Toxicokinetics/Toxicodynamics of \(\gamma\)-Hydroxybutyrate-Ethanol Intoxication: Evaluation of Potential Treatment Strategies

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Running Title: Treatment of GHB-ethanol intoxication

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Abbreviations: ABEC, area below the effect curve; AUC, area under the plasma concentration-time curve; Cl, clearance; Cl/F, oral clearance; Cl_m, metabolic clearance; Cl_R, renal clearance; E_max, maximum pharmacodynamic effect; GABA, γ-aminobutyric acid; GHB, γ-hydroxybutyrate; MCT, monocarboxylate transporter

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Abstract

γ-hydroxybutyrate (GHB), a common drug of abuse, is often co-ingested with ethanol. Increasing renal clearance via monocarboxylate transporter (MCT) inhibition represents a potential therapeutic strategy in GHB overdose, as does inhibition of GABA<sub>B</sub> receptors. In this research, we investigate toxicokinetic/toxicodynamic interactions between GHB-ethanol and efficacy of treatment options for GHB-ethanol intoxication in rats. Sedation was assessed using the endpoint of return-to-righting reflex. Respiration was assessed using plethysmography. Co-administration of 2.0 g/kg ethanol IV with GHB 600 mg/kg IV increased sleep time compared to GHB alone. Administration of ethanol to steady-state concentrations of 0.1-0.2% and 0.3-0.4% (w/v), did not affect toxicokinetics of GHB 600 mg/kg IV, nor respiratory rate, but resulted in significantly lower peak tidal volumes compared to GHB alone. Oral co-administration of ethanol 2.5 g/kg had no significant effect on toxicokinetics of GHB 1500 mg/kg PO. Pretreatment with specific receptor inhibitors indicated no effect of GABA<sub>A</sub> receptor inhibition on sleep time or respiratory depression in GHB-ethanol intoxication. GABA<sub>B</sub> receptor inhibition partially prevented sedation and completely prevented respiratory depression. Ethanol increased fatality when administered at 0.1-0.2% (4/10) and 0.3-0.4% (9/10) vs. GHB 1500 mg/kg IV alone (0/10). Treatment with the MCT inhibitor, L-lactate, significantly decreased sleep time following GHB-ethanol and decreased fatality at 0.1-0.2% (0/10) and 0.3-0.4% ethanol (5/10). Treatment with a GABA<sub>B</sub> receptor antagonist completely prevented fatality at 0.3-0.4% (0/10). These data indicate ethanol potentiates the sedative and respiratory depressant effects of GHB, increasing the risk of fatality. MCT and GABA<sub>B</sub> receptor inhibition represent potentially effective treatments in GHB-ethanol intoxication.
Introduction

Abuse of γ-hydroxybutyrate (GHB) and its precursors has become well-recognized in the U.S. and other countries. GHB overdose results in 1,000-2,000 emergency department visits annually in the U.S. alone (Substance Abuse and Mental Health Services Administration, 2011). GHB intoxication can result in adverse effects including bradycardia, hypothermia, coma and respiratory depression/arrest, which has lead to GHB-associated fatalities (Chin et al., 1998; Caldicott et al., 2004; Zvosec et al., 2011). In overdose cases, other drugs of abuse are commonly co-ingested with GHB, and numerous reports indicate ethanol to be the most common of these, some indicating ethanol co-ingestion in the majority of GHB overdose cases (Mason and Kerns, 2002; Galicia et al., 2011; Zvosec et al., 2011).

Although GHB abuse has been recognized as a significant issue in public health, both alone and with ethanol co-ingestion, there currently exists no pharmacologic treatment for GHB overdose. In our laboratory, we have evaluated a novel therapeutic strategy for the treatment of GHB overdose by increasing GHB elimination via inhibition of its active renal reabsorption. Renal reabsorption of GHB is saturable which can be attributed to transport by the group of transporters known as monocarboxylate transporters (MCTs) (Morris et al., 2005; Wang et al., 2006). We have demonstrated the utility of MCT inhibition as a therapeutic strategy for overdose of GHB alone in animal studies, in which administration of MCT inhibitors increases GHB total and renal clearance at high GHB doses, improving toxicodynamic endpoints (Wang et al., 2008a; Wang et al., 2008b; Morse et al., 2012). Similarly, in our pilot clinical study, MCT inhibition with L-lactate administered with osmotic diuresis increased the renal clearance of GHB in humans (Morris et al., 2011). Along with alteration of GHB toxicokinetics, direct inhibition of GHB toxicodynamics also represents a potential treatment strategy for GHB intoxication. The involvement of GABA_{b} receptors in many GHB toxicodynamic
endpoints has been demonstrated (Carai et al., 2001; Kaupmann et al., 2003; Carai et al., 2005; Morse et al., 2012), indicating GABA<sub>B</sub> receptor antagonists may also be effective as treatment for GHB overdose. However, unlike MCT inhibition with L-lactate, treatment with GABA<sub>B</sub> receptor antagonists is not currently a clinically available therapy, although the GABA<sub>B</sub> receptor antagonist SGS742 has undergone Phase II clinical trials for indications other than GHB intoxication (Froestl et al., 2004).

