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Gamma-Butyrolactone's Discriminability and Effect on Low Rates of Lever Pressing by Rats: Alone and in Combination With D-Amphetamine and Naloxone

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McINTIRE, K. D., J. CLEARY AND S. WEINFURTER. *Gamma-butyrolactone's discriminability and effect on low rates of lever pressing by rats: Alone and in combination with d-amphetamine and naloxone.* PHARMACOL BIOCHEM BEHAV **30**(1) 45–53, 1988.—Three studies examined gamma-butyrolactone (Gbl) for benzodiazepine-like effects on low rates of food reinforced lever pressing by rats. A fourth study established Gbl's discriminative properties. Additionally, d-amphetamine or naloxone was administered with Gbl to test hypotheses of Gbl's neurochemical mechanisms of action. In Experiment 1, Gbl caused a dose-related decrease in lever pressing during a fixed-interval reinforcement schedule. Contrary to previous reports, neither d-amphetamine nor naloxone reversed the depressive effects of a high dose of Gbl on behavior. In Experiment 2, Gbl increased lever pressing which had been suppressed in the presence of a tone correlated with response-independent foot-shock (conditioned suppression). These results are consistent with, and extend, previous findings of benzodiazepine-like antipunishment effects of Gbl. However, in Experiment 3, when brief electric shocks were presented after each lever press, Gbl did not increase lever pressing. These results show the limited generality of Gbl's antipunishment effect compared to broad spectrum anxiolytics. Experiment 4, a drug discrimination study, showed rats readily discriminated 150 and 125 mg/kg Gbl from saline. However, neither d-amphetamine nor naloxone generalized to the Gbl lever. Amphetamine partially blocked the discriminative properties of 150 mg/kg Gbl, whereas naloxone had little effect on Gbl's discriminative properties. Thus, there is some support for a direct catecholaminergic role in Gbl-related seizures and little support for opioid receptor participation. The results of Experiments 1 and 4 indicate that Gbl's effects on behavior are complex, and are not accounted for by hypotheses involving only catecholamine and/or opioid mechanisms of action.

Gamma-butyrolactone Lever pressing d-Amphetamine Naloxone

GAMMA-butyrolactone (Gbl) is converted by plasma and liver lactonases into gamma-hydroxybutyrate (Ghb) [13–15], which occurs naturally in the mammalian brain with the highest concentration in the hypothalamus [23]. Parenterally administered Ghb is biologically active in the central nervous system: Levels of dopamine [21] and acetylcholine [4] are increased, activity in dopamine containing neurons is depressed and EEGs are significantly altered [20,22]. High doses of Gbl or Ghb induce a trance-like cataleptic state and seizure-like EEG activity. Indeed, Ghb-induced seizures have been suggested as a model for certain forms of epilepsy [20,22]. Additionally, Gbl's effects on EEG and catalepsy are reversed by d-amphetamine [20] or naloxone [22].

Although much is known about the neurochemistry of Ghb, there is a lack of systematic information concerning Ghb's behavioral effects. The few available behavioral studies indicate that Ghb may affect memory processes and may have some anxiolytic properties. In one memory investigation [5], Ghb or diazepam interfered with recall of random digit strings by humans. Ghb's anxiolytic potential is indicated by two animal studies. First, defensive behavior in isolation-reared mice is decreased and contact behavior is increased by 50 mg/kg Ghb [7]. The authors compared the effects of Ghb to those of chlordiazepoxide and suggested possible anxiolytic properties for Ghb. Second, 37.5 to 150 mg/kg Gbl increases the rate of lever pressing in rats which

has been suppressed by intermittent punishment [8]. This antipunishment effect is significant because such effects are prominent behavioral properties of anxiolytic benzodiazepines and barbiturates.

Ghb's effects in the two animal studies cited above indicate possible clinical or research potential as an anxiolytic substance. However, there are other possible explanations for the results and a more substantial elucidation of Ghb's behavioral profile is required. The present studies are designed to clarify the nature of Ghb's effects on low rates of lever pressing in rats. Similar procedures have been useful in defining behavioral characteristics of anxiolytic substances. Additionally, two of the studies will explore the degree to which the behavioral effects of high doses of Gbl may be reversed by either d-amphetamine or naloxone. These results have implications for understanding the mechanisms underlying Ghb's behavioral effects.

EXPERIMENT 1

Experiment 1 examined the hypothesis that antipunishment effects reported for Gbl relate to a general rate-dependent effect. The behavioral effect of a drug may be related to the rate with which the behavior is emitted [2,9]. As punished responding is reduced below its unpunished rate, it is possible that Gbl's previously reported antipunishment effects are independent of the aversive contingencies used to maintain the low rates. Simply, Gbl may equally increase all low rates of responding. To determine whether Gbl increases low rates of responding not suppressed by punishment, a fixed interval (FI) schedule was used. FI schedules result in a range of response rates from low to high and are useful in assessing relations between drugs and rate.

