JPET Fast Forward. Published on January 26, 2005 as DOI: 10.1124/jpet.104.080101

Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs

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A. Running title
Self-administration of amphetamine analogs

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Text:	35 pages
Tables:	2 tables
Figures:	3 figures
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Abstract:	249 words
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Discussion:	1,393 words

D. Abbreviations

MPP:	1-methyl-4-phenylpyridinium
NET:	norepinephrine transporters
SERT:	serotonin transporters

E. Section: Behavioral pharmacology

Abstract

It has been reported that among drugs with mixed actions on CNS monoamine systems, increased serotonergic activity is associated with decreased potency as a reinforcer. The present experiment was designed to examine this relationship for amphetamine analogs that varied in serotonin releasing potency, and to evaluate whether serotonergic actions can affect reinforcing efficacy. Compounds PAL 313 and 314 are *para*- and *meta*-methylamphetamine, respectively. PAL 303 and 353 are *para*- and *meta*-fluoroamphetamine, respectively. All compounds had similar potencies as *in vitro* releasers of DA and NE but differed in potency for 5-HT release [EC50 (nM) PAL 313=53.4; PAL 314=218; PAL 303=939; PAL 353=1937]. When made available to rhesus monkeys (N=4) for self- administration under a fixed-ratio 25 schedule, all were positive reinforcers with biphasic dose-response functions (0.003 - 1.0 mg/kg), and were equipotent. PAL 313 was self-administered at a lower rate than the other compounds, which were indistinguishable. Under a progressive-ratio schedule (N=5), all drugs were positive reinforcers. Dose-response functions increased to a maximum or were biphasic (0.01 - 1.0 mg/kg), and drugs were equipotent. At maximum, PAL 313 maintained less responding than other PAL drugs, which maintained similar maxima. Thus, all compounds were positive reinforcers under both schedules, consistent with their potent DA actions. Responding was lower when 5-HT potency was higher and comparable to DA and NE potency. The results suggest that the mechanism for this effect involves a decrease in reinforcing potency and efficacy among monoamine releasing agents when 5-HT releasing potency is increased relative to DA.

Among drugs that enhance monoaminergic neurotransmission in the CNS, especially psychostimulants, there is considerable evidence that dopamine (DA) plays an integral part in the reinforcing effects that contribute to their self-administration and abuse (for reviews, see Wise, 1978; Woolverton and Johnson, 1992; Howell and Wilcox, 2002). Observations that are consistent with this hypothesis include the finding that the potency of cocaine-like drugs in self-administration is positively correlated with their affinity in binding at the dopamine transporters (DAT) *in vitro* (Ritz et al., 1987; Bergman et al., 1989; Wilcox et al., 2000). Simple potency as a reinforcer, however, does not seem to predict potential for abuse particularly well. Rather, abuse has been more strongly associated with efficacy or strength as a reinforcer (see, e.g., Brady and Griffiths, 1976). Cocaine, for example, has a relatively weak affinity at the DAT, but is one of the most efficacious reinforcers in laboratory animals and is highly abused by humans. It seems that factors other than potency for increasing DA neurotransmission must contribute to reinforcing efficacy and abuse of compounds that bind the DAT.

Previous research has suggested several pharmacological determinants of reinforcing efficacy. With regard to monoaminergic actions, enhanced serotonergic activity may be negatively related to the self-administration of psychostimulants. Compounds that selectively increase serotonin (5-HT) neurotransmission have been found not to maintain self-administration (Howell and Byrd, 1995; Tessel and Woods, 1975; Vanover et al., 1992). Among amphetamine-like drugs that are not as selective for 5-HT activity, Ritz and Kuhar (1989) reported a negative correlation between potency as a reinforcer and binding affinity at the 5-HT transporter (SERT). The depletion of 5-HT by medial forebrain bundle lesion with 5,7-dihydroxy-tryptamine appeared to increase the reinforcing efficacy of cocaine in rats (Loh and Roberts, 1990) whereas manipulations that increase CNS 5-HT function can decrease

cocaine self-administration (Carroll et al., 1990; Howell and Byrd, 1995; Smith et al., 1986). Studies using co-administered phentermine and fenfluramine as a prototypical DA/5-HT releasing agents demonstrated that compounds which increase both extracellular DA and 5-HT in rat nucleus accumbens are not self-administered by rodents (Glatz et al., 2002), do not induce conditioned place preference in rats (Rea et al., 1998) and have low potential for abuse in human subjects (Brauer et al., 1996). Reinforcing efficacy among a series of cocaine analogs was shown to be negatively related to the SERT potency relative to DAT (Roberts et al., 1999). In that study, cocaine analogs with the highest relative SERT affinity did not function as reinforcers.

