Effect of GABA Agonists and GABA-A Receptor Modulators on Cocaine- and Food-Maintained Responding and Cocaine Discrimination in Rats

Andrew C. Barrett, S. Stevens Negus, Nancy K. Mello, and S. Barak Caine
Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, Belmont, Massachusetts

Received March 9, 2005; accepted July 18, 2005

ABSTRACT
Recent studies indicate that GABAergic ligands modulate abuse-related effects of cocaine. The goal of this study was to evaluate the effects of a mechanistically diverse group of GABAergic ligands on the discriminative stimulus and reinforcing effects of cocaine in rats. One group of rats was trained to discriminate 5.6 mg/kg cocaine from saline in a two-lever, food-reinforced, drug discrimination procedure. In two other groups, responding was maintained by cocaine (0–3.2 mg/kg/injection) or liquid food (0–100%) under a fixed ratio 5 schedule. Six GABA agonists were tested: the GABA-A receptor agonist muscimol, the GABA-B receptor agonist baclofen, the GABA transaminase inhibitor /H9253-vinyl-GABA (GVG), and three GABA-A receptor modulators (the barbiturate pentobarbital, the high-efficacy benzodiazepine midazolam, and the low-efficacy benzodiazepine enazenil). When tested alone, none of the compounds substituted fully for the discriminative stimulus effects of cocaine. As acute pretreatments, select doses of midazolam and pentobarbital produced 2.2- to 3.6-fold rightward shifts in the cocaine dose-effect function. In contrast, muscimol, baclofen, GVG, and enazenil failed to alter the discriminative stimulus effects of cocaine. In assays of cocaine- and food-maintained responding, midazolam and pentobarbital decreased cocaine self-administration at doses 9.6- and 3.3-fold lower, respectively, than those that decreased food-maintained responding. In contrast, muscimol, baclofen, and GVG decreased cocaine self-administration at doses that also decreased food-maintained responding. Enazenil failed to alter cocaine self-administration. Together with previous studies, these data suggest that among mechanistically diverse GABA agonists, high-efficacy GABA-A modulators may be the most effective for modifying the abuse-related effects of cocaine.

Cocaine abuse and dependence remain serious health and economic concerns in the United States. Cocaine is an indirect agonist at dopaminergic, noradrenergic, and serotonergic receptors, although a substantial body of evidence indicates that its abuse-related effects are mediated by increases in dopamine in the terminal regions of mesolimbic dopamine neurons (Wise and Bozarth, 1987; Johanson and Fischman, 1989). Endogenous GABA or exogenous ligands can alter the activity of ventral tegmental area (VTA) dopaminergic neurons by binding to at least two pharmacologically distinct, inhibitory receptor subtypes, GABA-A and GABA-B receptors (Sieghart, 1995; Marshall et al., 1999).

Activation of GABA-A and GABA-B receptors located on VTA neurons has been shown to inhibit these neurons, reduce dopamine release, and reduce cocaine-induced increases in extracellular dopamine (Klitienick et al., 1992; Walters and Pucak, 1996; Fadda et al., 2003). GABA-A receptors are also located on GABAergic interneurons presynaptic to dopaminergic VTA neurons, and activation of these receptors would be predicted to inhibit GABAergic interneurons, disinhibit VTA neurons, enhance dopamine release, and enhance cocaine-induced increases in extracellular dopamine (Klitienick et al., 1992; Walters and Pucak, 1996; Xi and Stein, 1998). In addition, GABA receptors are widely distributed throughout the central nervous system, and GABA agonists also produce effects at sites other than the VTA (Bowery et al., 1987). Consequently, the net effect of GABA receptor activation can be difficult to predict and likely depends on the functional balance of activity at postsynaptic and presynaptic receptor sites (Walters and Pucak, 1996; Xi and Stein, 1998). Nevertheless, given the interactions between GABA and dopaminergic systems, GABAergic ligands may be useful for modifying some of the abuse-related effects of cocaine.

ABBREVIATIONS: VTA, ventral tegmental area; FR, fixed ratio; TO, time out; GVG, γ-vinyl-GABA; ANOVA, analysis of variance; SCH 39166, (−)-trans-6,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5H-benzo[d]naptho-(2,1-b)azepine.
Drug discrimination and self-administration assays have been useful for examining the abuse-related effects of cocaine and their modulation by GABAergic ligands. With regard to GABA-A allosteric modulators, triazolam and pentobarbital attenuated the discriminative stimulus effects of cocaine in rhesus monkeys, and alprazolam attenuated the discriminative stimulus and subjective effects of d-amphetamine in humans (Negus et al., 2000; Rush et al., 2004). Likewise, alprazolam and chlordiazepoxide decreased cocaine self-administration in rats (Goeders et al., 1989, 1993). More variable results have been obtained with direct-acting GABA agonists. For example, baclofen and muscimol failed to alter the discriminative stimulus effects of cocaine in rats or rhesus monkeys, and baclofen failed to alter the reinforcing or subject-rated effects of cocaine in humans (Munzar et al., 2000; Negus et al., 2000; Lile et al., 2004). However, numerous studies have documented the ability of baclofen to decrease cocaine self-administration in animals under a variety of conditions (Roberts et al., 1996; Roberts and Andrews, 1997; Shoabi et al., 1998; Campbell et al., 1999; Brebner et al., 2000a,b; Stafford and Glowa, 2000). Preliminary clinical studies in humans likewise have indicated that baclofen can decrease self-reported cocaine use and craving (Ling et al., 1998; Shoptaw et al., 2003). Moreover, baclofen may also attenuate cue-induced cocaine craving as assessed by brain imaging in humans (Childress et al., 2000). GVG, a GABA transaminase inhibitor that retards the metabolism of endogenous GABA, decreased cocaine self-administration, although its ability to modulate the discriminative stimulus effects of cocaine has not been examined (Kushner et al., 1999).

Together, the specific GABA receptors (e.g., GABA-A and GABA-B) involved in modulating dopamine and cocaine-induced increases in dopamine, the GABAergic drugs that would be most effective in this regard (e.g., allosteric modulators, direct and indirect agonists), and the behavioral effects of cocaine that are most altered by GABA interventions (e.g., discriminative stimulus or reinforcing effects) all remain to be clarified. Therefore, the purpose of the present study was to further evaluate and compare the effects of a series of mechanistically diverse GABA agonists in assays of cocaine discrimination and self-administration in rats. The following six GABA agonists were selected to include GABA-A and GABA-B direct agonists (muscimol and baclofen, respectively), a GABA indirect agonist (the GABA transaminase inhibitor GVG), and GABA-A allosteric modulators, including a barbiturate (pentobarbital), and both high- and low-efficacy benzodiazepines (midazolam and enazenil, respectively). The effects of these GABA agonists on cocaine self-administration could reflect either a change in the reinforcing effects of cocaine or a change in the ability of the subject to emit the operant response. To address this issue, the present study compared GABA agonist effects on responding maintained by a range of cocaine doses and by a range of concentrations of a liquid food reinforcer. This approach was used previously to dissociate selective and nonselective effects of dopaminergic compounds on cocaine self-administration in rats (Barrett et al., 2004; for a discussion, see Mello and Negus, 1996).

