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Interactions of γ-hydroxy butyrate with ethanol and NCS 382

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Abstract

We examined the effects of γ-hydroxy butyrate (GHB) alone and in combination with either ethanol or NCS 382 [(2*E*)-(5-hydroxy-5,7,8,9-tetrahydro-6*H*-benzo[a][7]annulen-6-ylidene], a purported antagonist at the GHB receptor. These effects were examined on the responding of rats under a fixed-ratio (FR) 10 schedule of sugar solution (14%, w/v; 0.1 ml) presentation. GHB dose-relatedly decreased responding. When GHB was combined with ethanol, the effects of the two drugs were less than additive. NCS 382 did not antagonize the rate-decreasing effects of GHB. These observations are consistent with the notion that many of the behavioral actions of exogenously administered GHB result from GHB's actions at sites other than the GHB receptor, and are inconsistent with the popular notion that the effects of GHB and ethanol are synergistic.

Keywords: GHB (γ-hydroxy butyrate); Ethanol; NCS 382; (Rat); Schedule-controlled behavior; Drug interaction

1. Introduction

In these experiments, we examine the effects of γ-hydroxy butyrate (GHB) alone and in combination with either ethanol or NCS 382 [(2*E*)-(5-hydroxy-5,7,8,9-tetrahydro-6*H*-benzo[a][7]annulen-6-ylidene] on the behavior of rats. GHB is a potential neuromodulator or neurotransmitter (Maitre, 1997; Cash, 1994). GHB, its synthetic machinery and binding sites are all differentially distributed in the central nervous system (Doherty et al., 1978; Hédou et al., 2000; Mehta et al., 2001). NCS 382 shares binding sites with GHB (Mehta et al., 2001) and has been reported to antagonize some (Colombo et al., 1995c; Godbout et al., 1995; Cook et al., 2002), but not all, of the effects of GHB (Godbout et al., 1995; Cook et al., 2002; Emri et al., 1996; Carai et al., 2001).

GHB has several potential therapeutic uses. GHB can promote normalization of sleep patterns and has been approved for the treatment of narcolepsy (Broughton and Mamelak, 1979; Scharf et al., 1985; Scrima et al., 1989; Lammers et al., 1993). Others have advocated the use of GHB in the treatment of alcoholism and in particular alcohol withdrawal (Gallimberti et al., 1989; Addolorato et al., 1999; Nimmerrichter et al., 2002). These therapeutic uses may result in occasions in which GHB is used when ethanol is also consumed. At the same time, there is increasing concern about the abuse of GHB and about the use of GHB in combination with ethanol in drug-facilitated sexual assault (Galloway et al., 1997; Carter et al., 1997; Graeme, 2000; WHO Expert Committee on Drug Dependence, 2001; Schwartz et al., 2000).

The combination of GHB and ethanol is described in the popular press as producing synergistic effects. There has, however, been little empirical investigation of this issue to our knowledge. McCabe et al. (1971) describe the combined effects of GHB and ethanol on the "sleep time" of mice as synergistic. Others (Colombo et al., 1995b) have reported that rats treated daily with GHB become tolerant to the impairing effects of GHB on rotorod performance and cross-

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tolerant to the rotorod impairing effects of ethanol; and further, that when ethanol is administered daily, cross-tolerance develops to the impairing effects of GHB. This cross-tolerance and other effects such as the ability of GHB to occasion ethanol-appropriate responding in rats trained to discriminate ethanol, and ethanol to occasion GHB-appropriate responding in rats trained to discriminate GHB at least under a narrow range of conditions (Colombo et al., 1995a) have been used to argue that GHB and ethanol may share common mechanisms of action for at least some of their effects.

Many, though certainly not all (see Tsai and Coyle, 1998), of the actions of ethanol appear to be mediated through $GABA_A$ receptors. GHB and γ-amino butyric acid (GABA) can be interconverted to each other in the brain (Vayer et al., 1985; Snead et al., 1989), and systemic administration of GHB is thought to increase brain GABA levels. This may provide a mechanism through which GHB and ethanol can produce additive or supra-additive effects. In this paper, we report the results of experiments that show the effects of GHB and ethanol on operant responding in the rat are additive or sub-additive, and that NCS 382 does not antagonize the rate-decreasing effects of GHB on operant responding in the rat.

