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Stimulus properties of 7-OH-DPAT versus auto- and postsynaptic receptor-specific doses of quinpirole

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Abstract

The five types of dopamine (DA) receptor subtypes have been grouped into two families, the D_1-like (D_1 and D_5 receptors) and D_2-like (D_2, D_3, and D_4 receptors). Experimental evidence indicates that D_2-like receptors can be located either presynaptically, where they modulate the synthesis and release of DA, or postsynaptically. Controversy exists, however, over the precise location and role of the D_3 subtype of DA receptor. To investigate this issue, rats were trained using standard operant drug discrimination procedures to discriminate 0.10 mg/kg of the putatively D_3 receptor-preferring agonist *R*(+)-7-hydroxy-*N*,*N*,-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) from saline. Patterns of generalization to D-amphetamine, AMPT, and SCH 23390 indicated a presynaptic action of 7-OH-DPAT, while apomorphine generalization patterns suggested a postsynaptic action; quinpirole generalization suggested both a pre- and postsynaptic action of 7-OH-DPAT. The ability of spiperone, eticlopride, SCH 23390, and UH 232 to partially antagonize the 7-OH-DPAT stimulus attests to its lack of receptor subtype specificity. These results suggest both pre- and postsynaptic actions of 7-OH-DPAT along with a lack of specificity of the various pharmacological compounds for the D_3 receptor.

Keywords: Dopamine; D3 receptor; Drug discrimination; Behavior; 7-OH-DPAT; Quinpirole

1. Introduction

To date, five distinct subtypes of dopamine (DA) receptors have been recognized and grouped into two major families, the D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4) receptors based on similarities in protein structure, pharmacological responsiveness, and coupling to second messenger pathways (Gingrich and Caron, 1993; Sibley et al., 1993). D_2-like receptors have been identified on both pre- and postsynaptic neurons. Stimulation of presynaptic D_2-like receptors, also referred to as autoreceptors, leads to a decrease in DA synthesis and release (Wolf and Roth, 1990), and pharmacological studies have found that low doses of the DA agonists quinpirole and *R*(−)-apomorphine hydrochloride (APO) decrease synaptic DA by preferentially stimulating D_2-like DA autoreceptors (Imperato et al., 1988; See et al., 1991; Skirboll et al., 1979). The gene that codes for the D_3 receptor subtype has been cloned and the expression of this receptor protein has been shown to be neuroanatomically restricted to limbic regions of the brain (Sokoloff et al., 1990). Since the protein is expressed in the substantia nigra and ventral tegmental area (Diaz et al., 1995), both of which contain DA cell bodies, it has been suggested that the D_3 receptor may act as an autoreceptor. However, while several studies have found that D_3 receptors are indeed capable of acting as autoreceptors to modulate the concentration of synaptic DA (Aretha et al., 1995; Gilbert et al., 1995; Meller et al., 1993), it has also been suggested that these receptors may act as both autoreceptors and postsynaptic receptors (Damsma et al., 1993; Elsworth and Roth, 1997; Spealman, 1996), or exclusively as postsynaptic receptors (Svensson et al., 1994; Waters et al., 1993).

Several pharmacological compounds have been developed which are reported to exhibit selectivity for the D_3 over the D_2 subtype, namely the agonists *R*(+)-7-hydroxy-*N*,*N*,-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) and PD128907, and the antagonists UH 232 and AJ 76. Interestingly, even before

Abbreviations: DA, dopamine; DAergic, dopaminergic; FR, fixed ratio; APO, apomorphine; AMPT, α-methyl-*para*-tyrosine; ANOVA, analysis of variance

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the discovery of the D_3 receptor, 7-OH-DPAT was used as a selective autoreceptor agonist as it was found to induce hypomotility and inhibit a γ-butyrolactone (GBL)-induced increase in DA (Feenstra et al., 1983), decrease tyrosine hydroxylase activity (El Mestikawy et al., 1986), and decrease DA overflow (Mulder et al., 1987). In vitro studies have suggested that 7-OH-DPAT binds selectively to the D_3 subtype of receptor (Freedman et al., 1994; Kreiss et al., 1995; Levant et al., 1995; Levesque et al., 1992), though caution is warranted because this apparent subtype specificity may be the result of in vitro assay conditions (Burris et al., 1995) and may not be as great in vivo.

In the present study, a group of rats was trained to discriminate the stimulus properties of the putatively D_3 receptor-preferring 7-OH-DPAT from saline and then subjected to various pharmacological manipulations designed to mimic pre- and postsynaptic DA receptor stimulation in order to investigate the hypothesis that the D_3 receptor is located presynaptically and acts as an autoreceptor to modulate the concentration of synaptic DA. For clarification, the results of this study are compared with data from rats trained to the discriminative effects of either a low, autoreceptor specific dose (0.05 mg/kg; Widzowski and Cory-Slechta, 1993) or a postsynaptic receptor specific dose (0.20 mg/kg; Cory-Slechta et al., 1996) of the D_2/D_3 agonist quinpirole. The data from these quinpirole-trained rats have been presented previously (Cory-Slechta et al., 1996; Widzowski and Cory-Slechta, 1993). Additionally, antagonists of D_1 and D_2-like receptors were administered in an attempt to further elucidate the role of the D_3 receptor.

2. Methods

2.1. Animals

Twelve male Long–Evans rats (Harlan Blue Spruce Farms, Altamont, NY) obtained at 21 days of age were trained with 7-OH-DPAT. Upon arrival, animals were housed three per cage and given free access to Purina Test Diet semipurified chow 57755C (Purina Mills, Richmond, IN). Animals were housed individually after approximately 50 days of age. Food access was restricted to maintain body weights at approximately 300 g. Animals were housed in a vivarium colony room maintained at 22°C with a 12/12 h light/dark cycle (lights on from 0630 to 1830 h).

One animal was eliminated from the study after approximately 5 months because of atypical 7-OH-DPAT generalization test performance. All procedures were performed in accordance with NIH regulations and University of Rochester Animal Use and Care Committee guidelines.

2.2. Apparatus

Behavioral sessions were conducted in operant chambers (Model E10-10, Coulbourn Instruments, Lehigh Valley, PA) housed within light and sound attenuating enclosures ventilated by a fan. Each chamber contained three response levers that were 3.8 cm above the grid floor of the chamber and separated by 3.5 cm. The center lever was inactive in this experiment. Each chamber had a houselight that remained on throughout the behavioral sessions.

