PROGRESS REPORT FROM THE TESTING PROGRAM

FOR STIMULANT AND DEPRESSANT DRUGS (2005)

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Supported, in part, by the College on Problems of Drug Dependence

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INTRODUCTION

A research group within the Drug Evaluation Committee has been involved in the evaluation of stimulant and depressant compounds for approximately 20 years. The group currently includes laboratories at the University of Mississippi Medical Center (UMMC; Woolverton), the University of Texas Health Science Center at San Antonio (UTHSCSA; France, McMahon) and the University of Michigan (UM; Winger, Woods). As part of the Drug Evaluation Committee (Woods, Chair) of the College on Problems of Drug Dependence (CPDD), research is supported by both the CPDD and the National Institute on Drug Abuse (NIDA). One of the purposes of this group is to evaluate new compounds, generally classified as either stimulants or depressants, for their abuse liability and physical dependence potential. Compounds are received, coded and distributed by the Biological Coordinator (Coop, University of Maryland School of Pharmacy at Baltimore) for blind testing in the various laboratories. Drugs are then evaluated for reinforcing effects in monkeys with histories of drug self-administration (UM), and for discriminative stimulus effects in monkeys that discriminate amphetamine (UMMC), midazolam (UTHSCSA), or flumazenil (UTHSCSA). This report includes the results of evaluation of CPDD 0067 and CPDD 0072. Data for CPDD 0067 are limited to amphetamine discrimination since data from the other assays were included in the 2004 report (France et al., 2004). CPDD 0072 was tested in all laboratories. All studies were conducted in accordance with the guidelines of the respective Institutional Animal Care and Use Committees and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

METHODS

Reinforcing Effects in Rhesus Monkeys (UM)

Subjects and Apparatus

Three adult male rhesus monkeys (Macaca mulatta) experienced with self-administration of cocaine and saline served as subjects. Animals were surgically prepared with indwelling silicone rubber catheters using 10 mg/kg i.m. ketamine and 2 mg/kg i.m. xylazine as anesthetic. Catheters were implanted in either a jugular (internal or external), femoral, or brachial vein as necessary. Catheters passed s.c. to the mid-scapular region, exited the body, and continued through a hollow restraining arm to the outside rear of the cage. Each animal wore a Teflon mesh jacket (Lomir, Quebec, Canada) connected to a flexible stainless steel spring tether attached to the rear of the cage. Animals were individually housed in 83.3 x 76.2 x 91.4 cm deep stainless steel cages. A side-mounted panel was present in each cage and was equipped with a row of three stimulus lights (red-green-red) across the top, and two response levers (one

mounted under each red light). Animals were fed between 10 and 12 Purina monkey chows twice per day, and water was available ad libitum. Daily fresh fruit and other treats supplemented this diet. In accordance with IACUC requirements, environmental enrichment was provided on a regular basis. Operation of the infusion pump delivered 1 ml of drug solution over 5 seconds.

Procedure

Two 60-minute experimental sessions were conducted each day, one starting at about 10:00 and another starting at about 16:00 PM. The onset of each session was signaled by illumination of a red stimulus light. In the presence of this light, the 10^{th} response of the lever beneath it resulted in the operation of the infusion pump (FR 10). During the 5-second injection, the red stimulus light was extinguished and the center green light was illuminated; lever presses had no programmed consequence during the injection. Immediately following each injection, all stimulus lights were extinguished for a 1-minute time out period (TO 1') during which lever presses had no programmed consequences. Each TO period was incorporated into the total 60-minute session time.

Under baseline conditions, animals were maintained on a dose of 0.01 mg/kg/injection of cocaine. Saline was substituted for cocaine approximately every third or fourth session, occasionally for two consecutive sessions. Substitutions of CPDD 0072 occurred no often than twice each week, and both saline and cocaine were available during the intervening sessions. The number of injections of cocaine or saline that were taken in the session before each substitution of CPDD 0072 was averaged for comparison with CPDD 0072. Each dose of CPDD 0072 was made available once, except for 0.03 mg/kg/injection, which was available twice for each monkey, and 0.3 mg/kg/injection, which was available three times for each monkey.

Drugs

CPDD 0072 was dissolved in sterile water. Cocaine was dissolved in sterile physiological saline. Doses of 0.001, 0.003, 0.01, 0.03, 0.1 and 0.3 mg/kg/injection were tested in ascending order.

Discriminative Stimulus Effects in Rhesus Monkeys (AMPH discrimination, UMMC)

Subjects and Apparatus

Adult rhesus monkeys (*Macaca mulatta*; n=3) served as subjects. All monkeys had received other test drugs prior to the start of the present study. Monkeys were individually housed in stainless-steel cages with water available continuously. Feeding consisted of 110 to 200 g of Teklad Monkey Chow immediately after each session and a chewable vitamin tablet 3 days/week.

