Published in final edited form as:

Behav Pharmacol. 2008 July ; 19(4): 317–324. doi:10.1097/FBP.0b013e328308f20d.

Gamma-hydroxybutyric acid in male and female cynomolgus monkeys trained to discriminate 1.0 or 2.0 g/kg ethanol

Christa M. Helms, Ph.D.², Laura S.M. Rogers, B.S.³, and Kathleen A. Grant, Ph.D.^{1,2,a}

¹Department of Behavioral Neuroscience, Oregon Health & Science University, 505 NW 185th Avenue, Beaverton, OR 97006-6448, Telephone (503) 614-3738, Fax (503) 690-5384

²Division of Neuroscience, Oregon National Primate Research Center, Oregon Health & Science University, 505 NW 185th Avenue, Beaverton, OR 97006-6448, Telephone (503) 614-3738, Fax (503) 690-5384

³Wake Forest University School of Medicine, Department of Physiology and Pharmacology, Medical Center Blvd, Winston-Salem NC 27157

Abstract

Gamma-hydroxybutyric acid has been proposed as a pharmacotherapy for alcoholism in part based on similar discriminative stimulus effects as ethanol. To date, drug discrimination studies with gamma-hydroxybutyric acid and ethanol have exclusively used rodents or pigeons as subjects. To evaluate possible differences between species, sex, and route of administration, the current study investigated the substitution of gamma-hydroxybutyric acid (i.g. or i.m.) for ethanol 30 or 60 min after administration in male ($n = 6$) and female ($n = 7$) cynomolgus monkeys trained to discriminate 1.0 and 2.0 g/kg ethanol. At least one dose gamma-hydroxybutyric acid completely or partially substituted for ethanol in 4 of the 13 monkeys tested, with each case occurring in female monkeys. Ethanol-appropriate responding did not increase with gamma-hydroxybutyric acid dose. Monkeys were more sensitive to the response rate decreasing effects of gamma-hydroxybutyric acid administered intramuscularly compared to intragastrically. The lack of gamma-hydroxybutyric acid substitution for ethanol suggests that these drugs have different receptor bases for discrimination. Furthermore, the data do not strongly support shared discriminative stimulus effects as the rationale for gamma-hydroxybutyric acid pharmacotherapy for alcoholism.

Keywords

ethanol; discriminative stimulus effects; monkey; gamma-hydroxybutyric acid; drug discrimination

Introduction

Gamma-hydroxybutyric acid (GHB) is a recreationally-abused drug that is also used to treat insomnia (Pardi and Black 2006). Like ethanol, GHB produces sedation, anesthesia (Cash 1994), anxiolysis (Schmidt-Mutter et al. 1998; Gessa et al. 2000), ataxia and hypothermia (Nicholson and Balster 2001). Ethanol and GHB are frequently co-abused. In one study, 64% of cases of GHB abuse reported in emergency rooms involved ethanol consumption (Drug Abuse Warning Network 2002; Ricuarte and McCann 2005). The apparent similarity between the effects of GHB and ethanol has prompted the assessment of GHB as a treatment for alcoholism (Addolorato et al. 1996). Endogenous GHB is a neuromodulator released in a Ca^{2+} -dependent manner, a minor metabolite of GABA, and product of 1,4-butanediol and γ -

aCorresponding author: E-mail: grantka@ohsu.edu.

butyrolactone (Nicholson and Balster, 2001). Peripherally-administered GHB crosses the blood-brain barrier and its effects are mediated by GABA_B (Lingenhoehl et al. 1999) and GHB receptors (Andriamampandry et al. 2003, 2006). In humans, GHB reduces craving for ethanol (Di Bello et al. 1995), ethanol consumption (Gallimberti et al. 2000), and ethanol withdrawal symptoms (Nimmerrichter et al. 2002). Some researchers have argued that the effectiveness of GHB in treating alcoholism is due to similar subjective effects of ethanol and GHB (Gessa et al. 2000; Agabio et al. 1998).

Drug discrimination is a highly-sensitive and pharmacologically-specific *in vivo* assay for the receptor mechanisms mediating the effects of drugs that individuals can detect and be trained to respond to differentially. These drug effects therefore act as discriminative stimuli (Colpaert 1986; Holtzmann 1990). Discriminative stimulus effects are believed to reflect the subjective effects of a drug. Although they are clearly dissociable (Kelly et al. 2003), “subjective effects” and “discriminative stimulus effects” are often used synonymously. In monkeys, drugs that positively modulate γ -aminobutyric acid (GABA)_A receptors completely substitute for ethanol (e.g., barbiturates, benzodiazepines: Grant et al. 2000; 3 α -hydroxy neuroactive steroids: Bowen et al. 1999; Grant et al. 1996). In monkeys trained to discriminate 1.0 g/kg ethanol from water, ethanol and pentobarbital substituted more potently compared to monkeys trained to discriminate 2.0 g/kg ethanol (Grant et al. 2000). In addition, antagonists of *N*-methyl-D-aspartate (NMDA) receptors (ketamine, phencyclidine, dizocilpine) substitute for ethanol, albeit in a smaller proportion of the population (Vivian et al. 2002). Specifically, dizocilpine, phencyclidine, and ketamine showed reduced efficacy to substitute for 2.0 g/kg ethanol, especially in male monkeys, compared to male monkeys trained to discriminate 1.0 g/kg ethanol or female monkeys trained to discriminate 1.0 or 2.0 g/kg ethanol. In contrast, neither the GABA_A agonist muscimol nor the μ -opioid receptor agonist morphine substitute for ethanol (Grant et al. 2000). Thus, GABA_A receptor positive modulators, but not GABA_A agonists, and non-competitive NMDA receptor antagonists produce ethanol-like discriminative stimulus effects when administered systemically. Pregnenolone, progesterone, and their metabolites that positively modulate GABA_A receptors are increased in the cortex and hippocampus of rats injected with 150–300 mg/kg GHB (i.p.) (Barbaccia et al. 2002). These metabolites include the steroid 3 α -hydroxy-5 α -pregnan-20-one (allopregnanolone), which substitutes for ethanol in cynomolgus monkeys (Grant et al. 1996). Furthermore, GHB potentiates GABA_A-mediated inhibitory post-synaptic currents as a result of increased neuroactive steroid biosynthesis in CA1 pyramidal neurons (Sanna et al. 2004). These data suggest that GHB may substitute for the discriminative stimulus effects of ethanol because of its steroidogenic properties.

