume. Nonspecific binding was determined using NCS-382 (500 μM). The binding reaction was stopped by centrifugation (50,000g, 10 min, 4°C). The supernatant was decanted, and the vials were rapidly rinsed twice with 4 ml of ice-cold Tris buffer (50 mM, pH 7.4) without disturbing the pelleted tissue. Pellets were solubilized with 0.3 ml of Soluene-350 (Canberra Industries, Meriden, CT) for 4 to 6 h. Scintillation liquid (3 ml) was added to the solubilized material in the biowials. Radioactivity was quantified by liquid scintillation spectrometry. For determination of IC\(_{50}\) values, \[^{3}H\]NCS-382 (16 nM) binding was carried out in the absence and presence of concentrations of unlabeled compounds. \[^{3}H\]GABA (10 nM) binding to GABA\(_A\) receptors in cerebral cortex was performed in a similar manner, using a 10-min incubation period at 4°C, and GABA (100 μM) to define nonspecific binding. Binding affinity for GABA\(_A\) receptors was measured in tissue from cerebellum, an area where GHB binding to GABA\(_A\) receptors has been shown previously (Mathivet et al., 1997; Wu et al., 2003). For \[^{3}H\]GABA (10 nM) binding to GABA\(_A\) receptors in rat cerebellum, all of the assay tubes contained 40 μg isoguvacine HCl (MP Biomedicals, Irvine, CA) to displace \[^{3}H\]GABA binding to GABA\(_A\) receptors. These assay tubes also contained calcium chloride (2.5 mM), and the incubation was carried out at 25°C for 10 min. GABA (100 μM) was used to define nonspecific binding. All other assay conditions for \[^{3}H\]GABA binding were the same as those used for the \[^{3}H\]NCS-382 binding assays.

#### Drug Discrimination

Discrimination experiments using rats were conducted in commercially available operant chambers (model no. ENV-008CT; MED Associates, St. Albans, VT) located within sound-attenuating, ventilated enclosures (model no. ENV-022M; MED Associates), described in detail elsewhere (Carter et al., 2003). Data were collected using MED-PC IV software and interface (MED Associates). Rats were previously trained to discriminate 200 mg/kg GHB i.p. from saline. Discrimination training and experimental sessions were carried out as described previously (Carter et al., 2003). Immediately prior to each daily session, rats received 200 mg/kg GHB or saline i.p. and were placed into the operant chamber. A 15-min pretreatment period, during which the chamber was dark and responses (i.e., lever presses) had no programmed consequence, was followed by a 15-min response period, during which the stimulus lights above both levers were transilluminated, and 10 consecutive responses (fixed ratio 10; FR10) 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 FR requirement on the correct lever. The response period ended after 15 min or the delivery of 100 food pellets, whichever occurred first. Experimental sessions were conducted 5 to 7 days a week, and the order of training sessions was generally double alternation (e.g., saline, saline, drug, drug). All rats had satisfied the following testing criteria 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 of seven sessions (Carter et al., 2003). Subsequently, rats were required to satisfy these 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). Test sessions were identical to training sessions, except that 10 consecutive responses on either lever resulted in the delivery of food. Test compounds (UMB72, UMB86, and 3-HPA) were studied alone (given immediately prior to the 15-min pretreatment period in the operant chamber) and 10 min before the training dose of GHB, up to a dose that markedly decreased responding in more than half of the subjects (UMB72 and UMB86) or at which other limitations were apparent (e.g., solubility; 3-HPA). The order of treatment with different doses was unsystematic.

