Influence of Cocaine History on the Behavioral Effects of Dopamine D₃ Receptor-Selective Compounds in Monkeys

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Although dopamine D₃ receptors have been associated with cocaine abuse, little is known about the consequences of chronic cocaine on functional activity of D₃ receptor-preferring compounds. This study examined the behavioral effects of D₃ receptor-selective 4-phenylpiperazines with differing *in vitro* functional profiles in adult male rhesus monkeys with a history of cocaine self-administration and controls. *In vitro* assays found that PG 619 (*N*-(3-hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide HCl) was a potent D₃ antagonist in the mitogenesis assay, but a fully efficacious agonist in the adenylyl cyclase assay, NGB 2904 (*N*-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-9*H*-fluorene-2-carboxamide HCl) was a selective D₃ antagonist, whereas CJB 090 (*N*-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide HCl) exhibited a partial agonist profile in both *in vitro* assays. In behavioral studies, the D₃ preferential agonist quinpirole (0.03–1.0 mg/kg, i.v.) dose-dependently elicited yawns in both groups of monkeys. PG 619 and CJB 090 elicited yawns only in monkeys with an extensive history of cocaine, whereas NGB 2904 did not elicit yawns, but did antagonize quinpirole and PG 619-elicited yawning in cocaine-history monkeys. In another experiment, doses of PG 619 that elicited yawns did not alter response rates in monkeys self-administering cocaine (0.03–0.3 mg/kg per injection). Following saline extinction, cocaine (0.1 mg/kg) and quinpirole (0.1 mg/kg), but not PG 619 (0.1 mg/kg), reinstated cocaine-seeking behavior. When given before a cocaine prime, PG 619 decreased cocaine-elicited reinstatement. These findings suggest that (1) an incongruence between *in vitro* and *in vivo* assays, and (2) a history of cocaine self-administration can affect *in vivo* efficacy of D₃ receptor-preferring compounds PG 619 and CJB 090, which appear to be dependent on the behavioral assay.

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INTRODUCTION

With over 2000 people initiating use every day and 1.9 million Americans reporting past-month use, cocaine abuse continues to pose a public health concern in the United States (Substance Abuse and Mental Health Services Administration SAMHSA Office of Applied Studies, 2008). Despite tremendous efforts to discover a pharmacotherapy to treat cocaine addiction, no approved medications are currently available (Mello and Negus, 1996; Carroll *et al*, 1999; Vocci *et al*, 2005; Karila *et al*, 2008).

Although many neurotransmitter systems are implicated in cocaine abuse, the dopamine (DA) system has been a primary target for treatment development owing to its extensive role in

cocaine abuse (see review by Xi and Gardner, 2008). Cocaine elevates extracellular DA (Di Chiara and Imperato, 1988; Ritz *et al*, 1987) that subsequently stimulates DA D1- and D2-like receptors (Kebabian and Caine, 1979; Missale *et al*, 1998). The DA D2-like family of receptors, comprised of the D₂, D₃, and D₄ receptor subtypes, are thought to mediate directly the reinforcing effects of cocaine (Caine *et al*, 2000). D₃ receptors not only play a significant role in mediating the reinforcing effects of cocaine (Pilla *et al*, 1999; Nader and Mach, 1996, Caine and Koob, 1993; Self *et al*, 1996), but are also implicated in chronic cocaine use by the demonstration of higher D₃ receptor densities in post-mortem cocaine-overdose victims compared with drug-naïve controls in autoradiography studies (Staley and Mash, 1996). Because D₃ receptors exhibit a restricted pattern of localization to limbic brain regions (Sokoloff *et al*, 1990; Levant, 1997), in theory, binding at this receptor should not lead to extrapyramidal side effects or disruption of natural behaviors such as feeding. As a result, the development of novel pharmacological tools has increased interest as potential therapeutic agents (Heidbreder and Newman, 2010).

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Extensive medicinal chemistry and structure–activity relationships have been described and reviewed recently (Boeckler and Gmeiner, 2006; Zhang et al, 2007), especially for the 4-phenylpiperazine series of D_3-selective ligands. Despite nearly 80% sequence homology in the transmembrane regions of the D_2 and D_3 receptor subtypes, the discovery of compounds with >100-fold D_3 selectivity over D_2 and other receptor systems has been achieved. These compounds are typically evaluated for intrinsic activity in cell-based assays using cell lines transfected with hD_3 or hD_2 receptors and most compounds in this structural class are antagonists or partial agonists. However, to date, tractable structure–activity relationships that predict intrinsic activity have remained elusive. Moreover, a correlation between intrinsic activity as measured in vitro and behavioral actions of these compounds has not been established (Heidbreder and Newman, 2010). The compounds studied in the present experiments, the D_3 compound PG 619 (N-(3-hydroxy-4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide HCl), the D_3 partial agonist CJB 090 (N-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide HCl), and the prototypical D_3 antagonist NGB 2904 (N-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-9H-fluorene-2-carboxamide HCl), had previously been characterized in vitro using mitogenesis assays (Grundt et al., 2005, 2007). To further characterize these compounds, another in vitro assay was utilized, adenylyl cylase inhibition (Taylor et al., 2010), followed by examination of the behavioral effects of each compound in several in vivo functional assays. Thus, one aim of this study was to directly compare D_3-selective compounds using two in vitro assays with several behavioral measures in monkeys to better determine if either in vitro assay was more predictive of D_3 activity in vivo.

