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Antagonism of the discriminative stimulus effects of (+)-7-OH-DPAT by remoxipride but not PNU-99194A

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Abstract

The dopamine (DA) agonist 7-hydroxy-*N,N*-di-*n*-propyl-2-amino-tetralin (7-OH-DPAT) has been used extensively as a tool to investigate the role of DA D_3 receptors in the reinforcing and discriminative stimulus properties of psychostimulant drugs. The present study examined the relative importance of D_3 vs. D_2 receptor actions in the discriminative stimulus effects of (+)-7-OH-DPAT (0.03 mg/kg, sc) in 16 male Sprague–Dawley rats trained to discriminate this compound from saline in a two-lever, water-reinforced operant procedure under a FR 20 schedule. Stimulus generalization and antagonism tests were conducted with cocaine and with various selective D_2 and D_3 receptor ligands. In contrast to previous findings that (+)-7-OH-DPAT substitutes for cocaine, the present results demonstrated that cocaine does not produce stimulus generalization in animals trained to discriminate (+)-7-OH-DPAT. Although two D_3-preferring agonists, PD-128907 and pramipexole, produced complete stimulus generalization to the training drug, two highly selective D_3 antagonists (PNU-99194A, PD 152255) failed to block the discriminative stimulus effects of (+)-7-OH-DPAT. However, the D_2 antagonist remoxipride (3.0 mg/kg) produced a rightward shift in the (+)-7-OH-DPAT dose–response curve. These findings suggest that D_2 receptors are critically involved in mediating the cue properties of (+)-7-OH-DPAT. However, alternative interpretations that PNU-99194A is not entirely D_3 receptor selective should also be considered.

Keywords: (+)-7-OH-DPAT; PNU-99194A; Remoxipride; D3 receptors; D2 receptors; Drug discrimination; Rats

1. Introduction

Recent investigations of the behavioral pharmacology of addictive psychostimulant drugs have focused on the dopamine (DA) D_3 receptor (Bevins et al., 1997; Caine and Koob, 1995; Lamas et al., 1996; Spealman, 1996), a subtype of the D2 family of DA receptors (Civelli et al., 1991; Sibley and Monsma, 1992). This receptor subtype is localized in limbic brain regions (Levesque et al., 1992), areas well known to be involved in the reinforcing and discriminative stimulus properties of psychostimulants. Increased understanding of the role of D_3 receptors in mediating the behavioral effects of psychostimulant drugs may benefit the development of pharmacological treatment interventions for stimulant abuse, as well as the treatment of central nervous system (CNS) diseases involving the DA system.

Because of their reported selectivity for the DA D_3 receptor, compounds such as 7-hydroxy-*N,N*-di-*n*-propyl-2-amino-tetralin (7-OH-DPAT) and PD-128907 have been used to characterize the function of this receptor subtype (Damsma et al., 1993; Levesque et al., 1992; Pugsley et al., 1995). These compounds have been reported to have as much as a 100-fold greater affinity for D_3 over D_2 receptors based on in vitro binding assays (Burris et al., 1995; Levesque et al., 1992; Pugsley et al., 1995). However, many of these assays utilized a D_2 receptor antagonist rather than a D_2 agonist as the competitive ligand. Moreover, functional assays such as mitogenesis in Chinese hamster ovary cells indicate that the D_3 selectivity of these compounds is approximately five- to sevenfold (Sautel et al., 1995). The D_3 selectivity regarding the behavioral effects of these compounds remains speculative.