Considering the high rate of GHB-ethanol co-ingestion, the toxicokinetic/toxicodynamic interactions between these two drugs are of interest, as is the treatment of GHB overdose in the presence of ethanol. Available literature supports minimal toxicokinetic interaction between GHB and ethanol when the two are administered at high doses intravenously or at low oral doses (Van Sassenbroeck et al., 2003; Thai et al., 2006; Fung et al., 2008). In contrast, significant toxicodynamic interactions between GHB and ethanol have been reported with an array of toxicodynamic endpoints (Van Sassenbroeck et al., 2003; Cook et al., 2006; Thai et al., 2006). These toxicodynamic interactions may be attributed to different pharmacological actions; while GHB’s effects are attributed to action at GABA<sub>B</sub> receptors, effects of ethanol are mainly attributed to action at GABA<sub>A</sub> receptors (Adinoff et al., 1988; Carai et al., 2001; Morse et al., 2012). Clinically, overdose of both GHB and ethanol can produce profound sedation and respiratory depression, potentially resulting in fatality (Altose and Hudgel, 1986; Adinoff et al., 1988; Zvosec et al., 2011). Although multiple studies have assessed the effect of GHB-ethanol administration on sedation (McCabe et al., 1971; Van Sassenbroeck et al., 2003; Cook et al., 2006), the effect of ethanol on respiration during GHB overdose has not been evaluated. We predict that, like sedation, GHB-induced respiratory depression is potentiated by the presence of ethanol. Additionally, while the toxicological effects of these agents alone may primarily involve different receptors, the identification of specific receptors involved in these effects with GHB-
ethanol co-administration has not been assessed. Due to their different pharmacological actions, it is likely that multiple mechanisms are involved in the toxicodynamics of GHB-ethanol intoxication. Finally, information is lacking on the efficacy of potential treatment options for GHB-ethanol intoxication using relevant toxicodynamic endpoints. We predict that, as with GHB alone, both MCT and GABA₉ receptor inhibition represent potential treatment strategies for improving sedation, respiratory depression, and mortality in GHB-ethanol intoxication.
Materials and Methods

Chemicals and Reagents. Sodium GHB and SGS742 (3-aminopropyl-n-butyl-phosphinic acid) were provided by the National Institute on Drug Abuse. Sodium L-lactate was obtained from Sigma-Aldrich (St. Louis, MO). Ethyl alcohol USP (200 proof) was purchased from PharmcoAAPER (Brookfield, CT). SCH50911 ((2S)-(+)5,5-dimethyl-2-morpholineacetic acid) was purchased from R&D Systems (Minneapolis, MN).

Animals and Animal Surgery. Male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 260-340 g were used for all experiments. Animals were housed under controlled temperature and humidity with an artificial 12 hour light/dark cycle and food was available ad libitum. All animal protocols were approved by the Institutional Animal Care and Use Committee at the University at Buffalo. Animals were allowed to acclimate to their environment for one week prior to surgical implantation of jugular and femoral vein cannulae under anesthesia with ketamine/xylazine. Cannulae were flushed daily with 40 IU/mL heparinized saline to maintain patency. Animals were allowed a minimum of 72 hours for recovery from surgery before drug administration.

Toxicokinetic/toxicodynamic Interaction Studies.

