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Protocol

GHB-C rats: The control line of GHB-sensitive (GHB-S) and GHB-resistant (GHB-R) rats

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Abstract

γ-Hydroxybutyric acid (GHB)-sensitive (GHB-S) and GHB-resistant (GHB-R) rats have been selectively bred for their opposite sensitivity to the sedative/hypnotic effect of GHB. This opposite sensitivity has been found to generalize to the $GABA_B$ receptor agonist, baclofen. A control line [named GHB-control (GHB-C)] has been derived from the foundation stock of GHB-S and GHB-R rats. GHB-C rats have been bred without any evaluation of their sensitivity to GHB. The experiments described here were designed to evaluate the sensitivity of GHB-C rats, from the 13th generation, to the sedative/hypnotic effect of GHB (1 g/kg, i.p.) and baclofen (20 mg/kg, i.p.). All measures (onset, sleep time and r = sleep time/onset) of sensitivity to GHB- and baclofen-induced sedation/hypnosis in GHB-C rats were significantly different from and intermediate to those recorded in GHB-S and GHB-R rats. Furthermore, these values were similar to those recorded in the foundation stock. These results suggest that GHB-C rats may constitute a valid control line for GHB-S and GHB-R rats, representing the "general population" from which GHB-S and GHB-R rats were derived. Furthermore, the relative equidistance of sensitivity to GHB- and baclofen-induced sedation/hypnosis of GHB-C rats from those of GHB-S and GHB-R rats suggests that genetic factors contributes to the development of both sensitivity in GHB-S rats and resistance in GHB-R rats.

Theme: Disorders of the nervous system
Topic: Genetic models

Keywords: γ-Hydroxybutyric acid (GHB); Baclofen; $GABA_B$ receptor; Sedative/hypnotic effect; Sensitivity; Selective outbreeding and control line; GHB-sensitive (GHB-S), GHB-control (GHB-C) and GHB-resistant (GHB-R) rats

1. Type of research

This laboratory has recently undertaken the bidirectional selective breeding of two rat lines, namely γ-hydroxybutyric acid (GHB)-sensitive (GHB-S) and GHB-resistant (GHB-R) rats, which display opposite sensitivity to the sedative/hypnotic effect of GHB [3]. The selective breeding of GHB-S and GHB-R rats started from a foundation stock of Wistar rats, from which those individuals with high and low sensitivity to sedation/hypnosis induced by 1 g/kg GHB were selected (selection criteria were set as the ratio r = sleep time/onset ≥ 8 and ≤ 2 for GHB-S and GHB-R rats, respectively, with "onset" and "sleep time" defined as onset and duration of loss of righting reflex after GHB injection) [3]. Remarkably, by the 10th generation, the selective breeding procedure of GHB-S and GHB-R rats was virtually completed, as all rats of both lines fulfilled the selection criteria [4].

The breeding project of GHB-S and GHB-R rats also includes a control line [GHB-control (GHB-C)]. These rats derive from the same foundation stock of Wistar rats from which GHB-S and GHB-R rats were generated; specifically, 5 male and 5 female rats of the foundation stock were randomly picked and assigned to the GHB-C line, without

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any evaluation of their sensitivity to GHB [3]. Over the 13 generations produced to date, the GHB-C rat colony has been kept at the same population size of the GHB-S and GHB-R rats (i.e., approximately 20 families/line). The GHB-C rat line is thought to account for the possible occurrence of spurious line differences between GHB-S and GHB-R rats which may be unrelated to the selected trait but secondary to random genetic drifts occurring on continuation of breeding.

Once the selective breeding of GHB-S and GHB-R rats had come to completion [4], it was of interest to ascertain the actual distance of GHB-S and GHB-R rats from the control line, which theoretically represents the "general population". To this aim, the present study compared the sensitivity of GHB-S, GHB-C, and GHB-R rats to the sedative/hypnotic effect of 1 g/kg GHB.

