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Gamma-Butyrolactone Increases the Rate of Punished Lever Pressing by Rats

KENNETH D. McINTIRE AND BARBARA J. LIDDELL

Psychology Department, University of Wisconsin-Eau Claire, Eau Claire, WI 54701

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McINTIRE, K. D. AND B. J. LIDDELL. *Gamma-butyrolactone increases the rate of punished lever pressing by rats.* PHARMACOL BIOCHEM BEHAV 20(2) 307–310, 1984.—Four rats lever pressed for food on a two component multiple FR-VI schedule of reinforcement. In the FR component a brief electric shock coincided with the presentation of food. After lever pressing stabilized in the presence of the shock, drugs were administered in two phases. In Phase 1, one of four doses of either gamma-butyrolactone or sodium pentobarbital was injected before sessions. Both drugs increased lever pressing rates during the shocked component of the schedule at doses which did not affect lever pressing rates during the unshocked component. In Phase 2, one of four doses of a mixture of the two compounds was injected. The drug mixture increased rates of punished lever-pressing to levels similar to those reached in Phase 1. These results confirm previous findings for sodium pentobarbital and indicate that gamma-butyrolactone warrants further investigation into its behavioral properties.

Gamma-butyrolactone Gamma-hydroxybutyrate Conflict Punishment Lever pressing
Pentobarbital Benzodiazepines Rats

GAMMA-BUTYROLACTONE (GBL) was administered to four rats that received food pellets and shocks for lever pressing during one component of a multiple reinforcement schedule. The effect of GBL was compared to that of sodium pentobarbital (PB), a substance which increases the rate of punished responding [5].

Positively reinforced lever-pressing that is suppressed by response-dependent shock (punishment) increases in rate following the administration of certain barbiturates, benzodiazepines, and meprobamate. Other compounds reported to increase the rate of punished responding do so only (a) to a very small extent, (b) over a limited dose range, or (c) at doses which reduce the rate of unpunished responding and/or induce ataxia [1, 3, 11]. In contrast, the increase in punished responding which occurs following PB [5] or chlordiazepoxide administration [6] is robust and reliable; it occurs at doses which do not affect unpunished responding; and it is not a simple manifestation of the rate dependence effect [10].

GBL is metabolized to gamma-hydroxybutyrate (GHB), a natural constituent of the mammalian brain, by liver and plasma lactonase [8,9]. Possibly as a result of GBL's ability to depress activity in dopaminergic neurons and increase dopamine levels, there has been conjecture as to behavioral therapeutic uses for GBL. Walters and Roth [15] reviewed several studies in which GBL or GHB was administered to psychiatric patients. They concluded that the studies were poorly controlled and little evidence indicated that GBL might be useful in the treatment of schizophrenia. However, they commented that it would be worthwile to investigate GHB further as a possible antianxiety agent.

Even though research on the neurochemistry of GBL and GHB typically uses animal subjects [13], there are few instances of behavioral research. The only related behavioral analysis demonstrated that when isolation reared mice were placed in the presence of other mice, 50 mg/kg GHB increased contact behavior and reduced defensive behavior [7]. The present experiment furthered the behavioral research by determining whether GBL would increase the rate of punished lever-pressing in rats using a procedure which is sensitive to the effects of some antianxiety agents.

METHOD

Subjects

The subjects were four male, albino rats approximately 150 days of age, cross-bred from Holtzman and 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 one week prior to the start of the experiment and was approximately 23 hr food deprived prior to the start of each session.

Apparatus

Each of two Gerbrands operant chambers for rodents with grid floors contained two adjustable-force 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 right lever. The left lever was inoperative. Each chamber was housed in its own sound attenuating enclosure with the noise of the exhaust fan constantly present. A Coulbourn solid-state system in an adjacent room controlled all experimental functions and collected data. Coulbourn solid-state scrambling shockers delivered shocks to the grid floors.

