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The effects of eticlopride and the selective D_3-antagonist PNU 99194-A on food- and cocaine-maintained responding in rhesus monkeys

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Abstract

The dopamine D_3 receptor is mainly expressed in regions of the brain associated with the limbic system. D_3 receptor blockade may antagonize cocaine reinforcement while producing less severe extrapyramidal side effects than blockade of D_2 receptors. The purpose of the present studies was to evaluate the effects of a selective D_3 receptor antagonist and a non-selective D_2/D_3 receptor antagonist on food- and cocaine-maintained responding under two schedules of cocaine self-administration. Adult male rhesus monkeys were trained to respond under multiple schedules of food (1.0 g pellets) and cocaine (0.01–0.3 mg/kg/injection) presentation. In one experiment ($n=4$), the schedule was a fixed-interval (FI) 3-min and a second study ($n=6$) was conducted using a second-order fixed-ratio 5 (FI 6-min:S) schedule. The D_3 antagonist PNU 99194-A (0.3–3.0 mg/kg), which is 14-fold selective for D_3 relative to D_2 receptors, or the D_2/D_3 antagonist eticlopride (0.001–0.03 mg/kg) was administered immediately prior to the experimental session for at least 5 consecutive sessions. Under the multiple FI 3-min schedule of food and cocaine presentation, PNU 99194-A and eticlopride decreased food- and cocaine-maintained responding in a dose-dependent manner and irrespective of cocaine dose. Under the multiple second-order schedule of food and cocaine presentation, at least one dose of PNU 99194-A and eticlopride decreased cocaine- and food-maintained responding. These findings indicate that PNU 99194-A can decrease operant responding in monkeys, but not in a manner that would suggest selectivity of cocaine- over food-maintained responding. Future studies with more selective D_3 antagonists are needed to better address the role of this receptor subtype in cocaine addiction.

Keywords: Dopamine D_3 receptor antagonist; Self-administration; Eticlopride; PNU 99194-A; Rhesus monkey

1. Introduction

Cocaine has multiple sites of action within the central nervous system, including dopamine (DA), serotonin, and norepinephrine transporters. There is substantial evidence that blockade of DA transporters (DAT) by cocaine, and the subsequent elevation of synaptic DA, is the prevailing mediator of the behavioral effects of cocaine (Ritz et al., 1987; Di Chiara and Imperato, 1988; Kuhar et al., 1991). Elevated DA acts at two superfamilies of DA receptors, D1- and D2-like. These are further divided into five subtypes classified as D_1 and D_5 (D1-like) and D_2, D_3, and D_4 (D2-like). In the search for novel treatment options, drugs that act preferentially at each DA receptor subtype have been investigated for their ability to affect behavior associated with cocaine administration. A great deal of research has focused on the D2 receptor superfamily as it relates to cocaine abuse (Volkow et al., 1993; Nader and Czoty, 2005; Newman et al., 2005). Agonists at D2-like receptors maintain intravenous (i.v.) self-administration (Woolverton et al., 1984; Nader and Mach, 1996; Caine et al., 1999), whereas D2-like antagonists decrease cocaine self-administration (Woolverton and Virus, 1989; Bergman et al., 1990; Caine et al., 1997, 2002; Nader et al., 1999), suggesting that this family of receptors is involved in the reinforcing effects of cocaine.

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There are several drawbacks to the use of D_2-selective antagonists as treatment agents. For example, the administration of neuroleptic drugs that are D2-like antagonists in humans is associated with extrapyramidal side effects including dystonia, akathisia, and parkinsonism, and more rarely perioral tremor, neuroleptic malignant syndrome and tardive dyskinesias (Jacobson et al., 1974; Baldessarini and Tarazi, 2001). In animal models of cocaine addiction, administration of D2-like antagonists has resulted in non-selective decreases in cocaine- and food-maintained responding, suggesting an overall depression in behavior (Woolverton and Virus, 1989; Caine and Koob, 1994; Nader et al., 2002; Barrett et al., 2004). Such an effect suggests that in humans, drug treatment might decrease other behaviors, in addition to drug seeking. Furthermore, these drugs do not have reinforcing effects, indicating that compliance would probably be poor (Mello and Negus, 1996).

There is growing interest in cocaine pharmacotherapies targeting the D_3 subtype of the D2 superfamily (reviewed by Newman et al., 2005). Drugs selective for the D_3 receptor may be more viable as treatments due to the localization of D_3 receptors primarily in limbic areas such as the nucleus accumbens, olfactory tubercle and islands of Calleja (Levant, 1997). Because of this region-specific localization, D_3 compounds might attenuate the reinforcing effects of cocaine but produce less motoric side effects. In support of the notion that D_3-selective antagonists may have a more favorable side-effect profile, SB 277011-A, KCH-1110, S33084, and GR218231 did not affect locomotor activity and/or were relatively inactive in models of extrapyramidal activity (e.g., catalepsy; Millan et al., 2000; Park et al., 2003; Reavill et al., 2000). With respect to the ability of D_3-selective antagonists to block cocaine self-administration, Di Ciano et al. (2003) found that the D_3-selective antagonist SB 277011-A attenuated cue-maintained behavior in rodents responding under a second-order schedule. This compound also decreased cocaine self-administration under a progressive-ratio schedule in rats (Xi et al., 2005). In studies with the D_3-selective partial agonist BP 897, Pilla et al. (1999) found decreases in cue-controlled cocaine seeking behavior under a second-order schedule in rats. These results suggest a role of D_3 receptors in cocaine reinforcement, especially in models of "drug-seeking" behavior.