In close agreement with an increasing amount of experimental data indicating that the $GABA_B$ receptor is the likely site of action of many effects produced by moderate to high doses of GHB (see [1]), the differential sensitivity to GHB of GHB-S and GHB-R rats has been found to generalize to the sedative/hypnotic effect of the prototypic $GABA_B$ receptor agonist, baclofen [2]. Accordingly, the present study also evaluated the sensitivity of GHB-S, GHB-C, and GHB-R rats to baclofen-induced sedation/hypnosis.

2. Time required

Maintenance of the GHB-C rat line required a minimal amount of time. After random selection of the 5 pairs of foundation stock to be used to start the line, the only workload regarded–at each of the 13 generations produced to date–mating and weaning of the offspring.

The time required for evaluation of the rat sensitivity to the sedative/hypnotic effect of GHB or baclofen is described in detail in a previous paper [3].

3. Materials

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".

3.1. Animals

Male and female GHB-S, GHB-C, and GHB-R rats from the F13 of each line were used. At the time of the experiments with GHB and baclofen, rats were approximately 10 weeks old. Rats were housed 4–5 per cage in standard plastic cages with wood chip bedding under an artificial light–dark cycle of 12/12 h (lights on at 7:00 a.m.) at a constant temperature of 22 ± 2 °C and relative humidity of approximately 60%. Water and standard rat chow (Mucedola, Settimo Milanese, MI, Italy) were always available in the homecage.

3.2. Drugs

GHB (sodium salt; donated by Laboratorio Farmaceutico C.T., Sanremo, IM, Italy) was dissolved in distilled water (3.4%, w/v) and injected intraperitoneally (i.p.) at the single dose of 1 g/kg. A large injection volume (29.4 ml/kg) was chosen to minimize tissue irritation at the injection site [3]. Baclofen [(±)-baclofen; donated by Dr. Wolfgang Froestl, Novartis, Basel, Switzerland] was dissolved in 6 ml/kg saline and injected i.p. at the single dose of 20 mg/kg.

4. Detailed procedure

Evaluation of the rat sensitivity to the sedative/hypnotic effect of GHB and baclofen was performed following the standard procedure employed in the selective breeding of GHB-S and GHB-R rats [3]. Briefly, on the test day, immediately after GHB or baclofen injection, each rat was placed singly in a cage. The loss of righting reflex was evaluated by gently placing each rat on its back or flank 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 rat was unable to right itself was defined as onset of the loss of righting reflex. Each rat was then left undisturbed on its back or flank until it spontaneously regained its righting reflex (determined as having at least 3 paws under its body). Complete recovery of the righting reflex was defined as the rat being able to turn itself upright twice more within 60 s from the time it spontaneously regained the righting reflex. If this criterion was not fulfilled, the rat was left undisturbed until it spontaneously regained its righting reflex. The time between loss and recovery of righting reflex was monitored in each rat as its sleep time. Assessment of onset and sleep time was performed by operators trained for the same evaluation criteria.

Statistical evaluation of data on onset, sleep time, and *r*-ratio were performed by 1-way ANOVAs followed by the Newman–Keuls test for post hoc comparisons.

5. Results

5.1. "*GHB*" *experiment*

All measures of sensitivity to GHB-induced sedation/hypnosis in GHB-C rats were significantly different from those recorded in GHB-S and GHB-R rats (Fig. 1). Values of onset, sleep time, and *r*-ratio in GHB-C rats were intermediate to those of GHB-S and GHB-R rats (Fig. 1).

Specifically, in male GHB-C rats (a) onset was ~3-fold longer than that of GHB-S and approximately half of that of GHB-R rats [$F(2,95) = 95.25$, $P < 0.0001$], (b) sleep time was 40% shorter than that of GHB-S rats and ~4-fold longer than that of GHB-R rats [$F(2,117) = 250.59$, $P < 0.0001$], and (c) *r*-ratio was 4-fold lower and 7-fold higher than that