Taken together, these data suggest that among drugs that increase monoaminergic neurotransmission in the CNS, 5-HT activity may reduce reinforcing efficacy and, thereby, reduce self-administration. In a recent study with rhesus monkeys, Lile et al. (2003) reported robust self-administration of a cocaine analog, HD-60, with at least 80-fold selectivity for the SERT relative to the DAT. This same compound was not a positive reinforcer in rats in the study of Roberts et al. (1999; called WF-60). Thus, it is possible that there are important species differences in the influence of 5-HT activity on self-administration. In addition, although Ritz and Kuhar (1989) proposed a negative relationship between 5-HT release and reinforcing potency, a prospective study testing this hypothesis, or the relationship of 5-HT release to reinforcing efficacy, has not been reported. The present experiments were, therefore, designed to evaluate the relationship between 5-HT releasing potency, relative to other monoamines, and reinforcing potency and efficacy in rhesus monkeys. A series of amphetamine analogs with similar *in vitro* potencies in releasing DA and NE, but varying potencies in releasing 5-HT, were selected for testing.

Methods

All animal use procedures were approved by the University of Mississippi Medical Center's Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines.

1. Self-administration studies

Animals and Apparatus

The subjects were seven male rhesus monkeys (*Macaca mulatta*; 88-14, AP01, AP78, M341 for the fixed-ratio study; AP01, M341, M1389, L500, RJu2 for the progressive-ratio study) weighing between 9.6 and 11.8 kg at the beginning of the study. All the monkeys except 88-14 had histories of self-administration of cocaine and/or other stimulants. Monkey 88-14 had previously been trained in a drug discrimination paradigm to discriminate pentobarbital from saline and was naive to drug self-administration. Monkey Rju2 became ill and had to be removed from the study before all drugs had been tested. All monkeys were provided with sufficient food to maintain stable body weight (120-180 g/day, Teklad 25% Monkey Diet, Herlan/Teklad, Madison, WI) and had unlimited access to water. Fresh fruit and a vitamin supplement were provided daily and three times a week, respectively. Lighting was cycled to maintain 16 hours of light and 8 hours of dark, with light on at 06:00 hours.

The monkeys were individually housed in the experimental cubicles (1.0 m^3, Plaslabs, Lansing, MI). Each monkey was fitted with a stainless-steel harness attached by a tether to the rear wall of the cubicle. The front door of the cubicle was made of transparent plastic and the remaining walls were opaque. Two response levers (PRL-001, BRS/LVE, Beltsville, MD) were mounted on the inside of the door. Four jeweled stimulus lights, two red and two white, were mounted above each lever. Drug injections were delivered by a peristaltic infusion pump (Cole-

Parmer Co., Chicago, IL). A Macintosh computer with custom interface and software controlled all events in an experimental session.

Procedure

Monkeys were implanted with a silastic catheter (0.26cm o.d.× 0.076cm i.d.; Cole-Parmer Co., Chicago, IL) into the jugular (internal or external) or femoral vein under isoflurane anesthesia. Brachial veins were implanted with a microrenethane catheter (0.08" o.d.× 0.04" i.d.; Braintree Scientific, Braintree, MA) heated and drawn to approximately half size. The proximal end of the catheter was inserted into the vein and terminated in the vena cava near the right atrium. The distal end was threaded subcutaneously to exit the back of the monkey, threaded through the spring arm, out the rear of the cubicle and connected to the peristaltic pump. In the event of catheter failure, surgery was repeated using another vein, after the veterinarian confirmed the health of the monkey.

Experimental sessions began at noon each day and were conducted seven days per week. Thirty minutes before each session started, catheters were filled with drugs for the sessions without infusing the drugs into monkeys. At the start of a session, the white lights were illuminated above both levers and pressing the right lever resulted in the delivery of a drug injection for 10 seconds. During the injection, the white lights were extinguished and the red lights were illuminated. Pressing the left lever was counted but had no other programmed consequence. After the session, catheters were filled with 0.9% saline containing heparin (40 units/ml).

In baseline sessions, cocaine or saline was available for an injection. The baseline dose of cocaine or saline was initially available under a double-alternation schedule, i.e., two consecutive daily cocaine sessions were followed by two consecutive daily saline sessions.

When responding was stable (see below) for at least two consecutive double-alternation sequences of cocaine and saline (i.e., eight sessions), test sessions were inserted to the daily sequence between two saline or two cocaine sessions. To prevent monkeys from learning this session sequence, a randomly determined saline or cocaine baseline session was inserted after every other test session. Thus, the daily sequence of sessions was C, S, T, S, C, T, R, C, S, T, S, C, T, R, where "C", "S", "R" and "T", respectively, represent a cocaine baseline, a saline, a randomly determined cocaine/saline and a test session.

Drugs were tested in a different order across monkeys. All doses of one compound were tested before moving on to the next compound. For the first monkey tested with a given drug, doses were available in an ascending order. For the other monkeys, doses were tested in a random order. After a test session, a monkey was returned to baseline conditions until responding for cocaine and saline again met stability criteria, or a new stable baseline was established. All doses were tested at least twice in each monkey, once with a saline session the day before and once with a cocaine session the day before.