The in vivo evaluation of novel D_3 compounds has included unconditioned and conditioned behaviors (see Newman et al, 2005; Xi and Gardner, 2008; Heidbreder and Newman, 2010). In this study, we utilize drug-elicited yawning as an unconditioned behavior that has been associated with D_3 receptor activation (eg, Collins et al, 2005; Martelle et al, 2007). Of particular interest is the observation that, in vivo, full agonists such as quinpirole elicit yawning, but partial agonists do not show agonist activity (Martelle et al, 2007). In this study, we examined the ability of quinpirole, PG 619, CJB 090, and NGB 2904 to elicit yawns in cocaine-naïve and cocaine-experienced monkeys. All of the earlier work used cocaine-naïve animals or animals with a minimal cocaine history. The hypothesis was that the extensive cocaine histories of the monkeys would lead to different behavioral effects of D_3 receptor-selective compounds compared with effects observed in cocaine-naïve monkeys. We also utilized the cocaine-experienced monkeys to examine these drugs on cocaine self-administration and reinstatement. An earlier study reported that CJB 090 decreased cocaine self-administration in rhesus monkeys self-administering cocaine on a second-order schedule (Martelle et al., 2007), while showing no effect on cocaine reinforcement or reinstatement in squirrel monkeys (Achat-Mendes et al, 2009). In this study, we extended this comparison to another 4-phenylpiperazine, PG 619, which binds to D_3 receptors with ~100-fold selectivity over D_2 compared with the ~50-fold D_3 selectivity of CJB 090 and ~56-fold D_3 selectivity of NGB 2904 (Newman et al, 2005; Grundt et al, 2007). The use of multiple behavioral baselines allowed for the assessment of D_3 receptor contribution to the behavioral effects of cocaine and show that D_3 receptor mediation is dependent on the behavioral assay.

MATERIALS AND METHODS

Experiment 1: In Vitro Studies

Adenylyl cyclase. Complete details are described in Taylor et al (2010). Briefly, a whole cell-cyclic AMP accumulation assay in stably transfected HEK cells expressing human DA D_2 or D_3 receptors was used. The endogenous ATP pool was radioactively labeled by preincubating the cells in serum-free DMEM/25 mM HEPES medium containing 2 μCi/ml of [2,8-³H]adenine (20–Ci/mmol) in a 5% CO_2 incubator at 37 °C for 75 min and the radioactive medium was removed by aspiration. Added to the flask was serum-free DMEM (5.6 ml) containing 25 mM HEPES, pH 7.4, and 0.1 mM of the phosphodiesterase inhibitor isobutylmethylxanthine. A 400-μl aliquot of cells was removed and added to 50 μl of forskolin (100 μM) and 50 μl of PG 619, CJB 090, NGB 2904, or quinpirole in DMEM-HEPES. The cells were incubated for 20 min at 37 °C (vortexing every 5 min) and the reaction was terminated by adding 0.5 ml of 10% trichloroacetic acid (w/v) containing 1.0 mM unlabeled cyclic AMP. As described by Saloman et al (1974), both the [³H]cyclic AMP and the [³H]ATP fractions were collected following separation using columns of Dowex AG-50W-X4 and alumina. The final yield of [³H]cyclic AMP was corrected for column recovery of unlabeled cyclic AMP determined spectrophotometrically (OD$_{259}$). The primary dependent variable is the percent conversion of [³H]ATP into [³H]cyclic AMP.

Experiments 2–4: In Vivo Studies

Subjects. For all experiments, adult male rhesus monkeys (Macaca mulatta) served as subjects (Table 1). At the start of this study, four monkeys (R-1374, R-1375, R-1377, R-1381) had a history of cocaine self-administration (Czoty et al, 2007), with intake ranging from 635 to 1385 mg/kg, and four monkeys were drug naïve. Monkeys were individually housed in stainless-steel cages with ad libitum access to water and visual and auditory contact with other monkeys. Body weights were maintained at 95% of free-feeding weights with standard laboratory chow (Purina LabDiet 5045) and fresh produce at least three times a week. All monkeys were fitted with an aluminum collar (Primate Products, Redwood City, CA) and trained to sit calmly in a standard primate chair (Primate Products). All monkeys were implanted with an indwelling intravenous catheter (internal or external jugular or femoral vein) and a vascular access port (Access Technologies) under sterile surgical conditions as described previously (Czoty et al, 2007). To ensure catheter patency, the catheter was flushed with heparinized saline (100 U/ml) following each experimental session. All manipulations were performed in accordance with the 2003 National Research Council *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* and were approved by the Wake Forest University Institutional Animal Care and Use Committee.

Table I Experimental Subjects

Subject	Age	Weight (kg)	Lifetime cocaine intake (mg/kg)	Experiments participated in
R-1374	13	11.2	1385	Experiments 2, 3, and 4
R-1375	13	10.1	635	Experiments 2 and 4
R-1377	12	9.7	1089	Experiments 3 and 4
R-1381	13	10.4	922	Experiments 2, 3, and 4
R-1424	11	10.1	0	Experiment 2
R-1661	13	9.0	0	Experiment 2
R-1662	13	7.2	0	Experiment 2
R-1663	13	8.2	0	Experiment 2

Apparatus. Cocaine self-administration and reinstatement studies were carried out in ventilated, sound-attenuating chambers ($1.5 \times 0.74 \times 0.76 \, \text{m}^3$; Med Associates, East Fairfield, VT). Each chamber consisted of two response keys (5 cm wide) located on one side of the chamber with a horizontal row of three stimulus lights 14 cm above each response key, a food receptacle located between the response keys for delivery of 1-g banana-flavored food pellets and an infusion pump (Cole-Parmer Inc, Chicago, IL) for delivering drugs.