In addition to examining the effects of Gbl, Experiment 1 extended the analysis of previous reports that the behavioral effects of Gbl are reversed by d-amphetamine [20] and by naloxone [22]. Two doses of d-amphetamine and two doses of naloxone were administered alone, and in combination, with 300 mg/kg Gbl to determine if behavioral depression resulting from a high dose of Gbl is reversible, and if the reversibility is related to response rate.

METHOD

Subjects

Six albino Sprague-Dawley rats approximately 120 days of age were individually caged under constant illumination. They were food deprived to approximately 80% of their free-feeding body weights and were approximately 23 hr food deprived prior to sessions.

Apparatus

Sessions were conducted in each of three Coulbourn Instruments model E10-10 operant chambers. One wall of each chamber contained 6.0×4.0 cm feeder aperture, centrally located, 2.0 cm above the grid floor. When 45 mg food pellets were dispensed, the feeder light was illuminated for 1.0 sec. Levers located on each side of the feeder required approximately 0.15 N to depress. The right lever had no programmed consequences. Each chamber had its own sound-attenuating enclosure and a white masking noise was constantly present. Stimuli were presented, and data were collected by electromechanical equipment in an adjacent room.

Procedure

Sessions, which were conducted five or six days per week, began with the onset of the chamber's houselight and terminated with its offset. Sessions were 90 min in duration for Sessions 1–17 and 60 min thereafter. Sessions ended with the first food pellet after the scheduled session duration. After left-lever training in Session 1, the reinforcement schedule was changed during the first three sessions to FI 90 sec and food pellets were presented for the first lever press occurring after the scheduled interval. A timer malfunction beginning in the early sessions lengthened the fixed interval for one rat to approximately 150 sec. After the time problem was corrected, the interval for the rat was maintained at 150 sec for the duration of the experiment.

Beginning with Session 20 until the end of the experiment, drugs or saline were administered, IP, 10 min prior to sessions, with a minimum of five days between drug sessions. Three doses of Gbl (75, 150, and 300 mg/kg), two doses of d-amphetamine sulfate (2.0 and 4.0 mg/kg) and two doses of naloxone (5.0 and 10 mg/kg) were administered. Gbl was administered rather than Ghb because of Gbl's greater lipid solubility and faster metabolism after parenteral administration. Each drug was thoroughly suspended or dissolved in a glass container with 0.9% saline solution using an ultrasonic cleaning instrument. Several concentrations were made of each drug so that the total injection volume was approximately 0.2 ml. Gbl (300 mg/kg) was also administered in combination with amphetamine or naloxone. The amphetamine and naloxone doses were selected based on previous reports of Gbl's effects on EEG's and of significant drug interactions between Ghb and amphetamine or naloxone [20–22]. When drug combinations were administered, amphetamine or naloxone was administered five min before Gbl was administered and 15 min before sessions began. The order of injections was quasi-randomized for each rat. Each dose was administered once with the exception of 300 mg/kg Gbl which was administered twice. Saline vehicle was administered three times, once at the beginning, once near the middle, and once near the end of drug testing.

The following data were analyzed: First, mean lever presses per min (rate) across the entire session. Second, rate during each of three equal FI segments of the FI (i.e., 30 or 50 sec bins). Timing for the segments began with (a) session onset or (b) reinforcement. Lever presses occurring after the completion of third segment (i.e., reinforced responses) were not counted in the third segment.

RESULTS

Panel A of Fig. 1 shows that Gbl resulted in a dose-related reduction in lever pressing rate [repeated measures ANOVA: $F(3,15)=6.63$, $p<0.004$]. Figure 1, Panel B, shows the dose-related effects during the three segments of the interval.

The high rate of lever pressing during the third segment showed a dose-related decrease much like the overall rate data in Fig. 1, Panel A. The moderate rate which occurred during the middle segment showed small, but nonsignificant elevations at the two lowest doses of Gbl. The low rates occurring during Segment 1 were elevated by Gbl, resulting in a narrow dose effect curve similar to the punished rates reported by McIntire and Liddell [8]. A 3×4 repeated measures ANOVA found significant dose, segment, and dose × segment interaction effects [Dose: $F(3,15)=6.257$, $p<0.006$;

