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Evaluation of the reinforcing and discriminative stimulus effects of 1,4-butanediol and γ-butyrolactone in rhesus monkeys

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Abstract

Metabolic precursors and prodrugs of γ-hydroxybutyrate (GHB), including 1,4-butanediol (BDL) and γ-butyrolactone (GBL), have sedative and anesthetic effects and might have positive reinforcing effects. BDL and GBL were evaluated using behavioral procedures that measure positive reinforcing effects and discriminative stimulus effects of drugs that modulate γ-aminobutyric acid (GABA) at the $GABA_A$ receptor complex. One group of rhesus monkeys could respond for saline or the barbiturate methohexital (i.v.) in a self-administration paradigm. Two other groups of monkeys discriminated the barbiturate pentobarbital (i.g.) or the benzodiazepine midazolam (s.c.) from saline in a drug discrimination paradigm; another group of monkeys was treated with the benzodiazepine diazepam (5.6 mg/kg/day, p.o.) and discriminated the benzodiazepine antagonist flumazenil (s.c.) from vehicle. In self-administration experiments, methohexital and not BDL (0.1–3.2 mg/kg/injection) or GBL (0.1–3.2 mg/kg/injection) reliably maintained responding above saline levels. BDL and GBL, up to doses that suppressed responding, did not substitute for pentobarbital, midazolam or flumazenil. The onset of action for both drugs to decrease response rate was delayed (90 min for GBL and 150 min for BDL). These results suggest that any abuse-related effects of BDL and GBL are qualitatively different from the abuse-related effects of $GABA_A$ receptor modulators and further indicate that BDL and GBL do not have positive reinforcing effects in rhesus monkeys experienced with self-administration of a short-acting sedative-hypnotic.

Keywords: Barbiturate; Benzodiazepine; 1,4-Butanediol; Drug discrimination; γ-Butyrolactone; Self-administration

1. Introduction

γ-Hydroxybutyrate (GHB) is sedative-hypnotic, increases growth hormone and until recently, was available in various products indicated for weight loss, muscle building and sleep induction (Laborit, 1973; Dyer, 1991; Mason and Kerns, 2002 for review). However, increasing reports of marked sedation, anesthesia and coma following ingestion of GHB have led to restrictions on its availability including classification in the United States as Schedule I (Controlled Substances Act). GHB can enter the brain and be metabolized to the inhibitory neurotransmitter γ-aminobutyric acid (GABA; Maitre, 1997 for review). In addition, GHB itself is thought to be a neurotransmitter with specific binding sites that are localized to rat hippocampus and cerebellum (Snead and Liu, 1984; Mehta et al., 2001). Metabolic precursors and prodrugs of GHB, including 1,4-butanediol (BDL) and γ-butyrolactone (GBL), also are sedative-hypnotic (Rambourg-Schepens et al., 1997; Zvosec et al., 2001). The behavioral effects of GHB and its precursors are similar though not identical (Winter, 1981), and conversion of the precursors to GHB in vivo might suggest that GHB is responsible for the behavioral effects of the precursors (Roth et al., 1966; Roth and Giarman, 1968; Winter, 1981).

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Few studies have examined GHB and its metabolic precursors in behavioral assays that measure abuse-related and therapeutic effects of drugs. Self-administration is a commonly used assay that can measure positive reinforcing effects (e.g., Schuster and Thompson, 1969). Drug discrimination is another assay that can be used to assess abuse liability by determining whether drugs are qualitatively the same or different; that is, a drug sharing discriminative stimulus effects with a drug of abuse might also have abuse liability. In addition, drug discrimination is useful for defining the mechanism(s) of drug action; that is, drugs that share discriminative stimulus effects typically have a common mechanism of action. For example, drugs that allosterically facilitate GABA at the $GABA_A$ receptor complex (e.g., positive $GABA_A$ receptor modulators pentobarbital and midazolam) generally share discriminative stimulus effects (Woolverton and Nader, 1995; McMahon et al., 2001; McMahon and France, 2003). In addition, drugs that allosterically inhibit GABA at the $GABA_A$ receptor complex (e.g., negative $GABA_A$ receptor modulators), or that antagonize benzodiazepines, share discriminative stimulus effects with flumazenil in diazepam-treated monkeys (Gerak and France, 1999).