During experimental sessions, each monkey was seated in a restraint chair and placed in a sound-attenuating cubicle that had two response levers and a white houselight mounted on the ceiling. Above each lever was a set of white and red jeweled lights. Shoes attached to the foot rest of the chairs were fitted with brass plates through which electric shocks could

be delivered to the bottoms of the feet. Experimental events were programmed and recorded using an Apple Macintosh computer in an adjacent room.

Procedure

All monkeys previously had been trained in a discrete-trials paradigm to discriminate *d*-amphetamine (AMPH; 1.0 mg/kg) from saline. Each monkey was placed in the chair and moved to the test room. In the test room their feet were placed into shoes and held in place with a Velcro strap. Each monkey was given an infusion of either saline (0.25 ml/kg) or the training drug, followed by a 2.0 ml saline flush, intragastrically via a nasogastric tube. Monkeys then remained in the chair in the test room. Fifty-five minutes after the infusion, monkeys were placed in the experimental chambers. The session then began with a 5-min timeout, at the end of which the houselight and lever lights were illuminated (trial) and responding on the correct lever avoided electric shock (8515 and Ou3) or delivered a 1-gram banana-flavored food pellet (M163), and extinguished the lights. Responding on the incorrect lever reset the response requirement on the correct lever. The correct lever was determined by the pre-session infusion (drug or saline). If the response requirement (FR2, 8515; FR 5, M163, Ou3) was not met on the correct lever within 10 sec of the onset of the lights, shock (250 msec duration, 5 mA intensity) was delivered (8515 and Ou3). If the response requirement was not met within 4 sec of this shock, a second shock was delivered and the trial automatically ended. For M163, if the response requirement was not met within 10 seconds, the trial ended. Two consecutive trials in which 2 shocks were received or food was not obtained automatically ended the session. Trials were separated by a 30-sec timeout, and sessions lasted for 30 trials or 20 min, whichever came first.

Training sessions were conducted five days a week according to the following two-week schedule: SDDSS, DSSDD, where S denotes sessions preceded by saline infusion and D denotes sessions preceded by drug infusion. Discrimination training continued until at least 80% of the responses in the first trial, and at least 90% of the total trials (27/30), were completed on the correct lever for seven out of eight consecutive sessions. In addition, responding in the eighth of these consecutive sessions had to meet training criteria. At this point testing began. During testing, sessions were conducted according to the following two-week schedule: SDTST, DSTDT, where T denotes test sessions. If the criteria for stimulus control were not met during the training sessions, test sessions were not conducted and the training sequence continued. Test sessions were identical to training sessions except that completion of the response requirement on either lever was reinforced. For test sessions that involved s.c. injections, i.g. infusions of saline were given at the usual pretreatment time (one hour pre-session), followed immediately by s.c. injections of the test drug.

Drugs

d-Amphetamine sulfate (AMPH; Abbott Laboratories, N. Chicago, IL) was dissolved in sterile 0.9% saline to an infusion volume of 0.25 ml/kg. CPDD 0067 was dissolved in water and administered in 0.25 ml/kg up to the dose of 1.0 mg/kg. A solution of 10 mg/ml was administered in the appropriate volume to test the dose of 3.0 mg/kg. Doses of CPDD 0067 were tested once. When there was evidence of AMPH-lever responding (Ou3, 3.0 mg/kg), the test session was repeated. Doses up to 1.0 mg/kg of CPDD 0072 were tested in

the volume of 0.25 ml/kg. For higher doses, the concentration was adjusted to a maximum of 11.2 mg/ml and volume adjusted accordingly. Doses of CPDD 0072 were tested once.

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil and midazolam discriminations, UTHSCSA)

Subjects and Apparatus

The subjects were three female (RO, LI and JI) and one male (LE) rhesus monkeys (Macaca mulatta) weighing between 4.7 and 9.7 kg. Monkeys were housed individually in stainless steel cages in which water was continuously available and they received primate chow (Harlan Teklad, Madison, WI) daily as well as fresh fruit and peanuts several days per week.

Monkeys were seated in chairs that provided restraint at the neck. During experimental sessions, chairs were located in sound-attenuating, ventilated chambers that were equipped with two response levers, lights and a food cup. Chairs were equipped with shoes containing brass electrodes, to which brief (250 ms) electric shock could be delivered from an a.c. shock generator.