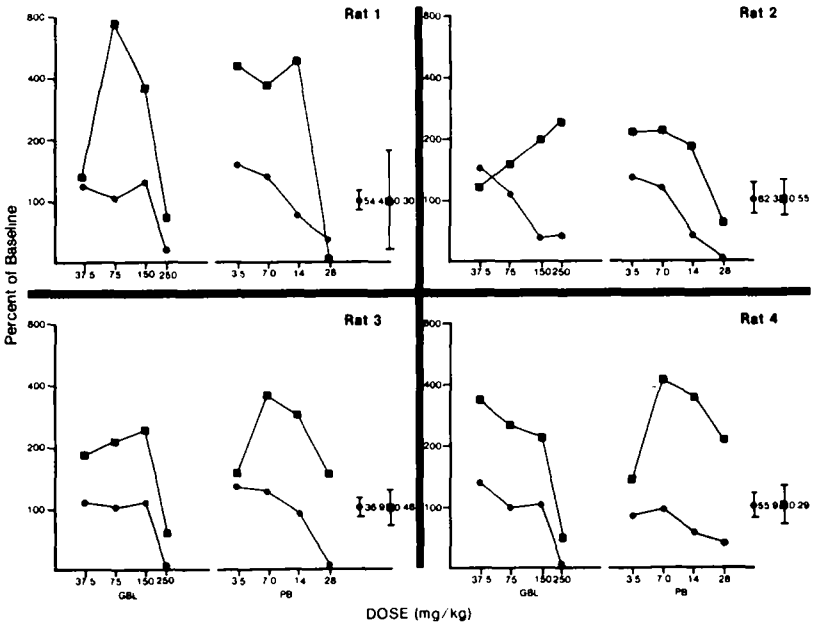

FIG. 1. Mean rate of lever pressing at each dose of GBL and PB for each rat as a percent of the mean of the saline sessions. Closed circles represent rate of responding on the VI component and squares represent responding on the punished FR component of the reinforcement schedule. Error bars represent ±1 S.E. for five or six saline sessions plotted as a percent of the mean of the saline sessions. The numbers adjacent to the error bars are mean lever presses per minute. The saline S.E.s (mean responses/min) for the VI and punished FR components of the reinforcement schedule respectively, for each rat were: Rat 1, 8.9 and 0.24; Rat 2, 16.7 and 0.16; Rat 3, 5.2 and 0.14; Rat 4, 11.7 and 0.10.

Procedure

Pre-drug training. There were 29 sessions of pre-drug training. After lever press training in the first session, all sessions were 48 min in duration and were conducted five days per week. Sessions began with the onset of the chamber's 28 V DC houselight and 90 dB white noise presented from a speaker located beneath the right lever. Session 2 initiated training on a two-component multiple schedule of reinforcement similar to one used by Davidson and Cook [4]. During each variable-interval (VI) component, lever pressing was reinforced with a mean interval between pellets of 30 sec with a range of two to 120 sec. During each fixed-ratio (FR) component, a 90 dB, 1000 Hz tone was present and food was presented after every tenth 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 20 to the end of the experiment a 0.5 mA, 0.25 sec foot shock coincided with the presentation of a food pellet during the FR components.

Drug testing—Phase 1. The first phase of drug testing began with Session 30. The rats were injected IP five min before each session with GBL, sodium PB, or 0.9% NaCl and water solution. The doses were 37.5, 75, 150, and 250 mg/kg GBL and 3.5, 7.0, 14.0, and 28.0 mg/kg sodium PB. The GBL was thoroughly suspended in a 0.9% saline solution before each injection. The sodium PB was administered as the commercially available, injectable product, Nembutal (Abbott) (50 mg/ml solution). The drug administration schedule was arranged in blocks of seven sessions, and sessions were conducted from three to five times per week. Each block began with a saline session. In the remaining six sessions of each block GBL and PB sessions alternated. Each drug was administered once or twice per week dependent upon (a) the number of sessions during the week and (b) whether saline was administered during the week. The order of doses for each rat was quasi-randomized with the restriction that no dose was repeated within a four day period. Each rat received three injections at the lowest dose of each drug and four or five injections at each remaining dose. Thirty-four days intervened between Sessions 56 and 57 in Phase 1 for reasons unrelated to the experiment. Prior to continued drug testing in Session 57, the rats were trained for five sessions in order to restabilize lever pressing.

Drug testing—Phase 2. The second phase of drug testing began five to seven days after the completion of Phase 1 with no intervening sessions. Each rat was injected five min before each session with a GBL/PB mixture or vehicle. GBL was added to the Nembutal solution such that each ml mixture contained 345 mg GBL and 34.5 mg sodium PB. The vehicle in Nembutal is water, alcohol 10%, and propylene glycol 40%. The doses of the mixture were: Dose 1, 17.5 mg/kg GBL and 1.75 mg/kg PB; Dose 2, 35 mg/kg GBL and 3.5 mg/kg PB; Dose 3, 70 mg/kg GBL and 7.0 mg/kg PB; and Dose 4, 140 mg/kg GBL and 14.0 mg/kg PB. Drug administration was arranged in two blocks of five sessions each. Each block began with a vehicle session and for the next four sessions, one of the four doses of the drug mixture was administered. The order of administration was quasi-randomized for each rat in each block. Sessions were conducted on consecutive days with two days intervening between blocks.