The abuse-related effects of GHB were examined previously in rhesus monkeys experienced with self-administration of a barbiturate and in rhesus monkeys trained to discriminate a positive $GABA_A$ receptor modulator (e.g., pentobarbital or triazolam) from saline (Woolverton et al., 1999). In that study, GHB was not self-administered and did not share discriminative stimulus effects with the barbiturate pentobarbital or the benzodiazepine triazolam in untreated monkeys; GHB also did not substitute for flumazenil in diazepam-treated monkeys. Collectively, these studies might suggest that GHB is not a positive $GABA_A$ receptor modulator and that its apparent abuse liability differs from short-acting sedative-hypnotics (but see Winter, 1981; Colombo et al., 1998). The present study used similar behavioral procedures to evaluate the abuse-related effects of BDL and GBL and to further evaluate whether the behavioral effects of BDL and GBL result from modulation of GABA at the $GABA_A$ receptor complex.

2. Methods

2.1. Reinforcing effects

2.1.1. Subjects

Four adult male rhesus monkeys (*Macaca mulatta*), weighing between 8.0 and 12.0 kg, were housed individually in stainless steel cages. Water was available continuously and Purina monkey chow was provided daily before sessions. Monkeys were surgically prepared with indwelling silicone rubber catheters using 10 mg/kg, i.m., ketamine and 2.0 mg/kg, i.m., xylazine as anesthetics. Catheters were implanted in jugular (internal or external), femoral or brachial veins as necessary. Catheters passed s.c. to the mid-scapular region, exited the body and continued, through a hollow restraining arm, to the outside rear of the cage.

2.1.2. Apparatus

The restraint and catheter protection devices have been described previously (Deneau et al., 1969). Each monkey wore a tubular stainless steel harness that protected the exit site of the catheter and allowed relatively unrestricted movement within the cage. A Teflon cloth jacket (Alice King Chatham Medical Arts, Los Angeles, CA) provided further protection of the catheter for some animals. The harness was connected to a flexible spring arm that carried the catheter to the back of the cage, where it joined tubing that passed through a roller infusion pump (Model MHRK 55; Watson and Marlow, Falmouth, UK).

Monkeys were individually housed in stainless steel cages, measuring $83.3 \times 76.2 \times 91.4$ cm deep. A 15.4-cm^2 stimulus panel was located on the side of each cage, approximately 10 cm from the front and 19 cm from the bottom of the cage. Across the top of the stimulus panel, 1.5 cm apart, were three circular, 2.5 cm in diameter, translucent plastic stimulus lights that could be illuminated by 5 W colored bulbs. The two side lights could be illuminated red and the center light green. Below each of the two red stimulus lights was a response lever (Model 121-07; BRS-LVE, Beltsville, MD) capable of being operated by a force of 0.010 to 0.015 N. Experimental control was provided by an IBM PS/2 computer programmed with Med-PC software (Med-Associates, Fairfield, VT) that was located in an adjacent room.

2.1.3. Procedure

Reinforcing effects of BDL and GBL were evaluated in a substitution self-administration procedure in monkeys who were experienced with i.v. self-administration of methohexital. Test sessions and baseline sessions had the same general structure. At the start of each session, a red light was illuminated over one of two levers. When a monkey completed the fixed-ratio requirement of 10 presses on that lever (fixed-ratio [FR] 10), a 5-s, 1.0-ml injection of saline, sodium methohexital (0.1 mg/kg) or a test compound was delivered. The red light was extinguished and a center green light was illuminated for the duration of the infusion. Each injection was followed by a 10-s timeout during which stimulus lights were extinguished and responding had no programmed consequence.

Twice daily experimental sessions lasted 130 min each. On approximately half of the baseline sessions, the monkeys could respond for saline. All animals showed clear and consistent differential responses to saline and methohexital before test compounds were evaluated. In test sessions, a dose of the test compound was made available for one session. Other conditions were similar to those of the baseline sessions.

