GAMMA-HYDROXYBUTYRIC ACID VERSUS ALCOHOL PREFERENCE IN SARDINIAN ALCOHOL-PREFERRING RATS

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Abstract — Previous experiments demonstrated that the selectively bred Sardinian alcohol-preferring (sP) rats possess a genetically based proclivity to consume pharmacologically relevant doses of gamma-hydroxybutyric acid (GHB). The present study was aimed at comparing the reinforcing properties of GHB and ethanol, measuring the propensity of sP rats to consume GHB and ethanol when both drugs were concomitantly available. Initially, two groups of sP rats (ethanol-naive and ethanol-experienced, respectively) were forced to consume GHB in order to help them discover the reinforcing properties, which could then prevail over the unpleasant taste of the GHB solution. Subsequently GHB (at concentrations increasing from 1 to 6% w/v) was offered in free choice with water and all rats consumed pharmacologically relevant amounts of GHB. Finally, under the free-choice regimen between GHB (presented to each rat at its preferred concentration), ethanol and water, daily ethanol intake averaged ~6 g/kg (i.e. the amount of ethanol usually consumed by sP rats), whereas GHB intake declined by ~75%. In the few rats showing a high intake of GHB, ethanol intake was not altered. No difference in GHB drinking behaviour was ever recorded between ethanol-naive and ethanol-experienced rats. The results of the present study demonstrate that freely available GHB is not capable of altering ethanol preference and consumption in sP rats and suggest that the postulated reciprocal substitutability of the two drugs does not completely include the reinforcing properties, at least in sP rats and when oral self-administration of GHB is considered. The results also provide a model of the low abuse liability of GHB observed in human alcoholics.

INTRODUCTION

Accumulating evidence features gamma-hydroxybutyric acid (GHB) as a promising agent in the pharmacotherapy of alcoholism. Indeed, GHB has been reported to reduce alcohol craving and consumption, promote abstinence and ameliorate the symptoms of the alcohol withdrawal syndrome in alcoholics (Gallimberti et al., 1989, 1992; Addolorato et al., 1996, 1998, 1999a; Walter et al., 1999; Moncini et al., 2000). Consistently, animal studies demonstrated that GHB administration reduced voluntary ethanol intake in ethanol-prefering rats and intensity of ethanol withdrawal signs in rats rendered physically dependent on ethanol (Fadda et al., 1989; June et al., 1995; Agabio et al., 1998). Further data, indicating that GHB and ethanol elicited similar discriminative stimulus effects in rats (Colombo et al., 1995b), suggested that GHB may exert its effects on alcohol dependence by mimicking alcohol actions in the central nervous system, i.e. a substitution therapy similar to that underlying methadone use in heroin addiction.

The high degree of similarity between the pharmacological profiles of GHB and ethanol intrinsically supports the possibility that GHB, like ethanol, can be abused in humans and produce positive reinforcing properties in laboratory animals. Accordingly, an increasing number of clinical observations have reported that GHB is used as a recreational drug, because of its capability, as the dose is increased, to produce feelings of euphoria, disinhibition, anxiolysis, relaxation and hypnosis (Galloway et al., 2000), i.e. a constellation of effects often described as closely resembling those of ethanol (Galloway et al., 1997). Furthermore, GHB has been reported to induce conditioned place preference (Martellotta et al., 1997) and be orally and intravenously self-administered in rats and mice (Colombo et al., 1995a; Martellotta et al., 1998). Interestingly, Sardinian alcohol-prefering (sP) rats, selectively bred for high ethanol preference and consumption, self-administered larger quantities of, and exhibited higher preference for, a GHB solution, than their alcohol-avoiding counterpart [namely, Sardinian alcohol non-prefering (sNP) rats] (Colombo et al., 1998), suggesting that, in this rat line, GHB may exert reinforcing properties similar to those of ethanol. Consistent with the postulated reciprocal substitutability of GHB and ethanol, acute administration of ethanol markedly reduces voluntary GHB intake in GHB-consuming sP rats (Colombo and Gessa, 2000).

In the wake of these results, the present study was designed to compare the reinforcing properties of GHB and ethanol, measuring the propensity of sP rats to consume GHB and/or ethanol when both drugs are concurrently available. In order to evaluate the possible influence of a prior exposure to ethanol, the present study included two separate groups of sP rats, ethanol-naive and ethanol-experienced, at the time of GHB presentation.

MATERIALS AND METHODS

Animals

Male sP rats, from the 47th generation and aged 75 days at the start of the study, were used. Rats derived from a population of sP rats which underwent Cesarean derivation at Charles River (Lyon, France) for production of specific pathogen-free individuals. Rats were individually housed in standard plastic cages [425 × 266 × 150 (h) mm] with wood chip bedding. The animal facility was under an inverted 12-h light:12-h dark cycle (lights on at 21.00), at a constant temperature of
22 ± 2°C and a relative humidity of ~60%. Standard laboratory rat chow (MIL Morini, San Polo d’Enza, RE, Italy) was provided ad libitum to the rats throughout the experimental period. Rat body weights were monitored twice per week. Prior to the start of the study, rats were both ethanol- and GHB-naive.

