

Attenuation of behavioral effects of cocaine by the metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)-pyridine in squirrel monkeys: Comparison with dizocilpine

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Running Title: MPEP Attenuates Behavioral Effects of Cocaine in Monkeys

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Text pages: 35

Tables: 4

Figures: 4

References: 50

Abstract: 248

Introduction: 570

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Abbreviations: metabotropic glutamate receptor, mGluR; ionotropic glutamate receptor, iGluR; 2-methyl-6-(phenylethynyl)-pyridine, MPEP; N-methyl-D-aspartate; NMDA; alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, AMPA; Discriminative stimulus, DS; norepinephrine transport, NET; CGP 37849, DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid

Recommended Section Assignment: Behavioral Pharmacology

ABSTRACT

Growing evidence suggests a role for metabotropic glutamate receptors (mGluRs) in the behavioral effects of cocaine related to its abuse. The mGluR5 subtype, in particular, has come under scrutiny due to its distribution in brain regions associated with drug addiction. This study investigated interactions between the selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) and cocaine in squirrel monkeys whose lever-pressing behavior was: 1) maintained under a second-order schedule of cocaine self-administration, 2) extinguished and then reinstated by cocaine priming, and 3) controlled by the discriminative stimulus (DS) effects of cocaine. Additional studies determined the effects of MPEP on unconditioned behaviors, coordination and muscle resistance. In each experiment, the effects of MPEP were compared with those of the N-methyl-D-aspartate (NMDA) antagonist dizocilpine. MPEP attenuated cocaine self-administration, cocaine-induced reinstatement of drug seeking, and the DS effects of cocaine at doses that did not markedly impair motor function or operant behavior in the context of drug discrimination. Dizocilpine also attenuated cocaine self-administration, but did not significantly alter cocaine-induced reinstatement of drug seeking, and it enhanced rather than attenuated the DS effects of cocaine. The findings point to a significant contribution of mGluR5 mechanisms in the behavioral effects of cocaine related to its abuse and suggest that MPEP has properties of a functional cocaine antagonist, which are not secondary to antagonism at NMDA receptors. The contrasting interactions of MPEP and dizocilpine with cocaine imply that glutamate acting through different metabotropic and ionotropic receptors may modulate the behavioral effects of cocaine in qualitatively different ways.

There is now a large body of literature documenting the importance of glutamate receptor mechanisms in the behavioral effects of cocaine related to its abuse. The majority of this research has focused on the contribution of ionotropic glutamate receptors (iGluRs), which are ligand-gated ion channels classified traditionally into three main subtypes [N-methyl-D-aspartate (NMDA) receptors, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and kainate receptors] based on structural homology and pharmacological specificity (Rockhold, 1998; Wolf, 1998). Drugs acting at iGluR subtypes have been found to modulate various behavioral effects of cocaine in animals, including i.v. self-administration, reinstatement of drug seeking, and development of behavioral sensitization (Li et al., 1997, Pierce et al., 1997; Cornish and Kalivas, 2001).

In addition to iGluRs, growing evidence suggests a role for metabotropic glutamate receptors (mGluRs) in the behavioral effects of cocaine related to its abuse (Vezina and Kim, 1999; Swanson and Kalivas, 2000, Epping-Jordan, 2002; Wang et al., 2002). The mGluRs are G-protein coupled receptors that have been classified into three main groups (Groups I-III) encompassing eight subtypes (mGluR 1-8) based on sequence homology, signal transduction pathways, and pharmacology (Conn and Pin, 1997). The mGluR5 subtype, in particular, has come under scrutiny due to its high level of expression in limbic and forebrain regions that are believed to be important neuroanatomical substrates underlying cocaine abuse (Spooren et al., 2001; Baker et al., 2002; Muly et al., 2003). Behavioral studies have shown that knock-out mice lacking the mGluR5 subtype do not acquire i.v. self-administration of cocaine and that wild-type mice treated with the selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) show reduced cocaine self-administration at doses that do not suppress operant behavior maintained by food reinforcement (Chiamulera et al., 2001). In line with these findings,

McGeehan and Olive (2003) reported that MPEP attenuates cocaine conditioned place preference in mice, and Kenny et al. (2003) reported that MPEP reduces i.v. self-administration of cocaine in rats. Collectively, these results point to a potentially important role for mGluR5 mechanisms in the reinforcing effects of cocaine and suggest that MPEP may have properties of a functional cocaine antagonist. The nature of this apparent antagonism and the effectiveness with which MPEP attenuates other effects of cocaine related to its abuse have not been explored systematically.

The purpose of the present study was to investigate the interactions of MPEP with cocaine in squirrel monkeys whose lever-pressing behavior was: 1) maintained under a second-order schedule of i.v. cocaine self-administration, 2) extinguished and then reinstated by priming with cocaine, and 3) controlled by the discriminative stimulus (DS) effects of cocaine. Additionally, observational studies characterized the effects of MPEP on a range of observable behaviors, coordination and muscle resistance. In each type of experiment, the behavioral effects of MPEP were compared with those of dizocilpine, as well as CGP 37849 [DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid; Chapman et al., 1991] in drug discrimination experiments. Comparative studies with these reference NMDA receptor antagonists were included to explore the possible contribution of NMDA receptor mechanisms in the interactions between MPEP and cocaine. Although MPEP exhibits high selectivity at mGluR5 receptors *in vitro* and *in vivo* (Gasparini et al., 1999; Thomas et al., 2001), MPEP has been reported to decrease NMDA-mediated neuronal toxicity (O'Leary et al., 2000) and to interact functionally with NMDA receptors in rats (Pisani et al., 2001; Homayoun et al., 2004). The latter findings suggest that the cocaine-attenuating effects of MPEP may be mediated, at least in part, via modulation of NMDA receptor activity.