Discrimination experiments using pigeons were conducted in sound-attenuating, ventilated operant chambers (BRS/LVE, Laurel-MD) equipped with two translucent response keys that could be transilluminated by red lights. Data were collected using MED-PC IV software and interface (MED Associates) to monitor and control inputs and outputs to the operant chambers and to record the data. The procedure has been described in detail elsewhere (Koek et al., 2004). Briefly, before each daily session, subjects received either 100 mg/kg GHB or saline (i.m.) and were immediately placed into the chamber. Sessions started with a pretreatment period of 15 min, during which the lights were off and key pecks had no programmed consequence. Subsequently, the left and right keys were transilluminated red, and 20 consecutive responses (FR20) on the injection-appropriate key resulted in the key lights being extinguished for 4 s, during which time a white light illuminated the hopper where food (Purina Pigeon Checkers; Purina, St. Louis, MO) was available. Responses on the incorrect key reset the FR requirement on the correct key. The response period ended after 30 food presentations or 15 min, whichever occurred first. Experimental sessions were conducted 5 to 7 days a week, and the order of training sessions was generally double alternation (e.g., saline, saline, drug, drug). All pigeons had satisfied the following testing criteria before this study: at least 90% of the total responses on the correct key and fewer than 20 responses on the incorrect key before the first food presentation for at least seven of nine consecutive sessions (Koek et al., 2004). Thereafter, tests were conducted when these criteria were satisfied during two consecutive (drug and saline) training sessions. Test sessions were the same as training sessions, except that food was available after completion of 20 consecutive responses on either key. Test compounds were studied under the same temporal parameters, available after completion of 20 consecutive responses on either key. Thereafter, tests were conducted when these criteria were satisfied for at least 90% of the total responses on the correct key and fewer than 10 responses on the incorrect lever before delivery of the first food pellet for five consecutive sessions or six of seven sessions (Carter et al., 2003). Subsequently, rats were required to satisfy these 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). Test sessions were identical to training sessions, except that 10 consecutive responses on either lever resulted in the delivery of food. Test compounds (UMB72, UMB86, and 3-HPA) were studied alone (given immediately prior to the 15-min pretreatment period in the operant chamber) and 10 min before the training dose of GHB, up to a dose that markedly decreased responding in more than half of the subjects (UMB72 and UMB86) or at which other limitations were apparent (e.g., solubility; 3-HPA). The order of treatment with different doses was unsystematic.

Behavioral Effects in Mice. Locomotor activity was assessed using four 30-×15×15-cm customized acrylic boxes (Instrumentation Services, University of Texas Health Science Center, San Antonio) that were separately enclosed in commercially available sound-attenuating chambers (model no. ENV-022M; MED Associates). Four infrared light beams were spaced 6 cm apart and located 2 cm above the floor of each box. Occlusions of the infrared light beams were counted using commercially available computer software (Multi-Varimax version 1.00, Columbus Instruments, Columbus, OH). The floor of the boxes consisted of a parallel grid of 2.3-mm stainless steel rods mounted 6.4 mm apart or of perforated 16-gauge stainless steel with 6.4-mm round holes (9.5-mm staggered centers). Floor types were counterbalanced between animals but were always the same for individual animals. Between tests, the floor and inside of the boxes were wiped with a damp sponge, and the litter paper beneath the floor was changed. Catalepsy was measured using a 1-cm-diameter horizontal bar supported 4 cm above the floor by two 8-×8-cm square pieces of Plexiglas. Ataxia was studied by means of an inverted screen apparatus (Instrumentation Services, University of Texas Health Science Center), consisting of four 13- ×13-cm wire screens (no. 4 mesh) located 23 cm above the floor of four Plexiglas containers. The four screens were connected to a rod and handle that could be rotated 180° to simultaneously invert the four screens.

Mice were tested one to three times per week with at least 48 h between tests. Individual mice were tested on average nine times (range = 1–16). Mice were not habituated to the locomotor activity boxes; however, the number of times that an animal had been tested did not significantly affect the mean number of beam breaks during the second half of the 30-min session under control conditions (i.e., after saline administration; \(F_{2,27} = 1.10, p = 0.37\)), indicating that repeated testing of the animals did not confound the measurement of locomotion. Doses used in this study were based on preliminary dose finding data. The order in which the drugs and doses were tested was randomized within groups of 20 mice (with the exception of the largest dose of each compound studied, which was studied last due to the potential toxicity of those doses). Two groups of 20 mice were used to study the test compounds (UMB72, UMB73, UMB86, 3-HPA) alone, and two groups of 20 mice were used for the antagonism studies. On test days, one subgroup of mice (\(n = 4\)) received 10 ml/kg saline, and four subgroups of mice (\(n = 4\) each) received a dose of a particular drug; saline and drug conditions were randomly assigned to individual subgroups, and no mouse received the same dose of drug twice. All drugs were administered i.p. in a volume of 0.1 to 1.0 ml.

