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Treatment of γ-Hydroxybutyrate Overdose with the $GABA_B$ Antagonist SGS742[S]

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ABSTRACT

High doses of the partial agonist of the $GABA_B$ receptor, γ-hydroxybutyric acid (GHB), cause respiratory depression that can lead to death. Previously, it has been shown that $GABA_B$ receptor antagonism is able to prevent respiratory depression and sedation when inhibitors are preadministered. To treat GHB overdoses, safety and efficacy of a treatment strategy at various times after GHB administration are necessary to more closely replicate a true overdose situation. Preliminary studies developed an assay for SGS742 and determined its pharmacokinetics in rats. The effects of SGS742 on GHB-induced respiratory depression were evaluated when SGS742 administration was delayed 1 and 2 hours after intravenous or oral administration of GHB or γ-butyrolactone, a GHB prodrug. SGS742 reversed GHB-induced respiratory depression in a dose-dependent manner at both time points tested, with no effects on its toxicokinetics. However, some of the dosing paradigms resulted in toxicity in the form of tremors, seizures, or abnormal movements. The tremors/seizures occurred in a manner that was dependent on both the dose and timing of SGS742 administration and were not altered with pretreatment with gabazine, a $GABA_A$ receptor inhibitor, and only partially reduced with pretreatment with NCS382, a selective GHB receptor antagonist. Additional studies with a second $GABA_B$ antagonist SCH50911 demonstrated similar effects, producing reversal of respiratory depression but producing tremors and abnormal movements. Further work is necessary to identify the potential use of $GABA_B$ antagonism as a treatment strategy for GHB overdoses.

SIGNIFICANCE STATEMENT

There is no current treatment for overdoses of the drug GHB. Since the toxicodynamic effects of GHB, namely sedation and respiratory depression, are mediated through $GABA_B$ receptor agonism, $GABA_B$ receptor antagonists may represent a therapeutic strategy to treat overdoses. This study demonstrates that although $GABA_B$ receptor antagonists are effective as a pretreatment, they are less effective when administered at times after GHB administration, and their administration is also associated with time- and dose-associated toxicity, namely tremors or seizures.

Introduction

γ-hydroxybutyric acid (GHB) is a structural analog of the major inhibitory neurotransmitter GABA (Waszkielewicz and Bojarski, 2004). Currently, GHB is marketed in the United States, Canada, and Europe as Xyrem for the treatment of narcolepsy; in Austria and Italy as Alcover for the treatment of alcohol withdrawal; and in Germany as an anesthetic Somsanit (Carter et al., 2009b). Despite these numerous clinical applications, the therapeutic utility of GHB has been limited by its high abuse potential.

GHB has several pharmacological effects that are exploited in abuse; these include euphoria, decreased inhibitions, and growth hormone release (White, 2017; Felmlee et al., 2021). The abuse of GHB carries the risk of severe adverse effects such as sedation, respiratory depression, hypothermia, coma, and even death (White, 2017; Trombley et al., 2019). GHB remains in the top five drugs involved with emergency department visits by the European Drug Emergencies Network (White, 2017). This demonstrates that GHB abuse still represents a threat to public health, as the risk of toxicity to users is high (Di Trana et al., 2021). Overdoses after the use of GHB to facilitate date rape, or chemsex-related intoxications, magnify the need for a treatment option that is specific to GHB overdose (Drevin et al., 2021; Felmlee et al., 2021). Additionally, the investigation of GHB overdose should also include γ-butyrolactone (GBL) overdose (White, 2017), since GBL is a widely used GHB prodrug (Tini and Del Rio, 2020; Di Trana et al., 2021) that is rapidly converted to GHB upon ingestion (Lettieri and Fung, 1978).

GHB is a weak agonist of $GABA_B$ and it has been demonstrated that many of the toxicological effects of GHB are mediated through the $GABA_B$ receptor (Carai et al., 2001; Kaupmann et al., 2003; Goodwin et al., 2005; Morse et al., 2012). This has implicated $GABA_B$ antagonism as a viable option for GHB overdose treatment. Currently, there are no

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ABBREVIATIONS: ABEC, area below the effect curve; E_{max}, maximum effect; GBL, γ-butyrolactone; GHB, gamma-hydroxybutyric acid; NMDA, N-methyl-D-aspartate; T_d, duration of effect; TD, toxicodynamics.

$GABA_B$ antagonists clinically available; however, one compound, SGS742 (CGP 36742; Fig. 1) with an IC_{50} of 38 μM (Froestl et al., 1995), has been studied in clinical trials for mild cognitive impairment; SGS742 was well tolerated in human subjects, with no serious adverse events (Gleiter et al., 1996; Froestl et al., 2004). SGS742 has also been used in clinical trials for the treatment of succinic semialdehyde dehydrogenase deficiency, a pediatric/adult epilepsy disorder with increased GABA and GHB plasma and brain concentrations (Schreiber et al., 2021). When administered prior to the concomitant intravenous administration of GHB and ethanol, an i.v. bolus of 500 or 1000 mg/kg SGS742 has been shown to be effective in reducing sleep time, a sedation endpoint, in rats (Morse and Morris, 2013b). Pretreatment with SGS742 reduces GHB-induced effects such as ataxia and muscle relaxation in baboons (Goodwin et al., 2005, 2006). A more potent $GABA_B$ antagonist, SCH50911, has also been effective in reducing sleep time in rats (Morse and Morris, 2013b), and treatment with SCH50911, prior to GHB administration or 5 minutes after GHB dosing, completely prevents the respiratory depressant effects observed with GHB (Morse et al., 2012). GHB has also been demonstrated to exert effects at $GABA_A$ and GHB receptors (Absalom et al., 2012; Bay et al., 2014). Pretreatment with the $GABA_A$ receptor antagonist bicuculline had no effect on GHB-induced sedation or respiratory depression (Morse et al., 2012); however, the impact of GHB at this receptor or at GHB receptors in the presence of $GABA_B$ antagonists has not been investigated and could play an important role in adverse effects after treatments for GHB overdose.

Our hypothesis was that reversal of $GABA_B$ receptor agonism due to GHB will result in decreased toxicity and death after overdoses, when the dosing paradigm more closely resembles an overdose situation. In a clinical overdose, pretreatment will not be possible, and immediate treatment is unlikely. The first aim of this research was to investigate the impact of the $GABA_B$ receptor antagonist SGS742 on GHB toxicokinetics and toxicodynamics (respiratory depression), when administered at times after GHB ingestion. The ideal GHB overdose treatment must be safe and effective when administered at various time points after GHB administration. Our studies demonstrated efficacy, but also toxicity in the form of tremors and seizures; therefore, our second aim was to investigate the role of $GABA_A$ and GHB receptors in the toxicity observed after GHB and $GABA_B$ receptor antagonist administration. Gabazine, a drug that acts as a $GABA_A$ receptor antagonist, and NCS382, a selective GHB receptor antagonist, were used in our studies (Castelli et al., 2004; Ainslie et al., 2016).

