Effects of Two Novel $D_3$-Selective 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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ABSTRACT

The present study examined the effects of two novel dopamine $D_3$ receptor compounds, NGB 2904 [N-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-9H-fluorene-2-carboxamide], an antagonist, and CJB 090 [N-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide], a partial agonist, in two models of cocaine abuse in rhesus monkeys. To establish a dose range and time course of effects, both compounds were shown to block quinpirole-induced yawning when administered i.m. 15, 30, or 120 min before quinpirole. Next, rhesus monkeys were trained to discriminate i.m. injections of saline (0.5 ml) and cocaine (0.3 mg/kg). Neither $D_3$ compound (0.03–3.0 mg/kg; $n = 3$) substituted for cocaine in any monkey. When given in combination with cocaine, CJB 090 but not NGB 2904 attenuated the discriminative stimulus effects of cocaine, shifting the cocaine dose-response curve to the right. In a separate group of monkeys, responding was maintained under a second-order schedule of either food (1.0-g pellets; $n = 3$) or cocaine (0.1 mg/kg/injection; $n = 4$) presentation. When responding was stable, a dose of NGB 2904 (1.0–5.6 mg/kg i.v.) or CJB 090 (0.3–3.0 mg/kg i.v.) was administered for 5 consecutive days, immediately before the session. CJB 090, but not NGB 2904, decreased cocaine- and food-maintained responding. These data indicate that compounds with relatively high affinity and selectivity for the $D_3$ receptor can attenuate the discriminative and reinforcing stimulus effects of cocaine while not producing cocaine-like effects. The present findings support the continued examination of $D_3$ compounds as pharmacological tools for better understanding the role of this receptor subtype in cocaine addiction and as potential lead compounds for novel therapeutic agents.

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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 D3 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 D3/D2 agonist 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; Beardsey 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 antagonist 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 D3 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-
a 0.5-ml injection. All solutions were delivered i.m. to 10 mg/ml, and filtered. If necessary, heat and sonication were synthesized as described in Newman et al. (2003) (see Fig. 1) 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: SSSCSSCSSC. 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.E.M.) 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

![Fig. 1. Chemical structures of NGB 2904 and CJB 090.](image-url)
dose-response curve determination; however, the percent cocaine-appropriate responding was not included in any analysis. Linear regression models were used to calculate ED₅₀ values for each subject and D₃ pretreatment dose. For all analyses, p < 0.05 was considered statistically significant. In addition, effects on unconditioned behaviors, such as not consuming food pellets earned or behaviors that may be considered stereotypic or suggestive of catastrophes, were noted at the end of each test session.

**Drugs.** (−)-Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) was dissolved in 0.9% saline and diluted to concentrations of 0.3 to 5.6 mg/ml. CJB 090 and NGB 2904 (NIDA-Intramural Research Program) were diluted to concentrations of 0.1 to 10 mg/ml. Haloperidol (McNeil Pharmaceuticals, Raritan, NJ) was dissolved in 0.9% saline and diluted to concentrations of 0.01 to 10 mg/ml. 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 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 presession, 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 ANOVA with cocaine dose as the factor. 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-
pirole representing the peak of the curve (17.5 ± 2.2 yawns). This dose of quinpirole 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 quinpirole-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 quinpirole (all p < 0.01). NGB 2904 (3.0 mg/kg) significantly attenuated quinpirole-induced yawning when administered 15 or 120 min before quinpirole (p < 0.05) but not when administered 30 min before quinpirole (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 quinpirole (Fig. 2B). No monkeys were administrated 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 dose-dependently 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 (0.06 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...
one monkey. 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-admini-
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 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

**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
A quinpirole dose-response curve was mediated via D₃ receptors. Collins et al. (2005) concluded that the ascending limb of the D₂/D₃ agonist quinpirole was shown to significantly induce yawning in monkeys (present study). In the present study, NGB 2904 had equivocal effects on cocaine-maintained behavior (Collins et al., 2005). Extending earlier results in rats, the D₃ receptor transfected cell lines (Grundt 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, 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,
D3 Receptors and Cocaine Abuse

581

 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). 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 (K1 = 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-stimulated mitogenesis in Chinese hamster ovary cells, would show any agonist activity in vivo. In summary, we found that the D3 partial agonist CJB 090, but not the D3 antagonist NGB 2904, was able to block the discriminative stimulus effects of cocaine and to decrease cocaine self-administration. There was no evidence that CJB 090 produced DA agonist effects in vivo. It remains possible that the behavioral effects observed with CJB 090 were due to D3 receptor antagonism, although blockade of the behavioral effects of cocaine occurred at doses that did not induce catalepsy. These findings support the continued examination of compounds acting at D3 receptors as pharmacological tools for further understanding the mechanistic underpinnings of cocaine addiction and as potential leads for therapeutic agents.

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