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Short communication

Protection by the $GABA_B$ receptor antagonist, SCH 50911, of γ-hydroxybutyric acid-induced mortality in mice

Mauro A.M. Carai[a,*], Giancarlo Colombo[b], Gian Luigi Gessa[a,b]

[a]"*Bernard B. Brodie*" *Department of Neuroscience, University of Cagliari, Viale Diaz 182, I-09126 Cagliari (CA), Italy*
[b]*C.N.R. Institute of Neuroscience, Section of Cagliari, Viale Diaz 182, I-09126 Cagliari (CA), Italy*

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Abstract

Different effects of moderate to high doses of γ-hydroxybutyric acid, including sedation/hypnosis, have been found to be blocked by γ-aminobutyric $acid_B$ ($GABA_B$) receptor antagonists. The present study investigated whether the protective effect of $GABA_B$ receptor antagonists extends also to γ-hydroxybutyric acid-induced mortality. To this aim, the present study investigated the effect of the $GABA_B$ receptor antagonist, (2S)(+)-5,5-dimethyl-2-morpholineacetic acid (SCH 50911; 100 mg/kg, ip), on mortality induced by γ-hydroxybutyric acid (1–6 g/kg, ip) in DBA mice. Pretreatment with SCH 50911 resulted in a significant shift to the right of the dose–response curve of γ-hydroxybutyric acid-induced mortality. Accordingly, the LD_{50} in SCH 50911-pretreated mice was significantly higher than that obtained in water-pretreated mice. The results of the present study support the hypothesis that (a) the $GABA_B$ receptor is a relevant site of action of γ-hydroxybutyric acid, and (b) $GABA_B$ receptor antagonists may constitute potentially effective therapeutic interventions for γ-hydroxybutyric acid intoxication.

Keywords: γ-Hydroxybutyric acid-induced mortality; $GABA_B$ receptor antagonist, SCH 50911; DBA, mouse

1. Introduction

Accumulating lines of experimental evidence suggest that several pharmacological effects of γ-hydroxybutyric acid (also known as GHB), the recreational drug gaining popularity—mostly in the U.S.—for its euphorigenic and relaxant properties, are mediated by activation of the γ-aminobutyric $acid_B$ ($GABA_B$) receptor (see Wong et al., 2004). As an example, sedation/hypnosis produced by relatively high doses of γ-hydroxybutyric acid in mice was completely prevented by the administration of $GABA_B$ receptor antagonists (Carai et al., 2001); the latter also readily reversed γ-hydroxybutyric acid-induced sedation/hypnosis once it was already present (Carai et al., 2001). Further, $GABA_B$ receptor antagonists prevented motor incoordination (Quang et al., 2002) and inhibition of locomotor activity (Nissbrandt and Engberg, 1996) produced by moderate to high doses of γ-hydroxybutyric acid in mice.

An increasing number of reports describe the fatalities associated with overdoses of γ-hydroxybutyric acid in humans (see Mason and Kerns, 2002). γ-Hydroxybutyric acid overdose has been reported to progressively produce ataxia, labile levels of consciousness, respiratory depression, coma and death (see Mason and Kerns, 2002). These signs are closely reproduced in rodents by the administration of high doses of γ-hydroxybutyric acid. However, to our knowledge, to date, no study has investigated the neurobiological bases of γ-hydroxybutyric acid-induced mortality or whether it can be prevented pharmacologically. Accordingly, the present study assessed the hypothesis that the selective $GABA_B$ receptor antagonist, (2S)(+)-5,5-dimethyl-2-morpholineacetic acid (SCH 50911) (Blythin et al., 1996; Snead, 1996), may provide

* Corresponding author. Tel.: +39 070 302227; fax: +39 070 302076.
E-mail address: macarai@unica.it (M.A.M. Carai).

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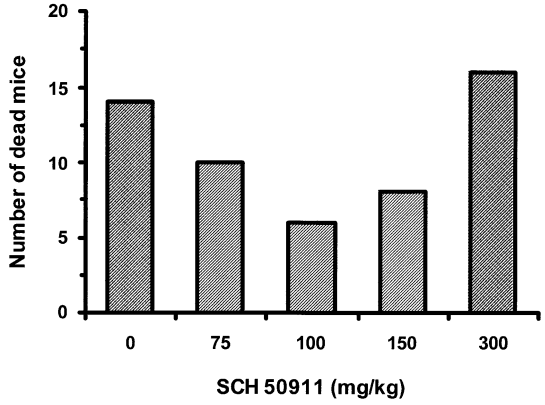

Fig. 1. Number of dead mice, out of 20 tested in each group, after the combination of different doses of the $GABA_B$ receptor antagonist, SCH 50911, with 4.3 g/kg γ-hydroxybutyric acid.

some degree of protection from γ-hydroxybutyric acid-induced mortality in mice.