Fixed-ratio schedule. Drugs were made available under a fixed-ratio (FR) schedule that has been described previously (Wee and Woolverton, 2004). The response requirement was 25 lever-presses per injection and each session lasted for 2 hours. The baseline dose of cocaine was the dose that maintained the maximum responding in a dose-response function in all monkeys, i.e., 0.03 mg/kg/injection. Responding was considered stable when the injections/session for cocaine varied no more than ±15% of the previous session and saline injections were less than 15 injections/session, and there were no trends in the data. During test sessions, one of various doses of the amphetamine analogs (0.003 - 1.0 mg/kg) was made available for self-administration under conditions identical to baseline conditions. When the

data in the two test sessions of a given dose were inconsistent (i.e., a dose was a reinforcer in one test and not in the other or a reinforcer in both tests but intake differed by more than 30 injections), the dose was made available for at least four consecutive sessions and until responding was stable.

Progressive-ratio schedule. Drugs were also made available to a second group of monkeys in which responding was maintained under a progressive-ratio (PR) schedule of reinforcement comparable to that described by Wilcox et al. (2000). The PR schedule consisted of 20 trials, with one injection available per trial. The response requirement started at 100 responses per injection and doubled after every fourth trial. A subject had 30 minutes to complete a trial (limited hold 30 min: LH 30'). A trial ended with a 10-sec drug injection or the expiration of the LH. There was a 30 minute-timeout (TO30') after each trial. If the response requirement was not completed for two consecutive trials (i.e., the LH expired), or the animal self-administered all 20 injections, the session ended.

The baseline dose of cocaine was the lowest dose that maintained the maximum injections in individual monkeys, i.e., 0.1 or 0.3 mg/kg/injection. Responding was considered stable in baseline sessions when injections/sessions varied by no more than two for both cocaine and saline for at least two consecutive double-alternation sequences. During test sessions, one of various doses of amphetamine and the amphetamine analogs (0.003 - 1.0 mg/kg) was available for monkeys under conditions identical to baseline sessions. When the two test sessions of a dose showed high variability (two determinations $\geq$ mean $\pm$ 3 injections) at the dose with the maximum mean injections, the dose was re-determined twice, once after saline session and once after cocaine baseline session. For drugs that exhibited biphasic dose-response functions, the TO after injection was increased to 60 minutes (TO 60') and the two highest doses

were tested again. This adjustment was made out of concern that, under the 30-minute TO, drug accumulation over the session may have acted to suppress responding and, thereby, decreased maximum responding.

Data analysis

The mean number of injections per session was calculated individually from the two test sessions as a function of dose. When a dose was tested in consecutive sessions under the FR schedule, the last two test sessions were used in data analysis. When a dose was re-tested under the PR schedule, the two re-test sessions were used in data analysis. The range of injections served as a measure of variability in individual subjects. A dose of a drug was considered to function as a reinforcer if the mean number of injections was above levels seen with saline and the ranges did not overlap.

For both schedules, the group mean dose-response functions for each drug were collapsed across the monkeys as to the dose of the maximum injections, regardless of the absolute values of the doses. This was done because each drug maintained qualitatively comparable dose-response functions across monkeys but with the maximum responding at a different dose, in particular under the FR schedule. Statistical analysis was done with these group means normalized as to dose. That is, the mean maximum number of injections was calculated for a drug by averaging the individual maximum mean injections for the drug, regardless of dose, across monkeys (e.g., Woolverton and Wang, 2004). Repeated measures one-way analysis of variance (ANOVA) with the Student-Newman-Keul as a post hoc test was then used to assess statistically significant differences among drugs in the maximum number of injections (GraphPad Prism 3.0).

Under the FR schedule, the potency of the compounds in self-administration was compared based on the doses that maintained the maximum injections in monkeys in which a drug served as a reinforcer. The doses of the maximum injections in individual monkeys were averaged for a given drug and compared using a repeated measures one-way ANOVA.

For the data under a PR schedule, the ED_{50} value was calculated for each animal in which a drug served as a reinforcer using the ascending limb of a dose-response function and non-linear regression analysis (GraphPad Prism 3.0). Mean ED_{50}s were calculated for each drug by averaging the log values of ED_{50}s in all monkeys in which the drug functioned as a reinforcer and taking the antilog of that value. Repeated measures one-way ANOVA, using multiple imputation to fill empty ED_{50} values in two monkeys for statistical purpose, was used to compare ED_{50} values (GraphPad Prism 3.0). The Student-Newman-Keul was used as a post hoc test.

2. *In vitro* monoamine release

In vitro release assays were conducted as described by Rothman et al. (2003) using [^{3}H]MPP^+ as the radioligand for both the DA and NE release assays. Rat caudate (for DA release) or whole brain minus cerebellum and caudate (for NE and 5-HT release) was homogenized in ice-cold 10% sucrose containing 1 μM reserpine. Nomifensine (100 nM) and GBR12935 (100 nM) were added to the sucrose solution for [^{3}H]5-HT release experiments to block any potential [^{3}H]5-HT reuptake into NE and DA nerve terminals. For the DA release assay, 100 nM desipramine and 100 nM citalopram were added to block [^{3}H]MPP^+ uptake into NE and 5-HT nerves. For the NE release assay, 50 nM GBR12935 and 100 nM citalopram were added to block [^{3}H]MPP^+ uptake into DA and 5-HT nerves. After 12 strokes with a

Potter-Elvehjem homogenizer, homogenates were centrifuged at 1000 x g for 10 min at 0-4 ^{o}C and the supernatants were retained on ice (synaptosomal preparation).

