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European Journal of Pharmacology 441 (2002) 157–163

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Central effects of 1,4-butanediol are mediated by $GABA_B$ receptors via its conversion into γ-hydroxybutyric acid

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Received 17 December 2001; received in revised form 27 February 2002; accepted 5 March 2002

Abstract

The aliphatic alcohol 1,4-butanediol in converted into γ-hydroxybutyric acid (GHB) via two enzymatic steps: first, it is oxidised by alcohol dehydrogenase in γ-hydroxybutyraldehyde; second, the latter is transformed, likely by aldehyde dehydrogenase, into GHB. Initially, the present study compared the sedative/hypnotic effect of GHB and 1,4-butanediol, measured as loss of righting reflex. 1,4-Butanediol was more potent than GHB, presumably because of a more rapid penetration of the blood brain barrier. Further alcohol dehydrogenase inhibitors, 4-methylpyrazole and ethanol, totally prevented the sedative/hypnotic effect of 1,4-butanediol; the aldehyde dehydrogenase inhibitor disulfiram partially blocked the sedative/hypnotic effect of 1,4-butanediol. Finally, the sedative/hypnotic effect of 1,4-butanediol was antagonised by the $GABA_B$ receptor antagonists, SCH 50911 [(2*S*)(+)-5,5-dimethyl-2-morpholineacetic acid] and CGP 46381 [(3-aminopropyl)(cyclohexylmethyl)phosphinic acid], but not by the putative GHB receptor antagonist NCS-382 (6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylideneacetic acid), indicating that it is mediated by $GABA_B$ but not GHB receptors. Taken together, these results suggest that the sedative/hypnotic effect of 1,4-butanediol is mediated by its conversion in vivo into GHB which, in turn, binds to $GABA_B$ receptors. Accordingly 1,4-butanediol, unlike GHB, failed to displace [^{3}H]GHB and [^{3}H]baclofen in brain membranes.

Keywords: 1,4-Butanediol; GHB (γ-hydroxybutyric acid); Sedation/hypnosis; 4-Methylpyrazole; Ethanol; Disulfiram; $GABA_B$ receptor antagonist; SCH 50911; CGP 46381; GHB receptor antagonist, NCS-382

1. Introduction

The aliphatic diol 1,4-butanediol, "pine needle oil", is a naturally occurring alcohol and a largely used industrial cleaner and solvent. In recent years, 1,4-butanediol has been marketed in the USA as a legal alternative to γ-hydroxybutyric acid (GHB), subsequent to the classification of GHB as a schedule I drug in March 2000 and to its withdrawal from the open market (DEA (Drug Enforcement Agency), 2000). 1,4-Butanediol is sold as dietary supplement to aid sleep, promote muscle growth and for its alcohol-like euphoriant properties (Zvosec et al., 2001). Cases of severe intoxication from 1,4-butanediol have been reported (Zvosec et al., 2001). 1,4-Butanediol is thought to exert its pharmacological effects via conversion into GHB (see below).

GHB is a putative neurotransmitter or neuromodulator that occurs in mammalian brain and possesses different physiological and pharmacological properties (see Maitre, 1997; Bernasconi et al., 1999). GHB is believed to play a role in the regulation of sleep and has been investigated for the treatment of narcolepsy, fatal familial insomnia and as an anaesthetic adjuvant (see Agabio and Gessa, in press). Specific GHB binding sites and metabolising enzymes have

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0014-2999/02/$ - see front matter
PII: S0014-2999(02)01502-9

been found in different brain areas of mammalians (Hechler et al., 1992; Castelli et al., 2000). However, GHB has been reported to interact also with $GABA_B$ receptors. Indeed, some GHB effects, such as sedation/hypnosis (Carai et al., 2001), are suppressed by $GABA_B$ receptor antagonists. Accordingly, millimolar concentrations of GHB have been shown to displace [^{3}H]baclofen from $GABA_B$ receptors in brain membranes (Bernasconi et al., 1992; Mathivet et al., 1997) and act as a $GABA_B$ receptor agonist at recombinant $GABA_B$ receptors in *Xenopus laevis oocytes* (Lingenhoehl et al., 1999).