2. Materials and methods

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. Animals

Male DBA mice (Charles River, Calco, LC, Italy), weighing 25–30 g, were used. DBA mice were chosen due to their particular sensitivity to the sedative/hypnotic effect of γ-hydroxybutyric acid (Carai et al., 2001). 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 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, MI, Italy) were provided ad libitum throughout the experimental period.

2.2. Procedure

In Experiment 1 (SCH 50911 dose-curve), mice were divided into groups of n=20 and treated with 0, 75, 100, 150 and 300 mg/kg SCH 50911 (these doses did not produce any clear sign of toxicity when given alone in preliminary experiments); 15 min later, mice were administered with 0 and 4.3 g/kg γ-hydroxybutyric acid (this dose of γ-hydroxybutyric acid was chosen because it corresponded to the calculated LD_{50} in a preliminary experiment). In Experiment 2 (γ-hydroxybutyric acid dose-curve), mice were divided into groups of n=10 and treated with 0 and 100 mg/kg SCH 50911; 15 min later, rats were administered with 1, 3, 3.3, 3.6, 4, 4.3, 4.6, 5 and 6 g/kg γ-hydroxybutyric acid. SCH 50911 (Tocris, Avonmouth, UK) was dissolved in 12.5 ml/kg distilled water; γ-hydroxybutyric acid (sodium salt; Laboratorio Farmaceutico C.T., Sanremo, IM, Italy) was dissolved in 29.4 ml/kg distilled water. Both drugs were injected ip. Immediately after drug injection, mice were housed singly and monitored once a day for 21 consecutive days.

2.3. Statistical analysis

In Experiment 1, data were analyzed by a chi-square test (dead; alive) (GraphPad Prism version 3.00, GraphPad Software, San Diego, CA, USA). In Experiment 2, LD_{50}s of γ-hydroxybutyric acid, with 95% confidence limits, were calculated using the above-mentioned computer program and analyzed by a two-tailed t-test.

3. Results

In Experiment 1 (SCH 50911 dose-curve), the number of mice which dead after the combination of different doses of SCH 50911 and a fixed dose of γ-hydroxybutyric acid was a U-shaped function of SCH 50911 dose (P<0.01, chi-square test) (Fig. 1). All deaths occurred during the first 3 days after γ-hydroxybutyric acid administration. Maximal protection by SCH 50911 was observed at the dose of 100 mg/kg (14/20 mice survived). On the basis of this result, this dose was chosen for the subsequent experiment.

In Experiment 2 (γ-hydroxybutyric acid dose-curve), pretreatment with 100 mg/kg SCH 50911 shifted the dose–response curve of γ-hydroxybutyric acid-induced mortality to the right (Fig. 2). The LD_{50}s of γ-hydroxybutyric acid in water- and SCH 50911-pretreated mice were 3.93±0.15 (95% confidence interval: 3.65–4.27) and 4.41±0.02 (95% confidence interval: 4.37–4.50) g/kg γ-hydroxybutyric acid, respectively (P<0.05, t-test). All deaths occurred during the first 3 days after γ-hydroxybutyric acid administration. SCH 50911 produced a complete protection from mortality

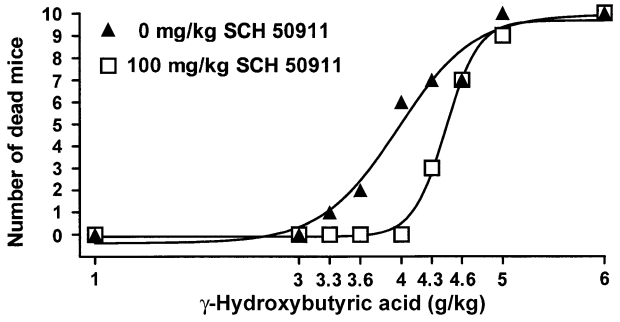

Fig. 2. Number of dead mice, out of 10 tested in each group, after the combination of the $GABA_B$ receptor antagonist, SCH 50911 (0 and 100 mg/kg), with different doses of γ-hydroxybutyric acid.

induced by doses of γ-hydroxybutyric acid lower than or equal to 4 g/kg (Fig. 2).