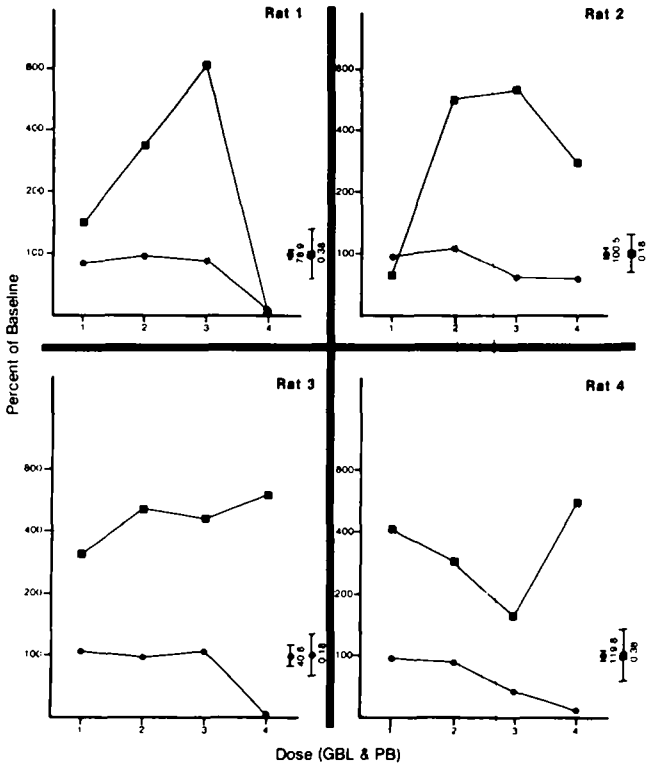

FIG. 2. Mean rate of lever pressing at each dose of the drug mixture presented as in Fig. 1 except that the error bars represent the range of the two vehicle sessions. The vehicle ranges (mean responses/min) for the VI and punished FR components of the reinforcement schedule respectively, for each rat were: Rat 1, 6.0 and 0.25; Rat 2, 2.0 and 0.125; Rat 3, 15.2 and 0.125; Rat 4, 8.5 and 0.25.

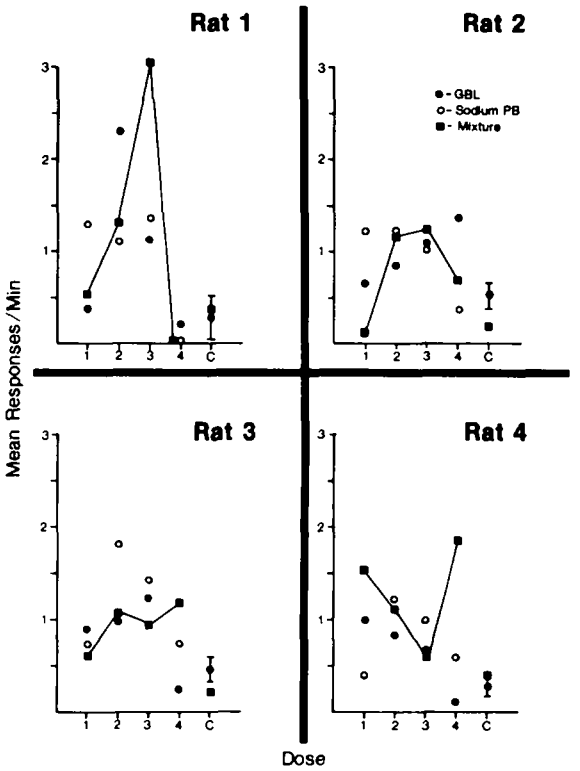

FIG. 3. Mean lever presses/min for each rat at each dose of each drug during the punished FR component of the reinforcement schedule. 1, 2, 3, and 4 represent doses in increasing magnitude as used in Phase 1 and Phase 2. C represents mean rate of lever pressing in the control condition of Phase 1 (closed circle) and Phase 2 (closed square). Error bar is ±1 S.E. for the saline sessions in Phase 1.

