Pharmacology Biochemistry and Behavior, Vol. 64, No. 2, pp. 363–365, 1999

0091-3057/99/$–see front matter

PII S0091-3057(99)00046-5

Contribution of $GABA_A$ and $GABA_B$ Receptors to the Discriminative Stimulus Produced by Gamma-Hydroxybutyric Acid

CARLA LOBINA,* ROBERTA AGABIO,* ROBERTA REALI,*,[1] GIAN LUIGI GESSA*† AND GIANCARLO COLOMBO†

Bernard B. Brodie Department of Neuroscience and †C.N.R. Center for Neuropharmacology, University of Cagliari, I-09124 Cagliari, Italy.

LOBINA, C., R. AGABIO, R. REALI, G. L. GESSA AND G. COLOMBO. *Contribution of $GABA_A$ and $GABA_B$ receptors to the discriminative stimulus produced by gamma-hydroxybutyric acid.* PHARMACOL BIOCHEM BEHAV **64**(2) 363–365, 1999.—The present study examined the involvement of $GABA_A$ and $GABA_B$ receptors in the discriminative stimulus effects of gamma-hydroxybutyric acid (GHB). Rats were trained to discriminate either 300 or 700 mg/kg GHB IG from water using a T-maze, food-reinforced drug-discrimination procedure. The direct $GABA_B$ agonist, baclofen, substituted completely for both training doses of GHB; its potency to substitute for GHB increased moderately as the training dose of GHB was increased. The positive $GABA_A$ modulator, diazepam, substituted partially for 300 mg/kg GHB, but failed to elicit GHB-appropriate responding in rats trained with the higher GHB dose. Finally, the $GABA_B$ antagonist, CGP 35348, completely blocked the discriminative stimulus effects of the high training dose of GHB, but only partially antagonized the effects of the low training dose. These results suggest that (a) GHB produces a compound stimulus, and (b) both $GABA_B$- and $GABA_A$-mediated cues are prominent components of this compound stimulus; the contribution of each component, however, appears to vary as the training dose of GHB is increased. © 1999 Elsevier Science Inc

Gamma-hydroxybutyric acid (GHB) Baclofen CGP 35348 Diazepam $GABA_B$ and $GABA_A$ receptors Compound stimulus Discriminative stimulus effects Drug discrimination T-maze Rat

THE exogenous administration of gamma-hydroxybutyric acid (GHB), a putative neurotransmitter in the mammalian brain, produces various neuropharmacological effects, such as changes in dopamine synthesis and release, anxiolysis, sedation, anesthesia, and EEG recordings, that resemble those of nonconvulsive epilepsy (4). Furthermore, GHB has been shown to reduce alcohol intake and attenuate the alcohol withdrawal syndrome in laboratory animals and alcoholics (3).

The receptor systems mediating the pharmacological effects of GHB, however, have not yet been fully identified. Different lines of evidence suggest that several physiological responses and pharmacological effects of GHB are mediated not only by interactions of GHB with its own receptors, but involve also the GABA ergic system (as a likely consequence of the conversion of GHB to GABA) (4).

The present study used a drug-discrimination procedure to further investigate the possible involvement of $GABA_A$ and $GABA_B$ receptors in the pharmacological profile of GHB. Diazepam (a positive modulator at the $GABA_A$/benzodiazepine receptor complex) and baclofen (a direct agonist at the $GABA_B$ receptor) were tested for their ability to produce GHB-like discriminative stimulus effects; the ability of the $GABA_B$ receptor antagonist, CGP 35348, to attenuate the GHB cue was tested also. Two different training doses of GHB (300 and 700 mg/kg) were used, because of the reported dose-dependent, biphasic behavioral effects of GHB, with low doses producing motor stimulation and high doses causing sedation and anesthesia (4).