Generally, however, GHB does not substitute for ethanol and ethanol does not substitute for GHB in drug discrimination studies, suggesting that the discriminative stimulus effects of GHB and ethanol are distinct (Nicholson and Balster, 2001). In female rats trained to discriminate 200 mg/kg GHB (i.p.) from saline, ethanol (630–1260 mg/kg) produced little or no substitution (Winter 1981). Ethanol (2.5 g/kg, i.p.) only partially substituted for 100 mg/kg GHB (s.c.) in male mice (Cook et al. 2006). However, symmetrical generalization between the discriminative stimulus effects of GHB and ethanol was observed within a narrow dose range. Specifically, 1.0 g/kg (i.g.) ethanol completely substituted in male rats trained to discriminate 300 mg/kg (i.g.) GHB, and vice versa. Symmetrical generalization was not observed with 2.0 g/kg ethanol (Colombo et al. 1995), suggesting that GHB may share discriminative stimulus only with lower training doses of ethanol. Using the same training doses, Metcalf et al. (2001) reported partial symmetrical generalization between 300–900 mg/kg GHB (i.g.) and 1.0 g/kg ethanol (i.g.) in male rats using a two-lever procedure in contrast to the t-maze used by Colombo et al. (1995). Recently, however, 1.0 to 4.0 g/kg ethanol at most partially substituted for 300 mg/kg (i.g. or i.p.) GHB in rats (Baker et al. 2004, 2005). A low dose of GHB that produces water-appropriate responding alone did not increase sensitivity to the discriminative stimulus effects

of ethanol in male mice (Cook et al. 2006), nor did GHB alter ethanol reinforcement in rhesus monkeys (Winger et al. 2007). These findings suggest that GHB and ethanol have non-additive receptor mechanisms that overlap to a very limited extent.

Furthermore, the discriminative stimulus effects of GHB appear to be influenced by whether water or food is used to train the discrimination. When rats are water-deprived and trained to discriminate 300 mg/kg GHB (i.g.) with water reinforcement, intraperitoneal administration of GHB does not substitute. In contrast, intragastric or intraperitoneal GHB partially substitutes for GHB (i.g.) in food-deprived, food-reinforced rats (Baker et al. 2004). Overall, the data from drug discrimination studies suggest a limited pharmacological basis for the similarity of GHB and ethanol. However, the apparent co-abuse of these substances by humans and the proposal that GHB can be an effective alcoholism pharmacotherapy suggests that primates may show greater overlap between the receptor mechanisms mediating GHB and ethanol compared to rodents. To address this, the current study evaluated the ethanol-like discriminative stimulus effects of GHB (i.g. or i.m.) 30 or 60 min after administration in male and female cynomolgus monkeys trained to discriminate 1.0 or 2.0 g/kg ethanol (i.g.), as the potency of test drugs to substitute for ethanol has been shown to vary with these training doses (Grant et al. 2000; Vivian et al. 2002).

Methods

Subjects

Male ($n = 6$, 4.9–7.1 kg) and female ($n = 7$, 2.9–5.1 kg) adult cynomolgus monkeys (*Macaca fascicularis*) were housed in a temperature ($23 \pm 1^\circ\text{C}$) and humidity- (40–60%) controlled room with a 12-h light dark cycle (lights on, 06.00 h). Stainless steel cages (76×60×70 cm) were modified for social housing. For training and feeding (3–4 h), the monkeys were housed individually. At all other times, the monkeys were housed in groups of two, three or four. The monkeys were fed a nutritionally-complete diet (Purina Mills, St Louis, MO, USA), daily fruit supplements, and the food delivered during experimental sessions. Water was available freely except during operant training and testing. Grant et al. (1996, 2000) describe the monkeys' origin and housing conditions. These studies were conducted according to the guidelines of the Wake Forest University Animal Care and Use Committee and the Guidelines of the Committee on the Care and Use of Laboratory Animal Resources (NRC 1996).