2.3. Drug discrimination procedure

Lever pressing was shaped using an automated overnight shaping procedure as described previously (Cory-Slechta et al., 1985). Rats were then trained to discriminate the stimulus properties of the putatively D_3 receptor-preferring agonist *R*(+)-7-OH-DPAT (0.10 mg/kg ip) from saline. The 10-min training sessions, which were initiated by the first response on a lever, were conducted at least 5 days/week. Injections of saline or 7-OH-DPAT occurred 15 min before the start of the training session. The presession injection time was initially 30 min, but was shortened to 15 min after approximately 40 training sessions in order to facilitate acquisition of the discrimination. Responding on one lever was reinforced with 45 mg food pellets after saline injections, while responding on the alternate lever was reinforced after 7-OH-DPAT injections. The levers defined as saline- or 7-OH-DPAT-appropriate were counterbalanced across chambers. Reinforcement was initially delivered on a fixed ratio (FR) 1 schedule and the response requirement was increased until an FR10 schedule was imposed. Training compounds were delivered in a random order across sessions, with the limitation that there could not be more than three consecutive 7-OH-DPAT or saline sessions.

Criterion accuracy for any session was defined as no more than three responses on the incorrect lever before the completion of the first ratio on the drug-appropriate lever. This ratio yields an accuracy value of 77%. Animals were considered to have acquired the discrimination when at least 8 of 10 consecutive sessions met this criterion accuracy.

After acquisition of the discrimination, generalization tests were carried out using doses of 7-OH-DPAT at and below the training dose of 0.10 mg/kg to generate a sensitivity dose–effect curve. Substitution and antagonism tests with other compounds were then conducted. All test sessions were separated by at least one criterion 7-OH-DPAT and saline training session. Additionally, during the generation of each substitution or antagonism dose–effect curve, generalization tests were conducted with both saline and the training dose of 7-OH-DPAT to ensure that discriminative performance was maintained across this period. Test sessions were response-initiated, and lasted 3 min. Responding during test sessions had no programmed consequence. Rather, three food pellets were delivered 0.5 s apart at the end of the test session independently of responding to prevent the development of any superstitious response patterns. At least two substitution tests at each dose of 7-OH-DPAT and one for each dose of each test compound were given to ensure reliability of results. However, not all

rats were included in each dose–effect curve determination. Sample sizes for each curve are indicated in corresponding figure legends.

2.4. Pharmacological manipulations

Various pharmacological tests were carried out after the determination of the 7-OH-DPAT sensitivity dose–effect curve to evaluate the nature of action and specificity of 7-OH-DPAT for the D_3 receptor. To assess whether 7-OH-DPAT may act as an autoreceptor agonist to modulate levels of synaptic DA, manipulations designed to mimic autoreceptor stimulation (Widzowski and Cory-Slechta, 1993) included: (1) substitution with low doses of the D_2-like receptor agonist (−)-quinpirole hydrochloride (LY 171555); (2) substitution with low doses of the mixed D_1/D_2 receptor agonist APO, which reportedly exhibits a greater D_2 than D_1 selectivity in vivo (Andersen and Jansen, 1990); (3) catecholaminergic depletion produced by DL-α-methyl-*p*-tyrosine (AMPT); and (4) postsynaptic D_1 receptor blockade produced by the D_1 receptor antagonist SCH 23390 (since presynaptic DA agonism should decrease D_1 receptor stimulation).

In order to evaluate the pharmacological specificity of the discrimination, manipulations designed to stimulate postsynaptic DA receptors included (Cory-Slechta et al., 1996): (1) substitution with high doses of quinpirole; (2) substitution with high doses of APO; and (3) substitution with the DA releaser and reuptake inhibitor D-amphetamine. Evaluation of the pharmacological specificity of dopaminergic (DAergic) antagonists for 7-OH-DPAT was assessed by administering prior to the training dose of 7-OH-DPAT: (1) the D_1 receptor antagonist SCH 23390; (2) the D_2 receptor-preferring antagonist spiperone; (3) the D_2 receptor antagonist eticlopride; (4) the putatively D_3 receptor-specific antagonist UH 232; and (5) a combination of the D_1 receptor antagonist SCH 23390 and the D_2 receptor antagonist eticlopride.

2.5. Drugs

7-OH-DPAT hydrobromide, LY 171555, AMPT, APO, *R*(+)-SCH 23390 hydrochloride, spiperone hydrochloride, and *S*(−)-eticlopride hydrochloride were obtained from Research Biochemicals International (Natick, MA). (+)-UH 232 maleate was obtained from Tocris Cookson (Ballwin, MO). D-Amphetamine sulfate was obtained from the University of Rochester Strong Memorial Hospital Pharmacy. With the exception of spiperone, which was dissolved in distilled, deionized water, all drugs were dissolved in 0.9% sterile saline. All drugs were injected intraperitoneally (ip), except for UH 232 which was administered subcutaneously (sc). All drugs were administered at an injection volume of 1 ml/kg with the exception of the 1 and 5 mg/kg doses of UH 232, which were administered at a volume of 2 ml/kg. Drug doses were calculated based on the salt. 7-OH-DPAT was administered 15 min before behavioral sessions. Quinpirole, D-amphetamine, SCH 23390, UH 232, and APO were all administered 30 min before behavioral test sessions, eticlopride 25 min, spiperone 1 h, and AMPT 3.5 h before test sessions. When SCH 23390 and eticlopride were administered together, SCH was given 30 min and eticlopride 25 min before the administration of the training dose of 7-OH-DPAT.

2.6. Data and statistical analyses

The accuracy of 7-OH-DPAT drug-lever responding in training sessions was calculated by dividing the number of responses on the 7-OH-DPAT lever during the first ratio by the total number of responses on both the 7-OH-DPAT and saline levers during the first ratio with the result multiplied by 100 to obtain a percentage. Accuracy in test sessions was calculated using data for lever presses up to the point that 10 responses had been made on either lever. Response rate was calculated by dividing the number of responses on both levers during the entire test session by the duration of the session in minutes. Because even a single response could markedly influence levels of drug lever responding in some test sessions, only those sessions with five or more responses were included in the statistical analysis of percent 7-OH-DPAT lever responding. All test results, however, were included in the analyses of response rate so that an accurate indication of drug-induced rate changes could be obtained.

To construct sensitivity dose–effect curves, the median value for accuracy of responding (percent 7-OH-DPAT lever responding) was used when more than two replications of a particular test had been performed because it was considered to most accurately reflect the actual drug lever response levels observed across replications for an individual subject. The mean of the response rate (responses per minute) across replications was used. Mean ± S.E.M. values were then derived across animals within each treatment group. In cases where only one determination of a test was undertaken, the calculation of response rates and 7-OH-DPAT lever responding represent the group mean of individual values across rats.

Since the criterion accuracy for 7-OH-DPAT drug-lever responding was 77%, any compound that engendered 7-OH-DPAT lever responding of 77% or greater was considered either to substitute fully for or not to antagonize the stimulus properties of the 7-OH-DPAT training dose. Compounds that engendered 7-OH-DPAT lever responding of 23% or less were either considered not to substitute for 7-OH-DPAT or to completely antagonize the stimulus properties of the 7-OH-DPAT training dose. Drugs that produced 7-OH-DPAT drug-lever responding between 23% and 77% were considered either to partially substitute for or partially antagonize the stimulus properties of 7-OH-DPAT.

