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γ-Aminobutyric Acid$_B$ (GABA$_B$)-Receptor Mediation of Different In Vivo Effects of γ-Butyrolactone

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Abstract. The endogenous brain constituent, γ-hydroxybutyric acid (GHB), as well as its prodrug, γ-butyrolactone (GBL), have recently gained interest in the drug addiction field due to their abuse potential and fatalities caused by overdose. It is known that GHB has two sites of actions: the γ-aminobutyric acid$_B$ (GABA$_B$) receptor and a specific-GHB binding site. The present study was designed to extend to GBL the investigations on the contribution of the GABA$_B$ receptor and the specific-GHB binding site to its in vivo effects. To this aim, DBA mice were pretreated either with GABA$_B$-receptor antagonists, (3-aminopropyl)(diethoxymethyl)phosphinic acid (CGP 35348) and (2*S*)(+)-5,5-dimethyl-2-morpholineacetic acid (SCH 50911), or a putative antagonist of the specific-GHB binding site, 6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylideneacetic acid (NCS-382), prior to the administration of doses of GBL that induced hypothermia, motor-incoordination (measured as motor-impairment at the Rota-Rod task), and sedation/hypnosis. The capability of SCH 50911 and NCS-382 to protect against GBL-induced lethality was also investigated. Pretreatment with either GABA$_B$-receptor antagonist completely prevented GBL-induced hypothermia, motor-incoordination, and sedation /hypnosis. SCH 50911 also provided complete protection against GBL-associated lethality. *Vice versa*, NCS-382 failed to exert any antagonistic or protective effect. These results suggest that the in vivo GBL effects tested in the present study are mediated by activation of the GABA$_B$ receptor.

Keywords: γ-butyrolactone (GBL), γ-hydroxybutyric acid (GHB), γ-aminobutyric acid$_B$ (GABA$_B$)-receptor antagonist (CGP 35348, SCH 50911), antagonist of the specific-GHB binding site, NCS-382

Introduction

γ-Hydroxybutyric acid (GHB) is an interesting endogenous molecule that is thought to act as a neuromodulator or neurotransmitter in the mammalian brain. When exogenously administered, GHB exerts a wide spectrum of psychopharmacological effects, up to motor-incoordination, sedation, hypnosis, and anaesthesia as the dose is progressively increased (1, 2).

Two major prodrugs of GHB have been identified: γ-butyrolactone (GBL) and 1,4-butanediol (1,4-BD). GBL is converted into GHB by peripheral lactonases or by nonenzymatic hydrolysis (2). 1,4-BD is converted into GHB probably through two enzymatic reactions: step one, 1,4-BD is metabolized by alcohol dehydrogenase and converted into γ-hydroxybutyraldehyde; step two, the latter is converted into GHB by aldehyde dehydrogenase (2).

GHB is also a recreational drug with high abuse potential, in view of its euphorigenic and relaxant properties (3 – 5). Since its banning and a series of warnings from different agencies, including the FDA and DEA, users' interest has shifted to GHB prodrugs, particularly GBL, due to its ready availability as a common solvent in numerous industrial processes (6). The few data available to date indicate a remarkably harmful potential (perhaps even higher than that of GHB) for GBL (6).

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The in vivo pharmacological effects of both 1,4-BD and GBL are thought to be secondary to their final conversion into GHB and the binding of the latter to the γ-aminobutyric acid$_B$ ($GABA_B$) receptor. As an example, 1,4-BD-induced sedation/hypnosis was completely prevented in mice by administration of either the alcohol dehydrogenase inhibitor 4-methyl-pyrazole or the $GABA_B$ receptor antagonists (2*S*)(+)-5,5-dimethyl-2-morpholineacetic acid (SCH 50911) and (3-aminopropyl)(cyclohexylmethyl) phosphinic acid (CGP 46381) (7). Also lethality produced by high doses of 1,4-BD has been abolished by SCH 50911 administration (8). Furthermore, SCH 50911 and the another $GABA_B$-receptor antagonist, (3-aminopropyl)(diethoxymethyl) phosphinic acid (CGP 35348), attenuated GBL-induced absence seizures in rats (9) and 1,4-BD-induced motor-incoordination in mice tested at the Rota-Rod (10).

Experimental data suggest that the behavioral effects of GHB and GBL may differ to some degree (11 – 13). It was therefore deemed of interest to investigate whether, and to what extent, the effects produced by a broad range of doses of GBL were antagonized, as observed for those of GHB, by $GABA_B$-receptor antagonists. The effects of GBL under examination varied from motor-incoordination, obtained with doses as low as 75 – 100 mg/kg, to lethality, induced by 2000 mg/kg.

Different lines of experimental evidence suggest the existence of a second GHB site of action, represented by a specific-GHB binding site (1, 14). In order to evaluate the possible contribution of this substrate to the above GBL effects, the present study also included a series of experiments with NCS-382, a putative antagonist of the specific-GHB binding site (15).

Materials and Methods

The experimental procedures employed in the present study were in accordance with the European Communities Council Directive (86/609/EEC) and the subsequent Italian Law on the "Protection of animals used for experimental and other scientific reasons".