Materials and Methods

Animals

Male Sprague-Dawley rats weighing approximately 350 g at the start of the study were purchased from a local vendor (Charles River Laboratories, Inc., Wilmington, MA) and acclimated to the laboratory for a minimum of 1 week. Rats were maintained in the range of 400 to 600 g with once-daily feedings of standard rat chow (approximately 17 g of Rat Diet 5012; PMI Feeds, Inc., St. Louis, MO). Bacon-flavored biscuits (Bio-Serve, Frenchtown, NJ) were also provided once or twice weekly, primarily for enrichment. Rats were typically housed individually with free access to water in a temperature- and humidity-controlled facility that was maintained on a 12-h light/dark cycle (lights on from 7:00 AM to 7:00 PM). Behavioral testing was generally conducted between 10:00 AM and 3:00 PM. Animal maintenance and research were conducted in accordance with the guidelines provided by the National Institutes of Health Committee on Laboratory Animal Resources, and all protocols were approved by the Institutional Animal Care and Use Committee. Data sets were generally completed with group sizes of six to eight rats (see figure legends for details).

Apparatus

All studies were conducted in experimental chambers (21 × 29.5 × 24.5 cm) placed within sound-attenuating cubicles equipped with a house light and an exhaust fan. Each chamber contained three response levers, with two situated on one wall of the chamber (3.0 cm above the grid floor and 1.5 cm from the side walls) and a third located at the center of the opposite wall (3.0 cm above the grid floor). A stimulus light was located above each lever. A steel molded cup was situated between the two levers and 2.0 cm above the floor for delivery of food pellets (45 mg of A/I Rodent Pellets; P. J. Noyes Co., Lancaster, NH) or liquid food solutions (Ensure protein drink diluted with water). An infusion pump (model PHM-100, MED Associates, Georgia, VT) mounted above each chamber was equipped with a 3.3-rpm motor to allow for multiple specified infusion durations and volumes. For studies of food-maintained responding, the infusion pump delivered solutions of Ensure protein drink via Tygon tubing into the food cup. All hardware components of the operant chambers and associated software were obtained from MED Associates. For studies of drug self-administration, the infusion pump was connected by Tygon tubing to a single channel fluid swivel (Lomir Biomedical, Malone, NY), which was mounted on a balance arm above the chamber. The output of the liquid swivel was attached to the externalized terminus of an i.v. catheter via a steel spring sleeve over a length of Tygon tubing (0.02 inches i.d. × 0.06 inches o.d.) to permit automated delivery of intravenous drug injections for specified durations (see below).

Drug Discrimination

Discrimination Training. After initial shaping of lever responding, rats were trained to discriminate 5.6 mg/kg cocaine from saline. On training days, rats received an i.p. injection of either saline or the training dose of cocaine. A random sequence was used to determine which injection was administered, with the two restrictions that 1) the same injection was usually not given for more than two consecutive sessions and 2) during each block of 30 training sessions, the numbers of saline and training drug sessions were approximately equal. Ten minutes after the injection of saline or cocaine, a 15-min response period began. During the response period, the house light and stimulus lights were illuminated, and 20 food pellets were available under a fixed ratio (FR) schedule of reinforcement. The fixed ratio was gradually increased from FR 1 to FR 20. When saline was administered, completion of the response requirement on one lever (saline-appropriate lever) resulted in food delivery. When the training dose of cocaine was administered, completion of the response requirement on the other lever (cocaine-appropriate lever) resulted
in food delivery. The positions of the saline- and cocaine-appropriate levers were counterbalanced across rats. Responses on the inappropriate lever reset the ratio requirement on the correct lever. If all 20 food pellets were delivered before the end of the 15-min response period, the house light and stimulus lights were turned off, and the session was terminated. Training sessions were conducted 5 days per week. Training continued until the following three criteria were met for seven of eight consecutive sessions: 1) percentage of injection-appropriate responding before delivery of the first reinforcer was \( \geq 90\% \); 2) percentage of injection-appropriate responding for the entire session was \( \geq 90\% \); and 3) all available food pellets were earned during saline training sessions.

After initial training under the single-cycle conditions described above, rats were subsequently trained under multiple-cycle conditions to permit administration of cumulative doses of cocaine or other test compounds during a single experimental session. For multiple-cycle training sessions, each 15-min cycle consisted of a 10-min time-out (TO) period followed by a 5-min response period. During the time-out periods, the stimulus lights over the levers were turned off, and responding had no scheduled consequences. During the response period, the stimulus lights over the response levers were turned on and rats could earn up to 10 food pellets under an FR 20 schedule of reinforcement. If all 10 food pellets were delivered before 5 min had elapsed, then the stimulus lights were turned off and responding had no scheduled consequences for the remainder of the 5-min period. Either saline or the training dose of cocaine was administered at the beginning of each cycle, with the provision that cocaine was administered only during the last cycle. During each cycle, responding on the injection-appropriate lever was reinforced as described above. Multiple-cycle training sessions were pseudorandomized so that during each block of 30 training sessions, approximately equal numbers of sessions were two, three, or four cycles in duration, with a cocaine injection during the last cycle. Occasionally, a session consisted of either one cycle with a cocaine injection or five cycles with only saline injections. Training continued until the three criteria described above were met during all cycles for seven of eight consecutive multiple cycle sessions.

**Discrimination Testing.** Once the training criteria were met, two types of test sessions were conducted: substitution tests and pretreatment tests. During substitution tests, the test compound was administered using cumulative dosing procedures. The following drugs were examined in substitution tests: cocaine (0.18–18.0 mg/kg), d-amphetamine (0.032–1.8 mg/kg), the dopaminergic D1 antagonist SCH 39166 (0.18 mg/kg), the GABA-A agonist muscimol (0.18–3.2 mg/kg), the GABA-B agonist baclofen (0.18–5.6 mg/kg), the GABA transaminase inhibitor GVG (180–320 mg/kg), the barbiturate pentobarbital (0.32–10.0 mg/kg), the high-efficacy benzodiazepine enazemil (0.10–3.2 mg/kg), and the low-efficacy benzodiazepine enazemil (0.10–3.2 mg/kg), and the low-efficacy benzodiazepine enazemil (0.10–3.2 mg/kg). Due to the long pretreatment interval required for GVG (180 min), dose-effect functions for this drug were determined with single-cycle tests. For each rat, the effects of most doses of a drug were determined two or three times in several cumulative dose tests with overlapping dose ranges. The lowest and highest doses of a drug; i.e., doses that typically had no effect or that completely eliminated responding, respectively, were sometimes tested only once in each rat. To determine appropriate pretreatment intervals for additional experiments with these drugs, the time course of the discriminative stimulus and rate-altering effects of the highest dose of each drug tested was determined once in a multiple-cycle test session. Tests were conducted at 10, 30, 100, and 300 min for all drugs with the exception of GVG, which was tested up to 3000 min. During pretreatment tests, either saline or a dose of a test compound was administered before the session, and saline and/or increasing doses of cocaine were administered during subsequent cycles of the test session. The following drugs were examined: SCH 39166 (0.10–0.18 mg/kg), muscimol (0.32–3.2 mg/kg), baclofen (1.8–5.6 mg/kg), GVG (180–320 mg/kg), pentobarbital (3.2–10.0 mg/kg), midazolam (0.10–3.2 mg/kg), and enazemil (0.32–1.0 mg/kg). These dose ranges of GABAergic drugs were selected to include doses that had no effect (lowest dose) or that completely eliminated responding (highest dose). All drugs were administered i.p. (1 ml/kg) 10 min before the session, with the exception of a 180-min pretreatment interval used for GVG. The effects of each pretreatment dose were typically determined twice in each rat with overlapping cocaine dose ranges. Throughout a test session, the completion of 20 responses on either of the two levers resulted in food delivery. Otherwise, conditions during test sessions were identical to those described during training sessions. During all phases of the study, testing usually occurred on Tuesdays and Fridays, whereas training sessions were continued on Mondays, Wednesdays, and Thursdays. A test session was conducted only if performance met the three criteria described above for accurate discrimination on the training day preceding the test day. If these criteria were not met, then the next scheduled test session was omitted and replaced by a training session.