Dose–effect curves and response rate data were analyzed by one-factor ANOVA (StatView, SAS Institute, Cary, NC). Subsequent post hoc analyses were based on Fisher's PLSD as appropriate. For all such analyses, P values $\leq .05$ were considered statistically significant.

Data from this study were compared to results obtained previously using comparable procedures in rats trained to discriminate either a low, autoreceptor-specific (0.05 mg/kg; Widzowski & Cory-Slechta, 1993) or a high, postsynaptic receptor-specific (0.20 mg/kg; Cory-Slechta et al., 1996) dose of quinpirole from saline.

3. Results

3.1. Training and performance stability

The mean (±S.E.M.) number of sessions to criterion following the imposition of the FR10 schedule of reinforcement was 52 (±7). Stability of the 7-OH-DPAT stimulus over the course of the experiment was indicated by group mean (±S.E.M.) 7-OH-DPAT lever response levels of 92.6 (±5.2%) and 12.9 (±5.0%) in redeterminations of 7-OH-DPAT and saline generalization test sessions, respectively, which occurred at the completion of all pharmacological manipulations.

3.2. 7-OH-DPAT dose–effect function

Rats learned to discriminate 7-OH-DPAT from saline, exhibiting greater than 90% 7-OH-DPAT lever responding following the training dose and less than 20% 7-OH-DPAT lever responding in the presence of saline. Doses of 7-OH-DPAT below the training dose engendered a dose-related decline in 7-OH-DPAT lever responding; response rates generally declined with increasing doses of 7-OH-DPAT (Fig. 1).

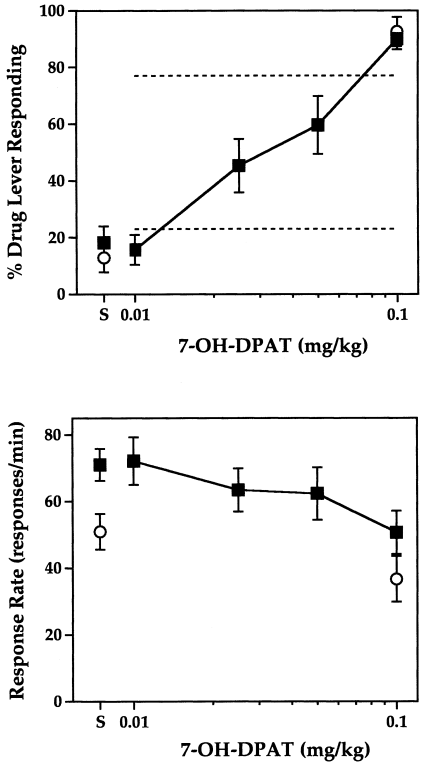

Fig. 1. Top: Percent 7-OH-DPAT lever responding as a function of 7-OH-DPAT dose after acquisition of the discrimination (closed squares, $n=11$). Also shown are results of generalization tests with saline and the training dose of 7-OH-DPAT (open circles, $n=11$) that were performed at the end of the study after all other pharmacological manipulations. Each point represents the mean±S.E.M. value based on the median value derived across replications of each dose for each rat. Bottom: Responses per minute as a function of 7-OH-DPAT dose as described above. Each data point represents the group mean±S.E.M. value based on the mean value derived across replications of each dose for each rat. In both the top and bottom panels, data from saline test sessions are denoted by "S."

3.3. Substitution tests

3.3.1. Cross-generalization between quinpirole and 7-OH-DPAT

As depicted in Fig. 2 (top), the D_2 receptor-preferring agonist quinpirole dose-dependently produced 7-OH-DPAT lever responding [$F(6,42)=20.53$, $P<.0001$]. A dose as low as 0.01 mg/kg engendered partial drug lever responding (54%) while full generalization (>77%) was produced by doses of 0.02 mg/kg and higher. In contrast, 7-OH-DPAT at best only partially generalized to the postsynaptic dose of quinpirole, with maximum drug lever responding of 55.7% and 51.8% at 0.15 and 0.20 mg/kg, respectively.

Response rates (Fig. 2, bottom) declined significantly relative to saline levels at all doses of quinpirole tested in 7-OH-DPAT-trained rats [$F(6,42)=4.526$, $P<.001$]; a similar decline in response rates relative to saline levels was observed following substitution with 7-OH-DPAT in 0.20 mg/kg quinpirole-trained rats.

3.3.2. Apomorphine

Drug lever responding to the D_1/D_2 receptor agonist APO increased in a significant [$F(7,32)=10.28$, $P<.0001$] and generally dose-related fashion in animals trained to 7-OH-DPAT. As shown in Fig. 3 (top), doses from 0.04 to 0.25 mg/kg produced mainly saline-appropriate responding, while doses of 0.75 and 1.0 mg/kg produced drug lever responding >85% and thus fully substituted for the 7-OH-DPAT cue.

This pattern of responding was quite different from that engendered by APO in animals trained to discriminate either a low or a high dose of quinpirole. In 0.20 mg/kg quinpirole-trained animals, 0.04 to 0.50 mg/kg APO elicited low levels of responding which were consistent with saline-appropriate responding. Only the 0.75 mg/kg dose of APO evoked partial substitution for quinpirole. In the 0.05 mg/kg quinpirole group, drug lever responding increased in a dose-related manner over the dose range of 0.04 to 0.167 mg/kg to a maximum of 69%. Interestingly, drug lever responding showed a dose-related decline with further increases in APO dose.

Response rates (Fig. 3, bottom) were relatively stable across doses of APO in 7-OH-DPAT-trained animals ($P>.05$). However, over the range of APO doses tested, response rates declined approximately 78% in the 0.20 mg/