Immediately after a test compound was administered, mice were placed in the locomotor activity chambers, and beam breaks were recorded for 30 min. At the end of the 30-min session, mice were removed from the chambers and tested for loss of righting. Mice were placed in a supine position, and loss of righting was defined as not placing the plantar surface of any paw on the floor within 15 s. After testing for loss of righting, mice were tested for catalepsy by placing their front paws on a horizontal metal bar located 4 cm above the floor. The time that both paws remained on the bar was measured up to 30 s. After these tests, animals were briefly returned to their home cage. Within 5 to 10 min after removal from the locomotor activity boxes, mice that had not exhibited loss of righting were tested for ataxia using the inverted screen test. Animals were placed on the screens, which were rotated 180° over a 1-s period; failure to remain on the apparatus for 60 s was scored as exhibiting ataxia.

For antagonism studies, a dose of 100 or 320 mg/kg CGP35348 was given 15 min prior to 1.0 mg/kg SKF97541, 17.8 mg/kg baclofen, 560 mg/kg GHB, 560 mg/kg UMB86, 1000 mg/kg UMB72, 1780 mg/kg UMB73, or 3200 mg/kg 3-HPA. These doses were chosen because they were the smallest doses that produced ataxia (an effect that was observed with all compounds) in more than 50% of the animals when tested alone. After administration of CGP35348, animals were returned to their home cage for 15 min; all other temporal parameters were the same as when test compounds were studied alone. Locomotor activity was not studied because CGP35348 decreased locomotion when given alone (data not shown).

Data Analysis. The radioligand binding data are expressed as the mean ± S.E.M. IC50 data were analyzed using DeltaGraph (DeltaPoint, Monterey, CA). These data were analyzed for each individual experiment, and the mean ± S.E.M. was then calculated. For drug discrimination studies, the mean percentage of responses on the drug lever or key [drug-appropriate responding (%DR)] ± 1 S.E.M. and the mean rate of responding ± 1 S.E.M. were plotted as a function of dose. If during a test 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. Mean percentages of responses on the drug lever or key were calculated only when they were based on at least half of the animals tested. One-tailed, paired Student’s test was conducted.
tests (NCSS, Kaysville, UT) were used to test for differences in GHB-appropriate responding between doses of GHB analogs and saline and between doses of GHB analogs given before the training dose of GHB and the training dose of GHB. Individual animals served as their own control, and one-tailed tests were used because GHB-appropriate responding can only increase when compounds are given alone and can only decrease when compounds are given together with the training dose of GHB.

In the locomotor activity assay, beam breaks were counted in 5-min periods, and the number of beam breaks occurring in the last three periods (between 15 and 30 min after the i.p. injection) were analyzed because preliminary results showed all drugs to be maximally active within this interval. The number of beam breaks from the last three (5-min) periods were averaged and transformed to a percentage of control where the control value was the average of the mean number of beam breaks from the last three (5-min) periods after all saline tests (n = 84). For graphical presentation, transformed data from individual animals were used to calculate the mean ± 1 S.E.M. at different doses of drug. The dependent variable in the calepsy assay was the time that both paws remained on the horizontal bar, up to 30 s, which was averaged across animals. Data for the inverted screen and loss of righting assays were quantal (i.e., present or absent) and were analyzed as the percentage of animals that exhibited ataxia or loss of righting.

The ED$_{50}$ values and 95% CL for the observational procedures in mice were calculated with the Litchfield-Wilcoxon procedure (PharmTools Pro version 1.1.27; The McCary Group, Elkins Park, PA); when compounds for which ED$_{50}$ values could not be calculated were excluded from individual analyses (NCSS).