**Data Analysis.** Cocaine-appropriate responding was calculated by dividing the number of responses on the cocaine-appropriate lever by the total number of responses on both levers. The percentage of cocaine-appropriate responding for each rat in each cycle of a test session was calculated only if the rat emitted at least five lever presses. The response rate was calculated by dividing the total number of responses by the total duration of the response period. For each rat, multiple determinations of the effects of saline or a drug dose were averaged. To determine whether the maximal level of cocaine-appropriate responding differed between cocaine and the GABA agonists, for each drug the maximal level of cocaine-appropriate responding observed at any dose was determined in each rat. These data were averaged, and one-way ANOVA was used to compare each drug alone to cocaine alone. When appropriate, the dose of a drug estimated to produce 50% cocaine-appropriate responses \( A_{50} \) was calculated for each rat by interpolation of the linear portion of the log-dose-effect function (one point below and one point above 50% cocaine-appropriate responding). Individual \( A_{50} \) values were averaged to yield a mean \( A_{50} \) value \( (\pm 95\% \) confidence limits). In pretreatment tests, \( A_{50} \) values for cocaine administered alone or after pretreatment were determined, and dose-effect functions were considered to be significantly different if the 95% confidence intervals did not overlap. In some pretreatment tests, all data points for cocaine-appropriate responding fell below or above 50%. In these instances, if the animal responded at the training dose of cocaine (5.6 mg/kg), \( A_{50} \) values were estimated by assuming that either 1) the next highest half-log unit dose of cocaine would have produced 100% cocaine-appropriate responding (in cases where all data points fell below 50%) or 2) the next lowest half-log dose would have produced 0% cocaine-appropriate responding (in cases where all points were above 50%). All drugs were tested up to doses that decreased response rates. For statistical analyses, the largest reduction in response rate observed at any dose of drug was determined in every rat. These values were averaged, and one-way ANOVA was used to compare these rates to rates during saline control tests.

**Cocaine- and Food-Maintained Responding**

**Initial Behavioral Training.** In all rats, lever pressing was initially shaped during training sessions up to 12 h in duration in which 45-mg food pellets reinforced responding under an FR 1 schedule of reinforcement. In subsequent sessions, the ratio requirement was increased to FR 5, and food training continued until rats earned a minimum of 50 food pellets in a single session under the FR 5 schedule of reinforcement. Rats were then assigned to groups in which responding was maintained by either cocaine (i.v.) or liquid food.

**Procedures for Evaluating Cocaine Self-Administration**

**Surgery.** Rats were anesthetized with an isofluorane/oxygen vapor mixture and prepared with chronic indwelling i.v. catheters as described previously (Barrett et al., 2004). Each catheter consisted of...
a 13-cm length of Silastic tubing fitted to a 22-gauge guide cannula that was bent at a right angle. The guide cannula was encased in dental cement anchored with a 0.5-inch-diameter circular nylon mesh. The tubing was passed s.c. from the back of the animal to the right external jugular vein. After surgery, buprenorphine (0.032 mg/kg) was administered once as an analgesic, and a prophylactic dose of ticarcillin (approximately 17 mg/kg i.v.) dissolved in saline containing heparin (3 USP U/0.1 ml) was delivered in a volume of 0.1 ml once daily for 5 days to prevent infection and maintain catheter patency. Thereafter, catheters were flushed daily with sterile physiological saline containing heparin (3 USP U/0.1 ml). If blood could not be withdrawn through the catheter, then approximately once per week or whenever behavior deviated from baseline parameters, catheter patency was tested by administering a solution containing 30 mg/ml ketamine and 1.5 mg/ml midazolam (0.1–0.2 ml i.v.). Animals with patent catheters exhibited prominent signs of sedation within 3 s after i.v. injection. Animals with faulty catheters were prepared with a new catheter in the left jugular vein.

**Initial Cocaine Self-Administration Training and Maintenance Procedures.** Daily training sessions of up to 3 h per day began 5 days after surgery. Rats were placed in the experimental chambers and received a noncontingent automated infusion to fill the catheter (17 μl) and to deliver one unit dose of cocaine (1.0 mg/kg). Thereafter, a cue light above the center lever was illuminated, and responding on that lever was maintained by i.v. cocaine injections under an FR 5 TO 20-s schedule of reinforcement. Under this schedule, completion of the response requirement produced an i.v. infusion of the unit dose of cocaine and initiated a 20-s TO period (including the time for drug infusion), during which the cue light was turned off and responses had no scheduled consequences. Training continued until cocaine self-administration behavior met the following criteria for at least three consecutive sessions: 1) a minimum of 5.0 mg/kg of cocaine self-administered every hour, and 2) less than 20% variability in the total number of cocaine reinforcers earned per session. After these criteria were met, extinction training commenced, in which either cocaine (1.0 mg/kg/injection) or saline (180 μl/kg) was alternately available during four successive sessions.

**Cocaine Dose-Effect Determinations.** A procedure for rapid assessment of responding maintained by different doses of cocaine was used (Caine et al., 1999). These multiple-component sessions consisted of three or four 20-min components separated by 2-min intercomponent time-out periods. Dose-effect functions were determined by increasing the volume of cocaine injections in successive components so that 0, 0.17, 0.56, or 1.78 μl injections were delivered in approximately 0, 1, 3, 2, and 10 s, respectively. Drug solutions consisted of 0.56 mg/ml cocaine, 1.78 mg/ml cocaine, or 5.6 mg/ml cocaine, yielding unit doses of 0, 0.01, 0.032, 0.10, 0.32, and 1.0 mg of total cocaine per injection (approximately 0.032–3.2 mg/kg/injection). The doses of cocaine available for self-administration during any given multiple-component session were determined by pseudorandom design, such that sessions began with the availability of 0.0 mg/kg cocaine followed by one of the following sequences of doses (in milligrams per kilogram per injection): 0.032, 0.1, and 0.3; 0.1, 0.32, and 1.0; and 0.32, 1.0, and 3.2. Two-hour single-component sessions of cocaine self-administration (1.0 mg/kg/injection) or multiple-component sessions of saline availability (0, 17, 56, and 178 μl) were periodically interspersed with multiple-component sessions of cocaine self-administration. Different doses of cocaine were always presented in an ascending order, and an injection of the cocaine dose available during each component was noncontingently administered at the beginning of the component. Pretreatment testing (see below) began once drug self-administration behavior stabilized, with stability defined as three consecutive multiple-component sessions during which the dose that maintained peak responding remained stable within a half-log unit range.

**Procedures for Evaluating Responding Maintained by Liquid Food.** In rats not prepared with i.v. catheters, a complementary set of studies was conducted in which responding was reinforced with liquid food (100% Ensure protein drink), water, or various concentrations of liquid food diluted in water. In daily 2-h training sessions, responding on the center lever was maintained by liquid food presentation (75 μl of 100% Ensure) under an FR 5 TO 20-s schedule of reinforcement. Criteria for initial training were 1) a minimum of 50 liquid food reinforcers earned in three consecutive sessions, and 2) less than 20% variability in the total number of reinforcers earned in three consecutive sessions. Once initial training criteria were met, extinction training commenced in which either liquid food (100% Ensure) or water was alternately available over four consecutive sessions.