Procedure

Initially (Phase 1), eight rats (termed ethanol-naive) had continuous access solely to water for 14 consecutive days. Conversely, nine rats (termed ethanol-experienced) were given ethanol and water under the two-bottle free-choice regimen with unlimited access for 24 h/day. Specifically, rats were offered two graduated bottles, containing 10% (v/v) ethanol, and water.

Subsequently, both ethanol-naive and ethanol-experienced rats were forced to consume 1% (w/v) GHB [sodium salt; Laboratorio Farmaceutico C.T., Sanremo (IM), Italy] dissolved in water, as the sole drinking fluid available, for 14 consecutive days (Phase 2). This initial no-choice phase proved necessary, because the unpleasant taste of the GHB solution would hamper rats in a free-choice regimen to discover the reinforcing properties of the drug.

Immediately after the forced drinking period, rats were moved to the ‘GHB vs water’ free-choice regimen (Phase 3). Two graduated bottles, containing water and the GHB solution, respectively, were continuously offered to ethanol-naive and ethanol-experienced rats. The concentration of the GHB solution was increased every 7 days from 1 to 2, 3, 4 and 6% (w/v); no interruption was interposed between each 7-day period.

All rats were then offered a free choice between 10% (v/v) ethanol, a GHB solution at the preferred concentration (see below) and water with unlimited access for 14 consecutive days (Phase 4). The preferred concentration of GHB was calculated individually for each rat from the data recorded in the 1–6% preference tests (Phase 3). Specifically, each value was based on the concentration at which each individual rat consumed the highest amount of GHB (in mg/kg) over the 7-day period during which each concentration was presented.

During all phases, fluid intakes were monitored daily. Bottles were refilled every day with fresh solution and their position changed daily on a random basis.

Data analyses

Data on daily ethanol and GHB intakes in ethanol-naive and ethanol-experienced rats, in Phases 2 and 4, as well as for each 7-day period with a specific GHB concentration in Phase 3, were compared by means of a two-way analysis of variance (rat groups × days).

RESULTS

In good agreement with the previously characterized ethanol drinking behavior of sP rats (Colombo, 1997), voluntary ethanol intake in Phase 1 in ethanol-experienced rats averaged 6.5 ± 0.1 g/kg; consistently, the preference ratio (defined as the ratio between the amount of ethanol solution consumed daily and total fluid intake) averaged 86.4 ± 0.9%.

During the 14-day no-choice period (Phase 2), daily GHB intake occurred at pharmacologically relevant doses (Colombo et al., 1995a) in both ethanol-naive and ethanol-experienced rats, averaging 755 ± 7 and 753 ± 11 mg/kg, respectively \( F(1,195) = 0.0031, P = 0.9563 \).

When GHB was offered in free choice with water (Phase 3), all rats showed days of preference for the GHB solution over water and alternate periods of high daily intake of GHB with temporarily self-imposed avoidance of the GHB solution. Daily GHB intake did not differ significantly between ethanol-naive and ethanol-experienced rats during any of the 7-day periods with a specific concentration of GHB (1%: \( F(1,90) = 0.9733, P = 0.2792 \); 2%: \( F(1,90) = 0.1879, P = 0.6709 \); 3%: \( F(1,90) = 0.0033, P = 0.9546 \); 4%: \( F(1,90) = 0.0026, P = 0.9570 \); 6%: \( F(1,90) = 0.0063, P = 0.9376 \)). The highest average daily GHB intake was recorded at the concentration of 2% in one ethanol-naive rat, 3% in one ethanol-naive rat, and 4% in six ethanol-naive rats, and in all the ethanol-experienced rats. When data from the preferred concentration of each rat were examined, daily GHB intake averaged 462 ± 29 and 390 ± 24 mg/kg in ethanol-naive and ethanol-experienced rats, respectively, with no significant group differences \( F(1,90) = 1.3032, P = 0.2715 \).

In Phase 4 (when ethanol, GHB and water were concurrently presented), ethanol intake in ethanol-experienced rats immediately resumed to the intake levels monitored in Phase 1 (Fig. 1, lower panel). In ethanol-naive rats, average ethanol intake was >4 g/kg from the first day of exposure and rose to the 5.5–6 g/kg range over the first 7 days (Fig. 1, upper panel). Daily ethanol intake did not diverge significantly between ethanol-naive and ethanol-experienced rats \( F(1,195) = 2.3063, P = 0.1496 \). Co-presentation of ethanol resulted in an immediate decline, by ~75%, in daily GHB intake in both ethanol-naive and ethanol-experienced rats; indeed, over the 14 days of Phase 4, GHB intake averaged 126 ± 14 and 93 ± 10 mg/kg in ethanol-naive and ethanol-experienced rats, respectively (Fig. 1), with no significant group differences \( F(1,195) = 1.3633, P = 0.2612 \). Only two and three rats in ethanol-naive and ethanol-experienced groups respectively, consumed occasionally amounts of GHB higher than the average consumption recorded at the preferred concentration.