The present experiments extend earlier work in several ways. First, we directly compared the effects of the D2-like antagonist eticlopride, which has approximately equal affinity at the D_3 and D_2 receptors (0.16 nM vs. 0.50 nM, respectively; Nader et al., 1999), on food and cocaine self-administration in rhesus monkeys responding under a fixed-interval (FI) schedule or a second-order schedule. An FI schedule was chosen in order to compare the present results with earlier work from our lab (Nader et al., 1999, 2002) and because the two main dependent variables (response rate and reinforcement frequency) are relatively independent measures (Zeiler, 1977; Johanson, 1978). A second-order schedule, in which responding is maintained by conditioned reinforcers, was used because these conditions may be more indicative of "drug seeking" and perhaps may be more sensitive to D_3 receptor compounds (e.g., Pilla et al., 1999; Schindler et al., 2002; Di Ciano et al., 2003). The present studies also compared the potency and efficacy of the putative D_3 antagonist PNU 99194-A (5,6-dimethoxy-indan-2-yl dipropylamine), which has 14-fold selectivity for D_3 over D_2 receptors (160 nM vs. 2281 nM, respectively; Audinot et al., 1998), with eticlopride. Because DA antagonists have been shown to non-selectively suppress behavior, a multiple schedule that included food and cocaine as the maintaining events was included to examine to what extent the effects of pretreatment drugs were specific to cocaine-maintained responding. Finally, to better assess the effects of each antagonist on changes in cocaine- and food-maintained responding over time, PNU 99194-A and eticlopride were administered for at least 5 consecutive sessions.

2. Materials and methods

2.1. Subjects

Ten adult male rhesus monkeys (*Macaca mulatta*) served as subjects. Monkeys R-1247, R-1249, R-1284, and R-1289 had an extensive history of cocaine self-administration (Nader et al., in preparation), but had not received other drugs prior to this study. Monkey R-1363 had previously received noncontingent i.m. cocaine injections and monkeys R-1346, R-1348, R-1349, R-1350, and R-1361 were drug-naïve at the beginning of the experiment. At the start of the study, monkeys' body weights were between 7 and 14 kg and were maintained at approximately 95% of free-feeding weights. Their diet consisted of 1.0-g banana-flavored pellets (Bio-Serv, Frenchtown, NJ) earned during the experimental sessions and supplemental feeding of Lab Diet Monkey Chow, given no sooner than 30 min post-session. In addition, they were given fresh fruit or peanuts at least three times per week. Each monkey was weighed once a week and, if necessary, their diets were adjusted to maintain stable weights. Monkeys were individually housed in stainless steel cages with water ad libitum and had visual and auditory contact with each other. All procedures were performed in accordance with established practices as described in the National Institutes of Health *Guide for Care and Use of Laboratory Animals*. In addition, all procedures were reviewed and approved by the Animal Care and Use Committee of Wake Forest University. Environmental enrichment was provided as outlined in the Animal Care and Use Committee of Wake Forest University Non-Human Primate Environmental Enrichment Plan.

2.2. Surgery

Under sterile conditions, each monkey was surgically prepared with an indwelling intravenous catheter and vascular access port (Access Technologies, Skokie, IL). Monkeys were anesthetized with a combination of ketamine (15 mg/kg, i.m.) and butorphanol (0.05 mg/kg, i.m.). After blunt dissection and isolation of the vein (internal jugular, external jugular, femoral or brachial), the proximal end of a polyurethane catheter (Access Technologies) was inserted into the vein for a distance calculated to terminate in the vena cava. The distal end of the

catheter was threaded subcutaneously to an incision made slightly off the midline of the back. The vascular access port was placed within a pocket formed by blunt dissection near the incision. After surgery, a topical antibiotic ointment, Bactroban cream (mupirocin calcium cream 2%, SmithKline Beecham Pharmaceuticals, Philadelphia, PA) was applied to the incision sites and analgesia (ketoprofen, 0.5 mg/kg, i.m.; Fort Dodge Animal Health, Fort Dodge, IA) was administered post-operatively as needed. Antibiotic (25 mg/kg kefzol; cefazolin sodium, Marsam Pharmaceuticals, Inc., Cherry Hill, NJ) was administered 5–7 days following surgery.

2.3. Apparatus

Experimental sessions were conducted in ventilated and sound-attenuated chambers ($150 \times 74 \times 76$ cm^3, Med Associates, East Fairfield, VT) designed to accommodate a primate chair (Primate Products, Redwood City, CA). An intelligence panel (48×69 cm), located on the right side of the chamber, contained two retractable levers (5 cm wide) with three small stimulus lights (red, white, and amber) centrally located 14 cm above each lever. The levers were positioned to be within easy reach of the monkey seated in the primate chair. About 1 g banana-flavored food pellets were delivered into a food receptacle located between the two levers on the intelligence panel. A peristaltic infusion pump (Cole-Parmer Co., Chicago, IL), for delivering drug injections at a rate of approximately 1.5 ml/10 s, was located on the top of the chamber.

