Comparison of the behavioral effects of gamma-hydroxybutyric acid (GHB) and its 4-methyl-substituted analog, gamma-hydroxyvaleric acid (GHV)

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Abstract

Gamma-hydroxybutyrate (GHB), a metabolite of GABA, is a drug of abuse and a therapeutic. The illicit use of GHB precursors and analogs reportedly has increased worldwide. Gamma-hydroxyvaleric (GHV) is a 4-methyl-substituted analog of GHB that reportedly is abused and is marketed as a dietary supplement and replacement for GHB. The purpose of these studies was to compare the pharmacological and behavioral profiles of GHV and GHB. In radioligand binding studies, GHV completely displaced [^{3}H]NCS-382 with approximately 2-fold lower affinity than GHB and did not markedly displace [^{3}H]GABA from GABA$_B$ receptors at a 20-fold larger concentration. In drug discrimination procedures, GHV did not share discriminative stimulus effects with GHB or baclofen. GHV shared other behavioral effects with GHB, such as sedation, catalepsy, and ataxia, although larger doses of GHV were required to produce these effects. Lethality (50%) was observed after the largest dose of GHV (5600 mg/kg), a dose that produced less-than-maximal catalepsy and ataxia. To the extent that large doses of GHV might be taken to in an attempt to produce GHB-like effects (e.g., hypnosis) GHV toxicity may pose a greater public health concern than GHB.
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1. Introduction

Gamma-hydroxybutyrate (GHB) is a metabolite of GABA (Doherty et al., 1975; Gold and Roth, 1977) and a putative neurotransmitter or neuromodulator in brain (Maitre, 1997). The endogenous GHB system has been shown to play an important role in several homeostatic processes, including the regulation of sleep (Mamelak et al., 1977; Lapierre et al., 1990) and metabolic oxygen demand (Boyd et al., 1992; Ottani et al., 2003). In addition to its role in maintaining homeostasis, exogenous administration of GHB has been re-

ported to be reinforcing in rodents. GHB has been shown to produce conditioned place preference in rats (Martellotta et al., 1997) and to maintain intravenous self-administration in mice (Martellotta et al., 1998). Consistent with these data is the worldwide increase in reports of GHB use, overdose, and withdrawal (Nicholson and Balster, 2001; Degenhardt et al., 2002; Freese et al., 2002; Mason and Kerns, 2002; McDonough et al., 2004).

With the placement of GHB into Schedule I of the Controlled Substances Act in March of 2000, the illicit use of GHB precursors reportedly has increased in the United States (Mason and Kerns, 2002; Lora-Tamayo et al., 2003; United States Department of Justice, 2003). Gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BDL) are two GHB precur-
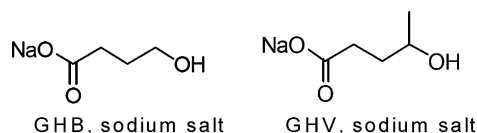

Fig. 1. Structures of gamma-hydroxybutyrate (GHB) and gamma-hydroxyvalerate (GHV) as sodium salts.

sors that share effects with GHB in vivo, including sedation (Carai et al., 2002; de Fiebre et al., 2004), hypothermia (de Fiebre et al., 2004), discriminative stimulus effects (Carter et al., 2003; Baker et al., 2004), EEG effects (absence seizures; Poldrugo and Snead, 1984; Snead, 1991), and neuroprotective effects against ischemia (MacMillan, 1980). GBL and 1,4-BDL do not bind to GHB receptors or GABA$_B$ receptors (Carai et al., 2002), rather, they are metabolized to GHB by peripheral lactonases and alcohol dehydrogenase, respectively (Roth and Giarman, 1966; Maxwell and Roth, 1972). Thus, the behavioral effects of GBL and 1,4-BDL are thought to be due to the metabolic conversion of these compounds to GHB (Carai et al., 2002; Quang et al., 2002).