**Liquid Food Concentration-Effect Determinations.** After initial training, a procedure for rapid assessment of responding maintained by different concentrations of liquid food was used (Barrett et al., 2004). During these sessions, liquid food was available under an FR 5 TO 20-s schedule in five sequential 20-min components, with each component separated by 2-min intercomponent time-out periods. Food concentrations (0, 3, 10, 32, and 100%) were tested in an ascending order, and each reinforcer was delivered in a volume of 75 μl over approximately 4.2 s. Each component began with noncontingent delivery of the reinforcer available under the FR schedule. Two-hour single-component sessions of food availability (32 or 100%) were periodically interspersed with multiple-component sessions of food-maintained responding. Criteria for stable responding under the multiple-component procedure were 1) peak levels of responding maintained by at least one concentration of food equaled or exceeded 20 reinforcers within a 20-min component, and 2) the concentration of liquid food that maintained peak responding varied by no more than one-half-log unit over three consecutive within-session determinations.

**Pretreatment Tests.** After stabilization of within-session dose-or concentration-effect functions for cocaine and food, respectively, pretreatment tests commenced. The following drugs and doses were evaluated as pretreatments: muscimol (0.10–3.2 mg/kg), baclofen (1.8–5.6 mg/kg), GVG (180–560 mg/kg), pentobarbital (3.2–32.0 mg/kg), midazolam (0.32–18.0 mg/kg), and enazemid (5.6–18.0 mg/kg). These doses were selected on the basis of preliminary results showing that lower doses were ineffective, whereas higher doses eliminated responding completely. All drugs were administered i.p. (1 ml/kg) 10 min before the session, with the exception of a 180-min pretreatment interval used for GVG. Pretreatment times for all drugs were determined on the basis of the time course of behavioral effects using the drug discrimination procedure. For each rat, each dose of a pretreatment drug was generally tested in at least two sessions. For the cocaine self-administration assay, full dose-effect functions were generated over at least two sessions by testing overlapping cocaine dose ranges. Each pretreatment test was preceded by a baseline session (i.e., a session during which the dose or cocaine concentration of food that maintained peak responding deviated by ≤0.5 log units from the most recent session in which no pretreatment was administered).

**Data Analysis.** Data from multiple-component test sessions were expressed as the total number of reinforcers earned in each 20-min component. In most instances, vehicle and each dose of each pretreatment drug were tested twice, and these data were averaged. Group mean values for the effects of pretreatment drugs on cocaine- and food-maintained responding were analyzed with two-way within-subjects ANOVA, with pretreatment dose and cocaine dose or food concentration as factors. Significant (p < 0.05) main effects or interactions were followed by pairwise comparisons (Duncan’s post hoc test) with each pretreatment drug compared with vehicle. When appropriate, ADI values were calculated by linear interpolation of a portion of the log dose-effect function for each rat. For cocaine self-administration data, ADI values were defined as the dose of each pretreatment drug that decreased responding maintained by 0.1 or 0.32 mg/kg/injection cocaine to 50% of vehicle pretreatment values. For food self-administration data, the ADI values were defined as the dose of each pretreatment drug that decreased responding main-
tained by 10, 32, or 100% food to 50% of vehicle pretreatment values. Individual $A_{50}$ values were averaged to yield a mean $A_{50}$ value ($\pm 95\%$ confidence limits). $A_{50}$ values for cocaine- versus food-maintained responding were considered to be significantly different if the 95% confidence intervals did not overlap. Relative potency estimates were also calculated for each pretreatment drug by determining the ratio of $A_{50}$ values for food to $A_{50}$ values for cocaine. Relative potency estimates were determined for cocaine- versus food-maintained responding under three conditions in which food and cocaine were selected to be as comparable as possible across the following different dimensions: 1) 32% food was compared with 0.32 mg/kg cocaine, because these were the lowest reinforcer magnitudes that maintained robust behavior in all rats tested; 2) 100% food was compared with 0.1 mg/kg cocaine, because these were the magnitudes of each reinforcer that maintained peak rates of responding under control conditions; and 3) 10% food was compared with 0.1 mg/kg cocaine, because these reinforcer magnitudes maintained comparable response rates under control conditions.

Drugs. Cocaine HCl was provided by the National Institute on Drug Abuse, National Institute (Bethesda, MD) and was dissolved in saline. $d$-Amphetamine sulfate, muscimol HBr, (±)-baclofen HCl, and pentobarbital sodium (Sigma-Aldrich, St. Louis, MO) were dissolved in water. Midazolam HCl (Abbott Laboratories, Abbott Park, IL) was obtained as a 5.0 mg/ml solution, with a saline vehicle. Enazenil (6-[bromophenyl]-N-ethyl-8-fluoro-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxamide) was generously provided by E. Costa and A. Guidotti (University of Illinois, Chicago, IL) and was dissolved in a vehicle of 10% ethanol, 40% water, and 50% polyethylene glycol. GVG was generously provided by M. Nader and R. Mach (Wake Forest University, Winston-Salem, NC) and was dissolved in water. SCH 39166 was generously provided by Schering-Plough Research Institute (Kenilworth, NJ) and was dissolved in a solution of 1% ethanol diluted with water. All drug doses refer to the weights of the salts.

**Results**

**Drug Discrimination**

**Effects of Cocaine and $d$-Amphetamine Alone.** Figure 1 shows discriminative stimulus effects of cocaine and $d$-amphetamine in rats trained to discriminate 5.6 mg/kg cocaine from saline. Cocaine and $d$-amphetamine produced dose-dependent increases in cocaine-appropriate responding, with full substitution obtained at doses of 5.6 and 0.56 mg/kg, respectively. Table 1 shows that maximal level of cocaine-appropriate responding produced by cocaine and $d$-amphetamine approached 100% and also indicates that at least one dose of these drugs engendered full substitution (≥80% cocaine-appropriate responding) in every rat tested. Whereas low-to-intermediate doses of both compounds tended to
Effects of GABA-A Modulators Alone. Figure 1 also shows the effects of the GABA-A receptor agonist muscimol, the GABA-B receptor agonist baclofen, and the GABA transaminase inhibitor GVG. All three ligands produced lower levels of cocaine-appropriate responding than cocaine, and none of the ligands produced full substitution in more than half the rats (Table 1). Nevertheless, muscimol and GVG, but not baclofen, produced dose-dependent partial substitution (between 20 and 80% cocaine-appropriate responding) for cocaine when tested up to doses that markedly reduced or completely eliminated response rates. All of these compounds produced dose-dependent decreases in response rates, with the highest dose of each compound significantly decreasing rates by at least 50% compared with saline control levels.

Effects of GABA-A Modulators Alone. Figure 1 also shows the effects of the barbiturate pentobarbital, the high-efficacy benzodiazepine midazolam, and the low-efficacy benzodiazepine enanzenil. All three ligands produced lower levels of cocaine-appropriate responding than cocaine, and none of the ligands produced full substitution in more than half the rats (Table 1). Nevertheless, these ligands produced dose-dependent partial substitution for cocaine when tested up to doses that markedly reduced or completely eliminated response rates. All of these compounds produced dose-dependent decreases in response rates, with the highest dose of each compound significantly decreasing rates by at least 50% compared with saline control levels.