Animals

Male DBA mice (Charles River Laboratories, Calco, Italy), weighing 25 – 30 g, were used. Mice were housed 20 per cage in standard plastic cages with wood chip bedding under a 12-h artificial light-dark cycle (lights on at 7:00 a.m.), at a constant temperature of 22 ± 2°C, and relative humidity of approximately 60%. Tap water and standard laboratory rodent chow (Mucedola, Settimo Milanese, Italy) were provided ad libitum.

Drugs

GBL was purchased from Sigma-Aldrich (Milan, Italy). SCH 50911 was purchased from Tocris Cookson, Ltd. (Avonmouth, UK). CGP 35348 was provided by Novartis (Basel, Switzerland). NCS-382 was provided by the National Institute on Drug Abuse (Bethesda, MD, USA).

GBL was diluted in bidistilled water when administered intragastrically and diluted in saline when administered intraperitoneally. SCH 50911 and CGP 35348 were dissolved in saline. NCS-382 was dissolved in saline containing 0.1 N sodium bicarbonate.

All solutions were administered at a volume of 12.5 ml/kg. All drugs were administered intraperitoneally, with the sole exception of GBL in the lethality experiments (see below) where it was administered intragastrically.

Procedures

GBL-induced hypothermia: Mice were initially habituated to the rectal probe (see below) measuring their body temperature once a day on the 3 days preceding the test. On the test day, mice were divided into 2 groups of n = 20 and treated acutely with CGP 35348 (0 and 300 mg/kg). Thirty minutes later, mice of each group were divided into 2 subgroups of n = 10 and treated acutely with GBL (0 and 100 mg/kg). This dose of GBL was chosen on the basis of literature data (12, 16). Body temperature was measured by a digital thermometer with rectal probe (Keithley, Cleveland, OH, USA) immediately before GBL administration and at 30, 60, 90, 120, and 180 min after GBL administration. The probe was left in place until steady readings were obtained (usually, 10 – 15 s). Body temperature measurements were conducted by an operator unaware of the drug treatment.

GBL-induced motor-incoordination: The present investigation employed a Rota-Rod procedure similar to that previously described by Hoffman and Tabakoff (17). Mice practiced on the apparatus for 3 daily training sessions prior to the test. The test session consisted of 2 trials on the Rota-Rod [accelerating Rota-Rod Treadmills for mice (Ugo Basile, Comerio, Italy)]: on the first trial (pre-drug performance), mice were placed on the revolving drum for 15 min. Rotation speed was kept constant (2 rpm) for 5 min, accelerated (from 2 to 20 rpm) over the following 5-min period and finally held at 20 rpm. The time each mouse managed to remain on the revolving drum was recorded. Time recording was initiated at the beginning of the acceleration phase. Only mice completing the first trial underwent the second trial.

In the "SCH 50911 *plus* GBL" experiment, one hour

after the first trial, mice were divided into 2 groups of n = 60 and treated acutely with SCH 50911 (0 and 150 mg/kg). Thirty minutes later, mice of each group were divided into 6 subgroups of n = 10 and treated acutely with GBL (0, 50, 75, 100, 125, and 150 mg/kg). GBL was administered 15 min before the second trial. In the second trial (post-drug performance), mice were required to perform the motor task on the drum for 11 min. The drum rotated at 2 rpm for 1 min, and then acceleration began (from 2 to 20 rpm, in 5 min). For the last 5 min, the speed was maintained at 20 rpm. Once again, the time spent by each mouse on the drum from the beginning of the acceleration phase was recorded.

In the "NCS-382 *plus* GBL" experiment, one hour after the first trial, mice were divided into 2 groups of n = 30 and treated acutely with NCS-382 (0 and 500 mg/kg). Thirty minutes later, mice of each group were divided into 3 subgroups of n = 10 and treated acutely with GBL (0, 75, and 150 mg/kg). GBL was administered 15 min before the second trial. The second trial was performed as described above.

In both experiments, the difference between first and second trial times, expressed as percentage of the first trial time, was calculated for each mouse and indicated its degree of motor impairment. Each mouse served as its own control. Recordings were conducted by an operator unaware of the drug treatment.

GBL-induced sedation/hypnosis: In the "GBL dose-response curve" experiment, mice were divided into 4 groups of n = 10 and treated acutely with GBL (100, 200, 300, and 400 mg/kg). According to the procedure used in previous studies (e.g., ref. 18), after GBL administration each mouse was placed on its back once every 60 s until it was unable to right itself within 60 s. The time between GBL administration and the start of the 60-s interval – when the mouse was unable to right itself – was measured as onset of the righting reflex loss. Each mouse was then left undisturbed lying on its back until it spontaneously regained its righting reflex (determined as having at least 3 paws under its body). Complete recovery of the righting reflex was defined as the mouse being able to turn itself upright twice more within 60 s. If this criterion was not fulfilled, the mouse was left undisturbed until it spontaneously regained its righting reflex. The time between loss and recovery of righting reflex was monitored in each mouse. Observations were conducted by an operator unaware of the drug treatment.

