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Cross-tolerance to ethanol and γ-hydroxybutyric acid

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Abstract

In the present study, the development of tolerance to the motor impairing effects of γ-hydroxybutyric acid (GHBA) and ethanol was compared (Experiment 1). Rats were required to perform a motor coordination task daily shortly after ethanol (3.5 g/kg) and GHBA (1.0 g/kg) administration for 9 consecutive days. Tolerance to the motor impairing effects of ethanol and GHBA developed to a similar extent but with different patterns. On the tenth day, the presence of cross-tolerance to the motor impairing effects of GHBA and ethanol was assessed (Experiment 2). Administration of 1.0 g/kg GHBA produced a significantly lower impairment in ethanol-tolerant rats than in ethanol-naive rats. Similarly, administration of 3.5 g/kg ethanol induced a significantly lower impairment in GHBA-tolerant rats than in GHBA-naive rats. The presence of cross-tolerance between GHBA and ethanol is discussed in terms of common pathways of neuroadaptation to chronic GHBA and ethanol.

Keywords: γ-Hydroxybutyric acid (GHBA); Ethanol; Motor-impairing effect; Cross-tolerance; (Rat)

1. Introduction

γ-Hydroxybutyric acid (GHBA), a naturally occurring compound of mammalian brain (Bessman and Fishbein, 1963; Roth and Giarman, 1969), has been found to be an effective agent in the pharmacotherapy of alcoholism. According to previous evidence for laboratory animals (Fadda et al., 1988, 1989), GHBA is reported to reduce alcohol consumption and alleviate symptoms of alcohol withdrawal in alcoholics (Gallimberti et al., 1989, 1992). It has been proposed that GHBA might exert its effects on ethanol dependence by mimicking ethanol's actions in the central nervous system (Fadda et al., 1989; Diana et al., 1991; Colombo et al., 1995).

The development of cross-tolerance and -dependence between ethanol and drugs commonly employed for reducing ethanol consumption and withdrawal has been a major issue in the clinical management of alcohol dependence (see Litten and Allen, 1991). Therefore, it is important to determine whether chronic treatment with ethanol or GHBA might confer tolerance to the other.

Ethanol and GHBA share some pharmacological similarities. For instance, acute administration of moderate to high doses of both ethanol and GHBA results in reduced motor coordination in laboratory animals (see Pohorecky, 1977; Mamelak, 1989). It has been extensively reported that tolerance to the motor impairing effects of ethanol occurs following chronic ethanol administration (see Lê et al., 1987). In contrast, to date no evidence of tolerance to the motor impairing effects of GHBA has been reported.

The present study was primarily designed to assess the development of tolerance to the ataxic effects of GHBA (Experiment 1). The existence of cross-tolerance between ethanol and GHBA was also examined (Experiment 2).

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Charles River, Calco, CO, Italy), weighing 250–300 g and 3 months old at the start of the experiment, were used. After delivery to our animal facilities, the rats were left undisturbed for

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10 days to acclimatize to the new housing conditions. The animals were housed 5 per cage with wood chip bedding under an artificial light-dark cycle of 12/12 h (light on at 7:00 a.m.), at constant temperature of 22 ± 2°C and relative humidity of 60%. An unlimited amount of standard laboratory food (MIL Morini, San Polo d'Enza, RE, Italy) was provided immediately after each daily session (see below). Since drugs were administered intragastrically, the rats were fasted for 12 h prior to the following session. Water was available ad libitum throughout the experiment.

2.2. Procedure

The possible development of tolerance to the motor incoordinating effects of GHBA and the presence of cross-tolerance to ethanol were assessed by using the accelerating Rota-Rod treadmills for rats (Ugo Basile, Comerio, VA, Italy). It has been extensively reported that tolerance to the motor impairing effects of ethanol develops at a greater rate and extent if animals had the opportunity to perform their task when intoxicated (see Lê, 1990). Therefore, the present study was designed so that rats practised the Rota-Rod task under ethanol and GHBA intoxication.

As previously described by Hoffman and Tabakoff (1984), each daily session consisted of 2 trials on the Rota-Rod. On the first trial (pre-drug performance), the rats were placed on the Rota-Rod 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. The animals which fell off the drum during the first 5 min period were excluded from the session.

Shortly after the first trial, the rats received either ethanol, GHBA or water. Thirty minutes after ethanol and water administration and 60 min after GHBA administration, the rats were then required to perform the motor task on the Rota-Rod for 11 min (second trial, post-drug performance). 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. Experimental design

In a preliminary study, dose-response curves for both ethanol and GHBA were assessed by administering 0 ($n = 6$), 1.4 ($n = 7$), 2.1 ($n = 8$), 2.8 ($n = 8$) and 3.5 ($n = 7$) g/kg ethanol and 0 ($n = 6$), 0.4 ($n = 7$), 0.6 ($n = 8$), 0.8 ($n = 7$) and 1.0 ($n = 7$) g/kg GHBA to drug-naive rats. This study was aimed at determining the doses of ethanol and GHBA which produce about 90–95% impairment at the Rota-Rod task.