MATERIALS AND METHODS

Subjects. A total of 19 adult male and female squirrel monkeys (*Saimiri sciureus*), weighing 0.6-1.2 kg, were studied in daily experimental sessions (typically, Monday-Friday). Between sessions monkeys were housed individually in a climate-controlled vivarium, where they had unlimited access to water. Monkeys participating in drug discrimination experiments were maintained at approximately 90% of their free-feeding body weights by adjusting their access to food (Teklad monkey chow supplemented with fresh fruit) in their home cages. Monkeys participating in other experiments had unrestricted access to food except during the experimental session. All monkeys were maintained in accordance with the guidelines of the Committee on Animals of Harvard Medical School and the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council; National Academy Press; Washington D.C., 1996). Research protocols were approved by the Harvard Medical School Institutional Animal Care and Use Committee.

Surgical Procedures. In studies involving cocaine self-administration and reinstatement of cocaine-seeking behavior, monkeys were implanted with chronic venous catheters (polyvinyl chloride, inside diameter: 0.38 mm, outside diameter: 0.76 mm) using the surgical procedures described by Carey and Spealman (1998). Briefly, under isoflurane anesthesia and aseptic conditions, one end of the catheter was passed to the level of the right atrium by way of a femoral or jugular vein. The distal end of the catheter was then passed subcutaneously and exited in the mid-scapular region. Catheters were flushed daily with heparinized saline solution (150-200 U/ml) and sealed with stainless steel obturators when not in use. Monkeys wore nylon mesh jackets (Lomir Biomedical, Toronto, Canada) at all times to protect the catheter.

Apparatus. In studies involving cocaine self-administration, reinstatement of cocaine seeking, and cocaine discrimination, daily sessions were conducted in ventilated, sound-attenuated chambers (Med Associates, St. Albans, VT), which were provided with white noise to mask extraneous sounds. Within the chambers, monkeys sat in Plexiglas chairs (MED Associates) facing an aluminum panel equipped with one or two response levers (depending on the type of study) and colored lights, which could be illuminated to serve as visual stimuli. Each press of a lever with a minimum downward force of approximately 0.25 N was recorded as a response. In experiments involving catheterized subjects, catheters were connected to syringe pumps (Med Associates) located outside the chamber. Each operation of the pump lasted 1 s and delivered a volume of 0.18 ml into the catheter. In experiments involving cocaine discrimination, 190-mg sucrose pellets (Bioserve, Frenchtown, NJ) could be delivered to a receptacle in the front panel of the chair.

Observational studies were conducted in a ventilated, transparent Plexiglas arena (114 x 122 x 213 cm) located in a lighted room isolated from other animals. The arena was equipped with perches, suspended plastic chains, and a wood-chip substrate to permit a range of species-typical behaviors. A video camera connected to a videocassette recorder was positioned approximately 1 m in front of the chamber and operated continuously during all sessions.

Cocaine Self-Administration. Monkeys were trained to self-administer cocaine under a second-order fixed-interval, fixed-ratio [FI(FR)] schedule of i.v. drug injection similar to the schedule described by Platt et al (2001). In the presence of a white light, completion of every 10th response (FR 10) during the 10-min interval (FI 10) resulted in a 2-s change in illumination from white to red light, and completion of the first FR after expiration of the FI resulted in an i.v. injection of cocaine paired with the 2-s light change. A 60-s timeout period, during which all

lights were off and responses had no scheduled consequences, followed each cocaine injection. If the FR requirement was not completed within 8 min following the expiration of the FI, the timeout period was started automatically without an injection (limited hold). Daily sessions ended after completion of five cycles of the second-order schedule or a maximum session length of 90 min. In preliminary studies, the dose of cocaine was varied over a 3-10-fold range to determine the dose that maintained maximum rates of responding for each monkey. These doses were 0.1 mg/kg/injection (one monkey), 0.18 mg/kg/injection (2 monkeys), and 0.3 mg/kg/injection (4 monkeys) and were held constant for the remainder of the study.

Testing began after stable baseline rates of responding were established (minimum of five sessions with no increasing or decreasing trend in response rate over at least three consecutive sessions). During test sessions, monkeys were pretreated i.m. with MPEP (0.1-1.0 mg/kg), dizocilpine (0.003-0.03 mg/kg), or vehicle 5 min before the session. Pretreatment times were based on previous behavioral studies with dizocilpine in squirrel monkeys (Wiley et al., 1997) and pilot time-course investigations with MPEP in squirrel monkeys (R.D. Spealman, unpublished observation) and rodents (Gasparini et al., 1999). Between test sessions with different drug pretreatments, baseline sessions were conducted until responding stabilized for at least 3 consecutive days as described above. Test sessions typically were conducted once per week, and the order of drug testing was varied across monkeys. Of the seven monkeys used in these studies, six were tested with all doses of MPEP and dizocilpine, one was tested only with MPEP, and one was tested only with dizocilpine.