Gamma-hydroxyvaleric acid (GHV) is a 4-methyl-substituted analog of GHB (Fig. 1) that binds to GHB receptors (Bourguignon et al., 1988) and, unlike GBL or 1,4-BDL, is not metabolized to GHB. Despite the lack of direct conversion to GHB, there is evidence that GHV shares some effects with GHB. Specifically, GHV is reported to be used recreationally as a substitute for GHB (United States Department of Justice, 2003), and several anecdotal comparisons of the effects of GHV and the GHV precursor gamma-valerolactone (GVL) to those of GHB and GBL can be found on the internet. Furthermore, the continued use of GVL as a substitute for GHB is apparent by the fact that GVL is listed as the (legal) active ingredient in GHB-related dietary supplements such as Tranquili-G (Smartbodyz Nutrition, patent pending).

Given a recent worldwide increase in clinical and forensic cases related to GHB intoxication and withdrawal (Degenhardt et al., 2002; Freese et al., 2002; Mason and Kerns, 2002; McDonough et al., 2004), there are compelling reasons to understand the mechanism of action, and the potential similarities and differences among GHB, GHB precursors, and GHB analogs that are used recreationally and therapeutically. The purpose of these studies was to compare the pharmacological and behavioral profiles of GHV and GHB. Radioligand binding was used to examine GHB and GHV binding to GHB, GABA$_A$, and GABA$_B$ receptors. Pharmacologically selective GHB discrimination procedures in rats and pigeons were used to determine whether GHV occasioned GHB-like discriminative stimulus effects in either of these species; the discriminative stimulus effects of GHB in rats have been shown to be mediated predominantly by GABA$_B$ receptors (Colombo et al., 1998; Carter et al., 2003), whereas the discriminative stimulus effects of GHB in pigeons appear to involve not only GABA$_B$ receptors, but also GABA$_A$ receptors (Koek et al., 2004). GHV was also studied in rats discriminating the GABA$_B$ agonist baclofen

because GHB and some GHB precursors share discriminative stimulus effects with baclofen (Carter et al., 2004). In addition to discriminative stimulus effects, the behavioral effects of larger doses of GHB and GHV were compared using locomotor activity, catalepsy, ataxia, and righting as dependent measures in C57/Bl6 mice. These procedures allowed for the comparison of several behavioral effects of these compounds over a broad range of doses.

2. Materials and methods

2.1. Subjects

All animals were housed individually on a 12 h light:12 h dark cycle (experiments conducted in 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 when they were sacrificed. Rats that had been trained to discriminate 200 mg/kg GHB ($n = 10$; Wu et al., 2003) or 3.2 mg/kg baclofen ($n = 11$; Carter et al., 2004) were maintained between 340 and 360 g by providing 5–16 g of 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) that were trained to discriminate 100 mg/kg GHB ($n = 6$; Koek et al., 2004), were maintained between 80 and 90% of their free-feeding weight, ranging from 590 to 620 g, by providing mixed grain in the home cage after daily sessions. Forty male C57/Bl6 mice (The Jackson Laboratory, Bar Harbor, ME) were used for observation experiments; all mice had free access to food (Rodent sterilizable diet, Harlan Teklad, Madison, WI) and were experimentally naïve before testing. On arrival, mice were allowed at least 5 days to habituate to the experimental room, then were handled for 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 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. Binding

2.2.1. Membrane preparation

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 1000 × *g* for 10 min at 4 °C. The supernatant was then centrifuged at 140,000 × *g* for 30 min at 4 °C to obtain the mito-

chondrial 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 $140,000 \times g$ for 30 min at $4\,°C$. The pellet was then resuspended in ice-cold Tris buffer (50 mM, pH 7.4) and centrifuged at $140,000 \times g$ for 30 min at $4\,°C$. This step was repeated twice. After the final centrifugation step, the pellet was suspended 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 ($140,000 \times g$, 30 min, $4\,°C$) and then resuspended in the buffer for use in the assay.