Several investigations have reported either complete or partial generalization to 7-OH-DPAT and (+)-PD-128907 in animals trained to discriminate cocaine (Acri et al., 1995; Lamas et al., 1996; Spealman, 1996) or D-amphetamine (Baker et al., 1998; Bevins et al., 1997). Based on these

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findings, some investigators have concluded that D_3 receptors may play an important role in the discriminative stimulus effects of cocaine and D-amphetamine. However, recent studies have demonstrated that the D_3-preferring antagonist, PNU-99194A fails to block the discriminative stimulus effects of these psychostimulants (Baker et al., 1997) or the stimulus generalization produced by 7-OH-DPAT in rats trained to discriminate D-amphetamine or cocaine (Baker et al., 1998; Garner and Baker, 1999). Preliminary investigations demonstrated that PNU-99194A only partially blocked the training dose of (+)-7-OH-DPAT, while greater antagonism was observed with the D_2 antagonist remoxipride (Baker et al., 1999). These results question the importance of D_3 receptors in the discriminative stimulus effects of (+)-7-OH-DPAT.

The present study further examined the antagonism of (+)-7-OH-DPAT with remoxipride, PNU-99194A and another D_3 antagonist, PD 152255, by testing their effects on the full dose–response function of (+)-7-OH-DPAT. Additionally, stimulus generalization was assessed with other putative D_3 agonists, (+)-PD-128907 and pramipexole, as well as with cocaine. Finally, PNU-99194A was also tested for stimulus generalization to (+)-7-OH-DPAT.

2. Method

2.1. Subjects

Sixteen male Sprague–Dawley rats (Harlan Breeding Laboratories, Indianapolis, IN) aged 50–60 days and weighing 250–300 g at the beginning of the study served as subjects. The animals had no previous operant training and were drug naive at the beginning of the present study. Animals were individually housed in plastic cages, in a colony maintained on a 12 h light/12 h dark cycle (lights on 07:00–19:00 hours) and at a relatively constant temperature (19–23°C) and humidity (50–60%). Commercial rat feed was freely available, and water was restricted to amounts received during 20-min training sessions and an additional 30 min/day. Free access to water was also 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 (IACUC) of Western Michigan University.

2.2. Apparatus

Training and testing sessions were conducted in eight standard operant chambers (ENV-001; MED Associates, Georgia, VT), housed in sound- and light-attenuating shells equipped with fans 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 removable levers on the front panel of the chamber. A center removable lever was located above the liquid delivery mechanism and used only during the initial autoshaping sessions. A Zenith 320-SX microcomputer programmed with MED-PC software (Version 2.0, Med Associates, Georgia, VT) was used to control experimental events and data collection.

2.3. Drugs

(+)-7-OH-DPAT-hydrobromide, pramipexole-hydrochloride, remoxipride-hydrochloride and PNU-99194A were generously provided by Pharmacia (Kalamazoo, MI). (+)-PD-128907-hydrochloride was purchased from Research Biochemicals International (Natick, MA). The National Institute on Drug Abuse (Bethesda, MD) provided cocaine-hydrochloride and Parke-Davis (Ann Arbor, MI) generously provided PD-152255. PNU-99194A was dissolved in sterile deionized water. PD 152255 was dissolved in a few drops of 0.1 N HCl and diluted with deionized water. All other drugs were dissolved in 0.9% bacteriostatic saline. All drugs were administered by subcutaneous (sc) injection, except cocaine-hydrochloride and PD-152255, which were administered by intraperitoneal (ip) injection. Injections were administered in a volume of 1 ml/kg and doses were calculated based on the salt form of each compound.

2.4. Autoshaping and errorless training procedures

Sixteen subjects were run in two squads of eight in 20-min training sessions 6 days a week (Monday–Saturday). Prior to discrimination training, lever presses were autoshaped on a single center lever under a schedule of continuous water reinforcement. No injections were given prior to these sessions. Once lever pressing was established in all animals, injections of either (+)-7-OH-DPAT (0.03 mg/kg) or saline were administered 15 min prior to errorless training sessions. Three errorless training sessions were conducted under each stimulus condition (SSDDSD) with only the stimulus-appropriate (drug or saline) lever present. Once the animals were exposed to both stimulus conditions under a FR 1 reinforcement schedule, the FR value was gradually incremented both within and across training sessions (see below).