Synaptosomal preparations were incubated to steady state with 5 nM [^{3}H]MPP^{+} (60 min) or 5 nM [^{3}H]5-HT (60 min) in Krebs-phosphate buffer [without bovine serum albumin (BSA); pH 7.4] which contained 154.4 mM NaCl, 2.9 mM KCl, 1.1 mM $CaCl_2$, 0.83 mM $MgCl_2$, 5 mM glucose, 1 mg/mL ascorbic acid, 50 µM pargyline plus 1 µM reserpine in a polypropylene beaker with stirring at 25^{o} C. with the appropriate blockers. After incubation to steady state, 850 µl of synaptosomes preloaded with [^{3}H]ligand were added to 12 x 75 mm polystyrene test tubes that contained 150 µl test drug in uptake buffer plus 1 mg/ml BSA. After 5 min ([^{3}H]5-HT) or 30 min (NE and DA assays) the release reaction was terminated by dilution with 4 ml wash buffer (10 mM Tris-HCl pH 7.4 containing 0.9% NaCl at 25 ^{o}C) followed by rapid vacuum filtration over Whatman GF/B filters using a Brandel Harvester. The filters were rinsed twice with 4 ml wash buffer using the Brandel Harvester, and the retained tritium was counted by a Taurus liquid scintillation counter at 40% efficiency after an overnight extraction in 3 ml Cytoscint (ICN Biomedical Inc., Costa Mesa, CA).

Data analysis and statistics

As previously described (Rothman et al., 1993), EC_{50} values for transporter assays were determined using the nonlinear least squares curve fitting program MLAB-PC (Civilized Software, Bethesda, MD). A correlation between *in vitro* measures and potency as a reinforcer under both FR and PR schedules or between *in vitro* measures and the maximum injections under both FR and PR schedules was evaluated using Pearson correlation coefficient (GraphPad, Prism 3.0 or Primer of Biostatistics). When the data violated the assumptions for parametric analysis (i.e., equal variance), Spearman correlation coefficient was used.

3. Drugs

Cocaine hydrochloride and amphetamine sulfate were provided by the National Institute on Drug Abuse (Rockville, MD). Amphetamine analogs PAL 314 (*m*-methylamphetamine), PAL 313 (*p*-methylamphetamine), PAL 353 (*m*-fluoroamphetamine), PAL 303 (*p*-fluoroamphetamine; Figure 1] were synthesized using methodology described by Monte et al. (1997). The appropriate aldehydes were converted to their nitroolefins using ammonium acetate and nitroethane. These crude nitroolefins were subsequently reduced with lithium aluminum hydride to obtain the desired compounds. Each compound was purified from the crude material as either a hydrochloride or fumarate salt. Purity was confirmed by combustion analysis and 1H NMR. For the self-administration study, drugs were dissolved in 0.9% saline. Doses were expressed as the salt forms of the drugs.

Results

Self-Administration under a fixed-ratio schedule

In test sessions, the baseline dose of cocaine (0.03 mg/kg/injection) maintained a mean of 54 injections/session (SEM = 10.3), while saline maintained a mean of 4 injections/session (SEM = 1.5; Figure 2). All PAL compounds maintained responding above saline levels at least at one dose in all monkeys and dose-response functions were biphasic. Monkey 88-14 was reliably more sensitive to all drugs, and consistently responded at higher rates, than the other monkeys. Based upon the dose that maintained maximum responding, the PAL compounds did not differ significantly in potency (Table 1; $F(3,9)=2.72$, $p > 0.1$). The mean maximum responding maintained by PAL 313 was significantly lower than that for PAL 314, PAL 303 and PAL 353 [Figure 2; $F(3,12)=6.13$, $p < 0.05$].

Self-Administration under a progressive-ratio schedule

The baseline dose of cocaine (0.1 or 0.3 mg/kg/injection) maintained a mean of 15.2 injections/session (SEM = 1.1), while saline maintained a mean of 1.6 injections/session (SEM = 0.2; Figure 3). *d*-Amphetamine, PAL 303, 314 and 353 functioned as positive reinforcers in all monkeys. PAL 313 functioned as a positive reinforcer in four of five monkeys (L500, M1389, AP78, RJu2). Monkey M341 initially showed high variability at two of four doses of PAL 313. When re-tested, this monkey did not self-administer PAL 313 at any dose. Dose-response functions increased with dose over low to moderate doses and were asymptotic or decreased again at higher doses. PAL 313, PAL 314, PAL 303 and PAL 353 did not differ significantly in potency, and *d*-amphetamine was about 6 to 12-fold more potent [Table 1; $F(4,16)=17.7$, $p < 0.0001$]. At maximum, *d*-amphetamine maintained more injections/session than the PAL compounds whereas PAL 313 maintained fewer injections/session than the other

compounds [Figure 3; F (3,12)=585, $p < 0.001$]. The maximum number of injections of PAL 314, PAL 303 and PAL 353 were indistinguishable. When drugs with biphasic dose-response functions, amphetamine, PAL 303 and PAL 353, were re-examined with a TO of 60 minutes, the shapes of the dose-response functions were unchanged, i.e., they were asymptotic or biphasic. Further, the maximum number of injections did not exceed those seen with the shorter TO for any drug.