2.2.2. [³H]NCS-382 and [³H]GABA binding assays

[³H]NCS-382 binding was measured using a centrifugation assay as described previously (Mehta et al., 2001). Briefly, aliquots (0.3–0.4 mg protein) of membrane-preparation in Tris buffer (50 mM, pH 7.4) were incubated with [³H]NCS-382 (16 nM) in triplicate at $4\,°C$ for 20 min in a 1 ml total volume. Non-specific binding was determined using NCS-382 (500 μM). The binding reaction was stopped by centrifugation ($50,000 \times g$, 10 min, $4\,°C$). The supernatant was decanted, and the vials were rapidly rinsed twice with 4 ml ice-cold Tris buffer (50 mM, pH 7.4) without disturbing the pelleted tissue. Pellets were solubilized with 0.3 ml of Soluene-350 (Packard, Meriden, CT) for 4–6 h. Scintillation liquid (3 ml) was added to the solubilized material in the bio-vials. Radioactivity was quantified by liquid scintillation spectrometry. For determination of IC_{50} values, [³H]NCS-382 (16 nM) binding was carried out in the absence and presence of concentrations of unlabeled GHB or GHV. [³H]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 non-specific binding. Binding affinity for $GABA_B$ receptors was measured in tissue from cerebellum, an area where GHB binding to $GABA_B$ receptors has been shown previously (Mathivet et al., 1997; Wu et al., 2003). For [³H]GABA (10 nM) binding to $GABA_B$ receptors in rat cerebellum, all of the assay tubes contained 40 μM isoguvacine HCl (ICN Biomedicals Inc., Costa Mesa, CA) to displace [³H]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 non-specific binding. All other assay conditions were similar to those used for the [³H]NCS-382 binding assays.

2.3. Discriminative stimulus effects

2.3.1. GHB discrimination—pigeon

Experiments were conducted in sound attenuating, ventilated chambers (BRS/LVE, Laurel, MD) equipped with two response keys that could be transilluminated by red lights. Chambers were connected by an interface (MED Associates Inc., St. Albans, VT) to a computer that used MED-PC IV software (MED Associates Inc.) to monitor and control inputs and outputs 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 the right keys were transilluminated red and 20 consecutive responses 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, St. Louis, MO) was available. Responses on the incorrect key reset the fixed ratio (FR) requirement on the correct key. The response period ended after 30 food presentations or 15 min, whichever occurred first. Experimental sessions were conducted 5–7 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.

2.3.2. GHB and baclofen discriminations—rat

Experimental sessions were conducted in commercially-available, sound-attenuating, ventilated enclosures (Model #ENV-022M and ENV-008CT; MED Associates Inc., St. Albans, VT), described in detail elsewhere (Carter et al., 2003). Data were collected using MED-PC IV software (MED Associates, St. Albans, VT) and a PC interface. Different groups of rats were trained to discriminate 200 mg/kg GHB or 3.2 mg/kg baclofen from saline. Discrimination training and experimental sessions were carried out as described previously (Carter et al., 2003; Carter et al., 2004). Immediately prior to each daily session, rats received an i.p. injection of saline or the training dose of a training drug and were placed into the operant chamber. A 15-min pretreatment period, during which the chamber was dark and responses had no programmed consequence, was followed by 15-min response period, during which the lights above both levers were transilluminated and 10 responses (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 5–7 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 out of seven sessions (Carter et al., 2003; Wu 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 with the exception that completion of the FR on either lever resulted in the delivery of food.

2.4. Behavioral effects in mice

Locomotor activity was assessed using four 30 cm × 15 cm × 15 cm customized acrylic boxes (Instrumentation Services, University of Texas Health Science Center at San Antonio) that were separately enclosed in commercially-available sound-attenuating chambers (Model #ENV-022M; MED Associates Inc., St. Albans, VT). Four infrared light beams were spaced 6 cm apart and located 2 cm from the floor of each box. Occlusions of the infrared light beams were counted using commercially available computer software (Multi-Varimex v1.00, Colombus Instruments, Colombus, 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. The floors and inside of the boxes were wiped with a damp sponge and the litter paper beneath the floors was changed between animals. Catalepsy was measured using a 1 cm diameter horizontal bar supported 4 cm above the floor by two 8 cm × 8 cm pieces of Plexiglas. The inverted screen apparatus (Instrumentation Services, University of Texas Health Science Center at San Antonio) consisted of four 13 cm × 13 cm wire screens (No. 4 mesh) located 23 cm above the floor of four Plexiglas containers. Screens were connected to a rod and handle, which could be rotated 180° to simultaneously invert the four screens.

