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European Journal of Pharmacology 484 (2004) 259–262
www.elsevier.com/locate/ejphar

Short communication

Chronic 1,4-butanediol treatment in rats: cross-tolerance to γ-hydroxybutyrate and (±)-baclofen

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Received 30 October 2003; accepted 7 November 2003

Abstract

The effects of 1,4-butanediol, γ-hydroxybutyrate (GHB), and (±)-baclofen on food-maintained responding in rats were assessed before, during, and after chronic treatment with 1,4-butanediol. Six weeks of treatment with 1,4-butanediol (twice daily, 320 mg/kg for 3 weeks followed by 560 mg/kg for 3 weeks) decreased sensitivity to the rate-decreasing effects of (±)-baclofen and GHB without changing sensitivity to 1,4-butanediol. Sensitivity to (±)-baclofen and GHB returned to control values 2–3 weeks after discontinuation of treatment. These data suggest that tolerance to the effects of GHB or its precursors might result from changes in $GABA_B$ mechanisms.

Keywords: 1,4-Butanediol; (±)-Baclofen; γ-GHB (γ-hydroxybutyrate); $GABA_B$ receptor; Cross-tolerance

1. Introduction

γ-Hydroxybutyrate (GHB) is an emerging drug of abuse, a purported neurotransmitter, and recently was approved for treating a severe form of narcolepsy (for review, see Nicholson and Balster, 2001). Availability of GHB has been restricted as a result of being designated Schedule I by the US Drug Enforcement Administration; one result appears to be increased recreational use of related substances, including metabolic precursors of GHB (Dyer et al., 1997). 1,4-Butanediol is metabolized to GHB by alcohol dehydrogenase and aldehyde dehydrogenase (Roth and Giarman, 1968; Snead et al., 1989). 1,4-Butanediol does not bind to GHB, γ-aminobutyric acid $(GABA)_A$, or $GABA_B$ receptors; conversion to GHB is thought to be the primary mechanism by which 1,4-butanediol exerts behavioral effects (Barker et al., 1985).

Converging lines of evidence implicate GABA mechanisms, in particular $GABA_B$, in the behavioral actions of GHB. GHB as well as the $GABA_B$ receptor-selective agonist (±)-baclofen decrease locomotor activity in mice and these effects are antagonized by the $GABA_B$ receptor-selective antagonist 3-aminopropyl (diethoxymethyl)phosphinic acid (CGP 35348; Nissbrandt and Engberg, 1996). Moreover, under conditions where $GABA_B$ receptor antagonists block the effects of GHB, the GHB receptor antagonist NCS-382 is not effective (Carai et al., 2002; Carter et al., 2003). Thus, $GABA_B$ mechanisms appear to be important in the behavioral effects of GHB and related compounds.

Prolonged use of (±)-baclofen (Chabal et al., 1992), GHB (Galloway et al., 1994; Schneir et al., 2001), or 1,4-butanediol (Zvosec et al., 2001) can produce tolerance or dependence in humans, although the magnitude and generality of these effects have not been thoroughly examined. While some studies failed to observe tolerance to GHB, other studies employing more frequent administration of large doses reported significant tolerance to GHB (Bania et al., 2003; Itzhak and Ali, 2002) or its metabolic precursor γ-butyrolactone (GBL; Van Sassenbroeck et al., 2003). It is not clear whether tolerance to GHB or related compounds is mediated by GHB, GABA, or other receptors. The current study examined the effects of chronic treatment with the long-acting GHB precursor 1,4-butanediol on the behavioral effects of 1,4-butanediol, GHB, and (±)-baclofen.

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doi:10.1016/j.ejphar.2003.11.016

2. Materials and methods

2.1. Animals and drugs

Seven adult experimentally naïve, male Sprague–Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were housed individually in plastic cages containing bedding (Sani-Chips, Harlan Teklad, Madison, WI) in a room maintained on a 14:10-h light/dark cycle with experiments conducted during the light period. Subjects were fed daily after sessions (Rat sterilizable diet, Harlan Teklad) and maintained at 80% of their free-feeding weight. Water was available continuously in the home cages. Experiments were performed in accordance with the IACUC at the University of Texas Health Science Center at San Antonio and the 1996 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences), and the European Community Guidelines.

GHB (sodium salt), (±)-baclofen, and 1,4-butanediol (Sigma-Aldrich, St. Louis, MO) were dissolved in sterile water or 0.9% saline and administered i.p. in a volume of 0.1–1.0 ml (pH 6–8).

2.2. Apparatus

Experiments were conducted in ventilated chambers equipped with two levers and associated lights (Model ENV-008CT; MED Associates, St. Albans, VT), and located within sound attenuating cubicles (Model ENV-022M; MED Associates). Experimental events were controlled and recorded with a microprocessor and commercially available software and interface (MED Associates). A hopper delivered food pellets (PJAI-0045; Research Diets, New Brunswick, NJ) to an opening located between the levers.

