JPET Fast Forward. Published on January 21, 2003 as DOI: 10.1124/jpet.102.046318

Mixed Cocaine Agonist/Antagonist Properties of (+)-Methyl 4β-(4-Chlorophenyl)-1-Methylpiperidine-3α-Carboxylate [(+)-CPCA], a Piperidine Based Analog of Cocaine

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JPET/2002/046318

Running title: Pharmacology of a novel mixed cocaine agonist/antagonist

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Number of text pages - 37; Number of figures – 6 (p 38-43); Number of tables – 2 (p 30,31)

Number of references - 45

Number of words: Abstract - 178; Introduction: 553; Discussion: 1293

Abbreviations: (+)-CPCA, (+)-methyl 4β-(4-chlorophenyl)-1-methylpiperidine-3α-carboxylic acid; DAT, dopamine transporter; NET, norepinephrine transporter; SERT, serotonin transporter; 5-HT, 5-hydroxytryptamine, serotonin.

ABSTRACT

The present studies 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 (DA) and norepinephrine uptake with high affinity, but was 33-fold less potent than cocaine in inhibiting serotonin (5-HT) uptake. Like cocaine, (+)-CPCA is a locomotor stimulant, though 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 three 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 to 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 Parades, 1987; Lago et al., 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 (DAT) (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., 1997; Rocha et al., 1998; Sora et al., 1998; Tella and Godberg, 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 (Spealman et al., 1993; Rocha et al., 1998; Tran-Nguyen et al., 1999; Belzung et al., 2000). Further, 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 evidence 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 relatively a 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 (Rothman and Glowa, 1995; Witkin et al., 1999; Menon et al., 1973). 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 like moderate cocaine-like effects (including limited reinforcing effects) as well as some cocaine antagonist properties.

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, while all other rodents were group-housed. The rhesus monkeys utilized in the self-administration experiment were housed for the duration of the study in $1m^3$ chambers with air filtration specifically designed for i.v. self-administration studies (Gold and Balster, 1996). All procedures were carried out in accordance with the National Research Councils Guide of the Care and Use of Laboratory Animals (National Academy Press, 1996).

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 previously described in detail (Wang et al., 2000). IC_{50} 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 K_i 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 NIMH Psychoactive Drug Screening Program as outlined at http://pdsp.cwru.edu/pdsp.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 elsewhere (Tella, 1994). Activity monitors (43.2 x44.4 cm) were enclosed in sound-attenuating chambers (BRS/LVE, Laurel, MD). A smaller Plexiglas chamber (40 x 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. Following 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-hr. 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 (NIDA) obtained locomotor activity data in male Swiss-Webster mice under a contractual agreement. These studies utilized similar equipment (40.5 x 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 (i.p., 10 ml/kg) or different doses of (+) CPCA and then twenty 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-hr.

Drug discrimination studies. The drug discrimination study was conducted using two groups of twelve male Sprague-Dawley rats, each lever-pressing for food reinforcement according to a procedure described elsewhere (Yasar et al., 1993; Munzar et al., 2000). One group of rats was trained to discriminate cocaine from saline, while the other group was trained to discriminate methamphetamine from saline. Daily food was restricted until body weights gradually stabilized

at about eighty-five percent 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 Valley, 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 operant chambers were controlled by microcomputers using MED-PC software (Med Associates Inc., East Fairfield, VT).

All drugs were administered 10 (cocaine-trained animals, N=7) or 15 (methamphetamine-trained animals, N=8) min prior to 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; FR10) on the lever appropriate to the pre-session 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 FR 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, which ever 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). The criterion for successful training completion was of 95% or more responses on the correct lever during the session and no more than 2 responses on the incorrect lever during the first trial. Once this behavior was maintained in seven out 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 while 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 elsewhere (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-hr daily sessions. Following lever–press training, rats were implanted with intravenous catheters in jugular or femoral veins under halothane anesthesia. Following seven days of post-operative 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 3 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. Following 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 prior to 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. Following 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, 1102, 1347, 1646, 2012, 2459, 3004, 3670, 4404, 5470, 6692, 8175, 9986, 12198, 14900. The failure of a rat to obtain a dose of cocaine for a period of 1 hour terminated the session, and the total number of infusions delivered during the entire 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 Figure 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 prior to different doses of cocaine injections (N=7-9/group). Ten min following 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-naïve mice received different doses of (+)-CPCA or saline 20-min prior to an i.p. injection of 20-mg/kg cocaine (N=8 mice/group). Mice were placed in activity monitors