Time Course of Cocaine-Like Discriminative Stimulus Effects. To determine appropriate pretreatment intervals for drug combination studies and to more thoroughly characterize the effects of the GABAergic ligands, the time course of cocaine-like discriminative stimulus and rate-altering effects of each drug was determined. Figure 2 shows the time course of the highest dose of each GABA agonist tested and, for comparison purposes, the effects of the training dose of cocaine (5.6 mg/kg) and the dopamine D1 receptor antagonist SCH 39166 (0.18 mg/kg). The training dose of cocaine produced full substitution at 10 min, partial substitution at 30 and 100 min, and only saline-appropriate responding at 300 min. In contrast, SCH 39166 produced predominantly saline-appropriate responding up to 100 min, with low levels of cocaine-appropriate responding (approximately 29%) observed at 300 min. Although cocaine failed to markedly alter response rates, SCH 39166 produced marked decreases in response rates at 10 to 30 min, with responding returning to saline control levels by 300 min.

Baclofen (5.6 mg/kg), muscimol (3.2 mg/kg), and GVG (320 mg/kg) produced low levels of cocaine-appropriate responding at each time point studied. These effects peaked at 10 and 100 min for baclofen and muscimol, respectively, and dissipated by 300 min. In contrast, the low levels of cocaine-appropriate responding produced by GVG emerged at approximately 180 min, remained at this level beyond 1800 min, and dissipated by approximately 3000 min. All three drugs produced time-dependent decreases in response rates that corresponded with their time-dependent increases in cocaine-like discriminative stimulus effects.

Midazolam (3.2 mg/kg) and enanzenil (3.2 mg/kg) produced partial substitution for cocaine that peaked at 30 min and decreased to negligible levels of cocaine-appropriate responding at 300 min. Pentobarbital (10 mg/kg) produced predominantly saline-appropriate responding from 30 to 300 min after administration. All three drugs produced time-dependent decreases in response rates, and the time point at which the maximal rate-decreasing effect occurred generally corresponded to the time point at which maximal levels of cocaine-appropriate responding occurred.

Effects of Pretreatment with Direct and Indirect GABA Agonists on Cocaine Discrimination. Figure 4 shows the effects of pretreatment with the dopamine D1 antagonist SCH 39166 on the discriminative stimulus effects of cocaine. A dose of 0.10 mg/kg SCH 39166 attenuated the effects of 1.8 and 5.6 mg/kg cocaine, resulting in a significant rightward shift in the cocaine dose-effect function. Table 2 shows mean $A_{50}$ values (95% confidence intervals) for cocaine alone and in combination with SCH 39166 and indicates that 0.10 mg/kg SCH 39166 increased the mean $A_{50}$ value for cocaine by approximately 3.6-fold. A higher dose (0.18 mg/kg) of SCH 39166 produced a 3.4-fold rightward shift in the cocaine dose-effect function. SCH 39166 alone produced marked reductions in response rates that were attenuated by intermediate doses of cocaine (1.8–5.6 mg/kg). Likewise, rate-decreasing effects produced by the highest dose of cocaine (18 mg/kg) were attenuated by SCH 39166 in a dose-dependent manner.

Effects of Pretreatment with Direct and Indirect GABA Agonists on Cocaine Discrimination. Figure 4 shows the effects of pretreatment with muscimol, baclofen, and GVG on the discriminative stimulus effects of cocaine. None of these compounds significantly altered the cocaine dose-effect curve when tested up to doses that alone produced marked reductions in response rates. Table 2 shows mean $A_{50}$ values (95% confidence intervals) for cocaine alone and in combination with various doses of GABA agonists. For every dose combination tested, the cocaine dose-effect function was altered by less than 2-fold. When administered alone, all of the GABA agonists produced dose-dependent reductions in response rates, such that responding was decreased by at least 50% compared with saline control conditions. For muscimol and baclofen, cocaine did not markedly alter the rate-decreasing effects of each drug. For GVG, select doses of

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Maximum % Cocaine-Appropriate Responding</th>
<th>Full Substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>100</td>
<td>n/a</td>
</tr>
<tr>
<td>d-Amphetamine</td>
<td>99</td>
<td>11/11</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>70$^a$</td>
<td>2/9</td>
</tr>
<tr>
<td>Enanzenil</td>
<td>62$^a$</td>
<td>4/10</td>
</tr>
<tr>
<td>Muscimol</td>
<td>58$^a$</td>
<td>4/9</td>
</tr>
<tr>
<td>GVG</td>
<td>42$^a$</td>
<td>1/5</td>
</tr>
<tr>
<td>Midazolam</td>
<td>40$^a$</td>
<td>4/9</td>
</tr>
<tr>
<td>Baclofen</td>
<td>20$^a$</td>
<td>0/11</td>
</tr>
</tbody>
</table>

$^a$ Significantly different from cocaine as determined by one-way ANOVA ($p < 0.05$).
cocaine (1.8 and 5.6 mg/kg) tended to attenuate the rate-decreasing effects of GVG.

Effects of Pretreatment with GABA-A Modulators on Cocaine Discrimination. Figure 5 shows the effects of pretreatment with pentobarbital, midazolam, and enazenil on the discriminative stimulus effects of cocaine. In contrast to the effects of the direct-acting GABA agonists and GVG, pretreatment with pentobarbital produced dose-dependent rightward shifts in the cocaine dose-effect function. Although the effects produced by doses of 3.2 and 5.6 mg/kg pentobarbital failed to reach statistical significance, a dose of 10 mg/kg produced a significant 3.6-fold rightward shift in the cocaine dose-effect function (Table 2). The effects of pretreatment with midazolam depended on the specific dose tested. Whereas a dose of 0.32 mg/kg produced a significant 2.2-fold rightward shift in the cocaine dose-effect function, a dose one-half-log unit higher (1.0 mg/kg) enhanced the effects of cocaine, resulting in a significant 2.1-fold leftward shift in the cocaine dose-effect function (Table 2). Enazenil produced yet a different profile, with both doses tested tending to increase the effects produced by low to intermediate doses of cocaine. Although these effects are suggestive of a leftward shift in the cocaine dose-effect function, the \( A_{50} \) value for cocaine was not significantly altered (Table 2). Pentobarbital and midazolam were tested up to doses that alone produced marked reductions in response rates, and the training dose of cocaine (5.6 mg/kg) tended to attenuate these response rate-decreasing effects. In addition, a low dose of midazolam (0.1 mg/kg) tended to increase response rates after injections of saline or low cocaine doses. Enazenil had only minimal effects on response rates up to the highest dose tested, and cocaine failed to markedly alter this profile.