2.4. Experiment 1: multiple fixed-interval schedule

2.4.1. Procedures

Monkeys R-1249, R-1284, R-1346, and R-1348 were initially trained to respond under an FI 3-min schedule of food presentation. Under this schedule, the first response after 3 min produced a 1.0-g banana-flavored food pellet followed by a 10-s timeout (TO). Next, responding was maintained under a two-ply multiple FI 3-min schedule of food presentation, in which the active lever alternated from the right to the left lever. Components were signaled by illumination of the amber light above the lever and lasted 20-min or until 5 reinforcers were obtained; components cycled twice per session. During delivery of a reinforcer, the amber light was extinguished and the red light above that lever was illuminated for 10 s. Illumination of the amber light signaled the availability of another food reinforcer under the FI 3-min schedule.

Following surgery, the schedule was changed to a two-ply multiple schedule of food (1.0-g banana-flavored pellets) and cocaine (0.03 mg/kg/injection) presentation, with each component cycling twice per session (i.e., food then cocaine); a 2-min TO separated components. Food reinforcement was available on the right lever and cocaine reinforcement was contingent on responding on the left lever. The components were designated, in order of occurrence, as FD1, COC1, FD2 and COC2. Prior to each experimental session, the area on the back of the animal containing the port was cleaned with 95% ETOH and betadine. Next a 22-gauge Huber Point Needle (Access Technologies) was inserted into the port, connecting the venous catheter to the cocaine solution. The pump was operated for approximately 3 s to fill the catheter and port with the concentration of cocaine that would be available during the experimental session. We have calculated that this infusion duration is sufficient to fill the catheter and port with the cocaine solution without administering a significant amount of drug to the animal. At the end of each session, the port and catheter were filled with heparinized saline (100 U/ml) to prevent clotting.

2.4.2. Dose–response curves and drug pretreatments

When responding maintained by the baseline dose of cocaine was stable (less than 20% variation in responding for at least 3 consecutive sessions, with no trends in responding), saline was substituted for cocaine for at least five consecutive sessions and until responding declined to less than 20% of baseline and was deemed stable. Following stable performance, the conditions were returned to baseline for at least five consecutive sessions and other cocaine doses (0.01–0.3 mg/kg/injection) were substituted for the baseline dose in random order. The minimum number of sessions that each dose was available for self-administration was individually determined and based upon the number of sessions that were required for responding to decline to less than 20% of baseline when saline was available. After a particular dose was evaluated, there was a return to baseline conditions for at least five sessions.

Once the cocaine dose–response curve was complete, the effects of PNU 99194-A (0.3–3.0 mg/kg; $n=4$) and eticlopride (0.001–0.03 mg/kg; $n=2$) were evaluated. Pretreatments were evaluated in combination with at least two self-administered cocaine doses; this typically represented a cocaine dose associated with peak response rates and a dose on the descending limb of the cocaine dose–response curve. A dose of DA antagonist was administered i.v. immediately before the session, for 5 consecutive sessions which is typically the minimum number of sessions required for responding to decline when saline is available. Cocaine self-administration was re-established for at least 5 consecutive sessions prior to evaluation of another DA antagonist dose. DA antagonist doses were tested in quasi-random order for each monkey; the highest dose was never tested first. If a monkey was tested with both PNU 99194-A and eticlopride, the former drug was tested first.

2.5. Experiment 2: multiple second-order schedule

2.5.1. Procedures

Monkeys R-1247, R-1289, R-1349, R-1350, R-1361, and R-1363 were initially trained under the multiple FI 3-min schedule of food and cocaine presentation as in Experiment 1. Next, the FI value was gradually increased to 6-min with one food and one cocaine component in each session. The conditions were then changed such that completion of an FI 6-min extinguished the amber and white lights and produced a 2-sec red stimulus light followed by another FI 6-min schedule [second-order fixed-ratio (FR) 2 (FI 6-min : S)]. Over approximately 1–2 months, the number of successive FI schedules required for

completion prior to reinforcer delivery was increased to an FR 5. The terminal schedule was a multiple second-order FR 5 (FI 6-min : S) schedule of food and cocaine presentation. Components lasted 45-min or until a reinforcer was delivered. In the first component, responding was maintained by food presentation (five 1.0-g banana-flavored pellets). Following a 2-min TO, responding in the second component was maintained by 0.1 mg/kg/injection cocaine (baseline dose). Illumination of the amber and white lights above the right lever signaled availability of food and illumination of the left lever lights signaled cocaine availability. During delivery of a reinforcer, the amber and white lights were extinguished and the red light above the appropriate lever was illuminated for 10 s.

2.5.2. Dose–response curves and drug pretreatments

When responding maintained by the baseline dose of cocaine (0.1 mg/kg/injection) was stable, saline was substituted until responding declined to less than 20% of baseline and was deemed stable. A cocaine dose–response function was determined in each monkey (0.03–0.3 mg/kg/injection), as described in Experiment 1, with each dose available for at least 5 consecutive sessions.

Once the cocaine dose–response curve was complete, the effects of PNU 99194-A (0.3–1.0 mg/kg; $n=4$) and eticlopride (0.001–0.003 mg/kg; $n=3$) were evaluated. Antagonists were administered immediately before experimental sessions for at least 5 consecutive sessions and until responding was deemed stable (less than 15% variation in responding for at least 3 consecutive sessions, with no trends in responding). If responding was not stable after 14 sessions, antagonist administration was terminated.