In the SCH 50911 *plus* GBL experiment, mice were divided into 5 groups of n = 10 – 12. Mice were treated acutely with SCH 50911 (0, 25, 50, 75, and 100 mg/kg). Fifteen minutes later, mice of all groups were treated acutely with GBL (400 mg/kg). This dose of GBL was chosen on the basis of the preceding experiment (GBL dose-response curve) in order to produce a sedative /hypnotic effect comparable to that produced by 1000 mg/kg GHB (18) and 350 mg/kg 1,4-BD (7). Onset and duration of loss of righting reflex were evaluated and recorded as described above.

In the "CGP 35348 *plus* GBL" experiment, mice were divided into 2 groups of n = 6. Mice were treated acutely with CGP 35348 (0 and 300 mg/kg). Fifteen minutes later, mice of both groups were treated acutely with GBL (400 mg/kg). Onset and duration of loss of righting reflex were evaluated and recorded as described above.

In the NCS-382 *plus* GBL experiment, mice were divided into 5 groups of n = 10. Mice were treated acutely with NCS-382 (0, 50, 100, 250, and 500 mg/kg). These doses of NCS-382 were identical to those tested in a previous antagonism experiment on GHB-induced sedation/hypnosis (18). Fifteen minutes later, mice of all groups were treated acutely with GBL (400 mg/kg). Onset and duration of loss of the righting reflex were evaluated and recorded as described above.

GBL-induced lethality: In the GBL *plus* SCH 50911 experiment, mice were divided into 4 groups of n = 10. Mice were treated acutely and intragastrically with GBL (2000 mg/kg). This dose of GBL was chosen on the basis of preliminary experiments in which it had proven to be the lowest dose to induce 100% death rate in mice within 24 h (this laboratory, unpublished results). Fifteen minutes after GBL administration – when all mice had already displayed marked signs of intoxication, including respiratory depression and loss of righting reflex – mice were treated acutely with SCH 50911 (0, 75, 150, and 300 mg/kg). These doses of SCH 50911 were chosen on the basis of previous experiments demonstrating that they completely protected against lethality induced by GHB (19) and 1,4-BD (8). At 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 18, and 24 h after GBL administration, the presence of vital signs (such as respiratory movements) was monitored.

In the GBL *plus* NCS-382 experiment, mice were divided into 4 groups of n = 10. Mice were treated acutely with GBL (2000 mg/kg). Fifteen minutes after GBL administration – when all mice had already displayed marked signs of intoxication – mice were treated acutely with NCS-382 (0, 50, 250, and 500 mg/kg). The highest dose of NCS-382 tested in the present study was up to 2.5 times higher than that tested in a previous antagonism experiment on lethality induced by GHB (19). Lethality was evaluated and recorded 1, 2, 3, 4, 5, and 6 h after GBL administration as described above.

Data analyses

Data on body temperature before GBL administration

were analyzed by a 1-way analysis of variance (ANOVA). Data on body temperature at the different time intervals after GBL administration were analyzed by a 2-way (drug treatment; time intervals) ANOVA, followed by the Newman-Keuls test for *post hoc* comparisons.

Data from motor-incoordination experiments were analyzed by a 2-way (GBL dose, antagonist dose) ANOVA, followed by the Newman-Keuls test for *post hoc* comparisons.

Data on onset and duration (both expressed in min) of loss of righting reflex from the sedation/hypnosis experiments were analyzed by a 1-way ANOVA. In the CGP 35348 *plus* GBL experiment, occurrence of loss of righting reflex was evaluated by a Fisher's exact probability test for a 2×2 Table [treatment (saline, drug) × loss of righting reflex (presence, absence)].

Survival curves from GBL-induced lethality were analyzed by the Logrank test for trend.

Results

GBL-induced hypothermia

Body temperature did not differ among mouse groups before the GBL treatment [$F(3,36) = 2.21$, $P>0.05$] ("Time 0" in Fig. 1). ANOVA revealed significant effects of both drug treatment [$F(3,36) = 15.09$, $P<0.0001$], time intervals [$F(4,144) = 25.75$, $P<0.0001$], and interaction between the two factors [$F(12,144) = 22.61$, $P<0.0001$]. The acute administration of 100 mg /kg GBL produced a profound decrease in body temperature: 30 min after GBL administration, body temperature in mice treated with 0 mg/kg CGP 35348 *plus* 100 mg/kg GBL was 3.6°C lower than that monitored in control mice (0 mg/kg CGP 35348 *plus* 0 mg/kg GBL) (Fig. 1). On continuing recording, body temperature in mice treated with 0 mg/kg CGP 35348 *plus* 100 mg/kg GBL returned to control values. Pretreatment with CGP 35348, given at a dose that did not alter body temperature *per se* (as indicated by the mouse group treated with 300 mg/kg CGP 35348 *plus* 0 mg/kg GBL), effectively antagonized GBL-induced hypothermia: at the 30-min time interval, the decreasing effect of GBL on body temperature (0 mg/kg CGP 35348 *plus* 100 mg/kg GBL) was reduced by approximately 70% by CGP 35348 pretreatment (300 mg/kg CGP 35348 *plus* 100 mg/kg GBL) (Fig. 1).