Drugs. (−)Cocaine HCl (NIDA, Bethesda, MD), quinpirole (Sigma-Aldrich, St Louis, MO), and PG 619 (NIDA-Intramural Research Program, Baltimore, MD and synthesized as described in Grundt *et al*, 2005) were dissolved in 0.9% sodium chloride. CJB 090 and NGB 2904 were synthesized as described in Grundt *et al* (2005) and were dissolved in 25% β-cyclodextrin. Quinpirole, PG 619, CJB 090, and NGB 2904 (see Figure 1) were diluted to concentrations of 1.0 and 5.0 mg/ml, whereas different doses of cocaine were made by adjusting the drug concentration. Heat and sonication were used for solubility purposes and all drugs were adjusted to pH 6–7 and filtered before administration.

Experiment 2: Effects of D$_3$ Compounds on Drug-Elicited Yawning

Procedure. Monkeys were seated in a primate restraint chair and placed in a testing room in front of a video camera. First, single injections of saline (1.0 ml, i.v.) and quinpirole (0.03–1.0 mg/kg, i.v.) were tested in all monkeys, with yawning measured over 30 min beginning immediately after the injection. Quinpirole did not elicit yawning in R-1377 and he was removed from the study. Next, the following drugs were tested at doses of 0.03–1.0 mg/kg (i.v.) and in the following order: PG 619, CJB 090, and NGB 2904. For each drug, doses were tested in random order with a minimum of a 24-h washout period between test sessions. After completion of all dose–response curves, antagonism studies were carried out in the three cocaine-history monkeys. For these studies, NGB 2904 (0.1–0.3 mg/kg) was administered 15 min before maximally effective doses of quinpirole and PG 619 (0.1 mg/kg), followed by a 1.0 ml saline flush. Antagonism of quinpirole-elicited yawning by
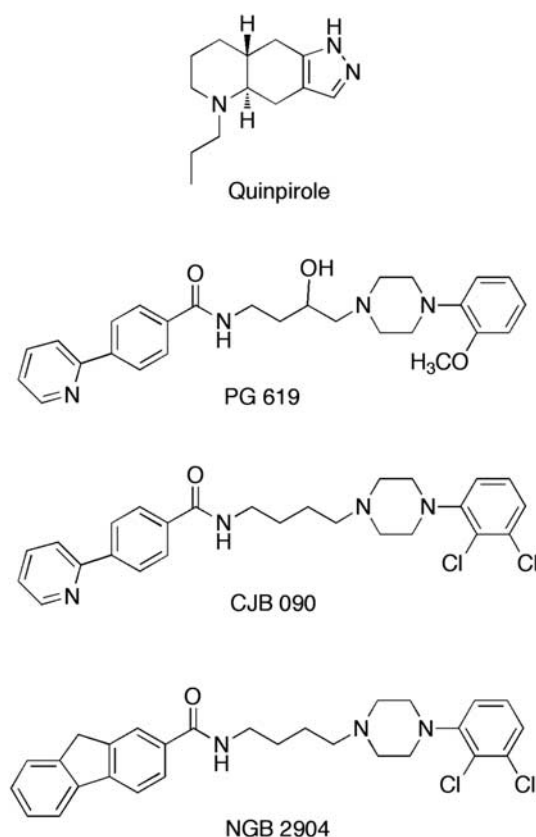
Figure 1 Chemical structure of quinpirole and the 4-phenylpiperazines PG 619 (N-(3-hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide HCl), CJB 090 (N-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide HCl), and NGB 2904 (N-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-9H-fluorene-2-carboxamide HCl).

CJB 090 has been reported by Martelle *et al* (2007). Immediately following the injection, the technician left the room and the monkey was filmed for 30 min. Tapes were later scored for occurrences of yawning, which was defined as full extension of the jaws, withdrawal of the lips, and exposure of the teeth (Code and Tang, 1991). The experimenter and another observer blind to experimental conditions had inter-observer variability <5%.

Data analysis. The primary dependent variable was the number of yawns during the 30-min observation period. Data were analyzed with a two-way repeated measures ANOVA using group (cocaine history and drug naive) and drug dose, followed by *post hoc* Bonferroni *t*-tests (significance level, $p < 0.05$).

Experiment 3: Effects of PG 619 on Cocaine Self-Administration

Procedure. Three cocaine-history monkeys (Table 1) were used in this experiment. Before the start of the session, the monkey was seated in a primate restraint chair, the animal's back containing the port was cleaned with betadine and 95% EtOH and a 22-G Huber Point Needle (Access Technologies) was inserted into the port. The Huber Point Needle was connected to an infusion pump that delivered drugs at a rate of approximately 1.5 ml/10 s. Each animal

received a 3-s prime of the cocaine dose available during the self-administration session. The session began with illumination of the white stimulus light located above one of the response keys. Cocaine reinforcement (0.03 mg/kg) was contingent upon responding on a fixed-ratio (FR) 30 schedule of reinforcement. Following completion of the FR requirement, the white light extinguished, a red light above the lever was illuminated for 10 s and cocaine was delivered; a 30-s time out followed each injection. The session was terminated once the monkey received a total of 15 injections or 2 h elapsed.

