Mixed Cocaine Agonist/Antagonist Properties of (+)-Methyl 4β-(4-Chlorophenyl)-1-methylpiperidine-3α-carboxylate, a Piperidine-Based Analog of Cocaine

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Received November 1, 2002; accepted December 6, 2002

ABSTRACT

The present study investigated the pharmacological properties of a piperidine-based novel cocaine analog, namely, (+)-methyl 4β-(4-chlorophenyl)-1-methylpiperidine-3α-carboxylic acid [(+)CPCA]. Like cocaine, (+)-CPCA inhibited rat synaptosomal dopamine and norepinephrine uptake with high affinity, but was 33-fold less potent than cocaine in inhibiting serotonin uptake. Like cocaine, (+)-CPCA is a locomotor stimulant, although it was less potent and efficacious than cocaine. Importantly, pretreatment with (+)-CPCA dose dependently blocked the locomotor stimulant effects of cocaine in rats. (+)-CPCA completely substituted for cocaine in drug discrimination tests, although it was about 3 times less potent than cocaine. It was also self-administered by rats. Unexpectedly, (+)-CPCA did not enhance cocaine-induced convulsions in mice. As expected from rodent studies, rhesus monkeys readily self-administered (+)-CPCA. However, compared with cocaine, (+)-CPCA showed limited reinforcing properties in rats as assessed by both fixed and progressive ratio intravenous drug self-administration tests. These results collectively suggest that (+)-CPCA has an atypical pharmacological profile having both cocaine-like “agonist” and some cocaine “antagonist” properties. These properties of (+)-CPCA suggest that it may have utility in the treatment of cocaine craving and dependence.

Developing an effective treatment for cocaine addiction continues to be a difficult task (Kosten et al., 1989; Grabowski et al., 1995; Batki et al., 1996; Pilla et al., 1999). The susceptibility to relapse to cocaine abuse is particularly high during the early weeks of drug withdrawal (Fischman and Schuster, 1982; Brower and Paredes, 1987; Lago and Kosten, 1994). There is an immediate need to develop a pharmacotherapeutic agent that will assist during this critical drug withdrawal phase. One approach that is being widely pursued is to develop a compound that partially mimics or reduces the effects of cocaine with minimal abuse liability of its own. Such a compound presumably would help to retain addicts in the treatment program during the vulnerable withdrawal phase.

The behavioral and reinforcing effects of cocaine are thought to be due mainly to its inhibitory effect on dopamine transporters (DATs) (Johanson and Fischman, 1989; Kuhar et al., 1991; Koob, 1992). There is some evidence suggesting the possible involvement of additional pharmacodynamic mechanisms in cocaine’s actions (Sherer et al., 1989; Price et al., 1995, 1997; Rothman and Glowa, 1995; Tella 1996; Stine et al., 1995; Rocha et al., 1998; Sora et al., 1998; Tella and Goldberg, 1998; Volkow et al., 1999). In this context, it has been suggested that serotonin transporter (SERT)-dependent effects may play some role in cocaine addiction (Rocha et al., 1998; Tran-Nguyen et al., 1999; Belzung et al., 2000). Furthermore, serotonergic drugs have been shown to modulate dopaminergic neurotransmission in the brain (Benloucif et al., 1993). Thus, it is possible that the full expression of cocaine’s pharmacological profile may require a high affinity for both DAT and SERT. Although there is conflicting evi-
dence suggesting an aversive role for serotonin in cocaine reinforcement (Richardson and Roberts, 1991; McGregor et al., 1993), we reasoned that a cocaine analog with a high affinity for DAT, but a relatively low affinity for SERT would have a pharmacological profile that would be only partially cocaine-like. In light of this, we have synthesized several piperidine-based cocaine analogs that have a relatively weak binding affinity for the SERT (Kozikowski et al., 1998). These piperidine-based molecules are truncated analogs of cocaine, or more precisely truncated analogs of the WIN series (tropane-based molecules) of compounds (Fig. 1). Given the reduced molecular size of these piperidines relative to the tropanes themselves, and the fact that they still embody cocaine’s "pharmacophoric elements", we were encouraged to explore their pharmacological effects.