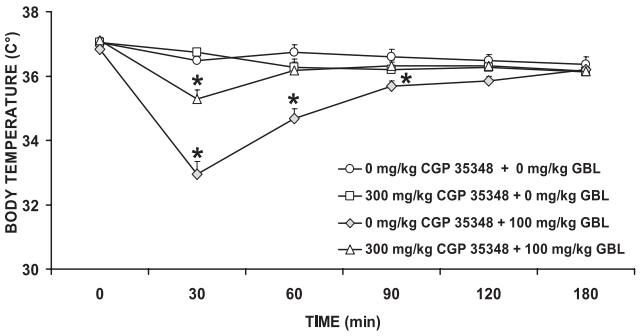

Fig. 1. Effect of the acute administration of γ-butyrolactone (GBL) and its antagonism by the $GABA_B$-receptor antagonist CGP 35348 on body temperature in DBA mice. Mice were initially treated with CGP 35348 (0 and 300 mg/kg). Thirty minutes later, mice were treated with GBL (0 and 100 mg/kg). Body temperature was measured by a digital thermometer with rectal probe immediately before GBL administration and at different time intervals after GBL administration. Each point is the mean ± S.E.M. of n = 10 mice. *: $P<0.05$, with respect to the mouse group treated with 0 mg/kg CGP 35348 *plus* 0 mg/kg GBL.

GBL-induced motor-incoordination

In the SCH 50911 *plus* GBL experiment, ANOVA revealed significant effects of both GBL dose [$F(5,107) = 39.61$, $P<0.0001$], SCH 50911 dose [$F(1,107) = 144.16$, $P<0.0001$], and interaction between the two factors [$F(5,107) = 11.78$, $P<0.0001$]. GBL administration resulted in a dose-dependent, complete impairment of the motor-coordination task, as indicated by approximately 100% impairment in both the group treated with 0 mg/kg SCH 50911 *plus* 125 mg/kg GBL and the group treated with 0 mg/kg SCH 50911 *plus* 150 mg/kg

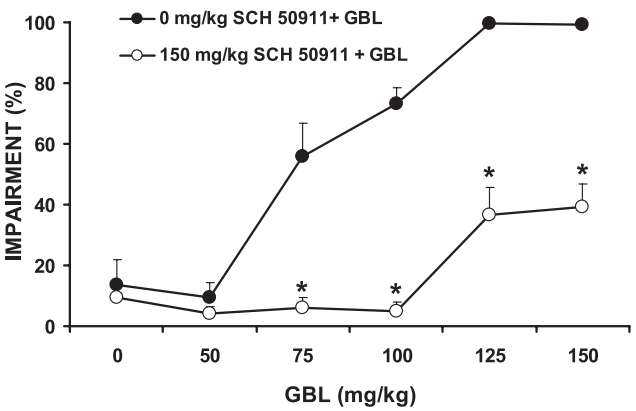

Fig. 2. Effect of the acute administration of different doses of γ-butyrolactone (GBL) and its antagonism by the $GABA_B$-receptor antagonist SCH 50911 on motor-coordination in DBA mice tested in the Rota-Rod task. Mice were initially treated with SCH 50911 (0 and 150 mg/kg). Thirty minutes later, mice were treated with GBL (0, 50, 75, 100, 125, and 150 mg/kg). Data were expressed as percent impairment of motor performance, defined as $[(T_1 - T_2) / T_1] \times 100\%$, where T_1 and T_2 are the amount of time each mouse remained on the rotating drum in the two trials conducted 60 min before SCH 50911 administration (pre-drug performance) and 15 min after GBL administration (post-drug performance), respectively]. Each point is the mean ± S.E.M. of n = 10 mice. *: $P<0.05$, with respect to mice treated with 0 mg/kg SCH 50911 and the corresponding dose of GBL (Newman-Keuls test).

GBL (Fig. 2). Pretreatment with SCH 50911 produced a significant antagonism of the motor-incoordinating effect of GBL, as indicated by a reduction of 60% – 90% of the impairment produced by doses of GBL equal to or greater than 75 mg/kg (Fig. 2).

In the NCS-382 *plus* GBL experiment, ANOVA revealed a significant effect of GBL dose [$F(1,58) = 102.34$, $P<0.0001$], but not of NCS-382 dose [$F(1,58) = 1.06$, $P>0.05$] and interaction between the two factors [$F(1,58) = 1.14$, $P>0.05$]. As expected, GBL administration produced a complete impairment of the motor-coordination task; however, this effect was virtually completely unaffected by pretreatment with 500 mg/kg NCS-382 (Fig. 3).