4. Discussion

The present study demonstrated that the acute administration of the $GABA_B$ receptor antagonist, SCH 50911, produced a significant protection from mortality induced in mice by high doses of γ-hydroxybutyric acid. These results extend to lethal doses of γ-hydroxybutyric acid the capability of $GABA_B$ receptor antagonists to antagonize γ-hydroxybutyric acid effects. Indeed, the $GABA_B$ receptor antagonists, (3-aminopropyl)(diethoxymethyl)phosphinic acid (CGP 35348), (3-aminopropyl)(cyclohexyl-methyl)-phosphinic acid (CGP 46381) and SCH 50911, prevented motor incoordination, sedation and hypnosis produced by moderate to high doses of γ-hydroxybutyric acid (Nissbrandt and Engberg, 1996; Carai et al., 2001; Quang et al., 2002). These in vivo results are in close agreement with biochemical data indicating that millimolar concentrations of γ-hydroxybutyric acid, comparable to those presumably achieved in brain after the administration of the doses producing the above effects, activate the $GABA_B$ receptor (Lingenhoehl et al., 1999).

The data generated by the present study are also consistent with those recently reported on the survival rate of mice lacking the gene encoding succinic semialdehyde dehydrogenase (SSADH), one of the enzymes involved in γ-hydroxybutyric acid degradation (Hogema et al., 2001). These mice are characterized by exceptionally high brain levels of γ-hydroxybutyric acid, which result in signs of ataxia, seizures and death within 4 weeks postnatally (Hogema et al., 2001). Interestingly, approximately 35% of these mice survived when treated daily with the $GABA_B$ receptor antagonist, CGP 35348 (Gupta et al., 2002).

The lack of complete protection, by $GABA_B$ receptor antagonists, of γ-hydroxybutyric acid-induced mortality in DBA mice (present study) and mortality associated to high brain levels of γ-hydroxybutyric acid in SSADH knockout mice (Gupta et al., 2002) suggests that other receptor systems are likely involved in this effect of γ-hydroxybutyric acid. γ-Hydroxybutyric acid has been found to bind to a specific receptor (Andriamampandry et al., 2003), and the repeated administration of its antagonist, NCS 382, protected approximately 60% of SSADH knockout mice from an early death (Gupta et al., 2002). Further, γ-hydroxybutyric acid has been reported to be converted into GABA which, in turn, binds to both $GABA_A$ and $GABA_B$ receptors (Hechler and Ratomponirina, 1997); subsequently, a contribution to the observed mortality may also derive from an inordinately elevated activation of the $GABA_A$ receptor. Accordingly, drugs capable of antagonizing the interaction of γ-hydroxybutyric acid with the $GABA_A$ receptor might provide additional protection from γ-hydroxybutyric acid-induced mortality.

Alternatively, $GABA_B$ receptor antagonists may bear an intrinsic toxicity which is added to that exerted by high doses of γ-hydroxybutyric acid. Accordingly, Experiment 1 of the present study found that increases in SCH 50911 dosage over 100 mg/kg vanished the protective effect on γ-hydroxybutyric acid-induced mortality. Further, the pro-convulsive activity of $GABA_B$ receptor antagonists under different experimental procedures (Vergnes et al., 1997; Motalli et al., 1999; Carai et al., 2002) may have limited their protective effect on γ-hydroxybutyric acid-induced mortality. It can also be hypothesized that $GABA_B$ receptor antagonists with higher receptor affinity than SCH 50911 and CGP 34358 might exert a greater protective effect on γ-hydroxybutyric acid-induced mortality.

Finally, factors other than γ-hydroxybutyric acid-induced activation of specific receptors might contribute to mortality associated to γ-hydroxybutyric acid. As an example, the elevated amount of sodium contained in the sodium salt of γ-hydroxybutyric acid (838 mg/kg at the dose of 4.6 g/kg γ-hydroxybutyric acid, being sodium the 18.23% of γ-hydroxybutyric acid molecular weight) might have contributed to the observed mortality by severely altering the electrolytic homeostasis.

These data need to be confirmed with other $GABA_B$ receptor antagonists. Their possible replication would support the hypothesis (Carai et al., 2001; Wong et al., 2004) that $GABA_B$ receptor antagonists may constitute potentially effective therapeutic interventions for the spreading number of cases of intoxication, often fatal, by γ-hydroxybutyric acid and its precursors (e.g.: Higgins and Borron, 1996; Zvosec et al., 2001; Mason and Kerns, 2002).

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References

Andriamampandry, C., Taleb, O., Viry, S., Muller, C., Humbert, J.P., Gobaille, S., Aunis, D., Maitre, M., 2003. Cloning and characterization of a rat brain receptor that binds the endogenous neuromodulator gamma-hydroxybutyrate (GHB). FASEB J. 17, 1691–1693.