Cocaine- and Food-Maintained Responding

Effects of Pretreatment with Direct and Indirect GABA Agonists on Cocaine- and Food-Maintained Responding. Figure 6 shows cocaine- and food-maintained responding after acute pretreatment with vehicle or various doses of muscimol, baclofen, and GVG. Muscimol produced dose-dependent and significant decreases in cocaine self-administration and food-maintained responding. Two-way ANOVA showed a significant main effect of muscimol dose on cocaine self-administration and food-maintained responding, and significant interactions between muscimol dose and cocaine dose, and between muscimol dose and food concentration. Whereas 0.32 mg/kg muscimol failed to alter cocaine self-administration, higher doses (1.0–3.2 mg/kg) decreased responding maintained by intermediate doses of cocaine (0.1–0.32 mg/kg/injection) without altering responding maintained by higher doses of cocaine (1.0–3.2 mg/kg/injection). In the assay of food-maintained responding, relatively low doses of 0.1 (data not shown) and 0.32 mg/kg muscimol failed to alter food-maintained responding, although there was a trend \((p = 0.06)\) for 0.32 mg/kg to increase food-maintained responding. The effects of higher muscimol doses were biphase, with 1.0 mg/kg increasing responding maintained by low
magnitude food concentrations (3–10%) and the highest dose (3.2 mg/kg) decreasing responding maintained by a wide range of food concentrations (10–100%). Table 3 shows A50 values and relative potency estimates for each drug to decrease responding maintained various reinforcer magnitudes for cocaine and food (for rationale, see “Data Analysis”).

Similar to muscimol, baclofen produced dose-dependent and significant decreases in cocaine self-administration and food-maintained responding. Two-way ANOVA showed a significant main effect of baclofen dose on cocaine self-administration and food-maintained responding, and significant interactions between baclofen dose and cocaine dose, and between baclofen dose and food concentration. Although 1.8 mg/kg baclofen failed to alter cocaine self-administration, 3.2 mg/kg baclofen decreased responding maintained by intermediate doses of cocaine (0.1–0.3 mg/kg/injection), without altering behavior maintained by higher doses of cocaine. The highest dose of baclofen (5.6 mg/kg) produced a near complete elimination of responding across the entire range of cocaine doses. In the assay of food-maintained behavior, 1.8 mg/kg baclofen failed to alter food-maintained responding, and doses of 3.2 and 5.6 mg/kg baclofen decreased responding maintained by a wide range of food concentrations (3–100%). The potencies of baclofen for decreasing cocaine- and food-maintained responding were comparable (Table 3).

Also shown in Fig. 6 are the effects of GVG on cocaine- and food-maintained responding. Two-way ANOVA showed a significant main effect of GVG dose on cocaine self-administration and food-maintained responding and significant interactions between GVG dose and cocaine dose and between GVG dose and food concentration. Although GVG decreased cocaine self-administration, the effects were not dose-dependent. For example, when responding was maintained by low doses of cocaine (0.032 mg/kg/injection), a low dose of GVG (180 mg/kg) produced the biggest decrease in responding. However, when a higher unit dose of cocaine was available (0.32 mg/kg/injection), only the intermediate dose of GVG (320 mg/kg) significantly decreased responding. In contrast to the lack of dose-dependent effects in the assay of cocaine

---

**TABLE 2**

Drug discrimination A50 values in milligrams per kilogram for cocaine administered alone or after acute pretreatment with SCH 39166 or GABAergic ligands

<table>
<thead>
<tr>
<th>Drug</th>
<th>A50 (±95% CI)</th>
<th>Dose Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCH 39166</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine alone</td>
<td>1.3 (0.8–2.0)</td>
<td></td>
</tr>
<tr>
<td>+0.10 SCH 39166</td>
<td>4.7 (2.5–8.8)</td>
<td>3.6</td>
</tr>
<tr>
<td>+0.18 SCH 39166</td>
<td>4.4 (2.5–7.8)</td>
<td>3.4</td>
</tr>
<tr>
<td>Muscimol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine alone</td>
<td>1.7 (1.1–2.8)</td>
<td></td>
</tr>
<tr>
<td>+0.32 Muscimol</td>
<td>2.6 (1.3–5.3)</td>
<td>1.5</td>
</tr>
<tr>
<td>+1.0 Muscimol</td>
<td>2.7 (1.7–4.4)</td>
<td>1.6</td>
</tr>
<tr>
<td>+3.2 Muscimol</td>
<td>2.0 (1.2–3.4)</td>
<td>1.2</td>
</tr>
<tr>
<td>Baclofen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine alone</td>
<td>1.8 (1.2–2.6)</td>
<td></td>
</tr>
<tr>
<td>+1.8 Baclofen</td>
<td>1.4 (0.8–2.4)</td>
<td>0.8</td>
</tr>
<tr>
<td>+3.2 Baclofen</td>
<td>2.0 (1.0–4.3)</td>
<td>1.1</td>
</tr>
<tr>
<td>+5.6 Baclofen</td>
<td>2.7 (0.9–8.1)</td>
<td>1.5</td>
</tr>
<tr>
<td>GVG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine alone</td>
<td>2.7 (2.2–3.2)</td>
<td></td>
</tr>
<tr>
<td>+180 GVG</td>
<td>1.4 (0.6–2.9)</td>
<td>0.5</td>
</tr>
<tr>
<td>+320 GVG</td>
<td>2.6 (0.8–5.5)</td>
<td>1.0</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine alone</td>
<td>1.6 (1.0–2.6)</td>
<td></td>
</tr>
<tr>
<td>+3.2 Pentobarbital</td>
<td>2.1 (1.1–4.0)</td>
<td>1.3</td>
</tr>
<tr>
<td>+5.6 Pentobarbital</td>
<td>3.1 (1.9–5.0)</td>
<td>1.9</td>
</tr>
<tr>
<td>+10 Pentobarbital</td>
<td>5.8 (3.9–8.6)</td>
<td>3.6</td>
</tr>
<tr>
<td>Midazolam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine alone</td>
<td>2.1 (1.6–2.7)</td>
<td></td>
</tr>
<tr>
<td>+0.1 Midazolam</td>
<td>1.9 (1.1–3.0)</td>
<td>0.9</td>
</tr>
<tr>
<td>+0.32 Midazolam</td>
<td>4.6 (2.9–7.4)</td>
<td>2.2</td>
</tr>
<tr>
<td>+1.0 Midazolam</td>
<td>1.0 (0.7–1.4)</td>
<td>0.5</td>
</tr>
<tr>
<td>Enazenil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine alone</td>
<td>1.7 (0.9–3.2)</td>
<td></td>
</tr>
<tr>
<td>+0.32 Enazenil</td>
<td>1.3 (0.5–3.7)</td>
<td>0.8</td>
</tr>
<tr>
<td>+1.0 Enazenil</td>
<td>1.0 (0.4–2.4)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

a Significant rightward shift as determined by nonoverlapping 95% confidence intervals (CI).

b Significant leftward shift as determined by nonoverlapping 95% confidence intervals.
self-administration, GVG produced dose-dependent decreases in responding in the assay of food-maintained behavior. Although 180 mg/kg GVG failed to alter responding at any food concentration, doses of 320 and 560 mg/kg decreased responding maintained by 32 to 100% and 10 to 100% food, respectively. \( A_{50} \) values indicate a similar potency for GVG to decrease cocaine- and food-maintained responding (Table 3).