A cocaine dose–response curve was generated for each animal before examining PG 619 as a pretreatment. When self-administration of the baseline dose (0.03 mg/kg) was deemed stable ($\pm 20\%$ of the mean response rate for three consecutive sessions, with no trends), saline was substituted for cocaine for at least five consecutive sessions and until responding declined to $< 20\%$ of baseline. After a return to baseline, different doses of cocaine were substituted for the baseline dose for a minimum of five sessions and until response rates were deemed stable; doses were tested in random order and there was a return to the baseline dose between substitution studies. After completion of the cocaine dose–response curve, pretreatment injections of PG 619 (0.1–1.0 mg/kg, i.v.) were administered 10 min before the self-administration session. This pretreatment time was based on the time course of drug-elicited yawning in these same animals. We also tested 3.0 mg/kg PG 619 in one monkey, but it induced catalepsy and hypothermia that persisted for nearly 3 h. Because these behaviors are attributed to D₂ receptors, this dose was not tested in any other animal (Collins et al, 2007; Achat-Mendes et al, 2010). All doses of PG 619 were tested in random order and double determined; at least four cocaine self-administration doses were examined in each monkey. The cocaine dose–response curve was redetermined following PG 619 evaluation to ensure a stable baseline for each animal.

Data analysis. The primary dependent variable was response rate (total responses per session divided by session length). For the analysis of the cocaine dose–response curve, mean data from the three sessions satisfying the stability criteria for cocaine self-administration were included in the analysis using a one-way ANOVA with

cocaine dose as a factor. Bonferroni *post hoc* testing was used to determine if PG 619 had significant effects on cocaine self-administration. For all analyses, $p < 0.05$ was considered statistically significant.

Experiment 4: Effects of Quinpirole and PG 619 on Cocaine-Elicited Reinstatement

Procedure. All four cocaine-history monkeys were used in this study. Saline was substituted for cocaine until response rates decreased to $< 20\%$ of baseline rates. Next, non-contingent injections (i.v.) of 0.1 mg/kg cocaine, PG 619, and quinpirole, administered immediately before the session, were examined. The doses of PG 619 and quinpirole were chosen because they elicited the maximum number of yawns in these monkeys, whereas the cocaine dose was the lowest dose that reliably reinstated cocaine seeking in these monkeys. There were at least two sessions of saline self-administration between each priming test session. PG 619 (0.03–1.0 mg/kg, i.v.) and quinpirole (0.1 mg/kg, i.v.) were also administered 10 min before the non-contingent cocaine prime to evaluate their effects on cocaine-elicited reinstatement.

Data analysis. Each monkey's response rate following the non-contingent 0.1 mg/kg cocaine injection was normalized to 100%. The primary dependent variable was percent cocaine-prime response rate for quinpirole and PG 619 alone and in combination with 0.1 mg/kg cocaine. Data were analyzed with a one-way ANOVA with Tukey's *post hoc* testing.

RESULTS

Experiment 1: *In Vitro* Comparison of PG 619, CJB 090 and NGB 2904

In vitro evaluation of the three 4-phenylpiperazines can be seen in Table 2. All three compounds were evaluated in competition binding assays in HEK 293 cells transfected with either human D₂L or D₃ DA receptors, as described previously (Grundt et al., 2005), and bind with high affinity to D₃ receptors and moderate affinity to D₂ receptors, with PG 619 having the highest D₃/D₂ selectivity (~ 100-fold;

Table 2 *In vitro* Data for 4-Phenylpiperazines

| Compound	D₃ K_i (nM ± SEM)	D₂ K_i (nM ± SEM)	D₃/D₂ affinity	D₃ mitogenesis IC₅₀ in nM ± SEM (% maximum stimulation)	D₂ mitogenesis IC₅₀ in nM ± SEM (% maximum stimulation)	D₃ adenylyl cyclase % maximum stimulation[d]	D₂ adenylyl cyclase % maximum stimulation[d]
PG 619	2.8 ± 0.8[a]	284 ± 28[a]	100	12.75 ± 1.26[a,b] (0)	ND[c]	106 ± 23.8	8.5 ± 6.6
CJB 090	0.5 ± 0.2[e]	24.8 ± 3.9[e]	62	6.3 ± 1.7[e] (29.7)	92.0 ± 4.9[e] (22.4)	39.9 ± 8.5[f]	38.6 ± 1.7[f]
NGB 2904	2.0 ± 0.4[e]	112 ± 22[e]	56	14.4 ± 0.5[b,e] (0)	1280 ± 270[b,e] (0)	5.5 ± 1.6[d]	42.7 ± 5.1[d]

[a]Grundt et al (2007).
[b]Antagonist.
[c]ND = not determined owing to low D₂ affinity.
[d]%Maximal inhibition of adenylyl cyclase was achieved using the test ligand at a concentration of $\sim 10 \times$ the K_i values using methods described in Taylor et al (2010).
[e]Grundt et al (2005).
[f]Taylor et al (2010).
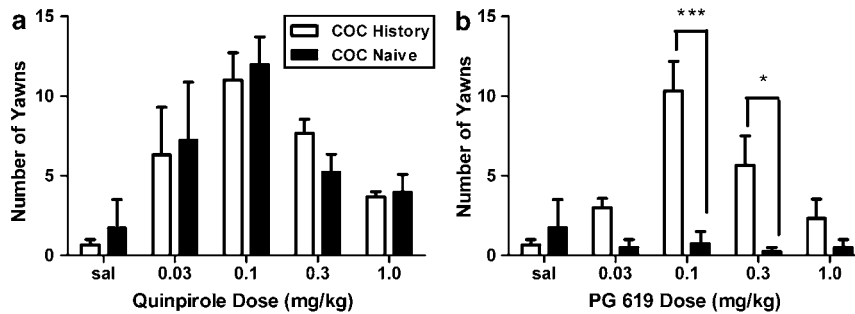
Figure 2 Dose-dependent yawning elicited by (a) the D_3 agonist quinpirole (0.03–1.0 mg/kg, intravenously (i.v.)) and (b) the D_3 receptor-selective compound PG 619 (N-(3-hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide HCl) (0.03–1.0 mg/kg, i.v.) in cocaine-history ($n = 3$, open bars) and cocaine-naïve monkeys ($n = 4$, closed bars). Data are represented as mean ± SEM number of yawns observed over a 30-min period. Asterisks (*$p < 0.05$; ***$p < 0.001$) indicate statistical differences between groups.