GBL-induced sedation/hypnosis

In the GBL dose-response curve experiment, the number of mice that lost the righting reflex after the administration of 100, 200, 300, and 400 mg/kg GBL was 0/10, 2/10, 10/10, and 10/10, respectively. GBL administration produced a dose-dependent reduction in the onset to loss of righting reflex [$F(2,19) = 40.12$, $P<0.0001$]; at the dose of 400 mg/kg GBL, onset averaged less than 11 min (Fig. 4, top panel). GBL administration also resulted in a dose-dependent increase in the duration of loss of righting reflex [$F(3,36) = 81.47$, $P<0.0001$], which peaked up to more than 100 min at the dose of 400 mg/kg GBL (Fig. 4, bottom panel).

In the SCH 50911 *plus* GBL experiment, the number of mice that lost the righting reflex after the combination of 0, 25, 50, 75, and 100 mg/kg SCH 50911 *plus* 400 mg/kg GBL were 10/10, 9/12, 4/12, 0/12, and 0/12, respectively. ANOVA for onset of loss of righting reflex – limited to those mice losing the righting reflex (consequently, the 75 mg/kg SCH 50911 *plus* 400 mg/kg GBL and 100 mg/kg SCH 50911 *plus* 400 mg/kg GBL mouse groups were excluded from analysis) – revealed a significant effect of SCH 50911 pretreatment [$F(2,20) = 5.15$, $P<0.05$] (Fig. 5, top panel). ANOVA for duration of loss of righting reflex – that included also the mice that did not lose the righting reflex, to which the value zero was assigned – revealed a signifi-

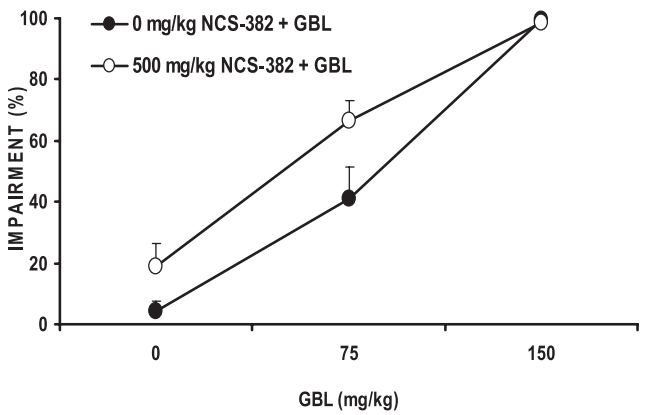

Fig. 3. Effect of the acute administration of different doses of γ-butyrolactone (GBL) and lack of blockade by the antagonist of the specific-gamma-hydroxybutyric acid (GHB) binding site, NCS-382, on motor-coordination in DBA mice tested in the Rota-Rod task. Mice were initially treated with NCS-382 (0 and 500 mg/kg). Thirty minutes later, mice were treated with GBL (0, 75, and 150 mg/kg). Data were expressed as percent impairment of motor performance, defined as $[(T_1 - T_2) / T_1] \times 100\%$, where T_1 and T_2 are the amount of time each mouse remained on the rotating drum in the two trials conducted 60 min before NCS-382 administration (pre-drug performance) and 15 min after GBL administration (post-drug performance), respectively]. Each point is the mean ± S.E.M. of n = 10 mice.

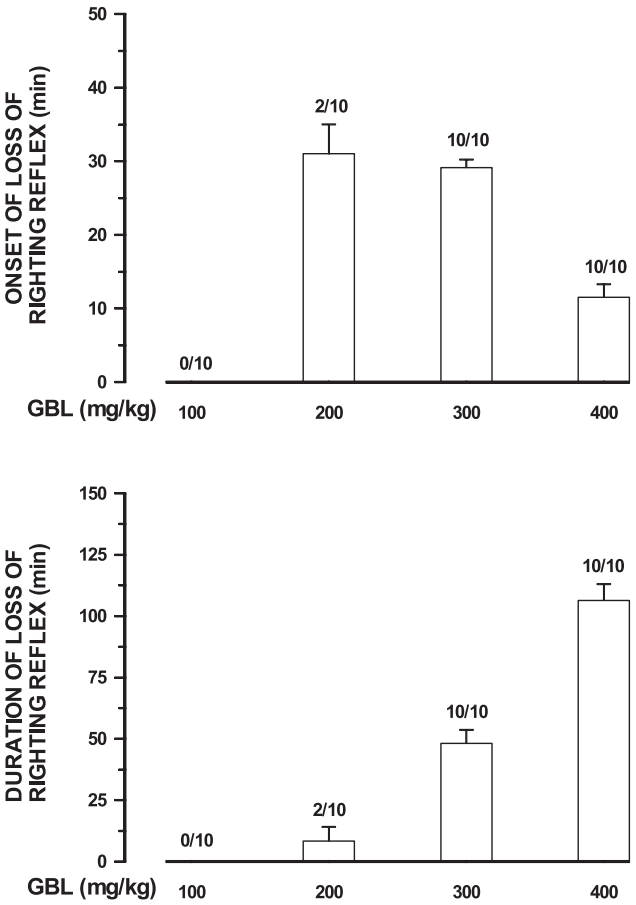

Fig. 4. Sedative/hypnotic effect produced by the acute administration of different doses of γ-butyrolactone (GBL) in DBA mice. Top and bottom panels illustrate the onset and duration, respectively, of loss of righting reflex. GBL was administered at the doses of 100, 200, 300, and 400 mg/kg. Figures on top of each bar indicate the number of mice losing the righting reflex over the total number of mice tested. In the top panel, each bar is the mean ± S.E.M. of the number of mice that lost the righting reflex; in the bottom panel, each bar is the mean ± S.E.M. of 10 mice (mice that did not lose the righting reflex were included assigning the value zero).