2.2. Discriminative stimulus effects

2.2.1. Pentobarbital discrimination

2.2.1.1. Subjects. Four adult male rhesus monkeys, weighing between 6.5 and 12.0 kg, were housed individually in stainless steel cages; water was available continuously. They received 120 to 200 g of Teklad monkey chow after each session and a chewable vitamin three times per week. The monkeys had been trained previously to discriminate pentobarbital from saline in a two-lever, discrete trial shock avoidance procedure. All monkeys had received other test drugs prior to BDL and GBL.

2.2.1.2. Apparatus. During experimental sessions animals were seated in primate restraint chairs and placed inside sound-attenuating cubicles. All chairs were fitted with shoes containing brass plates in the soles that permitted delivery of electric shock produced by a shock generator (SG 903 BRS/LVE, Laurel, MD). Chambers were equipped with two response levers (PRL-001, BRS/LVE) mounted on one wall. There were four white lights above each lever. Chambers were illuminated with ceiling-mounted 40 W incandescent lights. Experimental events were programmed and recorded with an Apple Macintosh II computer that was located in an adjacent room.

2.2.1.3. Procedure. The training and test procedures have been reported in detail elsewhere (Woolverton et al., 1994). A monkey was placed in the chair and either saline (1–2 ml) or pentobarbital (5.6 [one monkey] or 10.0 mg/kg) was administered intragastrically (i.g.) via a nasogastric tube, followed by a 1.5 ml saline flush. Fifty-five minutes after infusion, the monkey was placed into the experimental chamber. The session began with a 5-min timeout that was followed by 30 trials. On each trial, the house light and lever lights were illuminated and responding on the correct lever postponed scheduled shock and extinguished the lights. Incorrect responses reset the response requirement on the correct lever. The correct lever was determined by the presession infusion (drug or saline). If the response requirement (FR 5) was not satisfied on the correct lever within 10 s of the onset of the lights, shock (250 ms, 5 mA) was delivered. If the response requirement was not satisfied within 4 additional s, a second shock was delivered and the trial ended. The session was terminated when two shocks were delivered in two consecutive trials or after 30 trials. Consecutive trials were separated by a 30-s timeout.

Sessions were conducted 5 days a week according to the following schedule: SDDSS, DSSDD, where S denotes sessions preceded by saline and D denotes sessions preceded by drug. Discrimination training continued until at least 90% of the responses in the first trial were on the correct lever and subjects avoided shock on at least 90% of the trials (27/30) for seven out of eight consecutive sessions. When subjects failed these criteria, the training sequence was continued until the criteria were satisfied. Test sessions were identical to training sessions except that test drugs were administered and completing the response requirement on either lever postponed scheduled shock. Doses of GBL (10–300 mg/kg) were administered 60, 120 and 180 min before test sessions and doses of BDL (30–560 mg/kg) were administered 60, 120, 180 and 240 min before test sessions.

2.2.2. Midazolam and flumazenil discriminations

2.2.2.1. Subjects. Four female and two male adult rhesus monkeys, weighing between 3.5 and 10.0 kg, were housed individually in stainless steel cages. Water was continuously available and monkeys received primate chow (Harlan Teklad, Madison, WI) daily as well as fresh fruit and peanuts several days per week.

2.2.2.2. Apparatus. Monkeys were seated in chairs that provided restraint at the neck. For midazolam discriminating monkeys, chairs were equipped with shoes containing brass plates, to which brief (250 ms, 3 mA) electric shock could be delivered from a.c. generators located adjacent to the chambers. During experimental sessions, chairs were located in sound-attenuating, ventilated chambers that were equipped with several response levers, a food cup and an array of stimulus lights. Experimental control was provided by a computer and Med-PC software.

2.2.2.3. Procedure. For the midazolam discrimination ($N=3$), monkeys discriminated between s.c. injections of 0.32 mg/kg of midazolam and vehicle while responding under an FR 10 schedule of stimulus-shock termination (Lelas et al., 1999). Daily sessions comprised 2–8, 15-min cycles, each comprising a 10-min timeout, during which the chamber was dark and lever presses had no programmed consequence, followed by a response period, during which the chamber was illuminated red and shocks were scheduled to occur every 15 s. Monkeys could prevent scheduled shock for 30 s by responding 10 times on the correct lever as determined by the s.c. injection administered during the first min of the 10-min timeout (e.g., left lever after saline, right lever after midazolam). The response period ended after 5 min or the delivery of electric stimuli, whichever occurred first. Responses on the incorrect lever reset the response requirement on the correct lever.