Reinstatement of Cocaine Seeking. Five of the monkeys used in the experiments described above also were tested in experiments involving reinstatement of cocaine-seeking behavior using procedures similar to those described by Khroyan et al. (2000). Following a period of stable

responding under the second-order schedule of cocaine self-administration described above, cocaine-seeking behavior was extinguished by substituting saline for cocaine and omitting presentations of the cocaine-paired stimulus. Extinction sessions were otherwise identical to those described above for cocaine self-administration. Extinction sessions were conducted daily until responding declined to $\leq 10\%$ of the response rate maintained by cocaine self-administration (4-15 sessions depending on the subject), at which time reinstatement tests were started. Test sessions used the same procedures as i.v. cocaine self-administration, except that only saline was available for self-administration. During these tests, monkeys were pretreated i.m. with MPEP, dizocilpine, or vehicle 5 min prior to an i.v. priming injection of cocaine, which was administered immediately before the session. The effects of MPEP and dizocilpine were determined first on reinstatement of drug seeking induced by 0.3 mg/kg cocaine followed by additional tests of the effects of the drugs on reinstatement induced by a higher dose of cocaine (1.0 mg/kg). The effects of MPEP and dizocilpine in the absence of cocaine also were determined by administering the drugs individually without a subsequent priming injection. Four or five monkeys were used in each type of experiment. Between experiments with different drugs or drug combinations, self-administration of cocaine was re-established and subsequently extinguished using the procedures and criteria described above. Previous studies showed that periodically re-establishing and then extinguishing drug seeking between experiments permits reinstatement of drug seeking to be induced reliably by cocaine over >2 years of testing (Khroyan et al., 2000).

Cocaine Discrimination. Monkeys were trained to discriminate cocaine from saline using procedures described by Spealman et al. (1991). Briefly, each monkey was trained to respond differentially on the left and right levers depending on whether cocaine (0.3 mg/kg, i.m.) or

vehicle had been injected. During each training session, 10 consecutive responses on one lever (left for 4 monkeys, right for 3 monkeys) produced a pellet of food if cocaine had been injected, whereas 10 consecutive responses on the other lever produced a pellet of food if vehicle had been injected. Each response on the inappropriate lever (e.g., the vehicle lever when cocaine had been injected) reset the FR 10 requirement. Delivery of each food pellet was followed by a 10-s timeout period, during which the chamber was dark and responses had no scheduled consequences.

Daily training sessions consisted of a variable number of components ($n = 1-4$) of the basic procedure described above. The number of components per session was randomized from day-to-day with the restriction that each number occurred equally often within a block of 20 sessions. Each component ended after 10 food pellets had been delivered or after 5 min had elapsed, whichever occurred first. An extended (10-min) timeout, during which injections of cocaine or vehicle could be administered, preceded each component of a session. During most training sessions, vehicle was injected at the midpoint of the 10-min timeout preceding the first $n-1$ components, and cocaine was injected at the midpoint of the timeout preceding the last component of the session. Periodically, saline was injected during timeout periods preceding all four components of a session to prevent an invariant association between cocaine injection and the 4th component.

Drug testing began once monkeys made $\geq 90\%$ of responses on the injection-appropriate lever for at least five consecutive training sessions. Thereafter, test sessions were conducted once or twice per week, with training sessions on intervening days. Test sessions were conducted only if $\geq 90\%$ of responses were made on the injection-appropriate lever during at least four of the last five training sessions. Test sessions consisted of four components, each

preceded by a 10-min timeout period. During each component of a test session, completion of 10 consecutive responses on either lever resulted in delivery of a food pellet. Dose-response functions for cocaine and each test drug were determined using a cumulative-dosing procedure similar to the one described by Spealman et al. (1991). Briefly, incremental doses of a test drug were injected i.m. at the midpoint of the 10-min timeout period that preceded sequential components of a test session, permitting a 4-point cumulative dose-response function to be determined in a single session. Five or more different doses could be tested by administering overlapping ranges of cumulative doses during test sessions on different days. Cumulative dose-response functions were determined for cocaine (0.03-0.56 mg/kg), MPEP (0.03-1.8 mg/kg), dizocilpine (0.003-0.10 mg/kg) and CGP 37849 (1.0-18.0 mg/kg). Drug interaction experiments were then conducted for cocaine combined with MPEP (1.0 and 1.8 mg/kg), dizocilpine (0.01 and 0.03 mg/kg), CGP 37849 (1.0 and 10.0 mg/kg), and vehicle. In drug-interaction experiments, vehicle or a selected dose of MPEP, dizocilpine or CGP 37849 was administered 5 min before the first cocaine injection of the test session. Cumulative doses of cocaine were then administered during the session as described above. Four or five (out of a total of six) monkeys were tested in experiments with each drug or drug combination.