Sedation studies. The sedative effects of GHB and ethanol were measured using the endpoint of righting reflex, as in our previous studies (Wang et al., 2008a; Wang et al., 2008b; Felmlee et al., 2010b). To determine the effect of ethanol on the sedative effect of GHB, rats were administered ethanol 2.0 g/kg or ethanol 2.0 g/kg + GHB 600 mg/kg, intravenously over 5 minutes (n=3-4 in each group). This experiment was performed at a similar time and in a similar manner to our previous study assessing sedative effects of GHB alone, (Felmlee et al., 2010b); therefore, the same data at GHB 600 mg/kg was
used from the previous publication for comparison to GHB alone. To assess MCT inhibition as a treatment strategy for improving sedation in GHB-ethanol intoxication, the MCT inhibitor L-lactate (66 mg/kg bolus followed by a 302.5 mg/kg/hr infusion) was administered 5 minutes after GHB-ethanol. This dose of L-lactate was chosen to increase plasma lactate concentrations by 1-2 mM (Morse et al., 2012). To assess the receptors involved in the sedative effect of GHB-ethanol intoxication, GHB and ethanol were administered alone and concomitantly, and specific receptor inhibitors were also administered immediately prior to the concomitant administration of GHB-ethanol. Bicuculline (1mg/kg) was used to assess the role of GABA$_A$ receptors. SGS742 (500 and 1000 mg/kg) and the more potent GABA$_B$ receptor antagonist SCH50911 (100 and 200 mg/kg) were used to assess the role of GABA$_B$ receptors. SCH50911 at the lower dose has been demonstrated to completely prevent the sedative effect of GHB alone in mice (Carai et al., 2001). In all groups, the time of loss-of-righting reflex (LRR) and time of return-to-righting reflex (RRR) were recorded and sleep time determined as RRR-LRR. LRR was determined as the time at which the animal could not right itself after being placed on its back. Animals were left on their back following LRR and RRR was defined as the time at which the animal could right itself on its own. All animals were sacrificed at RRR at which time blood and brain samples were collected. Brain samples were immediately frozen in liquid nitrogen upon collection and all samples stored at -80 until analysis. In these studies, GHB was administered as a 200 or 300 mg/mL solution in sterile water and ethanol as a 50% (v/v) solution in sterile water. Bicuculline was dissolved in HCl, then diluted in saline to 1 mg/mL and pH 5.0. SCH50911 and SGS742 were administered as 100 mg/mL solutions in saline. All bolus doses were administered via the jugular vein cannula. L-lactate was administered as a 40 mg/mL solution in sterile water via the femoral vein cannula.
Respiratory depression/fatality studies. The effect of GHB-ethanol administration on respiration was measured using whole-body plethysmography, as in our previous study (Morse et al., 2012). Rats were placed in plethysmography chambers one hour prior to drug administration and allowed to acclimate to the chambers for 45 minutes before 5 baseline recordings were collected over 15 minutes. In all studies, GHB administration was considered time 0 and respiration recordings taken at 2.5, 5, 7.5, 10, 15, 20, 25, and 30 minutes and every 15 minutes thereafter for 6 hours. Measurements for the parameters of respiratory frequency (rate), tidal volume, and minute volume (rate \cdot tidal volume) were quantitated at each recording.

To assess the effect of ethanol on IV GHB toxicokinetics and GHB-induced respiratory depression, GHB 600 mg/kg was administered alone and concomitantly with ethanol administered to target moderate and high steady-state concentrations of 0.1-0.2% and 0.3-0.4% (w/v), respectively, n=5 in each group. Ethanol was administered as a 1.0 or 2.0 g/kg intravenous bolus over 5 minutes, right after the collection of baseline respiratory measurements. An infusion of ethanol was initiated 30 minutes later at a rate of 1.85 mg/min, the average $V_{\text{max}}$ rate of ethanol metabolism in male Sprague Dawley rats of this weight, to maintain target steady-state concentrations, a strategy previously described (Boje and Fung, 1989). Five respiratory measurements were again taken over 15 minutes starting 45 minutes after the ethanol bolus, to assess the effect of targeted ethanol concentrations alone on respiration. GHB was then administered intravenously at time 0 (60 min after the ethanol bolus). To determine the receptors involved in respiratory depression in GHB-ethanol intoxication, bicuculline, SCH50911, and SGS742 were administered immediately prior to GHB, at the higher ethanol dose (n=3-4 in each group). In all respiratory depression experiments, blood and urine samples were collected for 6 hours after GHB administration. GHB was administered as a 300 mg/mL solution in sterile water via the jugular vein cannula. The ethanol bolus was given as a
50% (v/v) solution in sterile water via the jugular vein cannula and ethanol infusion as a 20% (v/v) solution in sterile water via the femoral vein cannula. Bicuculline, SCH50911, and SGS742 were administered in saline as above.

To assess the effects of ethanol on GHB-associated fatality and the effects of potential treatment strategies for preventing fatality due to respiratory arrest in GHB-ethanol intoxication, GHB 1500 mg/kg IV was administered alone and with the same ethanol regimens as above (n=10 in each group). Treatment groups received 5 mg/kg SCH50911 or L-lactate (66 mg/kg bolus and 302.5 mg/kg/hr infusion), given 5 minutes after GHB. This dose of SCH50911 was the lowest dose demonstrated to significantly improve respiratory depression with GHB alone in our previous study (Morse et al., 2012). The dose of L-lactate was chosen to increase plasma lactate concentrations by 1-2 mM, as above. Animals were pronounced dead when respiration was ceased for several minutes. In animals alive at 8 hours, ethanol and L-lactate infusions were discontinued and animals were monitored up to 24 hours after GHB administration. For these experiments, GHB and ethanol were administered in a similar manner as in the respiratory depression experiments. SCH50911 was administered as a 5 mg/mL solution in saline via the jugular vein cannula. L-lactate was administered as a 40 mg/mL solution in sterile water via the femoral vein cannula. For the simultaneous infusion of ethanol/L-lactate, the concentration of L-lactate was maintained at 40 mg/mL and ethanol was added to the solution to maintain a 1.85 mg/min infusion of ethanol in each animal.

Oral toxicokinetic study. As the effect of high oral doses of ethanol on the toxicokinetics of high oral doses of GHB have not been previously evaluated, this potential interaction was assessed by administration of 1500 mg/kg GHB to rats, with and without 2.5 g/kg ethanol, by oral gavage (n=4-5). Rats were fasted for a minimum of 12 hours before drug administration. Blood and urine samples were collected for up to 15 hours following drug administration. GHB alone was administered as a 300 mg/mL solution in water.
combined GHB-ethanol administration, ethanol was added to 300 mg/mL GHB to result in an ethanol concentration of ~40% (v/v).