2.5. Discrimination training procedures

Discrimination training began on the seventh 20-min training session, with both levers present. Injections of either (+)-7-OH-DPAT (0.03 mg/kg) or saline were administered subcutaneously 15 min prior to daily sessions. For half the animals, left-lever responses were reinforced following saline and right-lever responses were reinforced following drug; conditions were reversed for the remaining

animals. Injections were administered in a pseudorandom order and neither condition (drug or saline) prevailed for more than two consecutive sessions. To reduce the influence of olfactory stimuli on lever choice, levers were wiped with isopropyl alcohol between the two squads of eight rats, which were also frequently run in different orders (Extance and Goudie, 1981).

The first training session under each stimulus condition began on the same reinforcement schedule that was in effect during the previous errorless training session with that condition. The reinforcement schedule was gradually incremented until all animals were responding reliably on a FR 20 resetting schedule, in which 20 consecutive correct responses were required for reinforcement and errors reset the response counter. During initial discrimination training sessions, the FR value was gradually incremented both within and across training sessions. Within training sessions, the FR value was programmed to increment following the delivery of five reinforcers at each FR value. The starting FR value at the beginning of each session and the value of the FR increment were determined for each individual subject based on performance during the previous training session under each condition. Once animals were reliably responding on a FR 20 schedule, this schedule remained in effect for all subsequent training sessions. The criterion for discrimination was a minimum of 80% condition-appropriate lever selection prior to the delivery of the first reinforcer and for the total session for at least nine of 10 consecutive training sessions.

2.6. Testing procedures

Stimulus generalization and antagonism tests were given after the aforementioned criteria were met. 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 compound tested, the order of doses was counterbalanced across subjects, and approximately 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. A dose–response curve was determined with the training compound in all 16 subjects (0.003–0.10 mg/kg). Stimulus generalization tests were conducted with several doses of (+)-PD-128907 (0.01–0.30 mg/kg) cocaine (1.5–10.0 mg/kg), pramipexole (0.1–1.0 mg/kg) and PNU-99194A (10.0–40.0 mg/kg). Antagonism tests were also administered with remoxipride (3.0 and 10.0 mg/kg), PNU-99194A (10.0 and 20.0 mg/kg) and PD152255 (1.0 and 3.0 mg/kg) in combination with each of several doses of (+)-7-OH-DPAT (0.01–0.10 mg/kg). PNU-99194A (10.0 mg/kg) and remoxipride (3.0 and 10.0 mg/kg) were also tested in combination with each of several doses of pramipexole. Each compound was tested in a minimum of eight animals.

2.7. Data analysis

Dose–response data are presented as the percentage of total responses made on the drug-appropriate lever during test sessions. Response rate is presented as the number of responses on either lever, per second, during test sessions. For each dose tested, the mean and standard error of the mean were calculated for each of these dependent measures. In the event that an animal did not complete at least 10 total responses during a test session, the percentage of drug-appropriate responses for that test was not included in the statistical analyses. Drug-lever selection that was 80% or greater was considered evidence for stimulus generalization. Complete antagonism was defined as drug-appropriate responding less than 20%. Drug-appropriate responding