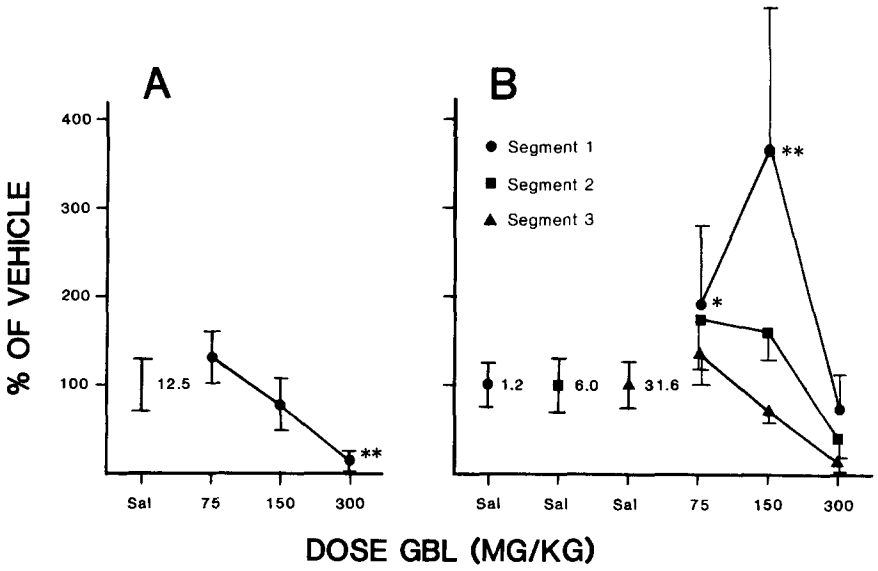

FIG. 1. (A) Mean FI lever presses per min at three doses of Gbl during Experiment 1. Data are grouped (n=6) and plotted as a percent of group mean responses per min during saline sessions. Error bars represent ±1.0 SE and are represented as a percent of mean saline response rate. Number next to saline error bar is overall mean lever presses per min during saline sessions. (B) Mean lever presses per min during each of three segments of the FI at three doses of Gbl. Data are grouped (n=6) and plotted as a percent of grouped mean lever presses per min during each segment for saline sessions. Error bars and saline lever pressing rates are presented as in panel A. *=$p<0.06$, **=$p<0.01$ (using Tukey's Method).

Segment: $F(2,10)=13.036, p<0.002$; Dose × Segment: $F(6,30)$, $p<0.009$].

Figure 2 shows the mean rate of lever pressing for both doses of d-amphetamine and naloxone as well as each drug combination. Figure 2 represents the drug combination data for only five rats. One rat died for reasons unrelated to the experiment. A 2×5 repeated measures ANOVA comparing the five Gbl-containing doses to five doses not containing Gbl was significant, $F(1,4)=30.6, p<0.006$. The other factor and the interaction comparisons were not significant. There was no evidence that d-amphetamine or naloxone elevated Gbl suppressed lever pressing.

DISCUSSION

The small but significant elevations in lever pressing rate during the first segment of the interval at 75 and 150 mg/kg Gbl may indicate that at least part of the antipunishment effect previously reported for Gbl can be related to a general tendency of subseizure and threshold seizure-inducing doses of Gbl to increase low rates of responding. However, many compounds may increase low response rates [2] without increasing punished responding, and it cannot be determined by the present study whether Gbl's antipunishment effect is part of a more general mechanism affecting all low response rates. This does not reduce the significance of these findings because anxiolytics such as benzodiazepines and barbiturates also increase low levels of unpunished responding [24] as well as punished responding.

The results of the drug combinations indicate limitations to previous reports that Gbl's behavioral effects may be reversed by d-amphetamine [20] or naloxone [22]. One or more of several procedural differences between this and previous studies may account for the differences in results. First, the operant lever pressing in the present study was observed across a range of rates and was maintained over a large number of experimental sessions. The 'behavioral' effects of Gbl reported to be reversed by d-amphetamine or naloxone are: visual response to light, visual and behavioral response to clicks of varying intensity, responses to tactile stimuli, electrical paroxysms and trance-like state [20,22]. The only behavior sufficiently quantified to allow evaluation demonstrated that 5 or 10 mg/kg naloxone reduced the catalepsy induced by 200 mg/kg Gbl in laboratory rats. Catalepsy was measured by "placing the animals hind legs on a cork 4 cm high and measuring the time the resultant posture was maintained" [22]. It may be that the reversibility of Gbl's behavioral effects by d-amphetamine or naloxone is limited to some relatively simple reflexive mechanisms and does not extend to schedule-controlled operant behavior.

In addition to response class, there are several other procedural differences between the present and previous studies which require consideration. The two most important of these are maximum Gbl dose and order of drug combination administration. Snead and Bearden [22] reported that 10 mg/kg naloxone administered IP to rats prior to (or after) 200 mg/kg Gbl prevented (or reversed) the behavioral effects of Gbl. Snead [20] found that 2–6 mg/kg d-amphetamine administered IV to rhesus monkeys prior to (or after) 400 mg/kg gamma-hydroxybutyrate prevented (or reversed) the behavioral effects of Ghb. Thus, the order of drug administration and the time between the first and second injection in the present study was likely insignificant. Although it is not possible to make direct dosage comparisons between studies using different species or even different ages and weights of the same species, the maximum dose of Gbl used in the present study was reasonably consistent with previous studies. Snead and Bearden [22] found 200 mg/kg Gbl caused immobility in rats in excess of five min duration. It is likely that a similar behavioral criterion was used by Snead [20]. In