In the present study, we evaluated the hypothesis that the behavioral pharmacology of one of these analogs lacking SERT activity [(+)-CPCA] would differ significantly from that of cocaine and that its pharmacological profile may be that of a "partial cocaine agonist", i.e., a monoamine uptake blocker with mild-to-moderate stimulant properties and limited reinforcing effects. Theoretically, such compounds may be of potential value in the treatment of addiction caused by psychomotor stimulants (Menon et al., 1973; Rothman and Glowa, 1995; Witkin et al., 1999). For the purpose of comparison, we also tested the cis-(-) isomer of (+)-CPCA and (-)-cocaine in several behavioral tests, including locomotor activity, drug discrimination, intravenous drug self-administration and modification of cocaine-induced convulsions. In this article, we present experimental evidence suggesting that (+)-CPCA has moderate cocaine-like effects (including limited reinforcing effects) as well as some cocaine antagonist properties.

Materials and Methods

Animals. Sprague-Dawley rats and Swiss-Webster albino mice (Charles River Laboratories, Wilmington, MA) were housed in temperature- and humidity-controlled rooms. The animals used for drug discrimination and drug self-administration study were housed individually, whereas all other rodents were group-housed. The rhesus monkeys used in the self-administration experiment were housed for 1-m³ chambers with air filtration systems themselves, and the fact that they still embody cocaine’s "pharmacophoric elements", we were encouraged to explore their pharmacological effects.

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In Vitro Transporter and Receptor Binding Studies. Transporter rich-specific regions were dissected from fresh brains taken from male Sprague-Dawley rats. Tritium-labeled dopamine, norepinephrine, and serotonin were used to measure specific, high-affinity uptake by synaptosomes prepared from the striatum, parietal and occipital cortices, and the midbrain, respectively, as described previously (Wang et al., 2000). IC₅₀ values were determined from analysis of dose-response curves by fitting the data to a four-parameter equation for sigmoidal curves; these values were then converted to Kᵦ values assuming competitive inhibition according to the Cheng-Prusoff equation. The activity of (+)-CPCA as an inhibitor of a large number of human neural receptors was assessed by standard protocols under equilibrium conditions by the National Institute of Mental Health Psychoactive Drug Screening Program as outlined at http://psdp.cwru.edu/psdp.htm.

Locomotor Activity Studies. Locomotor activity of male Sprague-Dawley rats was recorded using locomotor activity monitors (Columbus Instruments, Columbus, OH) and a computer as described previously (Tella, 1994). Activity monitors (43.2 × 44.4 cm) were enclosed in sound-attenuating chambers (BRS/LVE, Laurel, MD). A smaller Plexiglas chamber (40 × 40 cm) was situated inside each locomotor activity monitor. Horizontal activity was measured by a photocell array consisting of 15 infrared beams on both the x- and y-axes. The monitors were interfaced to a computer that tabulated distance traveled (in centimeters) using the software supplied by the manufacturer. After 30-min of habituation to test arenas, several groups of drug-naïve rats received i.p. injections of either saline, cocaine, (+)-CPCA, or (-)-cis-CPCA in a volume of 1 ml/kg. Locomotor activity was recorded in 10-min bins for the next 2 h. Each rat was used once only. The selection of the doses and drugs on any given test day was random (n = 8/group).

The National Institute on Drug Abuse obtained locomotor activity data in male Swiss-Webster mice under a contractual agreement. These studies used similar equipment (40.5 × 40.5-cm Digiscan test chambers with 16 infrared beams on the x- and y-axes) to measure locomotor activity in unhabituated mice. The mice were treated with either saline (10 ml/kg i.p.) or different doses of (+)-CPCA and then 20 min later they were given 20 mg/kg cocaine i.p. and placed in activity monitors. Locomotor activity was measured in 10-min bins for 1 h.

Drug Discrimination Studies. The drug discrimination study was conducted using two groups of male Sprague-Dawley rats, each lever-pressing for food reinforcement according to a procedure described previously (Yasar et al., 1993; Munzar et al., 2000). One group of rats was trained to discriminate cocaine from saline, whereas the other group was trained to discriminate methamphetamine from saline. Daily food was restricted until body weights gradually stabilized at about 85% of their free feeding body weights. Rats were trained to press a lever for 45-mg food pellets (F0021; Bioserv, Frenchtown, NJ) in standard operant-conditioning chambers (Coulbourn Instruments, Lehigh Instruments, PA). Each chamber was equipped with a house light and two levers separated by a recessed tray into which a dispenser could deliver food pellets. Chambers were enclosed within sound-attenuating boxes and supplied with white noise to mask extraneous sounds. The operand chambers were controlled by microcomputers using MED-PC software (MED Associates, East Fairfield, VT).