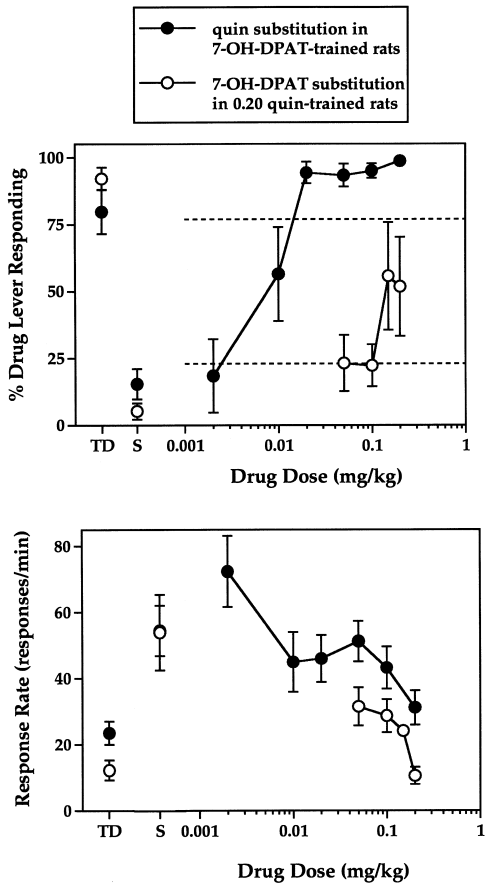

Fig. 2. Top: Percent drug lever responding as a function of quinpirole dose (0.002, 0.01, 0.02, 0.05, 0.10, or 0.20 mg/kg) in 7-OH-DPAT-trained rats (closed circle, $n=7$) or 7-OH-DPAT dose (0.05, 0.10, 0.15, or 0.20 mg/kg) in 0.20 mg/kg quinpirole-trained rats (open circle, $n=10$). Drug lever response levels greater than or equal to 77% (top dashed line) were defined as full substitution for the training drug; those at or below 23% (bottom dashed line) were defined as saline-appropriate responding and the area between defined as partial substitution. Bottom: Responses per minute as a function of drug dose as described above. Sample sizes are the same as described above. In both the top and bottom panels, values labeled "S" show data obtained during saline substitution tests performed during the acquisition of each drug dose–effect curve; data from drug training dose sessions are labeled "TD." Other details as in Fig. 1. Quinpirole data have been presented previously (Cory-Slechta et al., 1996).

kg quinpirole group and 48% in the 0.05 mg/kg quinpirole-trained group.

3.3.3. D-Amphetamine

Substitution testing with 0.25 to 6.0 mg/kg of the DA releaser/uptake inhibitor D-amphetamine produced, at best, only partial substitution in 7-OH-DPAT-trained animals (Fig. 4, top). Although increases in drug lever responding were generally dose-related, they were not significantly elevated relative to saline levels ($P>.05$). This pattern of responding was similar to that observed in both 0.20 and 0.05 mg/kg quinpirole-trained animals, with maximum levels of drug level responding in these two groups averaging approximately 35% at the highest dose of amphetamine tested.

Rates of responding were significantly affected by amphetamine administration in all three groups (Fig. 4, bottom). In 7-OH-DPAT-trained rats [$F(4,25)=12.14$, $P<.0001$] responding declined approximately 77% over the range of amphetamine doses tested. The 3 ($P<.01$) and 6 ($P<.0001$) mg/kg doses of amphetamine were particularly effective in this regard. In the quinpirole-trained groups responding also declined significantly relative to saline control rates of responding.

3.3.4. AMPT

Administration of the DA depleting drug AMPT significantly increased 7-OH-DPAT lever responding [$F(3,16)=3.83$, $P<.05$], but at most to levels consistent with partial substitution (Fig. 5, top). Levels of drug lever responding at

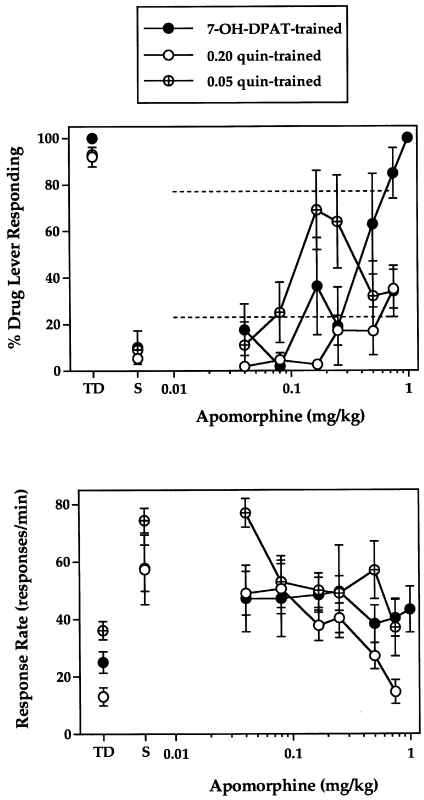

Fig. 3. Top: Percent responding on the drug lever as a function of the dose of APO (0.04, 0.08, 0.167, 0.25, 0.50, or 0.75 mg/kg; 1.0 mg/kg dose tested only in 7-OH-DPAT-trained rats) in rats trained to discriminate 7-OH-DPAT (closed circle, $n=3-5$), 0.20 mg/kg quinpirole (open circle, $n=8$), or 0.05 mg/kg quinpirole (crossed circle, $n=7-10$) from saline. Drug lever response levels greater than or equal to 77% (top dashed line) were defined as full substitution for the training drug; those at or below 23% (bottom dashed line) were defined as saline-appropriate responding and the area between defined as partial substitution. Bottom: Responses per minute as a function of dose of APO. Sample sizes are the same as described above. For both top and bottom panels, values labeled "S" show data obtained during saline substitution tests performed during the acquisition of each drug dose–effect curve; data from drug training dose sessions are labeled "TD." Other details as in Fig. 1. Quinpirole data have been presented previously (Cory-Slechta et al., 1996; Widzowski and Cory-Slechta, 1993).

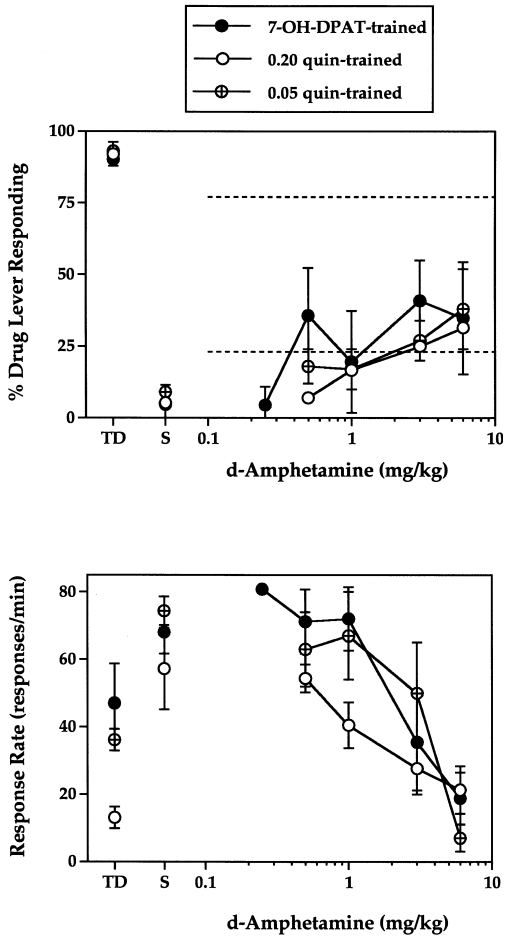

Fig. 4. Top: Percent responding on the drug lever as a function of the dose of D-amphetamine [0.25 (tested in 7-OH-DPAT-trained rats only), 0.50, 1.0, 3.0, or 6.0 mg/kg] in rats trained to discriminate 7-OH-DPAT (closed circle, $n=7-8$), 0.20 mg/kg quinpirole, (open circle, $n=7-10$) or 0.05 mg/kg quinpirole (crossed circle, $n=7-8$) from saline. Drug lever response levels greater than or equal to 77% (top dashed line) were defined as full substitution for the training drug; those at or below 23% (bottom dashed line) were defined as saline-appropriate responding and the area between defined as partial substitution. Bottom: responses per minute as a function of dose of D-amphetamine. Sample sizes are the same as described above. For both top and bottom panels, values labeled "S" show data obtained during saline substitution tests performed during the acquisition of each drug dose–effect curve; data from drug training dose sessions are labeled "TD." Other details as in Fig. 1. Quinpirole data have been presented previously (Cory-Slechta et al., 1996; Widzowski and Cory-Slechta, 1993).