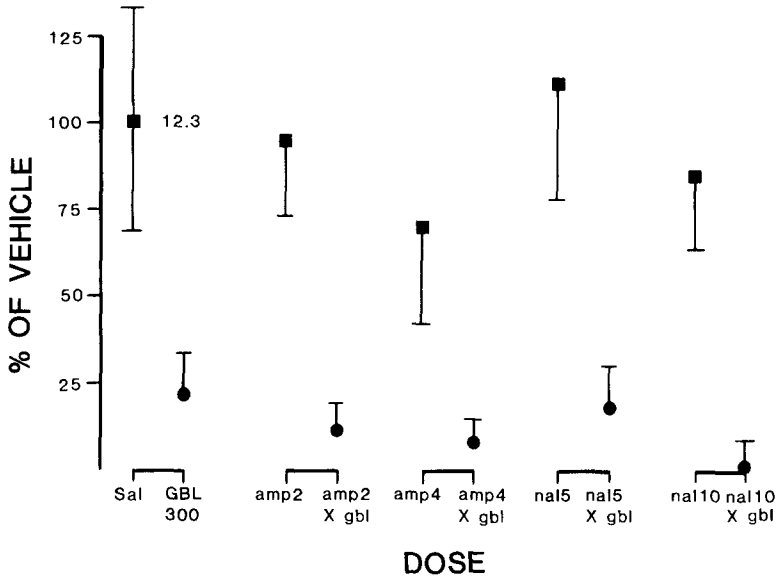

FIG. 2. Mean lever presses per min for saline, d-amphetamine, naloxone, Gbl and the drug combinations. Saline, 2.0 and 4.0 mg/kg d-amphetamine sulfate, and 5.0 and 10.0 mg/kg naloxone are presented as closed squares. Gbl 300 mg/kg and each dose of d-amphetamine or naloxone combined with Gbl are presented as closed circles. Data were grouped (n=5) and presented as a percent of the saline mean. Error bars and response rates represented as in Fig. 1.

the present study, 300 mg/kg Gbl was required to reduce responding appreciably below its base line. Only a moderate, unreliable overall rate reduction was evident with 150 mg/kg Gbl. Higher doses of Gbl were required in the present study to reduce behavior to levels comparable to those reported by Snead and Bearden, i.e., immobility. Thus, species differences, route of administration differences, dose variation, and Gbl vs. Gbh administration make direct comparisons among the studies difficult. Despite the differences, the 300 mg/kg doses of Gbl used in the present study was appropriate to induce behavioral depression and was behaviorally comparable to the doses used by Snead [20] and Snead and Bearden [22]. Additionally, the doses of amphetamine and naloxone were adequate to test for possible drug interactions on operant behavior. Five and 10 mg/kg naloxone are very high doses. Although Snead and Bearden [22] found that these doses reversed Gbl's behavioral effects, it is unlikely that any interaction is related to action at opiate receptors. Our amphetamine doses also covered the range of possible effective doses. Early on, we tried combining 8.0 mg/kg d-amphetamine with 300 mg/kg Gbl with several rats for one session. The combination resulted in complete behavioral depression for the entire session for all rats tested.

EXPERIMENT 2

Experiment 1 found that subseizure and threshold seizure-inducing doses of Gbl increase low rates of responding at the beginning of a FI reinforcement schedule. Experiment 2 extends the analysis of Gbl's effects on low response rates by using a conditioned suppression procedure. With a conditioned suppression procedure, a stimulus which is immediately followed by an aversive event (usually a brief shock) is presented independently of the ongoing behavior of the subject. Responding during the presence of the stimulus has no effect on the frequency of the aversive event. With repeated presentation, responding during the presence of the stimulus declines to near zero. Although procedural variations among experiments have complicated the interpretation of drug effects with the conditioned suppression procedure [6], Miczek [12] used a procedure similar to the one used here and clearly demonstrated that the anxiolytic chlordiazepoxide attenuates the suppressive effects of preshock stimuli. If Gbl increases responding with a conditioned suppression procedure, it will be the third demonstration of such increases under conditions in which anxiolytic compounds also increase responding.

METHOD

Subjects

The subjects were four 120-day-old hooded male rats bred from Blue Spruce stock at the University of Wisconsin-Eau Claire. The rats were individually caged, had free access to water, and were maintained on a reversed light-dark cycle (12 hr/12 hr). Each rat was food deprived to approximately 80% of its free-feeding body weight and was approximately 23 hr food deprived prior to each session.

Apparatus

Each of two Gerbrands operant chambers for rodents with grid floors contained two levers mounted 7.5 cm above the floor. The food hopper, into which 45 mg food pellets were dispensed, was centered on the wall between the levers 1.0 cm above the floor. Approximately 0.1 N was required to depress the left lever. The right lever was inoperative. Each chamber was housed in its own sound-attenuating enclosure with the sound of the exhaust fans constantly present. A Coulbourn solid-state digital-logic system in an adjacent room controlled all experimental functions and collected data. Coulbourn solid-state shockers delivered shocks to the grid floors.