**Sample analysis.** GHB plasma and urine concentrations were determined using previously validated LC/MS/MS assays (Felmlee et al., 2010a; Morse et al., 2012). Serum ethanol concentrations were determined using an enzymatic assay (Ethanol Assay Kit A-111, Biomedical Research Service Center, University at Buffalo, Buffalo, NY).

**Data/statistical analysis.** To determine GHB toxicokinetic parameters, noncompartmental analysis was performed using WinNonLin 5.2 (Pharsight, Palo Alto, CA). Area under the plasma concentration-time curve (AUC) was determined using the trapezoidal method. Total clearance (Cl) or oral clearance (Cl/F) was determined as Dose/AUC. Renal clearance (ClR) was determined as Ae/AUC, where Ae represents the total amount of GHB excreted in the urine. Metabolic or nonrenal clearance (Clm) was determined as Cl-ClR. To assess the effect of ethanol on GHB-induced respiratory depression, the pharmacodynamic descriptors of area below the effect curve (ABEC) and maximum effect (E_{max}) were used. ABEC was determined using WinNonLin. In all studies, for the detection of statistically significant differences in toxicokinetic/toxicodynamic parameters, mean values were compared using student’s t-tests or one-way ANOVA followed by Tukey’s or Dunnett’s post-hoc tests. Differences resulting in p<0.05 were considered significant.
Results

Effect of ethanol on GHB toxicokinetics. GHB plasma concentrations following the administration of GHB 600 mg/kg IV in the presence and absence of ethanol are displayed in Figure 1a. Serum ethanol concentrations obtained in this experiment are shown in Figure 1b. Mean steady-state ethanol concentrations were 0.18 ± .03% and 0.36 ± 0.06%, for the moderate and high dose of ethanol, respectively, indicating that the targeted steady-state ethanol concentrations were achieved with the doses administered. As shown in Table 1, at neither dose did ethanol co-administration result in a significant difference in GHB total, renal, or metabolic clearance compared to GHB alone. GHB plasma concentrations following the oral administration of GHB with and without ethanol are displayed in Figure 2. As shown in Table 2, no significant differences in mean toxicokinetic parameters were detected between groups.

Effect of ethanol, specific receptor inhibitors, and treatment with L-lactate on the sedative effect of GHB. As displayed in Figure 3, co-administration of ethanol 2.0 g/kg significantly increased the sleep time observed with GHB 600 mg/kg. Treatment with L-lactate 5 minutes after GHB-ethanol significantly decreased the sleep time compared to GHB-ethanol alone. As shown in Table 3, significantly lower GHB plasma and brain concentrations were determined at RRR with GHB-ethanol administration compared to GHB alone, however the brain:plasma ratio was unchanged. With L-lactate administration, brain concentrations of GHB were similar at RRR compared to GHB-ethanol alone, however the brain:plasma ratio was significantly decreased. Effects of pretreatment with specific receptor inhibitors are shown in Figure 4. Pretreatment with bicuculline 1 mg/kg resulted in similar sleep times as that of GHB-ethanol alone. (Higher doses of this agent were also evaluated, but resulted in fatalities.) Pretreatment with both SCH50911 and SGS742 significantly decreased the sleep time compared to GHB-ethanol alone; however, there were no significant differences between the higher and
lower dose groups of these agents. Receptor inhibitors had no effect on the blood-brain partitioning of GHB (data not shown).

Effect of ethanol, specific receptor inhibitors, and treatment with L-lactate on respiratory depression and fatality in GHB overdose. Plethysmography results following administration of GHB 600 mg/kg IV with and without ethanol are displayed in Figure 5. Targeted ethanol concentrations of 0.1-0.2% and 0.3-0.4% were maintained in this experiment (Figure 1b). No significant differences were detected for any respiratory parameter when assessed with ethanol alone at either dose compared to baseline. As shown in Table 4, the administration of ethanol with GHB did not significantly affect respiratory rate at either dose of ethanol compared to GHB alone; however both doses of ethanol significantly decreased the ABEC and E_{max} for tidal volume after GHB administration. No significant differences in minute volume E_{max} were detected at this GHB dose. Pretreatment with bicuculline did not affect respiratory rate, nor the tidal volume compared to GHB-ethanol alone. Pretreatment with 100 mg/kg SCH50911 completely prevented any decrease in respiratory rate. The compensatory increase in tidal volume in the SCH50911-treated group was also absent. Mean steady-state ethanol concentrations were maintained at target concentrations and were similar between control, bicuculline, and SCH50911 groups (0.36 ± 0.06, 0.31 ± 0.04, and 0.38 ± 0.07, respectively, P>0.05). Bicuculline and SCH50911 had no effect on GHB clearance. Results following pretreatment with SGS742 were highly variable, completely preventing respiratory depression in some animals while some animals still displayed effects similar to control, therefore this data is not shown. Administration of the same ethanol doses with GHB 1500 mg/kg resulted in increased rates of fatality with both ethanol doses compared to GHB alone, as shown in Table 5. Administration of L-lactate completely prevented fatality with GHB-ethanol 0.1-0.2%, and also decreased the rate of fatality observed with GHB-ethanol 0.3-0.4%, compared to that without treatment.
Administration of 5 mg/kg SCH50911 completely prevented fatality at the higher ethanol concentration of 0.3-0.4%.
Discussion

