ELSEVIER

European Journal of Pharmacology 358 (1998) 101–109

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The dopamine D_3 receptor antagonist PNU-99194A fails to block (+)-7-OH-DPAT substitution for D-amphetamine or cocaine

Lisa E. Baker [a,*], Kjell A. Svensson [b], Kelly J. Garner [a], Amy K. Goodwin [a]

[a] *Department of Psychology, Western Michigan University, Kalamazoo, MI 49008, USA*
[b] *Pharmacia & Upjohn, Kalamazoo, MI 49002, USA*

Received 23 April 1998; revised 31 July 1998; accepted 5 August 1998

Abstract

The present study examined the role of dopamine D_3 receptor actions in the stimulus generalization produced by (+)-7-OH-DPAT in rats trained to discriminate either D-amphetamine or cocaine from saline. Twelve male Sprague–Dawley rats were trained to discriminate D-amphetamine (1.0 mg/kg) and 12 rats were trained to discriminate cocaine (5.0 mg/kg) from saline in a two-choice, water-reinforced operant procedure. Stimulus generalization tests were administered with the D_3 receptor-preferring agonist, (+)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin ((+)-7-OH-DPAT, 0.01–1.0 mg/kg) as well as the D_3-preferring antagonist, 5,6-di-methoxy-2-(dipropylamino)indan-hydrochloride (PNU-99194A, 5–40 mg/kg). PNU-99194A (10–40 mg/kg) was also administered in combination with the training dose of D-amphetamine or cocaine to test for antagonism of each training drug cue. Finally, to assess the role of D_3 receptor actions in the stimulus generalization produced by (+)-7-OH-DPAT (0.1 mg/kg), PNU-99194A (10, 20 mg/kg) was tested in combination with this compound in each training group. The results showed complete stimulus generalization with (+)-7-OH-DPAT in rats trained to discriminate D-amphetamine, although only partial stimulus generalization was observed with this compound in rats trained to discriminate cocaine. PNU-99194A produced partial substitution for both training drugs, and failed to block the discriminative stimulus effects of either D-amphetamine or cocaine. Moreover, this compound failed to block the stimulus generalization produced by (+)-7-OH-DPAT in rats trained to discriminate D-amphetamine. These results question the importance of D_3 receptor actions in the discriminative stimulus effects of psychostimulants and their similarities to (+)-7-OH-DPAT.

Keywords: Dopamine D_3 receptor; (+)-7-OH-DPAT; PNU-99194A; Cocaine; D-Amphetamine; Drug discrimination

1. Introduction

Recent advances in molecular biology have modified the classification of dopamine receptors to include two subfamilies of G-protein coupled receptors: the D1 subfamily includes both D_1 and D_5 subtypes while the D2 subfamily consists of the D_{2S}, D_{2L}, D_3 and D_4 subtypes (Civelli et al., 1991; Sibley and Monsma, 1992; Missale et al., 1998). Research on the involvement of various dopamine receptors in the behavioral actions of psychostimulant drugs has recently targeted the D_3 receptor subtype. This subtype is highly concentrated in limbic regions of the brain (Sokoloff et al., 1990; Levesque et al., 1992), which are critical sites of action for the reinforcing properties of psychostimulant drugs. Interestingly, recent reports indicate that D_3 receptors appear to be increased in the brains of cocaine addicts (Staley and Mash, 1996; Mash, 1997; Segal et al., 1997). An understanding of the neuroadaptive changes that result from prolonged cocaine use may facilitate the development of pharmacological interventions to assist in the treatment of drug dependence. At the present time, little is known about the functions of D_3 receptors, largely because there are no highly selective D_3 receptor ligands generally available.

Despite the lack of highly selective ligands for the D_3 receptor, several compounds have been reported to bind with a moderate selectivity for D_3 over D_2 dopamine receptors. Behavioral investigations with these D_3 receptor-preferring ligands have led several researchers to suggest that D_3 receptors may modulate the reinforcing effects of cocaine. For example, D_3 receptor-preferring antagonists have been shown to reduce the breaking point in

* Corresponding author. Tel.: +1-616-387-4484; Fax: +1-616-387-4550; E-mail: lisa.baker@wmich.edu

0014-2999/98/$19.00
PII: S0014-2999(98)00582-2

animals maintained on a progressive ratio schedule of cocaine self-administration (Richardson et al., 1993; Smith et al., 1995; Roberts and Ranaldi, 1995), whereas D_3 receptor-preferring agonists appear to enhance the reinforcing efficacy of cocaine (Caine and Koob, 1995; Parsons et al., 1996). Of particular interest are recent findings that the D_3 receptor-preferring agonist, 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) maintains self-administration behavior in monkeys with a history of cocaine self-administration, but not in cocaine-naive monkeys (Nader and Mach, 1996). Neuroadaptive changes involving D_3 receptors may be implicated in these behavioral consequences of prolonged cocaine use. However, additional behavioral assays are required to further assess the involvement of D_3 receptor changes associated with cocaine administration. Such assays ought to involve behavioral measures that assess receptor specificity.