AMPT doses between 25 and 75 mg/kg ranged from 29% to 72% in 7-OH-DPAT-trained animals. The pattern of responding was similar in animals trained to 0.05 mg/kg quinpirole, where drug lever responding peaked at approximately 70% at the 75 mg/kg AMPT dose. In contrast, AMPT did not cross-generalize to the 0.20 mg/kg dose of quinpirole, producing maximal level drug lever responding of 12.8% in these rats.

AMPT administration decreased response rates relative to saline in 7-OH-DPAT-trained rats (Fig. 5, bottom), an effect which approached statistical significance [$F(3,16)=3.08$, $P=.057$]. Response rates were only minimally affected in 0.05 mg/kg quinpirole-trained rats, but significantly decreased in rats trained to 0.20 mg/kg quinpirole [$F(3,21)=5.03$, $P<.01$]. (This effect in 0.20 mg/kg quinpirole-trained rats derived from a significant difference between the 50 mg/kg dose and saline response rates.)

3.3.5. SCH 23390

Substitution of the D_1 receptor antagonist SCH 23390 for 7-OH-DPAT resulted in moderate levels of drug lever responding, which, even at the highest dose tested (0.25

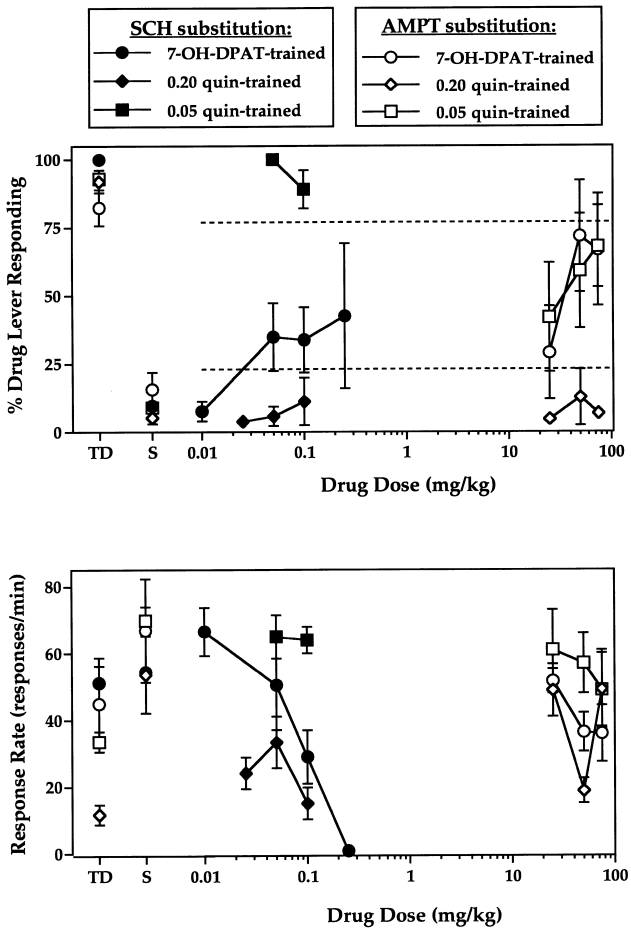

Fig. 5. Top: Percent responding on the drug lever as a function of the dose of SCH 23390 [0.01 (7-OH-DPAT-trained rats only), 0.025 (0.20 mg/kg quinpirole-trained rats only), 0.05, 0.10, or 0.25 (7-OH-DPAT-trained rats only) mg/kg; closed symbol] or AMPT (25, 50, or 75 mg/kg; open symbol) in rats trained to discriminate 7-OH-DPAT (circle, $n=5$), 0.20 mg/kg quinpirole (diamond, $n=8$), or 0.05 mg/kg quinpirole (square, $n=7-8$) from saline. Drug lever response levels greater than or equal to 77% (top dashed line) were defined as full substitution for the training drug; those at or below 23% (bottom dashed line) were defined as saline-appropriate responding and the area between defined as partial substitution. Bottom: Responses per minute as a function of drug dose. Sample sizes are the same as described above. For both top and bottom panels, values labeled "S" show data obtained during saline substitution tests performed during the acquisition of each drug dose–effect curve; data from drug training dose sessions are labeled "TD." Other details as in Fig. 1. Quinpirole data have been presented previously (Cory-Slechta et al., 1996; Widzowski and Cory-Slechta, 1993).

mg/kg), could only be considered partial substitution (Fig. 5, top). This pattern of substitution is intermediate to the substitution observed in 0.05 and 0.20 mg/kg quinpirole-trained animals. Negligible levels of drug lever responding were obtained in high dose quinpirole rats, whereas complete substitution was achieved with SCH in the group of low, autoreceptor dose-trained quinpirole rats.

SCH markedly decreased response rates relative to saline control levels in 7-OH-DPAT-trained rats [Fig. 5, bottom; $F(4,35) = 13.19$, $P < .0001$]. The 0.10 mg/kg dose significantly decreased ($P < .05$), and the 0.25 mg/kg dose almost completely suppressed ($P < .0001$) responding. As with accuracy measures, rates of responding were differentially affected in the two quinpirole-trained groups. Specifically, SCH 23390 administration induced a considerable decrease in response rates relative to saline levels in the 0.20 mg/kg quinpirole-trained group, but did not affect rates of responding in 0.05 mg/kg quinpirole-trained rats.