Although GHB abuse has been recognized as a significant public health issue, no specific treatment option exists for GHB overdose. Due to the high rate of ethanol co-ingestion in GHB overdose, evaluation of the toxicokinetic/toxicodynamic interaction between these agents is necessary to inform potential treatment for GHB intoxication. In this research we aimed to determine the nature of the toxicokinetic/toxicodynamic interactions between GHB and ethanol, using clinically relevant endpoints, and to assess the potential of a clinically available treatment, L-lactate, along with that of receptor inhibition, for improvement of these toxicodynamic measures in GHB-ethanol intoxication.

Regarding the toxicokinetic interaction between GHB and ethanol, our study indicates no effect of intravenous ethanol administration on the clearance of GHB, as has been previously reported (Van Sassenbroeck et al., 2003; Fung et al., 2008). A lack of effect of GHB on ethanol toxicokinetics has also been confirmed in these previous studies and was therefore not currently evaluated. GHB itself demonstrates several nonlinear pharmacokinetic properties, and previous research indicates oral GHB absorption to be dose-dependent and saturable at high oral doses (Lettieri and Fung, 1979; Arena and Fung, 1980). Our study using CaCo-2 cells also indicates saturable transport of GHB, and suggests this process to be MCT-mediated (Lam et al., 2010). It has been reported that transport of the MCT substrate, butyrate, in rat intestinal epithelial cells could be inhibited by high concentrations of ethanol, as well as its metabolite, acetaldehyde, which may be present in the intestine following high oral doses of ethanol (Goncalves et al., 2011). Due to a potential MCT-mediated intestinal interaction, we evaluated the effect of a high oral dose of ethanol on a high oral dose of GHB; however, our results from this experiment did not result in any significant effect on GHB oral clearance, although absorption appeared to be prolonged. It is possible that
inhibition of MCTs by ethanol simply delays GHB absorption without affecting total bioavailability, due to the continuous expression of MCTs throughout the GI tract, and therefore does not affect total oral clearance.

Results from our sedation study indicate that ethanol increases the sedative effect observed with GHB, which also concurs with previous reports. McCabe, et al. reported a longer sleep time with GHB-ethanol co-administration than the sum of the sleep times with either agent alone (McCabe et al., 1971), as we observed in our study. Van Sassenbroeck, et al. reported similar effects on sleep time, concluding a synergistic interaction between the two drugs at high concentrations of ethanol (Van Sassenbroeck et al., 2003). Studies in rodents have demonstrated the involvement of GABA_A receptors in the sedative effect of ethanol and the involvement of GABA_B receptors in the sedative effect of GHB (Liljequist and Engel, 1982; Beleslin et al., 1997; Carai et al., 2001).

Interestingly, in the current study, pretreatment with even the highest possible bicuculline dose did not significantly decrease sleep time compared to GHB-ethanol alone. This indicates that while sedative effects of ethanol alone may be partially attributed to action at GABA_A receptors, this receptor does not appear to be involved in the potentiation of GHB’s sedative effect by ethanol. Additionally, while pretreatment with both GABA_B receptor antagonists significantly decreased sleep time compared to GHB-ethanol alone, GABA_B inhibition was not completely effective in preventing sleep, and there was no difference between dose groups of either GABA_B receptor antagonist, suggesting maximal inhibition of GABA_B-mediated effects. In this experiment, co-administration of ethanol increased sleep time by approximately 60 minutes compared to GHB alone, which is similar to the sleep times observed with GHB-ethanol administration following GABA_B receptor inhibition. This suggests GABA_B receptor inhibition prevents the sedative effect of GHB, but not the potentiating effect of ethanol on this endpoint, and indicates effects of GHB-ethanol co-administration on sedation other than that mediated...
through direct action at GABA<sub>B</sub> receptors. It has also been postulated that the increased effect of ethanol on GHB-induced sedation may be due to an increase in GHB concentrations at the effect site, i.e. an effect of ethanol on GHB blood-brain barrier permeability (Van Sassenbroeck et al., 2003). When we measured GHB plasma and brain concentrations at RRR, both were lower with ethanol administration compared to GHB alone, but the brain:plasma ratio was maintained. These data indicate that ethanol co-administration alters the sedation concentration-effect relationship of GHB, but that this effect is not due to an increase in GHB brain partitioning. Interestingly, with administration of L-lactate following GHB-ethanol, the brain:plasma ratio was significantly lower compared to GHB-ethanol alone. While the primary effect of L-lactate on GHB toxicokinetics is mediated through inhibition of renal reabsorption, as shown in our previous publications (Morris et al., 2005; Wang et al., 2008a), this decreased brain partitioning, also presumably due to inhibition of MCTs at the blood-brain barrier, may play a role in the improvement of sedation with L-lactate treatment in the current study.