Materials and Methods

Chemical and Reagents. National Institute on Drug Abuse provided sodium GHB, SGS742, and 6,7,8,9-tetrahydro-5-hydroxy-5H-benzocyclohept-6-ylideneacetic acid (NCS382). Deuterated GHB (GHB-d6) was obtained from Cerilliant Corporation (Round Rock, TX). High-performance liquid chromatography grade acetonitrile and acetic acid were purchased from Honeywell Burdick & Jackson (Muskegon, MI). (2S)-(+)-5,5-Dimethyl-2-morpholineacetic acid (SCH50911) and gabazine were purchased from Tocris (Bristol, UK).

Animals and Surgery. Male Sprague–Dawley rats (Harlan, Indianapolis, IN), greater than 60 days of age (average of 300 g) were housed under controlled humidity and temperature, with an artificial 12-hour light/dark cycle; food was available ad libitum. Cannulae were surgically implanted in the jugular vein, under ketamine/xylazine anesthesia, and flushed daily with 40 IU/ml heparinized saline to maintain patency. Experiments were performed after a minimal recovery phase of 72 hours after surgery. All animal procedures were approved by University at Buffalo Institutional Animal Care and Use Committee.

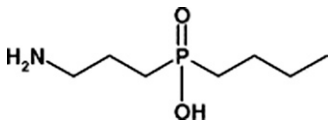

Fig. 1. Chemical structure of SGS742 (3-aminopropyl-*n*-butyl phosphinic acid).

Pharmacokinetic Studies of SGS742. Studies were performed to determine SGS742 pharmacokinetic and dosing regimens. Blood samples were collected over 8 hours, at time points of 3, 11, 21, 31, 61, 121, 181, 241, 301, 331, 361, and 481 minutes. Treatment groups consisted of 3 animals for each of the 100 and 500 mg/kg doses. SGS742 was administered as a sterile 100 or 200 mg/ml solution in normal saline via the jugular vein cannula.

Pharmacodynamic Studies for SGS742. The effect of SGS742 on respiration was studied using whole-body plethysmography (model PLY4213; Buxco Research Systems, Wilmington, NC), as previously performed in the Morris laboratory (Morse et al., 2012; Morse and Morris, 2013a; Vijay et al., 2015). Animals were allowed to acclimate for 45 minutes in plethysmography chambers prior to the collection of five baseline measurements of the respiratory parameters of interest (breathing frequency, tidal volume, and minute volume) over 15 minutes. SGS742 was administered as a sterile 200 or 100 mg/ml solution in normal saline intravenously via the implanted cannulae. Respiratory parameters were measured at 2.5, 5, 7.5, 10, 15, 20, 25, and 30 minutes and every 15 minutes thereafter until 8 hours.

Impact of SGS742 on GHB Toxicokinetics. The effect of SGS742 on the toxicokinetics of GHB was investigated. GHB was administered intravenously as a 1500 mg/kg bolus via a jugular vein cannula. The dose was selected to produce plasma concentrations in the range reported after clinical overdoses (Zvosec et al., 2011). The plasma concentration-time profiles in rats have been demonstrated in previous studies, as well as the toxicity observed at the doses used in this study (Morse et al., 2012; Morse and Morris, 2013a; Vijay et al., 2015). The time of administration for GHB was considered to be time 0. An i.v. bolus of 500 mg/kg SGS742 was administered 60 minutes after the GHB dose. Blood samples were collected for 8 hours after GHB administrations, including time points of 3, 11, 21, 31, 61, 121, 181, 241, 301, 331, 361, and 481 minutes. GHB was administered as a sterile 300 mg/ml solution in double distilled H_2O via the jugular vein cannula. SGS742 was administered as a sterile 300 mg/ml solution in normal saline. Treatment groups were compared with historical data where the same dose (1500 mg/kg) was administered in the same manner (Morse et al., 2012; Morse and Morris, 2013a; Vijay et al., 2015).

Impact of SGS742 and SCH50911 on GHB Toxicodynamics. The effect of SGS742 on the toxicodynamics (TD) of GHB was studied using whole-body plethysmography (model PLY4213; Buxco Research Systems, Wilmington, NC). Animals were allowed to acclimate for 45 minutes in plethysmography chambers prior to the collection of five baseline measurements of the respiratory parameters of interest over 15 minutes. GHB was then administered intravenously or orally via oral gavage, as a 14.4 mmol/kg bolus. For studies investigating GBL overdose, GBL was administered after baseline readings as a 5.77 mmol/kg oral dose via oral gavage. The time of administration for GHB/GBL was set as the reference time, 0 minutes. (Note that only GHB can be detected in plasma after GBL administration.) Additional studies were performed with a second $GABA_B$ receptor antagonist SCH50911 with doses of 25 or 50 mg/kg after the administration of intravenous GHB. The treatment compounds, SGS742 or SCH50911, were administered either 60 or 120 minutes after the GHB dose. Blood and urine samples were collected for 8 hours after intravenous GHB or

oral GBL administration and for 15 hours after oral GHB administration. The respiratory parameters breathing frequency, tidal volume, and minute volume were measured at time 2.5, 5, 7.5, 10, 15, 20, 25, and 30 minutes and every 15 minutes thereafter until 8 or 15 hours, along with extra measurements at 5 and 10 minutes after the administration of the treatment compounds. GHB or GBL was administered as a sterile 300 mg/ml solution in double distilled H_2O via the jugular vein cannula. SGS742 was administered as a sterile 300, 250, 100, or 50 mg/ml solution in normal saline via the jugular vein cannula. For studies involving pretreatment with gabazine or NCS382, these compounds were administered intravenously via the jugular cannula as sterile solutions in normal saline at concentrations of 1 or 100 mg/ml, respectively. All treatment groups consisted of 1–7 animals. Treatment groups were compared with historical data where the same dose (14.4 mmol/kg) of GHB was administered in the same manner (Morse et al., 2012; Morse and Morris, 2013a; Vijay et al., 2015).