Grundt *et al*, 2007). PG 619 and NGB 2904 antagonized quinpirole-induced mitogenesis in hD_3-transfected CHO cells with similar potencies, whereas CJB 090 was a partial agonist in this assay, showing ∼30% agonist activity as compared with quinpirole (Grundt *et al*, 2005). In the second adenylyl cyclase assay, a concentration of approximately 10 times the K_i at D_3 or D_2 receptors was used to stimulate adenylyl cyclase in hD_3- or hD_2-transfected HEK 293 cells. In the D_2 assay, PG 619 did not show a % maximum stimulation commensurate with an agonist profile (<20% maximal stimulation), but was fully efficacious in the D_3 assay (100%) and equivalent to quinpirole as a D_3 agonist. In contrast, CJB 090 was a partial agonist (by definition, between 20 and 70% maximal stimulation) at both D_2 and D_3 receptors and NGB 2904 was an antagonist at D_3.

Experiment 2: Effects of D_3 Compounds on Drug-Elicited Yawning

Following saline administration, monkeys yawned less than five times during the 30-min observation period (Figure 2a). Quinpirole (0.03–1.0 mg/kg) significantly ($F(4,20) = 9.45$, $p < 0.001$) elicited yawns in a dose-dependent manner as characterized by the inverted U-shaped function of dose (Figure 2a). There were no differences between groups, with peak yawns elicited by 0.1 mg/kg quinpirole. In contrast to the effects of quinpirole, PG 619 (0.03–1.0 mg/kg) significantly ($F(4,20) = 9.10$, $p < 0.001$) elicited yawns only in monkeys with an extensive history of cocaine self-administration (Figure 2b, open bars, and Figure 3, triangles). In these monkeys, the shape of the PG 619 dose–response curve was characterized as an inverted U-shaped function of dose, with peak yawns elicited following 0.1 mg/kg PG 619. In the cocaine-exposed monkeys, the frequency of yawns elicited by PG 619 was not different from the number of yawns elicited by quinpirole (Figure 3, triangles *vs* squares). In the cocaine-naïve monkeys, no dose of PG 619 elicited yawns (Figure 2b, closed bars). Another D_3 partial agonist, CJB 090 (0.03–1.0 mg/kg), also significantly ($F(4,40) = 27.11$, $p < 0.001$) elicited yawns in cocaine-history monkeys (Figure 3, diamonds), whereas the D_3 antagonist NGB 2904 (0.03–1.0 mg/kg) did not elicit yawns at the doses tested (Figure 3, inverted triangles). As with the quinpirole and PG 619 dose–response curves, the CJB 090

dose–response curve was characterized as an inverted U-shaped function of dose, with peak yawns elicited by 0.1 mg/kg. In drug combination studies in cocaine-history monkeys, NGB 2904 (1.0 mg/kg) significantly ($F(1,8) = 76.56$, $p < 0.001$) antagonized 0.1 mg/kg quinpirole- and 0.1 mg/kg PG 619-elicited yawning (Figure 3d).

Experiment 3: Effects of PG 619 on Cocaine Self-Administration

Cocaine maintained responding in all monkeys, with response rates significantly ($F(4,60) = 74.49$, $p < 0.001$) higher than rates of responding leading to saline injections (Figure 4, closed circles). Although the shape of the dose–response curves were generally characterized as an inverted U-shaped function of dose, peak rates of responding occurred following different doses in each monkey (0.01 mg/kg in R-1381, 0.03 mg/kg in R-1374, and 0.1 mg/kg in R-1377). When administered before sessions of cocaine availability, no dose of PG 619 (0.1–1.0 mg/kg) significantly affected cocaine-maintained responding across a range of cocaine doses (Figure 4).