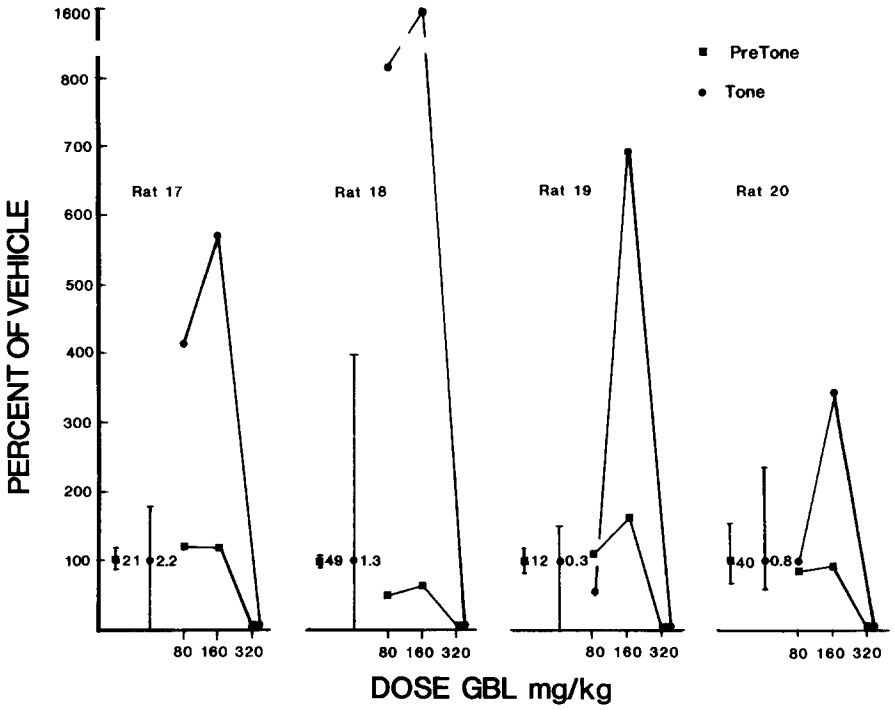

FIG. 3. Mean lever presses per min at each dose of Gbl for each rat presented as a percent of mean vehicle sessions. Brackets indicate range of Tone and PreTone rates during vehicle sessions. Numbers next to brackets are mean responses per min.

Procedure

Sessions were 48 to 50 min in duration and were conducted six or seven days per week. After lever press training in Session 1, the reinforcement schedule was changed to variable interval 30 sec for the remainder of the experiment. The mean interval between food pellets was 30 sec with a range of two to 120 sec. Beginning with Session 10, a 30 sec, 1.0 kHz tone of 85 dB was presented at 10 min intervals four times during a session. From Session 13 until the end of the experiment, the termination of the tone coincided with the presentation of a 0.3 mA shock of 0.25 sec duration. Behavior was allowed to stabilize under these conditions through Session 28. From Session 29 to the end of the experiment, Gbl, which has a $t^1/_2$ of approximately one hour in rats [13], was administered three times per week. The doses were 80, 160, and 320 mg/kg body weight Gbl. Each dose was administered three times, and the order of administration was randomized for each rat. All injections were IP, 10 min prior to sessions. The vehicle, 0.9% saline, was administered near the beginning, middle, and end of drug testing for each rat.

RESULTS

Figure 3 presents the mean rate of lever pressing during the presence of the 30 sec tone (Tone period) and during the 30 sec preceding the onset of the tone (PreTone period) as a percent of rate during vehicle control sessions for each rat. Gbl increased the rate of lever pressing during the Tone period at doses which had no effect on unsuppressed lever pressing during the PreTone period for all rats. The narrow dose response curve, with maximum effect at threshold seizure-inducing doses is similar to the results of Experiment 1 and the results of McIntire and Liddell [8] who used an intermittent punishment procedure. These results are significant because the rate increasing effects of Gbl generalize to conditions in which behavior is suppressed by stimuli associated with shocks presented independently of the subject's behavior. Moreover, previous reports of Gbl's antipunishment effects are extended.

EXPERIMENT 3

Gbl has been shown to increase low response rates under three conditions. First, rate increased during the initial segment of a FI reinforcement schedule in Experiment 1. Second, rate increased when responding was suppressed in the presence of an auditory stimulus which terminated with shock presentation in Experiment 2. Third, McIntire and Liddell [8] reported rate increases when responding was suppressed in the presence of an auditory stimulus during which every 10th lever press was followed by shock. In each of these three conditions, the Gbl-related rate increases had no substantial effect on response consequences (food or shock presentation). The present experiment compares Gbl's antipunishment effect with that of chlordiazepoxide HCl (Cdp) under conditions where shock is presented following *each* lever press. It is well established that Cdp and other anxiolytics increase responding under these conditions [3, 9–11, 16, 17, 24].

METHOD

Subjects

Three albino rats cross bred from Holtzman and Blue Spruce stock at the University of Wisconsin-Eau Claire were maintained as in Experiment 2.