Drug discrimination procedures are frequently employed to assess the neurochemical mechanisms involved in the behavioral effects of drugs. These procedures have been used extensively to assess the importance of D_1 and D_2 receptor subtypes in the actions of the psychostimulants, cocaine and amphetamine (Colpaert et al., 1978a,b, 1979; Woolverton et al., 1987; Kleven et al., 1988, 1990; Barrett and Appel, 1989; Spealman et al., 1991; Callahan et al., 1991; Callahan and Cunningham, 1993). Both D_1 and D_2 receptor antagonists have been reported to block the discriminative stimulus effects of these drugs (Woolverton et al., 1987; Kleven et al., 1988; Baker et al., 1993). However, neither D_1 nor D_2 receptor agonists produce complete stimulus generalization to cocaine (Barrett and Appel, 1989; Kleven et al., 1990; Spealman et al., 1991; Witkin et al., 1991). Several recent reports describe the effects of D_3 receptor-preferring agonists or antagonists in animals trained to discriminate cocaine or D-amphetamine (Callahan et al., 1992; Clark et al., 1995; Acri et al., 1995; Spealman, 1996; Lamas et al., 1996; Baker et al., 1997). The D_3 receptor-preferring agonists (±)-7-OH-DPAT and (+)-PD-128907 were reported to substitute for cocaine (Acri et al., 1995) and (±)-7-OH-DPAT was found to substitute for D-amphetamine in rats (Bevins et al., 1997), although at doses that markedly suppressed response rate. Other reports indicate that these compounds produce only partial generalization in monkeys trained to discriminate cocaine (Spealman, 1996; Lamas et al., 1996). Furthermore, D_3 receptor-preferring antagonists ((+)-AJ76, (+)-UH232, (−)-DS121) have been reported to only partially attenuate the discrimination of cocaine and amphetamine (Callahan et al., 1992; Clark et al., 1995).

PNU-99194A (5,6-di-methoxy-2-(dipropylamino) indan-hydrochloride) is currently the most selective D_3 receptor antagonist generally available. The in vitro binding profile of this compound was described by Cannon et al. (1982). PNU-99194A binds with nanomolar potency to the D_3 receptor and displays a 20-fold lower potency at the D_2 receptor in vitro (Haadsma-Svensson and Svensson, 1998). This compound produces behavioral activation in rodents at doses that do not increase dopamine release in the striatum or nucleus accumbens (Waters et al., 1993). Using drug discrimination procedures, we recently reported that PNU-99194A does not block cocaine discrimination in rats (Baker et al., 1997). However, this compound does appear to produce partial substitution for 10 mg/kg cocaine in rats (Baker et al., 1997). One aim of the present study was to determine whether PNU-99194A would substitute more fully in rats trained to discriminate a lower dose of cocaine (5.0 mg/kg). For comparison, this compound was also examined for stimulus generalization and antagonism in rats trained to discriminate D-amphetamine (1.0 mg/kg). In addition, the D_3/D_2 receptor agonist (+)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin ((+)-7-OH-DPAT) was tested for stimulus generalization in these animals. Finally, PNU-99194A was administered in combination with (+)-7-OH-DPAT to examine the importance of D_3 receptor-mediated actions in the substitution of (+)-7-OH-DPAT for either D-amphetamine or cocaine.

2. Materials and methods

2.1. Animals and housing

The subjects were 24 male Sprague–Dawley rats (Harlan Breeding Laboratories, Indianapolis, IN, USA) aged approximately 6 months and weighing approximately 400 g at the beginning of the study. These animals had previous operant training in an undergraduate learning lab, and were drug naïve at the beginning of the present study. For the duration of the study, rats were housed individually in wire mesh cages, in a colony maintained on a 12-h light/dark cycle (0700 to 1900) and at a relatively constant temperature (20–22°C) and humidity (50–65%). Commercial rat feed was provided ad libitum, and water was restricted to amounts received during 20-min training sessions and an additional 15 min per day. In addition, free access to water was given for 24 h approximately every 7 days. The animals were maintained in accordance with the general principles of animal husbandry outlined by the National Institutes of Health and the experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Western Michigan University.

2.2. Apparatus

Training and testing sessions were conducted in eight standard operant chambers (ENV-001, MED Associates, St. Albans, VT, USA), housed in sound and light attenuating shells to provide ventilation and masking noise. Each chamber contained an overhead 28 V house light and a

liquid reinforcer delivery mechanism (0.1 ml) that was mounted equidistant between two levers on the front panel of the chamber. A Zenith 320-SX microcomputer programmed with MED-PC instrumentation and software (MED Associates, version 2.0) was used to control experimental events.