Observational Studies. Six monkeys were studied in daily 30-min observational sessions, during which the animal's behavior was videotaped continuously. Scoring of videotapes was conducted by two observers, who were trained in the use of the behavioral scoring system described by Platt et al. (2000), but were not informed about the drugs under investigation. Before beginning the study, observers underwent at least 20 h of training until they met an inter-observer reliability criterion of at least 90%. The behavioral scoring system included ten categories (Table 1), which were scored by recording the presence or absence of each behavior in

15-s intervals during three 5-min observation periods across the session (0 - 5 min, 12 - 17 min, 24 - 29 min). Modified frequency scores (Platt et al., 2000) were calculated for each observation period as the number of 15-s intervals in which a particular behavior was observed (maximum score = 20).

Additionally, during the 6th, 18th and 30th min of each session, the monkey's leash was attached to a stainless steel transport pole, and it was removed briefly from the observation arena by a trained handler for evaluation of ataxia and muscle resistance (Platt et al., 2000). This technique effectively limited the monkey's access to the region of the transport pole farthest away from the handler and afforded safe evaluation of balance and resistance to leg extension. Ataxia was defined as the inability to balance on and/or grasp the pole (56.0 cm long, 1.0 cm in diameter) held in a horizontal plane. Muscle resistance was defined as increased resistance to manual extension of a hind limb and/or clinging to the grid floor of the observation arena. During each assessment, a score of 0, 1 or 2 was assigned to each measure. For ataxia, a score of 0 indicated that the monkey was able to balance normally on the pole, a score of 1 indicated inability to balance effectively, and a score of 2 indicated that the monkey could neither balance on nor grasp the pole. For muscle resistance, a score of 0 indicated no increased resistance to hind-limb extension, a score of 1 indicated either an increased resistance to extension of the hind limb or clinging to the grid floor, and a score of 2 indicated strong resistance to hind-leg extension and clinging to the grid floor. Test sessions with MPEP (0.1-1.8 mg/kg) and dizocilpine (0.003-0.03 mg/kg) were conducted once or twice per week, with vehicle control sessions on intervening days. All injections were administered i.m. 5 min prior to placing the subject in the observation arena.

Data Analysis. In studies involving cocaine self-administration, response rates for individual monkeys were computed by dividing the total number of responses by the total session time (excluding timeout periods). The number of self-administered injections (maximum: 5) also was recorded each session for individual monkeys. Individual data were then averaged across subjects (n=6 per experiment). In studies involving reinstatement of drug seeking, response rates for individual subjects (calculated as above) were converted to a percentage of the response rate induced by cocaine alone and then averaged across subjects (n=4 or 5 per experiment). In studies of drug discrimination, the percentage of responses on the cocaine lever was calculated for individual subjects in each component by dividing the number of responses on the cocaine lever by the total number of responses on both levers and multiplying the quotient by 100. The percentage of cocaine-lever responses was not calculated for an individual monkey if the response rate was < 0.1 responses/s. Response rates for individual subjects were computed in each component by dividing the total number of responses (regardless of lever) by the total component duration exclusive of timeout periods. Individual data were then averaged across subjects (n=4 or 5 per experiment). In observational experiments and assessments of ataxia and muscle resistance, individual scores were averaged across the three 5-min observation periods because no significant differences across the three periods were observed for any measure (as determined by repeated measures ANOVAs). Scores for individual subjects were then averaged across monkeys (n=6 per experiment). Data from all experiments were analyzed with repeated measures ANOVA; planned comparisons were performed with Dunnett's test or Bonferroni t-test, as appropriate, using the SigmaStat®3.0 statistical software package.

Drugs. Cocaine hydrochloride (Sigma-RBI, St. Louis, MO), MPEP hydrochloride and dizocilpine maleate (Tocris Cookson, Ellisville, MO), and CGP 37849 (NOVA Pharmaceuticals, Baltimore, MD) were dissolved in sterile water or 0.9% saline solution containing small amounts of ethanol, 2-hydroxypropyl- β -cyclodextrin, lactic acid or sodium hydroxide as required.

RESULTS

Cocaine Self-Administration. Intravenous injections of cocaine maintained consistent self-administration in all monkeys under the second-order schedule of i.v. drug injection. Baseline rates of responding ranged from 0.56 to 0.72 responses/s in individual subjects, with a mean rate of 0.64 (± 0.03 , S.E.M.) responses/s for the group. All subjects self-administered the maximum number of cocaine injections (5) during each baseline session. Pretreatment with MPEP produced dose-related decreases in the rate of responding maintained by self-administered cocaine (Fig.1, top left panel). Analysis of variance revealed a significant effect of MPEP ($F_{3,15} = 8.5$, $p < 0.05$), and planned comparisons showed a significant reduction in response rate after pretreatment with either 0.3 or 1.0 mg/kg MPEP compared to vehicle ($p < 0.05$, Dunnett's test). Pretreatment with 1.0 mg/kg MPEP also produced a significant reduction in the number of injections/session compared with vehicle (Fig. 1, bottom left panel; $p < 0.05$, Dunnett's test).