For the flumazenil discrimination ($N=3$), monkeys drank a solution containing 5.6 mg/kg of diazepam 3 h prior to daily sessions in which they discriminated between s.c. injections of vehicle and either 0.1 (one monkey) or 0.32 mg/kg of flumazenil while responding under an FR 5 schedule of food presentation (Gerak and France, 1999). Daily sessions comprised 2–8, 15-min cycles each comprising a 10-min timeout, during which the chamber was dark and lever presses had no programmed consequence, followed by a response period, during which the chamber was illuminated green and monkeys could receive food by

responding five times on the correct lever as determined by the s.c. injection administered during the first min of the 10-min timeout (e.g., left lever after vehicle, right lever after flumazenil). The response period ended after 5 min or the delivery of 10 food pellets, whichever occurred first. Responses on the incorrect lever reset the response requirement on the correct lever.

For the midazolam and flumazenil discriminations, test sessions were conducted following training sessions in which $\geq 80\%$ of the total responses occurred on the lever designated correct by the injection administered during the first min of the cycle and fewer than 10 (midazolam discrimination) or five (flumazenil discrimination) responses occurred on the incorrect lever prior to completion of the FR response requirement on the correct lever. Prior to each test, these criteria had to be satisfied for training sessions during which the training drug and saline injections were administered. The type of training session preceding test sessions varied nonsystematically. Test sessions were identical to training sessions except that various doses of the training drug were administered during the first min of each timeout (cumulative dosing procedure); otherwise, various doses of a test compound were administered prior to or during the first min of the first cycle followed by saline or sham injections during the first min of subsequent cycles (time course procedure). In monkeys discriminating midazolam, 10 consecutive responses on either lever postponed the shock schedule; in monkeys discriminating flumazenil, five consecutive responses on either lever resulted in food delivery. Substitution for the training drug was defined as $\geq 80\%$ responding on the drug-appropriate lever.

BDL (100–560 mg/kg in midazolam discriminating monkeys; 32–320 mg/kg in flumazenil discriminating monkeys) was administered at the beginning of and 120-min prior to 120-min sessions. GBL (100–560 in midazolam discriminating monkeys; 100–320 mg/kg in flumazenil discriminating monkeys) was administered s.c. at the beginning of 2-h sessions.

2.3. Drugs

For self-administration, four doses (0.1, 0.3, 1.0, and 3.2 mg/kg/injection) of BDL and GBL were studied i.v. in four and three monkeys, respectively. BDL and GBL were dissolved in saline.

For the pentobarbital discrimination, pentobarbital was mixed daily by diluting Nembutal (Abbott Laboratories, North Chicago, IL) with saline. Four doses (30, 100, 300 and 560 mg/kg) of BDL and four doses (10, 30, 100 and 300 mg/kg) of GBL were studied i.g. For the pentobarbital discrimination, BDL and GBL were dissolved in water and the infusion volumes were 0.25–0.5 ml/kg.

For the flumazenil discrimination, diazepam (Zenith Laboratories, Northvale, NJ) was suspended in 45–50 ml (depending on body weight) of fruit punch containing suspending Agent K to yield a dose of 5.6 mg/kg/daily drinking episode. Flumazenil (F. Hoffman LaRoche, Basel, Switzerland) was dissolved in a vehicle of 10% ethanol, 40% propylene glycol and 50% saline. For the midazolam discrimination, midazolam HCl was prepared commercially (Roche Pharma, Manati, PR). For the midazolam and flumazenil discriminations, BDL and GBL were dissolved in saline and injection volumes were 0.05–0.3 ml/kg.

2.4. Data analyses

Self-administration data are reported as the range of the number of injections from eight sessions for saline ($\pm$ S.E.M.) and methohexital ($\pm$ S.E.M.) and from two sessions for each dose of BDL or GBL across monkeys. For individual monkeys, self-administration of a drug is considered significantly different from saline when the number of drug injections is equal to or greater than three S.E.M. of the average number of saline injections.