Plasma Sample Analysis. GHB and SGS742 plasma concentrations were determined using a dual liquid chromatography coupled to tandem mass spectrometry assay developed and validated for this purpose. GHB-d6 (125 μg/ml) was used as an internal standard for GHB, and CGP 36216 (40 μg/ml) was used as an internal standard for SGS742. Dual standard stock solutions as well as a dual internal standard solution were used in sample preparation. To prepare a standard curve, 5 μl of dual internal standard and 5 μl of dual standard stock solution were added to 45 μl of blank plasma. For samples obtained at time points up to and including 241 minutes, 5 μl of sample and 5 μl of dual internal standard were added to 45 μl of blank plasma. For samples obtained after that time, 5 μl of dual internal standard were added to 50 μl of sample. To precipitate the plasma proteins, 800 μl of 0.1% formic acid in acetonitrile was added, and samples were vortexed and centrifuged at 10,000 rpm for 20 minutes. An aliquot (750 μl) of the supernatant was dried under a stream of nitrogen gas and then reconstituted in 1 ml of aqueous mobile phase.

The liquid chromatography coupled to tandem mass spectrometry assay was performed on Shimadzu Prominence high performance liquid chromatography system with binary pump and autosampler (Shimadzu Scientific Instruments, Portland, OR) in conjunction with a Sciex API 3000 triple-quadruple tandem mass spectrometer with a turbo ion spray (Applied Biosystems, Foster City, CA). Chromatographic separation was achieved by injecting 4 μl of sample on an Xterra MS C18 column (250 × 2.1 mm i.d., 5-μm particle size; Waters, Milford, MA). Mobile phase A was 5/95 acetonitrile/water with 0.1% acetic acid, and mobile phase B was 95/5 acetonitrile/water with 0.1% acetic acid. The flow rate was set to 230 μl/min with a gradient elution profile. The total run time for the procedure was 15 minutes. Positive ionization mode was used with multiple reaction monitoring. The Q1/Q3 m/z ratios were 105.1/87.2 and 111.1/93.2 for GHB and its internal standard (GHB-d6), respectively. For SGS742 and its internal standard (CGP 36216) the Q1/Q3 ratios were 180.0/163.0 and 152.0/134.9, respectively. The mass spectrometer parameters were optimized at a declustering potential of 15, focusing potential of 50, and entrance potential of 10. The collision energy and collision cell exit potential were specific to each analyte. For GHB, the collision energy and collision cell exit potential were 15 and 5, respectively. The same parameters were 18 and 8 for SGS742, respectively. The ion spray voltage was set at 5500 V and the temperature was 350°C. The nebulizer and curtain gas flow were set at 10 and 8 ml/min, respectively. The data were analyzed using Analyst software versions 1.4.2 (Applied Biosystems, Foster City, CA).

Regression analysis of GHB and SGS742 peak areas to their concentrations were used to assess the linearity of the curve. The intraday and interday precision and accuracy were determined using quality control samples at 10, 125, and 250 μg/ml for GHB and 5, 10, and 40 μg/ml for SGS742. Intraday precision and accuracy were evaluated by analyzing quality control samples in triplicate each day. Interday precision and accuracy were determined by performing quality control samples on 3 separate days. Each analysis consisted of a calibration curve and quality controls. Precision was determined by the relative standard deviation , and accuracy was determined by comparing the calculated concentration based on the calibration curve to the known concentration.

Data Analysis. Noncompartmental analyses for GHB and SGS742 were performed using the pharmacokinetic solver excel plugin published by Zhang et al. (2010). The primary toxicodynamic parameter was the area below the effect curve (ABEC) for breathing frequency. This was determined in GraphPad Prism 7, using the area under the curve function. The first observed negative peak was analyzed for all TD parameters. For studies including a delay in the administration of the treatment compound, TD parameters were assessed for the TD curve beginning at the time of administration of the treatment compound (60 or 120 minutes). Control TD parameters were derived from the control data from previous studies from the Morris laboratory (Morse and Morris, 2013a; Vijay et al., 2015). ABEC was identified using the individual baseline respiration for each animal, ignoring peaks that were less than 10% of the distance from the minimum to the maximum y-value. Duration of effect was defined as the length of time for the first negative peak. E_{max} was the minimum value for breathing frequency observed.

Statistical significance was determined using one-way ANOVA with Dunnett's post hoc for comparisons of multiple treatment groups to a control, or Tukey's post hoc when multiple groups were all compared against each other. For comparisons of a single group to a control a Student's t test was used. In cases where treatment was administered at 60 or 120 minutes for the same treatment compound, statistics were assessed separately for these two groups, as there were unique control parameters for comparison with these two times of administration. All statistics were performed in GraphPad Prism 7.

Results

SGS742/GHB Plasma Assay

The dual GHB/SGS742 assay exhibited acceptable analyte recovery, accuracy, and precision. The assay had a very consistent recovery of both GHB and SGS742; the recovery of SGS742 was nearly complete, ranging from 95.8%–102%, with a low limit of quantification, and excellent precision and accuracy (Supplemental Table 1). A representative spectrum is presented in Supplemental Fig. 1. The retention times for GHB and SGS742 were 3.62 and 4.23 minutes, respectively. The retention times for the internal standards, GHB-d6 and CGP 36216, were 3.61 and 2.48 minutes, respectively. The lower limits of GHB and SGS742 in plasma samples were 1 and 0.1 μg/ml, respectively. Validation of the assay demonstrated acceptable error in accuracy and precision with RSE values of 10% or less. The standard curve ranged from 0.1 μg/ml to 50 μg/ml for SGS742, and 1 μg/ml to 500 μg/ml for GHB. The inter- and intra- day precision and accuracy of the assay for SGS742 and GHB are presented in Supplemental Table 2.

Pharmacokinetics of SGS742. SGS742 was administered as an intravenous bolus at doses of 500 and 100 mg/kg. The plasma profiles for both doses are shown in Fig. 2. Clearance was linear, as the dose-normalized plasma profiles overlapped (data not shown). Overall, total clearance was 6.8 ml/min/kg, and the average observed half-life was 171 minutes or 2.84 hours (Table 1).

Impact of SGS742 on GHB Toxicokinetics. SGS742 was administered as a 500 mg/kg intravenous bolus 60 minutes after the intravenous administration of 1500 mg/kg GHB. There was no effect of SGS742 administration on the exposure, clearance, or half-life of GHB (Table 2). However, there was a small but statistically significant increase in the

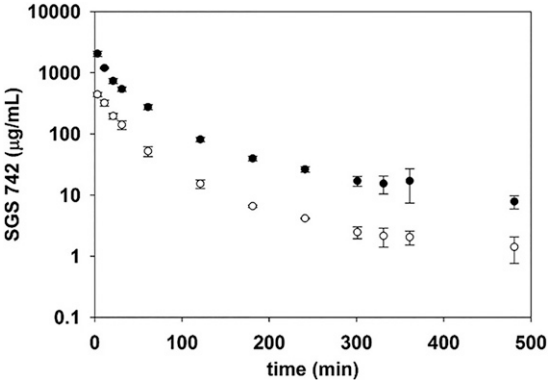

Fig. 2. SGS742 pharmacokinetics. SGS742 was administered as an i.v. bolus at a dose of 500 mg/kg (closed circles) and 100 mg/kg (open circles). Data are represented as mean ± standard deviation, with $n = 3$ for both doses.