Procedure

Flumazenil Discrimination. Monkeys JI and LE consumed 5.6 mg/kg of diazepam 3 h prior to daily sessions in which they discriminated between s.c. injections of 0.056 mg/kg of flumazenil and vehicle while responding under a FR 5 schedule of food presentation. Daily training sessions consisted of several discrete, 15-min cycles. Each cycle comprised a 10-min pretreatment period, during which the chamber was dark and lever presses had no programmed consequence, followed by a response period, during which the chamber was illuminated green and monkeys could receive a 300 mg banana-flavored food pellet by responding five times on the appropriate lever as determined by the s.c. injection administered during the first min of the 10-min timeout (e.g., left lever after vehicle, right lever after flumazenil). Responses on the incorrect lever reset the response requirement on the correct lever. Test sessions were identical to training sessions except that various doses of flumazenil or CPDD 0072 were administered during the first min of the timeout and 5 consecutive responses on either lever resulted in the delivery of food. CPDD 0072 was studied up to 2 h after administration (i.e., 8, 15-min cycles).

Midazolam Discrimination. Monkeys RO and LI discriminated between 0.32 mg/kg of midazolam (s.c.) and saline while responding under a FR 10 schedule of stimulus-shock termination. Daily sessions comprised multiple, 15-min cycles. Each cycle comprised a 10-min pretreatment period, during which the chamber was dark and lever presses had no programmed consequence, followed by a response period, during which the chamber was illuminated red and monkeys could postpone scheduled shock for 30 s by responding ten times on the appropriate lever as determined by the s.c. injection administered during the first min of the 10-min timeout (e.g., left lever after saline, right lever after midazolam). Failure to satisfy the response requirement within 15 s resulted in the delivery of a brief shock. The response period ended after 5 min or 4 shocks. Responses on the incorrect lever reset the response requirement on the correct lever. Test sessions were identical to training sessions except that various doses of midazolam or CPDD 0072 were administered during the first min of the timeout and 10

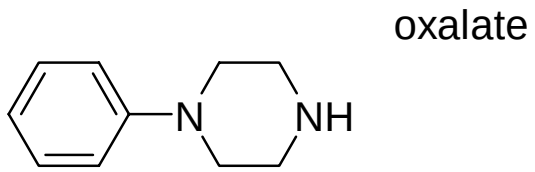
consecutive responses on either lever postponed the shock schedule. CPDD 0072 was studied every 15 min for up to 2 h after administration (i.e., 8, 15-min cycles).

Drugs

Diazepam (Zenith Laboratories, Northvale, NJ) was suspended in fruit punch (1 mg/ml) containing Suspending Agent K to yield a dose of 5.6 mg/kg/daily administration. Flumazenil (F. Hoffman LaRoche, LTD, Basel, Switzerland) was dissolved in a vehicle of 10% ethanol, 40% propylene glycol and 50% saline. Midazolam hydrochloride (Roche Pharma, Inc., Manati PR) was purchased as a commercially-prepared solution. CPDD 0072 was dissolved in saline and was studied up to a dose of 10 mg/kg s.c.

RESULTS

CPDD 0067: Phenylpiperazine oxalate



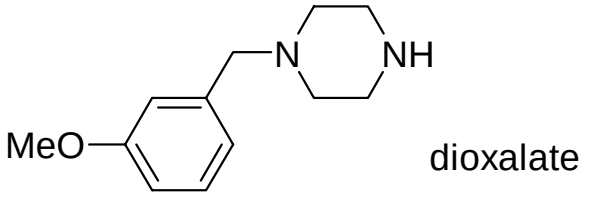
Discriminative Stimulus Effects in Rhesus Monkeys (AMPH discrimination)

When given i.g. 60 minutes before the session, CPDD 0067 lacked AMPH-like discriminative stimulus effects (Table 1). Monkey Ou3 exhibited full AMPH -lever responding when first given 3.0 mg/kg CPDD 0067, but responded exclusively on the saline lever in the second test session with this dose. There were no observable behavioral effects of changes in post-session food intake after any dose of CPDD 0067.