METHOD

Animals

Fourteen male Long–Evans rats (Harlan Nossan, Correzzana, Ml, Italy), were housed singly under standard condi-

Requests for reprints should be addressed to Giancarlo Colombo, C.N.R. Center for Neuropharmacology, c/o Bernard B. Brodie Department of Neuroscience, University of Cagliari, Via Porcell 4, I-09124 Cagliari, Italy.
[1]Present affiliation: Neuroscienze S.c.ar.l., Cagliari, Italy.

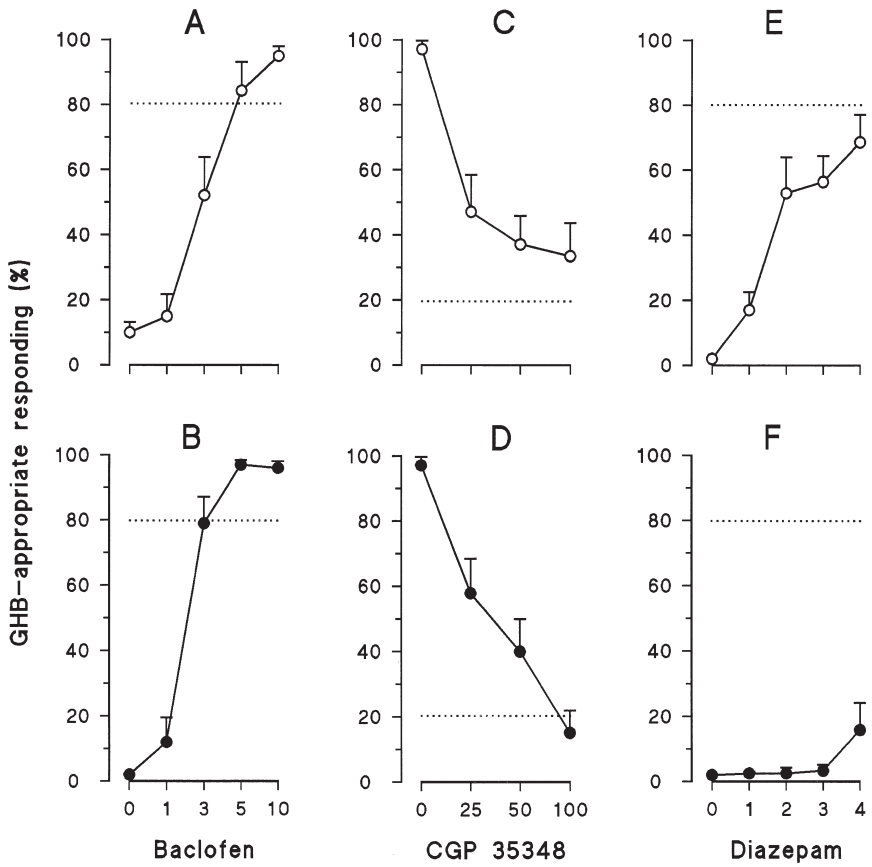

FIG 1. Average percent of entries into the GHB-appropriate arm of a T-maze following the administration of baclofen (A and B), the combination of CGP 35348 and the training doses of GHB (C and D), and diazepam (E and F) in rats trained to discriminate either 300 (○) or 700 (●) mg/kg GHB from water. The dashed lines indicate the limit of GHB-appropriate responding for complete substitution (80%) or blockade (20%) of the discriminative stimulus effects of GHB. Drug doses are expressed in mg/kg. Each point is the mean ± SEM of two determinations in five to seven rats.

tions, and maintained at 80% of their free-feeding body weight throughout the experiment. Standard rat chow was provided 4 h after each daily session (described below). Water was available ad lib in the home cage.

Apparatus and Procedure

A detailed description of the T-maze, drug-discrimination procedure has been reported elsewhere (1). Briefly, the T-shaped maze was made of black Plexiglas, and consisted of a central alley and two side runways; a recessed, plastic food cup was positioned on the floor at the far end (goal area) of each arm. Shelled sunflower seeds were used as reinforcement.