All drugs were administered 10 (cocaine-trained animals, n = 7) or 15 (methamphetamine-trained animals, n = 8) min before the testing. At the start of the session, a white house light was turned on and in its presence the rats were required to make 10 consecutive presses (fixed ratio 10 schedule of food presentation) on the lever appropriate to the presession treatment (cocaine, methamphetamine, or saline). The completion of 10 consecutive responses on the correct lever produced delivery of a 45-mg food pellet and started a 45-s time-out during which lever presses had no programmed consequences and the chamber was dark. Responses on the incorrect lever had no programmed consequences other than to reset the fixed ratio requirement on the correct lever. After each time-out the white house light was again turned on and the next trial began. Each session ended after 20 trials or after 30 min elapsed, whichever occurred first.

Discrimination-training sessions were conducted 5 days per week under a double alternation schedule (i.e., DDSSDDSS, etc., D, cocaine or methamphetamine; S, saline). For cocaine discrimination,
the criterion for successful training completion was of 95% or more responses on the correct lever during the session and no more than two responses on the incorrect lever during the first trial. For methamphetamine, rats were trained for six sessions during which training was conducted for different: 90% correct, with no more than four incorrect lever responses on the first trial. Once this behavior was maintained in seven of eight consecutive sessions, test sessions with other doses of training and other test drugs were then initiated. Test sessions were identical to training sessions with the exception that 10 consecutive responses on either lever produced a food pellet and ended the trial. Test sessions were conducted on Tuesdays and Fridays, whereas the training sessions continued on Mondays, Wednesdays, and Thursdays.

**Intravenous Drug Self-Administration Studies in Rats.** Fixed ratio intravenous drug self-administration experiments were performed according to the procedure described previously (Tella et al., 1996). Briefly, male Sprague-Dawley rats (n = 7–9/group) were trained to lever press for food under a fixed ratio 10 schedule during 1-h daily sessions. After lever press training, rats were implanted with intravenous catheters in jugular or femoral veins under halothane anesthesia. After 7 days of postoperative recovery period, rats were tested for i.v. cocaine self-administration by substituting cocaine (1 mg/kg/infusion) for food as a reinforcement. Experiments with saline and different doses of test drugs were begun when rats responded with less than 20% variability from the mean of three consecutive days. The self-administration of test drugs was studied by substituting the given dose of a test drug for cocaine for 5 days. The mean of the number of infusions delivered during the last 3 days of substitution was determined for each dose of test drug, and the means are presented in the figures. After the completion of each dose of test drug, animals were returned to the cocaine (1 mg/kg/infusion) training dose for three to five sessions before a change in the dose of the test drug. For the progressive ratio self-administration test, rats were initially trained to lever press for food followed by the fixed ratio cocaine self-administration procedure described above. After a stable pattern of cocaine self-administration, animals were switched to the progressive ratio procedure in which each delivery of cocaine or test solution is followed by an increase in the response requirement for the delivery of the next dose of cocaine. The sequence of progression of response requirement used was as follows: 1, 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 170, 219, 328, 402, 492, 603, 737, 901, 1,102, 1,347, 1,646, 2,012, 2,459, 3,004, 3,670, 4,404, 5,470, 6,692, 8,175, 9,986, 12,198, and 14,900. The failure of a rat to obtain a dose of cocaine for a period of 1 h terminated the session, and the infusions delivered during that period and that following session was termed as the break point of that session. Each dose of a given drug was tested for five sessions with one session per day, and the mean of the number of infusions delivered during the last three sessions was defined as the break point of the given dose of the test drug. Test sessions were alternated with cocaine (1 mg/kg/infusion) sessions so as to monitor the stability of behavior. Rats responding with less than 20% variability from the mean of the break points of the last three cocaine sessions were considered reliable responders. The data from these animals are presented in Fig. 4.

**Pretreatment Studies.** To study the modulatory effect of (+)-CPCA and (−)-cis-CPCA on cocaine’s discriminative stimulus effects, cocaine-trained rats that were previously tested in dose-response studies were used. These rats received (+)-CPCA (5.6 mg/kg i.p.), (−)-cis-CPCA (1.25 mg/kg), or saline 20 min before different doses of cocaine injections (n = 7–9/group). Ten minutes after cocaine injections, rats were tested for discriminative stimulus effects. Rats were randomly assigned to one of these three treatment groups and subgroups of each were randomly assigned to different cocaine dosage groups. To study the modulatory effect of (+)-CPCA on cocaine-induced locomotor activation, several groups of drug-naive mice received different doses of (+)-CPCA or saline 20 min before an i.p. injection of 20 mg/kg cocaine (n = 8 mice/group). Mice were placed in activity monitors immediately after cocaine injection, and data were recorded for 1 h. The group assignment and the dose selection were done randomly. Each animal was used only once. To study the modulatory effect of (+)-CPCA on cocaine-induced convulsions, separate groups of drug-naive male Swiss-Webster mice received different doses of (+)-CPCA (n = 15/dose group), (−)-cis-CPCA (n = 14–20), cocaine (n = 8–15), and saline (n = 43) 30 min before a 55-mg/kg dose of cocaine. The number of animals displaying tonic-clonic convulsions within 1 h of cocaine injection was noted for each group of animals. The data are presented as the percentage of animals that convulsed in each group. The group assignment and dose selection were done randomly. Each animal was used only once.