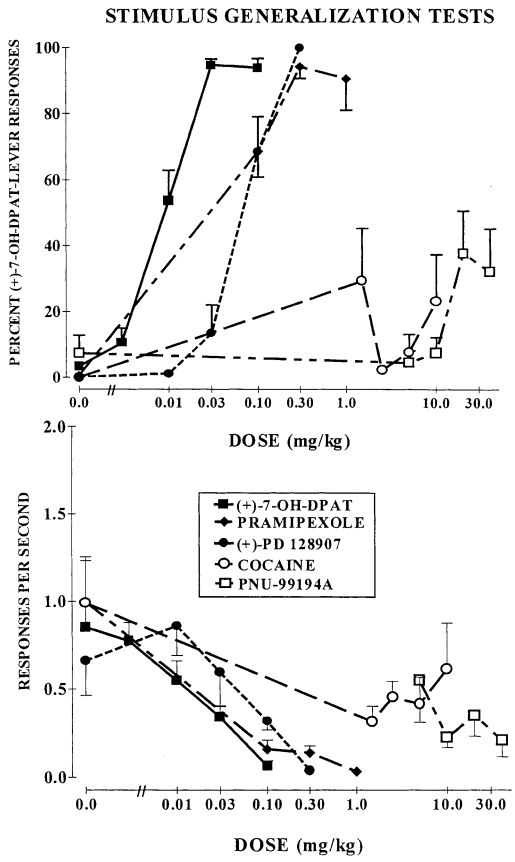

Fig. 1. Results of stimulus generalization tests with (+)-7-OH-DPAT ($n=16$), (+)-PD 128907 ($n=8$), pramipexole ($n=8$), cocaine ($n=8$) and PNU-99194A ($n=8$) in rats trained to discriminate (+)-7-OH-DPAT (0.03 mg/kg) from saline. The percentage of drug-lever responses is plotted in the top graph and response rate is plotted in the bottom graph (error bars indicate the S.E.M.).

between 20% and 80% was considered evidence for partial substitution or partial antagonism. Dose–response curves were also analyzed using a nonlinear regression, and ED_{50}'s and confidence intervals were calculated. Results of stimulus generalization tests were also analyzed by a one-way analyses of variance (ANOVAs). Two-way ANOVAs (antagonist dose, agonist dose) were conducted on the results of antagonism tests. Statistical analyses were conducted using the software GraphPad Prism (Version 2.0, GraphPad, San Diego, CA).

3. Results

(+)-7-OH-DPAT readily produced discriminative stimulus control in all 16 subjects. The mean number of sessions to criterion was 34 ± 11.75 (Range = 22–63; Median = 28). Results of stimulus generalization tests with (+)-7-OH-DPAT, (+)-PD-128907, pramipexole and cocaine are displayed in Fig. 1. (+)-7-OH-DPAT produced a dose-dependent increase in drug-appropriate responding with complete stimulus generalization at 0.03 and 0.10 mg/kg