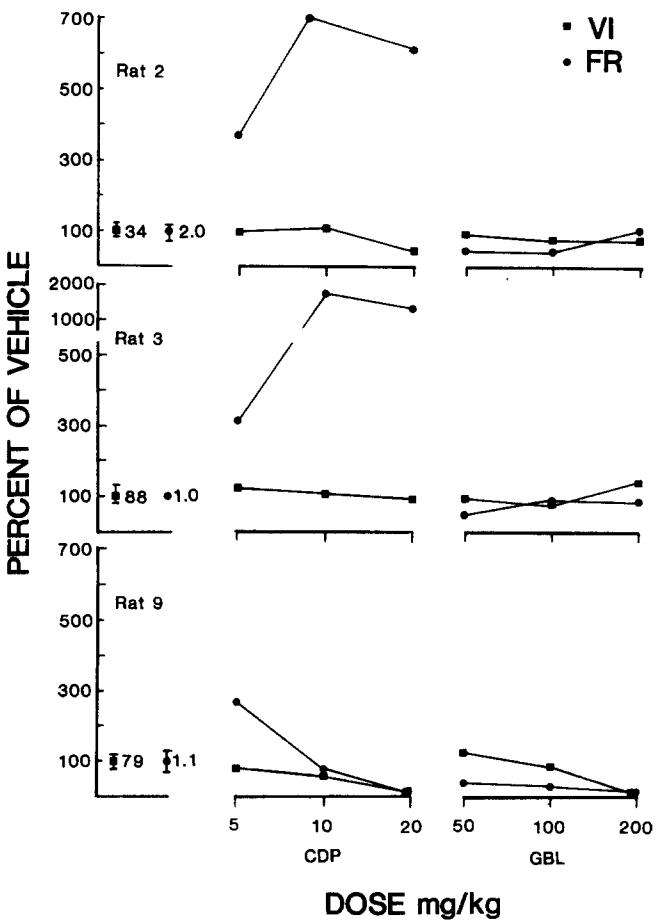

FIG. 4. Mean lever presses per min for each rat during each component of the reinforcement schedule at each dose of Gbl and chlordiazepoxide HCl. Brackets indicate range of vehicle sessions and numbers adjacent to brackets indicate mean responses per min.

Apparatus

Sessions were conducted in the same apparatus used in Experiment 2.

Procedure

After lever press training in the first session, all sessions were 48 min in duration and were conducted five or six days per week. Sessions began with the onset of the chamber's houselight and 90 dB white noise presented from a speaker mounted beneath the right lever. Session 2 initiated training on a two-component multiple schedule. During each variable interval (VI) component, lever pressing was reinforced with a mean interval of 30 sec between food pellets, with a range of two to 120 sec. During each fixed ratio (FR) component, a 90 dB, 1.0 kHz tone was present and food pellets were presented after every fifth lever press. Each VI component was present for ten min and alternated with FR components of two min duration. Sessions started with a VI component and ended with the termination of the fourth FR component. From Session 12 to the end of the experiment, a 0.3 mA foot shock coincided with each lever press during the FR components. This procedure differs from McIntire and Liddell [8] in two ways. First, the reinforcement schedule during the FR component in the present study was more dense. Second, shock amplitude was lower in the present study and shocks were presented more frequently. The reinforcement and shock values were selected to generate rates of lever pressing during the punished component which closely approximated those of McIntire and Liddell. Drug testing began after the performance of each rat stabilized, approximately 20 sessions.

During drug testing, the rats were injected 5–10 min prior to sessions with Gbl, Cdp, or vehicle (0.9% NaCl and water). Cdp solutions were mixed approximately 30 min prior to injections. The doses were 50, 100, or 200 mg/kg Gbl, and 5.0, 10.0 or 20.0 mg/kg Cdp. Each dose of each drug was administered once to each rat. The vehicle was administered three times: once at the beginning, near the middle, and near the end of testing. The order of doses for each rat was randomized and drugs were injected once or twice per week. Doses were selected from the mid-range of the estimated dose-response curve [8] for Gbl and extreme values were not observed.

RESULTS

Figure 4 shows the mean rate of lever pressing during each component of the reinforcement schedule for each rat at each dose of Gbl and Cdp.

Cdp showed the characteristic rate increasing effect for punished lever pressing at doses having little or no effect on unpunished lever pressing. Gbl had no rate increasing effect on punished lever pressing at any dose. These results contrast with Experiment 2 and with previous reports of Gbl related increases in suppressed lever pressing. Additionally, Gbl's lack of antipunishment activity in the present experiment contrasts with the robust antipunishment activity of Cdp and with several benzodiazepines, which typically increase rates of suppressed responding with schedules of continuous punishment [3].

EXPERIMENT 4

Experiments 1–3 clarified the effects of Gbl on low response rates. Additionally, Experiment 1 found that neither d-amphetamine nor naloxone reversed the rate-reducing effects of a high dose of Gbl. However, it is possible that the FI reinforcement schedule used in Experiment 1 controlled behavior in a manner which made the behavior insensitive to potential interactions of the drug combinations used. Experiment 4 addressed this issue using a drug discrimination procedure. Rats were trained to press one of two levers after injections of 150 mg/kg Gbl, and to press the other lever after injections of saline. A discriminability dose-response function was derived for Gbl. Additionally, d-amphetamine or naloxone was administered alone or in combination with 150 mg/kg Gbl. This discrimination procedure does not rely on response rate, and it has been shown to be sensitive to many drugs and drug combinations, e.g., [1]. Thus, it overcomes many of the limitations of Experiment 1 in assessing the interactions of Gbl with amphetamine and naloxone on operant behavior.