TABLE 1
Pharmacokinetic parameters of SGS742
Data presented as mean (standard deviation). No statistical significance between parameters (excluding $AUC_{0-\infty}$) as determined by a Student's t test.

SGS742 Dose	$AUC_{0-\infty}$ (mg*min/ml)	CL_T (ml/min/kg)	V_{ss} (ml/kg)	$t_{1/2}$ (min)
500 mg/kg	69.7 (3.11)	7.18 (0.312)	494 (30.6)	128 (51.2)
100 mg/kg	15.7 (1.46)	6.41 (0.581)	473 (198)	213 (133)

$AUC_{0-\infty}$, area under the curve extrapolated to infinity; CL_T, total clearance; $t_{1/2}$, half-life of elimination; V_{ss}, volume of distribution at steady state.

volume of distribution at steady state with SGS742 treatment (Table 2).

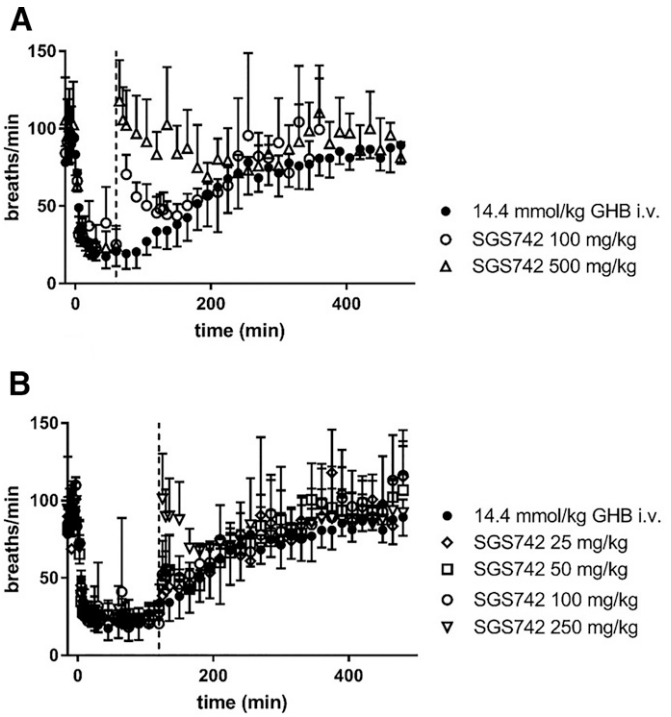

Fig. 3. Effect of SGS742 on intravenous GHB-induced respiratory depression. GHB was administered as an i.v. dose of 1500 mg/kg. SGS742 was administered as an i.v. bolus 60 (A) or 120 (B) minutes after GHB at a dose of 25 (open diamonds), 50 (open squares), 100 (open circles), 250 (open inverted triangles), or 500 mg/kg (open triangles). Breathing frequency control data (closed circles) were obtained from Morse et al. (2012) and Vijay et al. (2015). Data are represented as mean +/− standard deviation, with $n = 3$–6.

Impact of SGS742 on Intravenous GHB Toxicodynamics

The impact of SGS742 on GHB induced respiratory depression was dependent on the time of administration and dose of the treatment compound. Doses ranging from 25 to 500 mg/kg of SGS742 were investigated, with a 60- or 120-minute delay of treatment (Fig. 3). Although SGS742 had no direct effect on respiration (data not shown), SGS742 administered at the earlier treatment time and at the higher doses had the largest effect on GHB TD (Fig. 3; Table 3). Improvement in duration of effect (T_d) was observed for both doses of SGS742 (100 and 500 mg/kg) at the earliest time of administration (60 minutes) (Table 3). When the 500mg/kg dose was administered 120 minutes after GHB, a seizure was observed. Twitching movement was observed at the lower doses for SGS742 when administered at 120 minutes after GHB, with the exception of the lowest dose (25 mg/kg).

Impact of SCH50911 on Intravenous GHB Toxicodynamics. Studies examined the influence of a second $GABA_B$ antagonist SCH50911 on GHB TD to confirm the effects observed with SGS742. SCH50911 administration (25 or 50 mg/kg) reduced the effect of GHB induced respiratory depression when administered 60 or 120 minutes after intravenous GHB (Fig. 4). Breathing frequency increased at the time of SCH50911 administration with both doses tested (25 and 50 mg/kg SCH50911). Analysis of the TD parameters resulted in a significant improvement for ABEC only for the higher dose (50 mg/kg) of SCH50911 administered at the earlier treatment time (60 minutes) (Table 4). This dose elicited a seizure when it was administered 120 minutes after GHB in one animal and was not tested further. No seizures were observed when SCH50911 was administered 120 minutes after GHB at a dose of 25 mg/kg, and this dose also resulted in a significant reduction in both ABEC and t_d (Table 4).

Impact of SGS742 on Oral GHB Toxicodynamics. The effect of SGS742 on oral GHB TD was dose-dependent, as it was with intravenously administered GHB. Higher doses of SGS742 resulted in a greater reduction of the effect of GHB induced respiratory depression (Fig. 5; Table 5). Due to the

TABLE 2
Pharmacokinetic parameters describing the disposition of GHB with and without SGS742 treatment 60 minutes after GHB administration
Data presented as mean (standard deviation). Pharmacokinetic parameters abbreviations are described in Table 1 footnote.

Treatment	$AUC_{0-\infty}$ (mg*min/ml)	CL_T (ml/min/kg)	V_{ss} (ml/kg)	$t_{1/2}$ (min)
1500 mg/kg GHB[a]	295 (40.1)	5.15 (0.706)	400 (22.0)	36.5 (22.8)
1500 mg/kg GHB + SGS742 500 mg/kg 60 min Post GHB	274 (32.2)	5.78 (0.914)	485 (22.4)**	41.5 (11.6)

[a]Data obtained from Vijay et al. (2015).
**$P < 0.01$ as determined by a Student's t test.