TABLE 1
Discriminative stimulus effects of i.g. administration of CPDD 0067 in AMPH-trained monkeys

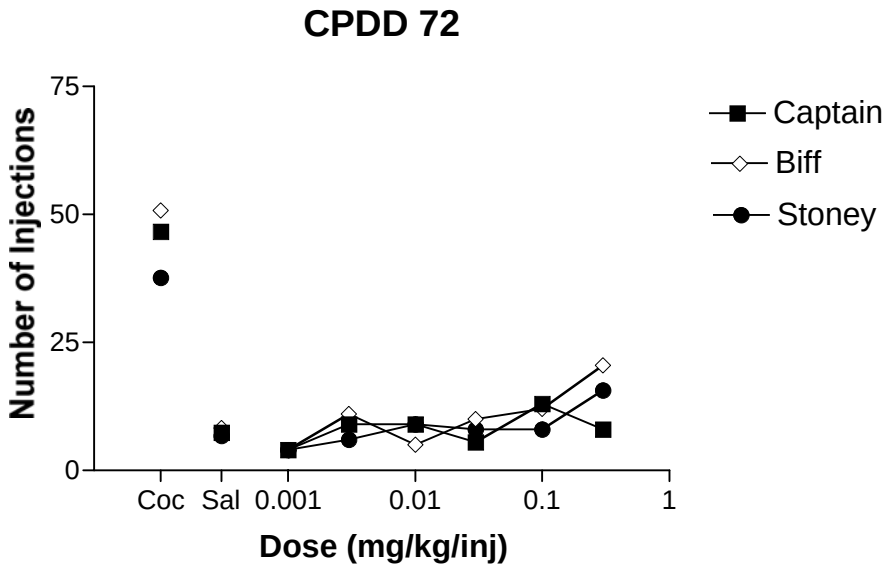
Subject	CPPD 0067 (mg/kg)				
	AMPH	**Saline**	**0.3**	**1.0**	**3.0**
8515	100/1.4	1.5/1.8	1/1.0	0/0.9	0/1.4
M163	100/1.8	5/1.4	0/1.8	0/1.9	0/1.7
Ou3	100/2.3	0/2.7	0/1.8	0/2.2	41/2.1

CPDD 0072: 1-(3-Methoxybenzyl)piperazine dioxalate



Reinforcing Effects in Rhesus Monkeys

As shown in the Figure below, animals took an average of between 37 and 50 injections of cocaine, and between 6.7 and 8.3 injections of saline prior to each substitution of CPDD 0072. Across a dose range of 0.001 to 0.1 mg/kg/injection of CPDD 72, the number of injections did not exceed those maintained by saline. At the largest dose of CPDD 0072 (0.3 mg/kg/injection) two of the three monkeys showed a tendency to respond above saline levels. This dose was tested three times in each animal. One monkey (Captain) did not take more than 9 injections on these three occasions. A second monkey (Stoney) took 25, 8, and 14 injections, and the third monkey (Biff) took the limit of 30 injections on the first exposure to 0.3 mg/kg/injection, and took 23 and 3 injections on the next two exposures, respectively. It should be noted that this type of variable drug intake is not unusual at doses slightly smaller than those that maintain relatively high and stable responses, suggesting that 0.3 mg/kg/injection of CPDD 72 was marginally reinforcing in two of the three monkeys. Unfortunately, this dose was at the limit of solubility for this drug, and a dose of 1.0 mg/kg could not be evaluated.



Discriminative Stimulus Effects in Rhesus Monkeys (AMPH discrimination)
When given i.g. 60 minutes before the session, CPDD 0072 lacked AMPH-like discriminative stimulus effects (Table 2). There were no observable behavioral effects of changes in post-session food intake after any dose of CPDD 0067.

TABLE 2
Discriminative stimulus effects of i.g. administration of CPDD 0072 in AMPH-trained monkeys

Subject	CPPD 0072 (mg/kg)						
	AMPH	**Saline**	**0.3**	**5.6**	**10**	**17**	**30**
8515	100/1.4	1.5/1.8	n.t.	0/2.3	0/2.5	n.t.	0/2.1
Ou3	100/2.3	0/2.7	0/2.7	0/2.9	n.t.	0/2.3	0/2.7

Discriminative Stimulus Effects in Rhesus Monkeys (flumazenil and midazolam discriminations)

Flumazenil Discrimination. In monkeys receiving 5.6 mg/kg/day of diazepam p.o. and discriminating between 0.056 mg/kg of flumazenil and vehicle, flumazenil dose-dependently increased responding on the drug (flumazenil)-associated lever with doses of 0.032 mg/kg (JI) and 0.1 mg/kg (LE) occasioning greater than 80% drug-lever responding (Table 3). Over the doses studied, flumazenil slightly decreased response rate in JI and had relatively little effect on response rate in LE.

TABLE 3
Flumazenil Dose (mg/kg)

Subject	Veh	0.0032	0.01	0.032	0.1
JI	0 / 1.92	0 / 1.91	0 / 2.16	100 / 1.44	NT
LE	2 / 1.05	2 / 1.23	7 / 1.22	10 / 1.25	98 / 1.10

Data represent percent drug-appropriate responding / response rate (responses / s)
Veh, vehicle
NT, not tested

Up to a dose of 10 mg/kg, CPDD 0072 did not substitute (i.e., did not occasion at least 80% drug-lever responding) for the flumazenil discriminative stimulus (Table 4) and did not markedly change rate of responding. Data shown are an average of 8 test cycles.