immediately following cocaine injection, and data were recorded for 1-hr. 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-naïve 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 prior to a 55-mg/kg dose of cocaine. The number of animals displaying tonic-clonic convulsions within 1-hr 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, 1996) 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-hr 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.

Drugs. (-)-Cocaine HCl, (+)-methamphetamine HCl (NIDA Drug Supply Source, Rockville, MD), (+)-CPCA and (-)-CPCA. (+)-CPCA and (-)-CPCA were synthesized starting from arecoline as described elsewhere (Kozikowski et al., 1998). The drugs were administered by dissolving their HCl salts in distilled water.

Data analysis. The raw data from locomotor study were converted to 30-min totals. The maximal activity occurred within the first hour following test drug injections. Therefore, the maximal 30-min activity within the first hour 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 percent 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 convulsion tests were analyzed by using the Chi-square test.

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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 affinity of (+)-CPCA is similar to that of cocaine in inhibiting [^{3}H] DA uptake, while (–)-cis-CPCA is 4.1-fold more potent than cocaine in this measure of affinity for the DAT (Table 1). Although all three test drugs have similar potencies in inhibiting [^{3}H] NE uptake, (+)-CPCA was considerably less potent than either cocaine or (–)-cis-CPCA in inhibiting [^{3}H] 5-HT uptake. In a broader screen, (+)-CPCA had moderate to low affinities for α_2 adrenoceptors (K_i s: 472 ± 69, 780 ± 48, and 425 ± 34 nM for α_{2A}, α_{2B}, and α_{2C} respectively), site 2 of sodium channel (K_i: 8.6 μM), 5-HT_7 receptors (K_i: 1.4 ± 0.2 μM), mu opiate receptors (K_i: 1.9 ± 0.8 μM) and $5HT_{2a}$ receptor (K_i: 778 ± 250 nM; cocaine > 10 μM). (+)-CPCA functions as a pure antagonist at the $5HT_{2A}$ receptor, and inhibits 5-HT-stimulated PI hydrolysis with a K_i of 11.9 ± 0.4 μM (about 1000-fold less potent than the selective antagonist, ketanserin).

Locomotor activity studies. Cocaine ($F_{3,52}$ = 29.2, P < 0.001), (+)-CPCA ($F_{4,48}$ = 14.738, P < 0.001) and (–)-cis-CPCA ($F_{4,68}$ = 14.0, P < 0.001) produced significant and dose-dependent locomotor activation in Sprague-Dawley rats (Fig. 2A, 2B and 2C). All three test drugs at a dose of 100-mg/kg produced convulsions. Within the range of non-convulsant 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, while (–)-cis-CPCA produced

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its maximal effects at 30 mg/kg, though this was not much in evidence until 60-120 min following administration (Fig 5C). The ED_{50} (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-dependency and lasted about 2 hr at the maximal dose (56-mg/kg) tested (Fig. 2A and 2B). 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) $_{3,34}$ = 31.6, $P < 0.001$; F (time) $_{3,102}$ = 16, $P < 0.001$; F (dose x time) $_{9,102}$ = 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 x 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 x time) $_{12,108}$ = 5.3, $P < 0.001$]. Similar to the locomotor effects observed in rats (Fig. 2B), both cocaine ($F_{4,35}$ = 13.73, $P < 0.001$) and (+)-CPCA ($F_{4,35}$ = 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 percent (ED_{50}) 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 ($F_{5,30}$ = 1.373, $P = 0.262$) and (-)-cis-CPCA ($F_{5,30}$ = 1.77, $P = 0.149$) did not alter rates

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of responding, while (+)-CPCA ($F_{4,24}$ = 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).