Drug discrimination data are expressed as the percentage of trials on the drug-appropriate lever (pentobarbital discrimination) or as the percentage of total responses occurring on the drug-appropriate lever (midazolam and flumazenil discriminations) averaged among monkeys ($\pm$ S.E.M.) and plotted as a function of dose. Control response rate for the pentobarbital discrimination represents the response rate after saline administration under test conditions. Control response rate for the midazolam and flumazenil discriminations represents the average of the five saline or vehicle training sessions immediately preceding a test. Doses of a compound required to decrease response rate to 50% of control (ED_{50}) were estimated using linear regression by using more than two appropriate data points, otherwise by interpolation. These values were determined first for individual monkeys and then averaged among all monkeys. The corresponding 95% confidence limits (95% CL) for ED_{50}s were calculated from the group average using the *t* statistic.

3. Results

3.1. Reinforcing effects

The range of injections of methohexital (0.1 mg/kg) per 130-min session was 75.3–136.3 across monkeys. For each animal, the number of injections of methohexital was significantly greater than the number of injections of saline (range for saline, 5.6–26.3). The range of injections for each dose of BDL was as follows: 5.5–21.5 for 0.1 mg/kg/injection; 4.5–61.0 for 0.32 mg/kg/injection; 4.5–19.5 for 1.0 mg/kg/injection and 7.0–11.0 for 3.2 mg/kg/injection. The number of injections of BDL (0.1–3.2 mg/kg/injection) was not statistically different from the number of injections of saline except for one monkey for 0.32 mg/kg/injection. The range of injections for each dose of GBL was as follows: 7.0–34.5 for 0.1 mg/kg/injection; 6.5–37.0 for 0.32 mg/kg/

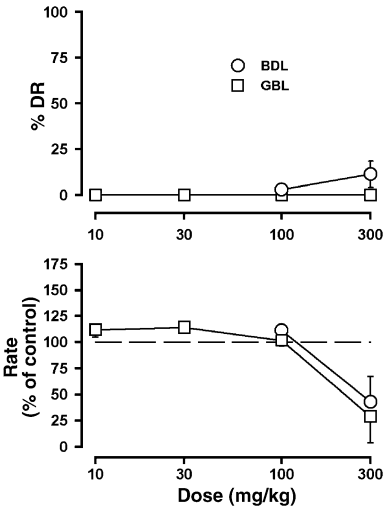

Fig. 1. Effects of BDL and GBL in rhesus monkeys discriminating pentobarbital. *Abscissae*: dose in milligrams per kilogram body weight. *Ordinates*: mean (±S.E.M.) percentage of responding on the pentobarbital lever (%DR = drug responding expressed as percent trials on the pentobarbital lever, top) and response rate expressed as percentage of control rate [rate (percent of control), bottom]. Data are averaged from four monkeys.

injection; 5.5–37.5 for 1.0 mg/kg/injection and 5.5–24.3 for 3.2 mg/kg/injection. The number of injections of GBL (0.1–3.2 mg/kg/injection) was not statistically different from the number of injections of saline.

3.2. Discriminative stimulus effects

3.2.1. Pentobarbital discrimination

Monkeys that discriminated between saline and pentobarbital responded ≥95% on the injection-appropriate lever during test sessions with the training drug or saline (data not shown). BDL administered 60 min before the session engendered little or no drug-appropriate responding up to a dose of 560 mg/kg (data not shown). Response rates were

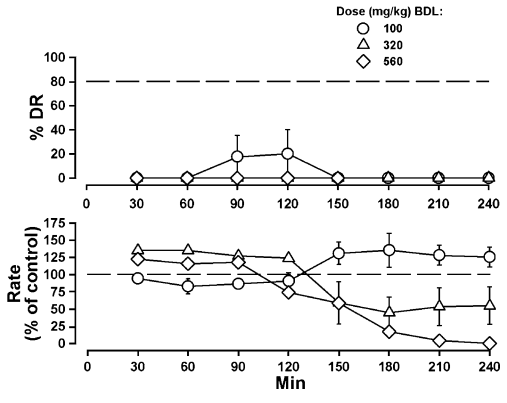

Fig. 2. Effects of BDL in rhesus monkeys discriminating midazolam. *Abscissae:* time in min. *Ordinates*: mean (±S.E.M.) percentage of responding on the midazolam lever (%DR = drug responding expressed as percent total responses on the midazolam lever, top) and mean (±S.E.M.) response rate expressed as percentage of control rate [rate (percent of control), bottom]. Data are averaged from three monkeys.