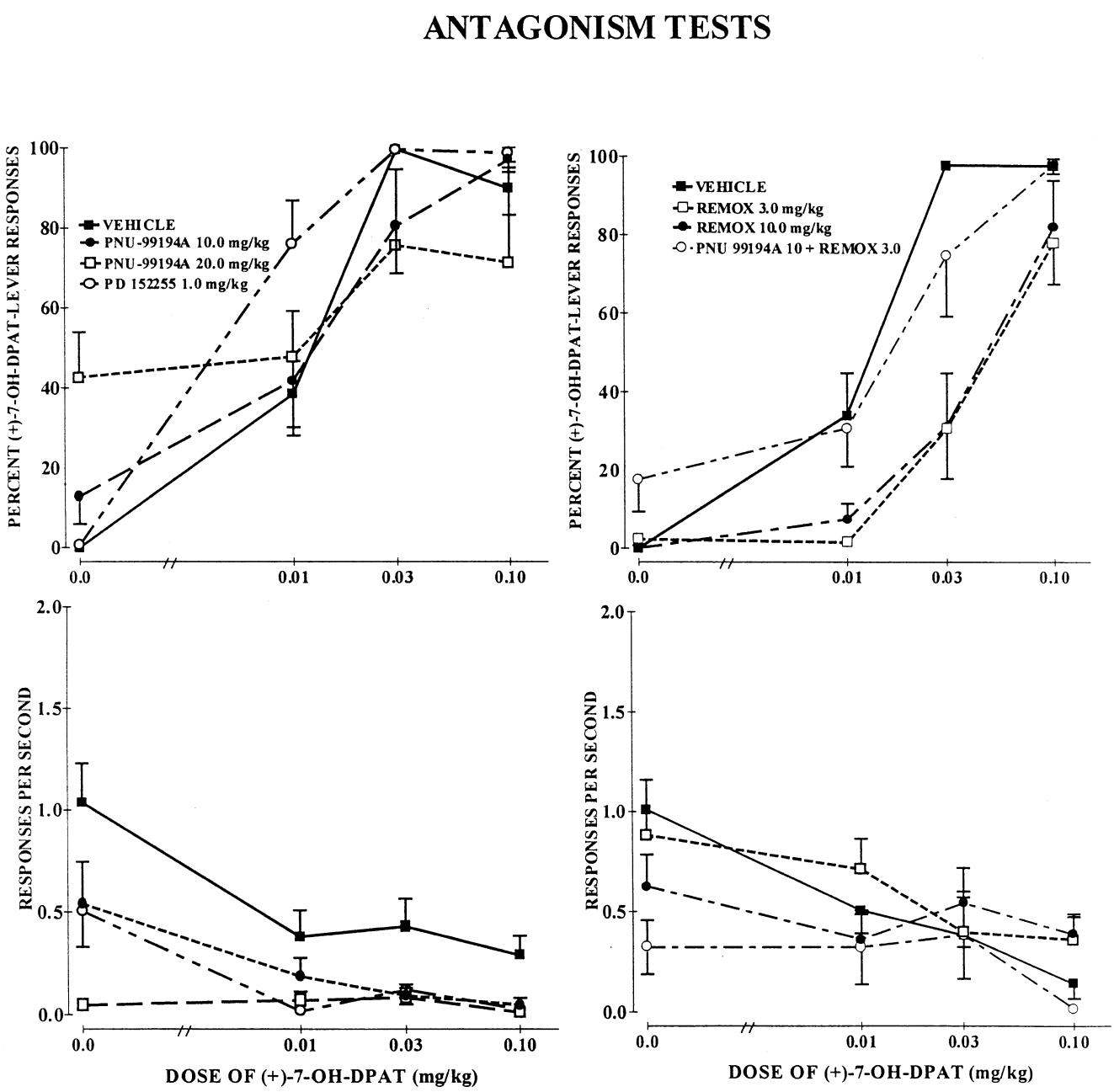

Fig. 2. Results of antagonism tests with PNU-99194A ($n = 8$), PD 152255 ($n = 8$), remoxipride ($n = 8$) and PNU-99194A + remoxipride ($n = 8$) administered in combination with (+)-7-OH-DPAT in animals trained to discriminate (+)-7-OH-DPAT (0.03 mg/kg) from saline. The percentage of drug-lever responses is plotted in the top graphs and response rate is plotted in the bottom graphs (error bars indicate the S.E.M.).

(ED_{50} = 0.01 mg/kg; 95% confidence intervals: 0.008–0.012 mg/kg). The percentage of drug-lever responses was significantly different across doses ($F_{3,63}$ = 61.27, P < .0001). Response rate was also significantly reduced by the training drug ($F_{3,63}$ = 6.27, P < .005); the response rate following the 0.10 mg/kg dose was significantly different from the saline response rate (P < .01).

(+)-PD 128907 produced a significant increase in drug-appropriate responding ($F_{4,39}$ = 24.86, P < .0001) with full substitution following a dose of 0.30 mg/kg (ED_{50} = 0.08 mg/kg; 95% confidence interval = 0.04–0.14). This compound also significantly disrupted responding in a dose-dependent manner ($F_{4,39}$ = 6.002, P < .005) with a statistically significant difference between the 0.30 mg/kg dose and saline (P < .05). Pramipexole also produced dose-dependent increases in drug-appropriate responding, with complete substitution for (+)-7-OH-DPAT following the 0.30 and 1.0 mg/kg doses. Response rate was severely depressed at all doses of pramipexole ($F_{3,31}$ = 13.13, P < .0001). Cocaine produced only partial substitution for (+)-7-OH-DPAT and did not produce a statistically significant increase in drug-appropriate responding. Cocaine also did not significantly affect response rate.