Like MPEP, pretreatment with dizocilpine produced a dose-related decrease in the rate of responding maintained by self-administered cocaine (Fig. 1, top right panel). There was a significant effect of dizocilpine dose ($F_{3,15} = 6.6$, $p < 0.05$) and a significant reduction in response rate after pretreatment with 0.03 mg/kg dizocilpine compared with vehicle ($p < 0.05$, Dunnett's test). Unlike MPEP, pretreatment with dizocilpine did not significantly reduce the number of cocaine injections/session regardless of dose. Higher doses of dizocilpine were not tested due to their prominent sedative and behavioral suppressant effects in squirrel monkeys (Rupniak et al., 1993; unpublished observations).

Reinstatement of Cocaine Seeking. During extinction sessions, in which saline was substituted for cocaine and the cocaine-paired stimulus was omitted, responding declined and

stabilized at low rates for all subjects, with a group mean of 0.01 (\pm 0.007) responses/s. Priming with cocaine immediately before the test session induced consistent reinstatement of drug-seeking behavior to average rates of 0.39 (\pm 0.14) responses/s following priming with 0.3 mg/kg cocaine and 0.52 (\pm 0.07) responses/s following priming with 1.0 mg/kg cocaine. As shown in Fig. 2, pretreatment with MPEP produced dose-related reductions in the reinstatement of drug seeking induced by either 0.3 mg/kg ($F_{3,9} = 11.0$, $p < 0.05$) or 1.0 mg/kg cocaine ($F_{3,9} = 5.6$, $p < 0.05$). Further analysis revealed a significant attenuation of drug seeking induced by 0.3 mg/kg cocaine after pretreatment with 0.3 and 1.0 mg/kg MPEP and a significant attenuation of drug seeking induced by 1.0 mg/kg cocaine after pretreatment with 1.8 mg/kg MPEP compared to vehicle ($p < 0.05$, Dunnett's test). Overall, the dose-response curve for MPEP combined with 1.0 mg/kg cocaine was displaced to the right of the dose-response curve for MPEP combined with 0.3 mg/kg cocaine, indicating that higher doses of MPEP were required to attenuate reinstatement of drug seeking when the dose of cocaine was increased.

Although there was a tendency for dizocilpine to reduce the rate of responding induced by cocaine priming, the effects were variable across subjects, ranging from a 48% increase in response rate to a 93% decrease in response rate depending on individual subject and dose. Averaged for the group of five monkeys, the mean response rate after pretreatment with the highest dose of dizocilpine (0.03 mg/kg) was 75% of the response rate induced by priming with 0.3 mg/kg cocaine and 55% of the response rate induced by priming with 1.0 mg/kg (Table 2). Neither of these effects differed significantly from those observed after vehicle pretreatment. In the absence of cocaine priming, neither MPEP (0.1-1.0 mg/kg) nor dizocilpine (0.003 -0.03 mg/kg) induced significant reinstatement of cocaine-seeking behavior (Table 3).

Cocaine Discrimination. Cocaine maintained consistent stimulus control over behavior throughout the study. During training sessions that preceded drug test sessions, individual monkeys made an average of $\geq 96\%$ responses on the cocaine lever after injection of the training dose of cocaine (0.3 mg/kg) and $\leq 5\%$ responses on the cocaine lever after injection of vehicle. Under test conditions, cocaine engendered dose-related increases in responding on the cocaine lever, reaching a maximum of nearly 100% cocaine-lever responses after cumulative doses of 0.3 mg/kg or greater. Pretreatment with MPEP (1.0 and 1.8 mg/kg) produced a dose-related attenuation of the DS effects of cocaine and an overall rightward shift in the cocaine dose-response function, (Fig. 3, top panel). Analysis of variance revealed a significant effect of MPEP ($F_{2,6} = 18.0, p < 0.05$), and planned comparisons showed a significant reduction in the percentage of drug-lever responses engendered by 0.1 and 0.3 mg/kg cocaine after pretreatment with either 1.0 or 1.8 mg/kg MPEP ($p < 0.05$, Bonferroni t-test). Attenuation of the DS effects of cocaine by MPEP was observed at doses that had little or no effect on the average response rate (Fig. 3, bottom panel).

In contrast to MPEP, pretreatment with dizocilpine (0.01 and 0.03 mg/kg) and CGP 37849 (1.0 and 10.0 mg/kg) either had little effect on or enhanced the DS effects of cocaine, resulting in an overall leftward shift of the cocaine dose-response curve (Fig 4, top panels). Analysis of variance revealed a significant effect of dizocilpine ($F_{2,8} = 10.3, p < 0.05$) and an effect approaching significance for CGP 37849 ($F_{2,8} = 4.0, p = 0.06$). Planned comparisons showed that there was a significant increase in drug-lever responding engendered by 0.03 and 0.1 mg/kg cocaine after pretreatment with 0.03 mg/kg dizocilpine and 0.03 mg/kg cocaine after pretreatment with 10.0 mg/kg CGP 37849 ($p < 0.05$, Bonferroni t-test). Enhancement of the DS effects of cocaine by dizocilpine and CGP 37849 was observed at doses that did not greatly

reduce the average rate of responding (Fig. 4, bottom panels). The highest dose of CGP 37849 (10.0 mg/kg), however, decreased the response rate significantly when combined with 0.3 mg/kg cocaine, and the highest dose of cocaine (1.0 mg/kg) decreased the response rate significantly when tested alone or combined with either dizocilpine or CGP 37849 ($p < 0.05$, Bonferroni t-test). In the absence of cocaine, none of the glutamate antagonists engendered significant cocaine-lever responding up to doses that induced emesis (1.8 mg/kg MPEP) or eliminated operant responding (0.1 mg/kg dizocilpine) in the majority of animals tested (Table 3).