**Effects of Pretreatment with GABA-A Modulators on Cocaine- and Food-Maintained Responding.** Figure 7 shows cocaine- and food-reinforced responding after acute pretreatment with pentobarbital, midazolam, and enazenil. Pentobarbital produced dose-dependent and significant decreases in cocaine self-administration and food-maintained responding. Two-way ANOVA showed a significant main effect of pentobarbital dose on cocaine self-administration and food-maintained responding, and significant interactions between pentobarbital dose and cocaine dose and, between pentobarbital dose and food concentration. Pentobarbital doses of 3.2 and 5.6 mg/kg decreased responding maintained by cocaine doses (0.032–0.1 mg/kg/injection) located on the ascending limb of the cocaine dose-effect function. The highest dose of pentobarbital tested (10 mg/kg) decreased responding maintained by low-to-intermediate doses of cocaine (0.032–0.32 mg/kg/injection) that comprised both the ascending and descending limbs of the cocaine dose-effect function. No dose of pentobarbital altered responding maintained by higher doses of cocaine (1.0–3.2 mg/kg/injection). In the assay of food-maintained responding, 10 mg/kg pentobarbital, a dose that markedly reduced cocaine self-administration, failed to alter responding maintained by any concentration of food. In contrast, doses of 18 and 32 mg/kg decreased responding maintained by 32% food and 10 to 100% food, respectively. The \( A_{50} \) values for pentobarbital to decrease cocaine- and food-maintained responding were significantly different, with pentobarbital being approximately 2.7–4.0-fold more potent to decrease cocaine-maintained responding (Table 3).

Midazolam also produced dose-dependent and significant decreases in cocaine self-administration and food-maintained responding. Two-way ANOVA indicated a significant main effect of midazolam on cocaine self-administration and food-maintained responding, and significant interaction between midazolam dose and food concentration. Midazolam doses of 0.32 and 1.0 mg/kg failed to alter responding maintained by any dose of cocaine. However, a higher dose (3.2 mg/kg) decreased responding maintained by intermediate doses of cocaine (0.1–0.32 mg/kg/injection). In the assay of food-maintained responding, the lowest dose of midazolam tested (6 mg/kg) increased responding maintained by 10% food, without significantly altering responding at other concentrations.
In contrast, higher doses of midazolam (10–18 mg/kg) decreased responding maintained by food concentrations ranging from 10 to 100%. When analyzed according to $A_{50}$ values, midazolam was 8.7- to 10.1-fold more potent to decrease cocaine- than food-maintained responding (Table 3).

In contrast to the effects of pentobarbital and midazolam, enazenil produced only minimal alterations in cocaine self-administration. Although some enazenil-cocaine combinations seemed to decrease cocaine self-administration, these effects were relatively small and not dose-dependent. Two-way ANOVA failed to indicate a main effect of enazenil on cocaine self-administration or an enazenil dose/cocaine dose interaction. In the assay of food-maintained responding, there was a significant main effect of enazenil dose and a significant interaction between enazenil dose and food concentration. All doses tested (5.6–18 mg/kg) decreased responding maintained by low to intermediate food concentrations (3–10%), without altering responding maintained by higher food concentrations.

Discussion

Summary. In the present study, pentobarbital and midazolam attenuated the discriminative stimulus effects of cocaine and selectively decreased cocaine self-administration in comparison with food-maintained responding. In contrast, muscimol, baclofen, GVG, and enazenil did not attenuate the discriminative stimulus effects of cocaine and failed to produce selective decreases in cocaine self-administration. These results suggest that high-efficacy GABA-A receptor modulators may attenuate the abuse-related effects of cocaine more effectively than direct GABA agonists, indirect GABA agonists, or low-efficacy GABA-A modulators.

Substitution of GABAergic Ligands in Cocaine Discrimination. When tested alone, none of the GABAergic ligands produced full substitution for the discriminative stimulus effects of cocaine. These findings are in agreement with studies demonstrating that direct-acting GABA agonists and GABA-A modulators fail to reproduce the discriminative stimulus effects of cocaine across a range of cocaine training doses (Witkin et al., 1991; Terry et al., 1994; Munzar et al., 2000; Negus et al., 2000). Although the GABAergic ligands generally produced dose- and time-dependent partial substitution for cocaine, several aspects of the data suggest that this partial substitution was not a robust phenomenon. For example, the maximal level of cocaine-appropriate responding ranged from 20 (baclofen) to 70% (pentobarbital) and generally occurred at doses of each drug that markedly reduced rates of responding. When analyzed according to
individual substitution patterns, less than half the animals tested with each drug exhibited full substitution for cocaine. Moreover, in time-course analyses, it was frequently observed that the maximal level of substitution was less than that observed in dose-response analyses. These results can be differentiated from the indirect dopamine agonists cocaine...
and d-amphetamine, for which the maximal level of cocaine-appropriate responding approached 100%, and full substitution occurred in all animals tested in both dose-response and time-course tests, and at doses that did not decrease response rates. The failure of these drugs to fully substitute for cocaine suggests that their mechanism of action for producing cocaine-like discriminative stimulus effects is different from that of indirect dopamine agonists.

Effects of Pretreatment with GABAergic Ligands on Cocaine Discrimination. The barbiturate GABA-A modulator pentobarbital dose dependently attenuated the discriminative stimulus effects of cocaine, with the highest dose producing an approximate 3.6-fold rightward shift in the cocaine dose-response function. These findings are consistent with those reported by Negus et al. (2000), in which pentobarbital dependence blocked the discriminative stimulus effects of cocaine in rhesus monkeys. Although the magnitude of the shift in the present study is modest, it is noteworthy that the dopamine D1 antagonist SCH 39166 produced a comparable 3.6-fold rightward shift in the cocaine dose-effect function. These findings indicate that under some conditions, GABA-A receptor modulation can produce effects on cocaine discrimination resembling dopamine receptor blockade.

An intermediate dose (0.32 mg/kg) of the high efficacy benzodiazepine GABA-A modulator midazolam also attenuated the discriminative stimulus effects of cocaine. Other studies also found that high-efficacy benzodiazepines attenuated the discriminative stimulus effects of cocaine or d-amphetamine (Druhan et al., 1991; Negus et al., 2000; Rush et al., 2004). In contrast to midazolam, the relatively low-efficacy benzodiazepine (“partial positive allosteric modulator”; Giusti et al., 1993) enazenil failed to alter the effects of cocaine. Similar efficacy-dependent effects were observed in rhesus monkeys (Negus et al., 2000) and together suggest that relative efficacy at benzodiazepine receptors may be a

Fig. 7. Effects of acute pretreatment (PT) with the GABA-A modulators pentobarbital, midazolam, and enazenil on responding maintained by food and cocaine. Abscissae, concentration of liquid food (log scale) (left) or unit dose of cocaine in milligrams per kilogram per injection (log scale) (right). Points above “0” show responding maintained by water (left) or the cocaine-associated cue light alone (right). Ordinates, total number of reinforcers earned during a 20-min period of availability. Values shown are group means (±S.E.M.) calculated from up to three determinations in each rat (n = 6–9 rats). Asterisks indicate significant differences from effects of vehicle injection by Duncan’s pairwise comparisons following a significant main effect or interaction by repeated measures ANOVA (p < 0.05). For enazenil effects on cocaine-maintained responding, no main effects or interactions were observed, and thus pairwise comparisons were not conducted.
critical determinant of GABAergic modulation of the discriminative stimulus effects of cocaine. Whereas an intermediate dose of midazolam produced a rightward shift in the cocaine dose-effect function, a dose only one-half-log unit higher (1.0 mg/kg) shifted the curve leftward. It is important to note that the higher dose of midazolam produced partial substitution for cocaine, and under such conditions, leftward shifts in the dose-response function may be more likely than rightward shifts.