2. Materials and methods

2.1. Subjects

Six male Lewis rats (Harlan, Indianapolis, IN), weighing between 275 and 300 g upon arrival to the laboratory, were used. Rats were individually housed in polycarbonate cages in a vivarium at the University of Texas Health Sciences Center at San Antonio. The animals had unlimited access to water, and access to food is limited to 12–15 g/day (on Fridays, rats received 45 g of food for the weekend). This food allotment was provided shortly following experimental sessions, maintaining bodyweights at 285–355 g during the course of the experiment. The vivarium had a 12-h light/12-h dark illumination cycle, and rats were trained and tested during the light phase. These experiments were conducted in accordance with the ‘‘Guide for the Care and Use of Laboratory Animals’’ (Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council, 1996) and approved by the Institutional Animal Use and Care Committee of the University of Texas Health Science Center at San Antonio.

2.2. Apparatus

Experimental sessions were conducted in operant chambers containing three levers. Only the right lever, which was opposite the dipper opening, was utilized in these experiments. Behavioral contingencies were controlled using Med-PC (Med-Associates, St. Albans, VT).

2.3. Procedures

Experimental sessions were conducted 5 days a week and consisted of a 10-min period of access to a sucrose solution under a fixed-ratio (FR) 10 schedule of reinforcement. During the period of sucrose solution availability, when the stimulus light above the lever was lighted, every 10th response on the active lever (FR 10) resulted in 10 s of access to a 14% (w/v) sucrose solution and the beginning of a 10-s timeout period. During this 10-s timeout period, the stimulus light above the lever was turned off, the house light was illuminated, and responses had no programmed consequences. Following this 10-s timeout period, the FR 10 schedule was again in effect, the houselight turned off, and the stimulus light above the lever turned on, unless the 10-min period of sucrose solution availability had expired. If the 10-min period of sucrose solution availability had expired, then the experimental session ended. When the rate of responding over the last 5 days was stable, animals were tested for drug-induced changes in responding. Drug tests were conducted on Tuesdays and Fridays. Regular training sessions were conducted on Mondays, Wednesdays, and Thursdays.

2.4. Drugs

GHB was purchased from Sigma Aldrich (St. Louis, MO), dissolved in a 0.9% saline solution, and injected intraperitoneally in a volume of 3 ml/kg 30 min before the beginning of the experimental session. Ethanol in a 10% (w/v) solution in sterile water was injected intraperitoneally 10 min before the beginning of the experimental session. Ethanol doses were achieved by varying volumes administered rather than by varying concentration (Linakis and Cunningham, 1979). NCS 382 [(2*E*)-(5-hydroxy-5,7,8,9-tetrahydro-6*H*-benzo[a][7]annulen-6-ylidene] was synthesized as described earlier (Maitre et al., 1990). NCS 382 was dissolved in 0.9% saline and injected intraperitoneally in a volume of 1 ml/kg 30 min before the beginning of the experimental session.

2.5. Data analysis

Response rate was the primary measure and was calculated by dividing the number of responses by the time available to make these responses (i.e., responses/s), i.e., session-time minus time-out time. Response rate was converted from responses/s to percent of the rate from the day before by dividing the rate of responding by the rate obtained on the previous day for that individual. The median rate of responding under this schedule was 0.656 responses/s on these days. Rates for the six individual rats had a broad range between rats but were fairly tightly controlled within a rat. The mean (S.E.M.) rate on the day before drug or vehicle injection for each of the six rats is as follows: 0.465 (0.051), 0.649 (0.070), 0.685 (0.082), 0.852 (0.098), 0.978