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 ($F_{4,35}$ = 5.8, $P < 0.001$), (-)-cis-CPCA ($F_{4,35}$ = 5.8, $P < 0.001$) and (+)-CPCA ($F_{4,35}$ = 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 as compared to 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 ($F_{6,36}$ = 9.3, $P < 0.001$) and (–)-cis-CPCA ($F_{7,14}$ = 2.75, P = 0.05; 4 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 ($F_{4,16}$ = 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 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 percent cocaine-lever responding [F (drug) $_{2,21}$ = 3.63, $P < 0.05$; F (dose) $_{4,84}$ = 15.94, $P < 0.001$; F (drug x 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.

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In order 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 (χ^2_3 = 12.25; $P < 0.01$) increased the percent of animals that convulsed following this high dose of cocaine (Fig. 5C). However, neither (+)-CPCA (χ^2_3 = 1.3; $P < 1$) nor (–)-cis-CPCA (χ^2_4 = 3.88; $P < 1$) enhanced the convulsant effects of cocaine. Furthermore, no other unusual behaviors were noted following administration of cocaine and (+)-CPCA.

Intravenous drug self-administration studies in monkeys. Various doses of (+)-CPCA were substituted for cocaine during 1-hour periods of daily access using a procedure used extensively for self-administration studies of various stimulant drugs (Johanson and Balster, 1978; Balster, 1991). (+)-CPCA was self-administered at rates comparable to 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, though 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 fashion as a function of the log of the dose (Fig 6, right).

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 analogues. 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. Further, pretreatment of mice with (+)-CPCA attenuates cocaine-induced locomotor stimulation. With regard to reinforcing effects, (-)-cis-CPCA appears 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, while (+)-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 analogues. The behavioral pharmacological profile of (+)-CPCA is especially intriguing and suggests that this piperidine analogue 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 (Johanson and Fischman, 1989; Koob, 1992; Kuhar et al., 1991; Ritz et al., 1987; Spealman et al., 1989), 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, though 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 non-identical 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, as both norepinephrine and dopamine-selective uptake inhibitors have been shown to potentiate cocaine discrimination (Callahan and Cunningham, 1997; Cunningham and Callahan, 1991; Herges and Taylor, 1998; Kleven and Koek, 1998).

GBR12909 is a high-affinity, low potency, inhibitor of DAT 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 (see 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 addition to the above considerations, it is possible that some other unknown pharmacodynamic properties may be critical for the unique behavioral profile of these agents. For example, the ability of (+)-CPCA to antagonize cocaine-induced increased locomotor activity in mice is not easily explained based on the known pharmacology of this compound. It is also possible that the apparent antagonism observed in the locomotor activity test is not true pharmacological antagonism. That is, this study does not rule out other possible factors such the transition from horizontal movements to stereotypic movements or some undefined aversive

property of (+)-CPCA may be involved in its antagonism of cocaine-induced locomotor effects. While it is conceivable that the apparent pharmacological differences between (+)-CPCA and cocaine are related to pharmacokinetic differences, it seems unlikely that they are due simply to differences in their duration of action, as the effects of high doses of both compounds appeared to have similar durations of action following i.p. administration (Fig. 2). On the other hand, there were apparent differences in time of peak effect that may play some role. These are issues that will need to be resolved in future studies.

Although the self-administration data in rats suggest that this analogue has limited reinforcing effects, studies in cocaine-experienced rhesus monkeys as assessed using a standard substitution procedure suggested that (+)-CPCA has dose-dependent reinforcing effects. However, this procedure is not designed to compare the relative reinforcing efficacy of cocaine and other stimulant test drugs (Balster, 1991). Even weak stimulants such as modafinil and ephedrine show reinforcing effects under essentially identical test conditions as these (Gold and Balster, 1996). In fact, the reinforcing effects of (+)-CPCA are consistent with its partial agonist profile. Mild reinforcing effects may be desirable in facilitating medication compliance and treatment acceptability. Human testing will be required to determine if the abuse potential of (+)-CPCA is compatible with its therapeutic use in cocaine addiction.

