

The Reinforcing Efficacy of Psychostimulants in Rhesus Monkeys: The Role of Pharmacokinetics and Pharmacodynamics

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ABSTRACT

This study was undertaken to investigate pharmacological variables that influence the reinforcing efficacy of psychostimulants. Rhesus monkeys ($n = 9$) responded under a within-session, exponentially increasing, progressive ratio schedule of cocaine reinforcement. Doses of cocaine, methylphenidate (MP), cocaine analogs [(±)-2β-propanoyl-3β-(2-naphthyl)-tropane (WF-23), HD-23; (±)-2β-propanoyl-3β-(2-isopropenyl)-tropane (WF-60), HD-60; and 2β-propanoyl-3β-(4-tolyl)-tropane (HD-11, WF-11), and 2β-propanoyl-3β-(4-tolyl)-tropane (HD-11, WF-11), PTT], and MP analogs [(αR,2R)-α-(2-naphthalenyl)-2-piperidineacetic acid methyl ester, HDMP-28; and (αR,2S)-α-(2-naphthalenyl)-2-pyrrolideneacetic acid methyl ester, HDMP-29] that varied in their pharmacokinetic and pharmacodynamic properties were substituted for cocaine. These drugs were chosen according to their selectivity for dopamine transporters (DAT) and 5-hydroxytryptamine (serotonin) transporters (5-HTT) as assessed in rodents and their duration of action. In addition, data pertaining to the rate of onset at DAT

were collected for the cocaine analogs using an ex vivo binding assay in rodent tissue. Finally, the pharmacodynamic profile of select drugs was confirmed in primate brain tissue. All drugs had reinforcing effects except HDMP-29. The rank ordering of the peak breaking points (BPs) was cocaine = MP = HDMP-28 ≥ HD-60 ≥ PTT ≥ HD-23 > HDMP-29. The time to peak DAT occupancy for the cocaine analogs was greater than 30 min. The potency to maintain peak BP was significantly correlated with DAT affinity. There was not a linear relationship between monoamine transporter affinity and reinforcing efficacy, but it appeared that in nonhuman primates there is a range of DAT affinity under which maximal responding is maintained. Interestingly, the 5-HTT-selective cocaine analog HD-60 functioned robustly as a reinforcer at several doses in all monkeys tested. These data question the dogma regarding the role of pharmacokinetic factors and the relative influence of DAT and 5-HTT in stimulant reinforcement.

Although the use of psychomotor stimulants such as cocaine and various amphetamine derivatives has remained stable over the past several years (SAMHSA, 2001), an effective

pharmacotherapy has yet to be developed. An understanding of the pharmacological properties that mediate the abuse liability of these drugs will assist in the design of treatments for psychostimulant abuse and dependence and result in a better understanding of addiction. Animal models of drug self-administration have allowed for the systematic investigation of drug-reinforced behavior; however, the variables that influence the reinforcing efficacy of psychostimulants are still not fully understood.

One pharmacological attribute common to psychostimu-

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ABBREVIATIONS: DA, dopamine; DAT, dopamine transporter; 5-HTT, 5-hydroxytryptamine transporter; NET, norepinephrine transporter; 5-HT, 5-hydroxytryptamine; HD-23, (±)-2β-propanoyl-3β-(2-naphthyl)-tropane (WF-23); HD-60, (±)-2β-propanoyl-3β-(2-isopropenyl)-tropane (WF-60); HDMP-28, (αR,2R)-α-(2-naphthalenyl)-2-piperidineacetic acid methyl ester; HDMP-29, (αR,2S)-α-(2-naphthalenyl)-2-pyrrolideneacetic acid methyl ester; PR, progressive ratio; BP, breaking point; PTT, 2β-propanoyl-3β-(4-tolyl)-tropane (HD-11, WF-11); TO, time-out; CFT, 2β-carbomethoxy-3β-(4-fluorophenyl)-tropane; S/C, striatal/cerebellar; ANOVA, analysis of variance; CI, confidence interval; MP, methylphenidate; RTI-55, 3β-(4-iodophenyl)tropane-2β-carboxylic acid (methyl ester tartrate; GBR 12909, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-3(phenylpropyl)piperazine.

lants is an increase in synaptic dopamine (DA) following their administration. The involvement of DA in the reinforcing effects of psychostimulants (and perhaps all other drugs of abuse) has been established across a wide range of experimental conditions (Leshner and Koob, 1999). Moreover, a positive relationship has been demonstrated between the binding affinity of psychostimulants at dopamine transporters (DAT) and their potency to maintain responding (Ritz et al., 1987; Bergman et al., 1989; Wilcox et al., 1999). However, there are recent reports of compounds with significant DAT affinity that have weaker reinforcing efficacy compared with cocaine (Lile et al., 2002; Woolverton et al., 2002), indicating that the neuropharmacology of psychostimulant abuse is not simply related to blockade of DA uptake.

Many commonly abused psychostimulants, including cocaine and the amphetamine derivatives, also bind with high affinity to 5-hydroxytryptamine (serotonin) and norepinephrine transporters (5-HTT and NET). It has been proposed that increased synaptic 5-HT acts as a negative modulator of the reinforcing effects of psychostimulants (Ritz and Kuhar, 1989; Richardson and Roberts, 1991). However, there are few data concerning the reinforcing efficacy of compounds selective for 5-HTT. In general, drugs that increase synaptic 5-HT do not maintain self-administration (Gold and Balster, 1991; Vanover et al., 1992; Roberts et al., 1999), although there may be exceptions (Fantegrossi et al., 2002). There is even less information available on the interactions of psychostimulants with the norepinephrine system. However, research suggests that although blockade of NET is involved in cocaine's discriminative stimulus effects, it is not a primary determinant of its reinforcing effects (Woolverton, 1987; Mello et al., 1990; Spealman, 1995).

In addition to pharmacodynamic variables, kinetic properties such as a drug's onset and offset of action may be important determinants of reinforcing effects. Using *N*-methyl-D-aspartate antagonists, Winger et al. (2002) reported that onset of action significantly influenced drug-maintained responding. In agreement with those findings, data from a recent study by Woolverton et al. (2002) suggested that differences in onset of action accounted for the reduced reinforcing efficacy of a novel DAT ligand compared with cocaine. With regard to duration of action, Ko et al. (2002) did not observe differences in the reinforcing efficacy of opiates with varied half-lives. One purpose of the experiments described here was to further extend these findings to psychostimulants to better understand the relationships between pharmacokinetic factors and reinforcing efficacy.

In the present study, the importance of pharmacological properties in determining the reinforcing efficacy of psychostimulants was evaluated in rhesus monkeys by comparing self-administration of cocaine, methylphenidate, and several of their analogs (HD-23, HD-60, HDMP-28, and HDMP-29) using a progressive-ratio (PR) schedule. Under this schedule, reinforcer delivery is contingent upon the completion of successively increasing ratio sizes. The final ratio completed by the animal is termed its "breaking point" (BP) and has been used to compare the reinforcing efficacy of cocaine to other psychostimulants (Stafford et al., 2001). Pharmacodynamic data on these drugs have been gathered previously from rodent tissue (Bennett et al., 1995; S. R. Childers, personal communication). Because relative binding affinity to mono-

amine reuptake inhibitors has been associated with differences in reinforcing efficacy (Roberts et al., 1999), the selectivity for monoamine transporters of chosen compounds was confirmed using an *in vitro* binding assay in monkey tissue to authenticate the comparisons between rodent binding data and monkey behavioral data. With respect to pharmacokinetics, the onset of action of the long-acting cocaine analogs (HD-23 and HD-60) was determined using an *ex vivo* binding assay. The onset of DAT binding for cocaine, MP, and the DAT-selective cocaine analog PTT was determined previously under similar conditions (Stathis et al., 1995; Lile et al., 2002) and is included here for comparison. As for duration of action, compounds were categorized into short- and long-acting based on structural differences and verified by locomotor assays in rodents (Porrino et al., 1995; Daunais et al., 1998; D. Morgan, personal communication).