TABLE 3
Impact of SGS742 administration on intravenous GHB toxicodynamics

Toxicodynamic Parameter	GHB 14.4 mmol/kg (60 min)	GHB 14.4 mmol/kg + 100 mg/kg SGS742 60 PD	GHB 14.4 mmol/kg + 500 mg/kg SGS742 60 PD	GHB 14.4 mmol/kg (120 min)	GHB 14.4 mmol/kg + 25 mg/kg SGS742 120 PD	GHB 14.4 mmol/kg + 50 mg/kg SGS742 120 PD	GHB 14.4 mmol/kg + 100 mg/kg SGS742 120 PD	GHB 14.4 mmol/kg + 250 mg/kg SGS742 120 PD
ABEC (breaths)	8.63×10^3 (2.64×10^3)	2.44×10^3 (3.95×10^3)	2.39×10^3 (4.57×10^3)	4.92×10^3 (2.42×10^3)	6.67×10^3 (2.96×10^3)	3.61×10^3 (1.97×10^3)	3.96×10^3 (1.65×10^3)	2.66×10^3 (2.29×10^3)
E_{max} (breaths/min)	18.0 (9.45)	25.2 (12.1)	24.3 (10.9)	32.9 (10.6)	26.5 (6.88)	29.9 (9.79)	20.6 (8.37)	24.4 (8.68)
T_d (min)	223 (77.5)	60.6 (88.8)*	32.0 (59.8)**	163 (77.5)	177 (76.1)	136 (75.5)	121 (35.1)	94.2 (79.6)

PD, minutes post GHB dose; T_d, duration of effect.
Data presented as mean (standard deviation). *$P < 0.05$; **$P < 0.01$ determined by one-way ANOVA with Dunnett's post hoc.

Fig. 4. Effect of SCH50911 on intravenous GHB-induced respiratory depression. GHB was administered at 0 minutes as a 1500 mg/kg i.v. bolus. Breathing frequency, tidal volume, and their product, minute volume, were recorded. SCH50911 was administered as an i.v. bolus 60 (A) or 120 (B) minutes after GHB at a dose of 25 (open circles) or 50 (open triangles) mg/kg. Breathing frequency control data (closed circles) were obtained from Morse et al. (2012) and Vijay et al. (2015). Data are represented as mean +/− standard deviation, with $n = 4–5$.

high variability, statistically significant improvements in ABEC and T_d were only achieved for the highest dose of SGS742 (250 mg/kg) when administered 120 minutes after oral GHB (Table 5). The lower doses did not result in an improvement in GHB TD, regardless of time of administration (Table 5). Some twitching was observed in the majority of animals administered 100 or 250 mg/kg SGS742 with both time points of administration.

Impact of SGS742 on Oral GBL Toxicodynamics. SGS742 was administered 120 minutes after oral GBL administration at a dose of 100 mg/kg. There was an increase in breathing frequency after the dose of SGS742 (Fig. 6; Table 6). All TD parameters were improved with SGS742 administered 120 minutes after dosing of GBL, but this was only statistically significant for E_{max} (Table 6).

Impact of Gabazine Pretreatment and SGS742 Treatment on Intravenous GHB Toxicodynamics. When the $GABA_A$ antagonist gabazine was administered 5 minutes prior to intravenous GHB, there was no effect on SGS742 treatment of GHB overdose (Fig. 7; Table 7). With gabazine pretreatment, there was a slight reduction in ABEC, further than what was observed with SGS742 treatment, but this was not statistically significant (Table 7). There was no effect of gabazine pretreatment on the observation of abnormal movements after SGS742 treatment. To assess the impact of gabazine pretreatment on GHB TD, the data were analyzed up to the time of administration of SGS742 at 120 minutes. There was no

TABLE 4
Impact of SCH50911 administration on intravenous GHB toxicodynamics
Data presented as mean (standard deviation). Parameter abbreviations are described in Table 3 footnote.

Toxicodynamic Parameter	GHB 14.4 mmol/kg (60 min)	GHB 14.4 mmol/kg + 25 mg/kg SCH50911 60 PD	GHB 14.4 mmol/kg + 50 mg/kg SCH50911 60 PD	GHB 14.4 mmol/kg (120 min)	GHB 14.4 mmol/kg + 25 mg/kg SCH50911 120 PD
ABEC (breaths)	8.63×10^3 (2.64×10^3)	3.27×10^3 (2.36×10^3)	889 (1.05×10^3)***	4.92×10^3 (2.42×10^3)	951 (1.43×10^3)*
E_{max} (breaths/min)	18.0 (9.45)	28.3 (19.4)**	17.3 (2.01)	32.9 (10.6)	34.9 (15.1)
T_d (min)	223 (77.5)	125 (107)	40.2 (40.6)**	163 (77.5)	38.9 (53.3)*

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ determined by one-way ANOVA with Dunnett's post hoc (60 min) or a Student's t test (120 min).

effect of gabazine pretreatment on GHB induced respiratory depression (data not shown).

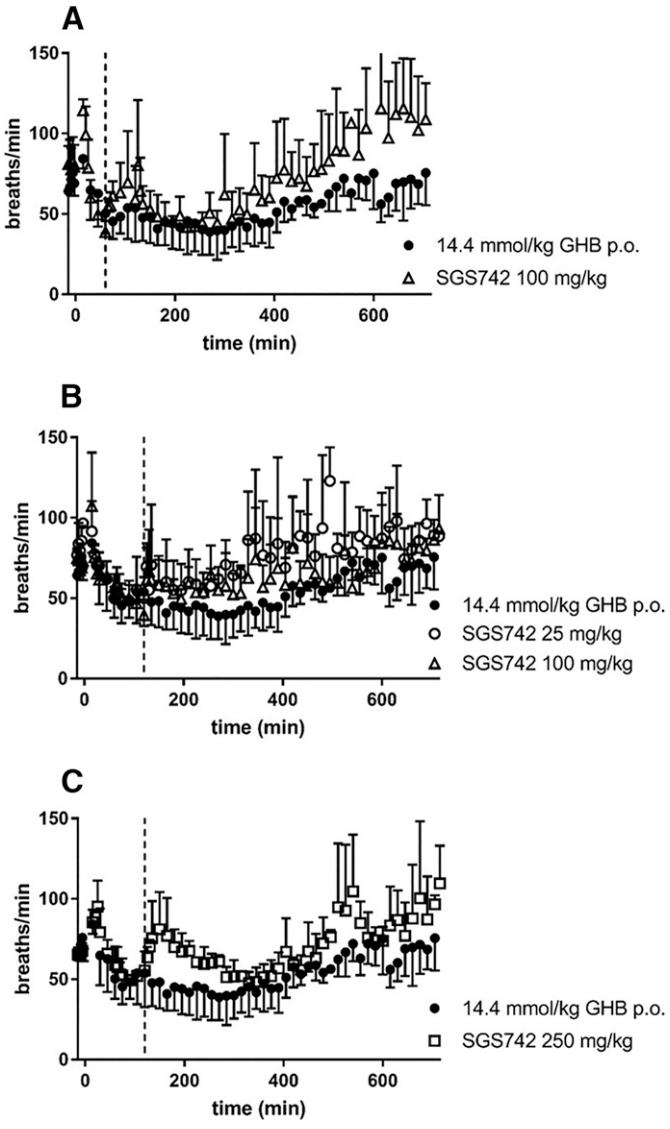

Fig. 5. Effect of SGS742 on oral GHB induced respiratory depression. GHB was administered at 0 minutes as a 1500 mg/kg oral bolus. SGS742 was administered as an i.v. bolus 60 (A) or 120 (B/C) minutes after GHB at a dose of 25 (open circles), 100 (open triangles), or 250 mg/kg (open squares). Breathing frequency control data (closed circles) were obtained from Morse and Morris (2013a). Data are represented as mean +/− standard deviation, with $n = 3$–4.