2.3. Drugs and injections

PNU-99194A hydrochloride and (+)-7-OH-DPAT were provided by Pharmacia & Upjohn, (Kalamazoo, MI, USA). Cocaine hydrochloride and D-amphetamine sulfate were provided by the National Institute on Drug Abuse (Rockville, MD, USA). PNU-99194A was dissolved in sterile distilled water and administered subcutaneously (s.c.). Cocaine, D-amphetamine, and (+)-7-OH-DPAT were dissolved in sterile saline (0.85%). Cocaine and D-amphetamine were administered intraperitoneally (i.p.) and (+)-7-OH-DPAT was administered s.c. All doses of each drug were calculated based on the salt.

2.4. Drug discrimination training procedures

Twelve rats were trained to discriminate cocaine (5.0 mg/kg, i.p.) from saline and 12 rats were trained to discriminate D-amphetamine (1.0 mg/kg, i.p.) from saline using a two-lever operant task under a fixed ratio 20 (FR 20) schedule of water reinforcement. Drug or saline injections were administered 15 min prior to the beginning of training sessions. Half the animals in each training group were reinforced with water (0.1 ml) for responding on the right lever after drug injections and on the left lever after saline injections. Conditions were reversed for the remaining animals. Levers were wiped with isopropyl alcohol before each session to reduce the influence of olfactory stimuli on lever pressing (Extance and Goudie, 1981). In addition, groups of animals were frequently run in different orders.

Training sessions lasted for 20 min and were conducted 6 days per week. Drug and saline injections were given in a pseudo-random order, with the limitation that no animal received more than two consecutive drug or saline conditions. Training under each condition began on a FR 1 schedule. Once responding was stable, the number of consecutive correct responses required for reinforcement was gradually increased until the final FR 20 was reached. The criterion for discrimination was set at a minimum of 80% correct lever responses before delivery of the first reinforcer for a minimum of nine out of 10 consecutive training sessions. Each subject was required to meet this criterion before testing began.

2.5. Testing procedures

Test sessions were conducted in a similar manner to training sessions with the exception that no reinforcers were delivered and the animal was removed from the chamber upon completion of 20 consecutive responses on either lever or when 20 min elapsed, whichever occurred first. For each drug tested, the order of doses was counterbalanced among subjects and half the animals received tests after drug maintenance sessions while the other half received tests after saline maintenance sessions. Subjects were administered at least two training sessions between test sessions and were required to maintain the 80% criterion under both training conditions before each test.

Stimulus generalization tests were conducted with several doses of cocaine (0, 1.25, 2.5, 5.0, 10.0 mg/kg) in those animals trained to discriminate cocaine from saline or several doses of D-amphetamine (0, 0.25, 0.50 and 1.0 mg/kg) in those animals trained to discriminate D-amphetamine from saline. Tests of stimulus generalization were also administered to both groups with the D_3 receptor-preferring agonist, (+)-7-OH-DPAT (0.01, 0.03, 0.10, 0.30, 1.0 mg/kg; 15 min) and the D_3 receptor-preferring antagonist, PNU-99194A (5.0, 10.0, 20.0, 40.0 mg/kg; 15 min). In addition, PNU-99194A (10.0, 20.0, 40.0 mg/kg; 30 min) was administered in combination with the training dose of cocaine (15 min) or D-amphetamine (15 min) to test for antagonism of each training drug. To assess the role of D_3 receptor actions in the stimulus generalization produced by (+)-7-OH-DPAT, two doses of PNU-99194A (10.0, 20.0 mg/kg; 30 min) were also tested in combination with (+)-7-OH-DPAT (0.1 mg/kg; 15 min).

2.6. Data analysis

A *t*-test was used to compare the number of sessions to criterion for each training condition. Dose response data were presented as the percent of total responses made on the drug-appropriate lever during test sessions. Response rate was presented as the number of responses made (on either lever) per second during test sessions. For each dose tested, the mean and S.E.M. were calculated for each group of rats. For animals that did not complete at least 20 total responses during test sessions, the data indicating percentage of responses on the drug-lever were not included in the analyses, although the response rate data from these animals were included. Drug-appropriate responding that was 80% or greater was considered evidence for stimulus generalization. A particular dose of a test compound was considered to produce antagonism if drug-appropriate responding was less than 20%. Drug-appropriate responding between 20% and 80% was considered evidence for partial substitution or partial antagonism. For each compound tested, the effect of dose on percentage drug-appropriate responses and response rate were analyzed by a one-way analysis of variance. For drugs that produced stimulus generalization, the dose response curve was also analyzed using a nonlinear regression (sigmoidal dose response function equation) and ED_{50}s and confidence intervals were calculated. Statistical analyses were

conducted with the software GraphPad Prism (GraphPad, San Diego, CA, USA).

3. Results

3.1. Discrimination and dose response functions with training drugs

All subjects met the discrimination criterion stated above. The animals trained to discriminate 1.0 mg/kg D-amphetamine reached this criterion in significantly fewer training sessions than the animals trained to discriminate 5.0 mg/kg cocaine ($t_{22} = 3.28$, $P < 0.001$). The mean number of sessions to criterion for the D-amphetamine group was 38 (± 2.40) with a range of 32 to 59 and a median of 34 sessions. The mean number of sessions to criterion for the cocaine group was 65 (± 7.82), with a range of 36 to 116 and a median of 59 sessions.