Experiment 4: Effects of Quinpirole and PG 619 on Cocaine-Elicited Reinstatement

When saline was substituted for cocaine, response rates decreased by at least 80% of baseline within five sessions. A dose of PG 619 that robustly elicited yawning in these monkeys (0.1 mg/kg) did not reinstate cocaine seeking in any monkey (Figure 5, left panel, open triangle). In contrast, 0.1 mg/kg cocaine resulted in significant ($t(3) = 5.59$, $p < 0.05$) increases in saline-contingent response rates (2163, 1454, 2100, and 2163% in R-1374, R-1375, R-1377, and R-1381, respectively). The mean increase of 1970% served as baseline for drug combination studies (Figure 5, open circle). Quinpirole (0.1 mg/kg) also reinstated drug-seeking behavior, although the effects were less than cocaine-induced reinstatement (Figure 5, left panel, open square). Although PG 619 did not induce reinstatement, it attenuated cocaine-induced reinstatement by approximately 50% (Figure 5, filled triangles). All doses of PG 619 were equally effective. Quinpirole (0.1 mg/kg) did not alter the ability of 0.1 mg/kg cocaine to reinstate drug seeking (Figure 5, filled square).
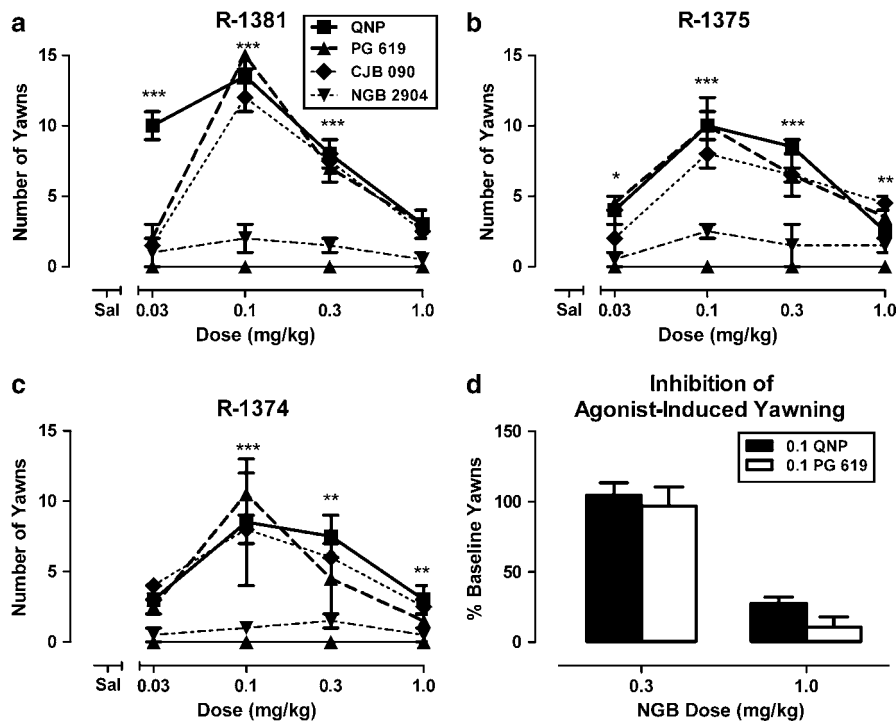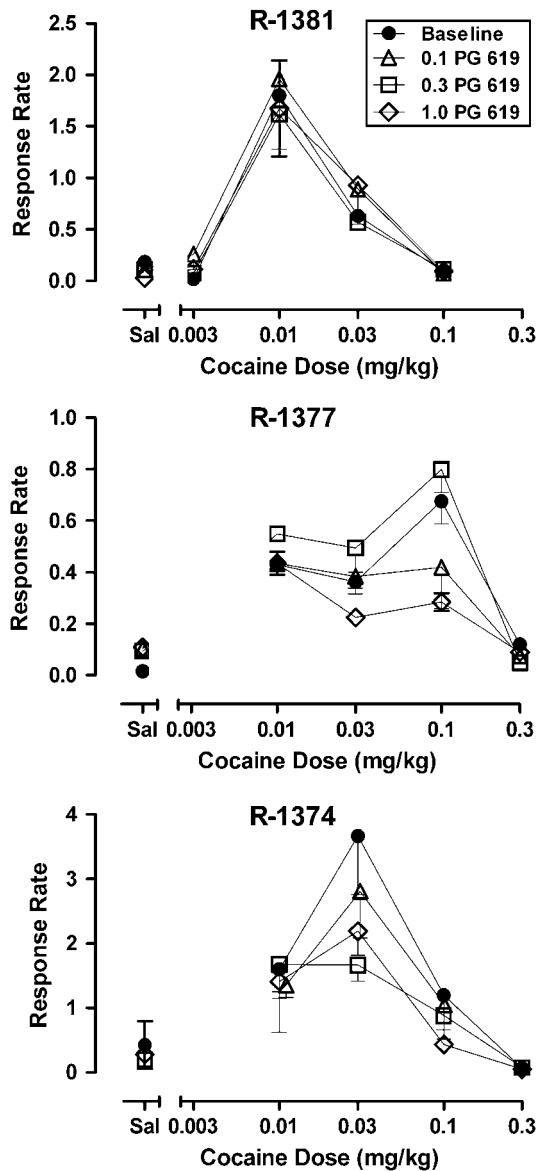
Figure 3 (a–c) Effects of quinpirole, PG 619 (*N*-(3-hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide HCl), CJB 090 (*N*-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide HCl), and NGB 2904 (*N*-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-9*H*-fluorene-2-carboxamide HCl) on drug-elicited yawning in cocaine-history monkeys. Data are represented as mean ± SEM number of yawns observed over a 30-min period. Asterisks (**$p < 0.01$; ***$p < 0.001$) indicate statistical difference from saline. (d) Effects of NGB 2904 (0.3–1.0 mg/kg) on quinpirole- and PG 619-elicited yawning in cocaine-history monkeys ($n = 3$). Asterisks (**$p < 0.01$) indicate statistical difference when monkeys were treated with NGB 2904 before quinpirole (QNP, closed bars) or PG 619 (open bars).