In vitro monoamine release assay

d-Amphetamine and the PAL compounds released DA, NE and 5-HT in a concentration-dependent manner, and concentration-effect functions were parallel (data not shown). All the compounds showed comparable potencies releasing DA with the EC_{50} values between 8.0 and 51.5 nM (Table 2). Similarly, the EC_{50} values for the PAL compounds did not differ for NE release with the EC_{50} values between 7.2 and 28.0 nM (Table 2). As for 5-HT release, the order of the potencies was PAL 313 > PAL 314 > PAL 303 > PAL 353 > *d*-amphetamine. Thus, the DA/NE potency ratio ranged between 1.1 and 2.0, while the DA/5-HT potency ratio ranged between 0.004 and 0.83.

Relationship between self-administration and in vitro effects

For the FR schedule, there was no significant correlation between potency as a reinforcer (Table 1) and *in vitro* potency (Table 2) releasing DA ($r = 0.55$, d.f. = 3, $p = 0.34$) or 5-HT ($r=-0.84$, d.f.=3, $p = 0.08$). However, potency as a reinforcer was positively correlated with the ratio of the *in vitro* potencies releasing DA/5-HT ($r = 0.98$, d.f. = 3, $p = 0.004$). There was no correlation with the ratio of the *in vitro* potencies releasing DA/NE ($r = 0.78$, d.f. = 3, $p = 0.12$). Maximum responding under the FR schedule was not correlated with any measure of *in vitro* potency. For the PR schedule, there was also a positive correlation between the ratio of

the *in vitro* potencies releasing DA/5-HT and reinforcing potency ($r = 0.97$, d.f. = 4, $p = 0.02$). However, potency as a reinforcer was not correlated with any other measure of *in vitro* potency. Efficacy as a reinforcer under the PR schedule was not correlated with any measure of *in vitro* potency.

Discussion

As predicted based upon their nanomolar potencies as releasers of DA, all of the tested compounds functioned as positive reinforcers in all monkeys responding under a FR schedule of reinforcement. Dose-response functions under the FR schedule were biphasic, as is typically seen under these conditions (Young and Herling, 1986; Bergman et al., 1989). Although not tested in the present study, *d*-amphetamine has been found to function as a reinforcer under conditions similar to those used here (Woolverton et al., 2001). Additionally, all but one of the compounds, PAL 313, served as a positive reinforcer in all monkeys responding under a PR schedule. PAL 313 was a reinforcer in four of the five monkeys tested. The compounds did not differ in potency in either self-administration assay and had comparable potencies as DA releasers *in vitro*, results that are at least consistent with the conclusion that DA release is involved in their reinforcing effects. Although a similar argument could be made for NE, NE agonists have not previously been reported to function as positive reinforcers (Woolverton, 1987; Wee and Woolverton, 2004). The reinforcing effects of these compounds in the present experiment are consistent with full or partial amphetamine-like discriminative stimulus effects that have been reported previously for *p*-fluoroamphetamine (PAL 303), *p*-methylamphetamine (PAL 313) and *m*-methylamphetamine (Higgs and Glennon, 1990; Marona-Lewicka et al., 1995). Together these results suggest amphetamine type of abuse potential of the compounds.

The lack of a significant negative correlation between potency as a reinforcer under the FR schedule and potency for releasing 5-HT *in vitro* is inconsistent with the hypothesis proposed by Ritz and Kuhar (1989). It is worth noting, nevertheless, that the correlation for this relationship approached statistical significance ($p = 0.08$) and may have achieved statistical significance with additional degrees of freedom. Additionally, the most potent 5-HT releaser,

PAL 313, was clearly less potent than the least potent 5-HT releaser, *d*-amphetamine. As an alternative view, the correlation between the ratio of DA/5-HT releasing potency *in vitro* and potency as a reinforcer was significant, suggesting that the mix of dopaminergic and serotonergic actions is a determinant of potency as a reinforcer. Results were similar for the PR schedule in that potency as a 5-HT releaser was not correlated with potency as a reinforcer among the PAL compounds, though again the least potent 5-HT releaser, *d*-amphetamine, was the most potent reinforcer. As for the FR schedule, the ratio of DA/5-HT *in vitro* potencies predicted potency as a reinforcer under the PR.