The dose response curves for each training drug are illustrated in Fig. 1. There was a clear dose dependent increase in drug-lever responses with increasing doses of

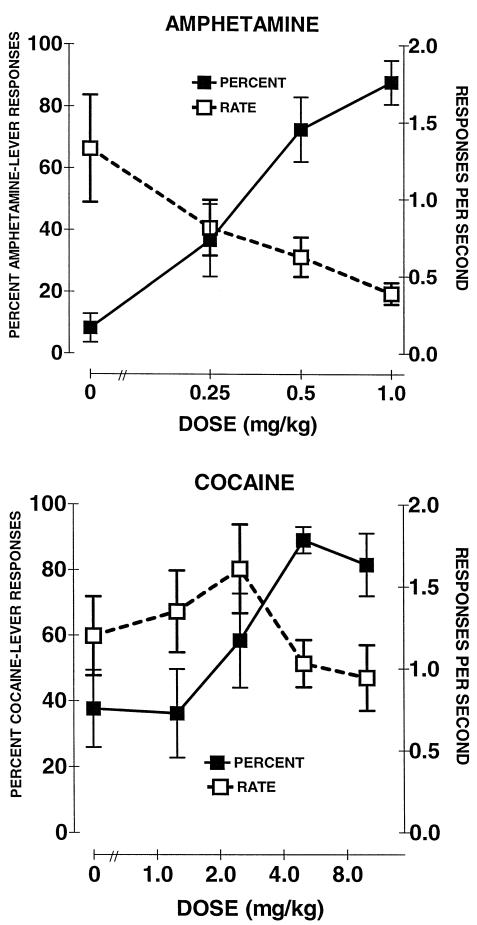

Fig. 1. D-Amphetamine (top) and cocaine (bottom) dose response curves. The percentage of drug-lever responses is indicated by the solid lines and response rate is indicated by the dashed lines. $n = 12$ at all doses.

D-amphetamine. The differences among the various doses in percent D-amphetamine-lever responses ($F_{3,47} = 15.06$, $P < 0.0001$) and response rate ($F_{3,47} = 3.40$, $P < 0.05$) were statistically significant. The ED_{50} for D-amphetamine was 0.30 mg/kg (95% confidence limits: 0.19–0.50 mg/kg). For the most part, there was also a dose dependent increase in drug-appropriate responding with increasing doses of cocaine. However, a 10 mg/kg dose of cocaine did not produce greater drug-appropriate responding than the training dose of 5 mg/kg. The difference among the various doses in percent cocaine-lever responses ($F_{3,46} = 4.63$, $P < 0.01$) was statistically significant. The effect of cocaine on response rate was not statistically significant. The ED_{50} for cocaine was 2.54 mg/kg (95% confidence intervals: 2.35–2.73 mg/kg).

3.2. Tests of generalization with PNU-99194A

Fig. 2 indicates that PNU-99194A produced only partial substitution for both training drugs. This compound produced an average of no more than 50% drug-appropriate responding in either group. Two of the animals trained to discriminate D-amphetamine generalized to PNU-99194A at both 10 and 20 mg/kg. One of these animals also generalized to the 40 mg/kg dose. The effects of this compound on percent D-amphetamine-lever responses ($F_{4,50} = 4.57$, $P < 0.005$) and response rate ($F_{4,54} = 4.89$, $P < 0.005$) were statistically significant.

The effect of PNU-99194A on percent cocaine-lever responses was not statistically significant. A total of six animals trained to discriminate cocaine exhibited stimulus generalization to only one dose of PNU-99194A. Two of these animals selected the drug lever after receiving the 2.5 mg/kg dose, one selected the drug lever following the 5.0 mg/kg dose, two following the 10 mg/kg dose and one following the 20 mg/kg dose. This compound produced significant reductions in response rate ($F_{4,58} = 3.11$, $P < 0.05$) and was completely disruptive in several rats at 40 mg/kg. Therefore, higher doses of PNU-99194A were not tested. Seven of the 12 cocaine-trained rats completed at least 20 responses following the administration of the 40 mg/kg dose. Six of these rats distributed their responses fairly equally between the two levers. Drug-appropriate responses of these six individual subjects ranged between 30 and 50%.