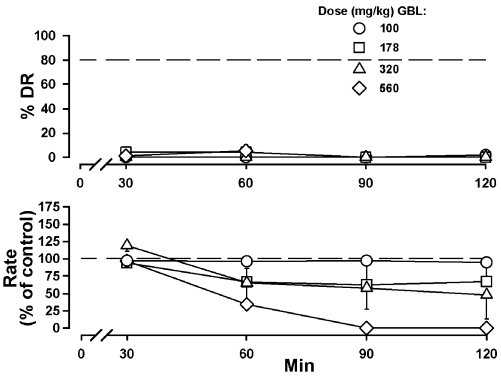

Fig. 3. Effects of GBL in rhesus monkeys discriminating midazolam. See Fig. 2 for details.

not systematically affected when BDL was administered 60 min before sessions except for a small decrease in the monkey discriminating 5.6 mg/kg of pentobarbital. When pretreatment time was varied between 120 (Fig. 1) and 240 min (data not shown) for doses of 100 and 300 mg/kg of BDL, no drug-appropriate responding was observed, and 100 mg/kg had no effect on response rate. However, 300 mg/kg of BDL decreased response rate to at least 50% of control in three of four monkeys when administered 120 min before the session (Fig. 1, circles), an effect that was diminished when the compound was administered 180 min before the session (data not shown). At 120 min after administration of BDL, the average ED_{50} (95% CL) for decreasing response rate calculated from three of four monkeys was 233 (170–318) mg/kg. After sessions in which BDL decreased response rate, monkeys were sedated and ataxic.

When administered 60 min before the session, GBL engendered no drug-appropriate responding in the pentobarbital-trained monkeys up to a dose (300 mg/kg) that decreased responding (Fig. 1). When pretreatment time was increased to 120 or 180 min for doses of 100 and 300 mg/kg, no drug-appropriate responding was observed, and 100 mg/kg had no effect on response rates (data not shown). In contrast, 300 mg/kg of GBL markedly decreased responding for up to 180 min (data not shown). At 60 min after

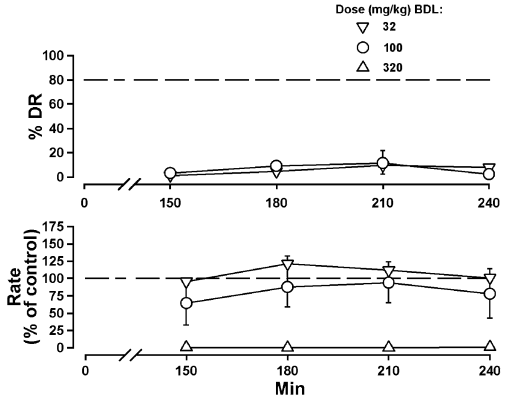

Fig. 4. Effects of BDL in diazepam-treated rhesus monkeys discriminating flumazenil. See Fig. 2 for details.

administration of GBL, the average ED_{50} (95% CL) for decreasing response rate from three of four monkeys was 174 (161–187) mg/kg. After sessions in which GBL decreased response rate, monkeys were sedated and ataxic.

3.2.2. Midazolam and flumazenil discriminations

In monkeys discriminating between midazolam and saline, midazolam produced dose-related increases in the percentage of responses on the midazolam lever with a dose of 0.1 or 0.32 mg/kg occasioning ≥80% midazolam lever responding across monkeys (data not shown). BDL (100–560 mg/kg) occasioned predominantly saline lever responding up to doses that suppressed responding (Fig. 2, top). BDL produced a dose- and time-related decrease in responding that became evident at 150 min and was maximal at 240 min (Fig. 2, bottom). In addition, 100 mg/kg of BDL slightly increased response rate between 150 and 240 min. At 240 min after administration of BDL, the group average ED_{50} (95% CL) was 305 (182–513) mg/kg. GBL (100–560 mg/kg), up to doses that suppressed responding, occasioned predominantly saline lever responding (Fig. 3, top). GBL produced a dose- and time-related decrease in responding that was maximal at 90 min (Fig. 3, bottom). With a 120-min pretreatment, the group average ED_{50} (95% CL) for rate-decreasing effects of GBL was 244 (119–502) mg/kg.