2.6. Data analysis

For both experiments, the primary dependent variable was response rate (responses/min). In addition, quarter-life (QL) values were also calculated. A QL value indicates when 25% of responding within the fixed-interval occurred and is an index of schedule-appropriate responding. For example, a QL value of 0.25 would indicate consistent responding throughout the interval, whereas QL values >0.25 indicate more responding later in the interval ("schedule appropriate"). QL values were only calculated from intervals that resulted in the completion of FI. Thus, when saline was substituted and response rates and reinforcement frequency were decreased, pattern of responding was based only on the intervals that resulted in a saline injection. Data represent the mean (±S.D.) of the last three sessions for each monkey. In Experiment 1, response rates were similar in both cocaine components (COC1 and COC2) and the effects of DA antagonist pretreatments were not different in the two components; therefore, the mean of both components was used for statistical analysis. Because the effects of pretreatment on rates of responding in FD1 should be the same regardless of the dose of cocaine available for self-administration later in the session, data were collapsed across cocaine doses for a given antagonist dose. Since food-maintained responding in FD2 was influenced not only by the antagonist pretreatment but also by the self-administered cocaine from COC1, only response rate data from the FD1 component are presented. Data presented in Figs. 1, 3 and 4 were analyzed by calculating the mean square error terms for data from each monkey and conducting planned pair-wise comparisons between means using Fischer's Least Significant Difference test. Effects were considered significant at $p<0.05$. The planned comparisons were based on the following hypotheses: 1) cocaine would maintain responding at rates significantly greater than saline; and 2) the dopamine antagonist pretreatment would dose-dependently decrease responding maintained by cocaine compared to when no

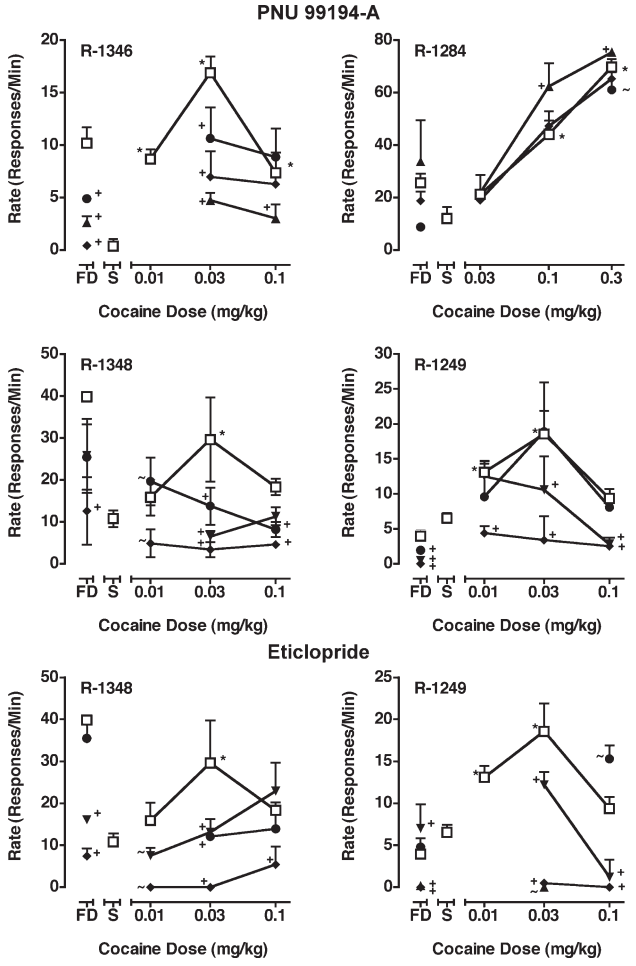

Fig. 1. Effects of PNU 99194-A (top panels) or eticlopride (bottom panels) on responding under a multiple FI 3-min schedule of food and cocaine presentation. Each panel represents mean (±S.D.) data from the last 3 days of responding for individual monkeys. Food data (FD) are from the FD1 component, collapsed across cocaine doses. Cocaine data are the average of both cocaine components (COC1 and COC2). Abscissa: dose of cocaine available in the drug component in mg/kg/injection; S: saline. Ordinate: response rate (responses/min). Different symbols represent different doses of antagonist. PNU 99194-A (top panel) doses (□ 0 mg/kg; ● 0.3 mg/kg; ▼ 0.56 mg/kg; ◆ 1.0 mg/kg; ▲ 3.0 mg/kg) or eticlopride (bottom panel) doses (□ 0 mg/kg; ● 0.001 mg/kg; ▼ 0.003 mg/kg; ◆ 0.01 mg/kg; ▲ 0.03 mg/kg). Note the difference in abscissa and ordinate scales across monkeys. Asterisks (*) indicate statistically significant difference in responding maintained by a dose of cocaine compared to saline. Plus symbols (+) indicate a statistically significant difference between baseline conditions and drug pretreatments for a particular dose of cocaine. Wave symbols (~) indicate that this dose condition was not included in the statistical analysis because not all cocaine-pretreatment dose combinations were tested.