### Results

**Binding.** GHB displaced $[^{3}H]$NCS-382 with an IC$_{50}$ of 25 µM (Table 1). Three ether analogs (UMB72, UMB73, and UMB87) had lower affinity at GHB receptors, with IC$_{50}$s ranging from 195 to 352 µM. 2-HPA, the 2-phenol corresponding to GHB, had similar affinity as the ether analogs at GHB receptors with an IC$_{50}$ of 146 µM, whereas the corresponding 3-phenol (3-HPA) had higher affinity, similar to that of GHB (IC$_{50}$ = 12 µM). The 4-phenyl analog (4-hydroxy-4-phenylbutyric acid) and the 4-naphthyl analog of GHB (UMB86) had IC$_{50}$ values (48 and 13 µM, respectively) similar to GHB, in the low micromolar range.

| Compound                  | $[^{3}H]$NCS-382: GHB Receptor | $[^{3}H]$GABA  
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<td>IC$_{50}$ ± S.E.M. (µM)</td>
<td>GABA$_{A}$ Receptor</td>
</tr>
<tr>
<td>3-HPA</td>
<td>12 ± 6</td>
<td>$-44 \pm 2$</td>
</tr>
<tr>
<td>UMB86</td>
<td>13 ± 6</td>
<td>$-30 \pm 2$</td>
</tr>
<tr>
<td>GHB</td>
<td>25 ± 9$^a$</td>
<td>$-36 \pm 4^b$</td>
</tr>
<tr>
<td>4-Hydroxy-4-phenylbutyric acid</td>
<td>48 ± 1</td>
<td>$-40 \pm 2$</td>
</tr>
<tr>
<td>2-HPA</td>
<td>146 ± 15</td>
<td>$-17 \pm 1$</td>
</tr>
<tr>
<td>UMB87</td>
<td>195 ± 28</td>
<td>$-50 \pm 4$</td>
</tr>
<tr>
<td>UMB73</td>
<td>294 ± 39</td>
<td>$18 \pm 11$</td>
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<tr>
<td>UMB72</td>
<td>352 ± 37</td>
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$^a$ Mehta et al. (2001).

$^b$ Wu et al. (2003).
period in the 15 to 30 min after saline administration for all saline tests was 99.7 ± 3.8 (n = 84). This value ranged from 93.2 ± 3.9 (25–30 min) to 105.5 ± 4.2 (15–20 min) and did not significantly differ between the three 5-min periods (F2,248 = 2.28, p = 0.05). All compounds dose-dependently and completely inhibited locomotion; the order of potency to decrease locomotion was SKF97541 > baclofen > GBL > 1,4-BDL > GHB > UMB86 > UMB72 > UMB73 > 3-HPA (Table 2; Fig. 3). At larger doses, catalepsy was observed with SKF97541, baclofen, GBL, 1,4-BDL, and GHB, and not with UMB86, UMB72, and 3-HPA alone (closed circles) and together with the training dose of GHB (open circles) in rats trained to discriminate 200 mg/kg GHB i.p. (A) and in pigeons trained to discriminate 100 mg/kg GHB i.m. (B) from saline. %DR and the rate of responding (responses per second) are plotted as a function of dose. Data points and error bars in A represent the mean ± 1 S.E.M. for at least eight of nine rats, with the exception of the following doses: 560 mg/kg UMB72 + 200 mg/kg GHB (n = 5), 560 mg/kg 3-HPA + 200 mg/kg GHB (%DR, n = 6; rate, n = 7), and 1780 mg/kg 3-HPA (n = 4). Data points and error bars in B represent the mean ± 1 S.E.M. for at least five of the six pigeons, with the exception of 178 mg/kg UMB86 (n = 4). Data above SAL or GHB show the effects of saline or the training dose of GHB, respectively.

**Fig. 2.** Effects of UMB86, UMB72, and 3-HPA alone (closed circles) and together with the training dose of GHB (open circles) in rats trained to discriminate 200 mg/kg GHB i.p. (A) and in pigeons trained to discriminate 100 mg/kg GHB i.m. (B) from saline. %DR and the rate of responding (responses per second) are plotted as a function of dose. Data points and error bars in A represent the mean ± 1 S.E.M. for at least eight of nine rats, with the exception of the following doses: 560 mg/kg UMB72 + 200 mg/kg GHB (n = 5), 560 mg/kg 3-HPA + 200 mg/kg GHB (%DR, n = 6; rate, n = 7), and 1780 mg/kg 3-HPA (n = 4). Data points and error bars in B represent the mean ± 1 S.E.M. for at least five of the six pigeons, with the exception of 178 mg/kg UMB86 (n = 4). Data above SAL or GHB show the effects of saline or the training dose of GHB, respectively.