3.3. Tests of antagonism with PNU-99194A

PNU-99194A did not block the discriminative stimulus effects of either cocaine or D-amphetamine (see Fig. 2). The combination of this compound with either D-amphetamine ($F_{3,40} = 7.64$, $P < 0.0005$) or cocaine ($F_{3,44} = 13.93$, $P < 0.0001$) also significantly suppressed response rate. Less than half the animals that were tested completed 20 responses when the 20 or 40 mg/kg dose of

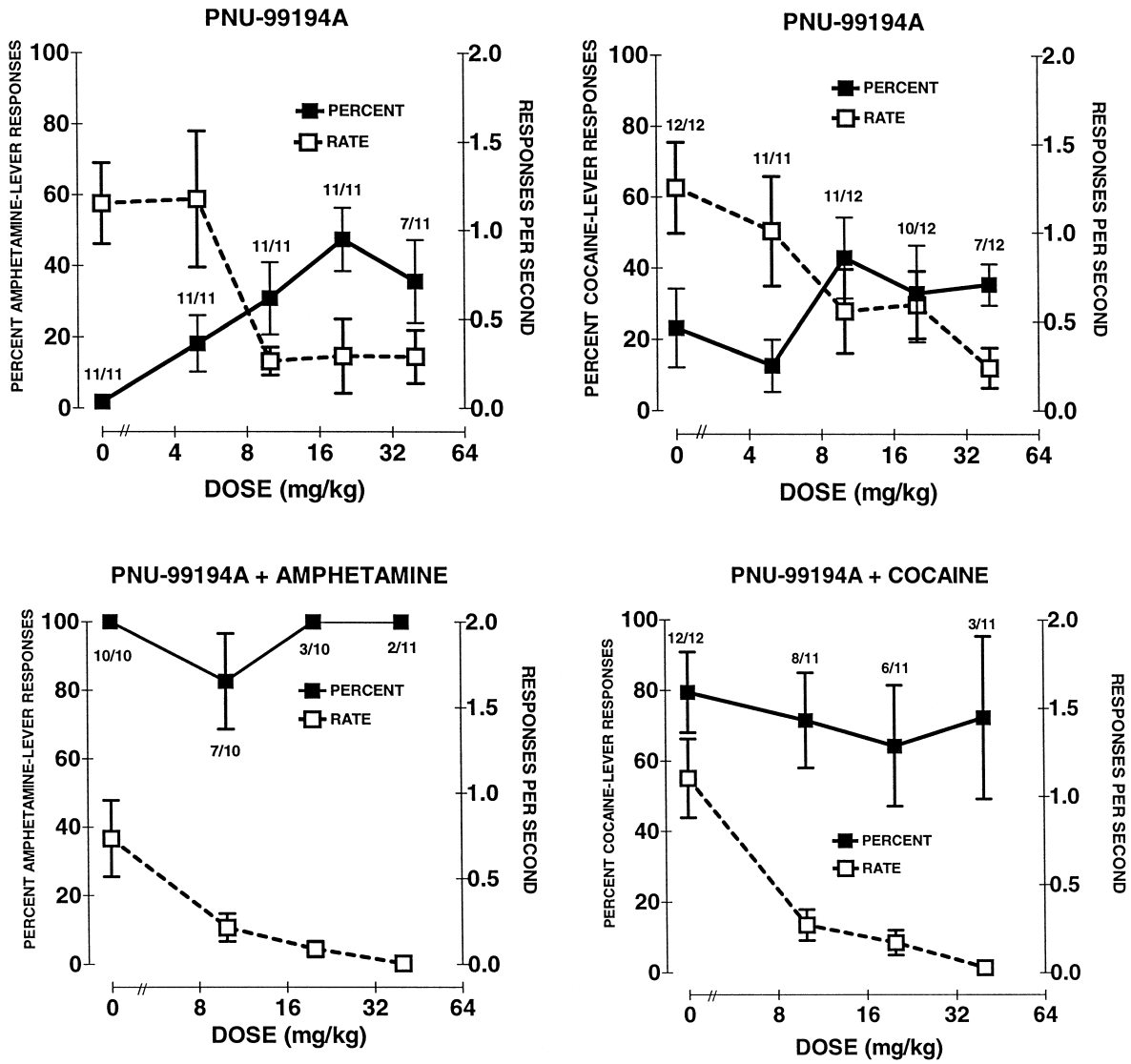

Fig. 2. Top: Results of stimulus generalization tests with PNU-99194A in rats trained to discriminate D-amphetamine 1.0 mg/kg (left) or cocaine 5.0 mg/kg (right). Bottom: Results of antagonist tests with PNU-99194A in rats trained to discriminate D-amphetamine (left) or cocaine (right). The percentage of drug-lever responses is indicated by the solid lines and response rate is indicated by the dashed lines. At each data point, n/N = number of animals that completed 20 responses/Number of animals that were tested.

PNU-99194A was given in combination with either training drug. The cocaine stimulus was attenuated in three out of eight animals that responded following 10 mg/kg PNU-99194A, two out of six animals that responded following 20 mg/kg, and 1 out of 3 animals that responded following 40 mg/kg. The D-amphetamine stimulus was attenuated by 10 mg/kg PNU-99194A in only one rat out of seven that responded following this dose.