Animals were tested 1–3 times per week with at least 48 h between tests. Doses used in this study were based on preliminary dose finding data. The order in which the doses were tested was randomized (with the exception of the largest dose of GHV, which was studied last due to the lethal effects of this dose). On test days, one group of mice ($n = 4$) received 10 ml/kg saline and four groups of mice ($n = 4$ each) received a dose of a particular drug; saline and drug conditions were randomly assigned to individual groups of mice and no mouse received the same dose of drug twice. All compounds were administered i.p. in a volume of 0.1–1.0 ml.

Immediately after the injection, mice were placed in the boxes. Locomotor activity was measured for 30 min in six, 5-min periods. At the end of the 30-min session, mice were removed from the boxes 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, the front paws of the mouse were placed 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 5–10 min following removal from the locomotor activity boxes, mice that did not exhibit 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 climb to the top or to cling to the bottom of the screen for 60 s was scored as failing the test.

2.5. Data analysis

The radioligand binding data are expressed as the mean ± 1 S.E.M. IC_{50} data were analyzed using DeltaGraph (DeltaPoint, USA). These data were analyzed for each individual experiment, and the mean ± 1 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 percentage of responses on the drug lever or key values were calculated only when they were based on at least half of the animals tested. In the locomotor activity assay, beam breaks occurring between 15 and 30 min after the i.p. injection were used as the dependent variable because preliminary results showed all drugs to be maximally active within this interval. For graphical presentation, data from the 15-min observation period were transformed to a percent of control values that represent the average number of beam breaks in the 15-min period after all saline tests ($n = 84$). Individual animal data were used to calculate the mean ± 1 S.E.M. at different doses of drug. The dependent variable in the catalepsy 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., pass or fail), and those data were analyzed as the percentage of animals that exhibited ataxia or loss of righting.

2.6. Drugs

The radioligand [^{3}H]NCS-382 (5-[^{3}H]-(2E)-(5-hydroxy-5,7,8,9-tetrahydro-6H-benzo[a][7] annulen-6-ylidene) ethanoic acid) was synthesized as described earlier (Mehta et al., 2001). [^{3}H]GABA was purchased from Perkin-Elmer Life Sci. (Boston, MA). All chemicals used in the synthesis of GHV were purchased from Sigma–Aldrich (USA). All compounds showed ^{1}H NMR (300 MHz, D_2O) and mass spectral (m/z, Finnegan LCQ, negative ion mode) spectra consistent with their assigned structures. Elemental analyses were performed by Atlantic Microlabs Inc. (USA), and were within

±0.4% of theory. GHB sodium salt and (±)baclofen were purchased from Sigma–Aldrich (USA).

For in vivo studies, drugs were dissolved in sterile water or physiological saline. The pH of each drug solution was adjusted to 5–9 with lactic acid or sodium hydroxide (Sigma–Aldrich, St. Louis, MO) and water, as necessary. Drugs were administered i.p. in rats and mice, and i.m. in pigeons. GHV was studied alone, and when given 10 min before the training dose of GHB or baclofen in drug discrimination studies. The order of treatment with different doses was unsystematic.

3. Results

3.1. Binding

GHB and GHV fully displaced [3H]NCS-382 from rat cerebrocortical membranes (Fig. 2). The affinity of GHV for [3H]NCS-382-labeled GHB receptors was about 2-fold lower than that of GHB; however, both compounds exhibited IC$_{50}$ values in the micromolar range (52 and 25 μM, respectively, Table 1). At 20–40-fold larger concentrations of
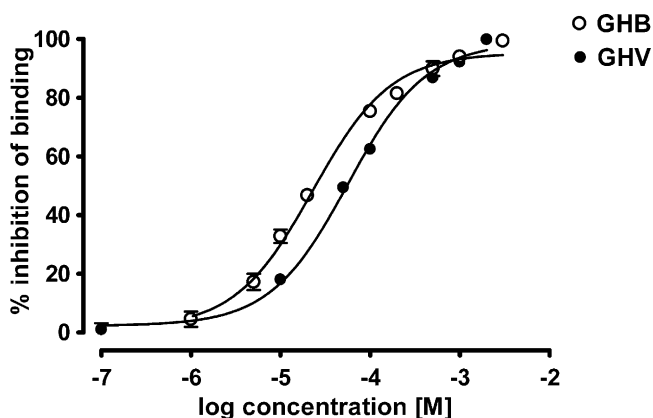

Fig. 2. Concentration-dependent inhibition of [3H]NCS-382 (16 nM) binding to rat cerebrocortical membranes by GHB or GHV. Each point represents the mean ± 1 S.E.M. of individual experiments (GHV, $n = 3$; GHB, $n = 5$). Each experiment was performed in triplicate. Data for GHB are from Mehta et al. (2001).