Materials and Methods

Experiment 1: Self-Administration under a PR Schedule in Rhesus Monkeys

Subjects. Nine adult male rhesus monkeys (*Macaca mulatta*), served as subjects. Monkeys R-1254, R-1347, and R-1362 were experimentally naive at the beginning of training under the PR schedule. Monkeys R-1241, R-1248, R-1268, R-1286, R-1322, and R-1326 had previous exposure to cocaine and other monoamine transporter ligands under various schedule conditions (Lile et al., 2000; J. A. Lile, unpublished observations). Monkeys R-1241, R-1248, R-1268, and R-1322 began training on the PR schedule immediately after the completion of prior experiments; R-1286 and R-1326 were drug abstinent for approximately 1 year before training under the PR schedule. Subjects weighed between 9 and 14 kg under free-feeding conditions. Their body weights were maintained at approximately 90 to 95% of free-feeding weights by supplemental feeding of Lab Diet high-protein monkey diet (100–150 g/day; PMI Nutrition International Inc., Brentwood, MO). Monkeys were weighed approximately once a month and, if necessary, their diet was adjusted to maintain stable weights. In addition, they were given fresh fruit or peanuts at least 3 days/week. Monkeys lived in a temperature- and humidity-controlled colony room; lighting was maintained on a 6:00 AM/8:00 PM on/off schedule. Environmental enrichment was provided as outlined in the Animal Care and Use Committee of Wake Forest University Nonhuman Primate Environmental Enrichment Plan.

Apparatus. Monkeys were individually housed in sound-attenuating cubicles (91 cm³; Plas Labs, Lansing, MI). The front wall of each cubicle was constructed of Plexiglas to allow the monkey visual access to the laboratory. Mounted on the Plexiglas wall were two response levers (BRS/LVE, Beltsville, MD), with two sets of jeweled stimulus lights covered with alternating red and white lens caps above each lever. In addition, cubicles were equipped with peristaltic infusion pumps (Cole-Parmer Instrument Co., Chicago, IL) for delivering drug infusions at a rate of approximately 1.5 ml/10 s. Each animal was fitted with a stainless steel restraint harness and spring arm (Restorations Unlimited, Chicago, IL) that was attached to the rear of the cubicle. Experimental events were controlled and counted by a Macintosh II computer and associated interfaces.

Surgery. Each animal was anesthetized with a combination of ketamine (15 mg/kg *i.m.*; Fort Dodge Animal Health, Fort Dodge, IA) and butorphanol (0.05 mg/kg *i.m.*; Fort Dodge Animal Health), and a chronic indwelling venous catheter was surgically implanted under sterile conditions. A two-component modified version of the typical single-lumen catheter was used. The proximal end of the catheter was composed of a "hydrocoat"-coated polyurethane catheter (Access Technologies, Skokie, IL) and the distal end consisted of a Broviac central venous silicone catheter with "Surecuff" tissue ingrowth cuff and "Vitacuff" antimicrobial cuff (Bard Access Systems, Salt Lake

City, UT). The proximal end of the catheter was inserted into a major vein (internal jugular, external jugular, femoral, or brachial), terminating in the vena cava. The distal end of the catheter was threaded subcutaneously to the back of the monkey to the point that the Surecuff tissue ingrowth cuff was positioned 3 to 5 cm below the skin exit site, and the antimicrobial Vitacuff was approximately 1 cm below the skin exit site. The remainder of the catheter exited the body through a small incision in the skin between the scapula on the back of the animal and was threaded through the spring arm to where it was connected to the infusion pump and associated hardware. The two catheter components were connected by a 20-gauge steel connecting pin. Antibiotics (Kefzol; 30 mg/kg i.m.) were administered prophylactically 1 h before surgery. In addition, topical antibiotic ointment (1% chloramphenicol; Allergan, Irvine, CA) was applied postsurgery to the surgical sites.

Procedure. Before the beginning of each test session, the catheter was flushed for approximately 20 s with the concentration of drug available for self-administration. We have calculated that this infusion duration is sufficient to fill the catheter with the drug solution available for that session without administering a significant amount of drug to the animal. All experimental sessions were typically conducted 7 days/week. Because session length was determined by individual session performance (see below), monkeys were fed at approximately 10:00 AM each day and sessions began at 2:00 PM, allowing for a maximum of a 20-h session. The next morning, each monkey's catheter was flushed with heparinized saline (100 U/ml) to help prevent clotting and the animals were fed.

Monkeys were initially trained to respond under a fixed-ratio 50 schedule of cocaine (0.03 mg/kg/injection) presentation with a 10-min TO after each injection. After acquisition of self-administration, a PR schedule was introduced. For two animals (R-1241 and R-1268), the baseline dose of cocaine was increased to 0.1 mg/kg/injection because the 0.03 mg/kg/injection of cocaine did not maintain stable responding under the PR schedule. For all monkeys, the first injection of cocaine was delivered after 50 responses, followed by a 10-min TO. The next ratio requirement was determined from the exponential equation used by Richardson and Roberts (1996): $\text{ratio} = 5 \cdot \text{exponent}(\text{S}^{\text{R}\#} \cdot 0.2) - 5$, where S^{R} is reinforcer.

For these studies, the first ratio requirement (i.e., 50 responses) corresponded to the 12th value given by this equation. A full listing of the ratio values, the corresponding injection number, and the cumulative number of responses for a given number of injections is shown in Table 1. The BP was defined as the final ratio completed when 2 h had elapsed without an injection delivered. In most cases,

TABLE 1
Ratio requirements and cumulative number of responses for the corresponding number of drug injections

Injection No.	Ratio Requirement	Cumulative Responses
1	50	50
2	62	112
3	77	189
4	95	284
5	117	401
6	144	545
7	177	722
8	218	940
9	267	1,207
10	328	1,535
11	402	1,937
12	492	2,429
13	602	3,031
14	737	3,768
15	901	4,669
16	1,102	5,771
17	1,347	7,118
18	1,646	8,764
19	2,012	10,776
20	2,458	13,234

the BP was reached within the 20-h session limit. There was an exception to this in monkey R-1362 at the highest dose of methylphenidate tested (0.3 mg/kg/injection). On the 1st day of substitution, the session length was approximately 30 h and the animal received 24 infusions of 0.3 mg/kg/injection methylphenidate. When responding had stabilized, however, session lengths were within the 20-h session limit.

When the BP for 0.03 or 0.1 mg/kg/injection cocaine was stable ($\pm 20\%$ of the mean number of injections for three consecutive sessions, with no trends in responding), saline was substituted for cocaine for at least five sessions and until the number of injections received declined and stabilized to less than 20% of baseline. After a return to baseline, various doses of cocaine (0.003–0.56 mg/kg/injection), HD-23 (0.0003–0.003 mg/kg/injection), HD-60 (0.001–0.3 mg/kg/injection), HDMP-28 (0.003–0.1 mg/kg/injection), HDMP-29 (0.01–0.3 mg/kg/injection), and methylphenidate (0.003–0.3 mg/kg/injection) were each made available for self-administration. Each drug was tested in four animals, with the exception of cocaine, which was tested at all doses in all animals. All doses and drugs were tested in random order and there was a return to baseline for at least three sessions between test doses. The minimum number of sessions that each dose was available for self-administration was individually determined and based upon the number of sessions required for responding to decline to less than 20% of baseline when saline was available. For all monkeys, test doses were available for at least five sessions. Various points in the cocaine dose-response curve were redetermined over the course of the experiment.

