ABSTRACT
The present study examined the effects of two novel dopamine D₃ receptor compounds, NGB 2904 \[N-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)-9H-fluorene-2-carboxamide\] and CJB 090 \[N-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide\], on the reinforcing and discriminative stimulus effects of cocaine in rhesus monkeys.

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ABBREVIATIONS: DA, dopamine; hD₂L, human D₂L receptor; hD₃, human D₃ receptor; CJB 090, \[N-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide\]; NGB 2904, \[N-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)-9H-fluorene-3-carboxamide\]; ANOVA, analysis of variance; TO, time-out; FR, fixed ratio; S, saline; FI, fixed interval; SB277011-A, \[N-[2-(6-cyano-3,4-dihydro-1H-isoquinolin-2-cyclohexyl)quinoline-4-carboxamide\]; BP 897, \[N-(4-(4-2-methoxyphenyl)piperazin-1-yl)butyl-2-naphthamide\] HCl; PNU 99194-A, 5,6-dimethoxy-N,N-dipropyl-2,3-dihydro-1H-inden-2-amine HCl.

Cocaine abuse is a major problem in the United States and worldwide [National Institute on Drug Abuse (NIDA), 2004; World Health Organization, 2004]. In 2002 alone, there were an estimated 1.1 million new users of cocaine, and the 2003 National Survey on Drug Use and Health reported that 34.9 million Americans 12 years of age and older confirmed using cocaine at least once in their lifetime (Substance Abuse and Mental Health Services Administration, 2004). Despite over 30 years of research, there is no safe and effective pharmacotherapy to offer those afflicted by cocaine addiction (Mello
and Negus, 1996; Carroll et al., 1999; Platt et al., 2002), although several clinical trials are currently underway (Gorelick et al., 2004; O’Brien, 2005; Vocci et al., 2005).

The dopamine (DA) system is thought to play a primary role in the behavioral and reinforcing effects of cocaine (Ritz et al., 1987). Within the DA system, two superfamilies of receptors have been identified, D1- and D2-like receptors, and preclinical data suggest that both are involved in the behavioral actions of cocaine (e.g., Caine et al., 2000). The D₃ receptor subtype belongs to the D₂-like superfamily of receptors and coexists with D₂ receptors in mesolimbic areas of the brain (Sokoloff et al., 1990). Because D₂ receptors are primarily localized in limbic brain regions, compounds selectively blocking D₃ receptors may be free of extrapyramidal effects (Sokoloff et al., 1990; Levant, 1997). Furthermore, autoradiographic studies have demonstrated higher densities of D₃ receptors in cocaine overdose victims compared with noncocaine-abusing controls (Staley and Mash, 1996). Taken together, these studies support a hypothesis that D₃ receptors may be an important therapeutic target in cocaine abuse.

Pharmacological tools provide evidence for a possible role of D₃ receptors in the discriminative and reinforcing effects of cocaine (e.g., Caine and Koob, 1993). Pretreatments with the D₂/D₃ agonists quinpirole or 7-hydroxy-N,N-di-n-propyl-2-aminotetralin produced dose-dependent decreases in cocaine self-administration in rats (Caine and Koob, 1993). More recently, the D₃-selective partial agonist BP 897 has been shown to decrease cocaine seeking by rats self-administering cocaine under a second-order schedule (Pilla et al., 1999). Unlike the full agonists, BP 897 does not maintain self-administration in rats or monkeys (Pilla et al., 1999; Beardsley et al., 2001) or mimic the discriminative stimulus effects of cocaine or methamphetamine in monkeys (Beardsley et al., 2001). One goal of the present study was to examine, in rhesus monkeys, another D₃ partial agonist, CJB 090, in cocaine discrimination and cocaine self-administration studies.

D₂-like receptor antagonists block discriminative and reinforcing effects of cocaine in rodents and monkeys (e.g., Kleven et al., 1990; Caine et al., 2000). Advances in medicinal chemistry have resulted in development of D₃-selective antagonists (Newman et al., 2005). In a rodent model of relapse, the D₃ antagonists SB-277011-A and NGB 2904 inhibited cocaine-induced drug seeking (Gilbert et al., 2005; Gal and Gyertyan, 2006). Also in rodents, SB-277011-A decreased cocaine self-administration under several conditions (Vorel et al., 2002; Di Ciano et al., 2003; Xi et al., 2005, 2006). A second goal of the present study was to assess the ability of another D₃-selective antagonist, NGB 2904, to alter the discriminative and reinforcing effects of cocaine in rhesus monkeys.