The central actions of 1,4-butanediol are considered to be mediated by its in vivo conversion into GHB (Roth and Giarman, 1968; Maxwell and Roth, 1972; Snead et al., 1989). Accordingly, administration of 1,4-butanediol produced an elevation in GHB concentration in rats (Roth and Giarman, 1968; Maxwell and Roth, 1972; Snead et al., 1982; Poldrugo and Snead, 1984). 1,4-Butanediol is converted into GHB probably through two enzymatic reactions: first, 1,4-butanediol is metabolised by alcohol dehydrogenase, an enzyme that may convert 1,4-butanediol into γ-hydroxybutyraldehyde dehydrogenaseeyde; second, the latter is converted into GHB and aldehyde dehydrogenase has been proposed to be one of the likely oxidising enzymes (Vayer et al., 1987; Snead et al., 1982; Barker et al., 1985; Taberner et al., 1972). The alcohol dehydrogenase inhibitors, ethanol and pyrazole, have been found to block the conversion of 1,4-butanediol into GHB in rat liver, but not in brain (Barker et al., 1985; Snead et al., 1989). The aldehyde dehydrogenase inhibitor, disulfiram, has been thought to be able to block the conversion of the intermediate γ-hydroxybutyraldehyde into GHB (Barker et al., 1985); however, similarly to pyrazole and ethanol, it exerted some inhibition only in liver but not in brain preparation (Barker et al., 1985). Thus, transformation of 1,4-butanediol into GHB in brain might be catalysed by enzymes other than alcohol dehydrogenase and aldehyde dehydrogenase (Barker et al., 1985; Snead et al., 1989).

The aims of this study were: (a) to assess the relative potency of 1,4-butanediol and GHB in producing sedation/hypnosis; (b) to investigate whether the sedative/hypnotic effect of 1,4-butanediol is secondary to its conversion into GHB; to this aim, the potent class I alcohol dehydrogenase inhibitors, 4-methylpyrazole (Blomstrand and Theorell, 1970; Farrés and Parés, 1987), ethanol, and disulfiram were used to block the two conversion steps; (c) to clarify whether 1,4-butanediol-induced sedative/hypnotic effect is mediated by activation of $GABA_B$ receptors after 1,4-butanediol conversion into GHB; accordingly, CGP 46381 [(3-aminopropyl)(cyclohexylmethyl)phosphinic acid], SCH 50911 [(2*S*)(+)-5,5-dimethyl-2-morpholineacetic acid], and the putative GHB receptor antagonist, NSC-382 (6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylideneacetic acid), were tested in combination with a sedative/hypnotic dose of 1,4-butanediol.

2. Methods

2.1. In vivo experiments

2.1.1. Animals

Male DBA/2JIco mice (Charles River, Calco, LC, Italy), weighing 25–30 g, were used. DBA mice were chosen because of their sensitivity to the sedative/hypnotic effect of GHB (Carai et al., 2001) and its prodrug, γ-butyrolactone (Dudek and Fanelli, 1980). After delivery to our animal facility, mice were left undisturbed for 7 days to adapt to the new housing conditions. Mice were housed 20 per cage in standard plastic cages [55 × 33 × 19 (h) cm] 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 (MIL Morini, San Polo d'Enza, RE, Italy) were provided ad libitum.

The experimental procedures employed in the present study were in accordance with the Italian Law on the "Protection of animals used for experimental and other scientific reasons" and approved by the Ethical Committee of the University of Cagliari.

2.1.2. Procedure

Initially, the relative potency of 1,4-butanediol and GHB in inducing sedation/hypnosis was tested. 1,4-Butanediol and GHB were acutely administered at the doses of 200, 500 and 1000 mg/kg to groups of $n = 6–12$ mice. Subsequently, the blockade tests on 1,4-butanediol-induced sedation/hypnosis were conducted. 4-Methylpyrazole (0 and 0.1 mg/kg), ethanol (0 and 1000 mg/kg), disulfiram (0, 1, 10 and 30 mg/kg), NCS-382 (0 and 250 mg/kg), SCH 50911 (0 and 100 mg/kg), and CGP 46381 (0 and 100 mg/kg) were acutely administered 15 min prior to the injection of 350 mg/kg 1,4-butanediol to groups of 6–10 mice.