Data Analysis. The primary dependent variable was the number of self-administered drug injections of each cocaine or methylphenidate analog. A separate mixed model was fit for each drug (including cocaine as a comparison), using monkey as a random effect to account for variations in responding between animals. A Dunnett's post hoc test was performed to compare each drug dose to saline. Next, a mixed model was used for a between drug comparison of the peak number of drug injections of all drugs tested. Post hoc multiple comparisons were performed using a Tukey-Kramer adjustment for pairwise comparisons. Finally, regression analyses were used to relate the logarithms of the relative behavioral potency or the relative reinforcing efficacy of these drugs with the logarithms of the relative concentrations to inhibit radiolabeled ligand binding in rodents for DAT alone (simple regression), 5-HTT alone (simple regression), and DAT and 5-HTT combined (multiple regression; Ritz et al., 1987). Relative behavioral potency refers to the ratio of the dose of the cocaine or methylphenidate analog that maintained peak BPs to the dose of cocaine that maintained peak BPs. Relative reinforcing efficacy refers to the ratio of the maximum number of cocaine or methylphenidate analog injections received to the maximum number of cocaine injections received. For all analyses, $p < 0.05$ was considered statistically significant. Self-administration data presented in figures and graphs and used in statistical analyses are the mean values from the last three sessions that each drug was available for self-administration and are presented as the group means (\pm S.E.M.). Baseline cocaine data (0.03 or 0.1 mg/kg/injection) represent the three sessions preceding the BP determination for each dose of cocaine.

Experiment 2: Ex Vivo DAT Binding in Rodents

Subjects and Apparatus. Male Sprague-Dawley rats weighing between 250 and 300 g were used. They were initially housed in groups of three in plastic cages and with a 12:12-light/dark cycle (lights on at 6:00 AM). Food and water were available ad libitum.

Procedure. DAT binding for the two cocaine analogs, HD-23 and HD-60, was studied ex vivo using methods similar to those previously published using mice (Scheffel et al., 1991; Stathis et al., 1995; Gatley et al., 1999). Drugs were given i.v. via a surgically implanted catheter. For surgery, rats were anesthetized with pentobarbital (50 mg/kg i.p.), and a femoral catheter was implanted using standard techniques. The exteriorized tip of the catheter was sealed by heat-

ing. After surgery, they were housed individually for 48 h and then used experimentally.

ED₅₀ Determination. To establish relative potency for DAT occupancy, injections of various doses of HD-23 or HD-60 were given before [³H]CFT to permit maximum (or equilibrium) binding of these cocaine analogs to DAT. Initially, catheterized rats were placed in a plastic restrainer and injected i.v. (0.4 ml/rat/10 s) with 1.0 μmol/kg HD-23 or 3.0 μmol/kg HD-60. These doses were selected based upon preliminary studies with the two tropanes and their in vitro potency ratio compared with cocaine. At various time points after drug injection (0.5–30 min), rats were injected intravenously with [³H]CFT (86 Ci/mmol; PerkinElmer Life Sciences, Boston, MA), 10 μCi/rat over 10 s. Because preliminary studies showed that the striatal/cerebellar (S/C) ratio of [³H]CFT reached a maximum 45 min after injection of [³H]CFT, rats were decapitated at this time point. After decapitation, brains were removed and the striatum (high DAT density) and the cerebellum (no DAT, nonspecific binding) were dissected. Striatum and cerebellum were weighed and placed into separate 5-ml glass vials. Solvable (10 μl/mg tissue) was added and the vial was allowed to sit for 24 h at room temperature. After 24 h, glacial acetic acid (1 μl/mg tissue) was added and 200 μl of the tissue solution was immediately pipetted into each well of 24-well scintillation plates (3–6 wells/sample). Microscint-20 cocktail (1,000 μl) was then added to each well and the plate was sealed. This preparation was allowed to sit for 4 h to further solubilize tissue and reduce chemiluminescence of the microscint-20 cocktail. Radioactivity was then counted. A complete dose-response function was then determined for HD-23 and HD-60 using the time point at which the decrease in [³H]CFT was maximal. The ED₅₀ value was calculated for reduction in [³H]CFT binding.

Time-Course Determination. To establish the rate of onset of DAT binding, an injection of selected doses of HD-23 or HD-60 was given at the time point at which [³H]CFT was asymptotic. The decrease in binding was measured at various time points after drug injection and compared with the same points after saline injection (Stathis et al., 1995). Specifically, saline or the ED₅₀ dose of the cocaine analogs were given 45 min after injection of [³H]CFT. Animals were decapitated at various time points (30 s–120 min) after injection of test drug.

Data Analysis. The S/C ratio was calculated. Data were normalized to S/C – 1 so that complete inhibition of binding approached zero. Transporter occupancy was calculated using the equation % occupancy = $(A - x/A - B) \times 100$ (Gatley et al., 1999). In this equation, A and x are S/C measured after injection of radioligand alone and drug plus radioligand, respectively. B is the S/C measured after a high dose of cold GBR 12909, a selective DAT ligand, which is assumed to reflect 100% occupancy of the transporter. The difference between B and 1.0 presumably reflects differences in nonspecific binding. ED₅₀ values (the dose of competing drug displacing half the specific binding) were calculated using iterative curve fitting (Prism 3.0; GraphPad Software Inc., San Diego, CA). Time-course data for inhibition of binding by HD-23 and HD-60 were converted to percentage of control with saline pretreatment data using the same time points as control. Data for HD-23 and HD-60 were compared with saline control groups using a one-way ANOVA followed by adjusted Bonferroni *t* tests; *p* < 0.05 was considered statistically significant.

Experiment 3: In Vitro Monoamine Transporter Binding in Rhesus Monkeys

Subjects and Apparatus. Frozen brain tissue from three rhesus monkeys was used for the in vitro studies, as described previously (Woolverton et al., 2000). Each monkey had a history of cocaine self-administration and had been drug-free for at least 2 months before sacrifice. A previous in vitro study investigating the efficacy of D₁ agonists using monkey brain tissue found no obvious difference between drug-naïve monkeys and animals that had been drug abstinent for at least 2 months (Weed et al., 1997). During the drug-free

period, they were maintained under standard conditions in stainless steel cages with water continuously available. Animals were fed a sufficient amount of Teklad Monkey Diet (Harlan, Indianapolis, IN) to maintain stable body weight and received fresh fruit 5 days/week and a chewable multiple vitamin tablet 3 days/week.

Procedure. No monkeys were sacrificed specifically for this experiment but had been euthanized previously after all accessible veins had been used in self-administration studies. For euthanasia, monkeys were sedated with ketamine then given an overdose of i.v. pentobarbital. Brains were collected immediately (within 10 min) after sacrifice, and the caudate nucleus, putamen, frontal cortex, and cerebellum were dissected (20–30 min) according to the atlas of Snider and Lee (1961). Immediately after dissection, tissue was frozen on aluminum foil over solid CO₂ for 30 min, with no additional preparation, and then placed into a –80°C freezer until assayed.

For binding studies, frozen tissue was thawed, homogenized in buffer (Woolverton et al., 2000), and centrifuged at 20,000g for 20 min at 4°C. The resulting pellet was resuspended in fresh buffer and centrifuged an additional one or two times, depending on the assay. After centrifugation, the pellet was suspended at the appropriate tissue concentration for displacement or saturation assays.

Specific conditions for displacement and saturation assays were as published previously with minor changes (Woolverton et al., 2000). For displacement studies, the tissue was added to assays containing the radioligand and various concentrations of PTT, HD-23, and HD-60 dissolved in assay buffer. For saturation studies, the tissue was incubated with varying concentrations of [³H]CFT (0.1–25.6 nM), [³H]nisoxetine (0.1–12.8 nM), or [³H]paroxetine (0.006–25.6 nM). For displacement studies, concentrations of HD-60 and PTT ranged between 0.01 nM and 100 μM and concentrations of HD-23 ranged between 0.01 nM and 10 μM. For both saturation and displacement studies, all assay tubes were brought to their final volume (1.0 ml for [³H]CFT and [³H]paroxetine; 500 μl for [³H]nisoxetine) with the addition of buffer. All assays were initiated with the addition of tissue. Assays were incubated under conditions described in Woolverton et al. (2000). Reactions were terminated by rapid vacuum filtration using a 24-well cell harvester (Brandel Inc., Gaithersburg, MD) through presoaked GF/C filters (Whatman, Maidstone, UK). The filters were rinsed with ice-cold buffer, and deposited into PerkinElmer TopCount deep-well plates. Five hundred microliters of Microscint-20 cocktail (PerkinElmer Life Sciences) was added to each well. Bound radioactivity was determined using a TopCount scintillation counter. Protein levels in tissue homogenate samples were determined using the bicinchoninic acid method (Smith et al., 1985; kits from Pierce Chemical, Rockford, IL). Absorbance (at 560 nm) was measured on a spectrophotometer (Beckman Coulter, Inc., Palo Alto, CA). All saturation and displacement assays were performed in triplicate.