The D₃ antagonist NGB 2904 binds with approximately 56-fold selectivity at the human D₃ receptor (hD₃) compared with the human D₂ receptor (hD₂), whereas the D₂ partial agonist CJB 090 has approximately 50-fold selectivity at the hD₃ compared with the hD₂ receptor (Grundt et al., 2005). Both NGB 2904 and CJB 090 are twice as selective for hD₃ receptors as is quinpirole, which has 23-fold selectivity for D₃ over D₂ receptors in HEK 293 cells (R. Luedtke, unpublished data). Because neither drug had been evaluated in nonhuman primates, the first experiment examined their ability to block quinpirole-induced yawning, suggested by behavioral experiments in rodents to be D₃ receptor mediated (Collins et al., 2005). The second experiment examined the ability of each compound to substitute for cocaine and to shift the cocaine dose-response curve in monkeys trained to discriminate cocaine. As a positive control, we also evaluated the nonselective D₃/D₂ antagonist haloperidol in these same monkeys. The third experiment assessed the ability of NGB 2904 and CJB 090 to alter cocaine- and food-reinforced responding using a second-order schedule that has been shown to be sensitive to D₃ partial agonist effects (Pilla et al., 1999).

**Materials and Methods**

**Subjects**

For all experiments, adult male rhesus monkeys (*Macaca mulatta*) served as subjects. Monkeys R-1360, R-1416, R-1425, R-1429, and R-1430 were drug naive at the start of the study. All other monkeys had a history of cocaine self-administration (Lile et al., 2003; Claytor et al., 2006). Each monkey was fitted with a nylon collar and trained to sit in a primate restraint chair as described previously (Claytor et al., 2006). At the start of the study, the body weights of the monkeys were between 8 and 14 kg and were maintained at approximately 95% of free-feeding weights. Their diet consisted of banana-flavored pellets (Bio-Serv, Frenchtown, NJ) earned during the experimental sessions (experiments 2 and 3) and supplemental feeding of Lab Diet Monkey Chow, given no sooner than 30 min postsession. 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.

**Experiment 1: Effects of NGB 2904 and CJB 090 on Quinpirole-Induced Yawning**

**Procedure.** For these studies, a total of seven monkeys with a prior cocaine history were used. In three monkeys a quinpirole dose-response curve was determined. Before each experimental session, the monkey was taken from its home cage, placed in a primate restraint chair, and given an i.m. injection of saline or quinpirole (0.0032–0.3 mg/kg), with doses tested in random order. Immediately after the injection, occurrences of yawning were counted in the ensuing 30 min. Full extension of the jaws, withdrawal of the lips, and exposure of the teeth distinguished yawning (Code and Tang, 1991). Each quinpirole dose was tested twice in each monkey. Following completion of the quinpirole dose-response curve, the effects of NGB 2904 (3.0–5.6 mg/kg) and CJB 090 (1.0–3.0 mg/kg) alone and in combination with quinpirole were examined. Because 0.1 mg/kg quinpirole elicited the greatest number of yawns, NGB 2904 and CJB 090 were examined in combination with that dose of quinpirole. On separate occasions, test compounds were administered 15, 30, or 120 min before quinpirole or saline. Each combination was tested once per animal at 15 and 30 min and twice per animal at 120 min (n = 3 per dose and time point, unless specified). The primary dependent variable was total yawns in 30 min. Experimental sessions were randomly videotaped, and a person other than the experimenter observed these sessions with an interobserver variability of <5%.

**Data Analysis.** Quinpirole dose-response curve data are presented as the mean (±S.E.M.) number of yawns during the 30-min observation period. A one-way, repeated-measures ANOVA was used to determine whether agonist-induced yawning was significantly different from saline, followed by planned comparisons (Fisher’s least significant difference test) of each dose to saline. Linear and nonlinear regression models (i.e., first versus second-order polyno-
were used to analyze the shape of the dose-effect curve. These data were analyzed using GB-Stat (Silver Spring, MD). The effects of NGB 2904 and CJB 090 on 0.1 mg/kg quinpirole-induced yawning, shown as a percentage of 0.1 mg/kg quinpirole baseline, were assessed using a two-way linear mixed-effects regression model for unbalanced repeated measures data (Jennrich and Schluchter, 1986), followed by planned comparisons. Data analyses were performed using SAS PROC MIXED version 8.2 (SAS Institute, Cary, NC). Statistical significance was defined as $p < 0.05$.