To assess the effect of ethanol on GHB-induced respiratory depression, we chose two steady-state ethanol concentrations, a moderate, clinically relevant concentration of 0.1-0.2% (w/v), similar to blood alcohol levels reported in GHB overdose cases (Caldicott et al., 2004; Couper et al., 2004), and a high concentration of 0.3-0.4%. Our previous research evaluating the effect of GHB alone on respiration indicates the primary effect of GHB to be a decrease in respiratory rate, which is accompanied by a compensatory increase in tidal volume, allowing minute volume to be maintained until doses approach lethality (Morse et al., 2012). The decrease in respiratory rate in this previous study was demonstrated to be completely prevented by GABA<sub>B</sub> receptor inhibition. The current data indicate the effect of ethanol on the concentration-effect relationship of GHB-induced respiratory depression to be prevention of this normally observed increase in tidal volume. Similar to results from our sedation
study, this effect of ethanol on respiration does not appear to be mediated through the GABA_\text{A} receptor, as pretreatment with bicuculline did not prevent the inhibition of tidal volume induced by ethanol. However, similar to our previous study with GHB alone, pretreatment with the GABA_\text{B} antagonist, SCH50911, was able to completely prevent any significant decrease in respiratory rate, precluding the need for the compensatory increase in tidal volume. Preclinical studies using the same dose of SCH50911 have reported no effect of this inhibitor alone on respiration (Bolser et al., 1995), indicating the complete lack of respiratory depression with SCH50911 pretreatment is due to prevention of GHB GABA_\text{B}-mediated effects.

The prevention of the compensatory increase in tidal volume observed with ethanol administration would be expected to translate into a lower minute volume with ethanol administration compared to GHB alone; however, at a GHB dose of 600 mg/kg, no significant difference in minute volume could be detected. Based upon our previous data, minute volume represents an insensitive measure of GHB-induced respiratory depression, due to little change in this measure before death (Morse et al., 2012). Therefore, in order to determine the overall effect of ethanol on GHB-induced respiratory depression, we chose to directly assess fatality, secondary to respiratory arrest, following the administration of GHB 1500 mg/kg. In this experiment, the prevention of the normal compensatory increase in tidal volume with ethanol administration translated into an increased risk of death from respiratory depression. L-lactate treatment decreased the fatality rate, at a dose determined in our previous study to result in clinically relevant increases in plasma lactate concentrations of about 1.5 mM, which result in no adverse effects (Morse et al., 2012). Due to results indicating complete prevention of respiratory depression with pretreatment of SCH50911, treatment with this inhibitor was also evaluated. SCH50911 pretreatment completely prevented fatality in GHB-ethanol intoxication, and was effective at a surprisingly low dose.
In summary, ethanol potentiates the sedative and respiratory effects of GHB, and inhibition of the compensatory increase in tidal volume with ethanol co-administration leads to an increased risk of death due to respiratory depression in GHB overdose. GABA\textsubscript{B} receptors appear to be involved in both sedation and respiratory depression in GHB-ethanol intoxication, while current data supports little involvement of GABA\textsubscript{A} receptors in GHB-ethanol toxicodynamics. Increasing GHB clearance via MCT inhibition with L-lactate results in improvement in toxicodynamic measures with GHB-ethanol administration, including the prevention of death from respiratory arrest. Therefore, MCT inhibition represents a potential clinical therapeutic strategy for the treatment of GHB overdose in the presence of ethanol. GABA\textsubscript{B} receptor inhibition may also be effective in GHB-ethanol intoxication, pending approval for use of GABA\textsubscript{B} receptor antagonists in the clinic.
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Authorship Contributions

*Participated in research design:* Morris and Morse

*Conducted experiments:* Morse

*Contributed new reagents or analytic tools:* Morris and Morse

*Performed data analysis:* Morse

*Wrote or contributed to the writing of the manuscript:* Morris and Morse
References


Footnotes

a) This work was supported by the National Institutes of Health National Institute on Drug Abuse [grant DA023223] and by a fellowship from Pfizer Global Research and Development.

b) A portion of this work was previously presented as an abstract: Respiratory Depression in \(\gamma\)-Hydroxybutyrate Overdose: Interaction with Ethanol and Treatment using Monocarboxylate Transporter Inhibition, American Association of Pharmaceutical Sciences Annual Meeting 2011, Washington DC.

c) Reprint requests should be submitted to:

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Figure Legends

Figure 1. GHB and ethanol concentrations following administration of GHB 600 mg/kg IV with and without ethanol. a) Plasma GHB concentrations b) serum ethanol concentrations. Ethanol was administered intravenously as a 1.0 or 2.0 g/kg bolus to reach target concentrations of 0.1-0.2% and 0.3-0.4% (w/v), followed by a 1.85 mg/min infusion for 6 hours after GHB administration. GHB was administered 60 minutes after the ethanol bolus, at time 0. Data presented as mean ± SD, n=5