Data Analysis. Radioligand binding data were initially reduced and analyzed using iterative curve fitting (Prism 3.0; GraphPad Software Inc.). *K_d* and *B_{max}* values, and their 95% confidence intervals (CIs), were derived from saturation studies. To compare the goodness-of-fit of one-site models to two-site models, one-site and two-site models with all Hill coefficients fixed to –1 were fit to displacement data. The one-site model was assumed unless the mean square error was significantly reduced by using a two-site model (*p* < 0.05 using a univariate *F* test). *K_i* values and their 95% CIs were calculated for each compound from displacement studies.

Drugs. The selected cocaine and methylphenidate analogs were chosen based on their pharmacodynamic and pharmacokinetic properties. The affinity of each of these drugs for DAT, 5-HTT, and NET was determined, under similar conditions (Bennett et al., 1995; S. R. Childers, personal communication), by displacement of [¹²⁵I]RTI-55 binding in rat striatal membranes, [³H]paroxetine binding in rat frontal cortex membranes, and [³H]nisoxetine in whole rat brains (minus cerebellum), respectively (Table 2). Behavioral and ex vivo data for PTT, obtained under conditions identical to those described in the present article, have been recently published (Lile et al., 2002)

TABLE 2

Pharmacodynamic profile of cocaine and methylphenidate analogs in rodent brain tissue

DAT and 5-HTT binding affinity data for cocaine, PTT, and HD-23 have been published previously (Bennett et al., 1995). Data for MP, MP analogs, and HD-60 were collected under similar conditions and obtained via personal communication with S. R. Childers. All numbers represent mean values of three to six separate determinations. The S.E.M. was less than 10% in all cases.

Compound	[¹²⁵ I]RTI-55 IC ₅₀	[³ H]PAR K _i	[³ H]NIS K _i
		<i>nM</i>	
Cocaine	173	302	404
MP	164	>10,000	256
HDMP-28	11.1	26.8	115
HDMP-29	465	2.5	797
PTT	8.2	131	65
HD-23	0.1	0.4	2.9
HD-60	15.8	0.1	94

PAR, paroxetine; NIS, nisoxetine.

and are included here for comparison purposes. These compounds can be divided according to their selectivity for DAT and 5-HTT into three categories: DAT-selective (PTT and MP), 5-HTT-selective (HD-60 and HDMP-29), and nonselective (cocaine, HD-23, and HDMP-28). In addition, they have been further subdivided into two categories based on their duration of action: short (cocaine, MP, HDMP-28, and HDMP-29) and long (HD-23, HD-60, and PTT). The cocaine analogs HD-23, HD-60, and PTT lack both of the ester bonds present on cocaine. Because approximately 90% of cocaine metabolism occurs by cleavage of these ester bonds, it would be predicted that these drugs would have a longer duration of action in vivo compared with shorter acting drugs such as cocaine and methylphenidate. This prediction has been verified by locomotor assays in rodents (Porrino et al., 1995; Daunais et al., 1998; D. Morgan, personal communication).

(-)-Cocaine HCl and (±)-methylphenidate HCl were provided by the National Institute on Drug Abuse (Bethesda, MD). HD-23 fumarate and HD-60 fumarate were synthesized according to the procedure described by Davies et al. (1993). The synthesis of HDMP-28 fumarate and HDMP-29 HCl have not been published; their chemical structures are shown in Fig. 1. All cocaine and MP analogs were the racemic mixtures. Molecular weights for each experimental drug (as the salt form) are as follows: 423 g/mol HD-23, 283.42 g/mol HD-60, 399.44 g/mol HDMP-28, 305.8 g/mol HDMP-29, and 387 g/mol PTT. Drug concentrations were calculated according to the salt form; all drugs were dissolved in sterile saline. Doses of cocaine and HD-23 were chosen based on previous behavioral studies in non-human primates from our laboratory (Nader et al., 1997; Lile et al., 2000). Methylphenidate doses were chosen based on previously published self-administration studies in rhesus monkeys (Johanson and Schuster, 1975). For the remaining cocaine and methylphenidate analogs, doses were chosen based on their affinity for DAT and 5-HTT, and on pilot data obtained during substitution in the first of the four monkeys to be tested with that particular drug.

Animal Welfare. Animal maintenance and research were conducted in accordance with guidelines provided by National Institutes of Health Office of Protection from Research Risks. The protocol for experiment 1 was reviewed and approved by the Wake Forest University Animal Care and Use Committee. Wake Forest University is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal

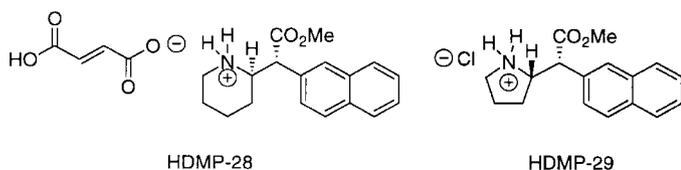


Fig. 1. Chemical structures for HDMP-28 and HDMP-29.

Care International. The protocols for experiments 2 and 3 were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

Results

Experiment 1: Self-Administration. The training dose of cocaine maintained stable self-administration in monkeys responding under the PR schedule. Initial substitution of saline for either 0.03 or 0.1 mg/kg/injection cocaine resulted in low levels of responding in all monkeys (range of 0–6 saline injections; Fig. 2). On average, 5.6 sessions (range of 4–9) of saline availability were required for the number of saline injections to stabilize below 20% of the mean number of injections maintained by the baseline dose of cocaine.

PR Performance: Nonselective DAT and 5-HTT Inhibitors. Cocaine (0.003–0.56 mg/kg/injection) functioned as a reinforcer in each monkey, and the resulting dose-response curves were characterized by an inverted U shape. When cocaine data were combined from all animals, statistical analysis indicated that the mean number of self-administered cocaine injections at each dose of cocaine was significantly greater than the number of saline injections received ($F_{6,596} = 75.36; p < 0.001$; followed by post hoc comparisons) (Fig. 2). Maximum mean BP for cocaine was approximately 1646 responses, corresponding to an average of 18 injections received at the 0.3-mg/kg/injection dose. Individual animal maximum BPs at this dose ranged from 492 to 8174 responses, corresponding to a range of 12 to 26 injections received before the ratio requirements failed to be completed. At the highest doses of cocaine tested (0.3 and 0.56 mg/kg/injection), food consumption was disrupted in some animals.