cant effect of SCH 50911 pretreatment [F(4,52) = 73.63, $P<0.0001$]. Duration of loss of righting reflex was significantly shorter in all mouse groups pretreated with SCH 50911 than in saline-pretreated group (0 mg/kg SCH 50911 *plus* 400 mg/kg GBL) (Fig. 5, bottom panel).

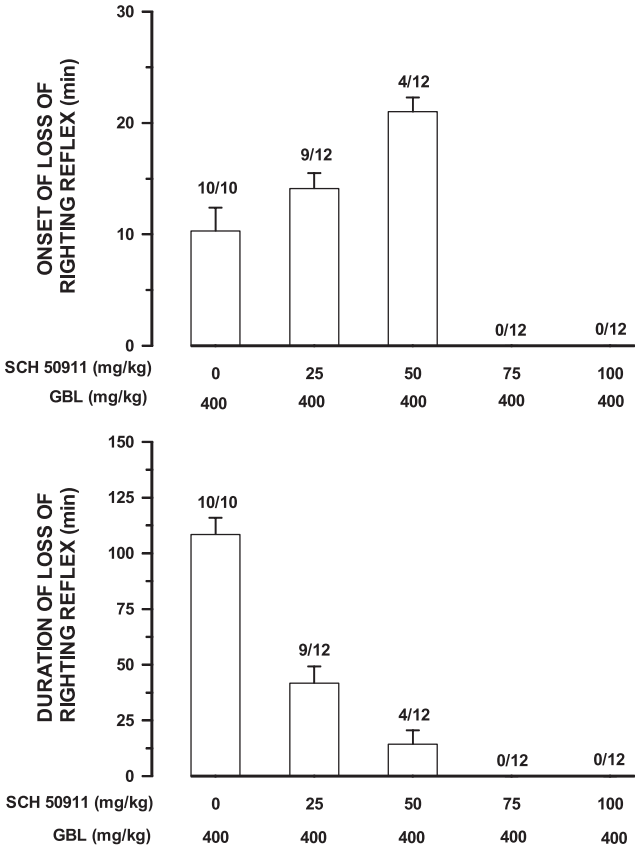

Fig. 5. Blockade by the $GABA_B$-receptor antagonist SCH 50911 of the sedative/hypnotic effect produced by the acute administration of different doses of γ-butyrolactone (GBL) in DBA mice. Top and bottom panels illustrate the onset and duration, respectively, of the loss of righting reflex. GBL was administered at the dose of 400 mg/kg. SCH 50911 (0, 25, 50, 75, and 100 mg/kg) was administered 15 min before GBL administration. Figures on top of each bar indicate the number of mice losing the righting reflex over the total number of mice tested. In the top panel, each bar is the mean ± S.E.M. of the number of mice that lost the righting reflex; in the bottom panel, each bar is the mean ± S.E.M. of 10 mice (mice that did not lose the righting reflex were included assigning the value zero).

In the CGP 35348 *plus* GBL experiment, pretreatment with 300 mg/kg CGP 35348 resulted in a complete prevention of the sedative/hypnotic effect in all mice treated with 400 mg/kg GBL ($P<0.005$, Fisher's exact test) (Table 1).

In the NCS-382 *plus* GBL experiment, all mice lost the righting reflex. ANOVA failed to reveal any significant effect of pretreatment with NCS-382 on both onset [F(4,45) = 1.39, $P>0.05$] (Fig. 6, top panel) and duration [F(4,45) = 1.84, $P>0.05$] (Fig. 6, bottom panel) of loss of righting reflex.

GBL-induced lethality

In the GBL *plus* SCH 50911 experiment, Logrank test for trend revealed the presence of significant differences ($P<0.0001$) among the mouse groups. Specifically, all mice from the 2000 mg/kg GBL *plus* 0 mg/kg SCH 50911 died within 4 h after GBL administration (Fig. 7). All three doses of SCH 50911 (75, 150, and 300 mg/kg) produced a virtually complete protection against GBL-induced lethality; indeed the number of mice that died was 2/10, 0/10, and 0/10 in the 2000 mg/kg GBL *plus* 75, 150, and 300 mg/kg SCH 50911-treated mouse groups, respectively (Fig. 7).

In the GBL *plus* NCS-382 experiment, Logrank test for trend revealed the absence of any significant difference ($P>0.05$) among the mouse groups. Specifically, all mice, irrespective of drug treatment, died within 5 h after GBL administration (Fig. 8).