**Drugs.** Quinpirole (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile saline to a concentration of 1.0 mg/ml. CJB 090 and NGB 2904 (NIDA-Intramural Research Program, Baltimore, MD) were synthesized as described in Newman et al. (2003) (see Fig. 1) and were dissolved in 25% β-cyclodextrin, diluted to concentrations of 0.1 to 10 mg/ml, and filtered. If necessary, heat and sonication were applied to assist with solubility. All solutions were delivered i.m. in a volume of approximately 0.5 ml/10 kg. Saline was administered as a 0.5-ml injection.

**Experiment 2: Effects of NGB 2904 and CJB 090 on Cocaine Discrimination**

**Apparatus.** For experimental sessions, an intelligence panel (48 × 36 cm) was attached to the front of the home cage. The panel contained two clear response keys located 8 cm apart along the top half of the panel and within easy access to the monkey sitting at the front of the cage. Three small red stimulus lights were located behind each response key. A pellet dispenser was mounted to the back of the intelligence panel and dispensed 300 mg of banana-flavored pellets (P.J. Noyes Co., Lancaster, NH). Experimental sessions began with illumination of all six stimulus lights. During food presentation, the stimulus lights extinguished for a 2-s time-out (TO), and responding had no scheduled consequence. Experimental sessions and data acquisition were accomplished via a Power Macintosh computer system and National Instruments interface.

**Training Procedure.** Six monkeys were trained to discriminate i.m. cocaine (0.3 mg/kg) from saline using a two-key, fixed-ratio (FR) drug discrimination procedure in which responding was maintained by food presentation. The FR value was individually determined to maintain reliable discrimination (FR 30 for R-1360, R-1416, R-1425, and R-1430 and FR 50 for R-1362 and R-1429). Sessions were conducted at approximately the same time each day, 6 to 7 days/week. One key represented the correct choice following saline administration, and the other key represented the correct choice following cocaine administration. Before the beginning of this experiment, key assignments were designated for each animal. For three monkeys, the right key corresponded with saline, and the left key corresponded with cocaine. For the other three monkeys, the response key assignments were reversed.

At the beginning of the training session, an i.m. injection of either saline or 0.3 mg/kg cocaine was administered and was followed by a 10-min TO period. During the TO period, all stimulus lights were extinguished, and responding had no consequence. Following the initial TO period, a 15-min response period was signaled by the illumination of all six stimulus lights. During the response period, food was available under an FR schedule in which 30 or 50 consecutive responses emitted on the appropriate key activated the pellet dispenser to deliver one pellet. Delivery of the pellet was accompanied by all stimulus lights extinguishing for a 2-s TO. Conversely, responses emitted on the inappropriate key reset the FR value for the correct lever. Furthermore, completion of an entire FR on the inappropriate key resulted in stimulus light extinguishment and a 2-s TO but no pellet delivery. The sequence of saline (S) or cocaine (C) presentation was quasi-random across days and occurred as follows: SSCSCSSCSC. Training continued until the following criteria for stimulus control were met for three of the last four training sessions: a) at least 80% of the responses emitted before delivery of the first reinforcer occurred on the injection-appropriate key, and b) at least 90% of the total session responses occurred on the injection-appropriate key.

**Testing Procedure.** Once performance met the above criteria for stimulus control, test sessions were implemented among continued training sessions. Test sessions were identical to training session with the exception that completion of the FR requirement on either key was reinforced with food presentation; switching between keys reset the FR value. Test sessions were conducted no more than twice per week and only if training criteria had been met for the two preceding sessions and if presentation of both saline and the cocaine training dose had occurred since the previous test session. In the event that a monkey's performance fell below criteria, the animal was returned to training conditions until discrimination again met criteria for at least two consecutive sessions. Presentation of either saline or cocaine during test sessions was randomized, and determination of each condition occurred at least twice in each monkey.