**Intravenous Drug Self-Administration Study in Rhesus Monkeys.** In view of the intriguing pharmacological profile of (+)-CPCA, we further tested this compound in rhesus monkeys. We assessed the reinforcing effects of (+)-CPCA. A standard substitution procedure as described elsewhere (Gold and Balster, 1986) was used to assess the reinforcing effects of (+)-CPCA. Four adult rhesus monkeys were prepared with intravenous catheters, catheter protection, and tethers that allowed nearly unrestricted movement within their living cages. During daily 1-h sessions of drug availability, lever presses under a fixed ratio 10 schedule resulted in intravenous delivery of cocaine (30 µg/kg/infusion), the positive control, saline, the negative control, and various test doses of (+)-CPCA. Between 4-day tests with saline and (+)-CPCA solutions animals were again given access to cocaine. The last 3 days of each substitution test were used for data analyses with the ranges for (+)-CPCA and saline compared.

**Data Analysis.** The raw data from locomotor study were converted to 30-min totals. The maximal activity occurred within the 1st h after test drug injections. Therefore, the maximal 30-min activity within the 1st h was selected for the dose-response analysis. The data were analyzed by analysis of variance followed by post hoc contrast tests for individual group comparisons. For drug discrimination data analysis, the response rate on both levers and the percentage of cocaine or methamphetamine lever-appropriate responding were calculated for each rat. These data were analyzed using analysis of variance followed by post hoc contrast tests. For self-administration data analysis, the total number of infusions was used as the variable for the analysis of variance. The data from convolution tests were analyzed by using the chi square test.

### Results

**In Vitro Receptor Binding Profile.** The (+)-trans-disubstituted piperidine, (+)-CPCA, and the (−)-cis-disubstituted piperidine, (−)-cis-CPCA were prepared from arecoline as described previously (Kozikowski et al., 1998). The drugs were administered by dissolving their HCl salts in distilled water.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(+)-CPCA and (−)-cis-CPCA</th>
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<tr>
<td>[3H]DA Uptake</td>
<td>[3H]NE Uptake</td>
</tr>
<tr>
<td>nmol</td>
<td>nmol</td>
</tr>
<tr>
<td>Cocaine</td>
<td>275 ± 24</td>
</tr>
<tr>
<td>(−)-CPCA</td>
<td>278 ± 33</td>
</tr>
<tr>
<td>(−)-cis-CPCA</td>
<td>67 ± 24</td>
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cies in inhibiting [3H]norepinephrine uptake, (+)-CPCA was considerably less potent than either cocaine or (−)-cis-CPCA in inhibiting [3H]5-HT uptake. In a broader screen, (+)-CPCA had moderate-to-low affinities for α2A adrenoceptors (Kᵢ values of 472 ± 69, 780 ± 48, and 425 ± 34 nM for α2A, α2B, and α2C, respectively), site 2 of sodium channel (Kᵢ value of 8.6 μM), 5-HT₁ receptors (Kᵢ value of 1.4 ± 0.2 μM), μ-opiate receptors (Kᵢ value of 1.9 ± 0.8 μM), and 5-HT₂A receptor (Kᵢ value of 778 ± 250 nM; cocaine >10 μM). (+)-CPCA functions as a pure antagonist at the 5-HT₂A receptor and inhibits 5-HT-stimulated phosphatidyl inositol hydrolysis with a Kᵢ value of 11.9 ± 0.4 μM (about 1000-fold less potent than the selective antagonist ketanserin).