Apparatus

Experimental sessions took place 5–6 days per week in ventilated and sound-attenuating chambers ($1.50 \times 0.74 \times 0.76$ m; Med Associates, Inc., St. Albans, VT, USA) designed to accommodate a primate chair ($1.17 \times 0.61 \times 0.61$ m; Plas Labs, Lansing, MI, USA). A panel (0.48×0.69 m) was mounted on one wall of the chamber within arm's reach of a monkey sitting in a primate chair. The panel contained two retractable levers, three lights (amber, green and red) above each lever, and a central white light. Two house lights were located near the ceiling at the rear of the chamber. A food tray was attached to the primate chair to receive 1-g banana-flavored pellets (P.J. Noyes, Lancaster, NH, USA). The pellets were delivered through vinyl tubing connected to a feeder set outside of the chamber. The scheduling of events and data acquisition was controlled by a PC- or Macintosh-compatible computer connected to an interface (Med Associates) programmed with LabView software (National Instruments, Austin, TX, USA).

Procedure

Experimental design—The experiment consisted of 4 groups of monkeys in a 2×2 experimental design with sex and ethanol training dose as the main group factors. Discrimination training with 1.0 g/kg ethanol (20% w/v, 5 ml/kg) versus water was conducted

with 3 male (4864, 4865, 4867) and 4 female (2830, 2852, 2913, 3123) monkeys. Discrimination training with 2.0 g/kg ethanol (20% w/v, 5 ml/kg) versus water was conducted with 3 male (4890, 4891, 4889) and 3 female (2835, 3220, 5999) monkeys.

Discrimination training—For each session, the monkey was seated in a primate chair that was wheeled into the chamber. The number of responses per reinforcer was increased from fixed ratio (FR)-1 to a terminal schedule of FR-15 to FR-100. The terminal FR was set for each subject so that all the pellets were delivered in approximately 5 min, with males receiving 20–25 pellets and females receiving 10–15 pellets per session. The duration over which behavior was measured was limited to minimize variability in brain concentrations of ethanol and GHB. A session ended after all the pellets were delivered or 30 min, whichever occurred first. Response training was complete after responding under the terminal FR schedule was stable (> 0.25 responses/s for 3 consecutive sessions). After response training, the monkeys were habituated to nasogastric (i.g.) gavage in which an infant feeding tube (5 French, 1.7×381 mm) was passed down one nostril, through the esophagus and into the stomach. For 5 consecutive sessions, the monkeys received tap water (5 ml/kg, i.g.), were wheeled into the chamber, and after a pre-treatment time of 30 min, the “water-appropriate” lever extended into the chamber coinciding with illumination of the lights above the lever. The next 5 sessions were identical, except the monkeys were administered a training dose of ethanol (1.0 or 2.0 g/kg, i.g.) and reinforced for responding on the “ethanol-appropriate” lever. After the 30-min pre-treatment time, the house and center lights illuminated and the appropriate lever extended into the chamber. Completion of a FR requirement resulted in the center light shutting off, immediate delivery of a banana pellet, and then re-illumination of the center light (< 1 s).

Discrimination training commenced following these 10 sessions. For discrimination training sessions, either ethanol or water was administered, and after 30 min, both sets of lights above the levers illuminated and both the water- and ethanol-appropriate levers extended into the chamber. Completion of consecutive responses on the appropriate lever satisfying the FR requirement resulted in delivery of a banana pellet. Responses on the inappropriate lever set the response count on the appropriate lever to zero. Ethanol and water training sessions alternated after two sessions with either condition (i.e., ethanol, ethanol, water, water). The training session condition was changed, however, if $\geq 90\%$ of the responses during the entire session and $\geq 70\%$ during the first FR were on the appropriate lever. Discrimination training was complete when these accuracy criteria were satisfied for 5 consecutive sessions.

Substitution testing—Substitution tests were conducted twice per week. A single dose of GHB (0, 30, 56, 100, 170, 300, 560 mg/kg, i.m. or i.g.) was administered 30 or 60 min before the water- and ethanol-appropriate levers extended into the chambers. The doses were selected to encompass the range of doses trained or tested in previous studies and the doses of GHB that increase hippocampal and cortical neuroactive steroids (see Introduction). Consecutive responses satisfying the FR requirement on either lever resulted in reinforcement during test sessions. If the monkey switched levers, however, the response count for the other lever was set to zero. Training sessions intervened between test sessions. If the accuracy criteria were not met during a training session, training continued until the criteria were met for 3 consecutive sessions. Each test drug dose was usually administered both after an ethanol training session and after a water training session. The range of test doses was selected for each monkey to include a minimal dose that did not alter rates of responding or substitute for ethanol and a maximal dose that decreased response rates by $\geq 50\%$ from the preceding training session. Typically, the order of doses tested began in the middle of the dose range. An equal distribution of higher and lower doses was subsequently tested. The maximum dose of intramuscular GHB tested was limited to the injection volume tolerated by the monkey and sedation. Higher doses were not tested if the monkey appeared to be in pain during an injection (e.g., grimace) or did not consume a full meal the next day.

Drugs

Anhydrous ethanol (1.0 or 2.0 g/kg; Warner-Graham Co., Cockeysville, MD, USA) was diluted to 20% (w/v) with tap water and administered in a 5–10 ml/kg (i.g.) volume after which 5 ml of water was administered 30 min before each training session. Before each session, gamma-hydroxybutyrate (sodium salt) (GHB; Sigma; St. Louis, MO, USA) was dissolved in saline and administered in intramuscular injection volumes of ≤ 2 ml/kg and intragastric infusion volumes of 3–5 ml/kg followed by a flush with 5 ml water. All drug doses were based on the salt form. Large intramuscular volumes were administered with multiple injections (up to 3), limiting the maximum dose tested due to tolerability. Vehicle was tested with equivalent volumes administered to matched intramuscular injection sites. All drugs were prepared fresh daily.