In all experiments, after injection of 1,4-butanediol or GHB each mouse was placed on its back once every 60 s until it was unable to right itself within 60 s. The time between drug injection 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. If a mouse did not lose the righting reflex within 120 min, it was excluded from the analysis of onset. Each mouse was then left undisturbed on its back until it spontaneously regained its righting reflex (determined as having at least three 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 as its sleep time.

2.1.3. Drugs

1,4-Butanediol (Sigma-Aldrich, Milan, Italy), 4-methylpyrazole (Sigma-Aldrich), SCH 50911 (synthesised as pre-

viously described by Blythin et al., 1996) and CGP 46381 (Tocris, Bristol, UK) were dissolved in 12.5 ml/kg saline. Ethanol (from a commercial source) was dissolved in 10 ml/kg saline. Disulfiram (Sigma-Aldrich) was suspended in 12.5 ml/kg saline plus 0.1% Tween 80. GHB (sodium salt; Laboratorio Farmaceutico CT, Sanremo, IM, Italy) and NCS-382 (synthesised by G Ci as previously described by Maitre et al., 1990) were dissolved in 12.5 ml/kg distilled water. All drugs were injected i.p.

2.1.4. Data analysis

Onset of the loss of righting reflex and sleep time after 1,4-butanediol and GHB administration were expressed in min and used as measures of their sedative/hypnotic effect. Data on onset and sleep time from the experiment comparing 1,4-butanediol and GHB were analysed by a two-way analysis of variance (ANOVA) (drug, dose), followed by the Newman–Keuls test for post hoc comparisons. Data from the blockade study with disulfiram were evaluated by a one-way ANOVA, followed by the Newman–Keuls test for post hoc comparisons. Data from the blockade tests with 4-methylpyrazole, ethanol, SCH 50911 and CGP 46381 were analysed by the Fisher's exact test for a 2×2 table [treatment (saline, drug) $\times$ loss of righting reflex (presence, absence)]. Data on onset and sleep time from the blockade study with NCS-382 were analysed by the unpaired, two-tailed Mann–Whitney test.

2.2. Binding experiments

2.2.1. Tissue preparation

Male Sprague–Dawley rats (Charles River, Calco, LC, Italy), weighing 200–250 g, were used. Rats had food and water ad libitum. Rats were killed by decapitation, their brains rapidly removed and cerebral cortices dissected on ice.

Cortical tissue was homogenised using an Ultrax turrax homogeniser (Janke and Kunkel, IKA Labortechnick, Stanfen, Germany) in 20 volumes (v/w) tissue of ice-cold 0.32 M sucrose containing 1 mM EDTA. The homogenate was centrifuged at $1000 \times g$ for 10 min and the supernatant collected and recentrifuged at $20{,}000 \times g$ for 20 min. The pellet was resuspended in 20 volumes (v/w) of ice-cold water, homogenised using a Polytron homogeniser and centrifuged at $8000 \times g$ for 20 min. The supernatant together with the buffy layer on the pellet was then centrifuged at $45{,}000 \times g$ for 20 min. The resulting pellet was resuspended in ice-cold distilled water and once more centrifuged at $45{,}000 \times g$ for 30 min. The final pellet was frozen and stored at -80 °C for at least 18 h before use for binding assay.