**Locomotor Activity Studies.** Cocaine (F₃,₆₂ = 29.2, P < 0.001), (+)-CPCA (F₄,₄₈ = 14.738, P < 0.001), and (−)-cis-CPCA (F₄,₆₈ = 14.0, P < 0.001) produced significant and dose-dependent locomotor activation in Sprague-Dawley rats (Fig. 2, A–C). All three test drugs at a dose of 100 mg/kg produced convulsions. Within the range of nonconvulsant doses, there were significant differences in the magnitudes of maximal locomotor effects of these drugs with (+)-CPCA and (−)-cis-CPCA having about 40 and 60%, respectively, of that of cocaine. The maximal effect occurred at 56 mg/kg for cocaine and (+)-CPCA, whereas (−)-cis-CPCA produced its maximal effects at 30 mg/kg, although this was not much in evidence until 60–120 min after administration (Fig. 2C). The ED₅₀ (95% confidence limits) doses of cocaine, (+)-CPCA, and (−)-cis-CPCA in producing locomotor stimulation were 19.9 (14.9–25.7), 15.9 (7.9–23.1), and 4.1 (0.96–7.2) mg/kg, respectively.

The duration of locomotor effects of both cocaine and (+)-CPCA also showed dose dependence and lasted about 2 h at the maximal dose (56 mg/kg) tested (Fig. 2, A and B). In contrast, the duration of locomotor effects of (−)-cis-CPCA followed a biphasic pattern with high doses (30 and 56 mg/kg) producing brief locomotor stimulation (Fig. 2C). A two-way analysis of variance revealed significant effects of dose, time, and the interaction of the two for cocaine [F(dose)₃,₄₈ = 31.6, P < 0.001; F(time)₃,₁₀₂ = 16, P < 0.001; F(dose × time)₃,₁₀₂ = 3.3, P = 0.001], (−)-cis-CPCA [F(dose)₄,₃₈ = 9.0, P < 0.001; F(time)₃,₁₁₄ = 23.6, P < 0.001; F(dose × time)₁₂,₁₁₄ = 2.3, P = 0.013], and (+)-CPCA [F(dose)₄,₃₆ = 9.0, P < 0.001; F(time)₃,₁₀₈ = 48.9, P < 0.001; F(dose × time)₁₂,₁₀₈ = 5.3, P < 0.001]. Similar to the locomotor effects observed in rats (Fig. 2B), both cocaine (F₄,₃₅ = 13.73, P < 0.001) and (+)-CPCA (F₄,₃₅ = 5.8, P < 0.001) produced significant locomotor activation in mice. The maximal increase in locomotor activity engendered by (+)-CPCA in mice was about one-half that caused by cocaine (Fig. 2D).

**Drug Discrimination Studies.** In rats trained to discriminate cocaine from saline, (−)-cis-CPCA and (+)-CPCA completely substituted for cocaine (Fig. 3A). The doses (95% confidence limits) of cocaine, (−)-cis-CPCA, and (+)-CPCA that produced 50% (ED₅₀) cocaine-appropriate lever responding were 4.1 (3.5–5.0), 2.87 (2.5–3.3), and 10.56 (8.49–14.02) mg/kg, respectively. Cocaine (F₅,₃₀ = 1.373, P = 0.262) and (−)-cis-CPCA (F₅,₃₀ = 1.77, P = 0.149) did not alter rates of responding, whereas (+)-CPCA (F₅,₂₄ = 3.02, P = 0.038) significantly diminished rates of responding at 15.6 (P < 0.05) and 30 mg/kg (P < 0.05) (Fig. 3B).

**Fig. 2.** Locomotor stimulant effects of cocaine (n = 8), (+)-CPCA (N = 7 or 8), and (−)-cis-CPCA (n = 12–15) in Sprague-Dawley rats (A–C) and mice (D). The dose-effect and time course for the locomotor effects of saline (closed triangles) and different doses (3 mg/kg, open squares; 10 mg/kg, open circles; 30 mg/kg, open diamonds; 56 mg/kg, closed squares) of either cocaine (A), (+)-CPCA (B), or (−)-cis-CPCA (C) in rats. (D) The dose-effect curves for ambulation counts (open circles) and (+)-CPCA (open triangles) on ambulation counts in mice. *P < 0.05; **P < 0.01, ***P < 0.001 compared with the saline control data (3136 ± 199 counts). Only the effect of 30 mg/kg (P < 0.05) (+)-CPCA was significantly different from the corresponding control response (2641 ± 233 counts) in mice, whereas all doses of cocaine significantly increased activity above control.