**TABLE 2**

<table>
<thead>
<tr>
<th>Locomotor Activity</th>
<th>Catalepsy</th>
<th>Ataxia</th>
<th>Loss of Righting</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHB**</td>
<td>ED50 220.8</td>
<td>98.9</td>
<td>215.0</td>
</tr>
<tr>
<td>UMB86</td>
<td>304.3</td>
<td>133.9</td>
<td>471.7</td>
</tr>
<tr>
<td>UMB72</td>
<td>422.8</td>
<td>288.3</td>
<td>619.3</td>
</tr>
<tr>
<td>UMB73</td>
<td>750.7</td>
<td>514.5</td>
<td>1098.4</td>
</tr>
<tr>
<td>3-HPA</td>
<td>1441.3</td>
<td>870.3</td>
<td>2025.5</td>
</tr>
</tbody>
</table>

N.D., value could not be determined.  
**From Carter et al. (2005).**
UMB72, UMB73, or 3-HPA (Fig. 3, middle column). Catalepsy was never observed under control conditions (data not shown). The cataleptic effects of SKF97541, baclofen, GBL, 1,4-BDL, and GHB were biphasic; catalepsy increased with smaller doses and subsequently decreased with larger doses. The order of potency of these compounds to produce catalepsy

**Fig. 3.** Locomotor activity, catalepsy, ataxia, and loss of righting in mice treated with GABAA agonists (SKF97541 and baclofen), GHB and GHB precursors (GBL and 1,4-BDL), and GHB analogs (UMB86, UMB72, UMB73, and 3-HPA). Compounds are arranged from most potent (SKF97541) to least potent (3-HPA) to decrease locomotor activity. Locomotor activity is plotted as the average ± S.E.M. percentage of control of the number of beam breaks that were observed under saline conditions for individual animals. The average (± S.E.M.) number of beam breaks per 5-min period in the 15 to 30 min after saline administration for all saline tests was 99.7 ± 3.8 (n = 84). Catalepsy is plotted as the average time ± S.E.M. that animals exhibited catalepsy up to 30 s. Ataxia and loss of righting (LOR) are plotted as the percentage of animals that exhibited these signs. All behavioral effects are shown as a function of dose of drug (milligrams/kilogram) using the same abscissa. n = 8 in all cases except 56 mg/kg baclofen (n = 4). GHB data are from Carter et al. (2005).
was SKF97541 > baclofen > GBL > 1,4-BDL > GHB and was highly correlated with their potency to decrease locomotion \((r = 0.998)\).

At larger doses, ataxia and loss of righting were observed, with average ED\(_{50}\) values that were 1.6 ± 0.2 and 4.5 ± 0.6 times greater than those for decreasing locomotion, respectively (Table 2). The potency to produce ataxia and loss of righting was highly correlated with the potency to decrease locomotion \((r = 0.993\) and 0.996, respectively). UMB73 and 3-HPA failed to produce loss of righting in more than 50% of the animals. Larger doses were not tested because 75 and 87% lethality was observed following 1780 mg/kg UMB73 and 3200 mg/kg 3-HPA, respectively (data not shown). Lethality was generally observed at least 24 h after administration and also occurred after doses of 1,4-BDL (25% at 1780 mg/kg), UMB72 (12.5% at 1000 mg/kg and 62.5% at 1780 mg/kg), and UMB86 (50% at 1000 mg/kg and 100% at 1780 mg/kg) but was never observed following administration of SKF97541, baclofen, GBL, or GHB.

The GABA\(_B\) receptor antagonist CGP35348 decreased the number of animals that exhibited ataxia after receiving 1 mg/kg SKF97541, 17.8 mg/kg baclofen, or 560 mg/kg GHB (Fig. 4). CGP35348 failed to attenuate the ataxia that was observed following administration of 560 mg/kg UMB86, 1000 mg/kg UMB72, 1780 mg/kg UMB73, or 3200 mg/kg 3-HPA. A dose of 320 mg/kg CGP35348 completely blocked catalepsy in mice treated with SKF97541 or baclofen (data not shown). Catalepsy increased from 15.6 ± 4.9 s to the maximum of 30.0 s when 100 mg/kg CGP35348 was given prior to 560 mg/kg GHB. A larger dose of CGP35348 (320 mg/kg) together with GHB decreased catalepsy to 8.3 ± 3.3 s (data not shown).