Table 1
IC$_{50}$ values of GHB and GHV using [3H]NCS-382 (16 nM) as a radioligand in rat cerebrocortical membranes and effect of GHB and GHV (1 mM) on [3H]GABA (10 nM) binding to GABA$_A$ receptors in rat cerebrocortical membranes and GABA$_B$ receptors in rat cerebellar membranes

Compound	[3H]NCS-382 IC$_{50}$ ± S.E.M. (μM)	[3H]GABA% change in binding	
		GABA$_A$ receptor	GABA$_B$ receptor
GHB	25 ± 2[a]	−36 ± 4[b]	−41 ± 3[b]
GHV	52 ± 5	−37 ± 5	−18 ± 5

Each value is the mean ± 1 S.E.M. of at least three experiments, and each experiment was performed in triplicate.
[a] Mehta et al. (2001).
[b] Wu et al. (2003).

GHB or GHV (1 mM), [3H]GABA binding to GABA$_A$ receptors was inhibited 36–37%. Similarly, GHB displaced 41% of [3H]GABA binding from GABA$_B$ receptors at the same concentration, though GHV had lower affinity for GABA$_B$ receptors, displacing 18% of binding at 1 mM.

3.2. Discriminative stimulus effects

Under test conditions, the training dose of GHB (100 mg/kg) occasioned 99.0 ± 0.7% drug-appropriate responding in pigeons (Fig. 3, top left panel, open circles). Saline did not occasion substantial drug-appropriate responding (2.6 ± 0.2%; data not shown). GHB dose-dependently increased responding on the drug-associated key. GHV occasioned, at most, 3% GHB-appropriate responding in pigeons (Fig. 3, top left panel, closed circles) up to doses that markedly decreased responding. Administration of GHB or GHV decreased the rate of responding (Fig. 3, bottom left panel). GHV was about 10-fold less potent than GHB to decrease food-maintained responding in pigeons.

In rats discriminating GHB or baclofen from saline, GHB dose-dependently occasioned responding on the drug-appropriate lever (Fig. 3, top center and right panels, open circles). Saline did not occasion substantial drug-appropriate responding in either group of animals (0.4 ± 0.2% and 0.1 ± 0.1%, respectively; data not shown). GHV occasioned, at most, 12% GHB-appropriate responding and 17% baclofen-appropriate responding when tested up to doses that markedly decreased responding (Fig. 3, top center and right panels, closed circles). GHV decreased food-maintained responding in rats across the range of doses studied, being about 3-fold less potent than GHB (Fig. 3, bottom center and right panels).

When GHV was given prior to the training dose of GHB in pigeons, at least 86% of responding occurred on the GHB-appropriate lever at all doses of GHV studied (Table 2). In rats trained to discriminate GHB, the training dose of GHB occasioned 84.8 ± 10.8% drug-appropriate responding. When 560 mg/kg GHV was given prior to the training dose of GHB, 79.3 ± 14.5% of responding occurred on the GHB-appropriate lever (Table 2). A larger dose of 1000 mg/kg GHV decreased the drug-appropriate responding elicited by 200 mg/kg GHB to 52.2 ± 18.9% without markedly altering rate of responding as compared to this dose of GHB administered alone. In rats trained to discriminate baclofen, the training dose of baclofen occasioned 89.2 ± 10.6% drug-appropriate responding. Doses of GHV (560–1780 mg/kg) given prior to the training dose of baclofen resulted in 56–60% drug-appropriate responding (Table 2).

