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SCIENCE DIRECT

Brain Research 1033 (2005) 109–112

BRAIN RESEARCH

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Research report

Different sensitivity to the motor incoordinating effects of γ-hydroxybutyric acid (GHB) and baclofen in GHB-sensitive and GHB-resistant rats

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Accepted 16 November 2004
Available online 6 January 2005

Abstract

The present study investigated whether the differential sensitivity of selectively bred γ-hydroxybutyric acid (GHB)-sensitive (GHB-S) and GHB-resistant (GHB-R) rats to GHB- and baclofen-induced sedation/hypnosis generalized to the motor incoordinating effect of the two drugs. To this aim, GHB-S and GHB-R rats were tested on a Rota-Rod after the acute administration of GHB (100–500 mg/kg, i.p.) and baclofen (1.25–5 mg/kg, i.p.). Significant line differences were observed in the dose–response curves for both GHB and baclofen, with GHB-S rats displaying a greater sensitivity to the motor incoordinating effects of both drugs than GHB-R rats. No line difference was observed in diazepam (1.25–5 mg/kg, i.p.), pentobarbital (5–15 mg/kg, i.p.), and ethanol (1–1.5 g/kg, i.p.) dose–response curves. These results suggest that the differential sensitivity of GHB-S and GHB-R rats to GHB and baclofen extends to the effects produced by doses of the two drugs which are 5–10 times lower than those for which rats have been selectively bred. These results are discussed in terms of the $GABA_B$ receptor being the likely neural substrate on which the differential sensitivity of GHB-S and GHB-R rats resides.

Theme: Disorders of the nervous system
Topic: Genetic models

Keywords: γ-Hydroxybutyric acid (GHB); Baclofen; GHB-sensitive (GHB-S) and GHB-resistant (GHB-R) rats; $GABA_B$ receptor; Motor incoordination; Rota-Rod

1. Introduction

This laboratory has recently set up to the bidirectional selective breeding of two lines of rats, named γ-hydroxybutyric acid (GHB)-sensitive (GHB-S) and GHB-resistant (GHB-R), which display opposite sensitivity to the sedative/hypnotic effect produced by the acute, intraperitoneal administration of 1 g/kg GHB [4,6]. The selective breeding of GHB-S and GHB-R rats started from the identification, among a foundation stock of Wistar rats, of those individuals with opposite sensitivity to GHB-induced sedation/hypnosis. Selection criteria were set as the ratio r = sleep time/onset being ≥8 and ≤2 for GHB-S and GHB-R rats, respectively (where "onset" and "sleep time" were defined as onset and duration of loss of righting reflex, respectively, after GHB injection). By the 10th generation, the selective breeding of GHB-S and GHB-R rats was completed, as indicated by all rats of both lines fulfilling the selection criterion [7].

Initial work found that the differential sensitivity to GHB of GHB-S and GHB-R rats generalized also to the sedative/hypnotic effect of the prototype $GABA_B$ receptor agonist, baclofen (20 mg/kg, i.p.) [4], in agreement with the hypothesis that the $GABA_B$ receptor is a major site of action of GHB in the CNS (see Ref. [1]).

The relatively few studies conducted to date with GHB-S and GHB-R rats have been focused exclusively on their differential sensitivity to GHB- and baclofen-induced

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doi:10.1016/j.brainres.2004.11.030

sedation/hypnosis. However, it is of interest to understand whether this differential sensitivity of GHB-S and GHB-R rats extends to other effects of GHB and baclofen. These lines of information would contribute to further characterize these two rat lines as well as to their validation as a possibly relevant experimental model for studies in the GHB and $GABA_B$ field. Accordingly, the present study evaluated whether GHB-S and GHB-R rats differed in terms of an effect (namely, motor incoordination) produced by doses of GHB and baclofen which were 5–10 lower than those for which GHB-S and GHB-R rats have been selectively bred and investigated to date.

2. 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" and approved by the Ethical Committee of the University of Cagliari.