DISCUSSION

The main purpose of this study was to characterize the behavioral effects of the DA D₃ compound PG 619 in rhesus monkeys. In addition, PG 619 and several DA D₃ receptor-selective compounds with varying intrinsic efficacies as measured *in vitro* were directly compared using two different assays and with subsequent behavioral characterization of these compounds as agonists, partial agonists, or antagonists. An additional aim was to characterize the effects of PG 619 in cocaine-naïve and cocaine-history monkeys. We had hypothesized that if chronic cocaine exposure altered D₃ receptor densities (Staley and Mash, 1996; Neisewander *et al*, 2004) and the effects of full D₃ agonists (Nader and Mach, 1996; Collins and Woods, 2009), then it is plausible that the behavioral effects of D₃ receptor-selective compounds would also be influenced by a history of cocaine self-administration. In control monkeys, PG 619 did not elicit yawning, but in cocaine-history monkeys it appeared to have unconditioned behavioral effects similar to the full agonist quinpirole. A similar behavioral profile was noted with the partial D₃ agonist CJB 090, but not the D₃ receptor-selective antagonist NGB 2904. Although PG 619 appeared to function as an agonist when using unconditioned behaviors, it did not reinstate cocaine seeking similar to the D₃ agonist quinpirole, but did attenuate cocaine-induced reinstatement. The behavioral model of cocaine abuse influenced the characterization of PG 619, because doses that attenuated reinstatement did not block cocaine self-administration.
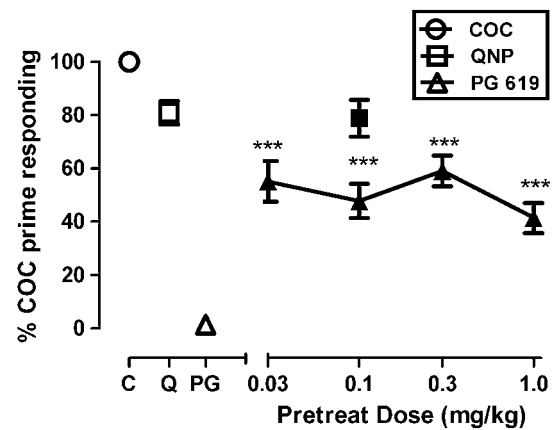
Initially, the *in vivo* effects of PG 619 were perplexing because based on the *in vitro* mitogenesis assay PG 619 is an antagonist at the DA D₃ receptor subtype. Therefore, it was hypothesized that *in vivo* PG 619 would have activity similar to NGB 2904. However, the results from the adenylyl cyclase inhibition assay indicated that PG 619 can also act as a fully efficacious agonist. We have previously published the structure–activity profile of a panel of substituted 4-phenylpiperazines structurally related to PG 619, which indicated that the efficacy of this class of compound can differ in these two functional assays (Taylor *et al*, 2010). That finding is consistent with the theory of functional selectivity, which proposes that a ligand can have differing efficacy and/or potency for different receptor-associated effector pathways (Gay *et al*, 2004; Kenakin, 2002; Clarke, 2005). Therefore, a compound that is an antagonist for one receptor-linked second messenger system might also show some degree of intrinsic efficacy for a second pathway. For the 4-phenylpiperazines that we have evaluated thus far, PG 619 is unique in that it appears to be a neutral antagonist for the pertussis toxin-sensitive mitogenic assay (Pilon *et al*, 1994), although being a full agonist for the adenylyl cyclase inhibition assay. This finding suggests that while both pathways are mediated by G proteins, it may be the adenylyl cyclase pathway that is directly involved in the yawning response observed in the monkeys with a cocaine history, which makes *in vivo* responses of PG 619 appear to be more like the partial agonist CJB 090.

In this study, the ability of the full agonist quinpirole to elicit yawning was not influenced by a history of cocaine

Figure 4 Effects of PG 619 (N-(3-hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide HCl) (0.1–1.0 mg/kg) on response rates (responses per s) maintained by cocaine (0.003–0.3 mg/kg) in cocaine-history monkeys ($n = 3$) responding under a fixed-ratio 30 schedule of reinforcement. Data are represented as mean ± SEM.

Figure 5 (Left panel) Effects of 0.1 mg/kg cocaine (C), quinpirole (Q), and PG 619 (N-(3-hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide HCl) (PG) administered alone (open symbols) to reinstate cocaine seeking. (Right panel) Effects of PG 619 (0.03–1.0 mg/kg) and quinpirole (0.1 mg/kg) to attenuate cocaine-induced reinstatement when administered 10 min before 0.1 mg/kg cocaine (filled symbols). Data are represented as mean ± SEM percent cocaine-prime responding. Asterisks (***$p < 0.001$) indicate statistical difference from cocaine-elicited responding.

exposure, nor was the inactivity of the D₃ receptor antagonist NGB 2904. However, both PG 619 and CJB 090 elicited yawns in monkeys with an extensive cocaine history, but not in cocaine-naïve monkeys. Earlier studies have consistently shown that D₃ partial agonists do not display agonist-like effects *in vivo* (see Heidbreder and Newman, 2010). Results from this study are the first data showing *in vivo* agonist activity of drugs that do not show full D₃ agonist profiles in the *in vitro* mitogenesis assay. The doses of PG 619 that were behaviorally active in yawning did not affect rates of cocaine self-administration. Interestingly, while the ability of PG 619 to elicit yawning was similar to quinpirole, only quinpirole reinstated cocaine seeking, whereas PG 619 attenuated cocaine-induced reinstatement,

suggesting differential modulation by D₃ receptors of the multiple stimulus effects of cocaine. Although we did not test CJB 090 and cocaine in reinstatement, Achat-Mendes et al (2009) found CJB 090 ineffective in attenuating cocaine-primed reinstatement in squirrel monkeys. Clearly, additional studies are warranted to better understand the relationship between functional selectivity at D₃ receptors, as determined *in vitro*, with behavioral effects involving cocaine.