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. Further, (+)-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, while 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 reinforcing 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, as it exhibits fundamental differences from other related dopamine uptake inhibitors.

Acknowledgements

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

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Footnotes

This work was supported by a grant from the National Institute on Drug Abuse and in part by the NIMH Psychoactive Drug Screening Program (NO1MH80005).

Table 1. Monoamine reuptake activity of cocaine, (+)-CPCA and (–)-cis-CPCA.

	K_i ± S.E. (nM)		
Compound	**[^{3}H] DA Uptake**	**[^{3}H] NE Uptake**	**[^{3}H] 5-HT Uptake**
Cocaine	275 ± 24	119 ± 38	177 ± 13
(+)-CPCA	276 ± 33	90 ± 5	5900 ± 400
(–) - cis-CPCA	67 ± 24	98 ± 7	390 ± 27

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Table 2. Intravenous self-administration testing of (+)-CPCA in cocaine-experienced rhesus monkeys.

Test Compound	Test Dose (μg/kg/infusion)	# of Monkeys Showing Reinforcement (out of 4)[a]
Cocaine	30	4
(+)-CPCA	1	2
(+)-CPCA	10	3
(+)-CPCA	30	4
(+)-CPCA	100	4
(+)-CPCA	300	3

[a] 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.

Figure legends:

Fig. 1. Structure of cocaine, a WIN analog, and the piperidines (+)-CPCA and (–)-cis-CPCA.

Fig. 2. Locomotor stimulant effects of cocaine (n = 8), (+)-CPCA (n = 7 or 8) and (–)-cis-CPCA (n = 12 to 15) in Sprague-Dawley rats (**A, B, 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 cocaine (open circles) and (+)-CPCA (open triangles) on ambulation counts in mice. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared to 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 on the percent cocaine-appropriate responding (**A**) and on 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). *$P < 0.05$; as compared to the corresponding vehicle control responses.

Fig. 4. Intravenous self-administration of cocaine (circles), (–)-cis-CPCA (squares) and (+)-CPCA (triangles) under fixed-ratio (**A**) and progressive-ratio (**B**) schedule procedures in Sprague-Dawley rats. The data points represent mean ± SEM of 7 to 9 rats. Four cases were deleted in the statistical analysis of fixed-ratio data due to missing data points at 0.0156 and

0.0312 mg/kg (–)-cis-CPCA. * P < 0.05; **P < 0.01; ***P < 0.001 as compared to the saline control data (3.4 $\pm$ 0.75 infusions/2h in fixed-ratio test and 2.7 ± 0.46 infusions/session in progressive-ratio test). Three cases were deleted in the analysis of progressive-ratio data due to missing data points at certain doses of cocaine, (+)-CPCA and (–)-cis-CPCA.

Fig. 5. Modification of discriminative-stimulus (**A**), locomotor (**B**) and convulsant (**C**) effects of cocaine. (**A)** The dose-discriminative stimulus effect curves of cocaine following pretreatment with (+)-CPCA (triangles, n =8), (–)-cis-CPCA (squares, n = 8) and saline (circles, n = 8) on the percent cocaine-appropriate responding in rats trained to discriminate cocaine (10 mg/kg) from saline. **P < 0.01 as compared to saline pretreatment condition. (**B**) The effect of (+)-CPCA on locomotor activation produced by 20 mg/kg cocaine. ***P < 0.001 as compared to the cocaine control. There were 8 animals in each dose group. (**C**) The effect of pretreatment with cocaine (solid bars, n = 8 to 15), (+)-CPCA (stippled bars, n = 15), and (–)-cis-CPCA (open bars, n = 14 to 20) on convulsions induced by 55 mg/kg cocaine. The 55 mg/kg cocaine following saline pretreatment produced convulsions in 44 percent of the animals tested (n = 43) and is presented as the *horizontal* striped bar in the figure. * P < 0.05; **P < 0.01 as compared to the saline pretreated cocaine control group.