2.3. Procedure

Daily sessions consisted of four 20-min cycles with each cycle comprising a 15-min timeout, during which the chamber was dark and responses had no programmed consequence, followed by a 5-min response period, during which stimulus lights above the levers were illuminated and responding on the active lever produced food. Over the initial six to nine sessions, the response requirement was increased from continuous reinforcement to a fixed ratio 10 with a maximum of 10 pellets delivered per cycle. Response rates (responses/second) were averaged across cycles for each session, and training was conducted until responding was stable: daily rates within ±20% of the overall average rate for 10 consecutive or 11 out of 12 sessions.

Dose–effect curves were determined for1,4-butanediol, GHB, and (±)-baclofen by administering increasing doses (cumulative dosing) of drug across cycles in the first min of timeouts, up to doses that decreased responding, and with at least two non-drug training sessions between consecutive tests. Next, 1,4-butanediol was administered 5 h before and 7 h after daily sessions: 320 mg/kg during weeks 1–3 and 560 mg/kg during weeks 4–6, twice daily. Changes in sensitivity to 1,4-butanediol were evaluated weekly, and those to GHB and (±)-baclofen during week 6. 1,4-Butanediol treatment was discontinued and dose–response curves for 1,4-butanediol, GHB, and (±)-baclofen were determined weekly for 3 weeks.

2.4. Data analysis

Response rates were determined by averaging the rates for all cycles within a session for individuals. Control rates determined prior to chronic treatment were calculated by averaging these daily rates for the 10 control sessions

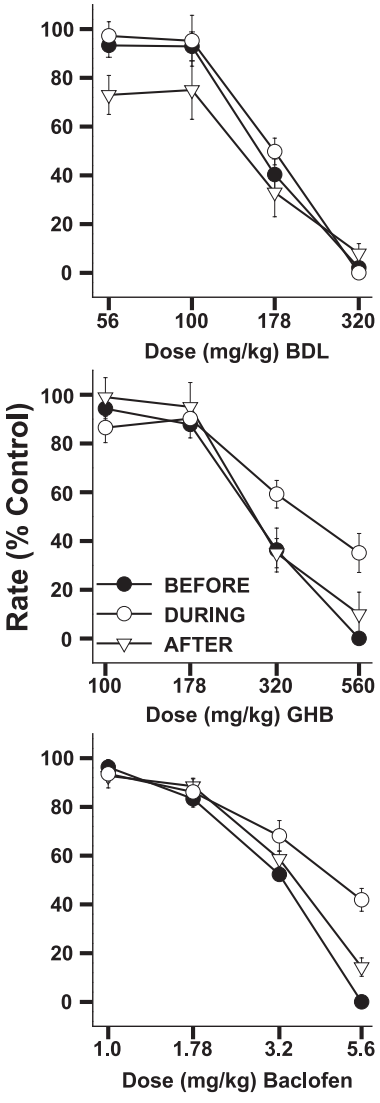

Fig. 1. Dose–effect curves for the rate-decreasing effects of 1,4-butanediol, GHB and (±)-baclofen before, during, and after chronic treatment with1,4-butanediol. Ordinate: Average response rate expressed as a percentage of control rates. Abscissa: Cumulative dose in mg/kg body weight.

Table 1
Mean ED_{50} in mg/kg (±95% CI) for rate-decreasing effects of 1,4-butanediol, GHB, and (±)-baclofen before, during, and after chronic treatment with BDL

Condition	1,4-Butanediol	GHB	(±)-Baclofen
Before	170.2 (151.1–191.8)	278.0 (253.1–305.4)	3.0 (2.7–3.4)
During	166.2 (154.0–179.3)	334.0 (307.7–362.5)[a]	5.1 (4.2–6.2)[a]
After (week 1)	176.1 (152.6–203.1)	321.8 (302.6–342.3)[a]	5.3 (4.1–6.7)[a]
After (week 2)	178.9 (157.7–203.0)	338.4 (298.5–383.7)[a]	3.3 (2.7–4.1)
After (week 3)	157.6 (131.7–188.4)	299.4 (266.0–337.0)	3.5 (3.0–4.1)

[a] Significant difference ($p<0.05$) from ED_{50} obtained before BDL treatment.

immediately preceding testing. Response rates during tests were calculated as a percentage of control rates for individuals then averaged among all subjects and plotted as a function of dose.

Doses required to decrease responding to 50% of control (ED_{50}) with 95% confidence intervals (95% CI) were estimated using linear regression; ED_{50} s were determined for individuals then averaged across all subjects. Mean log ED_{50} values were compared before, during, and after chronic treatment using a one-way ANOVA ($\alpha<0.05$), followed by Dunnett's test to compare experimental values with controls (before treatment).

