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Short communication

Drosophila metabolize 1,4-butanediol into γ-hydroxybutyric acid in vivo

Rosalba Satta, Nikola Dimitrijevic, Hari Manev*

Department of Psychiatry, The Psychiatric Institute, University of Illinois at Chicago, 1601 West Taylor Street, MC912, Chicago, IL 60612, USA

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Abstract

A solvent, 1,4-butanediol, is also abused as a recreational drug. In mammals, 1,4-butanediol is metabolized into γ-hydroxybutyric acid (GHB), which stimulates metabotropic γ-aminobutyric acid (GABA) $GABA_B$ and putative GHB receptors. Here we show that in vivo injection of 1,4-butanediol into adult *Drosophila* leads to GHB synthesis (GHB was detectable 5 min after 1,4-butanediol injection and increased dramatically 1–2 h later). This synthesis of GHB was accompanied by an impairment of locomotor activity that was mimicked by a direct injection of GHB into flies. We propose *Drosophila* as a model to study the molecular actions of 1,4-butanediol and GHB.

Keywords: Fruit fly; GHB (γ-hydroxybutyric acid); GABA (γ-aminoybutyric acid); Behavior

1. Introduction

Novel invertebrate models of drug abuse are being developed (Wolf and Heberlein, 2003), including the use of *Drosophila melanogaster* (fruit fly) in neuropharmacological research (Manev et al., 2003). *Drosophila* are remarkable in that they respond to drugs of abuse (e.g., cocaine and ethanol) with molecular and behavioral patterns similar to those in mammals. Compared to mammals, *Drosophila* are more amenable to gene manipulations; for example, mutations or gene silencing via RNA interference (Dzitoyeva et al., 2001), which can be combined with pharmacological tools (Dzitoyeva et al., 2003).

1,4-Butanediol is a naturally occurring alcohol used as an industrial solvent that is also abused as a recreational drug; prolonged human consumption of this substance leads to severe withdrawal symptoms (Mycyk et al., 2001). It appears that 1,4-butanediol produces central nervous system effects after it is metabolized into γ-hydroxybutyric acid (GHB), which acts as an agonist at metabotropic receptors for the neurotransmitter γ-aminobutyric acid (GABA), $GABA_B$ receptors (Carai et al., 2002), and also at putative GHB receptors (Castelli et al., 2002; Wu et al., 2003). Since *Drosophila* expresses $GABA_B$ receptors (Mezler et al., 2001; Dzitoyeva et al., 2003), fruit flies could be used to further characterize the interaction of 1,4-butanediol with this receptor system. As a first step in this research, we investigated whether in vivo administration of 1,4-butanediol leads to its conversion into GHB in flies.

2. Materials and methods

2.1. Drosophila and injections

Flies (male Canton S, 4–5 days old) were cultured at 25 °C, 50–60% humidity, 12 h/12 h light/dark cycle, on yeast, dark corn syrup, and agar food. For injections, flies were anesthetized by CO_2 (maximally for 5 min). Using custom-beveled glass pipettes coupled to a cell injector (Narashige) and a micromanipulator, under a stereo microscope, we injected a volume of 0.2 μl/fly by a pulse pressure of 300 kPa (Dzitoyeva et al., 2003). The drugs, that is, 3% 1,4-butanediol (Aldrich) and GHB (Sigma), were diluted/dissolved in Ringer solution (NaCl, KCl, $CaCl_2$; 7.5, 0.35, 0.21 g/l, respectively). Control flies were injected with Ringer solution only.

2.2. Gas chromatography/mass spectrometry (GC/MS) assay of GHB

Groups of five flies were homogenized in 200 μl of 100 mM phosphate buffer pH 6; d6-GHB (2,2,3,3,4,4-d6; Cam-

* Corresponding author. Tel.: +1-312-413-4558; fax: +1-312-413-4569.
E-mail address: HManev@psych.uic.edu (H. Manev).