The compounds differed in the maximum rate of self-administration under the FR schedule, to the extent that rate of responding was lower for PAL 313 than for the other compounds. PAL 313 was the most potent *in vitro* 5-HT releaser and had the highest *in vitro* DA/5-HT potency ratio. Although this result raises the possibility of a relationship between DA/5-HT activity and reinforcing efficacy, this conclusion cannot be drawn unambiguously. Rate of self-administration under a simple FR schedule is determined not only by the reinforcing effect of a drug but also by other effects of the drug that can affect rate. These have been termed "non-specific" effects and may include effects on motor, sensory or integrative function, satiety or punishing effects. Any or all of these might be influenced by an increase in 5-HT activity. The PR schedule, by increasing response requirement for successive injections, measures maximum behavioral output maintained by an injection. From this information an estimate of a drug's efficacy as a reinforcer, or its maximum reinforcing effect, can be inferred. The present PR, by arranging a TO after each injection, is designed to minimize the influence of non-specific effects on responding. The TO allows these effects to dissipate, at least partially, between injections. Results from the PR schedule are consistent with a conclusion that the

reinforcing efficacy of each of the PAL compounds was lower than that of *d*-amphetamine. Moreover, the reinforcing efficacy of PAL 313 was lower than that of the other compounds. This efficacy relationship was directly related to 5-HT potency, with *d*-amphetamine the least potent and PAL 313 the most potent 5-HT releaser. The implication is that increased potency as a 5-HT releaser is associated with diminished reinforcing efficacy.

Overall, results from both schedules of reinforcement lend support to the hypothesis that potency as a 5-HT releaser contributes negatively to potency as a reinforcer, but with the refinement that the DA/5-HT potency ratio may be a better predictor than 5-HT potency alone. The observation that the compound with the highest 5-HT releasing potency (PAL 313) was the least efficacious reinforcer, while the compound with the lowest 5-HT relasing potency (*d*-amphetamine) was the most efficacious reinforcer, supports the conclusion of Roberts et al. (1999) for DA uptake blockers. The observation that the compound (PAL 313) with the highest 5-HT potency had the lowest potency and efficacy as a reinforcer, while the other PAL compounds did not apparently differ on these measures, raises the possibility that there may be a threshold of 5-HT activity for these effects to be evident. Additional research would be required to determine whether this threshold is for absolute 5-HT potency or 5-HT potency relative to DA potency.

There are at least two possible accounts of the reduced reinforcing efficacy of compounds with increased 5-HT/DA potency. It is possible that increased 5-HT release opposes the activation of DA systems that are involved in the reinforcing effect. Such an effect would not be apparent in the *in vitro* results, but could be measured *in vivo*. Indeed, in an experiment using *in vivo* microdialysis in rats (Clark et al., 2004) PAL 313 showed diminished efficacy for increasing extracellular DA relative to the other compounds, even with comparable

DA releasing potency *in vitro*. In research with monoamine uptake blockers, Howell and colleagues have published data consistent with an opposing effect of 5-HT activity on DA activity. More specifically, cocaine-induced increases in extracellular DA concentrations, measured *in vivo,* were diminished after pretreatment with the selective 5-HT reuptake blocker alaproclate in monkeys (Czoty et al., 2002). Alaproclate also decreased the metabolic effects of cocaine in monkeys, as measured by PET (Howell et al., 2002). The present results are consistent with the conclusion that 5-HT actions oppose DA actions involved in reinforcing effects.

The possibility should also be considered that 5-HT activity could act to decrease self-administration of a DA agonist via a punishment mechanism. It has been demonstrated that i.v. drug injections can punish ongoing behavior maintained by food delivery (Katz and Goldberg, 1986; Woolverton, 2003). Delivery of response contingent electric shock can also decrease self-administration of cocaine (Bergman and Johanson, 1981). In preliminary experiments (Woolverton, unpublished) we have found that the addition of histamine to an injection of cocaine can act to punish cocaine self-administration. Considering the clinical reports of unpleasant side effects of drugs that increase 5-HT neurotransmission (Finfgeld, 2004; Birmes et al., 2003; Sternbach, 1991), it is conceivable that the addition of 5-HT actions could suppress self-administration of a DA agonist.

It is interesting to note that PAL 313 was approximately equipotent in releasing all three monoamines. In this, it parallels cocaine, which is equipotent as a blocker of uptake of DA, NE and 5-HT *in vitro*. Although cocaine dose-response functions were not determined in the present experiment, cocaine has been repeatedly tested under these conditions (e.g., Wilcox et al., 2000; Woolverton and Wang, 2004) and is clearly a more efficacious reinforcer than PAL

313. The reason(s) for this difference are unclear, although it does indicate the relationship between monoamine activity and reinforcing efficacy is not simple. This conclusion is underscored by the reports, noted previously, of self-administration of HD-60 by monkeys (Lile et al., 2003) but not by rats (Roberts et al., 1999). HD-60 is a high potency 5-HT uptake blocker relative to its potency as a DA uptake blocker. It has been reported that monoamine releasers can produce a robust increase in the extracellular monoamines *in vitro* whereas reuptake transporter inhibitors of similar potencies were less effective (Rothman and Bauman, 2002). Monoamine transporter inhibitors depend on the basal firing rates of neurons to increase extracellular monoamines and, at the same time, appear to stimulate the negative feedback inhibition, actions that may contribute to a relatively smaller increase in the neurotransmitters. This difference may contribute to the greater influence of serotonergic activity on the self-administration of PAL 313 than on the self-administration of cocaine.