When substituted for the baseline dose of cocaine, the nonselective DAT and 5-HTT inhibitor HDMP-28 (0.003–0.1 mg/kg/injection) functioned as a reinforcer in all four animals

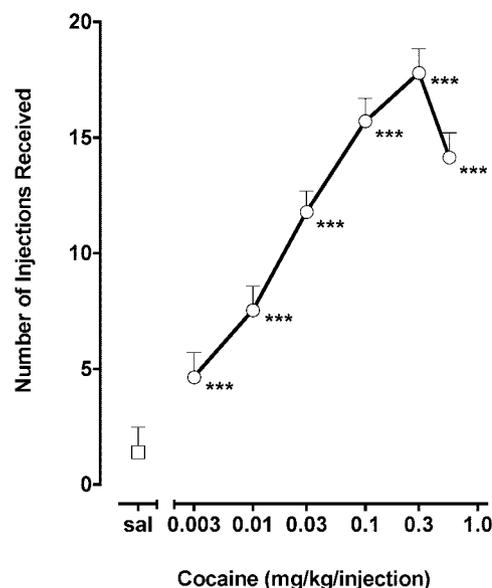


Fig. 2. Number of injections received when saline (sal) and various doses of cocaine (0.003–0.56 mg/kg/injection) were substituted for the training dose of cocaine. Data are the group ($n = 9$) mean (\pm S. E. M.) for the last three sessions under each condition as estimated by the model used for analysis. Asterisks (***) indicate a statistically significant difference ($p < 0.001$) in the group mean number of drug injections received compared with saline.

tested. One of the monkeys (R-1362) was removed from the experiment due to catheter-related complications and was only tested at the 0.01-mg/kg/injection dose of HDMP-28. At this dose, the maximum number of injections self-administered was 14, corresponding to 737 responses completed before the BP was reached. For the remaining three animals, the mean number of HDMP-28 injections self-administered at all but the lowest dose tested was significantly greater than the number of self-administered saline injections ($F_{10,173} = 28.21$; $p < 0.001$; followed by post hoc comparisons) (Fig. 3, filled squares). The highest BP maintained in individual monkeys ranged from 402 to 2012 responses (11–19 injections) at the 0.03 dose of HDMP-28. The mean BP at this dose of drug was approximately 737 responses, corresponding to 14 injections delivered before the response requirements were no longer completed. At the highest dose of HDMP-28 tested, changes in gross observable behavior included hyperactivity in monkey R-1347 and a decrease in appetite in R-1254.

HD-23 (0.0003–0.003 mg/kg/injection), a nonselective and extremely high-affinity, long-acting cocaine analog, maintained stable self-administration in two of four monkeys when substituted for cocaine. When group data were analyzed, the mean number of injections maintained by the 0.001-mg/kg/injection dose of HD-23 was significantly greater than that maintained by saline ($F_{10,229} = 74.92$; $p < 0.001$; followed by post hoc comparisons) (Fig. 3, open squares). The maximum mean BP maintained by HD-23 was 218 responses (range of 62–1102 responses), corresponding to eight injections (range of 2–16 injections) at the 0.001-mg/kg/injection dose. For two of the monkeys (R-1241 and R-1322), HD-23 did not function as a reinforcer when responding was allowed to stabilize (data not shown). In monkey R-1322, when HD-23 was initially substituted for the baseline cocaine dose, responding was maintained for the first few days of

availability at a maximum of 15 injections at the 0.0017-mg/kg/injection dose, corresponding to 901 responses before the BP was reached. HD-23 self-administration then decreased and stabilized to levels that were not different from saline. This decline in responding was more protracted than the pattern of responding typically seen when saline was substituted for the baseline dose of cocaine, which was characterized by a rapid drop in the number of self-administered injections. In monkey R-1241, HD-23 did not maintain responding at any point during substitution of the four doses of this analog. For the two monkeys in which HD-23 functioned as a reinforcer (R-1326 and R-1248), hyperactivity, stereotypy, and decreases in appetite were noted at the 0.0017- (R-1326) and 0.003 (R-1326 and R-1248)-mg/kg/injection doses.

PR Performance: DAT-Selective Inhibitors. The DAT-selective piperidine derivative MP (0.003–0.3 mg/kg/injection) maintained self-administration in all four animals tested. Data at the 0.3-mg/kg/injection dose of MP were available for only two of four monkeys and were therefore omitted from analysis. The mean number of MP injections received was significantly greater than saline-maintained responding at all doses ($F_{10,253} = 34.07$; $p < 0.001$; followed by post hoc comparisons) (Fig. 4, filled inverted triangles). The maximum mean BP for MP (approximately 1,102 responses) was maintained by the 0.03-mg/kg/injection dose and corresponded to 16 injections of MP at this dose. The maximum BPs for individual monkeys ranged from 328 to 3670 responses, corresponding to a range of 10 to 22 MP injections received before the animal failed to complete the response requirement. A decrease in appetite was observed at the 0.03-mg/kg/injection dose of MP in monkey R-1347 and at the 0.3-mg/kg/injection dose in monkey R-1326. In addition, hyperactivity was also seen at the 0.3-mg/kg/injection dose of MP in monkey R-1326.

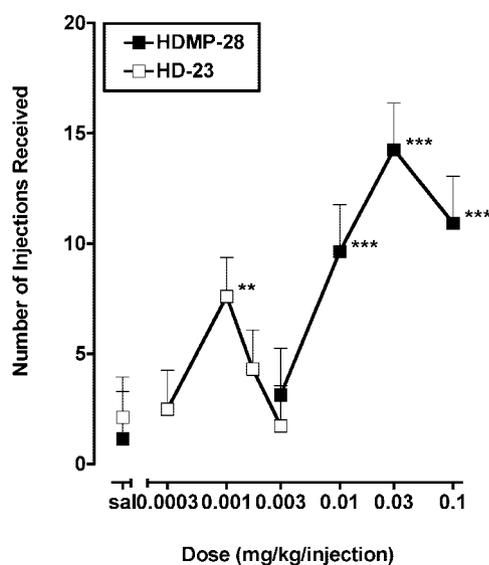


Fig. 3. Number of injections received when saline (sal), HDMP-28 (0.003–0.1 mg/kg/injection; filled squares), or HD-23 (0.0003–0.003 mg/kg/injection; open squares) was substituted for the training dose of cocaine. Data are the group ($n = 3$ for HDMP-28; $n = 4$ for HD-23) mean (\pm S. E. M.) as estimated by the model used for analysis. Asterisks (*) indicate a statistically significant difference in the group mean number of drug injections received compared with saline: *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

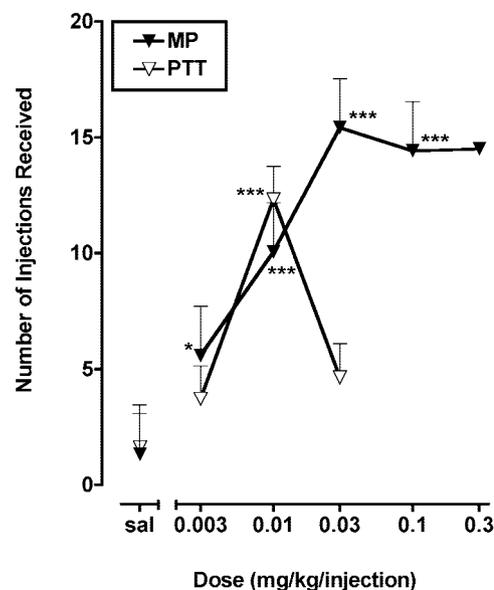


Fig. 4. Number of injections received when saline (sal), MP (0.003–0.3 mg/kg/injection; filled inverted triangles), or PTT (0.003–0.03 mg/kg/injection; open inverted triangles) was substituted for the training dose of cocaine. Data are the group ($n = 4$) mean (\pm S. E. M.) as estimated by the model used for analysis. Data for 0.3 mg/kg/injection MP were incomplete (mean of only two of four monkeys) and were omitted from analysis. Individual animal data for PTT have been published previously (Lile et al., 2002). All other details are as in Fig. 3.

The long-acting, slow onset DAT-selective cocaine analog, PTT, was recently evaluated under identical conditions (Lile et al., 2002) and was included here for comparison. When PTT was made available for self-administration under the PR schedule, a significant number of injections were self-administered at the 0.01-mg/kg/injection dose and maximum BPs ranged from 144 to 1102 responses (corresponding to a range of 6–16 injections; Fig. 4, open inverted triangles).