Blythin, D.J., Kuo, S.-C., Shue, H.-J., McPhail, A.T., Chapman, R.W., Kreutner, W., Rizzo, C., She, H.S., West, R., 1996. Substituted morpholine-2S-acetic acid derivatives: Sch 50911 and related compounds as novel $GABA_B$ antagonists. Bioorg. Med. Chem. Lett. 6, 1529–1534.

Carai, M.A.M., Colombo, G., Brunetti, G., Melis, S., Serra, S., Vacca, G., Mastinu, S., Pistuddi, A.M., Solinas, C., Cignarella, G., Minardi, G.,

Gessa, G.L., 2001. Role of $GABA_B$ receptors in the sedative/hypnotic effect of γ-hydroxybutyric acid. Eur. J. Pharmacol. 428, 315–321.

Carai, M.A.M., Brunetti, G., Lobina, C., Serra, S., Vacca, G., Minardi, G., Colombo, G., Gessa, G.L., 2002. Proconvulsive effect of the $GABA_B$ receptor antagonist, SCH 50911, in rats undergoing ethanol withdrawal syndrome. Eur. J. Pharmacol. 445, 195–199.

Gupta, M., Greven, R., Jansen, E.E., Jakobs, C., Hogema, B.M., Froestl, W., Snead, O.C., Bartels, H., Grompe, M., Gibson, K.M., 2002. Therapeutic intervention in mice deficient for succinate semialdehyde dehydrogenase (gamma-hydroxybutyric aciduria). J. Pharmacol. Exp. Ther. 302, 180–187.

Hechler, V., Ratomponirina, C., Maitre, M., 1997. γ-Hydroxybutyrate conversion into GABA induces displacement of $GABA_B$ binding that is blocked by valproate and ethosuximide. J. Pharmacol. Exp. Ther. 281, 753–760.

Higgins, T., Borron, S., 1996. Coma and respiratory arrest after exposure to butyrolactone. J. Emerg. Med. 14, 435–437.

Hogema, B.M., Gupta, M., Senephansiri, H., Burlingame, T.G., Taylor, M., Jakobs, C., Schutgens, R.B., Froestl, W., Snead, O.C., Diaz-Arrastia, R., Bottiglieri, T., Grompe, M., Gibson, K.M., 2001. Pharmacologic rescue of lethal seizures in mice deficient in succinate semialdehyde dehydrogenase. Nat. Genet. 29, 212–216.

Lingenhoehl, K., Brom, R., Heid, J., Beck, P., Froestl, W., Kaupmann, K., Bettler, B., Mosbacher, J., 1999. γ-Hydroxybutyrate is a weak agonist at recombinant $GABA_B$ receptors. Neuropharmacology 38, 1667–1673.

Mason, P., Kerns II, W.P., 2002. Gamma hydroxybutyric acid (GHB) intoxication. Acad. Emerg. Med. 9, 730–739.

Motalli, R., Louvel, J., Tancredi, V., Kurcewicz, I., Wan-Chow-Wah, D., Pumain, R., Avoli, M., 1999. GABA(B) receptor activation promotes seizure activity in the juvenile rat hippocampus. J. Neurophysiol. 82, 638–647.

Nissbrandt, H., Engberg, G., 1996. The $GABA_B$-receptor antagonist, CGP 35348, antagonizes γ-hydroxybutyrate- and baclofen-induced alterations in locomotor activity and forebrain dopamine levels in mice. J. Neural Transm. 103, 1255–1263.

Quang, L.S., Desai, M.C., Kraner, J.C., Shannon, M.W., Woolf, A.D., Maher, T.J., 2002. Enzymes and receptor antagonists for preventing toxicity from the gamma-hydroxybutyric acid precursor 1,4-butanediol in CD-1 mice. Ann. N.Y. Acad. Sci. 965, 461–472.

Snead III, O.C., 1996. Relation of the [^{3}Hγ]γ-hydroxybutyric acid (GHB) binding site to the γ-aminobutyric acidB ($GABA_B$) receptor in rat brain. Biochem. Pharmacol. 52, 1235–1243.

Vergnes, M., Boehrer, A., Simler, S., Bernasconi, R., Marescaux, C., 1997. Opposite effects of $GABA_B$ receptor antagonists on absences and convulsive seizures. Eur. J. Pharmacol. 332, 245–255.

Wong, C.G.T., Gibson, K.M., Snead III, O.C., 2004. From the street to the brain: neurobiology of the recreational drug γ-hydroxybutyric acid. Trends Pharmacol. Sci. 25, 29–34.

Zvosec, D.L., Smith, S.W., McCutcheon, J.R., Spillane, J., Bradley, J.H., Peacock, E.A., 2001. Adverse events including death, associated with the use of 1,4-butanediol. New Engl. J. Med. 344, 87–94.