Discussion

In the present study, four different effects of GBL (hypothermia, motor-incoordination, sedation/hypnosis, and lethality), produced in mice by doses varying between 75 and 2000 mg/kg, have been found to be completely blocked by administration of two $GABA_B$-receptor antagonists. These results strengthen the hypothesis that GBL, once injected, is rapidly converted into

Table 1. Prevention of the sedative/hypnotic effect of γ-butyrolactone (GBL) by the $GABA_B$-receptor antagonist CGP 35348 in DBA mice

CGP 35348 (mg/kg)	GBL (mg/kg)	Number of mice that lost the righting reflex *vs* the number of mice tested	Onset (min)	Duration (min)
0	400	6/6	6.5 ± 0.6	102.3 ± 23.2
300	400	0/6*	ND	0.0 ± 0.0

In the "Onset" column, values are the mean ± S.E.M. of the onset of loss of righting reflex; ND: not determined, since no mice from this group lost the righting reflex. In the "Duration" column, values are the mean ± S.E.M. of duration of loss of righting reflex; data from all mice were included since those not losing the righting reflex were assigned the value zero. *: $P<0.005$ (Fisher's exact test).

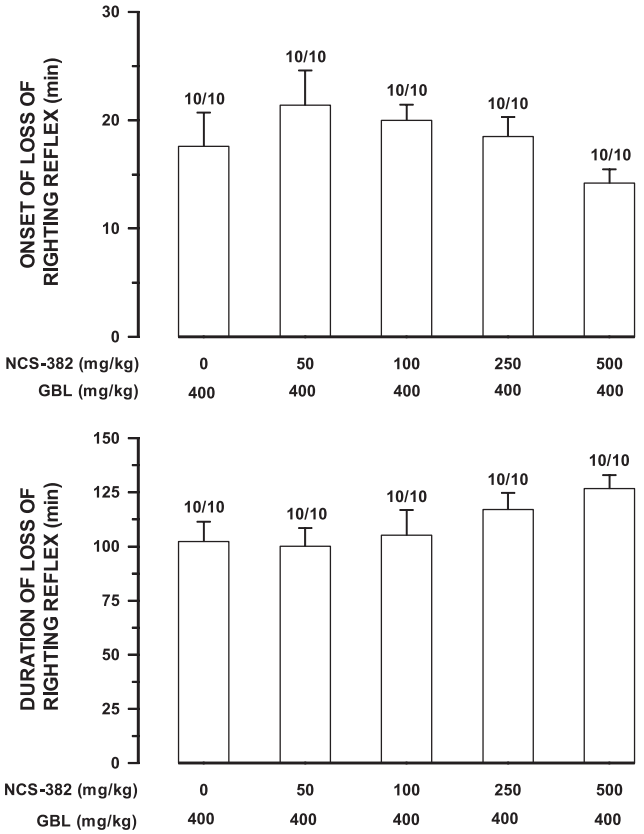

Fig. 6. Lack of blockade by the antagonist of the specific-γ-hydroxybutyric acid (GHB) binding site, NCS-382, of the sedative /hypnotic effect produced by the acute administration of different doses of γ-butyrolactone (GBL) in DBA mice. Top and bottom panels illustrate the onset and duration, respectively, of the loss of righting reflex. NCS-382 (0, 50, 100, 250, and 500 mg/kg) was administered 15 min before administration of GBL (400 mg/kg). Figures on top of each bar indicate the number of mice losing the righting reflex over the total number of mice tested. In both panels, each bar is the mean ± S.E.M. of 10 mice.

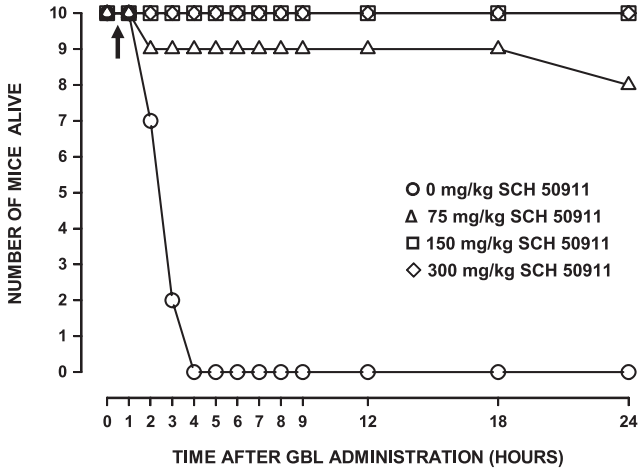

Fig. 7. Resuscitative effect of different doses of the $GABA_B$-receptor antagonist SCH 50911 on lethality induced by the acute administration of a single dose of γ-butyrolactone (GBL) in DBA mice. GBL (2000 mg/kg) was administered intragastrically at time 0. SCH 50911 (0, 75, 150, and 300 mg/kg) was administered 15 min after GBL administration (see the arrow), when all mice had already displayed severe signs of intoxication.