PR Performance: 5-HTT-Selective Inhibitors. The 5-HTT-selective methylphenidate analog HDMP-29 (0.01–0.3 mg/kg/injection) appeared to function as a reinforcer in two of the four monkeys (R-1286 and R-1322), but varied in the potency to maintain peak self-administration between these two animals (data not shown). The maximum BP maintained by HDMP-29 in individual animals ranged from 77 to 267 responses, corresponding to a range of three to nine injections of HDMP-29. The group mean number of injections maintained by cocaine for these four monkeys was significantly greater than saline; HDMP-29, however, was not significantly different from saline at any dose (Fig. 5, filled triangles). The only obvious change in behavior was an increased latency to consume chows in some monkeys at the highest dose tested.

The metabolically stable 5-HTT-selective cocaine analog HD-60 (0.003–0.3 mg/kg/injection) functioned as a reinforcer in all four monkeys when substituted for the baseline dose of cocaine. Data at the 0.3-mg/kg/injection dose of HD-60 were available for only two of four monkeys and were therefore omitted from analysis. The mean number of HD-60 injections received at the 0.03- and 0.1-mg/kg/injection doses was significantly greater than saline ($F_{10,235} = 39.69$; $p < 0.001$; followed by post hoc comparisons) (Fig. 5, open triangles). The peak BPs for HD-60 (range of 402–2012 responses) for each animal were maintained by the 0.1-mg/kg/injection dose, corresponding to a range of 11 to 19 injections received before the BP was reached. The maximum mean BP for the group was maintained by the 0.1-mg/kg/injection dose of

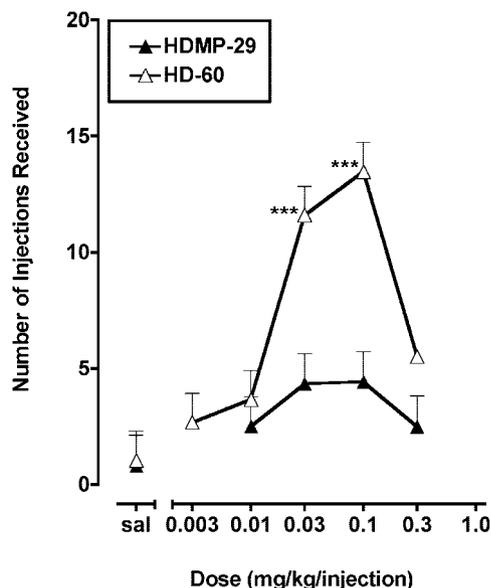


Fig. 5. Number of injections received when saline (sal), HDMP-29 (0.01–0.3 mg/kg/injection; filled triangles), or HD-60 (0.003–0.3 mg/kg/injection; open triangles) was substituted for the training dose of cocaine. Data for 0.3 mg/kg/injection HD-60 were incomplete (mean of only two of four monkeys) and were omitted from analysis. All other details are as in Fig. 4.

HD-60 and was approximately 737 responses, which corresponded to 14 injections. There were no noticeable changes in gross observable behavior at any dose of HD-60.

BP Comparisons. The group mean peak number of self-administered drug injections in descending order for each of the monoamine reuptake inhibitors was cocaine, 18; MP, 16; HDMP-28, 14; HD-60, 14; PTT, 12; HD-23, 8; and HDMP-29, 4. There was a statistically significant difference in the peak number of injections maintained by the seven drugs ($F_{6,98} = 37.96$; $p < 0.001$). Post hoc comparisons revealed that the peak number of injections of cocaine, MP, and HDMP-28 was not significantly different from one another; MP, HDMP-28, HD-60, and PTT injections did not differ significantly; and the mean number of self-administered injections of PTT and HD-23 was not different. The maximum number of injections of HDMP-29 received was significantly lower than for every other drug. The rank ordering of the peak number of injections (and therefore BPs) for these seven drugs based on these analyses was as follows: cocaine = MP = HDMP-28 \geq HD-60 \geq PTT \geq HD-23 > HDMP-29.

Monoamine Transporter Affinity and Behavioral Potency and Efficacy. These data were also analyzed using regression analyses to relate the logarithms of the relative (compared with cocaine) concentrations to inhibit radiolabeled ligand binding for DAT and 5-HTT with the logarithms of the relative behavioral potency of these drugs to maintain peak BPs (Ritz et al., 1987) and with their relative reinforcing efficacy. When a simple linear regression of the data for all compounds tested (including PTT) was calculated, binding affinity at DAT accounted for a significant proportion of the variance in the potency of these drugs to maintain peak BPs ($R^2 = 0.74$) (Fig. 6A). In contrast, binding affinity at 5-HTT did not account for a significant proportion of the variance in the potency of these drugs to maintain peak BPs ($R^2 = 0.04$). Inclusion of the logarithms of the relative inhibitory concentrations at DAT and 5-HTT in a multiple linear regression model resulted in a nonsignificant increase in goodness of fit ($R^2 = 0.79$) compared with when DAT binding data were used alone. Unlike potency measures, the relationship between relative reinforcing efficacy and the relative concentrations to inhibit radiolabeled ligand binding for DAT, 5-HTT, or DAT and 5-HTT combined was not described by a linear function. Instead, it seemed that there was a range of DAT affinity in which maximum responding was maintained (Fig. 6B). The R^2 values, y-intercepts, and slopes for the regression analyses are given in Table 3.

Experiment 2: Ex Vivo DAT Binding. Data regarding the maximum DAT occupancy and rate of onset for cocaine and PTT under these conditions have been reported elsewhere (Lile et al., 2002; Woolverton et al., 2002). These data have also been collected for MP under similar conditions in mice and were used for comparison (Stathis et al., 1995). The maximum decrease in [3 H]CFT binding by the cocaine analogs was seen when HD-23 was given 10 min before [3 H]CFT and when HD-60 was given 30 min before [3 H]CFT (data not shown). When various doses of HD-23 or HD-60 were administered at their respective pretreatment times, the tropanes inhibited the binding of [3 H]CFT in a dose-related manner with an ED_{50} value of 1.04 μ mol/kg for HD-23 and 6.95 μ mol/kg for HD-60 (compared with 8.82 μ mol/kg for cocaine and 1.82 μ mol/kg for PTT). When the ED_{50} dose of HD-23 was given at various times before sacrifice, DAT occupancy