The first set of experiments determined a cocaine dose-response curve (saline, 0.03–0.56 mg/kg i.m.) in all six monkeys. The second set of experiments evaluated the interaction between CJB 090 (0.03–3.0 mg/kg i.m.) and cocaine (saline, 0.03–0.56 mg/kg i.m.) and between NGB 2904 (0.03–3.0 mg/kg i.m.) and cocaine (saline, 0.03–0.56 mg/kg). For these test sessions, a pretreatment injection of CJB 090 or NGB 2904 was administered 5 min before administration of saline or cocaine, i.e., 15 min before the start of the response period. All other conditions were identical to the initial testing sessions. Presentation of the possible combinations of pretreatment dose and cocaine dose was randomized, and each test condition occurred at least twice in each monkey.

As a positive control, the DA D2-like antagonist haloperidol (0.003–0.03 mg/kg) was studied with the training dose of cocaine (0.3 mg/kg) in four monkeys (R-1416, R-1425, R-1429, and R-1430). The experimental parameters were identical to those used to evaluate CJB 090 and NGB 2904, with the exception that haloperidol pretreatments were only tested against the training dose of cocaine (0.3 mg/kg). Each dose was tested twice, in random order, and after completion of the CJB 090 or NGB 2904 experiments.

**Data Analysis.** For test sessions, the primary dependent variables were the percentage of responding that occurred on the cocaine-appropriate key and the response rate (total number of responses/length of the response period). All drug combinations were tested at least twice. Dose-response curves were determined for individual animals and are represented as the mean (±S.D.) of all determinations for that animal. In addition, group data are shown as the mean (±S.E.M.) of three monkeys per dose combination. Drug doses that engendered at least 80% cocaine-appropriate responding for the duration of a test session were considered to have substituted for the discriminative stimulus effects of cocaine. For doses that reduced the rate of responding to levels where no reinforcers were obtained, the rate of responding was calculated and included in the
Experiment 3: Effects of NGB 2904 and CJB 090 on Cocaine- and Food-Maintained Responding

**Surgery.** Under sterile conditions, each monkey was surgically prepared with an indwelling i.v. 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 s.c. to an incision made in the skin. The vascular access port was positioned to be within easy reach of the monkey seated in the primate chair (Primate Products, Redwood City, CA). An intelligence panel (48 cm; Med Associates, East Fairfield, VT) designed to accommodate a primate chair (Priclone, East Fairfield, VT) was applied to the incision sites.

**Apparatus.** Experimental sessions were conducted in ventilated and sound-attenuated chambers (150 × 74 × 76 cm; 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. One-gran 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.

**Procedures.** Seven monkeys responded under a two-component multiple second-order schedule of food and cocaine presentation as described previously (Claytor et al., 2006). The second-order schedule used was an FR 5 (FI 6-min: S) schedule. Under these conditions, the first response after 6 min (the FI) produced a brief 2-s stimulus, and completion of the fifth FI resulted in either food or cocaine presentation. Monkeys were initially trained under a multiple schedule in which responding in the first component was maintained by banana-flavored food pellets, and responding in the second component was maintained by cocaine (0.1 mg/kg/injection). For these studies, the conditions were changed such that the same reinforcer was presented in both components, and only one lever was active. Responding by monkeys R-1289, R-1349, R-1361, and R-1498 was maintained by 0.1 mg/kg/injection cocaine, whereas responding by monkeys R-1247, R-1350, and R-1363 was maintained by food (five 1.0-g banana-flavored pellets) presentation. Illumination of the amber lights above a lever signaled the beginning of a session; completion of an FI 6-min resulted in extinction of the amber stimulus light and illumination of the red stimulus light for 2 s followed by another FI 6-min schedule. During delivery of a reinforcer, the amber light was extinguished, and the red light above the appropriate lever was illuminated for 10 s. Components lasted 45 min or until a reinforcer was delivered; a 2-min TO separated components.

For all monkeys, before each experimental session, the area on the back of the animal containing the port was cleaned with 95% EtOH and Betadine, and a 22-gauge Huber Point Needle (Access Technologies) was inserted into the port. For monkeys in the food reinforcement group, the port was flushed with heparinized saline (or drug pretreatment), and the needle was removed before the session. For monkeys self-administering cocaine, the Huber Point Needle connected the venous catheter to an infusion pump. The pump was operated for approximately 3 s, filling the catheter and port with the concentration of cocaine available during the experimental session. If a test drug was administered pressession, this occurred before operation of the pump. After each session, the port and catheter were filled with heparinized saline (100 U/ml) to prevent clotting.