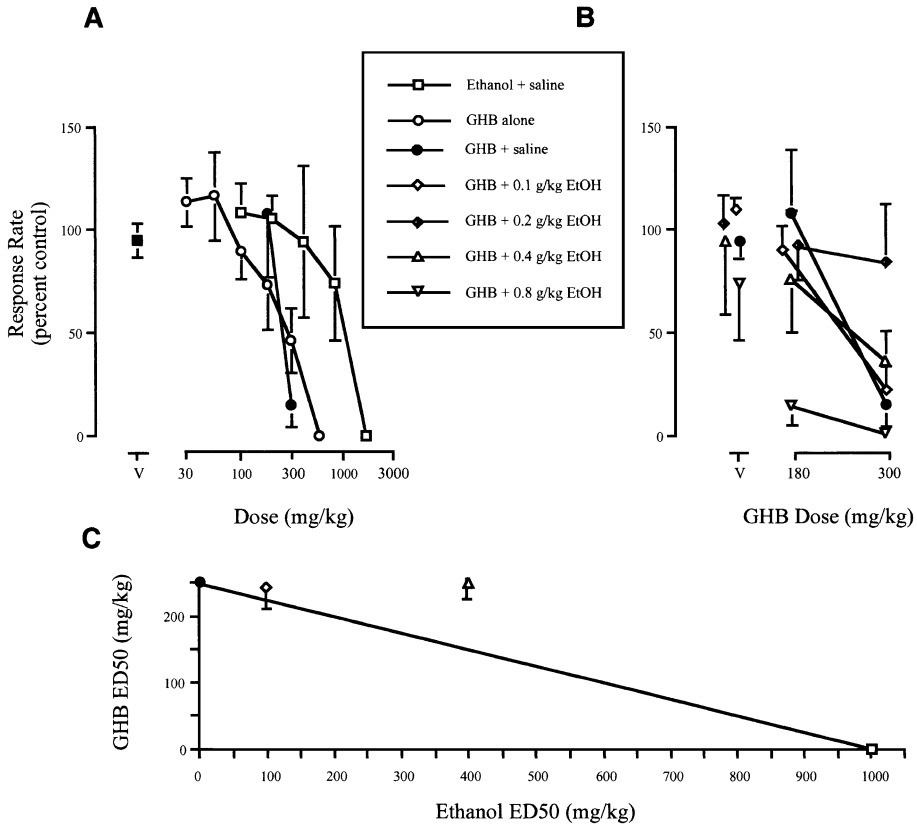

Fig. 1. The effects of GHB and ethanol alone (panel A) and in combination (panels B and C) on the responding of rats under an FR 10 schedule of 0.1 ml 14% sucrose solution presentation. In panels A and B, the *y*-axis is the mean rate of responding expressed as a percent of the preceding day's rate. The *x*-axis is dose in mg/kg on a log scale. Both drugs were administered intraperitoneally with GHB being given 30 min before, and ethanol or saline being given 10 min before the 10-min session. In panel A, the point above 'V' represents the effects of two saline injections—one 30 min and another 10 min before the session. In panel B, the points above "V" represent the effects of saline in combination with either another saline injection or a dose of ethanol. In panels A and B, the bars through the points are the standard errors of the mean for that point. Panel C is an isobologram for the effects of GHB and ethanol. The *y*-axis is the GHB dose for a given combination producing an ED_{50} effect and the *x*-axis is the ethanol dose for a given combination producing an ED_{50} effect. The point on the *y*-axis is the ED_{50} for GHB alone, 246 mg/kg (205–291; 95% CL), and the point on the *x*-axis is the ED_{50} for ethanol alone, 1000 mg/kg (783–1278). The bars through the two points are the 95% CLs for the GHB ED_{50} when given in combination with either 100 or 400 mg/kg ethanol [ED_{50}s of 242 (213–276) and 250 mg/kg (226–275), respectively]. No points for the effects of GHB in combination with either 0.2 or 0.8 g/kg ethanol are included, as the GHB ED_{50} for these combinations could not be calculated. No tested dose of GHB reduced responding below 50% in combination with 0.2 g/kg ethanol, and all tested doses of GHB reduced responding below 50% in combination with 0.8 g/kg ethanol.

(0.103), 1.441 (0.161). Rates of responding following a saline injection were very similar to the day before (95 (8)% of the rate of the day before; see Fig. 1A). ED_{50}s were calculated using linear regression upon the data points that were monotonic and produced between 15% and 85% of the maximal response (Tallarida and Murray, 1987). The confidence limits (CL) for these points were determined as suggested by Bliss (1967). Analysis of variance (ANOVA) and regressions were calculated using SYSTAT (version 5.2.1; Systat, Evanston, IL), run on a Macintosh G4.