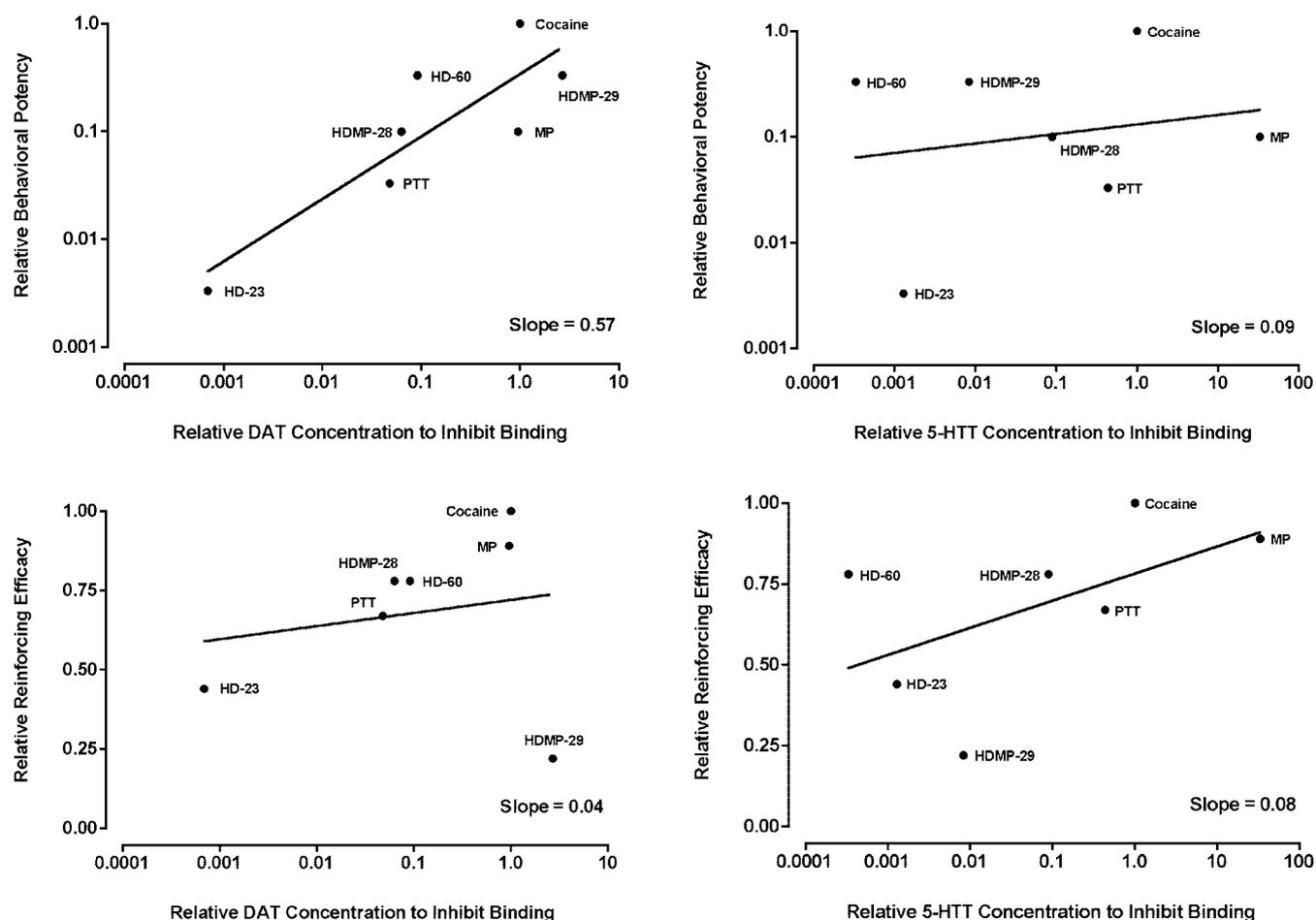


Fig. 6. Relation between the relative inhibitory concentrations for [¹²⁵I]RTI-55 binding at DAT (left) or 5-HTT (right) of cocaine and MP analogs and their relative behavioral potency (top) or their relative reinforcing efficacy (bottom). Regressions were calculated using logarithms but are presented as antilogarithms for clarity. The slope of the regression line is given in the bottom right corner of each graph. Data from the simple regression analyses, but not the multiple regression analyses, are presented in this figure.

TABLE 3

Results from linear regression analysis of the logarithms of the relative potency to inhibit binding at DAT, 5-HTT, and DAT and 5-HTT combined as predictors of the logarithms of the relative behavioral potency to maintain peak BP and of the relative reinforcing efficacy. Numbers in parentheses represent the 95% CI.

Binding Affinity Data Included	Relative Behavioral Potency			Relative Reinforcing Efficacy		
	R ²	y-Intercept	Slope	R ²	y-Intercept	Slope
DAT	0.74	-0.48 (-1.04 to 0.09)	0.57 (0.18 to 0.97)	0.04	0.72 (0.36 to 1.08)	0.04 (-0.21 to 0.29)
5-HTT	0.04	-0.88 (-1.93 to 0.17)	0.09 (-0.43 to 0.61)	0.30	0.78 (0.49 to 1.08)	0.08 (-0.06 to 0.23)
DAT and 5-HTT	0.79	-0.55 (-1.19 to 0.09)	DAT: 0.66 (0.18 to 1.14) 5-HTT: -0.12 (-0.46 to 0.21)	0.30	0.77 (0.38 to 1.16)	DAT: -0.02 (-0.32 to 0.27) 5-HTT: 0.09 (-0.11 to 0.29)

was significant ($F_{7,16} = 11.25$; $p < 0.001$) at the 60- and 120-min time points (Fig. 7, squares). When the ED₅₀ dose of HD-60 was given at various times before sacrifice, DAT occupancy was significant ($F_{7,16} = 15.12$; $p < 0.001$) at the 30-, 60-, and 120-min time points (Fig. 7, circles). For comparison, DAT occupancy was significant for the ED₅₀ dose of PTT and cocaine at the 30- and 2-min time points, respectively (Lile et al., 2002; Woolverton et al., 2002).

Experiment 3: In Vitro Monoamine Transporter Binding. All cocaine analogs inhibited binding of all three radioligands in monkey brain tissue in a concentration-related manner. Displacement was consistent with a one-site model for all three radioligands. HD-60 had high affinity for the [³H]paroxetine, and somewhat less affinity for the

[³H]CFT and [³H]nisoxetine sites (Table 4). HD-23 was relatively nonselective for the [³H]CFT and [³H]paroxetine binding sites (Table 4). The order of affinity for PTT from highest to lowest at the three radioligand binding sites in monkey brain tissue was [³H]CFT, [³H]nisoxetine and [³H]paroxetine (Table 4).

Discussion

A PR schedule was used to compare the reinforcing efficacy of a series of cocaine and methylphenidate analogs based on their pharmacokinetic and pharmacodynamic profiles. In the present study, six drugs were evaluated. For comparison, we have also included PTT, which was evaluated under identical

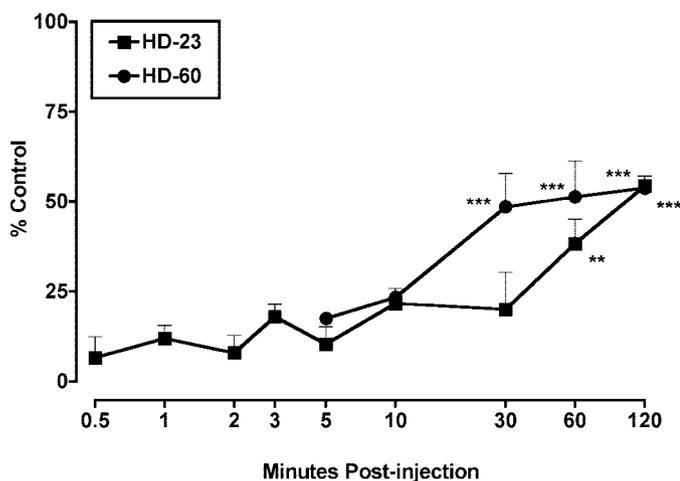


Fig. 7. Time course of DAT occupancy by HD-23 and HD-60 in rats. HD-23 (1.04 $\mu\text{mol/kg}$) or HD-60 (6.95 $\mu\text{mol/kg}$) was given 45 min after [^3H]CFT and rats were sacrificed at the indicated time points. Each point represents the percentage of saline-injected animals sacrificed at the same time points. Each point is the mean of three to five rats and vertical lines are the S.E.M. Asterisks (*) indicate a statistically significant difference above saline controls: **, $p < 0.01$; ***, $p < 0.001$.

conditions (Lile et al., 2002). Their rank for potency at DAT as assessed in rodents was HD-23 > PTT = HDMP-28 = HD-60 > MP = cocaine > HDMP-29 (Bennett et al., 1995; S. R. Childers, personal communication). In terms of their duration of action, these drugs can be divided into two groups: short (cocaine, MP, HDMP-28, and HDMP-29) and long (HD-23, HD-60, and PTT). Of the compounds that have been assayed for their rate of onset to bind DAT using an ex vivo binding assay, the order from fastest to slowest was cocaine = MP < HD-60 = PTT < HD-23 (Stathis et al., 1995; Lile et al., 2002; experiment 2). The rank ordering of the mean peak BPs for these seven drugs was cocaine = MP = HDMP-28 \geq HD-60 \geq PTT \geq HD-23 > HDMP-29. The only drug to not function as a reinforcer in this study was HDMP-29.