**Dose-Response Curves and Drug Pretreatments.** For the monkeys self-administering cocaine, a cocaine dose-response curve was determined before the beginning of pretreatment studies. When responding maintained by the baseline dose (0.1 mg/kg/injection) was stable (±20% of the mean for three 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. After a return to baseline for at least five consecutive sessions, a different dose of cocaine (0.03–0.3 mg/kg/injection) was substituted for the 0.1 mg/kg/injection cocaine dose; a dose of cocaine was available for at least five consecutive sessions. After a particular dose was evaluated, there was a return to baseline conditions for at least five sessions. Cocaine doses were tested in random order for each monkey.

For monkeys in both groups, the effects of NGB 2904 (1.0–5.6 mg/kg) and CJB 090 (0.3–3.0 mg/kg) were examined. Pretreatments were evaluated under baseline conditions (0.1 mg/kg/injection cocaine or five 1-g banana pellets). A dose of CJB 090 or NGB 2904 was administered i.v. immediately before the session for five consecutive sessions. Three doses were studied in each monkey, with doses tested in random order. There was a return to baseline (no treatment) for at least five consecutive sessions before evaluation of another dose of CJB 090 or NGB 2904.

**Data Analysis.** The primary dependent variable was response rate (responses per minute). For the analysis of the cocaine dose-response curve, mean data from the last three sessions for each monkey, at each cocaine dose, were included in the analysis using a one-way repeated-measures ANOVA with cocaine dose as the factor. For analysis of the effect of component on baseline responding maintained by cocaine or food, paired Student’s t tests were used to compare the mean rates of responding within subjects. For each test compound, mean data from the last three sessions were analyzed using two-way repeated measures ANOVAs (component × dose). For all analyses, p < 0.05 was considered statistically significant. If there was a significant main effect from the ANOVA, planned comparisons of each dose of D3 compound to baseline were conducted with Fisher’s least significant difference test.

**Drugs.** (-)Cocaine HCl was dissolved in 0.9% saline. Different doses were studied by changing the drug concentration. CJB 090 and NGB 2904 were prepared as described above and injected i.v. immediately before the session.

**Results**

Experiment 1: Effects of NGB 2904 and CJB 090 on Quinpirole-Induced Yawning

Following saline administration, monkeys did not yawn during the 30-min observation period (Fig. 2A, S). A one-way ANOVA revealed that quinpirole induced increases in yawning compared with vehicle [F(5,35) = 16.1, p < 0.05]. The overall shape of the dose-response curve was characterized as an inverted U-shaped function of dose with 0.1 mg/kg quin-


picrole representing the peak of the curve (17.5 ± 2.2 yawns). This dose of picrole was used in combination with CJB 090 and NGB 2904. CJB 090 (1.0 and 3.0 mg/kg) and NGB 2904 (3.0 and 5.6 mg/kg) significantly attenuated picrole-induced yawning (Fig. 2B). Post hoc tests revealed that both doses of CJB 090 significantly decreased yawning when administered 15, 30, or 120 min before picrole (all p < 0.01). NGB 2904 (3.0 mg/kg) significantly attenuated picrole-induced yawning when administered 15 or 120 min before picrole (p < 0.05) but not when administered 30 min before picrole (p = 0.08; Fig. 2B). Likewise, 5.6 mg/kg NGB 2904 attenuated yawning when administered 30 (p < 0.05) or 120 (p < 0.01) min before picrole (Fig. 2B). No monkeys were administered 5.6 mg/kg NGB 2904 at the 15-min time point. CJB 090 alone (1.0 and 3.0 mg/kg), given 2 h before recording, did not elicit significant yawning (data not shown), with peak effects occurring following 1.0 mg/kg (2.7 ± 3.3 yawns in 30 min).