3.4. Tests of generalization with (+)-7-OH-DPAT

Fig. 3 shows the effects of (+)-7-OH-DPAT in rats trained to discriminate D-amphetamine or cocaine. This compound produced complete stimulus generalization in rats trained to discriminate D-amphetamine (ED_{50} = 0.02 mg/kg; 95% confidence intervals: 0.015–0.022 mg/kg), but only partial stimulus generalization was observed in rats trained to discriminate 5.0 mg/kg cocaine. The effects of (+)-7-OH-DPAT on percent D-amphetamine-lever responses ($F_{5,38} = 104.7$, $P < 0.0001$) and response rate ($F_{5,47} = 14.14$, $P < 0.0001$) were statistically significant. Higher doses of this D_3/D_2 agonist severely suppressed responding, especially in cocaine-trained animals. Of the 11 cocaine-trained animals that were tested at each dose of this compound, only two completed 20 responses with 0.3 mg/kg (100%, 22%), and one completed 20 responses with the 1.0 mg/kg dose (74%). Thus, an ANOVA on percent cocaine-lever responses included only the 0, 0.03 and 0.1 mg/kg doses, which indicated a statistically sig-

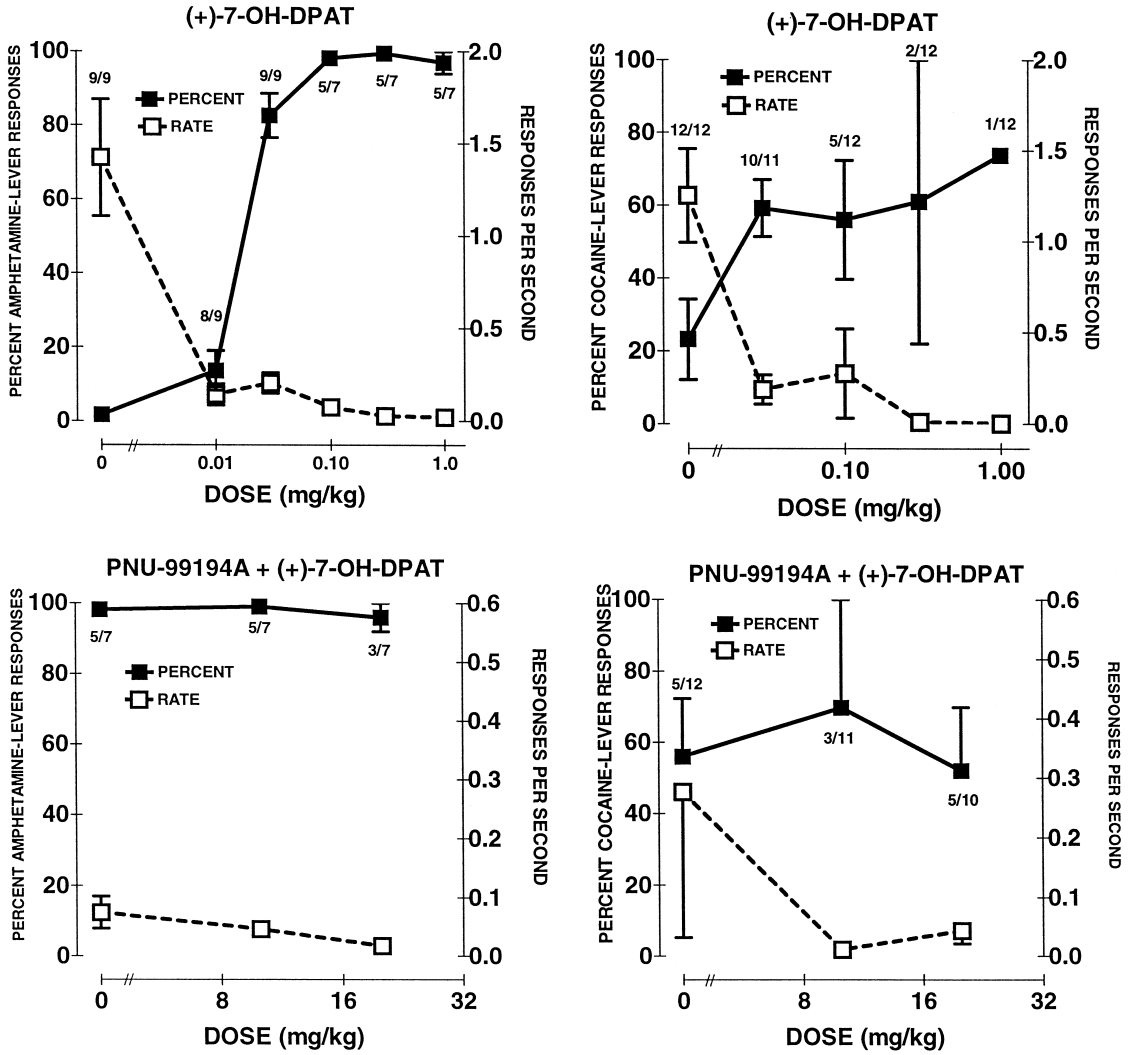

Fig. 3. Top: Results of stimulus generalization tests with (+) 7-OH-DPAT in rats trained to discriminate D-amphetamine 1.0 mg/kg (left) or cocaine 5.0 mg/kg (right). Bottom: Results of tests with PNU-99194A (0, 10, 20 mg/kg) administered in combination with (+)-7-OH-DPAT (0.1 mg/kg) in rats trained to discriminate D-amphetamine 1.0 mg/kg (left) or cocaine 5.0 mg/kg (right). The percentage of drug-lever responses is indicated by the solid lines and response rate is indicated by the dashed lines. At each data point, n/N = number of animals that completed 20 responses/Number of animals that were tested.