**Discussion**

The main finding of the present study is that selective GHB analogs shared some, but not all, of the effects of GHB. Three chemical approaches were taken to synthesize GHB analogs that could not be metabolized to GABAergic ligands. Radioligand binding studies identified several GHB analogs that were selective for GHB receptors, and a selective compound from each chemical class was studied in several behavioral procedures. Selective GHB analogs (UMB86, UMB72, and 3-HPA) did not occasion GHB-like discriminative stimulus effects in rats and pigeons, or catalepsy in mice. However, UMB86, UMB72, 3-HPA, and UMB73, like GHB, decreased locomotion and, at larger doses, produced ataxia. The ataxic effects of these compounds were not attenuated by CGP35348, suggesting that GHB receptors might mediate the effects of these compounds and contribute to the effects of GHB and GHB precursors.

Increasing evidence (e.g., cross-substitution between GHB and baclofen) in rats and pigeons suggests that GABAergic activity is a major component of the discriminative stimulus effects of GHB (Colombo et al., 1998; Lobina et al., 1999; Carter et al., 2003, 2004a; Baker et al., 2004; Koek et al., 2004). Thus, compounds that do not bind to GABA receptors might not be expected to produce or attenuate GHB-like discriminative stimulus effects, which is consistent with the relatively small amount of GHB-appropriate responding observed after administration of selective GHB receptor ligands (Wu et al., 2003; current study) and the failure of UMB86, UMB72, or 3-HPA to fully antagonize the discriminative stimulus effects of the training dose in either rats or pigeons. However, the apparent partial effects of 1780 mg/kg 3-HPA alone in rats or 178 mg/kg UMB72 together with the training dose of GHB in rats and pigeons might suggest a small
GHB receptor-specific analogs, which unlike baclofen, and extend these findings to structurally related analogs of GHB receptor agonists on catalepsy were biphasic is likely responsible for the effects that these compounds share with GHB receptors but rather to GABA<sub>B</sub> receptor-mediated inhibition of neurotransmitter release, a mechanism of action that is not shared with the selective GHB analogs used in this study.

The novel GHB receptor-selective analogs in this study have begun to serve as pharmacological tools to examine the structure-activity relationship of GHB receptors and the contribution of GHB receptors to the behavioral profile of GHB and related compounds. The present data support previous findings that the discriminative stimulus effects of GHB involve actions at GABA<sub>B</sub> receptors and further provide evidence that the cataleptic and ataxic effects of GHB are dependent on activity at GABA<sub>B</sub> receptors. UMB86, UMB72, UMB73, and 3-HPA lack affinity for GABA receptors; thus, activity at non-GABAergic (e.g., GHB) receptors might be responsible for the effects that these compounds share with GHB (e.g., ataxia) that are not attenuated by the GABA<sub>B</sub> receptor antagonist CGP35348. Because the affinity of some of these compounds for GHB receptors in vitro differs from their potency in vivo (e.g., 3-HPA), other factors such as bioavailability and efficacy might contribute to the differences observed between these compounds and GHB.

To the extent that different behavioral effects of GHB are mediated by different mechanisms (e.g., GHB, GABA<sub>B</sub>, or other receptors), compounds that are selective for GHB receptors might retain some of the therapeutic effects of GHB, while having fewer undesired effects. The present studies have identified UMB86, UMB72, and 3-HPA as selective GHB analogs that exhibit some, but not all, of the behavioral effects of GHB and its precursors GBL and 1,4-BDL; these compounds will be helpful tools to investigate the role of GHB receptors in the behavioral profile of GHB.