2.2.2. [^{3}H]Baclofen and [^{3}H]GHB binding assay

For binding assay, membrane pellets were allowed to thaw at 4 °C before resuspension in 20 volumes (v/w) of both 50 mM KH_2PO_4 buffer (pH 6.5) containing 1 mM EDTA and 50 mM Tris–HCl, pH 7.4, for [^{3}H]GHB and [^{3}H]baclofen, respectively. The suspension was incubated for 20 min at 20 °C before centrifugation at $7000 \times g$ for 10 min. The washing step was repeated three more times allowing 15 min incubation with each addition of the same buffer to remove the endogenous ligand GABA or GHB. The final pellet was then resuspended in appropriate binding buffer to a final concentration of 200–300 μg/ml of membrane protein for both [^{3}H]GHB and[^{3}H]baclofen, respectively. The incubation buffer used was (in mM): 50 Tris–HCl, pH 7.4, 2.5 $CaCl_2$, or 50 KH_2PO_4, pH 6.5, 1 EDTA for [^{3}H]baclofen and [^{3}H]GHB, respectively. [^{3}H]GHB binding assay was performed in triplicate in a volume of 0.6 ml at 4 °C for 30 min. Non-specific binding was estimated in the presence of 1 mM unlabelled GHB. [^{3}H]Baclofen binding was assayed in duplicate in a volume of 1 ml at 20–22 °C for 20 min, and *R*-(−)-baclofen (100 μM) was used to define non-specific binding. In both binding assays, free ligand was separated from bound ligand by rapid filtration

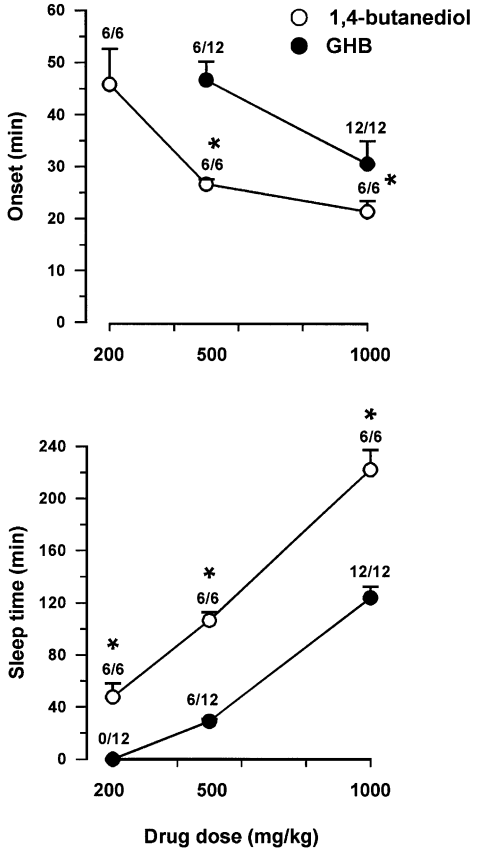

Fig. 1. Comparison of the sedative/hypnotic effect of 1,4-butanediol and γ-hydroxybutyric acid (GHB) in DBA mice. Top and bottom panels illustrate, respectively, the time to lose (onset) and regain (sleep time) the righting reflex after the i.p. administration of different doses of 1,4-butanediol or GHB. Figures on top of each point indicate the number of mice which lost the righting reflex over the total number of mice tested. In the top panel, each point is the mean ± S.E.M. of the onset of mice which lost the righting reflex; in the bottom panel, each point is the mean ± S.E.M. of the sleep time of 6–12 mice (mice that did not lose the righting reflex were included assigning the value zero). * $P < 0.05$ in comparison to the mouse group treated with the equal dose of GHB (Newman–Keuls test).

through Whatmann GF/B glass filters using a Brandell 96-sample harvester (Gaithesburg, USA). Filters were then rinsed twice with either ice-cold 50 mM Tris–HCl buffer (pH 7.4) containing 2.5 mM $CaCl_2$, or 50 mM KH_2PO_4 buffer (pH 6.5) for [^{3}H]baclofen and [^{3}H]GHB, respectively. Filter-bound radioactivity was counted in a liquid scintillation counter (Packard Tricarb 2100, Packard, Meridien, USA), using 3 ml of scintillation fluid (Packard Ultima Gold MV, Packard).

[^{3}H]GHB and [^{3}H]baclofen displacement curves were carried out using serial dilutions ranging from 10^{-9} to 10^{-4} M of the unlabelled compounds and either [^{3}H]GHB (60 nM) or [^{3}H]baclofen (20 nM). Independent experiments were repeated on membrane preparations from at least three different brains. The Bradford protein assay (Bradford, 1976) was used for protein determination with bovine serum albumin as a standard according to the protocol of the supplier (Bio-Rad, Milan, Italy).