Data analysis

For each monkey and test session, the percentage of total responding on the ethanol-appropriate lever (total responses on the ethanol-appropriate lever/total responses) and response rate (total responses/session time) was calculated. When ethanol-appropriate responding was double-determined, ethanol-appropriate responding was averaged from the values obtained after both the ethanol and water training sessions. Complete and partial substitution was operationally-defined as, respectively, $\geq 80\%$ and ≥ 20 –79% of the responses on the ethanol-appropriate lever. Baseline response rate was calculated by averaging the response rates for each ethanol and water training session immediately preceding a test session. Response rates were analyzed with 2 (sex) $\times$ 2 (training dose) mixed-factor ANOVAs with route of administration (i.g., i.m.) as a within-subjects factor. Percent of baseline response rate and percent ethanol-appropriate responding were analyzed with 2 (sex) $\times$ 2 (training dose) $\times$ 3 (GHB dose) $\times$ 2 (pre-treatment interval) mixed-factor repeated measures ANOVAs with GHB dose and pre-treatment interval as within-subjects factors. The dose at which response rate was decreased by 50% from baseline (ED_{50}) was computed via linear interpolation between the two doses that encompassed the 50% effect. When a drug completely substituted for ethanol, the ED_{50} was computed. Huynh-Feldt corrections were used for analyses with repeated factors violating the sphericity assumption (adjusted degrees of freedom are cited throughout the manuscript). Main effects and interactions were evaluated with Bonferroni-corrected pairwise comparisons. For all tests, α was 0.05.

Results

Percent ethanol-appropriate responding did not increase with GHB dose after intramuscular [$F(3, 12) = 0.52$] or intragastric [$F(1.61, 11.29) = 0.08$] administration. There were no main effects or interactions involving sex, pre-treatment interval, or training dose for percent ethanol-appropriate responding after intramuscular or intragastric GHB. Complete substitution of GHB for ethanol was observed in one case: after a 60-min pre-treatment, in a female trained to discriminate 1.0 g/kg ethanol (3123), the ED_{50} for GHB substitution was 92.8 mg/kg (i.g.) (98% ethanol-appropriate responding). Three cases of partial substitution were observed in female monkeys, two after intragastric GHB (Figure 1) and one after intramuscular GHB (Figure 2). There were no cases of complete or partial substitution of GHB for ethanol in male monkeys (Figure 3 and Figure 4).

A 2 (sex) $\times$ 2 (training dose) $\times$ 2 (route: i.m., i.g.) mixed factor repeated measures ANOVA indicated that the baseline response rates during sessions preceding tests with intramuscular and intragastric GHB did not significantly differ. Although males had slightly lower response rates, mean baseline response rate in sessions preceding GHB given intramuscularly did not differ significantly between the sex or training dose groups (1.0 g/kg: female, 2.3 ± 0.6 ; male, 1.8 ± 0.6 ; 2.0 g/kg: female, 2.4 ± 0.6 ; male, 1.2 ± 0.6 responses/s). The same result was obtained

when rates during the sessions preceding intragastric GHB were analyzed (1.0 g/kg: female, 2.5 ± 0.4 ; male, 1.8 ± 0.4 ; 2.0 g/kg: female, 1.6 ± 0.5 ; male, 1.1 ± 0.6 responses/s). Vehicle treatment decreased rates of responding below 100% of baseline in 6/12 cases with i.g. vehicle across both pre-treatment intervals, with mean ($\pm$ SEM) percent of baseline response rate 65.8 ± 9.3 for these 6 cases. Intramuscular vehicle injections decreased response rates to $61.9 \pm 8.3\%$ of baseline in the 7/15 cases in which rates of responding were $<100\%$ including both pre-treatment intervals. Overall, vehicle treatments did not systematically affect rates of responding.

Percent of baseline response rates decreased significantly with GHB dose after intramuscular [$F(3, 6) = 6.2, p \leq 0.05$] but not intragastric [$F(2, 14) = 0.6$] administration. Examination of Figure 1–Figure 4, however, reveals that rates of responding decreased from baseline in a majority of monkeys after intragastric GHB, with decreases $\leq 50\%$ of baseline response rate in 7/24 (29%) monkeys across both pre-treatment intervals. Intramuscular GHB showed slightly greater efficacy to reduce response rates, with 6/16 (37.5%) showing response rates $\leq 50\%$ across both pre-treatment intervals (Table 1). There were no significant interactions among the factors. With GHB given intramuscularly, the overall percent of baseline response rate was $> 90\%$ for 0, 170 and 300 mg/kg, but declined to $53.3 \pm 23.8\%$ after 560 mg/kg GHB (i.m.). Females trained to discriminate 2.0 g/kg ethanol and males trained to discriminate 1.0 g/kg ethanol were not tested with 0 mg/kg GHB (i.g.), so the previous analysis excluded this dose. A subsequent analysis included only the groups for which 0 mg/kg GHB (i.g.) was tested, i.e., females trained to discriminate 1.0 g/kg ethanol and males trained to discriminate 2.0 g/kg ethanol. A 2 (sex) $\times$ 2 (training dose) $\times$ 4 (dose: 0, 170, 300, 560 mg/kg) mixed factor ANOVA with dose as the within-subjects factor did not reveal a main effect of dose [$F(1.77, 7.07) = 0.74$] on percent of baseline response rate after intragastric GHB. Overall, both intragastric and intramuscular GHB decreased response rates albeit somewhat non-systematically and with moderate individual differences.