Impact of NCS382 Pretreatment and SGS742 Treatment on Intravenous GHB Toxicodynamics. Pretreatment with NCS382 (100 mg/kg) prior to administration of intravenous GHB did not have an effect on the SGS742-mediated reduction of GHB induced respiratory depression, when SGS742 (100 mg/kg) was administered 60 minutes after GHB (Fig. 8A; Table 8). When SGS742 treatment was delayed to 120 minutes after GHB, there was a statistically significant reduction in ABEC and T_d for the group that was pretreated with NCS382 (Fig. 8B; Table 8). There was a statistically significant increase in all TD parameters for the pretreated group, indicating a reduction in respiratory depression for the pretreated group, when it was compared with the group treated with SGS742 without pretreatment (Table 8). In animals with NCS382 pretreatment, fewer animals exhibited twitching movements that could be related to seizures, although one animal (out of five) still exhibited these movements.

The impact of pretreatment with NCS382 was assessed by analyzing the data prior to administration of SGS742. No differences were observed when data were analyzed for 60 minutes after GHB administration between pretreated and control groups (Table 9). When data were analyzed 120 minutes after GHB, there was a statistically significant reduction in ABEC for the group that was pretreated with NCS382. There was no difference in E_{max} or T_d for the pretreated group compared with the control.

Discussion

Abuse of GHB is associated with severe adverse effects including sedation, respiratory depression, hypothermia, coma, and even death, due to the steep concentration-toxicity relationship (White, 2017; Trombley et al., 2019). The majority of the toxicodynamic effects of exogenous GHB, including decreased respiration, locomotion, and body temperature, have been shown to be dependent on partial agonism at the $GABA_B$ receptor, and pretreatment with $GABA_B$ antagonists has been demonstrated to reverse sedation, respiratory depression, and lethality observed in mouse, rat, and baboon studies (Carai et al., 2001; Jensen and Mody, 2001; Kaupmann et al., 2003; Goodwin et al., 2005, 2006; Morse et al., 2012; Morse and Morris, 2013b). Since GHB exerts most of its pharmacological/toxicological effects as a partial agonist of $GABA_B$ receptors in the brain, this receptor represents a potential target for the treatment of GHB overdose. Two $GABA_B$ receptor antagonists, SCH50911 and SGS742, have been evaluated in preclinical studies. Although there are no commercially available $GABA_B$ receptor antagonists used clinically, SGS722 has been

TABLE 5
Impact of SGS742 on oral GHB toxicodynamics

Toxicodynamic Parameter	GHB 14.4 mmol/kg (60 min)	GHB 14.4 mmol/kg + 100 mg/kg SGS742 60 PD	GHB 14.4 mmol/kg (120 min)	GHB 14.4 mmol/kg + 25 mg/kg SGS742 120 PD	GHB 14.4 mmol/kg + 100 mg/kg SGS742 120 PD	GHB 14.4 mmol/kg + 250 mg/kg SGS742 120 PD
ABEC (breaths)	9.07×10^3 (6.55×10^3)	6.24×10^3 (6.32×10^3)	7.89×10^3 (5.78×10^3)	3.48×10^3 (4.09×10^3)	1.79×10^3 (1.35×10^3)	40 (36)*
E_{max} (breaths/min)	32.7 (9.02)	35.0 (2.76)	32.7 (9.02)	44.0 (8.60)	40.6 (5.51)	46.7 (6.75)
T_d (min)	341 (214)	165 (156)	300 (180)	101 (102)	116 (86.4)	4.8 (4.3)*

Data presented as mean (standard deviation). Abbreviations are described in Table 3 footnote. *$P < 0.05$ determined by Student's t test (60 min) one-way ANOVA with Dunnett's post hoc (120 min).

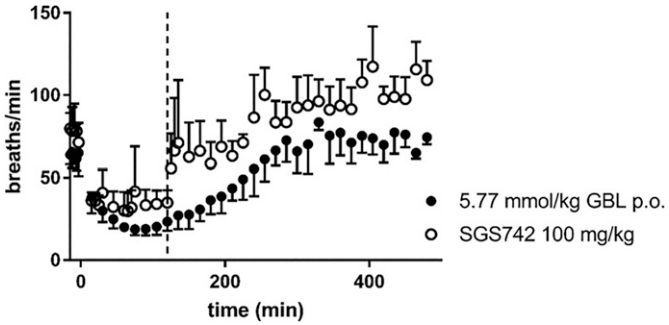

Fig. 6. Effect of SGS742 on GHB induced respiratory depression with oral GBL administration. GBL was administered at 0 minutes as a 5.77 mmol/kg oral bolus. An i.v. bolus of SGS742 was administered 120 minutes after GBL at a dose of 100 mg/kg (open circles). Breathing frequency control data (closed circles) were obtained from Morse and Morris (2013a). Data are represented as mean +/− standard deviation, with $n = 4$.

TABLE 6
Impact of SGS742 on oral GBL toxicodynamics
Data presented as mean (standard deviation). Abbreviations are described in Table 3 footnote.

Toxicodynamic Parameter	GBL 5.77 mmol/kg	GBL 5.77 mmol/kg + 100 mg/kg SGS742 120 PD
ABEC (breaths)	3.42×10^3 (1.62×10^3)	1.96×10^3 (1.82×10^3)
E_{max} (breaths/min)	23.4 (5.61)	33.8 (6.11)*
T_d (min)	125 (22.7)	71.7 (54.4)

PD, minutes post GHB dose.
*$P < 0.05$ as determined by a Student's t test.

investigated in clinical trials, demonstrating a lack of toxicity (Froestl et al., 2004), and therefore was the focus of the current studies.

Initial studies characterized the pharmacokinetics of SGS742 in rats after the administration of 100 and 500 mg/kg intravenously. The results indicated dose-proportional pharmacokinetics and minimal to no effects of SGS742 on the toxicokinetics of GHB. SGS742 also had no direct effect on respiration, our toxicological endpoint in these studies. Importantly, no adverse effects of SGS742 administration were observed in rats even at the highest doses used. Based on the plasma concentrations achieved in this study and the brain penetration of SGS742, as determined in brain microdialysis studies by Andrén et al. (1998), and the rapid reversal of respiratory depression observed at doses of 250 and 500 mg/kg, effective brain concentrations were achieved to reverse respiratory depression caused by GHB.