The calculation of IC_{50} (concentration which inhibits 50% of specific radioligand binding) was performed by non-linear curve fitting of the concentration–effect curves using GraphPad Prism Program (GraphPad Prism Software, San Diego, USA). The *F*-test was used to determine the best approximation of a non-linear curve fitting to one or two site model ($P<0.05$).

2.2.3. Drugs

[^{3}H]GHB or 4-hydroxy[2-3-3H]butyric acid, sodium salt (30–60 Ci/mmol) and [^{3}H]baclofen (38.7/Ci/mmol) were obtained from ARC (St. Louis, USA) and New England Nuclear (NEN), Boston, respectively. GHB (sodium salt) and 1,4-butanediol were purchased from Sigma-Aldrich. *R*-(−)-baclofen was obtained from Tocris.

3. Results

3.1. In vivo experiments

Administration of 1,4-butanediol and GHB produced a dose-dependent sedative/hypnotic effect (Fig. 1). ANOVA revealed a significant difference in potency between the two drugs in both onset [$F_{treatment}(1,42)=4.503$, $P<0.05$] and sleep time [$F_{treatment}(1,48)=97.031$, $P<0.000001$]. At

Table 1
Prevention of the sedative/hypnotic effect of 1,4-butanediol by the alcohol-dehydrogenase inhibitor, 4-methylpyrazole, in DBA mice

4-Methylpirazole (mg/kg)	1,4-Butanediol (mg/kg)	Number of mice that lost the righting reflex on total number of mice tested	Onset (min)	Sleep time (min)
0	350	10/10	26.0 ± 1.5	87.4 ± 4.0
0.1	350	0/10	ND	0 ± 0

In the Onset column, values are the mean ± S.E.M. of the onset time of mice which lost the righting reflex (ND: not determined, since no mouse lost the righting reflex); in the Sleep time column, values are the mean ± S.E.M. of the sleep time of 10 mice (mice that did not lose the righting reflex were included assigning the value 0).

Table 2
Prevention of the sedative/hypnotic effect of 1,4-butanediol by ethanol in DBA mice

Ethanol (mg/kg)	1,4-Butanediol (mg/kg)	Number of mice that lost the righting reflex on total number of mice tested	Onset (min)	Sleep time (min)
0	350	10/10	27.7 ± 1.8	81.1 ± 10.5
1000	350	0/10	ND	0 ± 0

In the Onset column, values are the mean ± S.E.M. of the onset time of mice which lost the righting reflex (ND: not determined, since no mouse lost the righting reflex); in the Sleep time column, values are the mean ± S.E.M. of the sleep time of 10 mice (mice that did not lose the righting reflex were included assigning the value 0).