Discussion

The current study found little evidence that the discriminative stimulus effects of pharmacologically-active doses of GHB (i.g. or i.m.) are similar to those of 1.0 or 2.0 g/kg ethanol (i.g.). All cases of complete or partial substitution occurred at sub-maximal doses. Some monkeys showed dose-dependent decreases in response rate indicating that the doses tested were pharmacologically-active, although these doses did not substitute for ethanol. The lack of dose-dependent substitution of GHB for ethanol in the current study is consistent with past drug discrimination studies reporting that GABA_A receptor positive modulators and NMDA receptor antagonists, at best, partially substitute for GHB in rats (Colombo et al. 1998; Baker et al. 2004, 2005) or pigeons (Koek et al. 2004). On the other hand, the results of the current study differ from the report that 300 mg/kg GHB and 1.0 g/kg ethanol show complete symmetrical generalization (Colombo et al. 1995). In the current study, 300 mg/kg (i.g.) GHB partially substituted for 1.0 g/kg ethanol in only one monkey tested (Figure 1). Indeed, rats can be trained to discriminate between 300 mg/kg (i.g.) GHB and 1.0 or 1.5 g/kg (i.g.) ethanol, suggesting dissociable discrimination stimulus effects (Baker et al. 2008). The discriminative stimulus effects of GHB appear to be mediated by agonism of GABA_B and GHB receptors (e.g., Baker et al. 2005; Carai et al. 2004; Carter et al. 2003; Lobina et al. 1999).

The few cases of complete or partial substitution of GHB for ethanol occurred in female monkeys. For example, in monkey 3123, 170 mg/kg GHB (i.g.) completely substituted for 1.0 g/kg ethanol (i.g.) (Figure 1). In this case, the dose of GHB is low compared to the complete substitution of 300 mg/kg GHB (i.g.) in male rats trained to discriminate 1.0 g/kg ethanol (i.g.) (Colombo et al. 1995). A female-specific factor, e.g., menstrual cyclicity, may influence the

substitution of GHB for ethanol. During the luteal phase of their menstrual cycle, when circulating progesterone peaks, cynomolgus monkeys are more sensitive to the discriminative stimulus effects of ethanol, and to the ethanol-like stimulus effects of allopregnanolone, compared to during their follicular phase (low circulating progesterone) (Grant et al. 1997). These findings suggest the possibility of additive ethanol-like effects of ovarian-derived and GHB-induced neuroactive steroids. Indeed, the lack of a dose-dependent effect of GHB on ethanol-appropriate responding could be accounted for by substitution testing irrespective of menstrual cycle phase. Retrospective evaluation of menses and progesterone records from the current data set, however, indicates that GHB partially or completely substituted for ethanol during the follicular phase of the menstrual cycle, when circulating progesterone was low or undetectable for monkey 2830 when tested with 300 mg/kg GHB (i.g.) and monkey 3123 when tested with 170 mg/kg GHB (i.g.). It is unlikely, therefore, that the few cases of GHB substitution for ethanol in the current study are due to additive effects of neuroactive steroids resulting from GHB administration and luteal phase progesterone derivatives.

The effects of GHB on response rate in the current study were influenced by route of administration, with intramuscular but not intragastric GHB significantly decreasing rates of responding as a function of dose. With both routes of administration, however, GHB decreased rates of responding with moderate efficacy. The rare tests in which GHB partially or completely substituted for ethanol occurred with both routes of administration. Brain concentrations of GHB could be slightly lower after intragastric compared to intramuscular administration due to slower absorption, although this has not yet been investigated. After intragastric administration, GHB is rapidly absorbed through the gastrointestinal tract and metabolized in the liver to a greater extent before entering circulation compared to intramuscular administration. In general, the discriminative stimulus effects of GHB appear to be independent of route of administration, although route of administration has been shown to interact with reinforcer type. Intragastric GHB only partially substitutes for intraperitoneal GHB in water-reinforced rats (Baker et al. 2004).

In humans, plasma concentrations of GHB are highly variable from 10–360 min after administration of 25 mg/kg GHB (p.o.), with mean plasma concentrations peaking at 30 min (Brenneisen et al. 2004). Previous studies have also reported a lack of or inconsistent decreases in response rate with GHB except with very high doses. For example, 1500 mg/kg (i.g.) GHB decreased response rates in rats (Colombo et al. 1995). On the other hand, Baker et al. (2004) reported minor decreases in rats' response rates after 75–400 mg/kg GHB (i.g.), with the greatest mean percent decrease approximating 50%. Percent of baseline response rate was nearly 50% with up to 300 mg/kg (s.c.) GHB in mice (Cook et al. 2006). Rates of responding were $\leq 53\%$ of baseline at 600–900 mg/kg (i.g.) GHB in rats (Metcalf et al. 2001). In baboons, 350 mg/kg GHB (i.g.) did not disrupt performance of a fine motor task (Weerts et al. 2005). Indeed, many effects of GHB are characterized by a steep dose-effect function. For example, visual analog scale ratings of "dizziness" were more than 6-fold greater 90 min after administration of 72 mg/kg GHB (p.o.) compared to 40 mg/kg GHB, a < 2 -fold dose difference (Abanades et al. 2006).