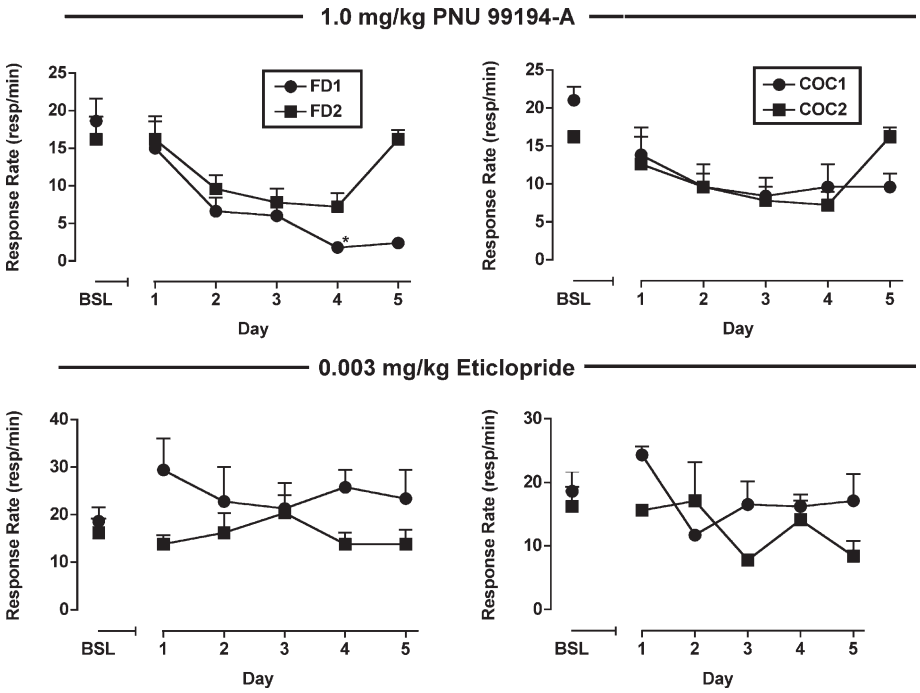

Fig. 2. Mean response rates (responses/min) for the 5-day treatment of PNU 99194-A (1.0 mg/kg; top panel) or eticlopride (0.003 mg/kg; bottom panel) in monkeys responding under the multiple fixed-interval 3-min schedule of food and cocaine presentation (FD1, COC1, FD2, COC2). Abscissa: day. Ordinate: response rate (responses/min). Each value represents mean data (±S.E.M.) from two to four monkeys tested when 0.03 mg/kg/injection cocaine was available for self-administration. Asterisk (*) indicates statistically significant difference in responding compared to the first session.

pretreatment was administered. To determine the effects of DA antagonist treatment over consecutive sessions, data presented in Figs. 2 and 5 were analyzed using a linear trend analysis as described in Stoops et al. (2003).

2.7. Drugs

(−)Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) was dissolved in sterile saline. Different doses were studied by changing the drug concentration. Stock solutions of (−)eticlopride (RBI, Natick, MA) and PNU 99194-A (generously donated by K. Svensson, Pharmacia and Upjohn, Kalamazoo, MI) were prepared in sterile saline up to concentrations of 1.0 mg/ml (eticlopride) or 10 mg/ml (PNU 99194-A).

3. Results

3.1. Experiment 1: multiple fixed-interval schedule

Under baseline conditions, responding was reliably maintained by 1.0-g food pellets and 0.03 mg/kg/injection cocaine. Cocaine-maintained responding varied as a function of dose (saline, 0.01–0.3 mg/kg/injection), with mean rates of responding being characterized as an inverted U-shaped function of dose, and peak rates occurring at 0.03 mg/kg/injection in three of four monkeys (Fig. 1, open squares). In all monkeys, response rates decreased when saline was substituted for cocaine (Fig. 1). Planned comparisons revealed that at least one dose of cocaine maintained significantly greater responding compared to saline in all monkeys. In three of four monkeys, food-reinforced response rates were lower than peak response rates in the cocaine components.

Pre-session administration of 1.0 mg/kg PNU 99194-A for 5 consecutive sessions resulted in session-dependent decreases in FD1 (Fig. 2 presents data from sessions in which 0.03 mg/kg/injection cocaine was available). In contrast, 1.0 mg/kg PNU 99194-A did not differentially affect FD2 responding across sessions. It can be seen from Fig. 2, that once responding was stable, the effects seen in COC1 were similar to those observed in COC2, and thus mean COC data will be presented for all PNU 99194-A doses. Similar effects from a trend analysis were observed when 0.1 mg/kg/injection cocaine was self-administered (data not shown). Planned comparisons revealed significant dose-dependent decreases in food-maintained responding (FD1) following pretreatment with at least one dose of PNU 99194-A in three of four monkeys (Fig. 1). In two subjects (R-1249 and R-1346), all doses of PNU 99194-A significantly decreased food-maintained responding. In three of four subjects, PNU 99194-A decreased cocaine-maintained responding, irrespective of the dose of cocaine available for self-administration, producing a downward shift in the cocaine dose–response curve (Fig. 1). In one monkey (R-1284), PNU 99194-A pretreatment resulted in statistically significant increases in cocaine-maintained responding.