DISCUSSION

The results of the present study indicate that presentation of an ethanol solution resulted in an immediate and virtually complete suppression of voluntary GHB intake in sP rats. Indeed, concurrent exposure to three bottles [containing a GHB solution (at the preferred concentration by each rat), 10% ethanol and water], resulted in: (1) no alteration in ethanol intake when it was extended to ethanol. The results of the present study demonstrated that sP rats, although possessing a genetically determined proclivity to consume daily quantities of GHB that are expected to produce neuropharmacological
Intake of GHB did not result in work, i.e. the reciprocal substitutability of GHB and ethanol administration are needed to elucidate this point. Of the 2 drugs, appropriate studies using the intragastric self-administration are presumably driven by the psychopharmacological effects ruled out. Although both ethanol and GHB intakes in sP rats over GHB. In other words, it can be proposed that, in sP rats, the central effects of ethanol that sustain voluntary ethanol drinking are more reinforcing than those of GHB that sustain its voluntary consumption.

The possible influence of taste (pre-ingestive) factors on the outcome of the present study cannot at present be completely ruled out. Although both ethanol and GHB intakes in sP rats are presumably driven by the psychopharmacological effects of the 2 drugs, appropriate studies using the intragastric self-administration are needed to elucidate this point.

In contrast with the guiding hypothesis of the present work, i.e. the reciprocal substitutability of GHB and ethanol (Colombo and Gessa, 2000), intake of GHB did not result in a proportional decline in ethanol consumption. Indeed, in the few rats exhibiting a pharmacologically relevant intake of GHB (>500 mg/kg/day), daily ethanol intake was not affected by the concomitant high intake of GHB. For example, rat no. 20 from the ethanol-naive group on day 13 of Phase 4 consumed the ‘usual’ amount of ethanol (5.5 g/kg/day), although it also self-administered a dose of GHB (1182 mg/kg/day) expected to produce robust neuropathological effects. Apparently, the central effects of ethanol that maintain ethanol drinking behaviour in sP rats are not closely reproduced by those elicited by GHB.

The negligible intake of GHB monitored in sP rats having concurrent access to ethanol may reproduce the low abuse liability of GHB observed in human alcoholics. Indeed, in some contrast with the increasing number of episodes of recreational use of GHB outside a therapeutic protocol (Galloway et al., 2000), self-directed intake of GHB appears to be a limited phenomenon among alcoholics: the percentage of alcoholics increasing voluntarily the dose of GHB over the recommended amount varied between 10 and 15 in the two studies addressing this issue (Addolorato et al., 1996; Gallimberti et al., 2000). The majority of these episodes were interpreted as an attempt to adjust the therapeutic dose, rather than the search for certain psychotropic effects of the drug; accordingly, no cases of abuse have been reported after a proper fractioning of the daily dose (Addolorato et al., 1998), and only a single case of shift from alcohol dependence to GHB dependence has been described (Addolorato et al., 1999b). These observations are in agreement with the favourable safety profile of GHB as a pharmacotherapy for alcoholism (Beghè and Carpanini, 2000).

In conclusion, the results of the present study demonstrate that voluntary ethanol intake surmounts GHB intake in sP rats that had previously consumed voluntarily relevant doses of GHB. In the few rats showing a high intake of GHB, ethanol intake was not concomitantly altered, suggesting that the postulated reciprocal substitutability of the two drugs does not completely include, at least in sP rats, the reinforcing properties.

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**Fig. 1.** Daily gamma-hydroxybutyric acid (GHB) and ethanol intakes in Sardinian alcohol-prefering (sP) rats.

Intakes were measured in ethanol-naive (top panel) and ethanol-experienced (bottom panel) rats during the 14-day ‘Ethanol vs GHB vs water’ free-choice period (corresponding to Phase 4). Ethanol intake is expressed on the left ordinate as g/kg body wt; GHB intake is expressed on the right ordinate as mg/kg body wt. Ethanol was offered at the concentration of 10% (v/v); GHB was offered at the preferred concentration for each rat [varying between 2 and 4% (w/v); see the text for details]. Each point is the mean ± SEM of eight or nine subjects. The ‘GHB vs water’ and ‘Ethanol vs water — GHB vs water’ points represent the mean daily ethanol and GHB intakes when either ethanol or GHB was offered in free choice with water (Phase 1 for ethanol intake; 7 days of Phase 3 at the preferred concentration by each rat for GHB intake). Each point is the mean ± SEM of eight or nine subjects for n = 7 days.