nificant effect of this compound ($F_{2,26} = 3.64$, $P < 0.05$). The effect of all doses on response rate were subject to an ANOVA, which was highly significant ($F_{4,58} = 10.11$, $P < 0.0001$).

3.5. Antagonist tests with PNU-99194A and (+)-7-OH-DPAT

PNU-99194A did not block the stimulus generalization produced by (+)-7-OH-DPAT (0.1 mg/kg) in rats trained to discriminate D-amphetamine (see Fig. 3). The combination of these compounds in rats trained to discriminate cocaine also did not produce less drug-appropriate responding than that observed with (+)-7-OH-DPAT alone. The results of ANOVA tests were nonsignificant for both training groups. Doses of PNU-99194A higher than 20 mg/kg were not assessed in combination with (+)-7-OH-DPAT because this drug combination severely suppressed response rate.

4. Discussion

The present results support previous findings (Baker et al., 1997) that the relatively selective dopamine D_3 receptor antagonist, PNU-99194A does not produce complete stimulus generalization in rats trained to discriminate cocaine and extends these findings to rats trained to discriminate D-amphetamine. These results also indicate that D_3 receptors do not appear to be critically involved in the discriminative stimulus effects of either cocaine or D-amphetamine, because PNU-99194A does not block the discrimination of these psychostimulants in rats.

This study also demonstrated that the D_3/D_2 receptor agonist, (+)-7-OH-DPAT substituted completely for D-amphetamine. These results are consistent with those of Bevins et al. (1997) but not with those of Varty and Higgins (1997) who reported that (±)-7-OH-DPAT failed to produce stimulus generalization in rats trained to discriminate D-amphetamine. It should be noted that Varty and Higgins (1997) used a 0.3 mg/kg training dose of D-amphetamine, whereas Bevins et al. (1997) and the present study used a 1.0 mg/kg training dose. A number of other methodological differences (rat strain, schedule of reinforcement, type of reinforcer) between the present study and that of Varty and Higgins (1997) could also account for the conflicting results. However, a comparison of the present findings with those of Acri et al. (1995) also indicate that training dose may be an important variable. The present study showed only partial stimulus generalization to 5.0 mg/kg cocaine, whereas Acri et al. (1995) reported complete substitution with (±)-7-OH-DPAT for 10 mg/kg cocaine. It should noted, however, that other researchers have reported only partial stimulus generalization with (±)-7-OH-DPAT in monkeys trained to discriminate cocaine (Spealman, 1996; Lamas et al., 1996). The data of Spealman (1996) do indicate that the discriminative stimulus effects of cocaine are enhanced in an additive manner by the D_3 receptor-preferring agonists, PD 128907, (±)-7-OH-DPAT and quinpirole. Clearly, the potential similarities between D_3 preferring agonists and psychostimulant drugs warrant further study. The importance of training dose should be examined in tests of stimulus generalization with 7-OH-DPAT and other compounds with similar receptor binding profiles.

The substitution of D_3 receptor-preferring agonists for cocaine in drug discrimination experiments have implicated D_3 receptor actions in the mediation of cocaine's discriminative stimulus effects (Acri et al., 1995; Spealman, 1996; Lamas et al., 1996). These reports are based primarily on investigations with D_3 receptor agonists or relatively nonselective antagonists. All of the D_3 receptor-preferring agonists and many of the D_3 receptor-preferring antagonists currently available also have a relatively high affinity for D_2 receptors. The D_3 receptor selectivity of (+)-7-OH-DPAT is largely dependent on the receptor binding assay employed. For example, in receptor binding experiments in which the antagonist ligand, [^{125}I]sulpiride was used, (+)-7-OH-DPAT was reported to be 38 to 98 fold more selective for the D_3 than the D_2 receptor (Levesque et al., 1992; Freedman et al., 1994; MacKenzie et al., 1994). However, in functional assays the D_3 to D_2 receptor selectivity of this compound is reported to be only 4 to 7 fold (Chio et al., 1994; Sautel et al., 1995).