**Experiment 2: Effects of NGB 2904 and CJB 090 on Cocaine Discrimination**

**Training and Control Performance.** For this study, monkeys R-1360, R-1416, R-1425, R-1430, R-1362, and R-1429 were trained to discriminate cocaine. The number of training sessions necessary to obtain stable and reliable cocaine discrimination ranged from 126 to 433 (R-1425, 126; R-1429, 150; R-1430, 155; R-1362, 172; R-1416, 221; and R-1360, 433 sessions). In all monkeys, cocaine dosage-dependent increased the percent cocaine-appropriate responding; full substitution was observed with the training dose and higher cocaine doses (Figs. 3 and 4, top, closed circles). During test sessions, response rates ranged from 0.94 to 2.28 responses/s following saline administration and from 1.47 to 2.84 responses/s following administration of the training dose (Figs. 3 and 4, bottom, closed circles). As a group, response rates did not vary as a function of cocaine dose, except when the highest dose of cocaine was administered (0.56 mg/kg).

**Effects of CJB 090, NGB 2904, and Haloperidol.** No doses of CJB 090 (0.1–3.0 mg/kg) substituted for cocaine in any of the monkeys tested (Fig. 3, top, left axis, open symbols). These doses of CJB 090 did not significantly affect response rates compared with rates observed following saline injections (Fig. 3, bottom). When given in combination with cocaine, lower doses of CJB 090 (0.1 and 0.3 mg/kg) did not shift the cocaine dose-response curves (data not shown). Both 1.0 and 3.0 mg/kg CJB 090 shifted the cocaine dose-response curve to the right at doses that did not affect response rates (Fig. 3). In the absence of CJB 090, the ED50 for cocaine varied from 0.11 to 0.25 mg/kg. Pretreatment with 1.0 and 3.0 mg/kg CJB 090 increased the ED50 dose of cocaine in all three monkeys (see Fig. 3).

No doses of NGB 2904 (0.1–3.0 mg/kg) substituted for cocaine in any of the monkeys tested (Fig. 4, top, above SAL, open symbols; lower doses not shown). These doses of NGB 2904 did not significantly affect response rates compared with rates observed following saline injections (Fig. 4, bottom, above SAL; lower doses not shown). When given in combination with cocaine, NGB 2904 (1.0–3.0 mg/kg) produced equivocal results, attenuating (R-1430), potentiating (R-1360), or not affecting (R-1429) the cocaine dose-response curve (Fig. 4). Only in monkey R-1430 did NGB 2904, in combination with cocaine, affect response rates (Fig. 4, bottom). As a positive control, the D2-like antagonist haloperidol was also tested in combination with the training dose of cocaine (0.3 mg/kg). Haloperidol (0.003–0.056 mg/kg) dose-dependently decreased the frequency of cocaine-appropriate responding in three (R-1416, R-1425, R-1429) of four monkeys (Table 1). In the fourth monkey (R-1430), haloperidol reduced cocaine-appropriate responding, but not in an orderly manner. In all animals, haloperidol produced some level of catalepsy and decreased response rates in all subjects.

**Experiment 3: Effects of NGB 2904 and CJB 090 on Cocaine- and Food-Maintained Responding**

**Control Performance.** For monkeys self-administering cocaine, response rates varied as a function of cocaine dose during the two components of the session (p < 0.05), and responding was above rates observed when saline was available (data not shown). The baseline dose of cocaine (0.1 mg/kg) was on the ascending limb or at the peak of the cocaine dose-response curve in both components, except in...
Cocaine-maintained responding was significantly higher in the second component compared with the first component \((p = 0.005)\), whereas food-maintained responding did not differ between components (Table 2). Food-maintained responding remained stable throughout the experiment, with mean rates of 6.0 and 11.0 responses/min in the first and second components, respectively, which were below rates of responding observed in the cocaine self-admin-
D3-selective partial agonist CJB 090 and the D3-selective evaluated in several nonhuman primate models. Both the self-administration. The current findings further support the reotypy, at doses that affected cocaine discrimination and drug produced D2-like side effects, such as catalepsy or steiveduced equivocal effects on cocaine discrimination and did not affect rates of cocaine- or food-maintained responding at doses that reversed quinpirole-induced yawning. Neither compound substituted for cocaine and decreased cocaine- and food-maintained responding. In contrast, NGB 2904 dose-dependently reversed quinpirole-induced yawning. Neither compound was potent at decreasing food-maintained responding [p < 0.01; Fig. 5B] compared with cocaine-maintained responding. Planned comparisons with baseline revealed that food-maintained responding was significantly reduced following 1.0 mg/kg CJB 090 in the second component and following 0.3 mg/kg NGB 2904 in the second component. In contrast, no dose of NGB 2904 (1.0–5.6 mg/kg) significantly affected cocaine-maintained responding. In contrast, NGB 2904 produced equivocal effects on cocaine discrimination and did not affect rates of cocaine- or food-maintained responding at doses that reversed quinpirole-induced yawning. Neither drug produced D2-like side effects, such as catalepsy or stereotypy, at doses that affected cocaine discrimination and self-administration. The current findings further support the continued development of compounds with high affinity at D3 receptors as potential treatment agents for cocaine abuse.