**Fig. 3.** Discriminative stimulus effects of cocaine (circles), (+)-CPCA (triangles), and (−)-cis-CPCA (squares) in Sprague-Dawley rats (A and B). The dose-effect curves are shown for the percentage of cocaine-appropriate responding (A) and for the response rates (B) in rats trained to discriminate cocaine (10 mg/kg) from saline (n = 7). The corresponding dose-effect curves in animals trained to discriminate methamphetamine (1 mg/kg) from saline are shown in panels C and D (n = 8). *P < 0.05 compared with the corresponding vehicle control responses.
Similar to the effects observed in cocaine-trained rats, cocaine and (-)-cis-CPCA completely generalized to the discriminative stimulus produced by methamphetamine (Fig. 3C). However, (+)-CPCA produced a maximal effect of 73% methamphetamine-appropriate responding at 18 mg/kg. The doses (95% confidence limits) of cocaine, (-)-cis-CPCA, and (+)-CPCA that produced 50% (ED$_{50}$) methamphetamine-appropriate lever responding were 3.0 (2.3–3.8), 2.97 (2.4–3.6), and 10.3 (8.1–14.5) mg/kg, respectively. Cocaine ($F_{3,35} = 5.8, P < 0.001$), (-)-cis-CPCA ($F_{3,35} = 5.8, P < 0.001$), and (+)-CPCA ($F_{3,35} = 5.8, P < 0.001$) significantly altered rates of responding in methamphetamine-trained animals. Cocaine and (-)-cis-CPCA, but not (+)-CPCA at low doses (1 and 3 mg/kg) significantly ($P < 0.05$) increased rates of responding. However, both (-)-cis-CPCA (10 mg/kg) and (+)-CPCA (18 and 30 mg/kg) at high end of doses significantly ($P < 0.05$) reduced rates of responding. This suggests that the low maximal substitution by (+)-CPCA compared with full substitution by (-)-cis-CPCA may not be due to its rate suppressant effects.

**Intravenous Drug Self-Administration Studies in Rats.** In fixed ratio self-administration test, rats consistently and significantly self-administered both cocaine ($F_{3,35} = 9.3, P < 0.001$) and (-)-cis-CPCA ($F_{3,14} = 2.75, P = 0.05$; four cases were deleted in the analysis due to missing data points for these animals at 0.0156 and 0.0312 mg/kg (-)-cis-CPCA). The dose-response curves of these two drugs were nearly identical with an inverted U-shaped dose-response pattern (Fig. 4A). In contrast, the self-administration of (+)-CPCA, although statistically significant ($F_{5,40} = 2.76, P < 0.05$), was limited and did not result in an inverted U-shaped dose-response curve. This suggests that (+)-CPCA may be less efficacious as a reinforcer, and the rats do not titrate the dose self-administered as they do with cocaine and (-)-cis-CPCA (Fig. 4A). In a progressive-ratio self-administration test designed to determine the relative strength of reinforcement of these drugs, cocaine ($F_{3,12} = 38.92, P < 0.001$) and (-)-cis-CPCA ($F_{3,16} = 20.9, P < 0.001$) dose dependently increased the break points at similar maximal levels (Fig. 4B). In contrast, the maximal break point of (+)-CPCA, although statistically significant ($F_{4,16} = 4.47, P < 0.05$), was about one-half of that of (-)-cis-CPCA and cocaine, and there was no clear dose-response relationship. These results further support the conclusions that (+)-CPCA has lower reinforcing efficacy than cocaine.

**Pretreatment Studies.** Pretreatment of rats with both (+)-CPCA (5.6 mg/kg) and (-)-cis-CPCA (1.25 mg/kg) significantly potentiated the discriminative stimulus effects of a low dose (1.25 mg/kg) of cocaine in cocaine-trained rats (Fig. 5A). A two-way analysis of variance revealed significant effects of drug, dose, and the interaction of the two for the percentage of cocaine-lever responding [$F$ (drug)$_{2,21}$ = 3.63, $P < 0.05$; $F$ (dose)$_{4,84}$ = 15.94, $P < 0.001$; $F$ (drug × dose)$_{8,84}$ = 3.16, $P = 0.01$].

In mice, (+)-CPCA produced dose-dependent attenuation ($F_{5,42} = 33.9, P < 0.001$) of locomotor activation produced by 20 mg/kg cocaine (Fig. 5B). Although 30 mg/kg (+)-CPCA per se produced significant locomotor stimulation, pretreatment of mice with this dose of (+)-CPCA completely abolished the locomotor stimulant effects of 20 mg/kg cocaine (Fig. 5B). This finding is reminiscent of the actions of a typical partial agonist in that partial agonists are known to produce both agonistic and antagonistic effects depending on the absence or presence of a higher efficacy agonist.