Woods, Collins, and co-workers have extensively characterized the role of DA D₃ and D₂ receptors in quinpirole-elicited yawning (Collins *et al*, 2005, 2007, 2008). Quinpirole, like other D₃ agonists, has affinity at both D₃ and D₂ receptors (Gehlert *et al*, 1992; Levant *et al*, 1992), and as such, the shape of the quinpirole dose–response curve is an inverted U-shaped function of dose, with the ascending limb mediated primarily by D₃ receptors and the descending limb by concomitant D₂ receptor stimulation (Collins *et al*, 2005, 2007). We had previously reported a similar shape of the quinpirole-elicited yawning dose–response curve in cocaine-naïve rhesus monkeys (Martelle *et al*, 2007; Hamilton *et al*, 2010), which was replicated in this study. Furthermore, unpublished data from our laboratory indicates that quinpirole-elicited yawning is specific to D₃ receptors as hypothermia is not elicited at the doses on the ascending limb (0.01–0.1 mg/kg). An extensive history of cocaine self-administration did not alter the shape, potency, or efficacy of quinpirole-elicited yawning. Interestingly, cocaine exposure does appear to alter the potency of low-efficacy D₃ compounds as lower doses of CJB 090 and NGB 2904 elicited and antagonized, respectively, agonist-elicited yawning in cocaine-exposed monkeys compared with the doses that inhibited quinpirole-elicited yawning in cocaine-naïve rhesus monkeys (Martelle *et al*, 2007). Therefore, the potency of low efficacy agonists may be differentially affected as a function of cocaine exposure. Earlier work in

monkeys (Nader and Mach, 1996) and rodents (Collins and Woods, 2007) has shown that a cocaine history is necessary for D_3 agonists to maintain drug self-administration. Thus, it appears that the influence of cocaine self-administration on the behavioral pharmacology of D_3 agonists, such as quinpirole, is dependent on the *in vivo* assay. These findings indicated that the consequences of cocaine exposure on the behavioral effects of the D_3 partial agonist CJB 090 and the mixed efficacy compound PG 619 were also influenced by the *in vivo* assay, although not in a manner similar to what had been observed with full agonists. In our earlier study, CJB 090 did not elicit yawns in cocaine-naïve monkeys and did not substitute for cocaine in monkeys trained to discriminate cocaine from saline (Martelle *et al*, 2007). However, in monkeys with an extensive cocaine history, both CJB 090 and PG 619 elicited yawns, the magnitude of effects were comparable to quinpirole-elicited yawns. Possible reasons for these 4-phenylpiperazines showing agonist activity following a cocaine history could be due to the reported increase in D_3 receptor densities (Staley and Mash, 1996; Neisewander *et al*, 2004) and/or the decreases in D2-like receptor densities following chronic cocaine self-administration (Moore *et al*, 1998; Nader *et al*, 2002). The fact that the quinpirole dose–response curve was not different in the cocaine-naïve and cocaine-exposed monkeys suggests that changes in receptor densities was unlikely the mechanism mediating this effect. Additional studies, perhaps combining other manipulations known to alter sensitivity to the behavioral effects of D_3 compounds (eg, food restriction *vs* food satiation; Baladi *et al*, 2010) as well as assessing the role of D_2 receptors by measuring body temperature (Collins *et al*, 2007) will be necessary to elucidate the changes in DA receptor function during chronic cocaine exposure. Alternatively, the functional selectivity of the 4-phenylpiperazines such as PG 619 might be affected by chronic cocaine use. However, further research is necessary to fully address this issue.

This study also investigated the ability of PG 619 to attenuate cocaine self-administration. As we had reported earlier with CJB 090 and the D_3 antagonist NGB 2904, PG 619 had no effect on cocaine self-administration. These findings in monkeys may be at odds with the view that D_3 compounds may be effective treatments for drug addiction (Le Foll *et al*, 2005). One possible reason for the lack of effect in this study was the schedule of reinforcement used in the cocaine self-administration paradigm. It has been hypothesized that ratio-based schedules are not as sensitive to the effects of D_3 partial agonists or antagonists as other reinforcement schedules, such as second-order schedules or progressive-ratio schedules (Pilla *et al*, 1999; Xi *et al*, 2006). Our earlier study (Martelle *et al*, 2007) used a second-order schedule and CJB 090 decreased cocaine self-administration. However, in that study, CJB 090 also decreased food-maintained responding under an identical second-order schedule of reinforcement. Thus, while the schedule of reinforcement may increase or decrease sensitivity of D_3 compounds to alter cocaine self-administration, the concurrent study of behavior maintained by a non-drug reinforcer suggests possible non-selectivity in decreasing cocaine-maintained behavior. Future studies should utilize reinforcement schedules that are less sensitive to rate-decreasing effects of the pretreatment drug, such as using cocaine-food choice paradigms (eg, Negus, 2003).

Although the unconditioned behavioral effects of PG 619 were altered by chronic cocaine exposure, the D_3 agonist-like effects of PG 619 were not apparent in reinstatement studies, in contrast to quinpirole, which reinstated cocaine seeking. Thus, it appears that not all behavioral assays deemed sensitive to D_3 agonists will be sensitive to D_3 partial agonists, nor are they equally sensitive to the effects of chronic cocaine exposure. From a cocaine addiction treatment perspective, the fact that PG 619 did not reinstate cocaine-seeking behavior, but did attenuate these effects of cocaine is intriguing and potentially promising from a drug discovery point of view (see also Cervo *et al*, 2003; Achat-Mendes *et al*, 2009).

DISCLOSURE

The authors declare no conflict of interest.

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