Figure 2. Plasma GHB concentrations following administration of GHB 1500 mg/kg PO with and without ethanol. GHB and ethanol (2.5 g/kg) were administered concomitantly to rats by oral gavage. Data presented as mean ± SD, n=4-5

Figure 3. Effect of ethanol co-administration and treatment with L-lactate on the sedative effect of GHB. GHB 600 mg/kg and ethanol 2.0 g/kg were administered intravenously. L-lactate 66 mg/kg + 302.5 mg/kg/hr infusion was administered 5 minutes after GHB-ethanol administration and continued until animal sacrifice at RRR. One-way ANOVA followed by Tukey’s post-hoc test was used to determine statistically significant differences in sleep time between groups. Data presented as mean ± SD, n=3-8. Data from GHB 600 mg/kg alone was used from a previous study (Felmlee et al., 2010b) # significantly different from ethanol alone (p<0.05) * significantly different from GHB-ethanol (p<0.05)

Figure 4. Effect of pretreatment with specific receptor inhibitors on sedation in GHB-ethanol intoxication. GHB 600 mg/kg and ethanol 2.0 g/kg were administered intravenously. Receptor inhibitors were administered intravenously just prior to GHB-ethanol. One-way ANOVA followed by Tukey’s post-hoc test was used to determine
statistically significant differences in sleep time between groups. Data presented as mean ± SD, n=3-4

* significantly different from GHB-ethanol (P<0.05)

Figure 5. Effect of ethanol and pretreatment with specific receptor antagonists on respiratory depression following administration of GHB 600 mg/kg IV. Ethanol was administered intravenously as a 1.0 or 2.0 g/kg bolus at -60 minutes to reach target concentrations of a) 0.1-0.2% and b) 0.3-0.4% (w/v), followed by a 1.85 mg/min infusion at -30 minutes, which continued until 360 minutes. GHB was administered at time 0. Receptor antagonists were administered in animals receiving 0.3-0.4% ethanol, just prior to GHB administration. Baseline measurements at -75 minutes represent baseline prior to any drug administration (measurements were taken at -15 minutes for GHB alone and are plotted at -75 minutes for comparison to ethanol-treated groups). Measurements at -15 minutes represent those in the presence of ethanol alone. Data presented as mean ± SD, n=3-5
Tables

Table 1. Toxicokinetics of IV GHB with and without ethanol co-administration

<table>
<thead>
<tr>
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<th>Cl (mL/kg/min)</th>
<th>ClR (mL/kg/min)</th>
<th>Clm (mL/kg/min)</th>
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<tr>
<td>GHB 600 mg/kg IV</td>
<td>5.84 (0.64)</td>
<td>1.61 (0.48)</td>
<td>4.22 (0.44)</td>
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<tr>
<td>GHB 600 mg/kg IV + EtOH 0.1-0.2%</td>
<td>5.30 (0.58)</td>
<td>1.76 (0.22)</td>
<td>3.54 (0.46)</td>
</tr>
<tr>
<td>GHB 600 mg/kg IV + EtOH 0.3-0.4%</td>
<td>5.91 (1.03)</td>
<td>2.09 (0.98)</td>
<td>3.82 (0.47)</td>
</tr>
</tbody>
</table>

Cl=total clearance    ClR=renal clearance    Clm=metabolic clearance

Data presented as mean (SD), n=5. Ethanol was administered as a 1.0 or 2.0 g/kg IV bolus injection, (followed by an infusion of 1.85 mg/min ), resulting in target concentrations of 0.1-0.2% and 0.3-0.4% (w/v), respectively. GHB was administered 60 minutes after ethanol bolus administration. Results of one-way ANOVA followed by Dunnett’s post-hoc test indicated no statistically significant differences between mean toxicokinetic parameters in either ethanol-treated group compared to GHB alone.
Table 2. Toxicokinetics of oral GHB with and without ethanol co-administration

<table>
<thead>
<tr>
<th></th>
<th>Cl/F (mL/kg/min)</th>
<th>C_{max} (μg/mL)</th>
<th>T_{max} (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHB 1500 mg/kg PO</td>
<td>7.49 (1.1)</td>
<td>492 (200)</td>
<td>6.0 (2.5)</td>
</tr>
<tr>
<td>GHB 1500 mg/kg PO + EtOH 2.5 g/kg</td>
<td>7.46 (1.6)</td>
<td>489 (240)</td>
<td>3.88 (4.4)</td>
</tr>
</tbody>
</table>

Cl/F = total clearance/bioavailability (oral clearance)

C_{max} = maximum plasma concentration  
T_{max} = time of maximum plasma concentration

Data presented as mean (SD), n=4-5. GHB and ethanol were administered concomitantly by oral gavage. Student’s t-tests indicated no statistically significant differences in mean toxicokinetic parameters between groups.
Table 3. Effect of ethanol and L-lactate treatment on GHB plasma and brain concentrations at RRR