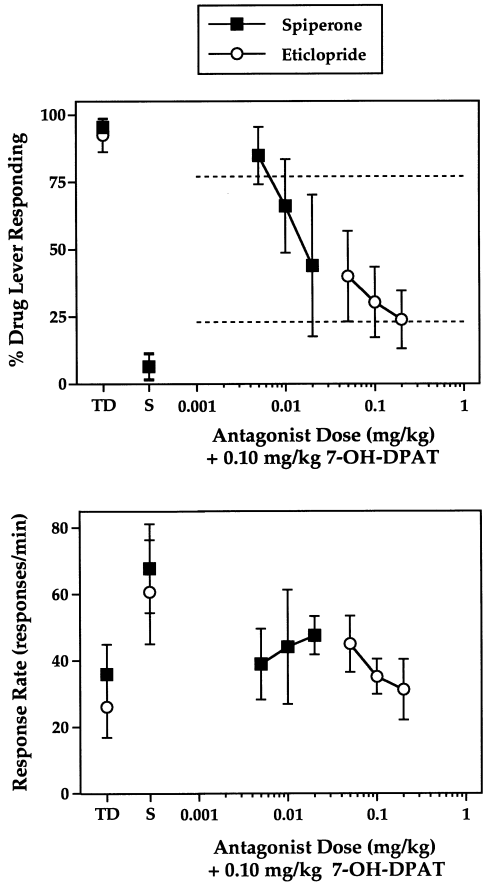

Fig. 6. Top: Percent responding on the 7-OH-DPAT lever as a function of the dose of spiperone (0.005, 0.01, or 0.02 mg/kg) or eticlopride (0.01, 0.05, 0.10, or 0.20 mg/kg) administered prior to the training dose of 7-OH-DPAT (0.10 mg/kg). The spiperone antagonism dose–effect curve (closed square) was derived from four rats and the eticlopride dose–effect curve (open circle) was derived from six rats. Drug lever response levels greater than or equal to 77% (top dashed line) were defined as 7-OH-DPAT-appropriate responding; those at or below 23% (bottom dashed line) were defined as complete antagonism of the 7-OH-DPAT training dose and the area between defined as partial antagonism. Bottom: Responses per minute as a function of dose of spiperone or eticlopride. Sample sizes are the same as described above. In both the top and bottom panels, values labeled "S" show data obtained during saline substitution tests performed during the acquisition of each drug dose–effect curve; data from drug training dose sessions are labeled "TD." Other details as in Fig. 1.

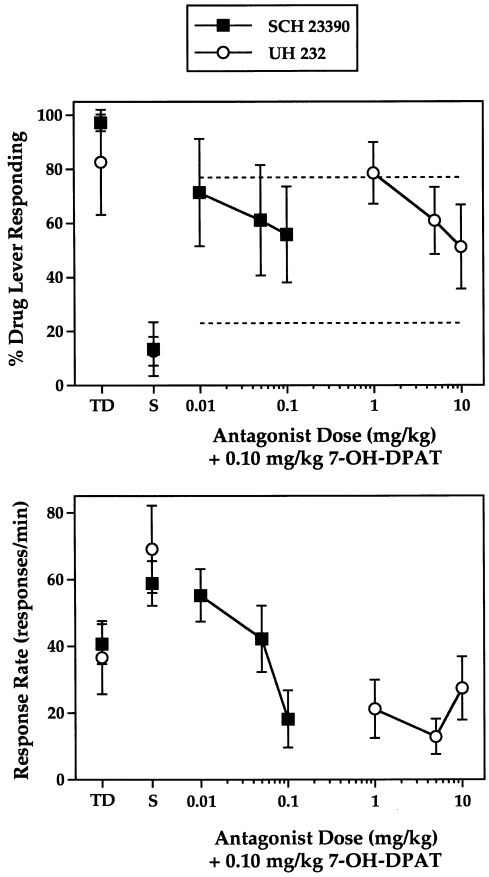

Fig. 7. Top: Percent responding on the 7-OH-DPAT lever as a function of the dose of SCH 23390 (0.01, 0.05, or 0.10 mg/kg) or UH 232 (1.0, 5.0, or 10.0 mg/kg) administered prior to the training dose of 7-OH-DPAT (0.10 mg/kg). The SCH 23390 antagonism dose–effect curve (closed square) was derived from six rats and the UH 232 dose–effect curve (open circle) was derived from five rats. Drug lever response levels greater than or equal to 77% (top dashed line) were defined as 7-OH-DPAT-appropriate responding; those at or below 23% (bottom dashed line) were defined as complete antagonism of the 7-OH-DPAT training dose and the area between defined as partial antagonism. Bottom: Responses per minute as a function of dose of SCH 23390 or UH 232. Sample sizes are the same as described above. In both the top and bottom panels, values labeled "S" show data obtained during saline substitution tests performed during the acquisition of each drug dose–effect curve; data from drug training dose sessions are labeled "TD." Other details as in Fig. 1.

3.4. Antagonism tests

3.4.1. Spiperone

Pretreatment with the putatively D_2 receptor-specific antagonist spiperone dose-relatedly, though nonsignificantly ($P > .05$), decreased 7-OH-DPAT lever responding such that partial antagonism of the stimulus properties of the training dose of 7-OH-DPAT was produced (Fig. 6, top).

Spiperone pretreatment did not significantly alter response rates relative to either saline ($P>.05$) or training dose ($P>.05$) rates (Fig. 6, bottom).

3.4.2. Eticlopride

The putatively D_2 receptor-specific antagonist eticlopride generally decreased 7-OH-DPAT lever responding in a dose-related manner as compared to the 7-OH-DPAT training dose [$F(3,20)=4.95$, $P<.01$]; all three doses were effective in this regard (all P's $<.05$). Despite the statistical significance of this antagonism, eticlopride did not fully antagonize the stimulus properties of 7-OH-DPAT (Fig. 6, top).

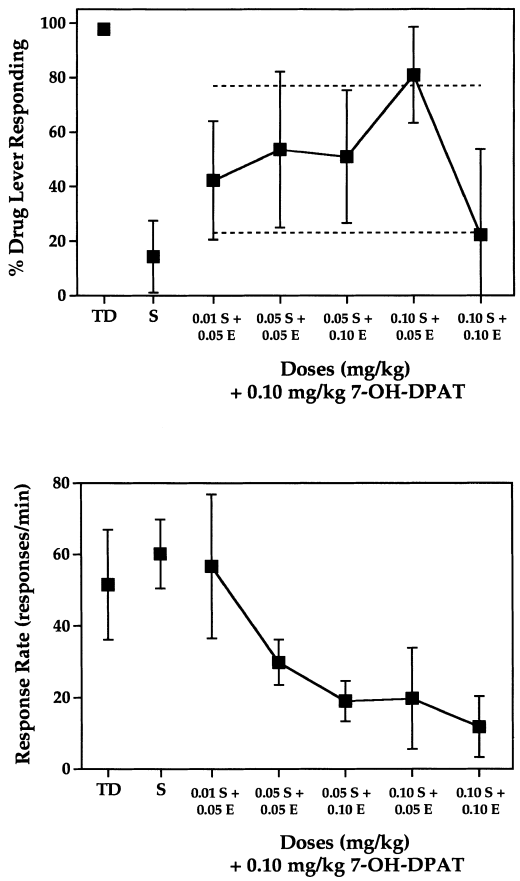

Fig. 8. Top: Percent responding on the 7-OH-DPAT lever as a function of the combined doses of SCH 23390 and eticlopride administered prior to the training dose of 7-OH-DPAT (0.10 mg/kg). Data were obtained from four rats except at the 0.10 mg/kg SCH/0.10 mg/kg eticlopride dose combination, where two rats were tested, and data from this combination were not included in statistical analyses due to insufficient responding. Drug lever response levels greater than or equal to 77% (top dashed line) were defined as 7-OH-DPAT-appropriate responding; those at or below 23% (bottom dashed line) were defined as complete antagonism of the 7-OH-DPAT training dose and the area between defined as partial antagonism. Bottom: Responses per minute as a function of the combined doses of SCH 23390 and eticlopride. Sample sizes are the same as described above. In both the top and bottom panels, values labeled "S" show data obtained during saline substitution tests performed during the acquisition of each drug dose–effect curve; data from drug training dose sessions are labeled "TD." Other details as in Fig. 1.