In the present study, there were three pharmacodynamic variables that were considered: DAT affinity, 5-HTT affinity, and the relative affinity between these transporters. Multiple regression analysis revealed that the potency of cocaine, MP and their analogs that maintained the peak BP was primarily related to DAT affinity, confirming the results from several other studies (Ritz et al., 1987; Bergman et al., 1989; Wilcox et al., 1999). However, there was not a direct relationship between DAT affinity and reinforcing efficacy. Instead, there seemed to be a range of DAT binding affinity at which maximal responding was maintained, in that the compounds with the highest and lowest DAT affinity supported the least amount of behavior. At one extreme, HD-23 maintained the lowest mean BPs that were still significantly different from saline, but had the highest affinity for DAT in both rat and monkey brain. It has been proposed that some drugs exhibit a "functional maximum reinforcing effect" which is limited by the direct, disruptive effects on behavior (Wilcox et al., 2000) and may be attributable to high levels of DA activity. Disruptive effects resulting from a complete and long-lasting inhibition of DA reuptake at doses that occupy the DAT to the degree necessary for reinforcement may impose a ceiling on the reinforcing efficacy of HD-23. Consistent with this hypothesis, a previous study demonstrated that administra-

tion of HD-23 to rodents resulted in more intense stereotypy and larger increases in locomotor behavior than cocaine (Daunais et al., 1998). At the other end of the spectrum, HDMP-29 failed to support BPs greater than saline, which could be due to an inability to increase synaptic DA concentrations to the extent required to reinforce behavior.

Although the potency of these drugs to maintain peak BP seems primarily related to DAT (present results; Ritz et al., 1987; Bergman et al., 1989; Wilcox et al., 1999), 5-HTT blockade may also influence the potency of stimulant drugs (present results; Ritz and Kuhar, 1989). Recently, Czoty et al. (2002) suggested that cocaine-induced increases in extracellular DA are antagonized by administration of drugs that enhance 5-HT activity. Although not statistically significant, the multiple regression analysis indicated that a model containing both DAT and 5-HTT affinity data accounted for a greater proportion of the variation in potency to maintain self-administration between drugs, which seemed to be due to an opposing influence of increased 5-HTT binding. For example, DAT affinities for cocaine and MP in rodents are similar (173 and 164 nM, respectively), but they differ in their affinity at 5-HTT (302 nM for cocaine versus >10,000 nM for MP). Consistent with the hypothesis that increased central 5-HT levels are a negative modulator of extracellular DA, the dose of cocaine that maintained peak BPs was 1.0 log unit less potent than MP. Nonetheless, it is possible that factors other than 5-HTT affinity that were not considered here (e.g., bioavailability) may have also influenced the behavioral potency of these drugs.

In previous studies, administration of *l*-tryptophan, a 5-HT precursor (McGregor et al., 1993), and fluoxetine, a selective 5-HT reuptake inhibitor (Richardson and Roberts, 1991), decreased cocaine-maintained BPs in rodents responding under a PR schedule. Furthermore, drugs that increase synaptic 5-HT typically do not maintain self-administration (Gold and Balster, 1991; Vanover et al., 1992). The data from those studies imply that increased extracellular 5-HT can have a negative impact on the reinforcing efficacy of psychostimulants. The selective 5-HT reuptake inhibitor HD-60, however, maintained high BPs in the present study. Although 5-HTT-selective, HD-60 does have considerable affinity for DAT, and falls within the range of DAT binding in which responding seems to be maximally maintained. HD-60 and HDMP-29 have nearly identical DAT/5-HTT ratios, yet only HD-60 functions as a reinforcer, suggesting that the higher DAT affinity accounts for this behavioral difference. In contrast, HD-60 and HD-23 have equal affinity at 5-HTT, but HD-23 has a 100-fold higher affinity at DAT and maintains lower BPs compared with HD-60. Although the present data do not allow for precise conclusions about the role of each monoamine transporter in cocaine reinforcement, these data do question existing dogma regarding the relative influence of DAT and 5-HTT. Clearly, additional studies are needed to better understand these important interactions.

Two pharmacokinetic variables that have been investigated for their effects on drug reinforcement are onset and offset of action (Balster and Schuster, 1973; Panlilio and Schindler, 2000). For the compounds that both duration and onset of action are known (cocaine, MP, HD-60, HD-23, and PTT), these two kinetic properties covaried. Cocaine and MP have a short onset and duration of action, whereas the cocaine analogs are long-acting and take a significantly longer

TABLE 4

K_i values for cocaine analogs at biogenic amine transporters in rhesus monkey brain tissue

Data represent the mean (95% CI); ($n = 3$).

	^3H]CFT (nM)	^3H]PAR (nM)	^3H]NIS (nM)
HD-60	48.0 (39.3–58.5)	0.60 (0.48–0.73)	12.1 (8.11–18.1)
HD-23	1.55 (1.27–1.89)	1.54 (1.00–2.38)	89.6 (31.4–255)
PTT	3.61 (2.80–4.67)	174 (109–276)	51.8 (5.16–519)

PAR, paroxetine; NIS, nisoxetine.

time to attain significant DAT binding. With the exception of HD-60, the more rapid onset and offset of action would seem to predict greater reinforcing efficacy. Cocaine and MP, which maintained the highest BPs of the seven drugs, enter the central nervous system quickly (Stathis et al., 1995), and compared with the cocaine analogs, are cleared more rapidly. In contrast, HD-23, which takes 60 min to attain significant DAT binding (experiment 2) and is present at the DAT for up to 5 days (Daunais et al., 1998), was not as efficacious a reinforcer compared with cocaine and MP. However, HD-60 and MP have very different pharmacokinetic profiles, but maintained qualitatively similar amounts of self-administration. It is therefore difficult to determine the influence of these pharmacokinetic variables on reinforcing efficacy, although previous research suggests that a drug's onset of action is a more important determinant (Ko et al., 2002; Winger et al., 2002; Woolverton et al., 2002). Further research is necessary to understand the relationship between pharmacokinetics and the ability of a drug to reinforce responding.

Previously, Roberts et al. (1999) evaluated the reinforcing efficacy of a series of cocaine analogs that included the three cocaine analogs compared here (PTT, HD-23 [referred to as WF-23] and HD-60 [WF-60]) in rats responding under a similar PR schedule. In comparing the present results with those from rodents, there was significant variance in the reinforcing effects of these three cocaine analogs between rats and monkeys. In rodents, PTT maintained higher BPs than cocaine, and HD-23 and cocaine were approximately equally efficacious as reinforcers. In monkeys, however, PTT and HD-23 maintained significantly lower BPs than cocaine. Interestingly, the 5-HTT-selective cocaine analog HD-60 functioned robustly as a reinforcer in monkeys, but did not maintain self-administration at any dose in rats. Because earlier studies demonstrated that drugs acting preferentially at 5-HTT typically do not maintain responding in nonhuman primates (Gold and Balster, 1991; Vanover et al., 1992), the selectivity of HD-60 for the three monoamine transporter types was assessed in vitro using monkey brain tissue (experiment 3). The results from that experiment confirm that the monoamine transporter selectivity of the cocaine analogs (including HD-60) in monkey brain is in agreement with the data from in vitro binding studies in rodent tissue (Bennett et al., 1995). These findings suggest species differences in the neurobiology of the reinforcing effects of monoamine reuptake inhibitors.

The exact pharmacological mechanisms by which psychostimulants differ in their efficacy as reinforcers are not completely clear, and it is difficult to study these variables independently because they are not mutually exclusive properties. However, these data provide additional information about the neuropharmacological substrates of behavior reinforced by psychomotor stimulants, and have further im-

plications concerning drug addiction in humans as well. The drastic differences between the behavioral effects of cocaine analogs in rats and monkeys that were observed under similar conditions provide a clear example of the importance in studying multiple species when using animal models to make predictions about the human condition. With respect to the use of monoamine transporter inhibitors as pharmacotherapies for cocaine addiction, these results suggest that psychostimulants with a range of pharmacodynamic and pharmacokinetic properties can still be expected to have significant reinforcing effects, which may aid in compliance to initiate and maintain treatment.

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