In monkeys discriminating between flumazenil and vehicle, flumazenil produced dose-related increases in the percentage of responses on the flumazenil lever with a dose of 0.032 or 0.1 mg/kg occasioning ≥80% flumazenil lever responding across monkeys (data not shown). BDL (100–560 mg/kg), up to doses that suppressed responding, occasioned predominantly vehicle lever responding (Fig. 4, top). BDL produced a dose-related decrease in responding from 150 to 240 min after administration (Fig. 4, bottom). At 240 min after administration of BDL, the ED_{50} (95% CL) for decreasing response rate was 126 (54–292) mg/kg in diazepam-treated monkeys. GBL (100–560 mg/kg), up to doses that suppressed responding, occasioned predominantly vehicle lever responding (Fig. 5, top). GBL produced a dose- and time-related decrease in responding that was maximal beginning 90 min after administration and was sustained for the duration of the session (120 min; Fig. 5, bottom). At 120 min after administration of GBL, the ED_{50} (95% CL) for decreasing response rate was 246 (244–249) mg/kg.

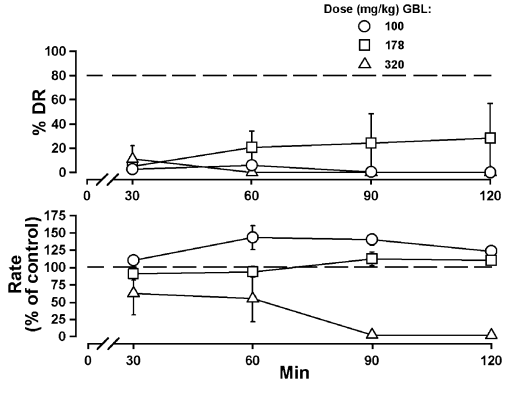

Fig. 5. Effects of GBL in diazepam-treated rhesus monkeys discriminating flumazenil. See Fig. 2 for details.

4. Discussion

This study demonstrates that i.v. BDL and GBL, up to doses of 3.2 mg/kg/injection, did not have positive reinforcing effects in rhesus monkeys that reliably self-administered the barbiturate methohexital. Only one dose of BDL in one monkey was self-administered to a greater extent than saline. Thus, it appears that BDL and GBL have, at most, weak positive reinforcing effects in rhesus monkeys. These data are consistent with a previous study demonstrating that GHB, a metabolite of BDL and GBL, is not reliably self-administered above saline levels under identical conditions in rhesus monkeys (Woolverton et al., 1999). Whereas these data indicate that BDL and GBL have little reinforcing effect when delivered i.v. to rhesus monkeys experienced with self-administration of a short-acting sedative-hypnotic, BDL and GBL are abused by humans (Rambourg-Schepens et al., 1997; Zvosec et al., 2001). Drugs with a more rapid onset of action are typically self-administered to a greater extent than drugs with a delayed onset of action (e.g., Winger et al., 2002); a delayed onset of action might be responsible for the apparent weak positive reinforcing effects of BDL and GBL in rhesus monkeys.