Eticlopride failed to reverse the 7-OH-DPAT training dose-related decrease in response rate at any of the doses tested [$F(1,10)=5.56$, $P<.05$; Fig. 6, bottom].

3.4.3. SCH 23390

The D_1 receptor-specific antagonist SCH 23390 partially antagonized the stimulus properties of 7-OH-DPAT, as evidenced by drug lever response values between 71% and 56% over the doses of SCH tested (Fig. 7, top). This effect was not statistically significant [$F(3,20)=1.59$, $P>.05$].

SCH administered prior to the 7-OH-DPAT training dose significantly decreased response rates relative to both saline [$F(3,20)=5.83$, $P<.01$] and 7-OH-DPAT training dose [$F(3,20)=4.21$, $P<.05$] levels (Fig. 7, bottom).

3.4.4. UH 232

Pretreatment with the putatively D_3 receptor-specific antagonist UH 232 resulted in a nonsignificant [$F(3,16)=1.21$, $P>.05$], though dose-related, decrease in 7-OH-DPAT responding to levels consistent with partial antagonism of the 7-OH-DPAT stimulus (Fig. 7, top).

UH 232 did decrease response rates, but this effect was not statistically significant with respect to 7-OH-DPAT training dose rates [$F(3,16)=1.61$, $P>.05$; Fig. 7, bottom].

3.4.5. SCH 23390/eticlopride combination

In general, combined pretreatment with a D_1-like and a D_2-like antagonist at best only partially antagonized the stimulus properties of 7-OH-DPAT; this effect was not statistically significant [Fig. 8, top; $F(4,15)=1.79$, $P>.05$]. The combination of the highest doses of each drug appeared to produce nearly complete antagonism, but these data could not be included in the statistical analysis due to the rate-suppressing effects of this combination.

Response rates were marginally decreased by the combined antagonists relative to training dose rates [Fig. 8, bottom; $F(5,18)=2.79$, $P<.05$]. This effect was primarily observed at the 0.05 mg/kg SCH/0.10 mg/kg eticlopride ($P=.05$) and 0.10 mg/kg SCH/0.10 mg/kg eticlopride ($P<.05$) dose combinations.

4. Discussion

Because it has been suggested that the D_3 receptor is capable of acting either as a presynaptic autoreceptor which participates in the control of DA synthesis and release (Aretha et al., 1995; Gilbert et al., 1995; Meller et al., 1993; O'Hara et al., 1996; Tang et al., 1994), as a postsynaptic receptor (Svensson et al., 1994; Waters et al., 1993), or as both a pre- and postsynaptic receptor (Damsma et al., 1993; Elsworth and Roth, 1997; Spealman, 1996), the present study sought to assess the functional properties of a D_3 receptor stimulus using the reportedly D_3-preferring agonist 7-OH-DPAT (Sanger et al., 1997). However, the hypothesis that the D_3 receptor was located presynaptically and func-

tioned as an autoreceptor was based on the assumption that the 7-OH-DPAT stimulus is mediated by the D_3 subtype of receptor (Chio et al., 1994; Levesque et al., 1992; MacKenzie et al., 1994; Sanger et al., 1997). As discussed below, this assumption may not have been entirely appropriate. Thus, rather than identifying the location and role of the D_3 subtype of receptor per se, the collective findings of this study support the conclusion that 7-OH-DPAT is able to act on receptors that are located both pre- and postsynaptically.

In discussing our results it is important to acknowledge that the poor receptor subtype specificity of 7-OH-DPAT may limit its use as a tool in the complex analysis of D_3 function at the level of the whole animal. Structural homology between the D_2 and D_3 DA receptors is 52% overall, but 75% in the transmembrane domain regions which are important for ligand recognition (Sokoloff et al., 1990), thereby complicating the ability of pharmacological ligands to be subtype specific. While Levant et al. (1996) have demonstrated that 7-OH-DPAT primarily stimulates the D_3 subtype of receptor at doses below 0.3 mg/kg (sc), other studies suggesting that 7-OH-DPAT is between 5- and 78-fold more selective for the D_3 than the D_2 subtype of DA receptor (Chio et al., 1994; Levesque et al., 1992; MacKenzie et al., 1994) utilized in vitro binding assays and thus may overestimate the receptor selectivity that can be achieved in an in vivo assay. In fact, Koshikawa et al. (1996a,b) concluded that intra-accumbens 7-OH-DPAT activated D_2 receptors, and Boulay et al. (1999a,b) and Xu et al. (1999) utilized a knockout mouse model to conclude that 7-OH-DPAT may act at D_2 receptors. Additionally, Millan et al. (2000) performed a drug discrimination study with several highly selective antagonists of the D_3 subtype and concluded that the discriminative stimulus effects of 7-OH-DPAT are mediated principally by the D_2 receptor subtype. Considering these reports, it is perhaps most appropriate to conclude, based on the results of the present study, that 7-OH-DPAT is an agonist capable of interacting with pre- and postsynaptic receptors of the D_2-like family.

The results of substitution tests with D-amphetamine and AMPT suggest that 7-OH-DPAT acts on DA autoreceptors. D-Amphetamine causes the release of vesicular DA and inhibits DA reuptake, thereby increasing the synaptic concentration of DA. If 7-OH-DPAT acts upon an autoreceptor to induce a decrease in the net concentration of synaptic DA, D-amphetamine would not be expected to fully generalize to the discriminative stimulus effects of 7-OH-DPAT. In fact, doses of D-amphetamine in the range of 0.25–6.0 mg/kg produced, at best, only partial substitution for the stimulus properties of 7-OH-DPAT (Fig. 5). Varty and Higgins (1997) were also unable to observe cross-generalization between 7-OH-DPAT and D-amphetamine. In contrast, however, Bevins et al. (1997) reported complete substitution of 7-OH-DPAT for the discriminative stimulus effects of D-amphetamine. Differences in training conditions and/or substitution testing may underlie such differential outcomes.