To further assess the atypical pharmacological profile of (+)-CPCA, both isomers and different doses of cocaine itself were tested for their ability to potentiate the convulsant effect of 55 mg/kg cocaine. As expected, pretreatment of mice with cocaine significantly ($\chi^2 = 12.25, P < 0.01$) increased the percentage of animals that convulsed after this high dose of cocaine (Fig. 5C). However, neither (+)-CPCA ($\chi^2 = 1.3, P < 1$) nor (-)-cis-CPCA ($\chi^2 = 3.88, P < 1$) enhanced the convulsant effects of cocaine. Furthermore, no other unusual behaviors were noted after administration of cocaine and (+)-CPCA.

**Intravenous Drug Self-Administration Studies in Monkeys.** Various doses of (+)-CPCA were substituted for cocaine during 1-h periods of daily access using a procedure used extensively for self-administration studies of various drugs.
stimulant drugs (Johanson and Balster, 1978; Balster, 1991). (+)-CPCA was self-administered at rates comparable with those maintained by cocaine and in excess of those maintained by saline (Table 2). Evidence for reinforcing effects were seen for at least two doses in each of the four monkeys tested. Intermediate test doses of 10, 30, and 100 μg/kg/infusion were reliably self-administered at rates above saline, although at 300 μg/kg/infusion, the infusion rate was low and similar to 1 μg/kg/infusion (Fig. 6, left). However, the total intake of (+)-CPCA increased in a classic sigmoidal manner as a function of the log of the dose (Fig. 6, right).

**TABLE 2**

Intravenous self-administration testing of (+)-CPCA in cocaine-experienced rhesus monkeys

<table>
<thead>
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<th>Test Compound</th>
<th>Test Dose (μg/kg/infusion)</th>
<th>No. of Monkeys Showing Reinforcement</th>
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<tr>
<td>Cocaine</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>(+)-CPCA</td>
<td>1</td>
<td>2</td>
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<td>(+)-CPCA</td>
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<tr>
<td>(+)-CPCA</td>
<td>300</td>
<td>4</td>
</tr>
</tbody>
</table>

* Monkeys were concluded to show reinforcement with a test solution when the mean number of infusions for the 3-day test period exceeded those for saline tests and their ranges did not overlap.

Discussion

The results of the present study suggest that there are similarities as well as subtle, but important differences between the pharmacological effects of cocaine and the present piperidine analogs. For example, like cocaine, (+)-CPCA and its isomer (-)-cis-CPCA bind to DAT and inhibit DA uptake, stimulate locomotor activity in rodents and completely substitute for cocaine in drug-discrimination tests. Pretreatment with either (+)-CPCA or (-)-cis-CPCA enhances discriminative stimulus effects of cocaine in rats. However, the maximal locomotor stimulant effects of (+)-CPCA and (-)-cis-CPCA are much less than that of cocaine. Interestingly, pretreatment of mice with either (+)-CPCA or (-)-cis-CPCA, unlike cocaine, do not produce an additive effect on cocaine-induced convulsions in mice. Furthermore, pretreatment of mice with (+)-CPCA attenuates cocaine-induced locomotor stimulation. With regard to reinforcing effects, (-)-cis-CPCA seems to be similar to cocaine as revealed by their nearly identical inverted U-shaped dose-response curves in fixed ratio self-administration test in rats. (+)-CPCA, however, has a flat dose-response curve in fixed ratio self-administration tests. Similarly, cocaine and (-)-cis-CPCA have nearly identical break points in progressive ratio self-administration test, whereas (+)-CPCA has a lower break point than either of these two drugs. These results suggest that there are subtle but distinct differences between cocaine and the present piperidine analogs. The behavioral pharmacological profile of (+)-CPCA is especially intriguing and suggests that this piperidine analog has properties that may be suitable for use as a medication for the treatment of cocaine addiction.