Fig 6. *Left:* Group mean infusions obtained for each unit dose (µg/kg/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 (ug/kg/infusion) of (+)-CPCA. Brackets represent the S.E.

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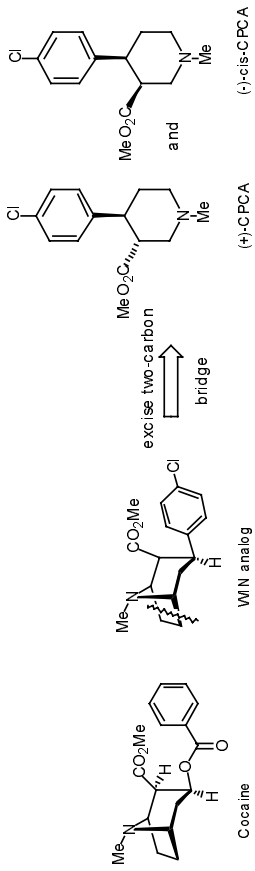

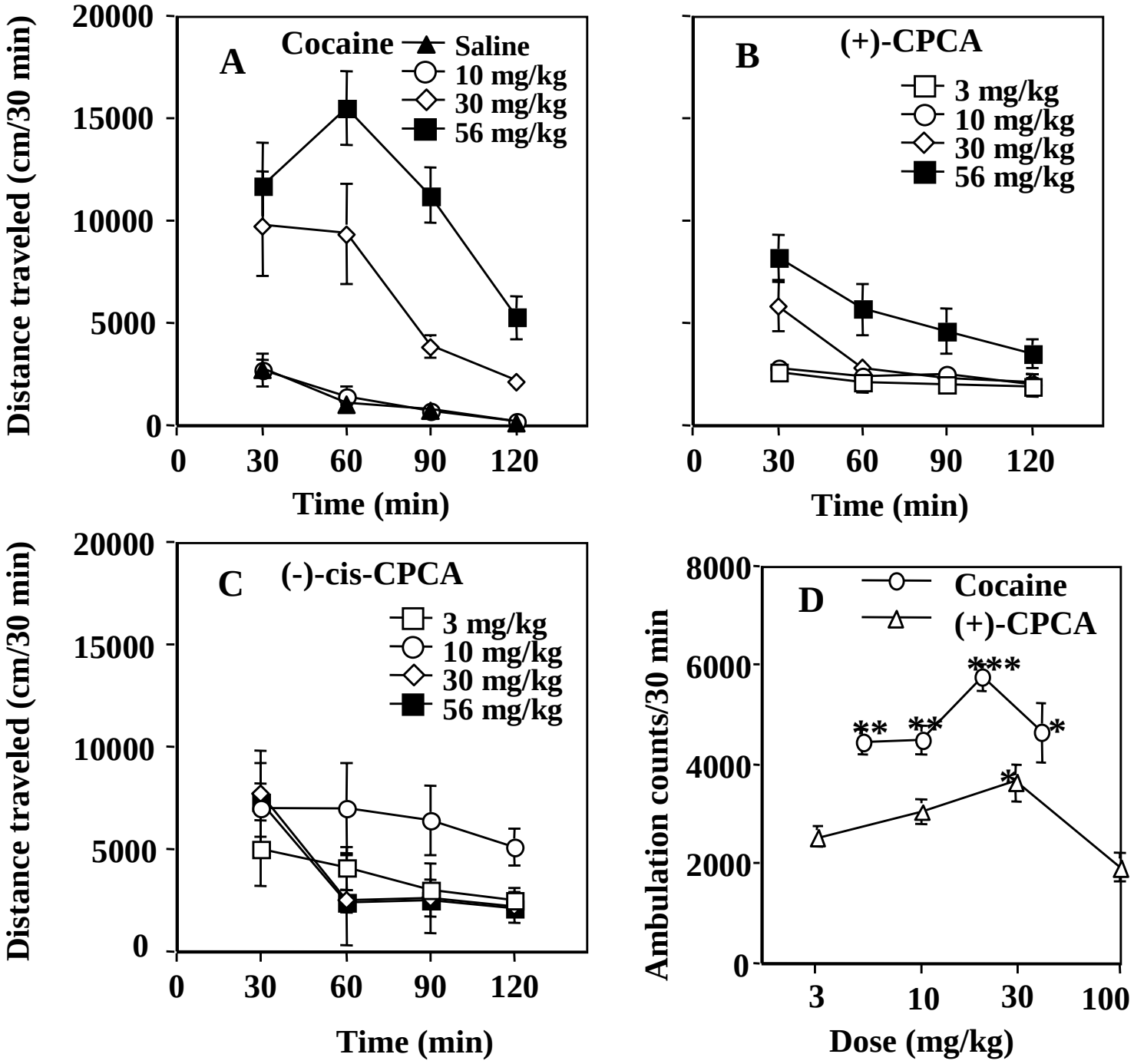

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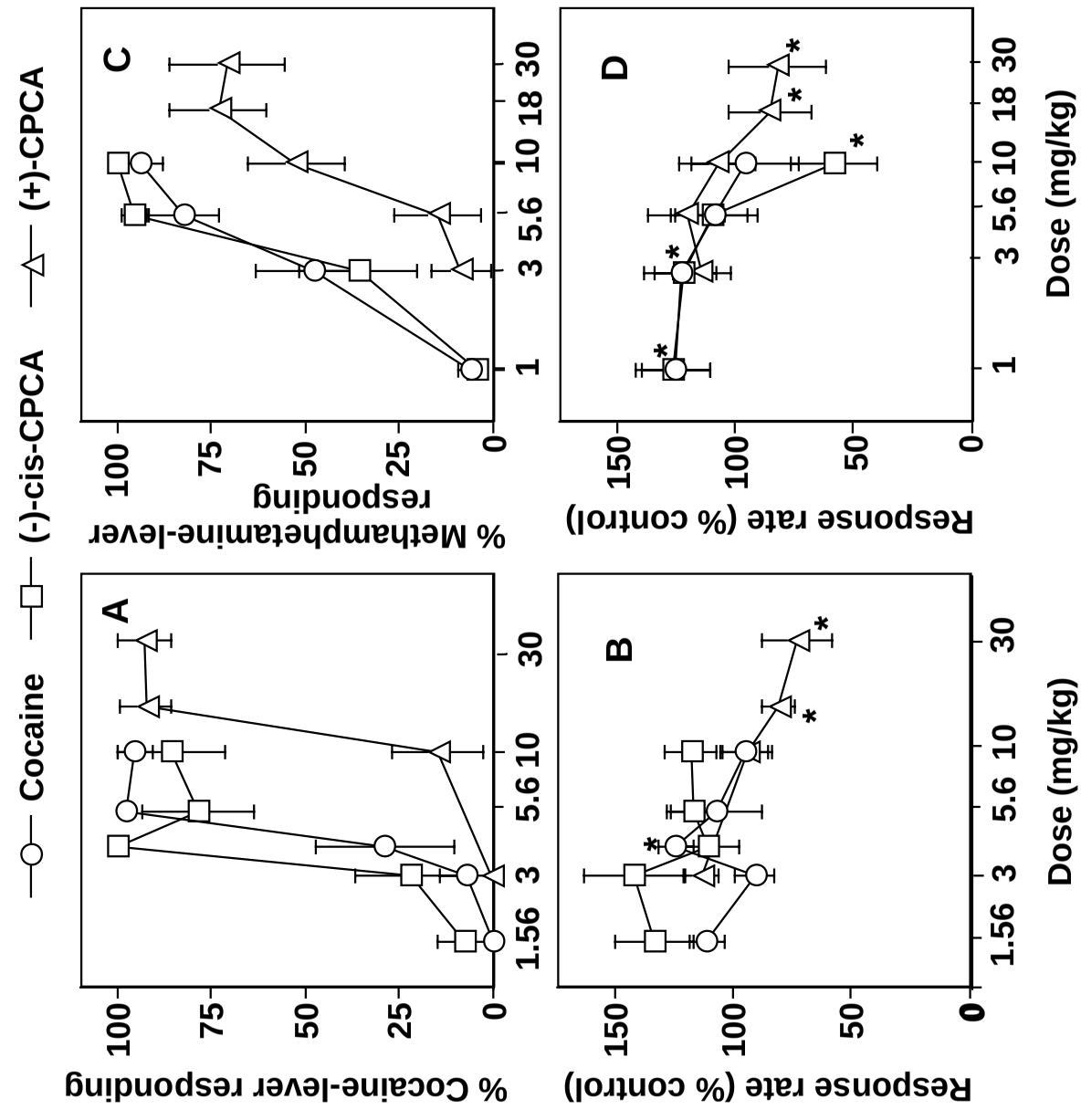