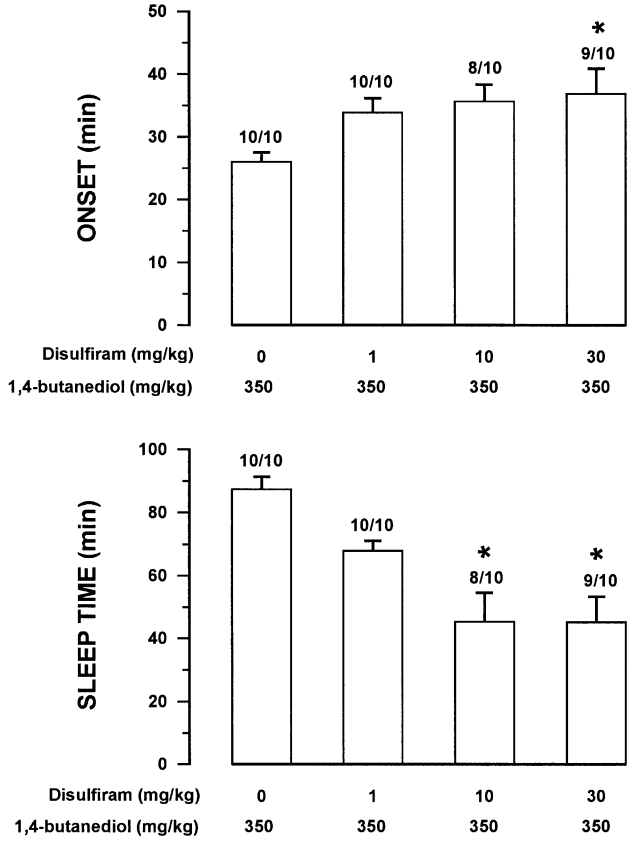

Fig. 2. Attenuation of the sedative/hypnotic effect of 1,4-butanediol by the aldehyde-dehydrogenase inhibitor, disulfiram, in DBA mice. Top and bottom panels illustrate, respectively, the time to lose (onset) and regain (sleep time) the righting reflex after administration of the combination of different doses of disulfiram and 350 mg/kg 1,4-butanediol. Disulfiram was administered i.p. 15 min prior to the i.p. injection of 1,4-butanediol. Figures on top of each bar indicate the number of mice which lost the righting reflex over the total number of mice tested. In the top panel, each bar is the mean ± S.E.M. of the onset of mice which lost the righting reflex; in the bottom panel, each bar is the mean ± S.E.M. of the sleep time of 10 mice (mice that did not lose the righting reflex were included assigning the value zero). * $P<0.05$ in comparison to 0 mg/kg disulfiram plus 350 mg/kg 1,4-butanediol group.

Table 3
Lack of prevention of the sedative/hypnotic effect of 1,4-butanediol by the γ-hydroxybutyric acid receptor antagonist, NCS-382, in DBA mice

NCS-382 (mg/kg)	1,4-Butanediol (mg/kg)	Number of mice that lost the righting reflex on total number of mice tested	Onset (min)	Sleep time (min)
0	350	10/10	31.3 ± 1.5	79.1 ± 3.7
250	350	10/10	29.3 ± 1.6	86.3 ± 3.8

In the Onset column, values are the mean ± S.E.M. of the onset time of $n = 10$ mice; in the Sleep time column, values are the mean ± S.E.M. of $n = 10$ mice.

doses of 1,4-butanediol equal to or greater than 200 mg/kg all mice lost the righting reflex. In contrast, 1000 mg/kg GHB were needed to induce loss of righting reflex in all mice.

Pretreatment with 0.1 mg/kg 4-methylpyrazole resulted in a complete antagonism of the sedative/hypnotic effects of 350 mg/kg 1,4-butanediol; indeed, while all mice of the control group (saline plus 1,4-butanediol) lost their righting reflex, no mouse in the 4-methylpyrazole plus 1,4-butanediol-group lost the latter ($P < 0.0001$, Fisher's exact test) (Table 1). Similarly, pretreatment with a non-hypnotic dose of ethanol (1000 mg/kg) resulted in a complete antagonism of the sedative/hypnotic effect of 350 mg/kg 1,4-butanediol; as shown in Table 2, no mouse of the ethanol plus 1,4-butanediol-group lost the righting reflex ($P < 0.0001$, Fisher's exact test).

Pretreatment with disulfiram partially blocked, in a dose-dependent manner, the sedative/hypnotic effect of 350 mg/kg 1,4-butanediol; onset and sleep time induced by 1,4-butanediol were increased by approximately 40% and reduced by approximately 50%, respectively, by administration of 10 or 30 mg/kg disulfiram [ANOVA for onset: $F(3,33) = 3.416$, $P < 0.05$; ANOVA for sleep time: $F(3,36) = 9.262$, $P < 0.001$] (Fig. 2).

All mice treated with the combination of 250 mg/kg NCS-382 [a dose that did not produce any sign of sedation (this lab, unpublished observation)] and 350 mg/kg 1,4-butanediol lost the righting reflex. Further, no significant difference ($P > 0.05$, Mann–Whitney test) in onset and sleep time was observed between saline plus 1,4-butanediol-treated rats and NCS-382 plus 1,4-butanediol-treated rats (Table 3).