3. Results

Responding was stable after 25.0 ± 2.7 (S.D.) sessions. 1,4-Butanediol, GHB, and (±)-baclofen decreased responding in a dose-related manner (closed symbols, Fig. 1). Twice daily treatment with 320 mg/kg 1,4-butanediol for 3 weeks followed by twice daily treatment with 560 mg/kg for 3 weeks did not alter sensitivity to 1,4-butanediol (open circles, upper panel, Fig. 1). However, this treatment decreased sensitivity to (±)-baclofen and GHB (open circles, middle and lower panels, Fig. 1); ED_{50} values determined before and during 1,4-butanediol treatment were significantly different for both drugs (Table 1). Sensitivity to the rate-decreasing effects of (±)-baclofen and GHB returned to control values 2 and 3 weeks, respectively, after discontinuation of treatment.

4. Discussion

GHB continues to be studied intensively due to its emergence as a drug of abuse in several different contexts (body building, date rape, club drug) as well as the recent approval of GHB for treating a severe form of narcolepsy. Recreationally and medicinally, GHB often is consumed daily over long periods and clinical reports suggest that tolerance and dependence can develop to GHB (Galloway et al., 1994); however, the generality of these effects as well as the mechanism(s) by which GHB produces tolerance or dependence are unknown. Reduced availability of GHB stimulated further interest in the abuse and dependence potential of metabolic precursors of GHB, including 1,4-butanediol and GBL, both of which share effects with GHB (Carai et al., 2002; Carter et al., 2003; Schneir et al., 2001; Zvosec et al., 2001). Because 1,4-butanediol has a significantly longer duration of action than GHB (Carter et al., 2003), it was studied for its ability to induce tolerance as well as cross-tolerance to the rate-decreasing effects of GHB and (±)-baclofen. After 6 weeks of treatment with 1,4-butanediol, sensitivity to (±)-baclofen and GHB was significantly decreased, as evidenced by rightward shifts of the dose–response curves. Sensitivity to (±)-baclofen and GHB returned to control values 2 and 3 weeks, respectively, after discontinuation of 1,4-butanediol treatment, demonstrating that changes in sensitivity were due to 1,4-butanediol treatment. Responding was not altered upon discontinuation of BDL treatment and there were no apparent changes in gross behavior of rats during several weeks after discontinuation.

While chronic treatment with BDL decreased sensitivity to GHB and (±)-baclofen, sensitivity to 1,4-butanediol was unchanged. 1,4-Butanediol does not bind to GHB or GABA receptors (Benavides et al., 1982; Snead and Liu, 1984; Roth and Giarman, 1968); however, 1,4-butanediol shares behavioral effects with GHB (Carter et al., 2003), presumably because of its conversion to GHB and subsequent actions on GABA and possibly GHB receptors. The slow onset and long duration of action of 1,4-butanediol (Carter et al., 2003) are consistent with its metabolic conversion to GHB. That sensitivity to 1,4-butanediol is not altered under conditions where cross-tolerance develops to GHB and (±)-baclofen could indicate that the rate-decreasing effects of 1,4-butanediol are not mediated by the same mechanism that mediates the rate decreasing effects of (±)-baclofen and GHB. Different configurations of $GABA_B$ receptor dimers vary in their affinity for different drugs (Bonanno and Raiteri, 1993) and some drugs indirectly modulate (Soudijn et al., 2002) $GABA_B$ receptor function; however, little is known about how receptor heterogeneity or modulation contribute to the behavioral actions of $GABA_B$ agonists or GHB (for review, see Ong and Kerr, 2000). Under the same conditions used in the current study, the $GABA_B$ receptor selective antagonist CGP 35348 attenuates the rate-decreasing effects of (±)-baclofen and not those of GHB (Carter, personal communication), further suggesting that these compounds might act at different $GABA_B$ receptors or at different sites on the $GABA_B$ heterodimer.

GHB has affinity for different receptors and is metabolized to GABA. While $GABA_B$ mechanisms appear to be particularly important for its behavioral actions, GHB receptors also might contribute to altered sensitivity during chronic treatment. For example, mice treated chronically with GBL were tolerant to GBL and cross-tolerant to (±)-

baclofen, whereas mice treated chronically with (±)-baclofen were tolerant to (±)-baclofen and not cross-tolerant to GBL (Gianutsos and Moore, 1978). It will be important to determine whether sensitivity to 1,4-butanediol decreases in animals rendered tolerant by chronic treatment with either GHB or (±)-baclofen.

Acknowledgements

Supported by USPHS DA14986 and RCA DA0211 (CPF). The authors thank Dr. L.R. McMahon and L.P. Carter for helpful suggestions as well as C. Cruz, H. Renteria, D. Mojica, and G. Phillips for excellent technical assistance.