There is growing interest in the use of D3 compounds in preclinical models of cocaine abuse (see Newman et al., 2005). Partial agonists may provide a unique versatility as a potential pharmacotherapeutic treatment because the efficacy of the compounds should vary with levels of extracellular DA competing for the receptor (Pulvirenti and Koob, 1994; Childress and O’Brien, 2000; Platt et al., 2002; Negus, 2006). In contrast, an antagonist would be expected to decrease cocaine seeking and decrease the positive subjective effects of cocaine, irrespective of levels of extracellular DA.

**Table 1**

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<td>Cacaine (0.1 mg/kg/injection)</td>
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<td>12.6 (6)</td>
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<td>Food (1.0 mg/kg/injection)</td>
<td>5.4 (4.2)</td>
<td>8.4 (3.6)</td>
<td>4.2 (2.4)</td>
<td>6.6 (3)</td>
</tr>
</tbody>
</table>

**Table 2**

Mean (± S.D.) rates of responding (resp/min) under baseline conditions in individual monkeys.

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Component 1</th>
<th>Component 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response (S.D.)</td>
<td>Response Rate (S.D.)</td>
<td></td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
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<td>---</td>
</tr>
<tr>
<td>R-1289</td>
<td>44.4 (22.2)</td>
<td>54.0 (13.8)</td>
</tr>
<tr>
<td>R-1327</td>
<td>5.4 (4.2)</td>
<td>8.4 (3.6)</td>
</tr>
</tbody>
</table>

**Discussion**

The effects of two novel DA D3-selective compounds were evaluated in several nonhuman primate models. Both the D3-selective partial agonist CJB 090 and the D3-selective antagonist NGB 2904 dose-dependently reversed quinpirole-induced yawning. Neither compound substituted for cocaine in drug discrimination studies. CJB 090 blocked the discriminative stimulus effects of cocaine and decreased cocaine- and food-maintained responding. In contrast, NGB 2904 produced equivocal effects on cocaine discrimination and did not affect rates of cocaine- or food-maintained responding at doses that reversed quinpirole-induced yawning. Neither drug produced D2-like side effects, such as catalepsy or stereotypy, at doses that affected cocaine discrimination and self-administration. The current findings further support the
quinpirole dose-response curve was mediated via D3 receptors. Extending earlier results in rats, the D2/D3 agonist quinpirole was shown to significantly induce yawning in monkeys (Collins et al., 2005). There seems to be some species differences because CJB 090 is nearly 100-fold selective for D3 receptors over D2 receptors in rat-transfected cell lines (Newman et al., 2003). Functional in vitro assays of agonist-induced mitogenesis established that CJB 090 induced only up to a 29.7% increase in mitogenesis, suggesting that CJB 090 functioned as a D3-selective partial agonist (Newman et al., 2003). However, CJB 090 did not show a behavioral profile that was indicative of a D3 agonist because it neither elicited significant yawning when administered alone nor substituted for cocaine in drug discrimination. This is similar to what has been reported with BP 897, a purported D3 partial agonist, which does not have cocaine-like discriminative stimulus effects or maintain self-administration in rats and monkeys (Beardsley et al., 2001). Taken together, these findings suggest that identification of partial agonists using in vitro functional assays may not predict agonist-like activity in vivo. However, when evaluated in combination with cocaine, CJB 090 was able to attenuate the discriminative-stimulus effects of cocaine, resulting in rightward shifts in the cocaine dose-response curve. Attenuation of the cocaine cue by CJB 090 was surmountable by increasing doses of cocaine. When tested in monkeys self-administering cocaine under a second-order schedule, CJB 090 also decreased cocaine-maintained responding. Doses of CJB 090 that decreased cocaine self-administration also decreased responding maintained by food, suggesting nonselective reductions in cocaine reinforcement (but see Nader et al., 2002). Likewise, BP 897 has been shown to reduce cue-induced reinstatement in rats, to attenuate the discriminative-stimulus effects of cocaine in mice, and to decrease cocaine seeking in rats responding under a second-order schedule (Pilla et al., 1999; Beardsley et al., 2001; Cervo et al., 2003; Gilbert et al., 2005). Taken together, these data suggest that in the presence of high dopaminergic tone, i.e., after cocaine administration, DA D3 receptors play a modulatory role in the production of cocaine-induced interoceptive cues and in the positive reinforcing effects of cocaine.