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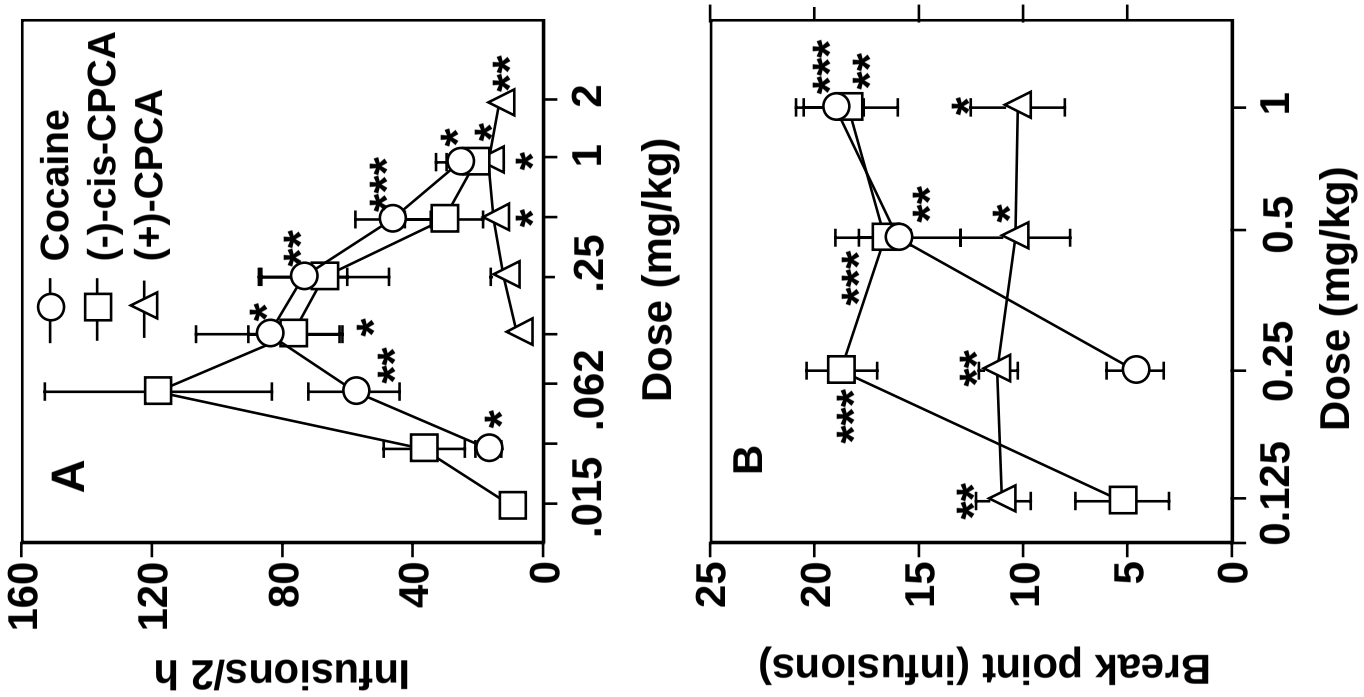