BDL and GBL did not share discriminative stimulus effects with the barbiturate pentobarbital or the benzodiazepine midazolam in rhesus monkeys, up to doses that decreased responding. These results suggest that BDL and GBL might have effects that differ qualitatively from barbiturates and benzodiazepines. Pentobarbital and midazolam allosterically facilitate GABA neurotransmission with high efficacy at the $GABA_A$ receptor complex and they share discriminative stimulus effects with most other high-efficacy positive $GABA_A$ receptor modulators (e.g., benzodiazepines, barbiturates and neuroactive steroids; Woolverton et al., 1994; Lelas et al., 1999; Rowlett et al., 1999; McMahon et al., 2001; McMahon and France, 2003). Thus, failure of BDL and GBL to substitute for midazolam or pentobarbital indicates that BDL and GBL are not high efficacy positive $GABA_A$ receptor modulators. BDL and GBL also did not share discriminative stimulus effects with the benzodiazepine antagonist flumazenil in diazepam-treated rhesus monkeys. Flumazenil has discriminative stimulus effects in diazepam-treated monkeys that are mimicked by some low efficacy positive $GABA_A$ receptor modulators (e.g., agonists) as well as neutral $GABA_A$ receptor modulators (e.g., antagonists; Gerak and France, 1999). Negative $GABA_A$ receptor modulators (e.g., inverse agonists) that inhibit Cl^- flux at different sites on the $GABA_A$ receptor

complex also share discriminative stimulus effects with flumazenil in diazepam-treated monkeys (Gerak and France, 1999). Collectively, the present results indicate that the behavioral effects of BDL and GBL do not predominantly result from modulation of GABA at the $GABA_A$ receptor complex. However, the present results cannot reject the possibility that direct stimulation of the $GABA_A$ receptor mediates the behavioral effects of BDL and GBL, perhaps by their eventual metabolism to GABA. If metabolism to GHB is important for the behavioral actions of BDL and GBL, then $GABA_B$ receptors would appear to be likely sites of action for BDL and GBL in vivo (Colombo et al., 1998).

There was a dose- and time-related decrease in response rate following administration of BDL and GBL in untreated rhesus monkeys discriminating pentobarbital or midazolam and in diazepam-treated monkeys discriminating flumazenil. The onset of action of GBL was more rapid than BDL regardless of treatment (untreated or diazepam-treated), route of administration (i.g. or s.c.) or reinforcer (SST or food). The more rapid onset of GBL relative to BDL supports the notion that conversion to GHB is responsible for the behavioral effects of these compounds because GBL is converted to GHB more rapidly than BDL (Snead et al., 1989). In untreated monkeys, the potency of BDL in decreasing response rate (ED_{50} in pentobarbital and midazolam discriminating monkeys was 233 and 305 mg/kg, respectively) was slightly less than the potency of GBL (ED_{50} in pentobarbital and midazolam discriminating monkeys was 174 and 244 mg/kg, respectively). To the extent that ED_{50}s were underestimated in pentobarbital discriminating monkeys (e.g., response rate was not decreased to $\leq 50\%$ of control for each compound in one monkey), route of administration did not appear to confer a difference in potency. However, the onset of action for both compounds was more rapid when administered i.g. as compared to s.c. The potency of BDL to decrease response rate was somewhat greater in diazepam-treated monkeys as compared to untreated monkeys, whereas the potency of GBL was the same in these two groups of monkeys. These results might suggest that the behavioral effects of BDL and GBL are differentially affected by diazepam treatment and might further suggest that the mechanisms of action of BDL and GBL are not identical.

In summary, i.v. BDL and GBL were not reinforcing in a self-administration paradigm in rhesus monkeys, up to doses of 3.2 mg/kg/injection. These results might suggest that BDL and GBL have low potential for abuse as compared to other drugs studied under these conditions (e.g., the barbiturate methohexital, opioids and stimulants; Woolverton et al., 1994; 1999; Anderson et al., 2001; Winger and Woods, 2001). In addition, BDL and GBL, when administered i.g. or s.c. up to doses that suppressed responding, did not share discriminative stimulus effects with positive $GABA_A$ receptor modulators in untreated monkeys or with flumazenil in diazepam-treated monkeys. These results suggest that BDL and GBL do not modulate GABA or otherwise inhibit Cl^- flux at the $GABA_A$ receptor complex. A previous study evaluating the effects of GHB under these conditions demonstrated that GHB did not have positive reinforcing effects and did not share discriminative stimulus effects with positive $GABA_A$ receptor modulators in untreated monkeys or with flumazenil in diazepam-treated monkeys (Woolverton et al., 1999). Collectively, data from rhesus monkeys suggest that BDL and GBL, such as GHB, are qualitatively different from sedative-hypnotics that positively modulate GABA at the $GABA_A$ receptor complex (e.g., barbiturates and benzodiazepines).

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