3.3. Behavioral effects in mice

In C57/Bl6 mice, GHB and GHV dose-dependently decreased locomotor activity, with GHV being about 10-fold less potent than GHB (Fig. 4). Across the same range of
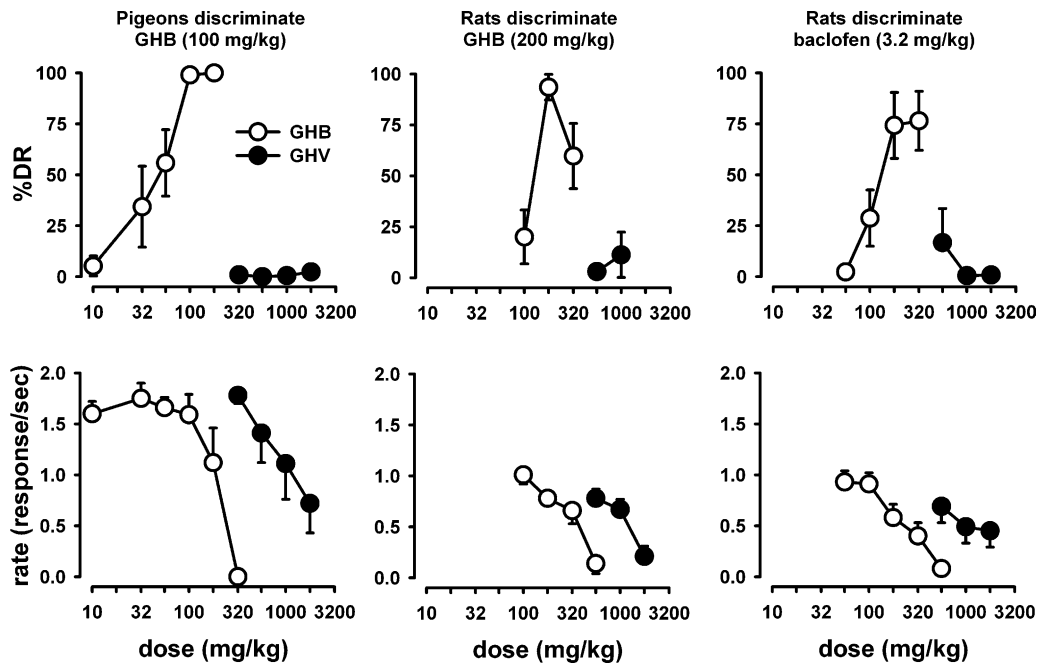
Fig. 3. Effects of GHB and GHV in pigeons that discriminate 100 mg/kg GHB (left column), in rats that discriminate 200 mg/kg GHB (middle column), and in rats that discriminate 3.2 mg/kg baclofen (right column) from saline. The percentage of responses on the drug-appropriate lever or key (%DR) and the rate of responding (responses/second) are plotted as a function of dose. In each discrimination procedure, administration of the training dose occasioned more than 84% drug-appropriate responding; administration of saline occasioned less than 3% drug-appropriate responding. Data points and error bars in the left column represent the mean ± 1 S.E.M. for at least 5 of the 6 pigeons, with the exception of 1780 mg/kg GHV ($n = 3$); data points and error bars in the middle column represent the mean ± 1 S.E.M. for at least 9 of the 10 rats tested; data points and error bars in the right column represent the mean ± 1 S.E.M. for at least 7 of the 11 rats with GHB and at least 5 of the 8 rats with GHV.

doses, GHB and GHV produced catalepsy. The cataleptic effects of GHB were biphasic; catalepsy increased with smaller doses and decreased with larger doses (Fig. 4). The potency of GHV to produce catalepsy was at least 10-fold less that that of GHB. The magnitude of catalepsy that was observed fol-

Table 2
Percent drug-appropriate responding and rate of responding following doses of GHV given prior to the training drug in three drug discrimination procedures

Discrimination	Dose (mg/kg) GHV	%DR ± S.E.M.	Rate ± S.E.M.	N
100 mg/kg	0	99.0 (0.7)	1.59 (0.20)	6
GHB—pigeon	560	99.3 (0.3)	1.75 (0.14)	6
	1000	98.0 (1.4)	1.35 (0.30)	5
	1780	86.2 (13.2)	1.35 (0.18)	6
200 mg/kg	0	84.8 (10.8)	0.61 (0.14)	9
GHB—rat	560	79.3 (14.5)	0.66 (0.12)	7
	1000	52.2 (18.9)	0.60 (0.12)	7
	1780	ND	0.20 (0.18)	3
3.2 mg/kg	0	89.2 (10.6)	1.00 (0.10)	9
Baclofen—rat	560	57.0 (20.0)	0.78 (0.14)	7
	1000	56.4 (19.7)	0.65 (0.15)	7
	1780	60.0 (24.3)	0.46 (0.15)	5

ND: not determined because less than half of the animals responded at this dose.

lowing GHV administration (13.6 ± 5.0 s) was less than that observed following administration of GHB (24.8 ± 3.8 s).