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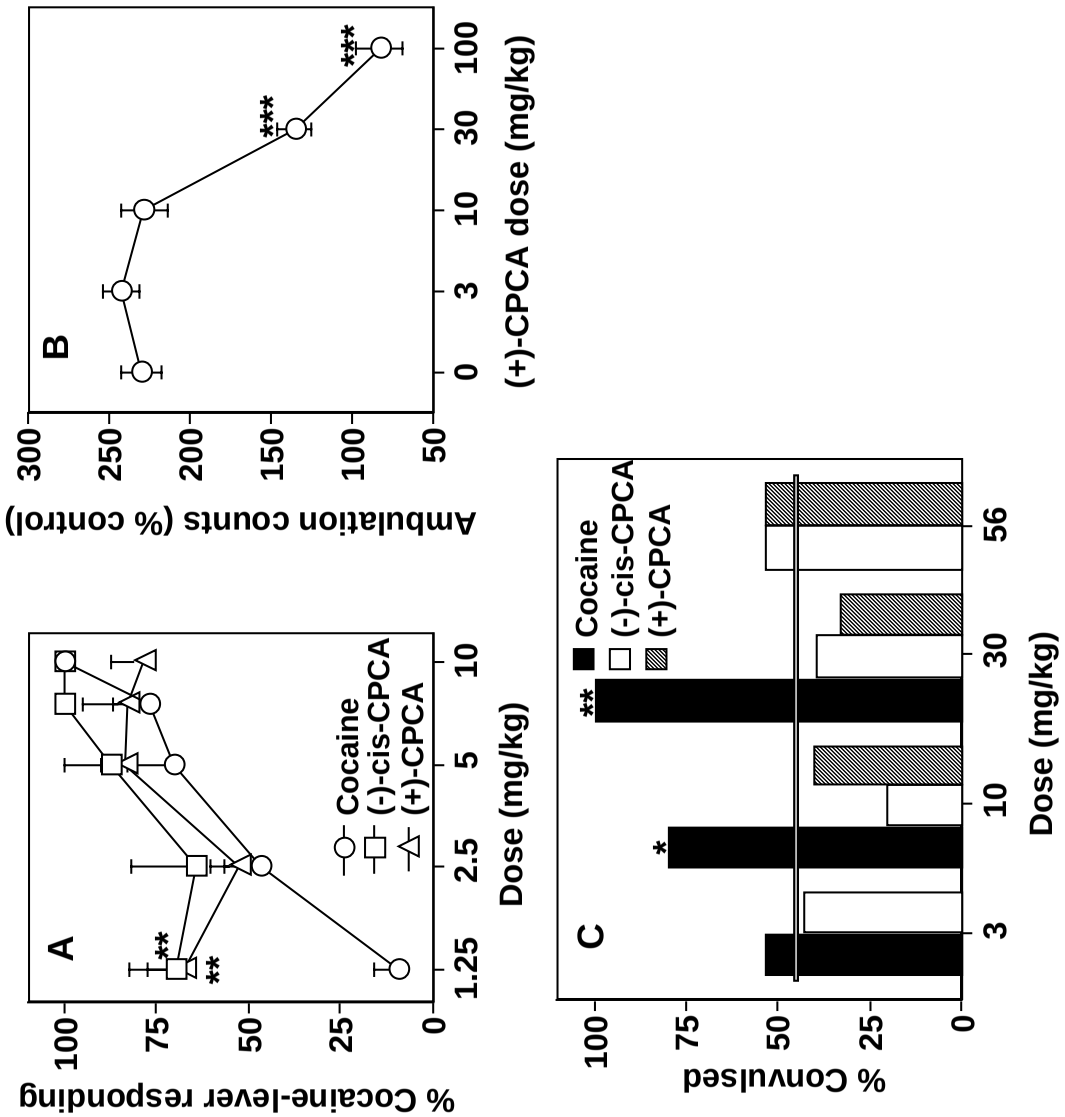

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