<table>
<thead>
<tr>
<th>Time of RRR (min)</th>
<th>C\textsubscript{plasma} (μg/mL)</th>
<th>C\textsubscript{brain} (μg/mL)</th>
<th>Brain:plasma ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHB</td>
<td>110 (17) *</td>
<td>352 (64) *</td>
<td>0.20 (0.03)</td>
</tr>
<tr>
<td>GHB + EtOH</td>
<td>162 (38)</td>
<td>178 (76)</td>
<td>0.21 (0.03)</td>
</tr>
<tr>
<td>GHB + EtOH + L-lactate</td>
<td>93 (8) *</td>
<td>257 (61)</td>
<td>0.15 (0.02) *</td>
</tr>
</tbody>
</table>

RRR=return-to-righting reflex

C\textsubscript{plasma}=plasma GHB concentration at RRR  
C\textsubscript{brain}=brain GHB concentration at RRR

Data presented as mean (SD), n=3-8. GHB (600 mg/kg) and ethanol (2.0 g/kg) were administered intravenously. L-lactate (66 mg/kg + 302.5 mg/kg/hr) was given intravenously 5 minutes after GHB/ethanol. Animals were sacrificed at RRR. One-way ANOVA followed by Dunnett’s post-hoc test was used to determine statistically significant differences compared to GHB + ethanol. Data from GHB 600 mg/kg alone was used from a previous study (Felmlee et al. 2010b)

* significantly different from GHB + ethanol (p<0.05)
Table 4. Effect of ethanol and pretreatment with specific receptor antagonists on GHB-induced respiratory depression

<table>
<thead>
<tr>
<th></th>
<th>GHB</th>
<th>GHB + EtOH 0.1-0.2%</th>
<th>GHB + EtOH 0.3-0.4%</th>
<th>GHB + EtOH 0.3-0.4% + bicuculline</th>
<th>GHB + EtOH 0.3-0.4% + SCH50911</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frequency ABEC</strong></td>
<td>5540 (1000)</td>
<td>4090 (1300)</td>
<td>5440 (2100)</td>
<td>5430 (1500)</td>
<td>------</td>
</tr>
<tr>
<td>(breaths)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Frequency E\text{max}</strong></td>
<td>31 (5)</td>
<td>38 (6)</td>
<td>39 (11)</td>
<td>45 (7)</td>
<td>------</td>
</tr>
<tr>
<td>(breaths/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tidal Volume ABEC</strong></td>
<td>207 (56)</td>
<td>132 (49) *</td>
<td>119 (32) *</td>
<td>123 (11) *</td>
<td>------</td>
</tr>
<tr>
<td>(mL/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tidal Volume E\text{max}</strong></td>
<td>3.63 (0.69)</td>
<td>2.65 (0.31) *</td>
<td>2.60 (0.38) *</td>
<td>2.60 (0.36) *</td>
<td>------</td>
</tr>
<tr>
<td>(mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Minute volume E\text{max}</strong></td>
<td>81 (16)</td>
<td>64 (11)</td>
<td>69 (23)</td>
<td>65 (5)</td>
<td>------</td>
</tr>
<tr>
<td>(mL/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ABEC=area below the effect curve  \( E_{\text{max}} \)=maximum pharmacodynamic effect

------ no significant changes in any respiratory parameters were determined compared to baseline with pretreatment of SCH50911

Data presented as mean, (SD), n=3-5. Ethanol was administered as a 1.0 or 2.0 g/kg IV bolus, followed by a 1.85 mg/min infusion. GHB 600 mg/kg IV was administered 60 minutes after ethanol bolus administration. One-way ANOVA followed by Tukey’s post-hoc test was used to determine statistically significant differences in mean toxicodynamic parameters between groups.

*significantly different from GHB alone (p<0.05)
Table 5. Effect of ethanol and potential treatment strategies on fatality following administration of GHB 1500 mg/kg IV

<table>
<thead>
<tr>
<th>Dose administered</th>
<th>No treatment</th>
<th>L-lactate</th>
<th>SCH50911</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHB</td>
<td>0/10</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>GHB + ethanol 0.1-0.2%</td>
<td>4/10</td>
<td>0/10</td>
<td>------</td>
</tr>
<tr>
<td>GHB + ethanol 0.3-0.4%</td>
<td>9/10</td>
<td>5/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

Ethanol was administered intravenously as a 1.0 or 2.0 g/kg bolus to reach target concentrations of 0.1-0.2% and 0.3-0.4% (w/v), followed by a 1.85 mg/min infusion until 8 hours after GHB administration. GHB administration was given 60 minutes after the ethanol bolus and was considered time 0. L-lactate (66mg/kg, followed by an infusion of 302.5 mg/kg/hr for 8 hours) and SCH50911 (5 mg/kg) were administered 5 minutes after GHB. Data presented as number of fatalities/total animals evaluated.