In the tolerance study, the rats were randomly assigned to 3 groups receiving either 3.5 g/kg ethanol ($n = 9$), 1.0 g/kg GHBA ($n = 10$) or tap water ($n = 12$) during each daily session. Sessions were continuously conducted for 9 consecutive days to determine the development of tolerance to ethanol and GHBA (Experiment 1). On the tenth day, the presence of cross-tolerance between ethanol and GHBA was tested (Experiment 2). This session did not differ from the preceding ones, except that the GHBA-treated rats received 3.5 g/kg ethanol and the ethanol-treated rats received 1.0 g/kg GHBA. Water-treated rats were subdivided into 2 groups ($n = 6$) and received either 3.5 g/kg ethanol or 1.0 g/kg GHBA. Ethanol and GHBA were administered 30 and 60 min prior to the start of the second trial, respectively.

2.4. Drugs

Ethanol solution (25%, v/v) was prepared from 95% ethanol in water. GHBA (sodium salt, donated by Laboratorio Farmaceutico C.T., Sanremo, IM, Italy) was dissolved in tap water (50 mg/ml). Ethanol and GHBA solutions were prepared shortly before use. Ethanol, GHBA and water were administered by intragastric intubation.

2.5. Data analyses

The difference between the first and second trial times, expressed as a percentage of the first trial time,

Table 1
Effect of different doses of ethanol and GHBA on motor performance of rats at the Rota-Rod task

Ethanol dose (in g/kg)	% Impairment of performance	GHBA dose (in g/kg)	% Impairment of performance
0	0 ± 0 ($n = 6$)	0	0 ± 0 ($n = 6$)
1.4	32.5 ± 11.3 ($n = 7$)	0.4	36.1 ± 11.9 ($n = 7$)
2.1	52.4 ± 9.3 ($n = 8$)	0.6	64.0 ± 12.8 ($n = 8$)
2.8	76.6 ± 6.1 ($n = 8$)	0.8	83.1 ± 6.8 ($n = 7$)
3.5	95.7 ± 2.0 ($n = 7$)	1.0	96.1 ± 1.6 ($n = 7$)

Percent impairment of performance is defined as $[(T_1 - T_2)/T_1] \times 100\%$, T_1 and T_2 being the amount of time each rat remained on the Rota-Rod drum before and after drug administration, respectively. Each value represents the mean ± S.E.M. of n subjects.

was calculated for each rat and indicated its degree of motor impairment for the session. Therefore, each rat served as its own control. When the second trial time was greater than the first, 0% impairment was scored.

In Experiment 1, between-group comparisons were performed by a two-way ANOVA for repeated measures to assess the development of tolerance. Differences in individual group means were evaluated by the Duncan's multiple range test. In Experiment 2, comparison of impairment between groups was performed using the Mann-Whitney two-sample test. Significance was maintained at $P < 0.05$ level.

3. Results

The dose-response study on the effect of different doses of ethanol and GHBA indicated that the acute administration of 3.5 g/kg ethanol and 1.0 g/kg GHBA resulted in 95.7 and 96.1% impairment of motor performance in the ethanol- and GHBA-naive rats, respectively (Table 1). These doses of ethanol and GHBA were chosen for tolerance testing.

Repeated exposure to 3.5 g/kg ethanol and 1.0 g/kg GHBA produced tolerance to the motor impairing effects of ethanol and GHBA, respectively (Experiment 1; Fig. 1). ANOVA revealed a significant main effect of treatments ($F_{\text{treatment}}(2,278) = 352.5$, $P < 0.001$), a significant main effect of time ($F_{\text{time}}(8,278) = 5.2$, $P < 0.001$) and a significant interaction between factors ($F_{\text{interaction}}(16,278) = 3.3$, $P < 0.001$). The tolerance to ethanol and GHBA differed in the pattern of development. The proportion of motor impairment was significantly ($P < 0.05$, Duncan's multiple range test) different between ethanol- and GHBA-treated groups from day 4 to day 7 of treatment. No sign of motor impairment was observed in the water-treated rats.