References

Bania, T.C., Ashar, T., Press, G., Carey, P.M., 2003. Gamma-hydroxybutyric acid tolerance and withdrawal in a rat model. Acad. Emerg. Med. 10, 697–704.

Barker, S.A., Snead, O.C., Poldrugo, F., Liu, C.C., Fish, F.P., Settine, R.L., 1985. Identification and quantitation of 1,4 butanediol in mammalian tissues: an alternative biosynthetic pathway for γ-hydroxybutyric acid. Biochem. Pharmacol. 34, 1849–1852.

Benavides, J., Rumigny, J.F., Bourguignon, J.J., Cash, C., Wermuth, C.G., Mandel, P., Vincenden, G., Maitre, M., 1982. High affinity binding sites for γ-hydroxybutyric acid in rat brain. Life Sci. 30, 953–961.

Bonanno, G., Raiteri, M., 1993. Multiple $GABA_B$ receptors. Trends Pharmacol. Sci. 14, 259–261.

Carai, M.A., Colombo, G., Reali, R., Serra, S., Mocci, I., Castelli, P., Cignarella, G., Gessa, G.L., 2002. Central effects of 1,4-butanediol are mediated by $GABA_B$ receptors via its conversion into γ-hydroxybutyric acid. Eur. J. Pharmacol. 441, 157–163.

Carter, L.P., Flores, L.R., Wu, H., Chen, W., Unzeitig, A., Coop, A., France, C.P., 2003. The role of $GABA_B$ receptors in the discriminative stimulus effects of γ-hydroxybutyrate in rats: time course and antagonism studies. J. Pharmacol. Exp. Ther. 305, 668–674.

Chabal, C., Jacobson, L., Terman, G., 1992. Intrathecal fentanyl alleviates spasticity in the presence of tolerance to intrathecal baclofen. Anesthesiology 76, 312–314.

Dyer, J., Galbo, M., Andrews, K., 1997. 1,4 Butanediol "pine needle oil": overdose mimics toxic profile of GHB. J. Toxicol., Clin. Toxicol. 35, 554.

Galloway, G.P., Frederick, S.L., Staggers, F., 1994. Physical dependence on sodium oxybate. Lancet 343, 57.

Gianutsos, G., Moore, K.E., 1978. Tolerance to the effects of baclofen and γ-butyrolactone on locomotor activity and dopaminergic neurons in the mouse. J. Pharmacol. Exp. Ther. 207, 859–869.

Itzhak, Y., Ali, S.F., 2002. Repeated administration of gamma-hydroxybutyric acid (GHB) to mice: assessment of the sedative and rewarding effects of GHB. Ann. N.Y. Acad. Sci. 965, 451–460.

Nicholson, K.L., Balster, R.L., 2001. GHB: a new and novel drug of abuse. Drug Alchol Depend. 63, 1–22.

Nissbrandt, H., Engberg, G., 1996. The $GABA_B$-receptor antagonist, CGP 35348, antagonises γ-hydroxybutyrate- and baclofen-induced alterations in locomotor activity and forebrain dopamine levels in mice. J. Neural Transm. 103, 56–63.

Ong, J., Kerr, D.I., 2000. Recent advances in $GABA_B$ receptors: from pharmacology to molecular biology. Acta Pharmacol. Sin. 21, 111–123.

Roth, R.H., Giarman, N.J, 1968. Evidence that central nervous system depression by 1,4 butanediol is mediated through a metabolite, γ-hydroxybutyrate. Biochem. Pharmacol. 17, 735–739.

Schneir, A.B., Ly, B.T., Clark, R.F., 2001. A case of withdrawal from the GHB precursors γ-butyrolactone and 1,4-butanediol. J. Emerg. Med. 21, 31–33.

Snead III, O.C., Liu, C.C. 1984. γ-hydroxybutyric acid binding sites in rat and human brain synaptosomal membranes. Biochem. Pharmacol. 33, 2587–2590.

Snead, O.C., Furner, R., Liu, C.C., 1989. In vivo conversion of γ-aminobutyric acid and 1,4-butanediol to γ-hydroxybutyric acid in rat brain. Biochem. Pharmacol. 38, 4375–4380.

Soudijn, W., van Wijngaarden, I., Ijzerman, A.P., 2002. Allosteric modulation of G protein-coupled receptors. Curr. Opin. Drug Discov. Dev. 5, 749–755.

Van Sassenbroeck, D.K., De Paepe, P., Belpaire, F.M., Boon, P.A., Buylaert, W.A., 2003. Tolerance to the hypnotic and electroencephalographic effect of gamma-hydroxybutyrate in the rat: pharmacokinetic and pharmacodynamic aspects. J. Pharm. Pharmacol. 55, 609–615.

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