All four monkeys showed schedule-appropriate responding under the FI schedule in all four components of the multiple schedule, as indicated by QL values greater than 0.25 (Table 1). In FD1, mean QL values ranged from 0.52 to 0.67 and were not affected by cocaine dose. Although high doses of self-

Table 1
Baseline quarter-life (QL) values under the multiple fixed-interval 3-min schedule[a]

Dose	FD1	FD2	COC1	COC2
Saline	0.60 (0.12)	0.49 (0.13)	0.42 (0.07)	0.43 (0.08)
0.01 Coc[§]	0.67 (0.12)	0.67 (0.06)	0.60 (0.08)	0.61 (0.05)
0.03 Coc	0.59 (0.09)	0.55 (0.10)	0.45 (0.13)	0.48 (0.13)
0.1 Coc	0.56 (0.08)	0.50 (0.13)	0.44 (0.10)	0.46 (0.12)
0.3 Coc[¶]	0.52 (0.07)	NR	0.42 (0.31)	0.36 (0.17)

$n=4$ except [§]$n=3$ and [¶]$n=2$.
NR=no responses; FD1=food-1; FD2=food-2; COC1=cocaine-1; COC2=cocaine-2.
[a] Values represent mean data (±S.E.M.) from all cocaine doses.

administered cocaine affected FD2 rates, FD2 QL values did not vary with cocaine dose, up to a dose that eliminated responding (Table 1). FD1 and FD2 QL values were not different from QL values determined from responding in COC1 and COC2 components. Administration of PNU 99194-A produced orderly changes in QL values (Table 2; top panel), which were not influenced by cocaine dose (data not shown). PNU 99194-A dose-dependently decreased QL values in each component, suggesting disruption in schedule control occurred.

A trend analysis indicated that the rate-decreasing effects of eticlopride did not significantly change across treatment days, as shown in Fig. 2 with 0.003 mg/kg eticlopride administered before sessions in which self-administration was maintained by 0.03 mg/kg/injection cocaine. Planned comparisons revealed that at least two doses of eticlopride significantly decreased food-maintained (FD1) responding in the two monkeys tested under these conditions; this effect was dose-dependent in R-1348 (Fig. 1). When the dose of cocaine that maintained peak response rates was available during the session, eticlopride dose-dependently decreased responding (Fig. 1). Increases in cocaine dose had little or no effect on the reductions in responding following eticlopride administration. Directly comparing the potency of eticlopride with PNU 99194-A in these two monkeys showed that eticlopride was approximately 2.0 log units more potent than PNU 99194-A. QL values decreased in a dose-dependent manner in both monkeys following eticlopride administration (Table 2; bottom panel), suggesting disruption in schedule control.

Table 2
Quarter-life (QL) values following PNU 91994-A or eticlopride in monkeys responding under the multiple fixed-interval 3-min schedule[a]

Dose	FD1	COC1	FD2	COC2
PNU 91994-A				
0.3 PNU	0.57 (0.10)	0.52 (0.08)	0.54 (0.29)	0.55 (0.08)
1.0 PNU	0.37 (0.05)	0.45 (0.14)	0.37 (0.20)	0.45 (0.14)
3.0 PNU	0.28 (0.03)	0.28 (0.20)	0.18 (0.13)	0.31 (0.11)
Eticlopride				
0.001 eticlopride	0.54 (0.08)	0.61 (0.03)	0.55 (0.06)	0.63 (0.04)
0.003 eticlopride	0.58 (0.03)	0.48 (0.02)	0.56 (0.03)	0.43 (0.03)
0.03 eticlopride[b]	0.24 (0)	NR	NR	0.40 (0)

[a] Each value represents mean data (±S.E.M.) from the last 3 sessions at all cocaine doses in four (PNU 91994-A) or two (eticlopride) monkeys.
[b] Represents one monkey.

3.2. Experiment 2: muliple second-order schedule

Under baseline conditions, responding was reliably maintained under the second-order FR 5 (FI 6-min:S) schedule of food and 0.1 mg/kg/injection cocaine presentation (Figs. 3 and 4). Planned comparisons revealed that in the drug component, cocaine-maintained responding was significantly greater than saline-maintained responding in all monkeys (Figs. 3 and 4). However, in only one of the four monkeys (R-1349) did cocaine-maintained responding vary as a function of dose in a manner that could be characterized as an inverted U-shape. Food-maintained responding was lower than cocaine-maintained responding in all monkeys. QL values in both components were indicative of schedule-appropriate FI responding, varying from 0.41 to 0.53 (data not shown).

Pretreatment drugs were administered for at least 5 consecutive sessions and up to 14 consecutive sessions. Fig. 5 shows mean data from the first 3 sessions of treatment (Days 1–3) and the last 3 sessions (Days 4–6) when 0.1 mg/kg/injection cocaine was available. There were no significant changes in response rates from the first day of treatment to the last day of treatment when the highest dose of PNU 99194-A was administered, although there did appear to be a trend downward in the cocaine component (Fig. 5). Similarly, the effects of the highest dose of eticlopride did not significantly change response rate from the first day to the last day throughout the chronic treatment (Fig. 5). When a lower cocaine dose maintained responding (0.03 mg/kg/injection), no significant differences across