3. Results

3.1. Effects GHB and ethanol

Fig. 1A shows the effects of GHB alone or in combination with saline, and the effects of ethanol in combination with saline on the rate of responding by rats for a sucrose solution. GHB dose-relatedly decreased FR responding. The ED_{50} for GHB alone was 278 mg/kg (95% CL; 140–555 mg/kg). Ethanol in combination with saline similarly decreased FR responding with an ED_{50} of 1 g/kg (0.78–1.28). Fig. 1B shows the effects of 180 and 300 mg/kg GHB in combination with saline, 0.1, 0.2, 0.4, and 0.8 g/kg ethanol. A dose of 300 mg/kg GHB in combination with a given dose of ethanol generally produced greater decreases than the same dose of ethanol combined with 180 mg/kg GHB. These dose combinations were at most additive and perhaps showed some small level of mutual antagonism. The GHB ED_{50} in combination with 0.1 g/kg ethanol (242 mg/kg [213–276]) and the ED_{50} for GHB in combination with 0.4 g/kg ethanol (250 mg/kg [226–275]) were similar to the ED_{50} for GHB in combination with saline (246 mg/kg [205–291]). The ED_{50} for GHB in combination with 0.2 g/kg ethanol could not be determined as neither 180 nor 300

mg/kg GHB in combination with 0.2 g/kg ethanol decreased responding below 50% of control. This is particularly noteworthy for the 300 mg/kg GHB dose, as this dose by itself or in combination with saline resulted in decreases in responding greater than 50%. The ED_{50} for GHB in combination with 0.8 g/kg ethanol also could not be determined as both 180 and 300 mg/kg GHB in combination with 0.8 g/kg ethanol produced a greater than a 50% decrease in responding. However, the ED_{50} for GHB in combination with 0.8 g/kg, based on simple additivity, would be much less than the lowest tested dose of 180 mg/kg GHB. In fact, the dose combination of 180 mg/kg GHB and 0.8 g/kg ethanol by simple additivity would be equivalent to either 484 mg/kg of GHB or 1.45 g/kg ethanol. Either of these would have produced rate-decreasing effects at least as great as was seen.

The expected ED_{50}s for the drug combinations can be derived from the line drawn in the isobologram shown in Fig. 1C. ED_{50}s representing additive effects would be expected to fall on this line, while those representing synergistic effects would be below this line. As can be seen, the ED_{50}s for the two dose combinations for which ED_{50}s could be determined were both above this line. Points above this line represent less than (infra) additive effects or antagonism. One should note that the confidence limits for the GHB ED_{50} in combination with 0.1 g/kg ethanol encompass this line. However, the ED_{50} for GHB in combination with 0.4 g/kg ethanol does not encompass this line and would not even encompass the line drawn from the upper bounds of the confidence limits for the ED_{50}s for ethanol and GHB in combination with saline. Thus, these results indicate that the effects of GHB in combination with ethanol on FR responding are at most additive and for some dose combinations mutually antagonistic.

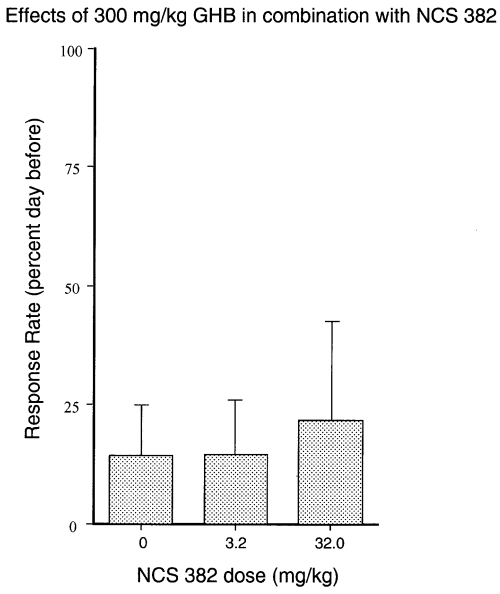

Fig. 2. Effects of 300 mg/kg GHB when given in combination with either saline, 3.2, or 32.0 mg/kg NCS 382 on the rate of responding by rats under a FR 10 schedule of sucrose solution presentation. The *y*-axis represents the mean rate of responding as a percent of the day before. Error bars represent the standard error of the mean. There were no significant differences between the effects of GHB alone or GHB in combination with NCS 382 ($F=0.07$, $df=2,15$, $P>0.1$).

3.2. Effects of GHB and NCS 382

Fig. 2 shows the effects of 300 mg/kg GHB in combination with saline, 3.2, or 32.0 mg/kg NCS 382. The rate of responding following GHB in combination with either dose of NCS 382 was similar to the rate of responding following GHB in combination with saline. Rates of responding were 14.7(10.5)%, 14.7(14.7)%, and 21.9(21.9)% of control [mean(S.E.M.)] following 300 mg/kg GHB in combination with saline, 3.2, or 32 mg/kg NCS 382, respectively. These differences were not significant ($F=0.07$, $df=2,15$, $P>0.10$).