The mechanisms accounting for the therapeutic effects of GHB in alcoholics remain speculative. First, GHB may antagonize the discriminative stimulus effects of ethanol. Antagonists of the discriminative stimulus effects of ethanol, Ro15-4513 and flumazenil (Ro15-1788) (rats: Gatto and Grant 1995; cynomolgus monkeys: Grant, Rogers, and Helms, unpublished observations), partially or completely substituted for GHB in pigeons trained to discriminate 56 or 100 mg/kg GHB (i.m.) (Koek et al. 2006). Second, GABA_B agonism may be important for the therapeutic effects of GHB. The GABA_B agonist, baclofen, reduced responding for ethanol in rats (Colombo et al. 2003) and craving in alcoholics (Addolorato et al. 2000). Baclofen did not, however, antagonize the discriminative stimulus effects of ethanol

(Shelton and Balster 1994). Third, GHB pharmacotherapy for alcoholism may be mediated by receptor mechanisms that indirectly affect the neural mechanisms mediating ethanol consumption. For example, GHB inhibits dopamine release (e.g., Howard and Feigenbaum 1997). Overall, the current data provide a convincing demonstration that, in non-human primates, GHB does not substitute for the discriminative stimulus effects of ethanol. Thus, the therapeutic effects of GHB in alcoholics is unlikely to be due to substitution for ethanol through a common receptor mechanism.

Acknowledgements

The authors thank Courtney A. Waters and Ken Szeliga for their technical expertise.

Sources of support: This research was supported by NIH/NIAAA AA10009, AA13860, and RR 000163.

References

- Abanades S, Farré M, Segura M, Pichini S, Barral D, Pacifici R, Pellegrini M, Fonseca F, Longohr K, de la Torre R. γ -hydroxybutyrate (GHB) in humans. Pharmacodynamics and pharmacokinetics. *Ann NY Acad Sci* 2006;1074:559–576. [PubMed: 17105953]
- Addolorato G, Castelli E, Stefanini GF, Casella G, Caputo F, Marsigli L, Bernardi M, Gasbarrini G. GHB Study Group. An open multicentric study evaluating 4-hydroxybutyric acid sodium salt in the medium-term treatment of 179 alcohol dependent subjects. *Alcohol Alcohol* 1996;31:341–345. [PubMed: 8879280]
- Addolorato G, Caputo F, Capristo E, Colombo G, Gessa GL, Gasbarrini G. Ability of baclofen in reducing alcohol craving and intake: II – preliminary clinical evidence. *Alcohol Clin Exp Res* 2000;24:67–71. [PubMed: 10656195]
- Agabio R, Colombo G, Loche A, Lobina C, Pani ML, Reali R, Gessa GL. γ -hydroxybutyric acid reducing effect on ethanol intake: evidence in favour of a substitution mechanism. *Alcohol Alcohol* 1998;33:1–10. [PubMed: 9539168]
- Andriamampandry C, Taleb O, Kemmel V, Humbert J-P, Aunis D, Maitre M. Cloning and functional characterization of a gamma-hydroxybutyrate receptor identified in the human brain. *FASEB J* 2006;21:1–11.
- Baker LE, Pynnonen D, Poling A. Influence of reinforcer type and route of administration on gamma-hydroxybutyrate discrimination in rats. *Psychopharmacology* 2004;174:220–227. [PubMed: 14991222]
- Baker LE, Searcy GD, Pynnonen DM, Poling A. Differentiating the discriminative stimulus effects of gamma-hydroxybutyrate and ethanol in a three-choice drug discrimination procedure in rats. *Pharmacol Biochem Behav.* 2008
- Baker LE, Van Tilburg TJ, Brandt AE, Poling A. Discriminative stimulus effects of gamma-hydroxybutyrate (GHB) and its metabolic precursor, gamma-butyrolactone (GBL) in rats. *Psychopharmacology* 2005;181:458–466. [PubMed: 16001124]
- Barbaccia ML, Colombo G, Affricano D, Carai MAM, Vacca G, Melis S, Purdy RH, Gessa GL. GABA_B receptor-mediated increase of neurosteroids by γ -hydroxybutyric acid. *Neuropharmacol* 2002;42:782–791.
- Bowen CA, Purdy RH, Grant KA. Ethanol-like discriminative stimulus effects of endogenous neuroactive steroids: effects of ethanol training dose and dosing procedure. *J Pharmacol Exp Ther* 1999;289:405–411. [PubMed: 10087031]
- Brenneisen R, ElSohly MA, Murphy TP, Passarelli J, Russmann S, Salamone SJ, Watson DE. Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. *J Anal Toxicol* 2004;28:625–630. [PubMed: 15538955]
- Carai MAM, Vacca G, Serra S, Colombo G, Froestl W, Gessa GL. Suppression of GABA_B receptor function in vivo by disulfide reducing agent, DL-dithiothreitol (DTT). *Psychopharmacology* 2004;174:283–290. [PubMed: 14760512]