Table 4
Prevention of the sedative/hypnotic effect of 1,4-butanediol by the $GABA_B$ receptor antagonist, SCH 50911, in DBA mice

SCH 50911 (mg/kg)	1,4-Butanediol (mg/kg)	Number of mice that lost the righting reflex on total number of mice tested	Onset (min)	Sleep time (min)
0	350	6/6	26.0 ± 1.8	104.0 ± 6.6
100	350	0/6	ND	0 ± 0

In the Onset column, values are the mean ± S.E.M. of the onset time of mice which lost the righting reflex (ND: not determined, since no mouse lost the righting reflex); in the Sleep time column, values are the mean ± S.E.M. of the sleep time of six mice (mice that did not lose the righting reflex were included assigning the value 0).

Table 5
Prevention of the sedative/hypnotic effect of 1,4-butanediol by the $GABA_B$ receptor antagonist, CGP 46381, in DBA mice

CGP 46381 (mg/kg)	1,4-Butanediol (mg/kg)	Number of mice that lost the righting reflex on total number of mice tested	Onset (min)	Sleep time (min)
0	350	6/6	33.3 ± 3.2	74.5 ± 7.4
100	350	0/6	ND	0 ± 0

In the Onset column, values are the mean ± S.E.M. of the onset time of mice which lost the righting reflex (ND: not determined, since no mouse lost the righting reflex); in the Sleep time column, values are the mean ± S.E.M. of the sleep time of six mice (mice that did not lose the righting reflex were included assigning the value 0).

The loss of righting reflex produced by 350 mg/kg 1,4-butanediol was completely suppressed by pretreatment with 100 mg/kg SCH 50911; as shown in Table 4, no mouse treated with SCH 50911 lost the righting reflex ($P < 0.01$, Fisher's exact test). Consistently, pretreatment with 100 mg/kg CGP 46381 resulted in a complete prevention of the sedative/hypnotic effect of 350 mg/kg 1,4-butanediol ($P < 0.01$, Fisher's exact test) (Table 5).

3.2. Binding experiments

As shown in Table 6, GHB inhibited [^{3}H]GHB and [^{3}H]baclofen binding with an IC_{50} of 105 ± 30 nM and 3 ± 0.8 μM (high and low affinity GHB recognition sites) and 65 ± 5 μM, respectively. In agreement with Benavides et al. (1982) and Snead (1996), baclofen failed to inhibit [^{3}H]GHB binding up to the concentration of 1 mM. Finally, 1,4-butanediol inhibited neither [^{3}H]GHB binding, as expected from a previous study (Bourguignon et al., 1988), nor [^{3}H]baclofen binding at concentrations up to 1 mM.

Table 6
Inhibition of [^{3}H]γ-hydroxybutyric acid (GHB) and [^{3}H]baclofen binding by GHB, baclofen and 1,4-butanediol in rat cortical membranes

	[^{3}H]GHB IC_{50}	[^{3}H]Baclofen IC_{50}
GHB	High-affinity (nM) 105 ± 30 Low-affinity (μM) 3 ± 0.8	65 ± 5 (μM)
Baclofen	n.s.	62 ± 3.5 (nM)
1,4-Butanediol	n.s.	n.s.

Cortical rat membranes were added to the incubation buffer containing either 60 nM [^{3}H]GHB or 20 nM [^{3}H]baclofen and serial dilutions ranging from 1 nM to 1 mM of the unlabelled compounds. Values are the mean ± S.E.M. The IC_{50} values were calculated using GraphPad Prism Program. The *F*-test was used to determine the best approximation of a non-linear curve fitting to one or two site model ($P < 0.05$). All IC_{50} values were obtained from three different dose–inhibition curves.
n.s.: non-significant inhibition up to 1 mM concentration.