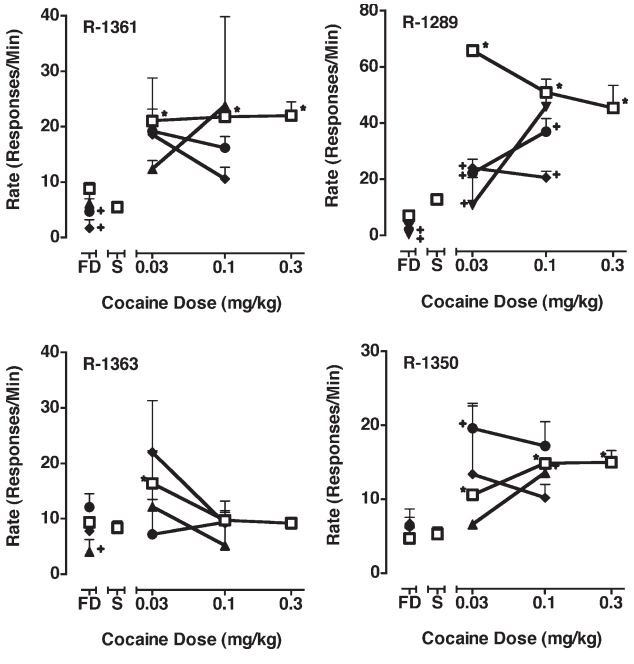

Fig. 3. Effects of PNU 99194-A on responding under a multiple second-order schedule of food and cocaine presentation. Each panel represents mean (±S.D.) data from the last 3 days of responding for individual monkeys. Abscissa: dose of cocaine available in the drug component in mg/kg/injection; FD: food component; S: saline. Ordinate: response rate (responses/min). Different symbols represent different PNU 99194-A doses. All other details are as described in Fig. 1.

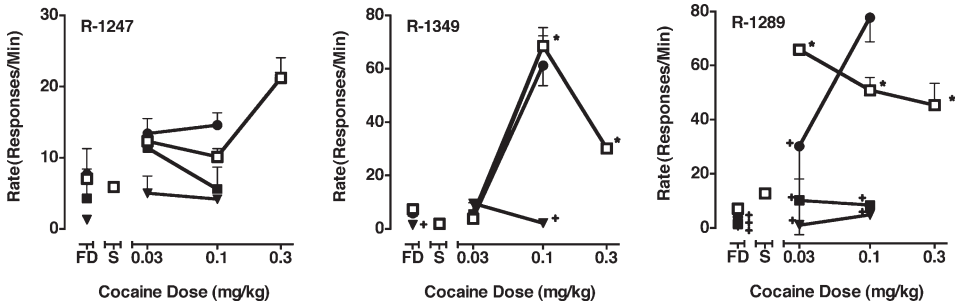

Fig. 4. Effects of eticlopride on responding under a multiple second-order schedule of food and cocaine presentation. Each panel represents mean (±S.D.) data from the last 3 days of responding for individual monkeys. Abscissa: dose of cocaine available in the drug component in mg/kg/injection; FD: food component; S: saline. Ordinate: response rate (responses/min). Different symbols represent different eticlopride doses. All other details are as described in Fig. 1.

treatment days was observed for either PNU 99194-A or eticlopride at any dose (data not shown).

In three of the four monkeys (R-1289, R-1363 and R-1361) tested with PNU 99194-A, statistically significant decreases in food-maintained responding were noted following at least one dose (Fig. 3). The lowest dose of PNU 99194-A that significantly decreased food-maintained responding differed in each monkey. Overall, the effects of PNU 99194-A on cocaine-maintained responding were modest and varied across monkeys. For example, in R-1289, PNU 99194-A significantly decreased cocaine-maintained responding, while in R-1350, a low PNU 99194-A dose in combination with 0.03 mg/kg cocaine resulted in increases in cocaine-maintained responding (Fig. 3). The effects of eticlopride were less variable between subjects. Eticlopride dose-dependently decreased or eliminated food- and cocaine-maintained responding in all monkeys (Fig. 4). There was no evidence of extrapyramidal side effects throughout the course of treatment with either drug.

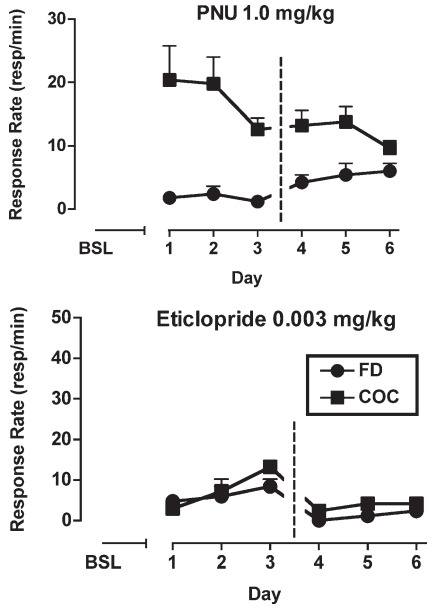

Fig. 5. Mean response rates (response/min) across days of treatment with PNU 99194-A (1.0 mg/kg; top panel) or eticlopride (0.003 mg/kg; bottom panel) in monkeys responding under a multiple second-order schedule. Abscissa: day. Ordinate: response rate (responses/min). Each value represents mean data (±S.E.M.) from three or four monkeys tested when 0.1 mg/kg/injection cocaine was available in the drug component. Days 1–3 represent the first 3 days of testing and Days 4–6 represent the last 3 sessions. For the group, the average number of treatment days was 10.75 for PNU 99194-A and 12 for eticlopride.