NGB 2904 has been classified as an antagonist at DA D3 receptors with high binding affinity (1.4 nM) at the hD3 receptor and >150-fold selectivity for primate D3 receptors over primate D2L receptors (Yuan et al., 1998). As was noted with CJB 090, species variation has been observed with NGB 2904. For example, NGB 2904 showed 830-fold selectivity for D3 over D2 receptors in cloned rat DA receptors and only 56-fold selectivity in cloned human DA receptors (Newman et al., 2003; Grundt et al., 2005). Irrespective of these binding affinities, NGB 2904 selectively and potently inhibited D3 receptors as demonstrated by antagonism of quinpirole-stimulated mitogenesis (Yuan et al., 1998), and it decreased quinpirole-induced yawning in monkeys (present study). In addition, NGB 2904 injections did not maintain self-administration in monkeys (J. L. Martelle, J. T. Ross, and M. Nader, unpublished data) and did not substitute for cocaine in drug discrimination, further suggesting a lack of agonist action.

In the present study, NGB 2904 had equivocal effects on cocaine discrimination and was inactive in decreasing cocaine-maintained responding under a second-order schedule. Doses that were effective in blocking quinpirole-induced yawning did not affect cocaine self-administration in rhesus monkeys. In an earlier study using the moderately selective D3 antagonist PNU 99194-A, which has an approximate 100-fold lower affinity at D3 receptors compared with NGB 2904,
decreases in cocaine- and food-maintained responding were observed (Claytor et al., 2006). The fact that we did not observe significant effects on cocaine self-administration following NGB 2904 administration under conditions in which PNU 99194-A did affect responding suggests that NGB 2904, despite its higher affinity and selectivity for D₃ receptors, may not be an effective pharmacotherapy for cocaine abuse. However, it should be pointed out that D₃ receptor antagonists have shown promise in rodent models of cocaine abuse (Ashby et al., 2003; Di Ciano et al., 2003; Gilbert et al., 2005; Xi et al., 2005, 2006). Previous experiments have demonstrated that higher doses of NGB 2904 produce diminished D₃ antagonist-like effects compared with intermediate doses (Gilbert et al., 2005). This finding could be related to the high lipophilicity of NGB 2904 leading to low bioavailability, poor pharmacokinetics, and significantly lower absolute levels in the brain after i.v. administration compared with another D₃-selective antagonist, SB-277011-A (Reavill et al., 2000), under the same conditions (Newman et al., 2005). Another possibility for the equivocal effects of NGB 2904 on cocaine discrimination, but not CJB 090, may be related to the affinity of each compound at D₂ receptors (Kᵢ = 112 versus 24 nM for NGB 2904 and CJB 090, respectively; Grundt et al., 2005). However, there was no evidence of catalepsy induced by any dose of CJB 090 or NGB 2904, which suggests that D₂ receptors are not likely involved in the behaviors observed in this study. Nevertheless, the precise contribution of D₂ and D₃ receptors in the behavioral effects of NGB 2904, CJB 090 and other “D₃-selective” compounds will require additional studies with highly selective D₂ and D₃ agonists, which are not currently available.

The present study aimed to examine: 1) the effects of a potent and selective D₃ antagonist (NGB 2904) and partial agonist (CJB 090) on the reinforcing and discriminative stimulus effects of cocaine in rhesus monkeys and 2) whether or not a D₃ partial agonist, as determined by quinpirole-stimulus effects of cocaine in rhesus monkeys and 2) whether or not a D₃ partial agonist, as determined by quinpirole-stimulated mitogenesis in Chinese hamster ovary cells, would affect responding suggest that positive N-methyl-D-aspartate receptor antagonists (e.g., ketamine) are effective in reversing cocaine withdrawal (Berk et al., 1997). However, there is a limited understanding of the mechanisms underlying these effects.

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References


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