In the present study, the administration of the $GABA_B$ receptor antagonists, SGS742 and SCH50911, were able to reduce the effects of GHB (after intravenous and oral administration) in a dose- and administration time- dependent manner. Higher doses were associated with both increased efficacy and increased toxicity of SGS742 and SCH50911. Similar efficacy was observed with the administration of the GHB prodrug, GBL. However, abnormal movements, tremors, or seizures were observed after the delayed administration of $GABA_B$ antagonists to animals receiving GHB or GBL, and effects were dose-dependent. These findings contrast with a lack of toxicity observed after pretreatment of $GABA_B$ receptor antagonists prior to GHB/GBL administration (Morse et al., 2012; Morse and Morris, 2013a; Vijay et al., 2015).

GHB is known to cause seizures on its own, and is even used to produce experimental models of absence epilepsy in rats (Venzi et al., 2015). Mice with succinic semialdehyde dehydrogenase deficiency, a condition that leads to elevated GHB and GABA brain concentrations, have been shown to present with rodent absence epilepsy (Cortez et al., 2004). In humans, succinic semialdehyde dehydrogenase deficiency can also lead to generation of seizures, and nearly half of patients develop epilepsy (Pearl et al., 2011). Although GHB has been clearly demonstrated to be influential in the processes that generate seizures, in this study the seizures were precipitated by the administration of the $GABA_B$ antagonist, not simply GHB alone. It may be that the rapid reversal of action of GHB at the $GABA_B$ receptor is causing these seizures. The interruption of balance between the action of GHB at $GABA_B$ receptors and the action of GHB at other receptor sites may also be the cause of toxicity.

Several other GHB binding sites have been identified in the central nervous system: these include a subset of $GABA_A$ receptors, characterized by the $\alpha 4$, δ, and $\beta 1$ subunits, as well as a novel GHB receptor (Absalom et al., 2012; Bay et al., 2014). The action of GHB at these other sites may be

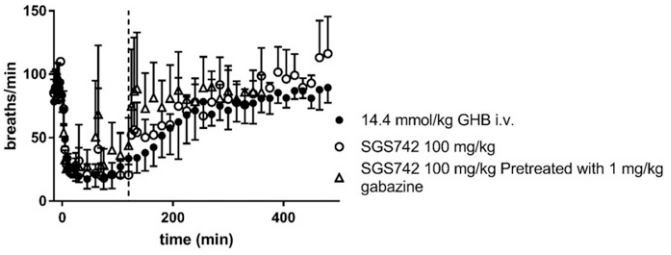

Fig. 7. Effect of SGS742 on intravenous GHB induced respiratory depression with gabazine pretreatment. GHB was administered at 0 minutes as a 14.4 mmol/kg i.v. bolus. SGS742 was administered as an i.v. bolus 120 minutes after GHB at a dose of 100 mg/kg with (open triangles) or without (open circles) 1 mg/kg gabazine administered 5 minutes prior to GHB administration. Breathing frequency control data (closed circles) were obtained from Morse et al. (2012) and Vijay et al. (2015). Data are represented as mean +/− standard deviation, with $n = 4$.

TABLE 7
Impact of SGS742 administration on intravenous GHB toxicodynamics with gabazine pretreatment

Toxicodynamic Parameter	GHB 14.4 mmol/kg	GHB 14.4 mmol/kg + 100 mg/kg SGS742 120 PD	GHB 14.4 mmol/kg + 100 mg/kg SGS742 120 PD with 1 mg/kg gabazine 5 min prior to GHB
ABEC (breaths)	4.92×10^3 (2.42×10^3)	3.96×10^3 (1.65×10^3)	1.47×10^3 (1.61×10^3)
E_{max} (breaths/min)	32.9 (10.6)	20.6 (8.37)	44.0 (21.6)
T_d (min)	163 (77.5)	121 (35.1)	61.2 (60.9)

Data presented as mean (standard deviation). Statistical significance determined by one-way ANOVA with Tukey's post hoc. Abbreviations are described in Table 3 footnote.

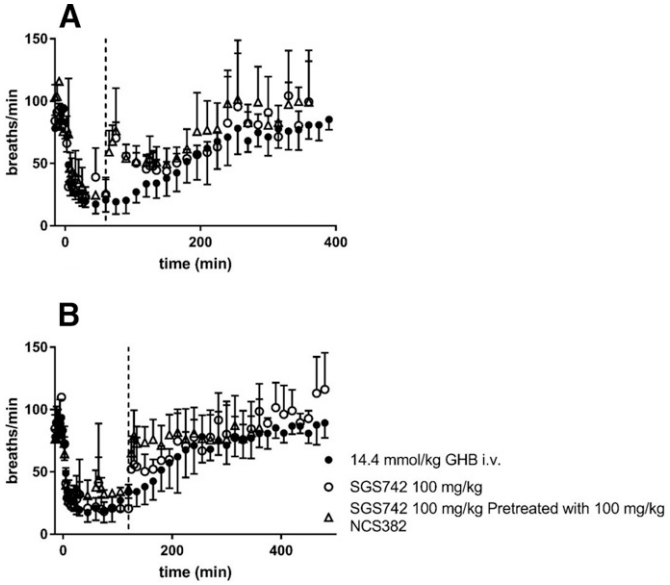

Fig. 8. Effect of SGS742 on intravenous GHB induced respiratory depression with NCS382 pretreatment. GHB was administered at 0 minutes as a 14.4 mmol/kg i.v. bolus. SGS742 was administered intravenously at 60 (A) or 120 (B) minutes after GHB, at a dose of 100 mg/kg with (open triangles) or without (open circles) 100 mg/kg NCSC382 administered 5 minutes prior to GHB administration. Control data (closed circles) for breathing frequency was obtained from Morse et al. (2012) and Vijay et al. (2015). Data are represented as mean +/– standard deviation, with $n = 4$–5.

contributing to the generation of seizures after $GABA_B$ antagonist administration. The impact of blocking the effects of GHB at these receptors by pretreatment with gabazine and NCS382, $GABA_A$, and GHB receptor antagonists, respectively, was investigated in the present study. Gabazine pretreatment had no effect of the efficacy of SGS742 treatment after intravenous GHB administration. When data prior to the administration of SGS742 was compared with control, it revealed there was no impact of $GABA_A$ pretreatment itself on the TD effects of GHB. There was also no difference in the abnormal movements associated with SGS742 treatment after gabazine pretreatment. These data suggest that $GABA_A$ receptors are not playing a role in GHB TD or the possible toxicity observed with SGS742 treatment. On the other hand, NCS382 pretreatment appeared to reduce respiratory depression and, after the delayed administration of SGS742, decreased the abnormal movements in animals; only one out of five animals with NSC382 pretreatment exhibited abnormal movements/tremors. These results suggest that the GHB receptor may play a role in GHB-induced respiratory depression, and may also be implicated in the potential seizure generation with SGS742 treatment. GHB receptors are generally associated with the physiologic effects of GHB, but Ainslie et al. (2016) reported that NCS382 may reverse loss of motor function after GHB administration, suggesting a potential reversal of toxicity.