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Table 1
Injection of 1,4-butanediol into adult male *Drosophila* leads to synthesis of γ-hydroxybutyric acid (GHB)

Time after butanediol injection (min)	GHB (ng/fly)
Control (Ringer-injected)	0
5	11
60	348
120	613

Similar results were obtained in two independent experiments.

bridge Isotope Laboratories) was added (2.5 ng/μl) to each sample as the internal standard. Each sample was applied onto 1 ml of a AG1X8 formate ion exchange column preconditioned with successive additions of 10 ml 20% ethanol in 0.5 M formic acid and 10 ml distilled water. GHB was eluted with the successive addition of 4 ml 20% ethanol in 0.5 M formic acid, dried under vacuum, and the residue was dissolved in 200 μl dimethylformamide. After two extractions with 1 ml of hexane, the dimethylformamide layer was dried by evaporation under nitrogen. The samples were derivatized with 100 μl ethyl acetate and 100 μl BSTFA [*N*,*O*,-bis(trimethylsilyl)trifluoroacetamide] with 1% trimethylchlorosilane for 10 min at 60 °C, and analyzed on a GC/MS system (HP 5890 gas chromatograph coupled to a HP 5971 mass selective detector) equipped with a HP-5 column (30 m × 0.25 mm × 0.25 μm). Two-microliter samples were injected manually. The temperature of the injection port was 250 °C. The oven was initially held at 70 °C for 4 min; thereafter, it was programmed to increase at a rate of 8 °C/min to 100 °C and at a rate of 25 °C/min to 175 °C. The instrument was operated in the selected ion monitoring mode (SIM); the ions 233 and 239 were used for quantitation of GHB and d6-GHB, respectively. The retention time for GHB and d6-GHB was approximately 9.1 min. The calibration curve ranged from 0.5 to 5 ng/μl GHB; the detection limit for the standard prepared in water was 0.25 ng/μl.

2.3. Behavioral assay

A *Drosophila* Activity Monitoring System (Trikinetics, Waltham, MA) coupled to a computer was used to monitor the locomotor activity of individual flies as described elsewhere (Dzitoyeva et al., 2003). Briefly, after the injection, flies remain immobile (due to CO_2 anesthesia) for a period of time. To quantify not only the total locomotor activity but also the duration of immobility, which can be affected by drugs, the system was slightly modified; that is, the space in each individual recording tube was restricted to a length of 8 mm in the center of the photo beam. Flies (six to eight per group) were placed in the recording tubes within 2 min of injection and the sampling time was set at 1-min intervals. No food was present during the recording period. After flies recovered from the injection, they gradually resumed locomotor activity. In this study, we observed an average daytime activity greater than four counts per minute, and we set the time of recovery from anesthesia as the first 1-min interval in which a fly produces four movements. Thereafter, the total locomotor activity was measured over the next >2 h period. The time to the first interval with more than four movements was analyzed with respect to the drug treatment variable; the level of locomotor activity also was analyzed.

2.4. Statistics

Analysis of variance (ANOVA) was followed by the Student's *t*-test. $P < 0.05$ was taken as significant.

3. Results

The GC/MC method we used in this study did not detect GHB in samples prepared from control flies. How-