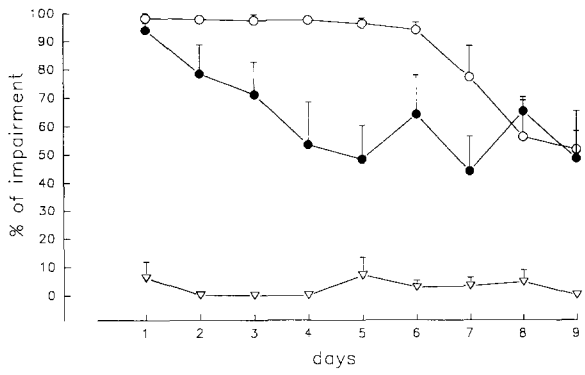

Fig. 1. Effect of repeated daily administrations of 3.5 g/kg ethanol (●, $n = 9$), 1.0 g/kg GHBA (○, $n = 10$) and water (▽, $n = 12$) on motor performance at the Rota-Rod task. Percent impairment of performance is defined as $[(T_1 - T_2)/T_1] \times 100\%$, T_1 and T_2 being the amount of time each rat remained on the Rota-Rod drum before and after drug administration, respectively. Each point represents the mean ± S.E.M. of n subjects.

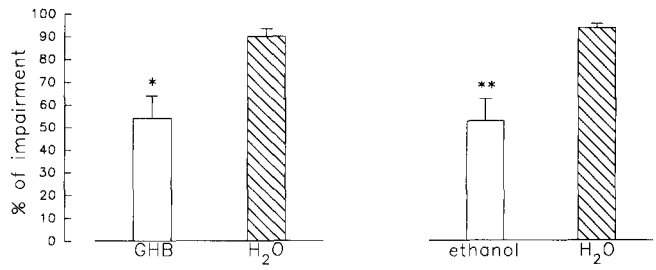

Fig. 2. Effect of the administration of 3.5 g/kg ethanol (left panel) and 1.0 g/kg GHBA (right panel) in GHBA- and ethanol-tolerant rats ($n = 9$), respectively. Control rats ($n = 6$, for each group) received tap water throughout Experiment 1, i.e. they were drug-naive on the test day. Percent impairment of performance is defined as $[(T_1 - T_2)/T_1] \times 100\%$, T_1 and T_2 being the amount of time each rat remained on the Rota-Rod drum before and after drug administration, respectively. Each bar represents the mean ± S.E.M. of n subjects. * $P < 0.05$ and ** $P < 0.005$ in comparison to drug-naive rats.

The development of cross-tolerance between ethanol and GHBA (Experiment 2) is shown in Fig. 2. In comparison to the control animals, administration of ethanol and GHBA resulted in a significantly lower impairment of motor performance in the GHBA- and ethanol-tolerant rats, respectively. Administration of 3.5 g/kg ethanol produced 54 and 90% impairment in the GHBA- and water-treated groups, respectively ($P < 0.05$, Mann-Whitney two-sample test; Fig. 2, left panel). Similarly, 1.0 g/kg GHBA induced 55 and 98% impairment in the ethanol- and water-treated groups, respectively ($P < 0.005$, Mann-Whitney two-sample test; Fig. 2, right panel).

4. Discussion

The results of the present study demonstrate that repeated exposure to a sub-anesthetic dose of GHBA resulted in the development of tolerance to its motor impairing effects. These findings are in close agreement with those reported by Nowycky and Roth (1979), indicating the development of tolerance to the hypnotic effects of γ-butyrolactone, the precursor of GHBA. The data of the present study also show that chronic treatment with ethanol or GHBA confers cross-tolerance to the other.

Tolerance to a specific drug can be viewed as the result of adaptive changes in neuronal structures and functioning induced by repeated exposure to that drug (see Grant et al., 1990). Therefore, the development of cross-tolerance between ethanol and GHBA might indicate the presence of common pathways of neuroadaptation to chronic ethanol and GHBA. This interpretation is consistent with evidence suggesting that GHBA and ethanol share several pharmacological

properties and might act through similar neurochemical mechanisms (Fadda et al., 1989; Diana et al., 1991; Colombo et al., 1995). Therefore, any elucidation of the mechanisms of the tolerance to GHBA might provide useful information for clarifying the neurotrasmitter systems associated with ethanol tolerance.

Data from Experiment 1 show that tolerance to the motor impairing effects of ethanol and GHBA developed to a similar extent. However, the pattern of tolerance acquisition was different between the ethanol- and GHBA-treated rats over sessions 1–9. In keeping with previous reports (Gallaher et al., 1982; Lê and Kiianmaa, 1988; Bitrán and Kalant, 1991), tolerance to ethanol was rapidly produced after a limited number of treatments with ethanol. In contrast, repeated exposure to GHBA was necessary before tolerance to GHBA developed. The initial exposure to ethanol and GHBA might lead to different responses of the neuronal systems which mediate their effects, thus determining the different pattern of development to tolerance which was observed in the present study.

The finding that cross-tolerance between ethanol and GHBA developed after chronic treatment suggests that these two compounds share common mechanisms of central action. This phenomenon may explain the lack of sedative effects of GHBA in alcoholic patients (Gallimberti et al., 1989) and may constitute the rationale for the use of GHBA in the treatment of alcohol dependence. It might also explain the abuse liability of GHBA in non-alcoholic subjects (Food and Drug Administration, 1991).

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