2.1. Animals

Female GHB-S and GHB-R rats, from the 12th generation and weighing 240–270 g, were used. Rats were housed 4 per cage, in standard plastic cages and 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.

2.2. Procedure

The present investigation employed a Rota-Rod procedure similar to that previously described by Hoffman and Tabakoff [5]. Rats practiced on the apparatus for 7 daily training sessions prior to the test. The test session consisted of 2 trials on the Rota-Rod [accelerating Rota-Rod Treadmills for rats (Ugo Basile, Comerio, VA, Italy)]: on the first trial (pre-drug performance), rats 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 rat managed to remain on the revolving drum was recorded. Time recording was initiated at the beginning of the acceleration phase. Only the rats which completed the first trial (600 s) underwent the second trial.

One hour after the first trial, independent groups of rats (n = 8–11) were treated with GHB (0, 100, 187, 250, 375, and 500 mg/kg), baclofen (0, 1.25, 1.87, 2.5, 3.75, and 5 mg/kg), diazepam (0, 1.25, 2.5, and 5 mg/kg), pentobarbital (0, 5, 10, and 15 mg/kg) and ethanol (0, 1, 1.25, and 1.5 g/kg). GHB, baclofen, and pentobarbital were administered 30 min before the second trial; diazepam and ethanol were administered 15 and 20 min, respectively, before the second trial. In the second trial (post-drug performance), rats were required to perform the motor task on the drum for 11 min. The drum rotated at 2 rpm for 1 min, 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 rat on the drum from the beginning of the acceleration phase was recorded.

2.3. Drugs

GHB (sodium salt; Laboratorio Farmaceutico C.T., Sanremo, IM, Italy), was dissolved in distilled water [injection volume: 29.4 ml/kg (this large injection volume was chosen to minimize tissue irritation at the injection site)]. Baclofen (Tocris Cookson Ltd., Avonmouth, U.K.) was dissolved in saline (injection volume: 2 ml/kg). Diazepam (Valium®, Roche S.p.A., Milan, Italy) was dissolved in propanediol (injection volume: 2 ml/kg). Pentobarbital (Sigma Chemical Co, St. Louis, MO, U.S.A.) was dissolved in saline (injection volume: 5 ml/kg). Ethanol (commercial source) was dissolved in saline (20% w/v; injection volume: 5–7.5 ml/kg). All drugs were injected intraperitoneally.

2.4. Data analysis

The difference between the first and second trial times, expressed as percentage of the first trial time, was calculated for each rat and indicated its degree of motor impairment. Each rat served as its own control. Data were analyzed by 2-way (rat line; drug treatment) ANOVAs, followed by the Newman–Keuls test for post hoc comparisons.

3. Results

With the sole exception of 100 mg/kg, all doses of GHB produced some degree of impairment in GHB-S rats; at doses equal to or greater than 250 mg/kg, GHB-induced impairment in GHB-S rats was virtually complete (Fig. 1). In contrast, only the two highest GHB doses affected motor coordination in GHB-R rats (Fig. 1) [$F_{line}(1,84) = 67.39$, $P < 0.0001$; $F_{treatment}(5,84) = 92.30$, $P < 0.0001$; $F_{interaction}(5,84) = 12.65$, $P < 0.0001$]. Post hoc analysis revealed statistical differences between GHB-S and GHB-R rats at the doses of 187, 250, and 375 mg/kg GHB (Fig. 1).

In GHB-S rats, doses of baclofen equal to or greater than 1.87 mg/kg impaired the rat performance on the Rota-Rod (Fig. 2). In contrast, only 3.75 and 5 mg/kg baclofen produced appreciable degrees of impairment in GHB-R rats (Fig. 2) [$F_{line}(1,84) = 21.57$, $P < 0.0001$; $F_{treatment}(5,84) = 83.91$, $P < 0.0001$; $F_{interaction}(5,84) = 7.14$, $P < 0.0001$]. Post