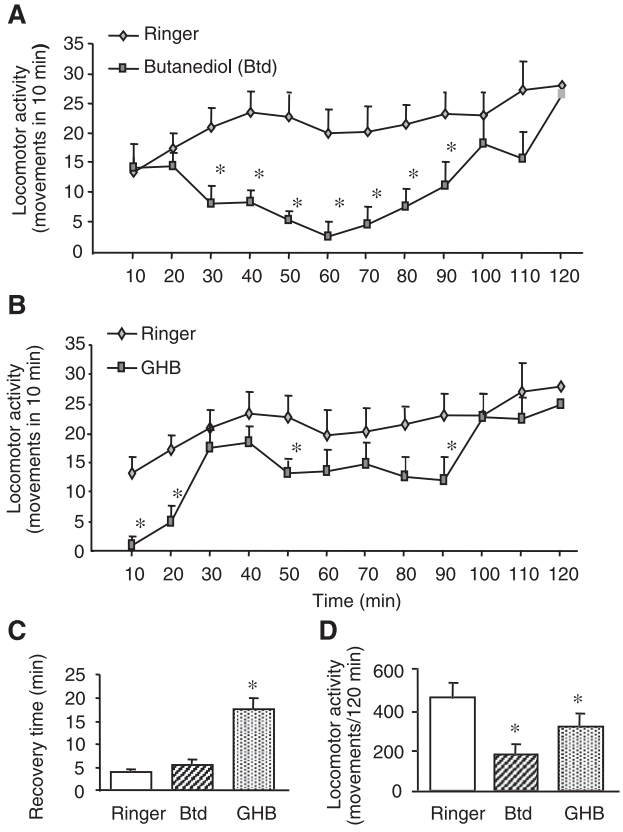

Fig. 1. Behavioral effects of 1,4-butanediol and GHB injection into adult flies. Shown is the locomotor activity of flies during a 2-h recording period: (A and B) Each time point represents the average activity ± S.E. measured during a 10-min period. Drug-treated groups were compared to vehicle-treated control (*$P < 0.05$; for clarity, the results are presented in two separate panels). (A) Note the initial period when both control and 1,4-butanediol (Btd; 3%)-injected flies show similar activity, followed by the delayed decreased activity of Btd group; (B) note the immediate locomotor impairment in GHB (600 ng/fly)-injected flies followed by a continued lower level of activity. (C) Quantitative analysis of recovery after injection. (D) Quantitative analysis of the extent of total locomotor activity after recovery. Results are the mean ± S.E. (*$P < 0.01$ vs. control).

ever, 5 min after 1,4-butanediol injection, GHB was already detectable, and the concentration of GHB increased significantly in samples obtained 1 and 2 h post-injection (Table 1).

Behaviorally, injection of 1,4-butanediol into the flies did not produce any significant effect on the time of their recovery from anesthesia, and the extent of locomotor activity in the immediate post-injection period did not differ between the vehicle- and the 1,4-butanediol-injected flies (Fig. 1). However, after this initial period, the 1,4-butanediol-injected flies showed progressive impairment in locomotor activity but tended to recover normal activity toward the end of the recording period.

We also analyzed the behavioral effects of a direct injection of GHB into adult flies. Injection of 10 ng/fly of GHB, the amount of GHB we found in flies 5 min after 1,4-butanediol administration, did not produce any significant behavioral alterations (data not shown). On the other hand, injection of 600 ng/fly of GHB, the amount we found in 1,4-butanediol-injected flies after the initial period, produced a significant behavioral impairment. Thus, in contrast to 1,4-butanediol, GHB produced an acute effect evidenced as a significant prolongation of recovery time from anesthesia. Thereafter, similar to 1,4-butanediol, GHB injection also produced a longer-lasting decrease in the extent of locomotor activity (Fig. 1).

4. Discussion

Our results demonstrate for the first time that *Drosophila* metabolize 1,4-butanediol into GHB in vivo. In mammals, the conversion of 1,4-butanediol into GHB may involve alcohol dehydrogenase, aldehyde dehydrogenase, and possibly some other enzymes (Carai et al., 2002; Snead et al., 1989; Quang et al., 2002), whereas endogenous GHB is primarily synthesized from GABA (Maitre et al., 2000). These pathways may be operative in *Drosophila*. Although we did not detect endogenous GHB in control flies (five flies in 200 μl; level of detection 0.25 ng GHB/μl), further studies are needed to investigate a possible putative endogenous GHB synthesis in *Drosophila*. Nevertheless, we demonstrated that fruit flies, similar to mice (Carai et al., 2002), metabolize 1,4-butanediol into GHB.

Our behavioral observations suggest that the behavior-impairing effects of 1,4-butanediol are due to its metabolism into GHB. Hence, both 1,4-butanediol and GHB trigger a significant impairment of *Drosophila* locomotor activity; however, whereas this effect occurs immediately upon GHB administration, it is delayed upon 1,4-butanediol administration. This observation is consistent with the gradual synthesis and accumulation of GHB found following injection of 1-4-butanediol.

A rapid injection with GHB resulted in immediate impairment of locomotor activity, a brief recovery, and prolonged diminished locomotor activity. The observed recovery period might be due to the rapid metabolism of GHB or the desensitization of target receptors. For example, repeated administration of GHB to mice causes tolerance to its sedative effects (Itzhak and Ali, 2002). Moreover, the motor-impairing effects of 1-4-butanediol tended to remit 2 h after injection, that is, when GHB levels are still high.

In this work, we demonstrated that 1,4-butanediol is metabolized into GHB in *Drosophila* in vivo and that both compounds produce significant and measurable behavioral effects. Recently, we demonstrated that adult fruit flies express functional $GABA_B$ receptors (Dzitoyeva et al., 2003), which along with putative GHB receptors (Castelli et al., 2002; Wu et al., 2003), might participate in the pharmacological actions of 1,4-butanediol and GHB (Carai et al., 2002). Thus, we propose that *Drosophila* could be used as a model organism to study the molecular and behavioral actions of 1,4-butanediol and GHB.

Acknowledgements

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