The results of antagonism tests with the D_3 antagonists, PNU-99194A and PD 152255 and the D_2 antagonist, remoxipride are illustrated in Fig. 2. PNU-99194A (Fig. 2, upper left-hand panel) failed to block the discriminative stimulus effects of (+)-7-OH-DPAT. The combination of 10.0 mg/kg PNU-99194A with (+)-7-OH-DPAT did not alter the (+)-7-OH-DPAT dose–response function. The 20.0 mg/kg dose of PNU-99194A reduced drug-appropriate responding following 0.03 and 0.10 mg/kg (+)-7-OH-DPAT, but not significantly. The combination of PNU-99194A and (+)-7-OH-DPAT markedly suppressed response rate (Fig. 2, lower left-hand panel). The main effects of PNU-99194A dose ($F_{2,84}$ = 20.24, P < .0001) and 7-OH-DPAT dose ($F_{3,84}$ = 8.82, P < .0001) on response rate were statistically significant as was the interaction ($F_{6,84}$ = 2.58, P < .05).

PD 152255 (Fig. 2, upper left-hand panel) also failed to block (+)-7-OH-DPAT discrimination. The 1.0 mg/kg dose of PD 152255 actually appeared to potentiate the 0.01 mg/kg dose of (+)-7-OH-DPAT (Fig. 2). The 3.0 mg/kg dose of PD 152255 (Fig. 2, lower left-hand panel) completely disrupted responding in all rats tested, which precluded the testing of higher doses.

Remoxipride (Fig. 2, upper right-hand panel) produced a rightward shift in the (+)-7-OH-DPAT dose–response curve. The ED_{50} of (+)-7-OH-DPAT alone was 0.01 mg/kg (95% confidence intervals = 0.01–0.05 mg/kg). When 3.0 mg/kg remoxipride was administered in combination with (+)-7-OH-DPAT, the ED_{50} was 0.03 mg/kg (95% confidence intervals = 0.01–0.24 mg/kg). Remoxipride 10.0 mg/kg shifted the ED_{50} of (+)-7-OH-DPAT to 0.06 mg/kg (95% confidence intervals = 0.01–0.12 mg/kg). However, because the confidence intervals overlapped, these ED_{50}s were not significantly different.

In an effort to determine whether the combination of a D_3 and a D_2 antagonist would block (+)-7-OH-DPAT's stimulus effects more fully, PNU-99194A and remoxipride (Fig. 2, upper right-hand panel) were tested in combination with a range of (+)-7-OH-DPAT doses. As noted in Fig. 2, this combination actually produced less antagonism than remoxipride alone.

Fig. 3 illustrates the results of antagonism tests with PNU-99194A and remoxipride administered in combination with pramipexole. PNU-99194A failed to block the stimulus generalization produced by pramipexole. Remoxipride shifted the pramipexole dose–response curve to the right and significantly attenuated the response rate suppression produced by pramipexole ($F_{2,84}$ = 4.83, P < .05).

Because PNU-99194A (20.0 mg/kg) appeared to produce partial substitution when tested in combination with vehicle, PNU-99194A (5.0–40.0 mg/kg) was subsequently tested for stimulus generalization. Again, partial substitution for the training drug was observed with PNU-99194A (see Fig. 1).