Observable Behavior. As shown in Table 4, MPEP induced a significant reduction in overall locomotion ($F_{4,16} = 16.5$, $p < 0.05$), and a significant increase in visual scanning ($F_{4,16} = 11.6$, $p < 0.05$) and muscle resistance ($F_{4,16} = 11.0$, $p < 0.05$), but had no significant effect on other motor behaviors, including foraging, object exploration, grooming and scratching (not shown). MPEP also did not induce ataxia or affect vocalization, static or resting postures. Dizocilpine had no significant effects on observable behavior with the exception of muscle resistance, which was increased by the highest dose of dizocilpine ($p < 0.05$, Dunnett's test). In addition to scored behaviors, emesis was observed in the majority of monkeys after 1.8 mg/kg MPEP, but was not observed after any dose of dizocilpine.

DISCUSSION

In the present study, MPEP attenuated cocaine self-administration by squirrel monkeys under a second-order schedule of i.v. drug injection, which resulted in dose-related decreases in response rate and the number of cocaine injections/session. These findings are consistent with recent reports that MPEP reduces i.v. cocaine self-administration in rodents (Chiamulera et al., 2001; Kenny et al., 2003). In the study by Chiamulera and coworkers, significant reductions in cocaine self-administration were observed at doses of MPEP that did not suppress operant responding maintained by food reinforcement. Similarly, in our study MPEP significantly reduced responding maintained by i.v. cocaine injections at doses that reduced locomotion but did not impair other motor behaviors in observational studies or disrupt operant response rate in the context of cocaine discrimination. Despite emetic effects at high doses, our findings suggest that MPEP-induced decrements in cocaine self-administration reflect functional antagonism of the reinforcing effects of cocaine and not a generalized disruption of operant behavior or motor function.

MPEP also produced a dose-related attenuation of drug seeking induced by priming with 0.3 and 1.0 mg/kg cocaine. Overall, the dose-response curve for MPEP with 1.0 mg/kg cocaine priming was shifted to the right of the dose-response curve for MPEP with 0.3 mg/kg cocaine priming. These dose-dependent differences imply that the cocaine-antagonist effects of MPEP are surmountable and that higher doses of MPEP are required to block reinstatement of drug seeking as the dose of cocaine is increased. Surmountable antagonism also was observed in animals trained to discriminate cocaine from vehicle as shown by an overall rightward shift in the dose-response curve for cocaine's DS effects. Significant attenuation of the DS effects of cocaine was seen at doses of MPEP that did not markedly affect the rate of lever pressing, again

implying that the interactions between MPEP and cocaine were pharmacologically specific and not the result of a generalized degradation of task performance or a simple masking of the effects of one drug by another (cf. Gauvin and Young, 1989).

Although MPEP displays high selectivity for mGluR5 receptors (Gasparini et al., 1999, Thomas et al., 2001), it also has been reported to decrease NMDA-mediated neurotoxicity (O'Leary et al., 2000) and to interact functionally with NMDA receptors in rodents (Pisani et al., 2001; Homayoun et al., 2004). These findings suggest a potential role for NMDA receptor mechanisms in the behavioral effects of MPEP in our study. To explore this possibility, we compared the effects of MPEP with those of the NMDA antagonist dizocilpine. The two drugs had qualitatively similar effects on response rate under the second-order schedule of i.v. cocaine injection, but differed with respect to their effects on cocaine-induced reinstatement of drug seeking and cocaine's DS properties. Whereas MPEP attenuated reinstatement of cocaine-seeking behavior and the DS effects of cocaine in a dose-related manner, dizocilpine did not significantly alter cocaine-induced reinstatement of drug seeking, and it enhanced rather than attenuated the DS effects of cocaine. These qualitatively different pharmacological profiles make it unlikely that the cocaine-antagonist effects of MPEP were mediated principally via attenuation of NMDA receptor activity.

MPEP also has been reported to inhibit norepinephrine transport (NET) and to mimic the effects of prototypic NET inhibitors on cellular activity in the amygdala and locus coeruleus of rats (Heidbreder et al., 2003). Although inhibition of NET could have contributed to some of the effects of MPEP in our study (e.g., reduced locomotion), it is not likely to be the mechanism underlying MPEP-induced attenuation of cocaine's behavioral effects. In this regard, selective NET inhibitors such as nisoxetine and talsupram have been found to partially substitute for the

DS effects of cocaine (Spealman, 1995) and to mimic rather than attenuate cocaine-induced reinstatement of drug-seeking behavior in squirrel monkeys (Spealman et al., 2004).