Further supporting a presynaptic mechanism of 7-OH-DPAT action is the fact that the DA depleting compound AMPT partially substituted for 7-OH-DPAT at doses between 50 and 75 mg/kg (Fig. 4). By causing DA depletion via inhibition of catecholamine synthesis (Moore and Dominic, 1971) AMPT would be expected to have functional consequences similar to those of an autoreceptor agonist and did produce levels of 7-OH-DPAT responding as high as 72%. AMPT produced levels of generalization of about 75% for the low, autoreceptor-specific dose of quinpirole, but evoked minimal levels of drug lever responding following training to the higher, presumably postsynaptic dose of quinpirole in our previous studies based on this same range of AMPT doses (Cory-Slechta et al., 1996; Widzowski and Cory-Slechta, 1993).

More equivocal results with respect to pre- versus postsynaptic action of 7-OH-DPAT were provided by substitution tests with the D_1 antagonist SCH 23390. This compound should deprive postsynaptic D_1 receptors of endogenous DA, as would a net decrease in synaptic DA in response to autoreceptor agonism. SCH 23390 produced approximately 90% drug responding for the presynaptic dose of quinpirole (0.05 mg/kg; Widzowski and Cory-Slechta, 1993), but levels of drug responding to these same doses of SCH 23390 never exceeded 11% for the postsynaptic dose of quinpirole (0.20 mg/kg; Cory-Slechta et al., 1996). The current findings show substitution levels intermediate to these two studies, up to 40% 7-OH-DPAT responding. Full substitution with SCH 23390 may have been achieved at doses higher than those tested here, but the severe rate reductions associated with 0.25 mg/kg SCH 23390 precluded testing higher doses of this compound.

Substitution testing with the D_2-like agonist quinpirole provides evidence for both pre- and postsynaptic mediation of the 7-OH-DPAT discriminative stimulus. Previous studies have demonstrated that low doses of quinpirole selectively stimulate DA autoreceptors involved in the control of DA release (Imperato et al., 1988; See et al., 1991; Widzowski and Cory-Slechta, 1993), while higher doses act upon postsynaptic D_2-like receptors (Cory-Slechta et al., 1996). In the present experiment, quinpirole fully substituted for the stimulus properties of 7-OH-DPAT at doses presumably ranging from pre- to postsynaptic (between 0.02 and 0.20 mg/kg). Additionally, Sanger et al. (1997) reported that quinpirole substituted for 7-OH-DPAT across a broad dose range, i.e. from 0.01 to 0.10 mg/kg, and Varty and Higgins (1997) observed full substitution of quinpirole for 7-OH-DPAT at quinpirole doses of 0.01 and 0.03 mg/kg.

Uncertainty over the receptor subtype selectivity of quinpirole complicates the interpretation of the present results. While some studies have reported that quinpirole has a 14- to 113-fold higher affinity for the D_3 over the D_2 receptor (Chio et al., 1994; Freedman et al., 1994; Griffon et al., 1996; Sokoloff et al., 1990), others have found that quinpirole exhibits a higher affinity for the D_2 than D_3 receptor (Seeman and Schaus, 1991) or is approximately

equipotent at the two receptor subtypes (Burris et al., 1995; Potenza et al., 1994). However, these estimates of receptor specificity may be highly dependent on assay conditions and the affinity state of the receptors (Burris et al., 1995; Chio et al., 1994; Seeman and Van Tol, 1994). Furthermore, the majority of these studies were performed in vitro, which may also complicate the direct comparison of their findings with the current in vivo results. As seen in Fig. 2, the substitution of 7-OH-DPAT for the postsynaptic dose of quinpirole (0.20 mg/kg) yielded only partial generalization at 7-OH-DPAT doses between 0.025 and 0.10 mg/kg (Cory-Slechta et al., 1996), suggesting that in vivo, postsynaptic receptors bound by quinpirole and 7-OH-DPAT are indeed distinct. This conclusion is further supported by an intracranial self-stimulation study in which quinpirole and 7-OH-DPAT were shown to have different behavioral effects (Hatcher and Hagan, 1998).

The results of substitution testing with the D_1/D_2 agonist APO suggest a postsynaptic site of action of 7-OH-DPAT. Like quinpirole, APO has been shown to act specifically on DA autoreceptors at low doses and on postsynaptic receptors at higher doses (Cory-Slechta et al., 1989, 1996; Skirboll et al., 1979; Strombom, 1976). In the present study, only relatively high APO doses (0.75–1.0 mg/kg) engendered full substitution for 7-OH-DPAT. Animals trained to the presynaptic receptor dose of quinpirole (0.05 mg/kg) exhibited biphasic responding to APO, with increasing levels of substitution at low to intermediate doses of APO (0.04–0.25 mg/kg), while higher APO doses (0.50–1.0 mg/kg) actually resulted in a decrease in drug lever responding (Widzowski and Cory-Slechta, 1993). In contrast, rats trained to the postsynaptic receptor dose of quinpirole (0.20 mg/kg) exhibited partial substitution only at the highest APO dose tested (0.75 mg/kg; Cory-Slechta et al., 1996). These results thus suggest that 7-OH-DPAT may act on a postsynaptic receptor population.

Data from testing with DAergic antagonists further establish the complex nature of 7-OH-DPAT action. The reportedly D_2 receptor-preferring antagonist eticlopride partially antagonized 7-OH-DPAT stimulus properties at doses between 0.05 and 0.20 mg/kg. Despite its use as a D_2 receptor antagonist, MacKenzie et al. (1994) found that eticlopride binds with a similar affinity to D_2 and D_3 receptor subtypes. Eticlopride was able to block the effects of 7-OH-DPAT in other species (Sokoloff et al., 1990; Yoshida et al., 1995; Yoshikawa et al., 1996), and was also effective in D_3 receptor-transfected cell lines (Griffon et al., 1996; Tang et al., 1994). The partial antagonism of the stimulus properties of 7-OH-DPAT by eticlopride shown here and by others (Bevins et al., 1997) corroborate these experimental findings and indicate that eticlopride is able to modulate the effects of 7-OH-DPAT. The putatively D_3 receptor-specific ligand UH 232 likewise produced only a partial antagonism of the stimulus properties of 7-OH-DPAT. Varty and Higgins (1997) have also reported that UH 232 only partially antagonized 7-OH-DPAT responding. As with the other antagonists tested in the present study, this may indicate the poor pharmacological specificity of this compound in vivo.

In conclusion, our results indicate that 7-OH-DPAT is capable of acting upon both pre- and postsynaptic receptors in vivo. However, as mentioned previously, the precise receptor subtype basis of action is rather difficult to ascertain due to the presumed lack of selectivity of various agonists and antagonists of the DA receptor subtypes. The future development of compounds with greater receptor subtype specificity will undoubtedly aid in the study of differential functions of D_2 versus D_3 receptors using in vivo model systems.

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