Each daily session consisted of 10 consecutive trials. Rats were trained to go to one of the two arms (GHB-appropriate arm) after administration of either 300 (n = 7) or 700 (n = 7) mg/kg GHB, and to go to the opposite arm (water-appropriate arm) after treatment with 10 ml/kg tap water. Water and GHB were administered by gavage 30 min before the beginning of the session. Rats were reinforced with the sunflower seeds only in the goal area of the arm appropriate to the treatment condition. Performance during a training session was considered correct when the correct arm was selected during the first trial and during at least eight of the nine subsequent trials. Five consecutive correct training sessions defined the criterion for learning the discrimination.

Once the discrimination criterion was attained, test sessions were interspersed among training sessions (testing phase). During test sessions, selection of either arm was reinforced. Baclofen (0, 1, 3, 5, and 10 mg/kg) and diazepam (0, 1, 2, 3, and 4 mg/kg) were injected IP 30 and 20 min before the test, respectively. CGP 35348 (0, 25, 50, and 100 mg/kg) was injected IP 15 min before the administration of the GHB training dose. Each drug dose was tested twice, counterbalancing for the training condition of the previous session.

Complete substitution was defined as an average of 80% or more of total session entries in the "GHB-appropriate arm." Complete blockade was defined as an average of 20% or less of total session entries in the "GHB-appropriate arm." Total session time was defined as the time taken to perform all trials of a single session, and was considered as an index of possible drug-induced motor impairments.

Drugs

GHB (sodium salt, Laboratorio Farmaceutico C.T., Sanremo, Italy) was dissolved in tap water and administered in a 10-ml/kg volume; (±)-baclofen (Research Biochemical International, Natick, MA) and CGP 35348 (donated by Ciba-Geigy Ltd, Basel, Switzerland) were dissolved in saline and injected in a 1-ml/kg volume; diazepam (Valium®, Roche SpA, Milan, Italy) was injected in 0.2–0.8-ml/kg volumes.

RESULTS

Administration of different doses of GHB resulted in a dose-dependent increase in the selection of the GHB-appropriate arm in both training groups (data not shown). Complete substitution occurred at doses of GHB equal to and greater than the training dose in the 300-mg/kg GHB group and at doses of GHB equal to and greater than 500 mg/kg in the 700-mg/kg GHB group.

Administration of different doses of baclofen resulted in a dose-dependent increase in the selection of the GHB-appropriate arm in both training groups, with doses of 5 and 10 mg/kg inducing complete substitution (Fig. 1A and B). The higher GHB training dose resulted in a small shift to the left of the baclofen substitution curve (ED_{50} = 2.9 and 2.0 mg/kg in the 300- and 700-mg/kg GHB training groups, respectively). A significant [$p < 0.05$ with respect to the preceding training session (Wilcoxon test)] increase in total session time was observed only in the 700-mg/kg GHB group after the administration of 10 mg/kg baclofen.

Combination of the $GABA_B$ antagonist, CGP 35348, with the training doses of GHB resulted in a dose-dependent reduction in the selection of the GHB-appropriate arm in both rat groups (Fig. 1C and D). At 100 mg/kg, CGP 35348 completely blocked the effects of the high training dose, but only partially those of the low training dose. No dose of CGP 35348 significantly affected the total session time in either group.

In the 300-mg/kg GHB group, administration of diazepam resulted in partial substitution for the GHB cue (68.6% selection of the GHB-appropriate arm after 4 mg/kg diazepam); in contrast, diazepam produced at most 20% selection of the GHB-appropriate arm in the 700-mg/kg GHB group (Fig. 1E and F). Motor performance was significantly ($p < 0.05$ with respect to the preceding training session) affected by doses of diazepam equal to or higher than 2 mg/kg, in both groups.