Although the neuropharmacological mechanisms underlying MPEP-induced attenuation of cocaine's behavioral effects have not been established, accumulating evidence points to both neuroanatomical and functional links between the dopamine and glutamate receptor systems in brain regions associated with cocaine abuse (Vezina and Kim, 1999; Spooen et al., 2001; Baker et al., 2003). The mGluR5 subtype is likely associated with dopamine neurons (Paquet and Smith, 2003) and might be expected to modulate the behavioral effects of cocaine by affecting dopamine neurotransmission. Previous research has shown that both presynaptic and postsynaptic dopamine functions play a critical role in the behavioral effects of cocaine related to its abuse (Johanson and Fischman, 1989; Platt et al., 2002). In this regard, the reinforcing, relapse-inducing, and DS effects of cocaine appear to be mediated by cocaine's ability to inhibit dopamine uptake, resulting in increased extracellular concentrations of dopamine at both D₁-like and D₂-like receptors (Spealman et al., 1991; Mello and Negus, 1996; Khroyan et al., 2000; Platt et al., 2002). MPEP might modulate dopamine neurotransmission by blocking mGluR5s involved in the regulation of D₁-like or D₂-like cell signaling, dopamine release, and/or dopamine transport (Liu et al., 2001; Page et al., 2001; Prisco et al., 2002). A recent study by McGeehan et al. (2004) suggests that there is a complex interaction between the mGluR5 and dopamine transporter with respect to stimulation of locomotor activity in mice. MPEP also might attenuate the behavioral effects of cocaine by blocking mGluR5s that regulate glutamate release and consequent iGluR neurotransmission (Conn and Pin, 1997; Thomas et al., 2001), presumably via AMPA/kainite receptors (Cornish and Kalivas, 2000; Park et al., 2002), rather than NMDA receptors (see above). Resolution of these possibilities will require systematic

study of MPEP combined with drugs that target specific elements of the dopamine and AMPA/kainate receptor systems.

Our finding that dizocilpine attenuated self-administration of cocaine is consistent with a previous report that dizocilpine attenuates cocaine self-administration under a FR schedule in rats (Pierce et al., 1997). Dizocilpine also has been reported to increase the break point for self-administered cocaine under a progressive-ratio schedule in rats (Ranaldi et al., 1996). These findings have been interpreted as evidence for enhancement of the reinforcing effects of cocaine by dizocilpine. A similar interpretation could apply to our results, as the doses of self-administered cocaine used in the present study were those that maintained maximum response rates in individual monkeys (i.e., the peak of the inverted U-shaped dose-response curve for cocaine self-administration). Under such conditions, decreases in self-administration could reflect either enhancement or inhibition of cocaine's reinforcing effects (cf. Mello and Negus, 1996). Direct comparison of the effects of MPEP and dizocilpine over a wider range of doses of self-administered cocaine will be necessary to provide a more definitive characterization of the effects of the glutamate antagonists on cocaine reinforcement.

In the absence of cocaine, neither MPEP nor dizocilpine reinstated cocaine-seeking behavior or engendered cocaine-like DS effects. Given the cocaine-antagonist properties of MPEP in our study, its lack of cocaine-like activity was not unexpected. Dizocilpine, on the other hand, has been shown to reinstate cocaine-seeking behavior in rats (De Vries et al., 1998), and both dizocilpine and CGP 37849 substitute partially for the DS effects of cocaine in this species (Kantak et al., 1995, 1998). None of these effects were observed in the present study. The reasons for these contrasting findings in squirrel monkeys and rats could not be determined from our study, but may reflect species differences in pharmacokinetics (Hucker et al., 1983),

differences in regional distribution of key NMDA receptor subunits in rodent and primate brain (Paquet et al., 1997; Meoni et al., 1998), or differences in experimental procedure. It is unlikely that the failure of dizocilpine to induce significant cocaine-like effects was the result of using inadequate doses, as 0.1 mg/kg dizocilpine eliminated operant responding in the majority of subjects trained to discriminate cocaine (cf. Table 3) and similarly high doses have been found to induce sedation and ataxia in squirrel monkeys (Rupniak et al., 1993).

To summarize our principal findings, MPEP attenuated cocaine self-administration, cocaine-induced reinstatement of drug seeking, and the DS effects of cocaine at doses that did not severely impair motor behavior or operant responding maintained by food delivery. Dizocilpine also attenuated self-administration of cocaine, but did not block reinstatement of cocaine seeking and enhanced the DS effects of cocaine. These findings point to a potentially important contribution of mGluR5 mechanisms to the behavioral effects of cocaine related to its abuse and suggest that MPEP has properties of a functional cocaine antagonist, which are not secondary to antagonism at NMDA receptors. Moreover, the contrasting interactions of MPEP and dizocilpine with cocaine imply that glutamate acting through different metabotropic and ionotropic receptors can modulate the behavioral effects of cocaine in qualitatively different ways. Our observations also raise the possibility that mGluR5 receptors may be viable pharmacological targets for treatment of cocaine addiction. Although the emetic effects of MPEP and the surmountability of its cocaine-antagonist effects appear to exclude MPEP as a therapeutic candidate, other mGluR5 ligands with more favorable profiles might provide suitable prototypes for medications development targeting cocaine abuse and relapse.

ACKNOWLEDGEMENTS

We gratefully acknowledge the technical contributions of K. Bano, A. Duggan, M. Duggan, S. Elkind, and D. Reed.