In addition to potential action of GHB at receptors other than $GABA_B$, there are other factors that could be playing a role in the generation of seizures. One of the metabolites of GHB, d-2-hydroxyglutarate (D2HG), has been shown to activate the recombinant N-methyl-D-aspartate (NMDA) receptors (NR1/NR2A, NR1/NR2B) in HEK293 cells (Kolker et al., 2002). D2HG is of interest as this compound is elevated in the inherited neurometabolic disease D-2-hydroxyglutaric aciduria, which is characterized by hypotonia, epilepsy, and psychomotor retardation (Kolker et al., 2002). It has also been shown that hypoxia, which would be induced by the respiratory depression with GHB, can lead to a rise in extracellular glutamate levels in the dorsal hippocampus of rats (Lopez-Perez et al., 2012). GHB itself has also been shown to modulate extracellular glutamate levels in a concentration-dependent manner in the CA1 region of the hippocampus in rats (Ferraro et al., 2001). Dose-dependent modulation of glutamate levels in rat hippocampus appears to be a result of differential activation of the various receptors with which GHB interacts. At high (mM) concentrations, the interaction of GHB at $GABA_B$ receptors dominates, leading to a decrease in extracellular

TABLE 8
Impact of SGS742 administration on intravenous GHB toxicodynamics with NCS382 pretreatment
Data presented as mean (standard deviation). Abbreviations are described in Fig. 3 footnote.

Toxicodynamic Parameter	GHB 14.4 mmol/kg (60 min)	GHB 14.4 mmol/kg + 100 mg/kg SGS742 60 PD	GHB 14.4 mmol/kg + 100 mg/kg SGS742 60 PD with 100 mg/kg NCS382 5 min prior to GHB	GHB 14.4 mmol/kg (120 min)	GHB 14.4 mmol/kg + 100 mg/kg SGS742 120 PD	GHB 14.4 mmol/kg + 100 mg/kg SGS742 120 PD with 100 mg/kg NCS382 5 min prior to GHB
ABEC (breaths)	8.63×10^3 (2.64×10^3)	2.44×10^3 (3.95×10^3)	5.65×10^3 (3.45×10^3)	4.92×10^3 (2.42×10^3)	3.96×10^3 (1.65×10^3)	417 (586)**†
E_{max} (breaths/min)	18.0 (9.45)	25.2 (12.1)	26.7 (12.2)	32.9 (10.6)	20.6 (8.37)	36.5 (3.28)†
T_d (min)	223 (77.5)	60.6 (88.8)	147 (106)	163 (77.5)	121 (35.1)	23.1 (32.9)**†

**$P < 0.01$ compared with control; † $P < 0.05$ compared with no pretreatment; determined by one-way ANOVA with Tukey's post hoc.

TABLE 9
Impact of NCS382 pretreatment on intravenous GHB toxicodynamics

	60 minutes		120 minutes	
Toxicodynamic Parameter	GHB 14.4 mmol/kg	100 mg/kg NCS Pretreatment	GHB 14.4 mmol/kg	100 mg/kg NCS Pretreatment
ABEC (breaths)	3.51×10^3 (313)	3.17×10^3 (928)	7.28×10^3 (587)	5.04×10^3 (887)**
E_{max} (breaths/min)	16.1 (6.98)	22.6 (5.37)	15.6 (7.05)	23.4 (5.81)
T_d (min)	57.5 (0.00)	57.1 (1.11)	118 (0.00)	117 (0.244)
N	4	9	4	5

Data presented as mean (standard deviation). Parameter abbreviations are described in Fig. 3 footnote. **$P < 0.05$ compared with control determined by one-way ANOVA with Dunnett's post hoc; data presented as mean (standard deviation).

glutamate. When $GABA_B$ receptors are blocked, the same concentration of GHB leads to an increase in extracellular glutamate. Although brain concentrations of GHB were not determined in this study, previous studies have shown that frontal cortex extracellular fluid concentrations are in the mM range for a dose of 800 mg/kg (7.68 mmol/kg) GHB, and the dose used in the current study was higher at 1500 mg/kg (14.4 mmol/kg) (Roiko et al., 2012). Therefore, it is highly likely that mM concentrations of GHB were present in the brain extracellular fluid in this study. The complex effects of GHB on the extracellular concentration of glutamate, the major excitatory neurotransmitter and agonist of the NMDA receptor, as well as the possible involvement of the GHB metabolite and NMDA agonist D2HG, may both be significant with regards to the generation of seizures in this study.

The current study demonstrated that the $GABA_B$ antagonists SGS742 and SCH50911 can reverse GHB induced respiratory depression, even when administered 60 or 120 minutes after GHB administration. In addition, SGS742 had no effect on respiration in the absence of GHB. However, the potential toxicity observed in the form of tremors or seizures for these compounds presents a major challenge to utilizing $GABA_B$ antagonism for the treatment of GHB overdose. The generation of seizure-like activity with the administration of SGS742 and SCH50911 was dependent on the dose and timing of administration of the antagonist with respect to GHB administration. The doses used of the two $GABA_B$ receptor antagonists differed in this study, reflecting their differences in potency for the inhibition of $GABA_B$ receptors. Both compounds are selective, competitive, and orally-active first generation $GABA_B$ inhibitors that block both central and peripheral $GABA_B$ receptors (Bolser et al., 1995; Froestl, 2010). Although both compounds have been reported to have minimal effects on other neuroreceptors, there are $GABA_B$ receptor subtypes with different distribution and function, and the specificity of these two $GABA_B$ inhibitors for the receptor subtypes is not known (Carter et al., 2009a).

There are many possible explanations for the dose- and time-dependent toxicity observed with $GABA_B$ receptor antagonists, which include but are not limited to the action of GHB at other receptors and the modulation of extracellular glutamate levels by GHB. Further mechanistic studies are necessary to elucidate which pathways are contributing to the generation of seizures to assess the potential of $GABA_B$ receptor antagonism as a treatment option for GHB overdose.

Authorship Contributions

Participated in research design: Follman, Morris.
Conducted experiments: Follman.
Contributed new reagents or analytic tools: Follman, Morris.
Performed data analysis: Follman, Morris.
Wrote or contributed to the writing of the manuscript: Follman, Morris.

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