RESULTS

Figure 1 plots the rate of lever pressing during each component of the reinforcement schedule in Phase 1. Each point is plotted as a percent of the saline condition. Despite the subject differences, at some doses all rats lever pressed at a higher rate during the punished FR component after drug administration. Most of the increases occurred at doses which did not affect the rate of unpunished responding during the VI component. However, the effective range of doses for both compounds was relatively narrow (2–3 doublings) when compared to the dose range of chlordiazepoxide [11]. Despite the large relative increases in punished lever-pressing rate, absolute rate did not approach prepunishment levels for any rat (55, 116, 107, and 61 responses/min for Rats 1–4 respectively). The PB data were analyzed for tolerance effects resulting from repeated drug administration. Mean rates of lever-pressing at each dose during the first half of the experiment (seven or eight injections per rat) were compared to mean rates at each dose during the second half of the experiment (seven or eight injections). There were no consistent trends toward either increased *or* decreased rates of lever pressing at any dose in either component of the reinforcement schedule. The results of Phase 1 confirm previous findings for PB [5] and extend previous research by showing that GBL was as effective as PB in increasing the rate of punished lever pressing.

Figure 2 shows the rate of lever pressing during each component of the reinforcement schedule in Phase 2. Rate is plotted as a percent of the vehicle condition. The rats lever pressed at a higher rate during the punished FR component after the administration of most doses of the drug mixture. Informal observation of the effects of the drug mixture before and after sessions showed activity levels to be greatly increased, even at the highest dose where the rats had difficulty maintaining balance. This increase was particularly apparent in Rat 4, whose dose response curve for punished lever pressing shows a sharp reversal at Dose 4. GBL and PB have pharmacologically different effects [16] and the mechanism of the interaction is unknown.

It is clear that the drug mixture increased the rate of punished lever-pressing for all rats. However, the control rate was lower in Phase 2 than in Phase 1 for two rats (Rats 2 and 3). This difference in control rate makes it difficult to clearly interpret the drug relationship. The lower rate was not a function of an unstable baseline in Phase 1, i.e., a training effect. First, Rats 1 and 4 maintained a mean rate of punished lever-pressing within the control S.E.s obtained during Phase 1. Second, no rat consistently increased or decreased rate of lever pressing across the five or six saline sessions in Phase 1. The reduced rate of punished lever-pressing for Rats 2 and 3 was probably not the result of the control vehicle used in Phase 2. Ethanol *increases* the rate of punished responding, but only at doses which induce ataxia and reduce the rate of unpunished responding, e.g., 1 or 2 g/kg [2]. The doses of ethanol in our vehicle didn't exceed approximately 0.04 mg/kg. The most probable explanations for the change in control rate from Phase 1 to Phase 2 are that five to seven days without sessions intervened between Phase 1 and

Phase 2 and there were only two sessions at each dose in Phase 2.

Figure 3 replots punished lever-pressing rate for each rat as mean responses per min. The large relative rate increases in Phase 2 for Rats 2 and 3 were a function of the lower control rates in Phase 2. Despite subject differences, the absolute rate for each rat in Phase 2 was generally comparable to the rate obtained for GBL and PB separately in Phase 1. The exceptions being the reversals at Dose 4 for Rats 3 and 4. Though the specific nature of the relationship between the compounds remains to be clarified, Figs. 2 and 3 clearly indicate that there was not a strong synergistic effect, nor did the compounds inhibit each others effect of increasing the rate of punished lever-pressing.

DISCUSSION

GBL, PB, and the mixture all increased the rate of punished lever-pressing above control vehicle levels. These results are significant because GHB occurs naturally in the mammalian brain [9] and a growing body of evidence indicates that GHB is metabolically active [13,15]. In addition to its effect on dopaminergic neurons, GHB increases brain levels of GABA and acetylcholine. The latter may result from the depression in activity in dopamine neurons [13]. Morphine increases GHB levels in the brain and the effect is blocked by naloxone [13]. Additionally, naloxone pretreatment abolishes the seizure activity, behavioral abnormalities, and increased striatal dopamine content produced by GBL [14]. The present findings indicate that GHB may have some interesting behavioral effects in addition to its neurochemical properties. Though the scope of the present experiment was relatively limited, the results indicate that a further analysis of the behavioral properties of GBL is warranted because it may help to elucidate the behavioral mechanisms of other frequently investigated compounds.

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