DISCUSSION

The results of the present study indicate that the $GABA_B$ receptor agonist, baclofen, produced discriminative stimulus effects that were similar to those of both a high (700 mg/kg) and a low (300 mg/kg) dose of GHB; its potency to produce these effects appeared to be somewhat higher in the high than in the low training dose group. The present report also demonstrates that the positive $GABA_A$ modulator, diazepam, partially substituted for the low GHB training dose, but failed to elicit GHB-appropriate responding in the high GHB training dose group. Together, these results suggest that (a) GHB produces a compound stimulus involving both $GABA_A$- and $GABA_B$-mediated cues; (b) in addition to varying quantitatively (i.e., with respect to intensity), the discriminative stimulus effects of GHB may also vary qualitatively (i.e., involving different proportions of the component cues) as the training dose of GHB is increased; (c) the $GABA_B$ component appears to be more salient at 700 than at 300 mg/kg GHB; (d) positive modulation of the $GABA_A$ receptor is also a relevant part of the interoceptive stimuli produced by 300 mg/kg GHB. The present results are consistent with data from drug mixture studies indicating that, as the relative amount of one of the components is increased, its contribution to the discriminative stimulus effects of the mixture increases, while that of the other component decreases (2).

The $GABA_B$ receptor antagonist, CGP 35348, completely blocked the discriminative stimulus effects of 700 mg/kg GHB, but blocked only partially the effects of 300 mg/kg GHB. It has been shown that complete blockade of the discriminative stimulus effects of a drug mixture occurs only when the stimuli produced by each component are blocked; indeed, blockade of only one component at most only partially attenuates the discriminative stimulus effects of the mixture, and such attenuation is proportional to the prominence of the component (5). Thus, the complete blockade of the discriminative stimulus effects of 700 mg/kg GHB is consistent with the hypothesized prominence of the $GABA_B$ component of the GHB cue at this dose, which may overshadow the perception of the other components. That is, the behavior of rats trained to discriminate 700 mg/kg GHB from water appeared to be guided predominantly by $GABA_B$-mediated cues; once these cues were blocked, overshadowed components could become apparent, but because the rats were unable to recognize the cues to which they were trained, they selected the water-associated arm of the maze. At the lower GHB training dose, CGP 35348 pretreatment resulted in at most partial blockade of the discriminative stimulus effects of GHB; this result is consistent with the somewhat lower potency of baclofen to substitute for 300 mg/kg GHB, and is further evidence that the $GABA_B$ component may be more salient at the higher than at the lower GHB training dose.

The results of the present study confirm and extend those previously reported by Winter (6), who formulated the hypothesis that the discriminative stimulus effects of GHB are composed of multiple cues, each one mediated by a specific receptor system. In that study, muscimol and chlordiazepoxide (agonist and positive modulator at the $GABA_A$ receptor, respectively), and baclofen partially substituted for GHB in rats trained to discriminate 200 mg/kg GHB, administered intraperitoneally, from saline in a two-lever operant procedure (6).

REFERENCES

1. Colombo, G.; Agabio, R.; Balaklievskaia, N.; Lobina, C.; Reali, R.; Fadda, F.; Gessa, G. L.: T-maze and food-reinforcement: An inexpensive drug discrimination procedure. J. Neurosci. Methods 67:83–87; 1996.
2. Garcha, H. S.; Stolerman, I. P.: Discrimination of a drug mixture in rats: Role of training dose, and specificity. Behav. Pharmacol. 1:25–31; 1989.
3. Gessa, G. L.; Agabio, R.; Carai, M. A. M.; Lobina, C.; Pani, M.; Reali, R.; Colombo, G.: Mechanism of the anti-alcohol effect of gamma-hydroxybutyric acid (GHB). Alcohol (in press).
4. Maitre, M.: The γ-hydroxybutyrate signalling system in brain: Organization and functional implications. Prog. Neurobiol. 51:337–361; 1997.
5. White, J.-A. W.; Stolerman, I. P.: Antagonism of a nicotine plus midazolam discriminative cue in rats. Behav. Pharmacol. 5:351–355; 1994.
6. Winter, J. C.: The stimulus properties of gamma-hydroxybutyrate. Psychopharmacology (Berlin) 73:371–375; 1981.