In contrast to the effects of the GABA-A modulators, direct (muscimol and baclofen) and indirect (GVG) GABA agonists failed to alter the discriminative stimulus effects of cocaine. This was probably not a result of inadequate dosing, because all drugs were tested up to doses that decreased response rates. In agreement with the present results, muscimol and baclofen also failed to alter the discriminative stimulus effects of cocaine in rhesus monkeys and rats (Munzar et al., 2000; Negus et al., 2000), and baclofen failed to alter the subject-rated effects of cocaine in humans (Lile et al., 2004). In accounting for the differences between the effects of GABA-A modulators and direct GABA agonists, it is important to note that the discriminative stimulus properties of these two classes of compounds are dissociable (Greech and Balster, 1993, 1997). Moreover, their mechanisms of action at the GABA receptor can also be differentiated. Whereas GABA-A modulators enhance basal activity at the receptor, the effects of direct GABA agonists are independent of basal rates of GABA receptor function.

**Effects of Pretreatment with GABAergic Ligands on Cocaine- and Food-Maintained Responding.** In general, the GABAergic compounds decreased responding maintained by low-to-intermediate doses of cocaine, with minimal effects on behavior maintained by higher unit doses of cocaine. As a result, the ascending limb of the cocaine dose-effect function was shifted rightward. This profile is consistent with numerous other studies documenting the ability of GABA-A modulators and direct and indirect GABA agonists to decrease cocaine self-administration (Goeders et al., 1989, 1993; Roberts et al., 1996; Shoaiib et al., 1998; Campbell et al., 1999; Kushner et al., 1999; Brebner et al., 2000a,b; Stafford and Gowa, 2000). The GABA-A modulators midazolam and pentobarbital were on average 9.6- and 3.3-fold more potent in decreasing cocaine- than food-maintained behavior, respectively. The selective effects of GABA-A modulators agree with a report by Goeders et al. (1993), in which alprazolam produced a selective decrease in cocaine-maintained responding in rats. It should be noted, however, that the selective effects of alprazolam were obtained only after a period of initial exposure in which alprazolam produced nonselective decreases in cocaine- and food-maintained responding. Moreover, Herling et al. (1979) found that the potency of pentobarbital to decrease response rates in rhesus monkeys was very similar whether cocaine or food was the reinforcing event. Nevertheless, the present data agree with at least some prior data documenting selective effects of GABA-A modulators on cocaine self-administration.

In contrast to the GABA-A modulators, the potency of direct (muscimol and baclofen) and indirect (GVG) GABA agonists to decrease cocaine- and food-maintained responding was similar, differing by less than 1.5-fold. The findings with GVG are consistent with a report indicating a similar potency for this compound to alter cocaine- and food-maintained behavior (Kushner et al., 1999). However, regarding GABA-B agonists such as baclofen, a number of cocaine self-administration studies have indicated little or no effect of GABA-B agonists on food-maintained responding when food is made available alone or concurrently with cocaine or during alternate components of a multiple schedule (Roberts et al., 1996; Roberts and Andrews, 1997; Shoaiib et al., 1998; Brebner et al., 2000a,b). Differences between these prior reports and the present one could be accounted for by the different experimental conditions under which behavior was maintained. Most importantly, in prior experiments, only one magnitude of food reinforcer was available, whereas a range of reinforcer magnitudes was made available in the present study. In addition, in prior studies, responding for cocaine and food was maintained under different schedules of reinforcement, rendering potency comparisons between the two consequent events difficult (Roberts and Andrews, 1997; Brebner et al., 2000a,b). Testing the effects of candidate medications across a wide range of reinforcer magnitudes and under identical schedules of reinforcement may help identify the conditions under which selective effects are observed (Barrett et al., 2004).

Midazolam produced the most selective decrease in cocaine self-administration in comparison to food-maintained responding, and midazolam was one of only two drugs to produce rate-increasing effects on food-maintained responding. A number of studies have documented the ability of midazolam to increase feeding behavior (e.g., Cooper and Yerbury, 1986), suggesting that the apparent selectivity was due, at least in part, to a selective increase in food-maintained behavior. That muscimol also increased feeding behavior (Stratford and Kelley, 1997; present study) but was equally potent in decreasing cocaine- and food-maintained responding suggests that a selective effect on feeding can not completely account for these results. Nevertheless, these data highlight one complication of using food-maintained responding as a control to evaluate the selectivity of pretreatment drug effects on cocaine self-administration (Barrett et al., 2004).

Another complication regarding comparisons of the effects of pretreatment drugs on cocaine- and food-maintained responding is that the shape and position of the reinforcer magnitude-effect curves and the absolute response rates differed across these assays. Both of these factors may be important determinants of pretreatment drug effects on behavior. These issues were addressed in the following manner. First, increasing the magnitude of the food reinforcer beyond the maximum studied here resulted in a descending limb under the FR schedule (unpublished observations). Thus, the highest food reinforcer magnitude studied here likely represents the peak of an inverted U-shaped function relating magnitude of food reinforcer to responding, and can be compared with the dose at the peak of the inverted U-shaped cocaine dose-effect function. Second, absolute rates of responding maintained by one dose of cocaine (0.1 mg/kg/injection) and one concentration of food (10%) were comparable. Accordingly, A_{50} values were compared across the following three dimensions: equivalent positions on the magnitude-effect functions, equivalent absolute response rates, and the lowest magnitude of each reinforcer that reliably maintained behavior in every rat. Interestingly, the relative potency of a
drug to decrease cocaine- versus food-maintained behavior did not change across the three dimensions. A caveat to the higher relative potency observed with high efficacy GABA-A modulators for cocaine- versus food-maintained behavior is that the drug pretreatment intervals were sometimes shorter for cocaine- versus food-maintained responding. In this regard, midazolam and pentobarbital displayed the greatest selectivity and were also the shortest-acting compounds (Fig. 2). There are three reasons that these temporal factors probably cannot fully account for our results. First, when \( A_{\text{xy}} \) values for decreasing food- and cocaine-maintained responding were calculated in different components of the test sessions, the selectivity of the pretreatment drugs was unaltered (Table 3). Second, the two compounds (midazolam and pentobarbital) that selectively decreased cocaine self-administration were the same two compounds that attenuated the discriminative stimulus effects of cocaine. Third, the same or similar high efficacy GABA-A modulators also displayed a favorable profile in previous studies on the attenuation of cocaine’s reinforcing and discriminative stimulus effects (Goeders et al., 1993; Negus et al., 2000).

Clinical Implications. Preclinical studies in rats may be useful for providing an impetus for clinical evaluations but are especially preliminary when only acute and not chronic treatments are evaluated. Accordingly, the present preclinical results with acute treatments should be extended to include chronic treatment studies. Nevertheless, the present findings are paralleled by recent clinical studies, where 9 to 10 weeks of treatment with GABA agonists decreased the number of patients testing positive for cocaine (Ling et al., 1998; Gonzalez et al., 2003; Shoptaw et al., 2003; Brodie et al., 2005). Interestingly, those reports did not include studies of GABA-A modulators. Insofar as the present preclinical results may have implications for the treatment of cocaine dependence, the present findings suggest that GABA-A modulators may be worthy of clinical evaluation for cocaine dependence.

Acknowledgments

We thank Jennifer M. Dohrmann for outstanding technical assistance. We thank Mike Nader and Rob Mach for the gift of GVG. We thank E. Costa and A. Guidotti for the gift of enaneth.

References

Hering S, Downs DA, and Woods JD (1979) Cocaine, d-amphetamine and pentobarbital effects on responding maintained by food or cocaine in rhesus monkeys. *Psychopharmacology (Berlin) 64*:261–269.