Subject	TABLE 4 CPDD 0072 Dose (mg/kg) Veh	3.2	10.0
JI	0 / 1.98	7 / 1.89	0 / 1.46
LE	4 / 1.07	4 / 1.52	13 / 1.15

See Table 3 for details

Midazolam Discrimination. In monkeys discriminating between 0.32 mg/kg of midazolam and vehicle, midazolam dose-dependently increased responding on the drug (midazolam)-associated lever with doses of 0.32 mg/kg (RO) and 0.1 mg/kg (LI) occasioning greater than 80% drug-lever responding (Table 5). The largest dose of midazolam (0.32 mg/kg) slightly decreased response rate.

Subject	TABLE 5 Midazolam Dose (mg/kg) Veh	0.01	0.032	0.1	0.32
RO	0 / 2.56	0 / 2.61	0 / 3.01	56 / 2.39	100 / 1.89
LI	0 / 1.76	0 / 1.63	11 / 1.58	100 / 1.86	100 / 1.35

See Table 3 for details

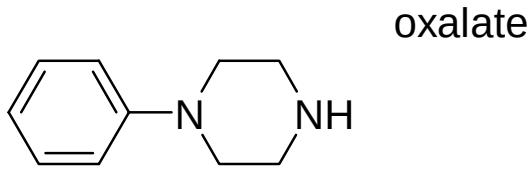
Up to a dose 0f 10 mg/kg, CPDD 0072 did not substitute (i.e., occasion at least 80% drug-lever responding) for the midazolam discriminative stimulus (Table 6); effects of CPDD 0072 on rate of responding were neither marked nor consistent between monkeys. Data shown are an average of 8 test cycles.

Subject	TABLE 6 CPDD 0072 Dose (mg/kg) Veh	3.2	10.0
RO	0 / 1.87	0 / 2.36	0 / 2.45
LI	0 / 2.97	0 / 1.38	0 / 1.27

See Table 3 for other details

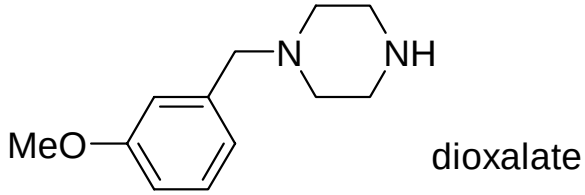
CONCLUSIONS

CPDD 0067: Phenylpiperazine oxalate



Last year (Fantegrossi et al., 2004), we reported CPDD 0067 was not self administered by rhesus monkeys and, up to doses that decreased rates of responding, did not substitute for midazolam or flumazenil in monkeys or for LSD in rats. Although CPDD 0067 had affinity for 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors, it did not share behavioral actions with LSD. CPDD 0067 is structurally similar to benzylpiperazine (BZP; CPDD 0063); however, unlike BZP (Fantegrossi et al., 2004; 2005) it was not self-administered by monkeys. In the present study CPDD 0067 did not share discriminative stimulus effects with AMPH in monkeys and would not, therefore, be predicted to have AMPH-like subjective effects.

CPDD 0072: 1-(3-Methoxybenzyl)piperazine dioxalate



Like CPDD 0067, CPDD 0072 is structurally similar to benzylpiperazine (BZP; CPDD 0063). Unlike CPDD 0067, CPDD 0072 was not self administered by monkeys and did not substitute for AMPH, midazolam or flumazenil. Collectively, these data suggest that this compound lacks abuse potential of the stimulant- or depressant-type.

REFERENCE

France CP, McMahon LR, Fantegrossi WE, Woolverton WL, Winter JC and Woods JH (2005) Progress report from the testing program for stimulant and depressant drugs (2004). In W.L. Dewey (Ed.), Problems of Drug Dependence, 2003: Proceeding of the 65th Annual Scientific Meeting, The College on Problems of Drug Dependence, Inc., National Institute on Drug Abuse Research Monograph #184, pp. 201-221, Washington D.C.: U.S. Government Printing Office.

Fantegrossi WE, Woolverton WL, Winger G, Coop A and Woods JH (2005) Reinforcing and discriminative stimulus effects of *l*-benzylpiperazine and trifluoromethylphenylpiperazine in rhesus monkeys. Drug Alcohol Depend 77: 161-168.

ACKNOWLEDGEMENTS

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