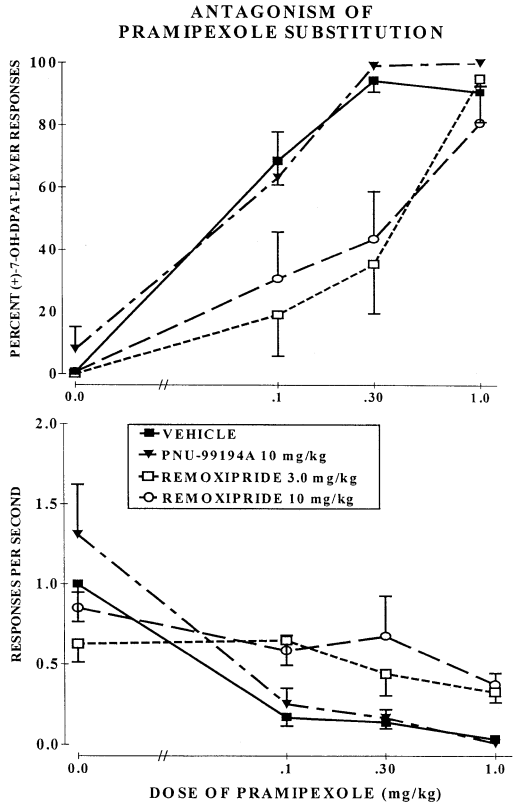

Fig. 3. Results of antagonism tests with PNU-99194A (n = 8) and remoxipride (n = 8) administered in combination with pramipexole in animals trained to discriminate (+)-7-OH-DPAT (0.03 mg/kg) from saline. The percentage of drug-lever responses is plotted in the top graph and response rate is plotted in the bottom graph (error bars indicate the S.E.M.).

4. Discussion

The results of the present study support previous findings that discriminative stimulus control is readily established in rats by 7-OH-DPAT (Depoortere et al., 2000; McElroy, 1994; Sanger and Depoortere, 1997; Varty and Higgins, 1997) or its (+)-isomer (Baker et al., 1999). Previous studies have indicated a potential modulatory role of the D_3 receptor subtype in the stimulus effects of 7-OH-DPAT (Bevins et al., 1997; Sanger and Depoortere, 1997; Varty and Higgins, 1997). Specifically, the potencies of cue substitution by several DA agonists were more strongly correlated with D_3 than D_2 in vitro potencies (Sanger and Depoortere, 1997). A study by Varty and Higgins (1997) supports the results of Sanger and Depoortere (1997) with evidence for D_3 receptor mediation of the discriminative stimulus effects of 7-OH-DPAT. Their data suggest that the pharmacological profile of the various dopaminergic compounds that substitute for the 7-OH-DPAT cue was more consistent with D_3 than D_2 receptor interactions. However, these conclusions are based on the use of agonists with only moderate selectivity for the D_3 receptor. Demonstrations that selective D_3 antagonists block the discriminative stimulus effects of 7-OH-DPAT are required to confirm these tentative conclusions.

Bevins et al. (1997) reported a rightward shift in the 7-OH-DPAT dose–response curve with eticlopride pretreatment (0.01, 0.05 mg/kg) in animals trained to discriminate D-amphetamine. However, because eticlopride is a D_2/D_3 antagonist, these results did not clearly distinguish the relative importance of D_3 vs. D_2 receptors in 7-OH-DPAT's substitution for D-amphetamine. Indeed, the putative D_3 antagonist, PNU-99194A did not block stimulus generalization produced by (+)-7-OH-DPAT or 7-OH-DPAT in rats trained to discriminate D-amphetamine or cocaine (Baker et al., 1998; Garner and Baker, 1999).

In animals trained to discriminate (+)-7-OH-DPAT, preliminary findings from our laboratory indicated that PNU-99194A only partially attenuated discrimination of the training dose (Baker et al., 1999). Moreover, a highly selective D_2 agonist, PNU-91356A, produced complete generalization to (+)-7-OH-DPAT (Baker et al., 1999). The present study expanded these preliminary findings by assessing the effects of PNU-99194A and another D_3 selective antagonist PD 152255 on a range of (+)-7-OH-DPAT doses (0.01–0.10 mg/kg). Neither of these compounds shifted the (+)-7-OH-DPAT dose–response curve to the right. In fact, PD 152255 (1.0 mg/kg) appeared to potentiate a low dose of (+)-7-OH-DPAT (0.01 mg/kg). It is also of interest to note that the combined administration of remoxipride (3.0 mg/kg) and PNU-99194A (10.0 mg/kg) actually produced less antagonism of (+)-7-OH-DPAT than did remoxipride alone. Perhaps this is due to some pharmacokinetic interaction between PNU-99194A and remoxipride that decreases the bioavailability of remoxipride. Alternatively, these findings could be interpreted as potentiation of (+)-7-OH-DPAT by PNU-99194A. Consistent with this notion is the observation that PNU-99194A actually produced partial substitution for (+)-7-OH-DPAT (see Fig. 1). In fact, while the present study was in progress, Depoortere et al. (2000) reported that PNU-99194A failed to block the discrimination of racemic 7-OH-DPAT and actually potentiated the discriminative cue produced by this compound. However, the present study did not show as great a potentiation by PNU-99194A as that demonstrated by Depoortere et al. (2000), which may be due to differences in the training drug or other methodological variables. Nonetheless, the present results are consistent with those of Depoortere et al. (2000) regarding the lack of 7-OH-DPAT antagonism observed with PNU-99194A.