METHOD

Subjects

Eleven male Wistar rats between 150 and 200 days of age at the beginning of the experiment were maintained at 80% of the free-feeding weights as in Experiment 1. They were indi-

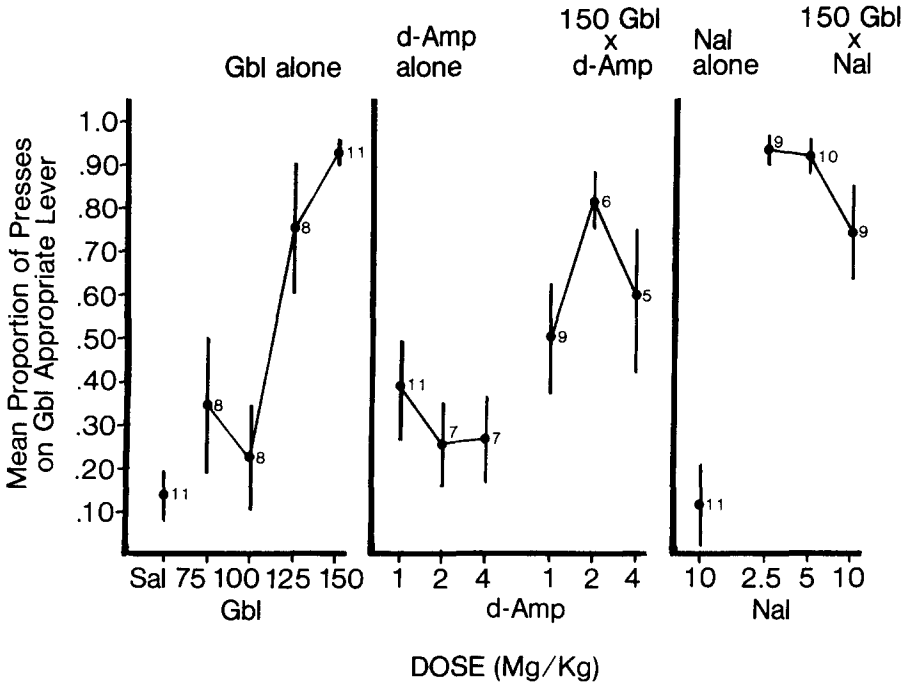

FIG. 5. Mean proportion of lever presses on the Gbl appropriate lever during each drug testing condition. Total lever presses for each rat on each lever in each session were converted to the following proportion: total presses on the "Gbl-appropriate" lever/total presses on both levers. The proportions for all rats were grouped for each session and means (●) and SE's (brackets) were calculated. Numerals next to means indicate number of rats emitting more than five presses on either lever and upon which the summary statistics are based. Only eight rats were tested at the intermediate doses of Gbl (75, 100 and 125 mg/kg). The saline value is calculated from the last three saline training sessions prior to the initiation of test sessions.

vidually caged under constant illumination and temperature with water freely available.

Apparatus

Sessions were conducted in two lever Coulbourn Instruments Model E10-10 operant chambers described in Experiment 1.

Procedure

Training. The rats were initially trained to press each lever under a continuous reinforcement schedule. Pressing on one of the levers was reinforced for an entire session and the reinforced lever alternated daily. Beginning with Session 7, the number of lever presses required for reinforcement was gradually increased to 20 (FR 20). When all rats were reliably pressing each lever, they were injected 30 min prior to sessions with either saline (0.9%) or 150 mg/kg Gbl suspended in saline. For six rats, pressing the left lever was reinforced following Gbl injection, and pressing the right lever was reinforced following a saline injection. For five rats the relationship between the injected substance and the reinforced lever was reversed. Reinforcement was programmed on only one of the two levers, the substance-"appropriate" lever. Pressing on the other lever has no programmed consequences. Gbl or saline sessions were randomly ordered with the restriction that no condition was repeated more than two sessions in succession. Sessions began at the onset of the chamber's 28 V houselight and terminated with the offset of the houselight and after presentation of 30 reinforcers.

Testing. Drug test sessions were intermittently scheduled among the training sessions for the remainder of the study. Test sessions followed three successive training sessions in which 62.5% of the lever presses were on the substance-appropriate lever prior to the presentation of the first reinforcer. Simply, if a rat emitted no more than 12 lever presses on the nonreinforced lever prior to reinforcement for three sessions in a row, the next session was a test session. For test sessions, all drugs or drug combinations were injected 30 min prior to the session and sessions terminated with the twentieth press on either lever or 60 min, whichever came first. Food pellets were not presented during test sessions. Injection volumes for all training and testing sessions were 1.0 ml/kg. The following drugs and drug combinations were tested: (a) Gbl at 75.0, 100.0, 125.0, and 150 mg/kg; (b) d-amphetamine at 1.0, 2.0, and 4.0 mg/kg; (c) naloxone at 10 mg/kg; (d) Gbl at 150 mg/kg in combination with 1.0, 2.0, and 4.0 mg/kg d-amphetamine; and (e) Gbl at 150 mg/kg in combination with 2.5, 5.0, and 10 mg/kg naloxone. d-Amphetamine and naloxone doses are in terms of the total salt. All drugs were mixed and stored separately as in Experiment 1. When drug combinations were administered, proper amounts of each suspension/solution were carefully drawn from the separate containers into a common syringe just prior to injection. Drug combinations were administered in a single injection to eliminate any potential discriminative stimuli which may have resulted from the procedural changes inherent in administering two injections as in Exper-

iment 1. Ghb's recorded effects have been reversed by preceding *or* following Ghb administration with amphetamine *or* naloxone [20,22]. As such, it is unlikely that any of the substances compete for a common site of action or receptor. Thus, there is no apparent pharmacological reason for using the common procedure of administering one compound prior to the second.