At very large doses, GHB and GHV dose-dependently produced ataxia. GHV was approximately 10-fold less potent than GHB in producing ataxia; 62.5% of the subjects failed the inverted screen test following a dose of 5600 mg/kg GHV, whereas all of the animals failed the inverted screen test after 1000 mg/kg GHB. At larger doses, GHB produced loss of righting with all of the animals failing the righting test at a dose of 3200 mg/kg GHB. In contrast, GHV did not produce loss of righting at any dose tested, up to doses that resulted in lethality (Fig. 4). Lethality was generally observed at least 24 h after administration and occurred in 50% of the animals tested with a dose of 5600 mg/kg GHV.

4. Discussion

The importance of GHB and the GHB system in regulating central nervous system function is an emerging concept in neurobiology (Cash, 1996; Howard and Feigenbaum, 1997; Maitre, 1997; Wong et al., 2004). GHB is used therapeutically to treat the sleep disorder narcolepsy in the United States (Fuller and Hornfeldt, 2003) and to treat alcoholism in Europe (Caputo et al., 2003; Poldrugo and Addolorato, 1999); however, the precise mechanisms by which GHB produces its therapeutic and abuse-related effects remain unclear.
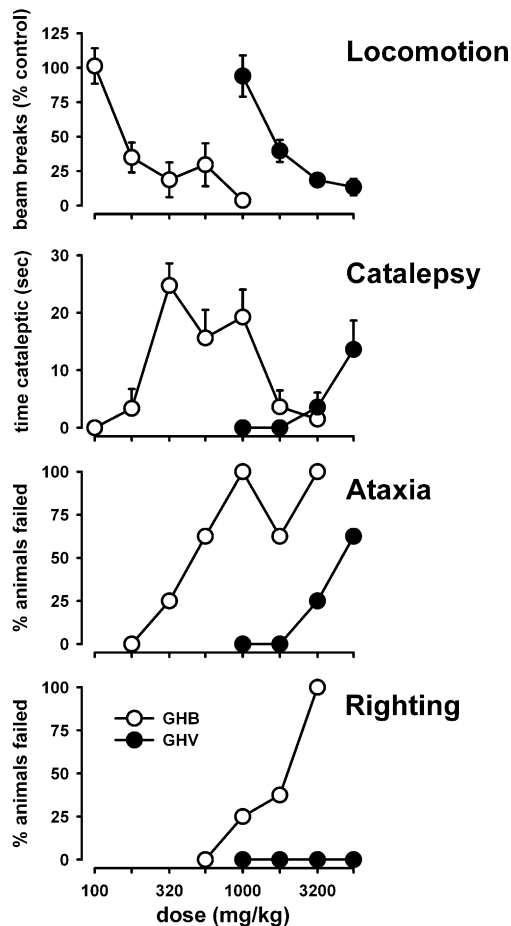
Fig. 4. Locomotion, catalepsy, ataxia, and righting in mice treated with GHB or GHV. Locomotor activity is plotted as the mean ± 1 S.E.M. percent control of the number of beam breaks that were observed under saline conditions. Catalepsy is plotted as the mean ± 1 S.E.M. time that animals exhibited cataleptic behavior up to 30 s. Ataxia and loss of righting are plotted as the percent of animals that failed the test. All behavioral effects are shown as a function of dose of drug (mg/kg) using the same abscissa. $N = 8$ for all doses studied.