The agonist PD128907 is reported to be display greater D_3 receptor selectivity than 7-OH-DPAT in binding experiments as well as in functional tests (DeMattos et al., 1993; Pugsley et al., 1995; Sautel et al., 1995). Recent investigations by Spealman (1996) examined the substitution of PD 128907 for cocaine in monkeys, then assessed three D_2 receptor antagonists (nemonapride, eticlopride, YM-43611) with differing affinities for D_3 receptors in combination with PD 128907. He reported that the order of potency with which these antagonists attenuated the substitution of PD 128907 for cocaine corresponded to the order of affinity of these compounds at cloned human D_3 receptors. Bevins et al. (1997) also assessed the effects of eticlopride on the stimulus generalization produced by (±)-7-OH-DPAT in rats trained to discriminate D-amphetamine and reported that (±)-7-OH-DPAT substitution was partially blocked by this D_2/D_3 receptor antagonist.

This brief review of the relevant literature suggests that there is sufficient evidence that D_2/D_3 receptor agonists are capable of mimicking the discriminative stimulus effects of cocaine (Acri et al., 1995; Spealman, 1996; Lamas et al., 1996) and D-amphetamine (Bevins et al., 1997; present results), and that stimulus generalization produced by these agonists are at least partially attenuated by D_2/D_3 receptor antagonists. However, none of the compounds that have been tested are completely selective for the D_3 receptor. More selective compounds are required to assess the importance of D_3 receptors in the psychostimulant-like effects of D_2/D_3 receptor agonists.

The most selective D_3 receptor antagonist currently available is PNU-99194A. This compound was observed to displace [^{3}H]spiperone from cloned rat D_3 receptors expressed in chinese hamster ovary (CHO) cells with a 20-fold higher potency than it displaced the dopamine agonist [^{3}H]PNU-86170 from cloned rat D_2 receptors expressed in CHO cells (Haadsma-Svensson and Svensson, 1998). Moreover, in various in vitro assays for intrinsic activity, PNU-99194A was found to be inactive (Haadsma-Svensson and Svensson, 1998).

In vivo assays of PNU-99194A include behavioral, neurochemical and electrophysiological investigations. This compound was reported to produce locomotor stimulation in rats that were habituated to their environment, and this effect was blocked by other dopamine antagonists (Waters et al., 1993; Clifford and Waddington, 1998). Additionally, PNU-99194A blocked the hypolocomotion induced by the D_3 receptor-preferring agonist, pramipexole (Waters, 1995). The behavioral activation produced by PNU-99194A appears to be mediated via postsynaptic dopamine D_3 receptors since local injections of this compound into the nucleus accumbens produced locomotor stimulation, whereas local injections into the ventral tegmental area did not increase locomotor activity (Kling-Petersen et al., 1995a). Other D_3 receptor-preferring antagonists which also produce behavioral activation (e.g., (+)-AJ-76, (+)-UH-232, (−)-DS-121) have been reported to facilitate dopamine synthesis and release (Svensson et al., 1986, 1993). However, PNU-99194A increases locomotor activity in habituated rats at doses that do not significantly increase dopamine release in the striatum or nucleus accumbens (Waters et al., 1993). Thus, the partial substitu-

tion of this compound for the discriminative effects of psychostimulant drugs is not likely mediated by dopamine release.

The present results clearly demonstrate that the stimulus generalization observed with (+)-7-OH-DPAT in rats trained to discriminate D-amphetamine was not attenuated by PNU-99194A. Additionally, in animals trained to discriminate cocaine, the combination of PNU-99194A and (+)-7-OH-DPAT did not produce less drug-lever responding than (+)-7-OH-DPAT alone. These results question the importance of D_3 receptor actions in the similarities between (+)-7-OH-DPAT and psychostimulants. However, more extensive testing of multiple dose combinations of PNU-99194A and (+)-7-OH-DPAT as well as tests with PNU-99194A in combination with more selective D_3 receptor agonists (e.g., PD 128907) are required to confirm these conclusions. More selective D_3 receptor antagonists will also be examined as they become available.

Several investigators have commented on the potential therapeutic uses of 7-OH-DPAT and other putative D_3 receptor-preferring agonists in the treatment of drug dependence (Acri et al., 1995; Caine and Koob, 1995; Bevins et al., 1997). The potential use of selective dopamine D_3 receptor antagonists in the rehabilitation of drug addicts is also intriguing. Although PNU-99194A has been shown to produce locomotor stimulation and conditioned place preference in rats (Waters et al., 1993; Kling-Petersen et al., 1995b), this compound fails to facilitate intracranial self-stimulation (Kling-Petersen et al., 1995b) and it only partially substitutes for D-amphetamine or cocaine in drug discrimination tests (Baker et al., 1997; present study). This suggests that PNU-99194A by itself lacks strong positive reinforcing properties and thereby abuse liability. On the other hand, its weak behavioral stimulant properties in combination with partial cocaine substitution may prevent anhedonia and relapse to cocaine use.

Acknowledgements

This work was funded by a grant from the National Institute on Drug Abuse (1RO3DA11291-1). The authors wish to thank William H. Darlington, Susane R. Haadsma-Svensson, Chiu-Hong Lin and Arthur G. Romero of Pharmacia & Upjohn, for the synthesis of (+)-7-OH-DPAT and PNU-91994A.

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