4. Discussion

These results confirm previous data (Roth and Giarman, 1968) indicating that 1,4-butanediol, similarly to its metabolite GHB, induced sedation/hypnosis in rodents. The comparison of the two drugs showed that 1,4-butanediol possesses a higher potency: indeed, comparable sleep times were obtained with 500 mg/kg 1,4-butanediol and 1000 mg/kg GHB. The higher potency of 1,4-butanediol, with respect to GHB, might be explained by the fact that GHB is a relatively polar compound that might penetrate the blood–brain barrier with difficulty, while 1,4-butanediol, a less polar molecule, readily crosses the barrier and exerts its pharmacological effects.

Further, the results of the present study suggest that the 1,4-butanediol-induced sedation/hypnosis is due to its in vivo conversion into GHB. Indeed, the sedative/hypnotic effect of 1,4-butanediol was totally prevented by 4-methylpyrazole and ethanol that inhibited alcohol dehydrogenase, subsequently abolishing the conversion of 1,4-butanediol into γ-hydroxybutyraldehyde, which in turn is converted into GHB. These results are in agreement with those reported by Poldrugo and Snead (1984), who demonstrated the ability of ethanol to block the electroencephalographic changes produced by 1,4-butanediol in rats. Inhibition of aldehyde dehydrogenase by disulfiram, which should block the conversion of the intermediate γ-hydroxybutyraldehyde into GHB, resulted in a significant reduction of the sedative/hypnotic effect of 1,4-butanediol. Consistently, an in vitro study demonstrated the partial blockade by disulfiram of the conversion of 1,4-butanediol into GHB in the rat liver (Poldrugo and Snead, 1986). The lack of complete antagonism with disulfiram of the sedative/hypnotic effect of 1,4-butanediol (present study) as well as of the in vitro conversion of 1,4-butanediol into GHB (Poldrugo and Snead, 1986) might be explained by the presence of alternative pathways of degradation of γ-hydroxybutyraldehyde (Poldrugo and Snead, 1986).

A recent study from this laboratory demonstrated that the sedative/hypnotic effect of GHB is mediated by stimulation of $GABA_B$ but not GHB receptors (Carai et al., 2001); indeed, treatment with the $GABA_B$ receptor antagonists, CGP 46381 and SCH 50911, but not with the putative GHB receptor antagonist, NCS-382, completely prevented and reversed the sedative/hypnotic effect produced by 1000 mg/kg GHB in DBA mice. In this light, the finding of the present study that the sedative/hypnotic effect of 1,4-butanediol was suppressed by $GABA_B$ receptor antagonists but not by NCS-382 constitutes further confirmation of the conversion of 1,4-butanediol into GHB. In turn, the GHB portion deriving from the conversion of 1,4-butanediol binds to the $GABA_B$ receptor and exerts its sedative/hypnotic effect. This hypothesis is also supported by the results of the binding study, showing the lack of any affinity of 1,4-butanediol for both GHB (Bourguignon et al., 1988; present study) and $GABA_B$ (present study) receptors. Interestingly, brain concentrations of GHB elicited by a sedative/hypnotic dose of 1,4-butanediol (Roth and Giarman, 1968) were found to be comparable to those reported to displace $GABA_B$ receptor agonists in in vitro assays (Mathivet et al., 1997; Lingenhoehl et al., 1999).

Since GHB and 1,4-butanediol are abused and their toxic effects include fatal coma (Thomas et al., 1997; Li et al., 1998; Ingels et al., 2000; Zvosec et al., 2001), $GABA_B$ receptor antagonists may offer resuscitative agents in these conditions. 4-Methylpyrazole is currently used in ethylene glycol poisoning (Brent et al., 2001; Borron et al., 1999); future studies might be designed to assess its potential utility to reverse the episodes of coma, respiratory depression and loss of consciousness occurring after the ingestion of high quantities of 1,4-butanediol. Finally, since GHB is clinically used in the treatment of alcoholism and narcolepsy (see Agabio and Gessa, in press), the finding that its precursor 1,4-butanediol is more effective than GHB itself suggests the possibility of evaluating its therapeutic potential in these pathologies.

Acknowledgements

The authors are grateful to Mrs. Anne Farmer for language editing of the manuscript. The present study was partially supported by the European Community, the Italian Government and the Regione Autonoma della Sardegna through "Programma Operativo Plurifondo–Sardegna".

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