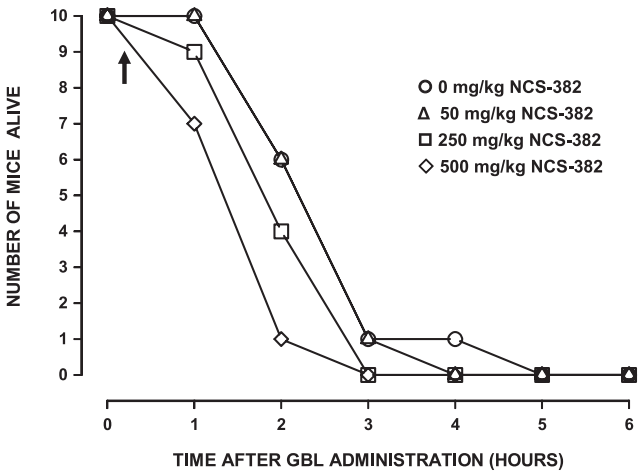

Fig. 8. Lack of resuscitative effect of different doses of the antagonist of the specific-γ-hydroxybutyric acid (GHB) binding site NCS-382 on lethality induced by the acute administration of a single dose of γ-butyrolactone (GBL) in DBA mice. GBL (2000 mg/kg) was administered intragastrically at time 0. NCS-382 (0, 50, 250, and 500) was administered 15 min after GBL administration (see the arrow), when all mice had already displayed severe signs of intoxication.

GHB (2). The derived GHB may a) directly bind to the $GABA_B$ receptor, for which it has a weak affinity; b) be converted into GABA, forming a GABA pool that, in turn, binds to $GABA_B$ receptors; and c) stimulate GABA release in some brain regions, again activating $GABA_B$ receptors (20). Accordingly, GBL has been reported not to bind to the $GABA_B$ receptor (20), and several effects of GHB and 1,4-BD, including motor-incoordination, sedation/hypnosis, and lethality, are blocked by treatment with $GABA_B$-receptor antagonists (7 – 10). Furthermore, different effects of GHB and GBL are completely absent in $GABA_B$-knockout mice (16, 21).

Literature data suggest the existence of differences in the pharmacological profile of GHB and GBL, as that they could have, at least in part, independent mechanisms of action (12, 13, 22). An explanation for these discrepancies may reside in the different pharmacokinetic characteristics of the two drugs. Specifically, because of the relatively low lipophilicity of GHB, its absorption may be relatively slow; *vice versa*, GBL displays a much greater lipid solubility that may make its absorption easier (23). However, to our understanding, it is difficult to hypothesize independent mechanisms of

action for GHB and GBL, as GBL does not bind to the $GABA_B$ receptor (20) and the complete blockade of its effects by $GABA_B$-receptor antagonists (present study; 9, 24) can be explained by its conversion into GHB and the subsequent binding of the latter to the $GABA_B$ receptor.

In recent years, GHB has become an illicit recreational drug because of its ability to produce feelings of euphoria, disinhibition, anxiolysis, emotional warmth, and sedation (2, 5). The current regulation of GHB in many Western Countries has rendered GHB more difficult to obtain; this has resulted in an increased trafficking and abuse of its precursors, GBL and 1,4-BD, which remain legally available because of their wide industrial uses (6). In this light, the results of the lethality experiment of the present study – demonstrating that SCH 50911 reversed GBL intoxications leading to lethality – acquire some interest as no specific antidote to GBL overdose is presently available (2). Blockade of GBL conversion into GHB does not seem to be feasible due to the rapidity of this reaction (25) and of the lack of in vivo evidence of the possibility of blocking the lactonases that catalyze this reaction. Conversely, the recently reported availability of $GABA_B$-receptor antagonists for human use (26) suggests that this class of agents might be tested as a possible pharmacotherapy for overdoses by GHB, 1,4-BD, and GBL.

The results of the experiments performed with the putative antagonist of the specific-GHB binding site, NCS-382, indicated that the drug was totally ineffective, if not displaying a tendency toward a potentiation, on GBL-induced motor-incoordination, sedation/hypnosis, and lethality. These results are in close agreement with previous data demonstrating the lack of any effect, or even a potentiation, of pretreatment with NCS-382 on hypomotility, sedation/hypnosis, and lethality induced by GHB and/or 1,4-BD (7,18,19, 27). Taken together, these results suggest that the specific-GHB binding site contributes minimally to the above effects of GHB and its precursors. Alternatively, it can be hypothesized that NCS-382 may not be particularly selective for the specific-GHB binding site (15) or even possess agonistic properties at this binding site (28), leaving the contribution of this binding site on GHB, 1,4-BD, and GBL pharmacology an open issue. Accordingly, it is worthy of note that the recently cloned specific-GHB binding site was insensitive to NCS-382, suggesting the possible existence of different subtypes of this binding site (29).

To summarize, the results of the present study indicate that different in vivo effects of GBL, produced by doses varying between 75 and 2000 mg/kg, have been antagonized by administration of $GABA_B$-receptor antagonists. The present results suggest that these GBL effects may be secondary to its conversion into GHB and the binding of the latter with $GABA_B$ receptors.

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