4. Discussion

The purpose of the present study was to compare the effects of the putative D_3 receptor antagonist PNU 99194-A and the D_2/D_3 receptor antagonist eticlopride on food- and cocaine-maintained responding in rhesus monkeys. To more fully characterize the behavioral effects of these DA antagonists, two different schedules of reinforcement were examined. When responding was maintained under an FI schedule and multiple presentations of food and cocaine occurred throughout the session, neither PNU 99194-A nor eticlopride showed selectivity in decreasing cocaine- relative to food-maintained responding. However, eticlopride appeared to produce greater overall reductions in responding compared to PNU 99194-A and was approximately 2.0 log units more potent than PNU 99194-A. When the conditions were changed such that responding was maintained by a conditioned reinforcer and the primary reinforcer occurred only once at the end of the component (i.e., second-order schedule), comparable results were obtained; neither drug had selective effects on cocaine- relative to food-maintained responding. These findings suggest that a DA receptor antagonist with 14-fold greater affinity for D_3 over D_2 receptors is not selective enough to support our hypothesis that blockade of D_3 receptors would attenuate cocaine-maintained responding relative to responding maintained by a non-drug reinforcer.

A unique characteristic of FI schedules is the relative independence between changes in response rates and reinforcement frequency (Zeiler, 1977). In an earlier study involving rhesus monkeys and seven D_2/D_3 antagonists, compounds with higher D_3 affinities were more potent at decreasing reinforcement frequency (i.e., cocaine intake) relative to response rates under an FI schedule (Nader et al., 1999). In the present study, both drugs disrupted patterns of responding as measured by QL values, suggesting that at the high doses these drugs affected schedule control. The present study extended our earlier work by using a multiple FI schedule to assess selectivity of the antagonists on cocaine self-administration compared to behavior maintained by a non-drug reinforcer. Support for the use of a

multiple schedule comes from previous studies using multiple schedules of food and cocaine presentation which showed that some drugs selectively decreased cocaine self-administration compared to food-maintained responding (Mello et al., 1992; Caine and Koob, 1994; Wojnicki et al., 1999). The present study also extended earlier work to include a compound with greater D_3 selectivity over D_2 receptors. The present findings using an FI schedule are similar to results in which responding was maintained under multiple FR schedules of food and cocaine presentation (Woolverton and Virus, 1989; Caine et al., 1994) and suggest that antagonists that are not selective, or have relatively low selectivity for D_3 compared to D_2 receptors, do not differentially affect responding maintained by drug and non-drug reinforcers, irrespective of the reinforcement schedule conditions.

A particular advantage of second-order schedules is that behavior can be maintained by only one or a few injections of cocaine, which allows for the study of "cocaine seeking" in the absence of accumulating effects of cocaine. In the present study, eticlopride decreased food- and cocaine-maintained responding in all subjects. In contrast, PNU 99194-A decreased responding maintained by food in 3 of the 4 monkeys but was less effective at decreasing cocaine-maintained responding. Results of previous research with rats responding under second-order schedules of cocaine presentation have strongly implicated the D_3 receptor in the reinforcing effects of cocaine (Pilla et al., 1999; Di Ciano et al., 2003). The disparity between these studies may be due to species differences or the D_3 compounds used. For example, a differential distribution of D_3 receptors has been found across species (Levant, 1998). In addition, the D_3 compounds used in the prior studies, BP 897 and SB 277011-A are 70 and 100 times more selective for D_3 compared to D_2 receptors, respectively (Pilla et al., 1999; Reavill et al., 2000). It remains to be determined whether the D_3 compounds used in those studies can selectively attenuate cocaine seeking relative to food-maintained responding in non-human primates.

A third possible reason for disparate results between studies is the dosing regimen used. Most studies, including those described above, administered drugs acutely, while the present study evaluated the effects of chronic (at least 5 consecutive sessions) treatments. One hypothesis involving antagonist treatments is that responding will decrease in a manner similar to extinction. Because extinction rarely occurs within one session, multiple assessments of the effects of treatment drugs may be necessary to mimic extinction. There was some evidence of reductions in responding from the first to the last day of treatment with PNU 99194-A in monkeys responding under the second-order schedule. Because under these conditions only one cocaine injection occurred at the end of the session, these findings further support the hypothesis that these drugs were blocking the reinforcing effects of cocaine (rather than the rate-altering effects of cocaine). Chronic drug treatment also has the advantage in that it allows for the determination of whether the initial acute reductions are attenuated over time. As it relates to pharmacotherapies, tolerance to treatment-induced decreases in cocaine self-administration is an important consideration. In the present study, there was no evidence of tolerance developing to the rate-decreasing effects of either dopamine antagonist.

The development of highly selective D_3 antagonists has been challenging (reviewed in Newman et al., 2005) and early preclinical studies were limited by the available pharmacological tools. While the present study did not support the hypothesis that D_3 receptor blockade would selectively decrease cocaine- relative to food-reinforced responding, more selective pharmacological tools are now available. The present findings highlight the importance of using multiple schedules, in which responding is maintained by a non-drug reinforcer as well as cocaine, and the importance of studying chronic treatment regimens.

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