There are several possible interpretations of these results. One interpretation is that D_3 receptor actions are not important in mediating the discriminative stimulus effects of 7-OH-DPAT, and D_2 receptor actions are more critically involved in these effects. This interpretation is supported by previous findings that the highly selective D_2 agonist PNU-91356 exhibited complete stimulus generalization to (+)-7-OH-DPAT (Baker et al., 1999) and by the present findings that the D_2 antagonist, remoxipride produced a rightward shift in the (+)-7-OH-DPAT dose–response curve. However, the ED_{50} confidence intervals (with and without remoxipride) overlapped and a higher dose of remoxipride did not further shift the dose–response curve. Thus, the discriminative stimulus effects may also not be solely mediated by D_2 receptors. In fact, two other moderately selective D_3 agonists, (+)-PD-128907 and pramipexole exhibited stimulus generalization in a similar manner to the D_2 agonist PNU-91356. It is also possible that the attenuation of (+)-7-OH-DPAT by remoxipride is not solely due to D_2 receptor antagonism. Although remoxipride is reported to have a much greater affinity for D_2 receptors than D_3 receptors, there are several metabolites of remoxipride that exhibit a relatively high affinity for D_3 receptors in the rat (Mohell et al., 1993). It is possible that one or more of these metabolites contributed to the antagonism of (+)-7-OH-DPAT's discriminative stimulus effects.

Another possible interpretation of the present data and those of Depoortere et al. (2000) is that PNU-99194A is not a selective D_3 antagonist, but is acting through some other mechanism. Results of recent investigations in our laboratory support this hypothesis. In rats trained to discriminate PNU-99194A, PD 152255 fails to produce generalization, whereas the atypical antipsychotic clozapine, and the anti-muscarinic compounds scopolamine and trihexyphenidyl do substitute for PNU-99194A (unpublished findings).

In summary, the present study attempted to delineate the relative importance of D_3 vs. D_2 receptor mediation of (+)-7-OH-DPAT's discriminative stimulus effects by comparing the effects of putative D_3 antagonists with that of a fairly selective D_2 antagonist on the (+)-7-OH-DPAT dose–response function. While the results suggest that the (+)-7-OH-DPAT cue is attenuated to a greater extent by D_2 receptor blockade, these results are still somewhat open to interpretation. However, it is at least clear that the discrimi-

nation of this compound is not based on a nonselective dopaminergic cue, because cocaine (present results) and D-amphetamine (Baker et al., 1999) both fail to substitute for (+)-7-OH-DPAT.

Finally, recent research using moderately selective D_3 receptor ligands have led to tentative conclusions regarding the functional importance of this receptor subtype. Clinical implications of this research are of considerable importance, as behavioral pharmacology research with D_3 receptor ligands may impact the development of more effective treatments for a variety of CNS diseases, such as Parkinson's Disease, schizophrenia and psychostimulant addiction. Results of recent studies using 7-OH-DPAT and similar compounds should be considered cautiously until more selective D_3 receptor ligands become readily available for investigation.

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