- Carter LP, Flores LR, Wu H, Chen W, Unzeitig AW, Coop A, France CP. The role of GABA_B receptors in the discriminative stimulus effects of γ -hydroxybutyrate in rats: time course and antagonism studies. *J Pharmacol Exp Ther* 2003;305:668–674. [PubMed: 12606639]
- Cash CD. Gammahydroxybutyrate: an overview of the pros and cons for it being a neurotransmitter and/or a useful therapeutic agent. *Neurosci Biobehav Rev* 1994;18:291–304. [PubMed: 7914688]
- Colombo G, Agabio R, Lobina C, Reali R, Fadda F, Gessa GL. Symmetrical generalization between the discriminative stimulus effects of gamma-hydroxybutyric acid and ethanol: occurrence within narrow dose ranges. *Physiol Behav* 1995;57:105–111. [PubMed: 7878101]
- Colombo G, Agabio R, Lobina C, Reali R, Gessa GL. Involvement of GABA_A and GABA_B receptors in the mediation of discriminative stimulus effects of γ -hydroxybutyric acid. *Physiol Behav* 1998;64:293–302. [PubMed: 9748096]
- Colombo G, Vacca G, Serra S, Brunetti G, Carai MAM, Gessa GL. Baclofen suppresses motivation to consume alcohol in rats. *Psychopharmacology* 2003;167:221–224. [PubMed: 12669176]
- Colpaert, FC. Drug discrimination: behavioral, pharmacological, and molecular mechanisms of discriminative drug effects. In: Goldman, SR.; Stoleran, IP., editors. *Behavioral analysis of drug dependence*. New York: Academic Press; 1986. p. 161-193.
- Cook CD, Biddlestone L, Coop A, Beardsley PM. Effects of combining ethanol (EtOH) with gamma-hydroxybutyrate (GHB) on the discriminative stimulus, locomotor, and motor-impairing function of GHB in mice. *Psychopharmacology* 2006;185:112–122. [PubMed: 16453156]
- Di Bello MG, Gambassi F, Mugnai L, Masini E, Mannaioni PF. Gamma-hydroxybutyric acid induced suppression and prevention of alcohol withdrawal syndrome and relief of craving in alcohol dependent patients. *Alcolologia* 1995;7:111–118.
- Drug Abuse Warning Network. The DAWN Report: club drugs, 2002 update. Washington, DC: US Department of Health and Human Services; 2002. p. 1-4.
- Gallimberti L, Spella MR, Soncini CA, Gessa GL. Gamma-hydroxybutyric acid in the treatment of alcohol and heroin dependence. *Alcohol* 2000;20:257–262. [PubMed: 10869867]
- Gatto GJ, Grant KA. Attenuation of the discriminative stimulus effects of ethanol by the benzodiazepine partial inverse agonist Ro 15-4513. *Behav Pharmacol* 1997;8:139–146. [PubMed: 9833009]
- Gessa GL, Agabio R, Carai MAM, Lobina C, Pani M, Reali R, Colombo G. Mechanism of the antialcohol effect of gamma-hydroxybutyric acid. *Alcohol* 2000;20:271–276. [PubMed: 10869869]
- Grant KA, Azarov A, Bowen CA, Mirkis S, Purdy RH. Ethanol-like discriminative stimulus effects of the neurosteroid 3 α -hydroxy-5 α -pregnan-20-one in female *Macaca fascicularis* monkeys. *Psychopharmacology* 1996;124:340–346. [PubMed: 8739549]
- Grant KA, Azarov A, Shively CA, Purdy RH. Discriminative stimulus effects of ethanol and 3 α -hydroxy-5 α -pregnan-20-one in relation to menstrual cycle phase in cynomolgus monkeys (*Macaca fascicularis*). *Psychopharmacology* 1997;130:59–68. [PubMed: 9089848]
- Grant KA, Waters CA, Green-Jordan K, Azarov A, Szeliga KT. Characterization of the discriminative stimulus effects of GABA_A receptor ligands in *Macaca fascicularis* monkeys under different ethanol training conditions. *Psychopharmacology* 2000;152:181–188. [PubMed: 11057522]
- Holtzman, SG. Discriminative stimulus effects of drugs: relationship to potential for abuse. In: Adler, MW.; Cowan, A., editors. *Modern Methods in Pharmacology, Testing and Evaluation of Drugs of Abuse*. New York: Wiley-Liss; 1990. p. 193-210.
- Howard SG, Feigenbaum JJ. Effect of gamma-hydroxybutyrate on central dopamine release in vivo. A microdialysis study in awake and anesthetized animals. *Biochem Pharmacol* 1997;53:103–110. [PubMed: 8960069]
- Kelly TH, Stoops WW, Perry AS, Prendergast MA, Rush CR. Clinical neuropharmacology of drugs of abuse: a comparison of drug-discrimination and subject-report measures. *Behav Cog Neurosci Rev* 2003;2:227–260.
- Koek W, Flores LR, Carter LP, Lamb RJ, Chen W, Wu H, Coop A, France CP. Discriminative stimulus effects of γ -hydroxybutyrate in pigeons: role of diazepam-sensitive and -insensitive GABA_A and GABA_B receptors. *J Pharmacol Exp Ther* 2004;308:904–911. [PubMed: 14718595]
- Koek W, Chen W, Mercer SL, Coop A, France CP. Discriminative stimulus effects of γ -hydroxybutyrate: role of training dose. *J Pharmacol Exp Ther* 2006;317:409–417. [PubMed: 16330491]