GHB remains a popular drug of abuse (Degenhardt et al., 2002; Freese et al., 2002; Mason and Kerns, 2002; McDonough et al., 2004), although with its placement into Schedule I of the Controlled Substances Act in 2000, the illicit use of GHB precursors and analogs in the Unites States has reportedly increased (Mason and Kerns, 2002; Lora-Tamayo et al., 2003; United States Department of Justice, 2003). Compounds that are converted to GHB in vivo (i.e., GHB precursors, GBL and 1,4-BDL) share some behavioral effects with GHB, including sedation (Carai et al., 2002; de Fiebre et al., 2004) and discriminative stimulus effects (Carter et al., 2003; Baker et al., 2004), and are reportedly used recreationally as substitutes for GHB (Nicholson and Balster, 2001; Mason and Kerns, 2002; United States Department of Justice, 2003). Less is known regarding the effects of other compounds that are not converted to GHB, but bind to the same receptors (Castelli et al., 2003; Wu et al., 2003).

GHB binds to $GABA_B$ receptors (Xie and Smart, 1992; Mathivet et al., 1997; Madden and Johnson, 1998) and shares some behavioral effects with $GABA_B$ receptor agonists, including catalepsy (Mehta and Ticku, 1987; Sevak et al., 2004), discriminative stimulus effects (Carter et al., 2004), and sedative/hypnotic effects (Carai et al., 2001; Itzhak and Ali, 2002). However, $GABA_B$ receptor agonists are not abused, and have even been suggested for the treatment of drug abuse (see Cousins et al., 2002, for review). GHV, on the other hand, binds to GHB receptors (Bourguignon et al., 1988; present study) and is used recreationally, suggesting that the GHB receptor may be important for the abuse-related effects of GHV and GHB. In this study, GHV did not occasion baclofen-like responding, consistent with its low binding affinity at $GABA_B$ receptors.

GHV did not have GHB-like discriminative stimulus effects in rats or pigeons, suggesting that the abuse liability of GHV is not identical to that of GHB. That GHV neither substituted for nor attenuated the GHB or the baclofen discriminative stimulus confirms that GHV has no activity at $GABA_B$ receptors. While discriminative stimulus effects of drugs can be predictive of their abuse liability, it is possible that the discriminative stimulus effects of GHB and GHV are not directly related to their abuse. Rather, other shared effects of GHV and GHB (i.e., muscle relaxation, sedation) might be important to their abuse. GHV decreased food-maintained responding in rats and pigeons, and decreased locomotor activity in mice. These effects are likely due to overall sedation and may also be a function of the cataleptic effects that are observed over the same dose range. At larger doses, both GHB and GHV produced ataxia, an effect which is likely due to their sedative and muscle relaxant properties.

Loss of righting was not observed following GHV administration at any dose tested and was limited (i.e., larger doses were not studied) by the lethality associated with GHV. This finding is consistent with the lack of effect of other GHB receptor-selective analogs on righting in (DBA/2Jico) mice (Castelli et al., 2003), and with GHV being less potent than GHB in each of the behavioral procedures used in this study. It should be noted that 50% of the mice died 24–48 h following the largest dose of GHV (5600 mg/kg), whereas lethality was never observed following doses of GHB (up to 3200 mg/kg) that produced loss of righting in all subjects. Thus, people who use GHV as a legal substitute for GHB could self-administer doses of GHV that approach the toxic or lethal effects of the drug, especially if the desired effect is one that is associated with larger doses of GHV or other GHB-like compounds.

GHV is used recreationally, presumably because it is legally available and because it is thought to have effects similar to GHB (United States Department of Justice, 2003). These studies showed that GHV shares some behavioral effects with GHB, such as sedation, catalepsy, and ataxia, although comparatively larger doses of GHV were required to produce these effects. In drug discrimination procedures, rats

and pigeons that discriminate GHB and rats that discriminate baclofen did not respond on the training dose-appropriate lever or key following administration of GHV. These studies indicate that GHV, like some other GHB receptor-selective ligands, does not share discriminative stimulus effects with GHB or baclofen (Wu et al., 2003), and that the abuse-related effects of GHV may be related to other (GABAergic or non-GABAergic) effects of the drug (e.g., muscle relaxation, sedation) that occur at larger doses. GHV was about 10-fold less potent than GHB in each of the behavioral procedures employed in this study, suggesting that comparatively larger doses of GHV may be required to mimic the effects of GHB (e.g., hypnosis). As a consequence, the deliberate or inadvertent consumption of GHV with the intention of producing GHB-like effects may require near toxic doses of GHV. Conversely, the identification of GHB receptor antagonists may be useful for the treatment of GHV and GHB overdose and toxicity.

Acknowledgements

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