Acknowledgements We gratefully acknowledge the expert advice of Dr. Warren May on the statistical analysis and the technical assistance of Hamilton McGee, Stephanie Dyson, and Joy Flenner.

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Footnotes

This study was supported by National Institute on Drug Abuse grants DA-10352 (to W.L.W) and DA-12970 (B.E.B.). W.L.W. is the recipient of NIDA grant K05-DA15343. This work was previously presented at the 2004 meeting of the College on Problems of Drug Dependence, San Juan, PR.

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Figure legends

Figure 1. Chemical structures of amphetamine and the substituted amphetamines that were tested.

Figure 2. Self-administration of PAL compounds under a FR 25 schedule of reinforcement. Drugs were available for self-administration for two hours/day. Each data point represents the mean injections/session of each dose for four rhesus monkeys and vertical error bars represent the S.E.M. values. The point above Sal or Coc represents self-administration of saline or the baseline dose of cocaine in test sessions, respectively. Data were normalized as to dose to adjust for individual differences in sensitivity. Max: the dose that maintained maximum injections in each animal; Max-1: half-log dose lower than Max; Max+1: half-log dose higher than Max.. *$p < 0.05$ compared with PAL 314, PAL 303 or PAL 353. Inset: dose-response functions of PAL compounds. The drugs were tested in a different dose range in one monkey because of different sensitivity to the drugs. Thus, data points are the mean of three or four monkeys. When the data point was from less than three monkeys, the number of subjects is indicated in parenthesis.

Figure 3. Self-administration of *d*-amphetamine and PAL compounds under a progressive-ratio schedule of reinforcement. Each data point represents the mean injections/session for four (*d*-amphetamine) or five monkeys (all PAL compounds) and vertical error bars represent the S.E.M. values. The point above Sal or Coc represents self-administration of saline in test sessions or cocaine during baseline sessions, respectively. Data were normalized as to dose to adjust for individual differences in sensitivity. Max: the dose of the maximum injections in each animal.

Max-1: half-log dose lower than Max. Max-2: full-log dose lower than Max. *** $p < 0.001$ compared with each of the other compounds. Inset: dose-response function of amphetamine and PAL compounds. The drugs were tested in different dose ranges in monkeys because of different sensitivities to the drugs. Thus, data points are the mean of four or five monkeys. When the data point was pooled from less than four monkeys, the number of subjects is indicated in parenthesis.

JPET Fast Forward. Published on January 26, 2005 as DOI: 10.1124/jpet.104.080101
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Table 1. Potencies and maximum responding of PAL compounds in self-administration.

Schedule		PAL 313	PAL 314	PAL 303	PAL 353	d-amphetamine
FR	potency	0.5 ± 0.22 (4)	0.23 ± 0.09 (4)	0.13 ± 0.03 (4)	0.09 ± 0.02 (4)	0.05 ± 0.03[#] (4)
	max. injection	16.8 ± 3.0 (4)	43.8 ± 12.2 (4)	56.1 ± 13.9 (4)	56.1 ± 17.7 (4)	37.3 ± 9.2[#] (4)
PR	potency	0.48 (-0.9±0.18) (4)	0.46 (-0.9±0.13) (5)	0.26 (-1.3±0.14) (5)	0.26 (-1.2±0.18) (5)	0.04[***] (-1.8 ± 0.11) (4)
	max. injection	8.9 ± 1.7 (5)	11.6 ± 1.2 (5)	12.6 ± 1.3 (5)	11.9 ± 1.1 (5)	13.8 ± 0.6 (5)

Data are mean ± SEM and, in parenthesis, the number of animals tested. For the FR schedule, the mean dose that maintained responding at the peak of the biphasic curve was calculated for potency. For the PR schedule, an ED_{50} dose of a dose-response function was obtained in individual monkey and was averaged across the monkeys for the mean. Potency is expressed as μmol/kg/injection. For the PR potency, log mean ED_{50} (mg/kg/injection) ± SEM was added in parenthesis to indicate variation. The maximum injection indicates the maximum injections per session. [***] $p < 0.0001$ compared with each of the PAL compounds. [#] Data from Woolverton et al., 2001

Table 2. *In vitro* potency as releasers of monoamine neurotransmitters.

	EC_{50} (nM)				
	[^{3}H] DA	[^{3}H] NE	[^{3}H] 5-HT	DA/NE	DA/5-HT
d-Amphetamine	8.0 ± 0.43	7.2 ± 0.44	1756 ± 94[#]	1.1	0.004
PAL 353	24.2 ± 1.1	16.1 ± 1.7	1937 ± 202	1.5	0.01
PAL 303	51.5 ± 1.7	28.0 ± 1.8	939 ± 76	1.8	0.05
PAL 314	33.3 ± 1.3	18.3 ± 1.4	218 ± 22	1.8	0.15
PAL 313	44.1 ± 2.6	22.2 ± 1.3	53.4 ± 4.1	2.0	0.83

Values are mean ± SD for three experiments.

[#] The value for 5-HT release was previously published (Rothman et al., 2001).