The mechanism underlying the observed behavioral similarities and differences between (+)-CPCA versus (-)-cis-CPCA and cocaine may relate to their pharmacodynamic differences. The binding of cocaine to dopamine transporter sites and the subsequent increase in synaptic dopamine in mesolimbic regions of the brain is thought to be the main mechanism underlying its reinforcing and other behavioral effects (Ritz et al., 1987; Johanson and Fischman, 1989; Spealman et al., 1989; Kuhar et al., 1991; Koob, 1992), although serotonin systems are also known to play some role (Walsh and Cunningham, 1997). In this respect (-)-cis-CPCA is pharmacologically similar to cocaine, although it has a somewhat higher affinity for the DAT than cocaine. Relative to cocaine and (-)-cis-CPCA, (+)-CPCA is similar in potency as an inhibitor of norepinephrine uptake and somewhat less potent at the dopamine transporter. However, it is much less potent as an inhibitor of serotonin uptake with (+)-CPCA being 15- and 33-fold less potent at the SERT relative to (-)-cis-CPCA and cocaine, respectively. This suggests that the degree of inhibition of 5-HT transport may account for some of the differences between these two piperidine isomers. There have been recent reports suggesting the possible critical involvement of 5-HT in cocaine’s behavioral and reinforcing effects, although the precise mechanism is not clear. The generally lower efficacy of (+)-CPCA in both locomotor and methamphetamine discrimination tests could result from the differential selectivity of the two isomers for the dopamine transporter relative to the serotonin transporter. That is, if 5-HT receptor activation is requisite for maximal efficacy, the difference in affinities for the SERT exhibited by (+)-CPCA and (-)-cis-CPCA may be so large.
that 5-HT transport is little affected at the doses tested. This difference in SERT affinity could also play a role in the suppression of response rates by doses of (+)-CPCA that engender cocaine-lever pressing. The rate effect, as well as that of partial generalization (as observed with methamphetamine), has been attributed to incomplete coincidence of state produced by the training stimulus and test drug (Koek et al., 1993). This interpretation supports the notion that (+)-CPCA is similar, but nonidentical to cocaine and methamphetamine. On the other hand, the ability of both piperidine isomers to potentiate the discriminability of a low dose of cocaine (1.25 mg/kg) could be a consequence of their ability to inhibit dopamine and norepinephrine reuptake, because both norepinephrine- and dopamine-selective uptake inhibitors have been shown to potentiate cocaine discrimination (Cunningham and Callahan, 1991; Callahan and Cunningham, 1997; Herges and Taylor, 1998; Kleven and Koek, 1998).

GBR12909 is a high-affinity, low-potency inhibitor of DAT that has been demonstrated to blunt the effects of cocaine in a variety of paradigms (Rothman and Glowa, 1995). Inasmuch as GBR12909 and (+)-CPCA have a similar difference in their affinities for the DAT and SERT, it is possible that the mechanism by which GBR12909 blunts the effects of cocaine may involve its relative lack of affinity for SERT. However, there is reasonably compelling evidence that the “antagonistic” pharmacology of GBR12909 is most likely due to its lipophilicity and slow onset of action at the dopamine nerve terminal, rather than its relative lack of affinity for the SERT (Rothman and Glowa, 1995). Interestingly, it has recently been shown that (+)-CPCA also has a slower rate of DAT occupancy in the first few minutes after administration than does cocaine (Woolverton et al., 2002).

In summary, (+)-CPCA has lower potency and efficacy than cocaine in increasing locomotor activity in rodents. (+)-CPCA, unlike (−)-cis-CPCA and cocaine, produces partial methamphetamine-like discriminative stimulus effects, although it is fully cocaine-like in cocaine-trained animals. (+)-CPCA has lower reinforcing potential than cocaine as assessed by fixed and progressive ratio intravenous drug self-administration tests in rats, with its reinforcing effects confirmed in rhesus monkeys. Furthermore, (+)-CPCA dose dependently antagonizes cocaine-induced locomotor activation and potentiates the discriminative stimulus effects of a low dose of cocaine. (+)-CPCA, unlike cocaine, does not enhance cocaine-induced convulsions. These results suggest that (+)-CPCA completely mimics certain behavioral actions of cocaine, whereas acting like a weak partial agonist in others, including its ability to attenuate cocaine-induced increase in locomotor activity and to serve as a positive rein-

Fig. 6. Left, group mean infusions obtained for each unit dose (micrograms per kilogram per infusion) of (+)-CPCA, and for saline (S) and cocaine (C). Each value for (+)-CPCA and saline represents the mean number of infusions of the last three sessions obtained by the monkeys (n = 4). The data points for cocaine were obtained from the three sessions of 30 μg/kg cocaine maintenance immediately preceding substitution tests with (+)-CPCA. Brackets represent the S.E. Right, group mean intake (μg/kg/1-h session) as a function of unit dose (micrograms per kilogram per infusion) of (+)-CPCA. Brackets represent the S.E.
forcing agent in rodents. Thus, the present pharmacological profile of (+)-CPCA is suggestive of potential utility in the treatment of cocaine addiction. This drug may also offer a valuable pharmacological tool for furthering our understanding of cocaine’s mechanism of action, because it exhibits fundamental differences from other related dopamine uptake inhibitors.

Acknowledgments

We appreciate the technical assistance of Mei Zhang in the transporter studies and Jian Rong Zhang in the chemical synthesis.

References


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