- Lingenhoehl K, Brom R, Heid J, Beck P, Froestl W, Kaupmann K, Bettler B, Mosbacher J. Gamma-hydroxybutyrate is a weak agonist at recombinant GABA(B) receptors. *Neuropharmacology* 1999;38:1667–1673. [PubMed: 10587082]
- Lobina C, Agabio R, Reali R, Gessa GL, Colombo G. Contribution of GABA_A and GABA_B receptors to the discriminative stimulus produced by gamma-hydroxybutyric acid. *Pharmacol Biochem Behav* 1999;64:363–365. [PubMed: 10515314]
- Metcalf BR, Stahl JM, Allen JD, Woolfolk DR, Soto PL. Discrimination of gamma-hydroxybutyrate and ethanol administered separately and as a mixture in rats. *Pharmacol Biochem Behav* 2001;70:31–41. [PubMed: 11566140]
- Nicholson KL, Balster RL. GHB: a new and novel drug of abuse. *Drug Alcohol Dep* 2001;63:1–22.
- Nimmerrichter AA, Walter H, Gutierrez-Lobos KE, Lesch OM. Double-blind controlled trial of γ -hydroxybutyrate and clomethiazole in the treatment of alcohol withdrawal. *Alcohol Alcohol* 2002;37:67–73. [PubMed: 11825860]
- Pardi D, Black J. γ -hydroxybutyrate/sodium oxybate. Neurobiology, and impact on sleep and wakefulness. *CNS Drugs* 2006;20:993–1018. [PubMed: 17140279]
- Ricuarte GA, McCann UD. Recognition and management of complications of new recreational drug use. *Lancet* 2005;365:2137–2145. [PubMed: 15964451]
- Sanna E, Talani G, Busonero F, Pisu MG, Purdy RH, Serra M, Biggio G. Brain steroidogenesis mediates ethanol modulation of GABA_A receptor activity in rat hippocampus. *J Neurosci* 2004;24:6521–6530. [PubMed: 15269263]
- Schmidt-Mutter C, Pain L, Sandner G, Gobaille S, Maitre M. The anxiolytic effect of γ -hydroxybutyrate in the elevated plus maze is reversed by the benzodiazepine receptor antagonist, flumazenil. *Eur J Pharmacol* 1998;342:21–27. [PubMed: 9544788]
- Shelton KL, Balster RL. Ethanol drug discrimination in rats: substitution with GABA agonists and NMDA antagonists. *Behav Pharmacol* 1994;5:441–450. [PubMed: 11224296]
- Vivian JA, Waters CA, Szeliga KT, Jordan K, Grant KA. Characterization of the discriminative stimulus effects of *N*-methyl-D-aspartate ligands under different ethanol training conditions in the cynomolgus monkey (*Macaca fascicularis*). *Psychopharmacology* 2002;162:273–281. [PubMed: 12122485]
- Weerts EM, Goodwin AK, Griffiths RR, Brown PR, Froestl W, Jakobs C, Gibson KM. Spontaneous and precipitated withdrawal after chronic intragastric administration of gamma-hydroxybutyrate (GHB) in baboons. *Psychopharmacology* 2005;179:678–687. [PubMed: 15645222]
- Winger G, Galuska CM, Hursh SR. Modification of ethanol's reinforcing effectiveness in rhesus monkeys by cocaine, flunitrazepam, or gamma-hydroxybutyrate. *Psychopharmacology* 2007;193:587–298
- Winter JC. The stimulus properties of γ -hydroxybutyrate. *Psychopharmacology* 1981;73:372–375. [PubMed: 6789360]

Figure 1. Percent ethanol-appropriate responding and percent decrease in response rate (RR) from baseline in individual female cynomolgus monkeys trained to discriminate 1.0 g/kg ethanol from water with a 30-min pre-treatment interval 30- or 60-min after administration of GHB (i.g. or i.m.). Data points may overlap.

Figure 2.

Percent ethanol-appropriate responding and percent decrease in response rate (RR) from baseline in individual female cynomolgus monkeys trained to discriminate 2.0 g/kg ethanol from water with a 30-min pre-treatment interval 30- or 60-min after administration of GHB (i.g. or i.m.). Intragastric vehicle treatments were not tested. Data points may overlap.

Figure 3.

Percent ethanol-appropriate responding and percent decrease in response rate (RR) from baseline in individual male cynomolgus monkeys trained to discriminate 1.0 g/kg ethanol from water with a 30-min pre-treatment interval 30- or 60-min after administration of GHB (i.g. or i.m.). Intragastric vehicle treatments were not tested. Data points may overlap.

Figure 4.

Percent ethanol-appropriate responding and percent decrease in response rate (RR) from baseline in individual male cynomolgus monkeys trained to discriminate 2.0 g/kg ethanol from water with a 30-min pre-treatment interval 30- or 60-min after administration of GHB (i.g. or i.m.). Data points may overlap.

Table 1

The ratio of monkeys showing $\leq 50\%$ of their training session response rate after GHB administration with sex and training dose presented separately. The mean ($\pm$ SEM) potency (ED_{50} , mg/kg) of GHB to decrease response rate is listed in parentheses where appropriate.

Route	Pretreatment (min)	1.0 g/kg Ethanol training		2.0 g/kg Ethanol training	
		Females	Males	Females	Males
i.g.	30	1/4 (184.7)	0/3	0/3	1/2 (314.6)
	60	1/4 (170)	0/3	2/3 (406.1 $\pm$ 22.7)	2/2 (412 $\pm$ 144)
i.m.	30	1/2 (219.6)	1/2 (285.5)	1/2 (236.0)	0/2
	60	0/2	1/2 (295.9)	1/2 (105.8)	1/2 (384.7)