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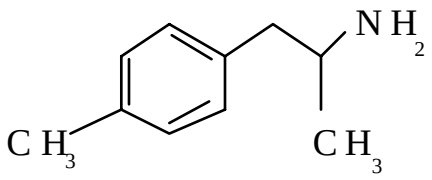

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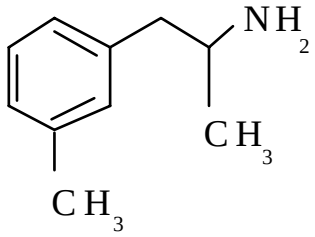

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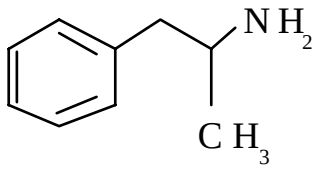

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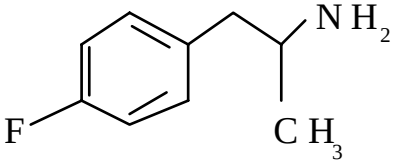

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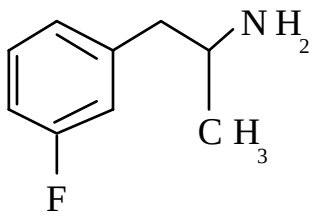

m-fluoroamphetamine (PAL 353)

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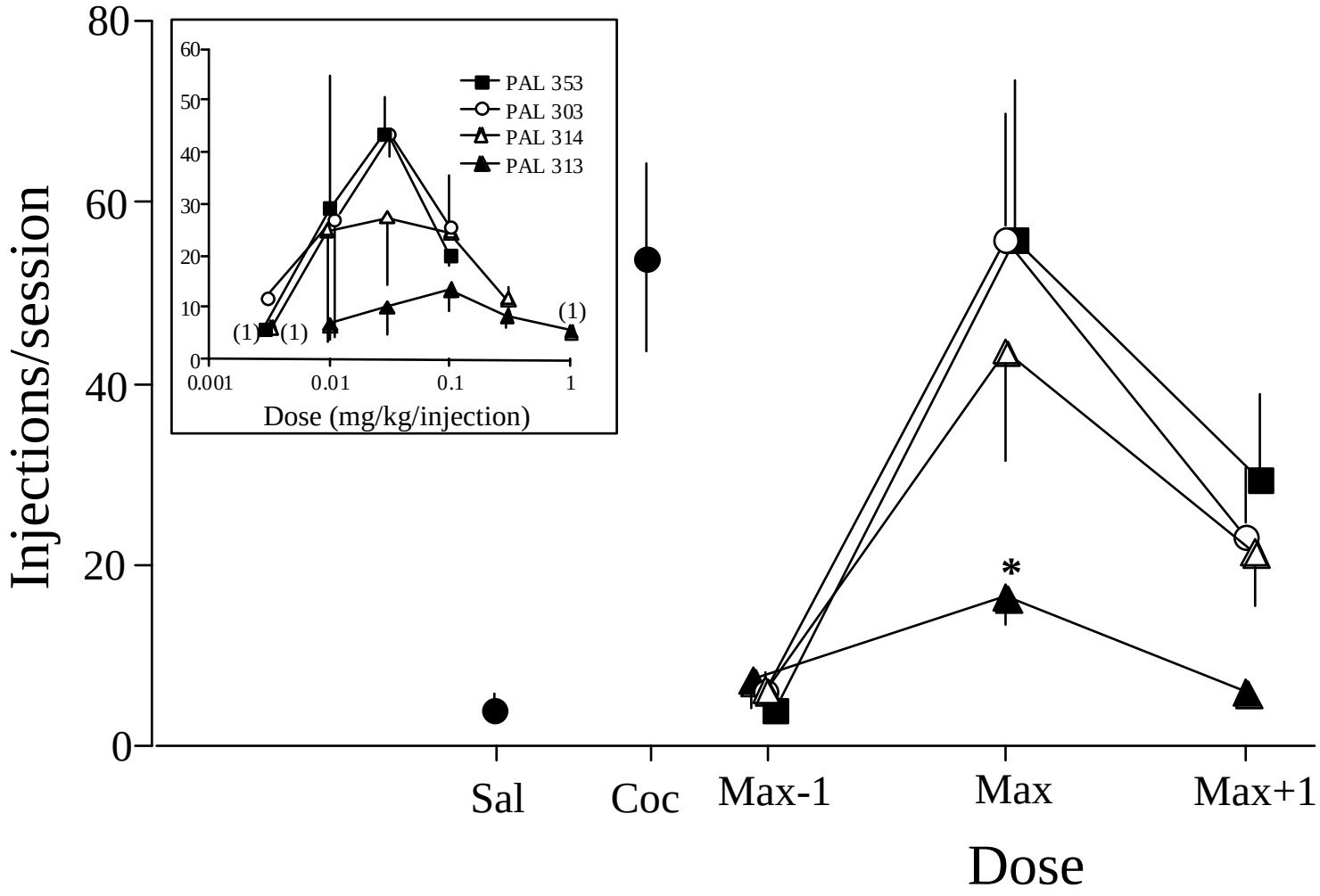

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