4. Discussion

Both GHB and ethanol produced dose-related decreases in FR responding in rats. When these two compounds were jointly administered, their effects were at most additive. When GHB was administered in combination with the purported GHB-antagonist NCS 382, no evidence of antagonism was observed. This later observation is consistent with the notion that many of the behavioral actions of exogenously administered GHB result from GHB's actions at sites other than the GHB receptor.

A previous study in mice reported that GHB produced dose-related decreases in FR responding (Cook et al., 2002), and the present study extends this to rats. Previous studies have also found that ethanol can produce dose-related decreases in FR responding in a variety of species including rats (e.g., Holloway and Vardiman, 1971). The behavioral effects of combinations of GHB and ethanol have, however, been little studied. McCabe et al. (1971) studied the effects of GHB and ethanol on the "sleep time" of mice. They report the effects of the GHB and ethanol in combination as being synergistic.

In the present study, the effects of GHB and ethanol were clearly not additive. The ED_{50} of GHB in combination with 0.1 or 0.4 g/kg ethanol was virtually identical to the ED_{50} of GHB alone or in combination with saline. When GHB was combined with 0.2 g/kg ethanol, an ED_{50} for GHB could not be determined as neither dose of GHB decreased responding to less than 50%, despite the 300 mg/kg dose doing so when given alone. While GHB in combination with 0.8 g/kg ethanol produced decreases in responding greater than those of GHB alone, these effects were less than predicted by additivity. Thus, across a wide range of dose conditions, the effects of GHB and ethanol were sub-additive.

There are several possible reasons for this apparent discrepancy between the results of McCabe et al. and our

results. Both the primary measures (sleep time versus operant responding) and the species used differed between the two studies, and either of these factors could account for the difference seen. However, McCabe et al. may also have not had the synergistic effects that they report. In this study (McCabe et al., 1971), they report the effects of 6.51 mmol/100 g ethanol alone and in combination with 0.25, 0.33, and 0.41 mmol/100 g GHB (the effects of these doses of GHB alone are also reported). The sleep times seen following any of these doses given alone was 30 min or less, and the sleep times following the ethanol plus GHB combinations were all greater than 60 min.

Unfortunately, one cannot determine whether the effects of the drug combination are synergistic or simply additive without more complete information about the GHB and ethanol dose–response curves when these drugs are given alone. While it seems possible from the data presented by McCabe et al. that they had synergistic effects, when dose–response curves for two drugs are very steep (like these curves are for ethanol or for GHB), the effects of doses of the two drugs that have little effect alone can have a very large effect when given in combination (e.g., 0.8 g/kg ethanol and 180 mg/kg GHB in this study; see Fig. 1B) from simple additivity. The steep dose–response curves of both ethanol and GHB for many of their behavioral effects may account for the serious effects seen clinically when seemingly "moderate" doses of the two drugs are combined (Graeme, 2000) rather than any synergistic actions of the drug combination.

We saw no evidence that NCS 382 could antagonize the rate-decreasing effects of GHB on FR responding in the rat. This is consistent with the results of a recent study by Cook et al. (2002) in mice. They studied the effects of 30 and 56 mg/kg NCS 382 in combination with various doses of GHB. Similar to our results with 3.2 and 32 mg/kg NCS 382 in combination with 300 mg/kg GHB, the effects of 30 mg/kg NCS 382 in combination with GHB were nearly identical to the effects of GHB alone. When the higher dose of NCS 382 was tested in combination with various doses of GHB, greater rate decreases were seen with the combination at lower GHB doses than were seen with these doses of GHB alone, indicating an additive or perhaps synergistic effect, but certainly not antagonism. Our results and the results of Cook et al. (2002) indicate that the ability of NCS 382 to antagonize the effects of GHB may be limited to a rather narrow range of conditions.

GHB is a drug with a complex pharmacology. GHB has potentially important therapeutic uses. On the other hand, GHB is emerging as an abused drug. The extent to which the potential therapeutic actions of GHB-like drugs can be separated from the abuse of GHB-like drugs will require the development of compounds that act specifically at the GHB receptor while not undergoing conversion to a GABA-active compound. Development of such compounds will permit us to begin to sort out which of GHB's many actions are the result of its actions at GHB, $GABA_A$, or $GABA_B$ receptors or result from actions at more than one of these receptors.

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