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Footnotes

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This research was supported by grants from the National Institute on Drug Abuse (DA17700 and DA11054) and the National Institute for Research Resources (RR00168).

Figure Legends

Fig. 1. Effects of MPEP and dizocilpine on response rate (top panels) and injections/session (bottom panels) under a second-order FI(FR) schedule of i.v. cocaine self-administration in squirrel monkeys. Points above “V” show effects of vehicle pretreatment. Data are means (\pm S.E.M., n=6). Asterisks show significant effects of pretreatment with MPEP or dizocilpine ($p < 0.05$, Dunnett’s test).

Fig. 2. Effects of MPEP on reinstatement of drug seeking induced by priming with 0.3 mg/kg cocaine (circles) and 1.0 mg/kg cocaine (triangles) in squirrel monkeys. Data are means (\pm S.E.M., n=4) expressed as a percentage of the response rate induced by cocaine priming alone. Points above “V” show effects of vehicle pretreatment. Asterisks show significant effects of pretreatment with MPEP ($p < 0.05$, Dunnett’s test).

Fig. 3. Effects of MPEP on the DS effects of cocaine (top panel) and response rate (bottom panel) in squirrel monkeys trained to discriminate 0.3 mg/kg cocaine from saline under a FR 10 schedule of food delivery. Data are means (\pm S.E.M., n=4). Asterisks show significant effects of pretreatment with MPEP ($p < 0.05$, Dunnett’s test).

Fig. 4. Effects of dizocilpine and CGP 37849 on the DS effects of cocaine (top panels) and response rate (bottom panels). Data are means (\pm S.E.M., n=5). Asterisks show significant effects of pretreatment with dizocilpine or CGP 37849. Other details are as in Fig. 3.

Table 1. Behavioral categories (adapted from Platt et al., 2000)

Locomotion	Any two or more directed steps in the horizontal or vertical plane
Object exploration	Any tactile or oral manipulation of features of the observational arena
Foraging	Sweeping and/or picking through wood-chip substrate
Self-grooming	Picking, scraping, spreading or licking of fur
Scratching	Rapid movement of digits through fur in a rhythmic motion
Visual scanning	Directed eye and/or head movements, usually from a sitting position
Rest posture	Species-typical posture: crouched on hind legs, hunched back, tail wrapped around upper body
Static posture	Maintenance of a fixed, rigid posture
Vocalization	Any utterance including chirps, twitters, peeps, etc.
Other	Any notable behavior not defined above (e.g., yawn, sneeze)

Table 2. Effects of dizocilpine on cocaine-induced reinstatement of drug seeking. Data are means (\pm S.E.M., n=5) expressed as a percentage of the response rate induced by cocaine priming.

Cocaine Priming Dose (mg/kg)	Dizocilpine Dose (mg/kg)	Reinstatement (% cocaine prime)
0.3	vehicle	92 (\pm 14)
0.3	0.003	73 (\pm 10)
0.3	0.03	75 (\pm 20)
1.0	vehicle	81 (\pm 13)
1.0	0.03	55 (\pm 20)

Table 3. Maximum effects of glutamate antagonists on reinstatement of cocaine seeking and DS effects of cocaine. Data are means (\pm S.E.M., n = 4 or 5).

Drug	Reinstatement		Drug Discrimination	
	Dose Range (mg/kg)	Max. Response Rate (responses/s)	Dose Range (mg/kg)	Max. Cocaine Lever (% responses)
Vehicle	—	0.009 \pm 0.007	—	4 \pm 3
MPEP	0.03 - 1.0	0.006 \pm 0.005	0.03 - 1.8 ^a	12 \pm 10
Dizocilpine	0.003 - 0.03	0.004 \pm 0.003	0.003 - 0.1 ^b	6 \pm 4
CGP 37849	—	—	1.0 - 18.0	9 \pm 10

^a Emesis in 4 of 5 monkeys after 1.8 mg/kg

^b Lever pressing eliminated in 3 of 5 monkeys after 0.1 mg/kg

Table 4. Effects of MPEP and dizocilpine on observable behaviors of squirrel monkeys. Data are means (\pm SEM; n = 5).

	Dose (mg/kg)	Behavioral score ¹		
		Locomotion (max. score: 20)	Visual scan (max. score: 20)	Muscle resistance (max. score: 2.0)
Vehicle	--	8.4 \pm 3.0	15.5 \pm 2.2	0 \pm 0
MPEP	0.1	5.2 \pm 3.5	18.7 \pm 0.9	0.4 \pm 0.2*
	0.3	3.7 \pm 2.8*	18.7 \pm 0.7	0.3 \pm 0.1
	1.0	4.2 \pm 2.3*	19.2 \pm 0.6*	0.7 \pm 0.1*
	1.8	4.9 \pm 3.3	18.9 \pm 0.6*	0.7 \pm 0.1*
Dizocilpine	0.003	10.7 \pm 3.9	14.2 \pm 2.8	0 \pm 0
	0.01	6.4 \pm 3.2	16.0 \pm 2.2	0.1 \pm 0.1
	0.03	6.9 \pm 2.9	16.5 \pm 2.2	0.5 \pm 0.2*

* p<0.05, Dunnett's test

¹See Methods and Materials