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**References**


Barbaccia ML, Colombo G, Africano D, Carai MA, Vasca G, Melis S, Purdy RH, and

It is well established that GABA<sub>B</sub> receptor activation inhibits neurotransmitter release in brain and spinal cord (for review, see Bowery et al., 1980, 2002). Administration of GHB (Feigenbaum and Howard, 1996) or baclofen (Bowery et al., 1980; Balsara et al., 1981) inhibits presynaptic dopamine release in unanesthetized animals, which is likely to account for their neuroleptic-like behavioral effects (e.g., catalepsy; Balsara et al., 1981; Hechler et al., 1993; Itzhak and Ali, 2002). Catalepsy produced by neuroleptics is enhanced by GHB (Navarro et al., 1998) or baclofen (Davies and Williams, 1978; Richardson and Richardson, 1982), although the cataleptic effects of neuroleptics and GABA<sub>B</sub> receptor agonists involve different mechanisms (Sevak et al., 2004). Haloperidol-induced catalepsy is attenuated by the N-methyl-D-aspartate receptor antagonist dizocilpine, whereas dizocilpine enhances GHB-induced catalepsy (Sevak et al., 2004). Together, these findings suggest that GHB- and baclofen-induced catalepsy is not due to antagonism at dopamine receptors but rather to GABA<sub>B</sub> receptor-mediated inhibition of neurotransmitter release, a mechanism of action that is not shared with the selective GHB analogs used in this study.

The absence of catalepsy following the administration of GHB to rats (Winter, 1981; Wu et al., 2003; Carter et al., 2004a) could play a more predominant role in mediating the effects of GHB that are observed at larger doses.

In observational studies in mice, SKF97541, baclofen, GBL, 1,4-BDL, GHB, UMB86, UMB72, UMB73, and 3-HPA dose-dependently decreased locomotor activity, produced ataxia, and produced loss of righting. These results are consistent with the sedative and muscle relaxant properties of GHB, GHB precursors, and GABA<sub>B</sub> receptor agonists (Carai et al., 2001, 2002a,b; Cook et al., 2002; Quang et al., 2002) and extend these findings to structurally related analogs of GHB. These GHB receptor-specific analogs, which unlike GHB and GABA<sub>B</sub> receptor agonists do not readily bind to GABA<sub>B</sub> receptors, did not produce catalepsy when studied up to lethal doses. This finding is similar to other studies showing that transgenic mice that do not express functional GABA<sub>B</sub> receptors do not exhibit hypolocomotion or hypothermia after administration of baclofen, GHB, or GBL (Kaufmann et al., 2003; Queva et al., 2003). The order of potency of SKF97541, baclofen, GBL, 1,4-BDL, GHB, UMB86, UMB72, UMB73, and 3-HPA was the same across the assays employed in this study, including the potency to produce catalepsy, when it was observed. In general, doses required to produce ataxia and loss of righting were larger than those needed to decrease locomotor activity or produce catalepsy.

It is known that peripheral (i.p.; Mehta and Ticku, 1987) and central (ventromedial thalamic nucleus; Wüllner et al., 1987) administration of baclofen produces catalepsy in rats. Baclofen-induced catalepsy is blocked by the GABA<sub>B</sub> receptor antagonist δ-aminovaleic acid, and not by bicuculline, bromocriptine, or scopolamine, indicating that the effect is mediated by GABA<sub>B</sub> receptors (Mehta and Ticku, 1987). The presence of catalepsy following a dose of 320 mg/kg GHB i.p. in this study is consistent with GHB having agonist-like activity at GABA<sub>B</sub> receptors (Lingenhoehl et al., 1999) and with previous reports of catalepsy following a dose of 300 mg/kg GHB i.p. in Swiss-Webster mice (Itzhak and Ali, 2002) and 560 mg/kg GHB i.p. in Sprague-Dawley rats (Sevak et al., 2004). That the effects of GHB, GHB precursors, and GABA<sub>B</sub> receptor agonists on catalepsy were biphasic is likely due to other effects of these compounds that interfere with catalepsy at larger doses, such as ataxia and muscle atonia.

The absence of catalepsy following the administration of selective GHB analogs UMB86, UMB72, UMB73, and 3-HPA and the attenuation of catalepsy produced by SKF97541, baclofen, and GHB by CGP35348 provides strong evidence that activity at GABA<sub>B</sub> receptors is necessary for catalepsy produced by GHB and its precursors in mice.


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