RESULTS

Each rat's total lever presses during test sessions were converted to a proportion of total presses emitted on the Gbl-appropriate lever. Figure 5 summarizes the individual proportions which were grouped and averaged for all rats emitting more than five lever presses on either lever during a session.

Doses of 150 and 125 mg/kg Gbl are clearly discriminable from saline. The function is steep and it is unclear whether doses of 75 to 100 mg/kg are discriminable from saline. However, the training dose (150 mg/kg) did not reduce responding appreciably below saline levels, i.e., there was no apparent seizure-related catalepsy.

Three doses of d-amphetamine were overall more saline-like than Gbl-like and 10 mg/kg naloxone was clearly indiscriminable from saline. The d-amphetamine × Gbl interaction provided only marginal support for catecholaminergic hypotheses of Gbl's behavioral effects, and was difficult to evaluate. One difficulty arises from the fact that high doses of amphetamine, alone or in combination with Gbl, depressed lever pressing by several rats to zero. Of the animals which did respond to the presence of the drug combinations, there is evidence that Gbl's discriminative properties may be partially reversed by d-amphetamine, at least at moderate doses. Second, the nature of the results are open to several interpretations. If the interactions had clearly been Gbl-like or amphetamine-like, the interpretation would have been relatively direct. However, lever pressing in the presence of the d-amphetamine × Gbl combinations was overall intermediate between Gbl-like and amphetamine-like lever pressing. There are at least three possibilities. First, d-amphetamine may have partially reversed the stimulus properties of Gbl. Second, an amphetamine-like stimulus may have been induced by the doses used. Third, the interaction may have resulted in a third state, unlike Gbl, amphetamine, or saline and the intermediate responding is a function of a novel stimulus being introduced (e.g., see Colpaert [1] for a recent discussion of drug discrimination procedures). The data for the naloxone combination are more easily interpreted. Two doses (2.5 and 5.0 mg/kg) of naloxone had no effect on the Gbl's discriminative properties and the highest dose of naloxone (10 mg/kg) reduced the total responding on the Gbl-appropriate lever to about 74%. This compares to an overall 93% Gbl-appropriate responding with the 150 mg/kg Gbl dose. These results are consistent with those of Experiment 1 which showed naloxone to be ineffective in reversing the effects of high doses of Gbl.

GENERAL DISCUSSION

Experiment 2 extended previous antipunishment reports for Gbl by showing that Gbl attenuates the suppressive effects of a stimulus followed by the presentation of shock. However, Experiment 3 showed no antipunishment effect when each response was punished. These results indicate that Gbl's antipunishment effects are not general or robust as compared to the benzodiazepines. Additionally, Experiment 1 showed that Gbl increases low rates of nonpunished responding, which indicates that Gbl's antipunishment effect may be related to a nonspecific rate-dependence. In conclusion, threshold seizure-inducing and subseizure doses of Gbl appear to increase low rates of operant responding, except where the low rate is maintained by frequent punishment. This profile contrasts with that of anxiolytic benzodiazepines and barbiturates which increase responding under schedules of continuous punishment. These results indicate that Gbl's or Ghb's anxiolytic potential are probably very limited and the mechanism underlying Gbl's antipunishment action differs from that of the benzodiazepines.

Experiment 1 provided no support for reports that amphetamine or naloxone reverse Gbl's depressive effects on behavior [20,22]. Overall, lever pressing rates remained the same with the Gbl × amphetamine combinations as with Gbl alone. These findings indicate that the well-identified catecholamine-related action of Gbl may not be solely or even primarily responsible for its behavioral effects. As with d-amphetamine, naloxone administered in combination with Gbl did not attenuate Gbl's rate reducing effects. Ten mg/kg naloxone combined with 300 mg/kg Gbl reduced rate to zero responses per min for all rats. The nature of the interaction remains unclear. Ghb levels increase with morphine administration and the increase is blocked by naloxone [18]. However, the very high dose of naloxone used (10 mg/kg) here, and in previous reports [22], makes it uncertain that the drug interaction is related to opiate receptors.

Experiment 4 demonstrated that subseizure-inducing doses of Gbl have discriminative properties which are probably related to seizure-inducing mechanisms. The dose-response function is steep and there is little to support a previous claim that doses as low as 10 mg/kg Ghb are discriminable [4]. Experiment 4 was consistent with Experiment 1 in that neither d-amphetamine nor naloxone clearly reversed the discriminative properties of 150 mg/kg Gbl. These results show limitations to catecholaminergic and opiate receptor explanation of Gbl's behavioral effects and indicate that other, perhaps GABA-related mechanisms, should be more thoroughly explored.

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